



KMJ



KUWAIT MEDICAL JOURNAL

The Official Journal of The Kuwait Medical Association

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Review Article

COVID-19 in nephrologist practice: A review of current knowledge

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ABSTRACT

Objective: To highlight the various aspects of kidney involvement in the context of COVID-19 and focus on the preventive policies that should be considered in vulnerable patients with renal diseases

Design: A comprehensive electronic search of the terms relevant to this review to identify the relevant studies

Setting: An electronic search was conducted in MEDLINE, PubMed, ISI Web of Science and Scopus scientific databases. We also searched the conference proceedings and clinicaltrials.gov database.

Subjects: Articles focusing on COVID-19 and kidney diseases, published up to July 2020

Interventions: Retrieved articles were subtly studied. Data obtained included the mutual relationship between the virus and kidney diseases.

Main outcome measures: The impact of COVID-19 on the kidney and the vulnerability of patients with pre-existing

kidney diseases were analyzed, with special focus on prevention.

Results: The pathogenesis of kidney injury in the setting of COVID-19 is largely explained by two main theories; either direct renal cellular injury or systemic viral effects. Acute kidney injury is frequently reported in patients with severe COVID-19 disease. Glomerulopathies have been reported as well. Patients with pre-existing kidney diseases experienced more severe disease. Several preventive strategies are proposed.

Conclusion: Evidence is growing that kidneys are a potential target for COVID-19. COVID-19 has been found to impair the kidney function in several ways. On the other hand, patients with preexisting kidney disease, those on long-term renal replacement therapy and recipients of kidney transplant are particularly vulnerable to infection and accordingly at risk for fatal morbidity and mortality.

KEY WORDS: acute kidney injury, COVID-19, haemodialysis, transplant infection, viral pneumonia

INTRODUCTION

A novel type of coronavirus, identified as SARS-CoV-2, appeared in Wuhan, China in late 2019 and has been declared by the World Health Organization as a global pandemic in 2020. The first described cases were found to be exposed to the Huanan Seafood Wholesale Market of Wuhan. Accordingly, animal to human transmission has been thought as the principal mode of transmission; however, human to human transmission has also been confirmed via either droplet or airborne transmission^[1]. As of 7th August, 2020, this outbreak caused over 18,902,735 reported infection

cases worldwide and around 709,511 confirmed cases of death^[2]. The diagnosis of patients infected with SARS-CoV-2 relies on the use of real-time reverse transcription polymerase chain reaction on sputum samples or samples obtained by a nasopharyngeal swab. Detection of IgM and IgG antibodies to SARS-CoV-2 can help in screening to detect the percentage of the population that has contracted the infection and that is therefore may be immune^[3]. Chest computed tomography scans may also be beneficial to diagnose COVID-19 in individuals with a high clinical suspicion of infection. The presence of bilateral multilobar

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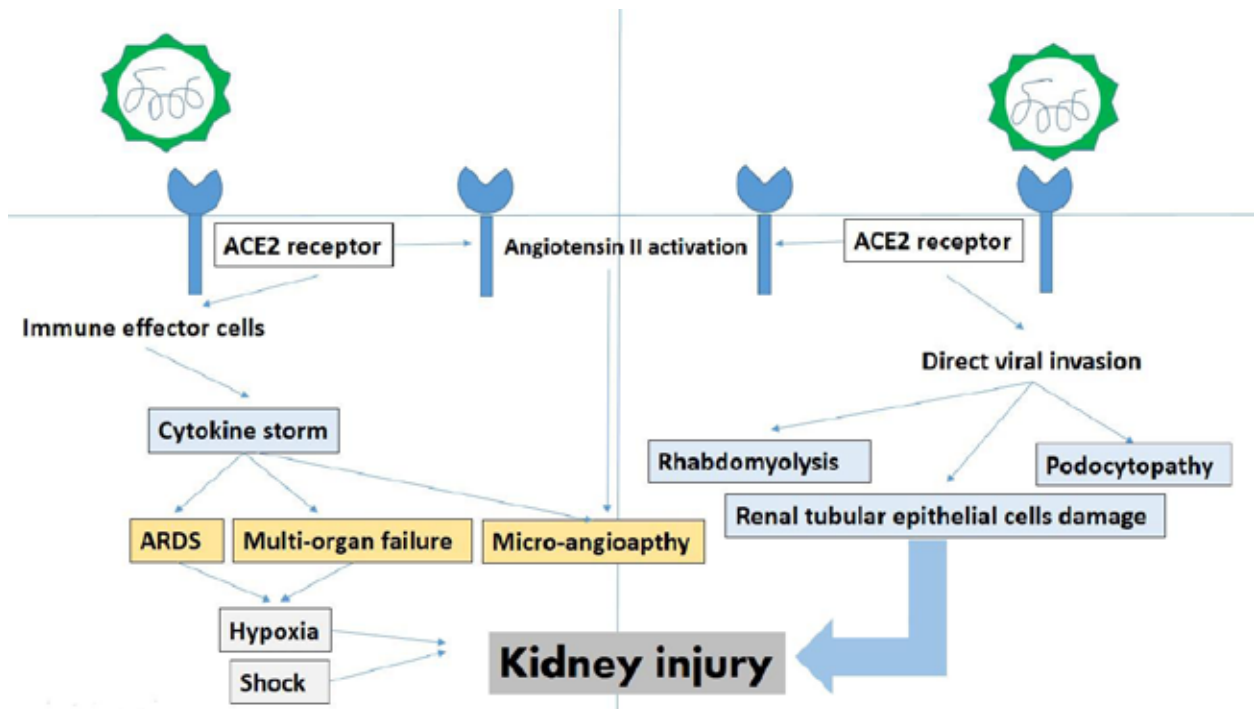


Fig 1: Mechanism of kidney injury in patients with COVID-19. It illustrates the effect of SARS-CoV-2 on the kidney directly via viral invasion into renal tubular epithelial, podocyte and skeletal muscle cells causing renal cell damage and rhabdomyolysis, and indirectly via stimulation of cytokine storm with consequent acute respiratory distress syndrome, microangiopathy and multi-organ failure; all of which is moderated by angiotensin converting enzyme 2 receptor binding and angiotensin II activation.

ground-glass opacities with a peripheral, asymmetric and posterior distribution is highly suggestive of SARS-CoV-2 infection^[4]. Other laboratory tests may be helpful in diagnosis of SARS-CoV-2 syndrome; lymphopenia, elevated aminotransferases, lactate dehydrogenase and muscle enzymes can be found. C-reactive protein and D-dimer values may also be increased. Laboratory alterations of multiorgan imbalance (high amylase, coagulation disorders, etc.) have been identified in patients with severe diseases^[5].

LITERATURE REVIEW

COVID-19 and kidney diseases

A. COVID-19 and acute kidney injury (AKI) (incidence, pathogenesis, and management)

Kidney involvement in the context of COVID-19 has been frequently reported. One of the commonly reported clinical presentations delineating kidney affliction is AKI, which is frequently associated with increased mortality, especially among patients with critical illness. In a multicenter study conducted by Li *et al* comprising 193 patients hospitalized for COVID-19, the incidence of AKI among the studied population was about 10%, associated with a risk factor 5.3 times higher for mortality compared to patients who were not complicated by AKI^[6]. Other studies reported an incidence of 0.5% to 23% of AKI among COVID-19 infected patients and up to 66% in severe cases^[7,8].

The incidence of AKI among non survivors of another cohort of 191 COVID-19 infected patients was 50%^[9]. Cheng *et al* analyzed the proportional risks of having elevated blood urea nitrogen (hazard ratio (HR): 7.15, 95% CI: 4.92-10.39) and elevated serum creatinine (HR: 2.99, 95% CI: 2.00-447), and found statistically significant results^[10].

The pathogenesis of kidney injury in the setting of COVID-19 infection (Figure 1) is largely explained by two main theories; [1] direct renal cellular injury caused by viral entry through the angiotensin converting enzyme 2 (ACE2) cell receptor which is abundantly expressed in renal tubular epithelial cells; and [2] systemic viral effects in an environment of massive cytokine release inducing tissue damage and organ failure^[11]. The reported isolation of SARS-CoV-2 from urine samples of infected patients supports the presence of direct viral cytopathic effect on kidney tissue^[12]. At the same time, AKI might develop in case of cytokine release syndrome attributable to intra-renal inflammation, increased vascular permeability, intravascular volume contraction and cardiomyopathy, which result in cardio-renal syndrome type 1^[13]. Cytokine release syndrome cardiomyopathy and acute viral myocarditis can both lead to renal vein congestion, hypotension and therefore, renal hypo-perfusion, causing a decrease in glomerular filtration rate^[14]. In addition, positive

fluid balance that may occur in COVID-19 infected patients results in worsening of renal vein congestion, leading to renal compartment syndrome. Other metabolic, acid/base and electrolyte disturbances can also develop in patients with COVID-19, as well as rhabdomyolysis, which can all aggravate kidney injury and haemodynamic instability^[14].

The management of AKI in the setting of COVID-19 infection seems to be the same as in other settings. Currently, there is no specific treatment for COVID-19 induced AKI. The indications, modalities as well as the timing of initiation of renal replacement therapy basically rely on non-specific data concerning critically ill patients with sepsis^[15]. Continuous renal replacement therapy is the modality of choice based on current statements and recommendations^[16,17]. Prolonged intermittent renal replacement therapy, also termed as slow low efficiency daily dialysis has been recognized as superior to intermittent hemodialysis (HD) in terms of safety. Both continuous renal replacement therapy and prolonged intermittent renal replacement therapy do not require a dedicated HD nurse, accordingly the risk of viral transmission to health care providers is less compared to intermittent HD^[17]. The high incidence of hypercoagulability associated with COVID-19 warrants adequate anticoagulation. Citrate anticoagulation represents the most favourable option; if not feasible, heparin might be considered^[15].

Peritoneal dialysis (PD) has been identified as a suitable treatment option in critically ill patients. Multiple advantages support the use of PD in the COVID-19 pandemic; it does not require expensive supplies as in HD and can be easily conducted in peripheral isolation units with few resources^[18]. In addition, the training of medical staff can be safely and easily applied during the pandemic^[19]. PD does not also require a water system for dialysate supply^[18] and there is no need for vascular access, otherwise a PD catheter can be easily inserted at the bedside^[20,21].

Extracorporeal therapies can also be used to remove cytokines in critically ill patients with cytokine release syndrome and therefore prevent consequent organ damage^[22].

B. COVID-19 and glomerular injury

Proteinuria and haematuria have been reported in the early studies of SARS-CoV-2 infected patients in Wuhan, China. Cheng *et al* reported an incidence of 44% for proteinuria and 27% for haematuria^[10]. The incidence of proteinuria was 36% in another studied case series of 198 patients from Shanghai^[23]. However, the majority of the reported cases exhibited mild proteinuria (being only 1+/2+ on dipstick), kidney imaging suggested presence of inflammation and oedema of the kidney^[6]. Although mild proteinuria

can be attributed to the febrile illness characterizing COVID-19, other reports suggest presence of direct viral effect on podocytes^[24]. The histopathological examination of renal biopsies obtained from patients infected with SARS-CoV-2 revealed severe acute tubular necrosis with CD68+ macrophage infiltration of the tubule-interstitium, but very little deposition was seen in glomeruli. Immunohistochemistry identified SARS-CoV-2 nucleocapsid protein in the kidneys^[8].

Collapsing focal segmental glomerulosclerosis which is a known complication of certain viral infections, in particular HIV, was also reported in SARS-CoV-2 infected patient presenting with AKI and nephrotic-range proteinuria^[25]. Another case of crescentic proliferative glomerulonephritis was also reported in a previously healthy patient infected with SARS-CoV-2 without pulmonary manifestations; the patient underwent plasmapheresis and intravenous immunoglobulin injection and dramatically improved^[26].

In SARS-CoV-2 infected patients, the use of immunosuppressive agents in the management of glomerular diseases represents a dilemma balancing the risk of infections against the aim of disease control. These immunosuppressives can impair lymphocyte function and/or exhibit lymphopenia^[27]. The decision to start immunosuppression in SARS-CoV-2 infected patients with de novo or relapsing glomerulonephritis should basically rely on the clinical, laboratory and histopathological findings as well as consider the coexisting comorbidities on an individual basis. Immunosuppression should be generally reduced to the least effective and safe levels. However, this is not feasible for newly diagnosed patients of immune-mediated kidney disease or those with relapsing disease^[27]. The use of extracorporeal therapies such as plasma exchange can help reduce the dose of steroids and improve the outcome. In addition, convalescent plasma may be helpful in patients with active SARS-CoV-2 infection^[28].

C. COVID-19 and chronic kidney disease (CKD)

Comorbidities, including CKD, are associated with more severe COVID-19 in patients. Henry and Lippi carried out a meta-analysis of four studies including 1389 COVID-19 patients and found that the prevalence of underlying CKD was higher in those with severe illness^[29]. One study showed increased mortality among CKD patients with COVID-19^[30]. Also, the AKI incidence was higher in CKD patients with COVID-19^[10].

Renin angiotensin system (RAS) inhibitors

Since ACE2 is a receptor for SARS-CoV-2^[31] and RAS inhibitors may increase levels of ACE2^[32,33],

there exists a debate regarding the use of ACE inhibitors and angiotensin receptor blockers in patients with COVID-19. However, the current evidence on discontinuation of ACE inhibitors or angiotensin receptor blockers in COVID-19 patients is not encouraging^[34,35]. Several studies were carried out and concluded that treatment with ACEI or angiotensin receptor blockers was not associated with a worse clinical outcome in hypertensive COVID-19 patients^[36,37]. Additionally, patients complaining of cardiovascular disease, hypertension and diabetes (diseases in which RAS inhibitors are indicated) often have a more severe course if they developed SARS-CoV-2 infection. Moreover, cessation of these drugs in some patients may aggravate comorbid cardiovascular or kidney disease and lead to increased mortality in those patients^[38,39]. Thus, we do not encourage discontinuation of RAS inhibitors in those patients except when adverse outcomes of these agents occur.

D. COVID-19 and end stage renal disease patients on maintenance HD

Unfortunately, HD patients are very susceptible to SARS-CoV-2 infection and are more likely to develop severe illness and a higher mortality. Goicoechea *et al* reported 30.5% mortality rate among the 36 COVID-19 infected HD patients included in their study^[40], which is higher than the reported mortality estimates in the general population that range from 1.4% to 8%^[41]. On the contrary, Wang *et al* found that HD patients with COVID-19 are clinically mild and unlikely to progress to severe pneumonia due to their impaired cellular immunity and inability of growing cytokines storm^[42]. Ideally, infection prevention and control instructions of the Centers for Disease Control should be followed in outpatient HD facilities, especially when there is suspected or confirmed COVID-19 cases^[43].

For early recognition of individuals with respiratory infection, active screening for body temperature and other symptoms of health care personnel (HCP) before, during and after dialysis shifts is mandatory. If a HCP develop symptoms during work, they should wear a mask and return home for self-quarantine^[44]. Facilities should recognize patients with any symptoms of respiratory infection (*e.g.*, fever or cough) before their entrance to the HD centre by instructing patients to call them. Accordingly, patients who have respiratory symptoms should keep facemasks on until they leave the facility.

Patients should be instructed to use disposable three-layer surgical mask filtering 95% of the particulate matter during dialysis to cover nose and mouth. Tissue paper should be used for coughing and sneezing, and must be discarded in plastic-lined trash cans^[45]. Washing hands with soap and water for

at least 20 seconds should be encouraged; otherwise, a hand sanitizer containing at least 60% alcohol can be used^[46]. Adequate spacing of at least 6 feet between masked, symptomatic patients and other patients during dialysis treatment is mandatory.

As a general rule, HCPs dealing with patients who have undiagnosed respiratory infections should follow the standard precautions to protect themselves against infection with the use of facemasks, gloves, eye protection (*e.g.*, goggles, face shields), and isolation gowns which should be worn over or instead of the cover gown and when gowns are removed.

When suspected or confirmed COVID-19 patients receive HD at the facility, additional measures should be applied^[43]. It is perfect to limit the time of contact between dialysis staff and COVID-19 infected patients in the room. The facility should provide telemedicine for monitoring the patient by dialysis staff through a glass door or camera. Dialysis effluent from patients with COVID-19 can be disposed of per standard facility protocols. Ideally, if the dialysis treatment is indicated for more than one patient with confirmed COVID-19, it is better to cohort these patients and the HCP caring for them together in the last shift of the day, considering a higher nurse to patient ratio^[45].

E. COVID-19 and renal transplantation

Transplant recipients are at higher risk because of immunosuppression, underlying CKD and other co-morbidities, especially hypertension and diabetes, which are important factors that affect outcomes in COVID-19 patients^[9]. There is a challenge regarding immunosuppression management in these patients and this should consider severity of COVID-19 infection, age, associated co-morbidities and the time post-transplant. Majority have relied on monotherapy with corticosteroid for maintenance of immunosuppression while treating renal transplant recipients with COVID-19; nevertheless, there is no agreement to routinely use corticosteroids in treatment of COVID-19 patients. Successful treatment of renal transplant recipients with moderate COVID-19 infection may be done with steroid sparing adjustments to immunosuppressive drugs including modest decreases in calcineurin inhibitor trough levels and antiproliferative doses. Further research is needed for determination of optimal management of immunosuppressive drugs in this group of patients including management of patients who present with severe COVID-19 infection and those that require ventilator support^[47]. Trials of anti-interleukin 6 monoclonal antibody tocilizumab are encouraged in COVID-19 patients, particularly for patients with severe respiratory disease and acute respiratory distress syndrome. To continue low dose tacrolimus,

more evidence is required before reporting firm conclusions. Fear of decreasing immunosuppression is due to risk of rejection, but it carries the risk of high rate of mortality of hospitalized COVID-19 patients, so physicians should concentrate on saving the patients' lives with a careful case by case evaluation of risks versus benefits of continuing immunosuppressive drugs^[48]. Transplantation is a high-risk operation during COVID-19 pandemic because of the risk of transmission of COVID-19 infection from the donor to the recipient as well as higher susceptibility of the recipient to develop severe disease while receiving aggressive immunosuppression in the early post-transplant period. It is suggested that transplantation operation is not encouraged during this pandemic^[48].

Management strategies of COVID-19: special focus on renal patients

In general terms, there is debate in the optimal management of COVID-19 as significant evidence of therapy is still lacking. Also, there is uncertainty in the indication of anti-retroviral therapy and there is no approved therapy for the treatment of COVID-19 infection. Till now, no clear guidelines present for the management of these patients^[49].

A. Non-hospitalised patients

Asymptomatic patients or those with mild infection (*e.g.*, fever, cough, and/or myalgias without dyspnoea) can be managed in the home^[50]; however, patients with risk factors for developing severe disease (*e.g.*, CKD and HD patients) should remain in frequent contact with their health care provider to closely monitor for any symptoms or signs suggestive of clinical worsening. The patient's home isolation should be a separate area for the patient to satisfactorily isolate from other home residents with available access to food and other items of daily living without transmission of infection to others and monitoring for clinical deterioration (*e.g.*, development of dyspnoea, confusion and persistent chest pain). The management is mainly supportive with antipyretics, good hydration and analgesics. With regard to anti-retroviral agents for outpatients, data are extremely limited and there may be potential toxicity in an unmonitored setting^[51,52].

Though there is uncertainty about ideal duration of home isolation, the Centers for Disease Control recommendations about discontinuing home isolation include both strategies of test-based and non-test-based. When using a test-based strategy, patients may discontinue isolation in home when fever resolves without the use of anti-pyretic drugs, respiratory symptoms improve, and at least two successive negative tests of nasopharyngeal swab specimens obtained ≥ 24 hours apart are confirmed. If the non-

test-based strategy is used, patients may discontinue home isolation when all the following criteria are met: (1) at least one week has passed since symptoms first appeared; and (2) at least three days have passed since recovery of symptoms^[53].

B. Hospitalised patients

Patients who have more severe disease or are at risk for more severe COVID-19 disease (suspected or documented) warrant hospitalization. Management of those patients consists of ensuring suitable infection control, supportive care and possible use of drugs with potential activity against SARS-CoV-2^[54].

CONCLUSION

The current evidence advocates that COVID-19 interferes with kidney function itself as well as it significantly affects the outcome of patients with various renal disease. The literature is rapidly changing while a large body of evidence is made regarding COVID-19. However, further studies are needed to provide better preventive and management policies, particularly for vulnerable patients with renal disease.

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Original Article

Ursodeoxycholic acid treatment for duodenogastric reflux in childhood

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ABSTRACT

Objective: The purpose of this study was to determine the clinical and histopathological features of duodenogastric reflux (DGR) in children and the effectiveness of ursodeoxycholic acid (UDCA) therapy.

Design: Prospective

Setting: Cukurova University Medical Faculty Pediatric Gastroenterology Clinic and Necmettin Erbakan University Medical Faculty Pediatric Gastroenterology Clinic, Adana and Konya, Turkey

Subjects: One hundred and four children

Intervention: One hundred and four patients with DGR were assessed in terms of their history, physical examination, endoscopy, histopathology and response to UDCA therapy.

Main outcome measure: Positive results were obtained by administering UDCA treatment in addition to stomach drugs.

Results: Eighty-two (79%) patients had previously used various antacids and proton pump inhibitors for dyspeptic symptoms. Gastritis was detected with upper gastrointestinal system endoscopy in all patients. Symptoms decreased by more than half or resolved completely in 97 patients (93%) at the 3-month follow-up interval.

Conclusion: DGR should be considered in the etiology of patients with dyspeptic symptoms who fail to respond to antacid and proton pump inhibitor therapy. The majority of patients with DGR responded well to three months of UDCA therapy.

KEY WORDS: children, duodenogastric reflux, dyspepsia, endoscopy, ursodeoxycholic acid

INTRODUCTION

Duodenogastric reflux (DGR) is the reflux of duodenal content from the duodenum back into the stomach^[1]. The reflux of bile and pancreatic and small intestinal secretions back into the stomach damage the stomach and esophageal mucosa, and this may also lead to gastritis, peptic ulcer, intestinal metaplasia, esophageal stricture and esophageal cancers^[2,3]. The gastritis, which develops due to duodenogastric bile reflux gastritis, is one of the common diseases of the gastrointestinal tract; and it accounts for 12.3% of all of the gastritis types^[4].

Bile acids dissolve the membrane of mucosa cells via their detergent effect, pass through the mucosa due to their lipophilic characteristics and accumulate in the cell, which causes mucosal harm by damaging the structure and function of the cell.

The most important cause of DGR is pyloric dysfunction^[5]. DGR occurs frequently after cholecystectomy, pyloroplasty and stomach surgery^[6]. Bile reflux that develops due to pyloric insufficiency in patients who have not previously undergone stomach surgery is defined as primary DGR^[7].

Patients with DGR may be asymptomatic or have

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mild or severe dyspeptic symptoms such as severe nausea after eating, vomiting bile, epigastric pain, epigastric or substernal pain, indigestion and lack of appetite. The pain that is associated with DGR can be differentiated from known gastritis pain as continuous epigastric pain and burning that increases after meals and generally does not respond to antacids. In some patients, anemia and weight loss might be observed^[8-11]. Examining the patient's history is very important for the diagnosis of DGR. In addition, radiological and scintigraphy examinations, bile acid and trypsin measurement in the aspiration fluid of the stomach and esophagus, bilirubin detection with bilitec, an alkali perfusion test, upper gastrointestinal system (GIS) endoscopy and histopathological examinations can be performed. Although DGR does not have a gold standard diagnosis, seeing ulcer, erosion, fragility, mucosal erythema and abundant bile pool in the stomach in the endoscopy, especially in patients who have abdominal pain, epigastric pain, nausea and vomiting, are the characteristics of DGR^[3,12,13].

At present, an ideal treatment plan for patients who have DGR is still not available. Proton pump inhibitors (PPI), histamine-2 receptor antagonists, drugs that increase the resistance of the stomach mucosa such as sucralfate and prostaglandin, antacids that contain aluminum, acid binder drugs such as cholestyramine, drugs that increase the stomach clearance such as methoclopramide/domperidone and cisapride, antibiotics and medical treatments that modify the structure of reflux material, such as ursodeoxycholic acid (UDCA), are used. In some patients, surgical treatments such as choledochojejunostomy can be conducted^[14]. In recent years, animal studies have shown that UDCA can alter the composition of bile acids in bile to prevent the development of experimental bile reflux gastritis^[14].

UDCA is a cytoprotective drug. With its polar characteristics, it stabilizes the cell membrane and protects against attacks from cytotoxic micella. It also causes the constitution of non-toxic micella by surrounding toxic bile acids. There is no information in the literature about the efficiency of UDCA in the treatment of DGR in children. This study aimed to evaluate children with DGR in terms of their clinical and histopathological findings and their response to UDCA treatment.

SUBJECTS AND METHODS

The study's protocol was approved by the appropriate Ethics Committee of our institution. Informed consent was obtained once the parents had been informed about the procedures.

One hundred and four patients with DGR who were followed at the Pediatric Gastroenterology,

Hepatology and Nutrition Departments at our institution were examined prospectively. There were 79 girls (average age: 13.1±2.5 years [range: 5-17.5]) and 25 boys (average age: 12.8±2.5 years [range: 5-17]). The patients were evaluated with clinical, laboratory and imaging methods, upper GIS endoscopy and histopathological findings, and we evaluated their responses to UDCA treatment. After a minimum 8 hour fast, sedation with midazolam 0.1 mg/kg and propofol 1 mg/kg per dose was applied by the anesthesiologist, and endoscopy was performed on all of the patients using a Pentax EG-2730K gastroscope (Pentax, Tokyo, Japan). DGR was diagnosed based on the observation of widespread bile in the stomach and obvious bile reflux from the pylorus during upper GIS endoscopy. During the endoscopic examination, gastritis findings such as hyperemia, fragility, edema, gastric and duodenal ulcers and erosions, masses, hemorrhage, hiatal hernia, strictures and stenosis in the inferior esophageal sphincter, bile pool and duodenogastric bile reflux were noted. For the histopathological examination, in all patients, multiple biopsies were taken from the stomach, duodenum and esophagus in patients with suspicious lesions and placed into a 10% formol solution. The biopsy specimens were stained with hematoxylin and eosin and examined by a pathologist who is a specialist in the field of gastroenterology.

Before presenting to our clinic, 82 of the patients (79%) received antacid and PPI treatments multiple times, and 25 (24%) of them received *Helicobacter pylori* (*H. pylori*) eradication treatment. Patients who had received non-steroidal anti-inflammatory drugs or corticosteroids within the last month were excluded from the study. The patients in the study were started on PPI (1 mg/kg) + sucralfate (50 mg/kg per day) + UDCA (20 mg/kg per day) treatment. The sucralfate treatment was stopped one month after it began, but the PPI and UDCA treatments were continued. The patients in whom *H. pylori* was detected were also given amoxicillin 50 mg/kg bid and claritromycin 14 mg/kg bid for 14 days. Recommendations were made to avoid fried foods, hot (spicy) and sour foods, chocolate, coffee, black tea, lemon, spicy foods, gaseous foods, fruit juices with acid and concentrated desserts, very fatty and very salty foods, and to have an intermittent and regular nutrition. The patients were followed for one year at intervals of three months.

The cases were classified as: a) those whose symptoms improved completely; b) those whose symptoms improved partly; and c) those whose symptoms did not improve at all by questioning their symptoms. The treatment of the patients whose symptoms improved was discontinued in the follow-ups.

Statistical analysis

Data was analyzed using Stata software (Stata v11.1. Statacorp, College Station, Texas. USA). Categorical variables were described as percentages.

RESULTS

The mean duration from the beginning of symptoms and the patients' presentation to our clinic was 10.5 ± 9.6 months. The most frequent symptoms of these patients are shown in Table 1. None of the patients reported a history of malignancy in the esophagus or stomach. Twenty-six patients were previously diagnosed with a chronic disease Table 2. Six patients with familial Mediterranean fever used colchicine, five patients with portal hypertension used propranolol, one patient with ulcerative colitis used mesalazine and one patient with malnutrition used a multivitamin, zinc and an enteral nutrition product.

Table 1: Symptoms of patients with duodenogastric reflux

Symptom	Number of patients, n (%)
Pain	92 (88)
Nausea	65 (63)
Vomiting	41 (39)
Regurgitation	27 (26)
Belching	7 (7)
Gagging	2 (2)
Panicula	2 (2)
Melena	1 (1)

Upon physical examination, 58 (56%) of the patients were found to have epigastric tenderness, two had hepatosplenomegaly, two had only splenomegaly and 42 (40%) were found to be normal. The weight percentile (p) was <5 p in 10 (10%) patients and >95 p

Table 2: Chronic diseases accompanying cases with duodenogastric reflux

Accompanying diseases	Number of patients n (%)
Familial Mediterranean Fever	6
Portal vein thrombosis	3
Cholelithiasis	3
*COACH syndrome	2
Ankylosing spondylitis	2
Osteoporosis	1
Cerebral palsy	1
Celiac disease	1
Immunoglobulin G deficiency	1
Aplastic anemia	1
Congenital absence of the portal vein	1
Chronic liver disease	1
Glycogen storage disease	1
Ulcerative colitis	1
Henoch-Schönlein purpura	1

*COACH syndrome is characterized by hypoplasia of the cerebellar vermis, oligophrenia, congenital ataxia, coloboma and hepatic fibrosis

in 17 (16%). The height was <5 p in 8 (8%) patients and >95 p in 6 (6%).

Antral gastritis was detected with upper GIS endoscopy in all patients. Seventeen patients had pangastritis, 18 had esophagitis and nine (8%) had duodenitis. During the histopathologic examination, *H. pylori* was detected in 26 patients. An ulcer was detected in the bulbous of two patients and in the antrum of one. Barrett's esophagus with *H. pylori* (-) was detected in two patients (Figure 1A and 1B) through both endoscopy and histopathology (Figure 2A and 2B).

At the third month of follow-up, full or half recovery of symptoms was observed in 97/104 of the



Fig 1A, 1B: Barrett's metaplasia of the esophagus in two patients

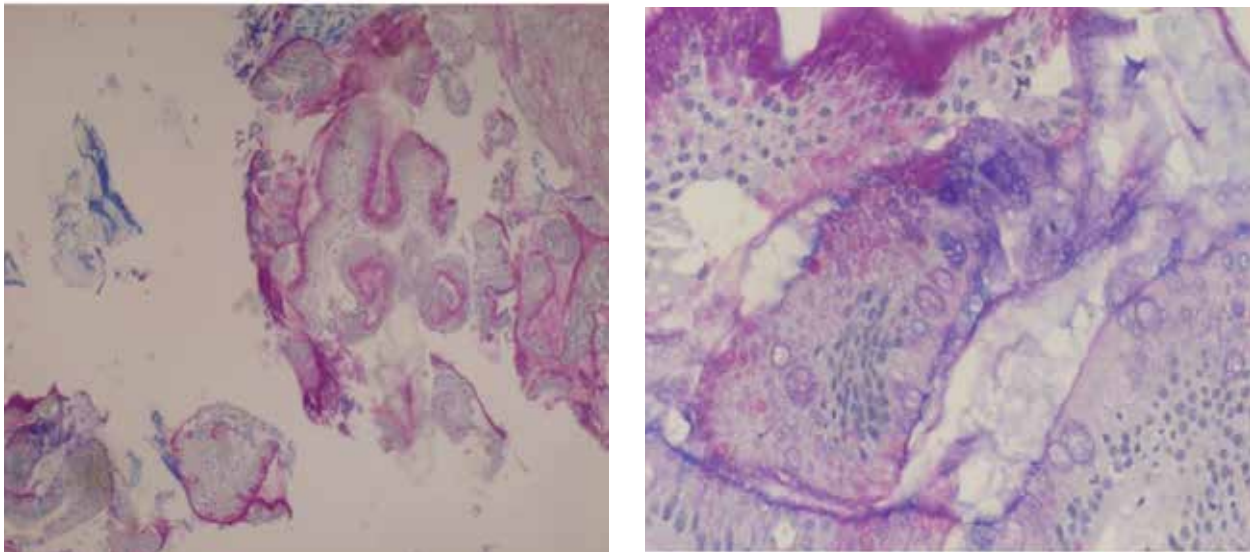


Fig 2A, 2B: Acidic mucin areas on histochemical Alcian blue-PAS staining (A: 40 \times and B: 200 \times)

patients (93%). At the sixth month of follow-up, five of seven patients who had not recovered at the end of the third month were observed to be fully recovered. It was observed in the 9th month follow-up of two patients that there were improvements in their symptoms although there were no improvements in their 6th month follow-ups. No other diseases were detected in these two patients. There was no statistically significant difference between the symptoms of *H. pylori* (-) and *H. pylori* (+) patients with DGR in terms of their response to the treatment ($P=1.0$). At 5.7 ± 2.3 months after the UDCA treatment was stopped, relapse was observed in 23

patients. Although relapse was observed more often in the *H. pylori* (+) group than in the *H. pylori* (-) group, a statistically significant difference was not observed between the *H. pylori* (+) and (-) groups ($P=.22$). None of the patients experienced side effects of the drugs or toxicity (Figure 3).

DISCUSSION

Gastroesophageal reflux and dyspeptic complaints can be observed frequently in childhood as well as in adulthood. Due to such complaints, many patients frequently use stomach-related drugs such as antacids

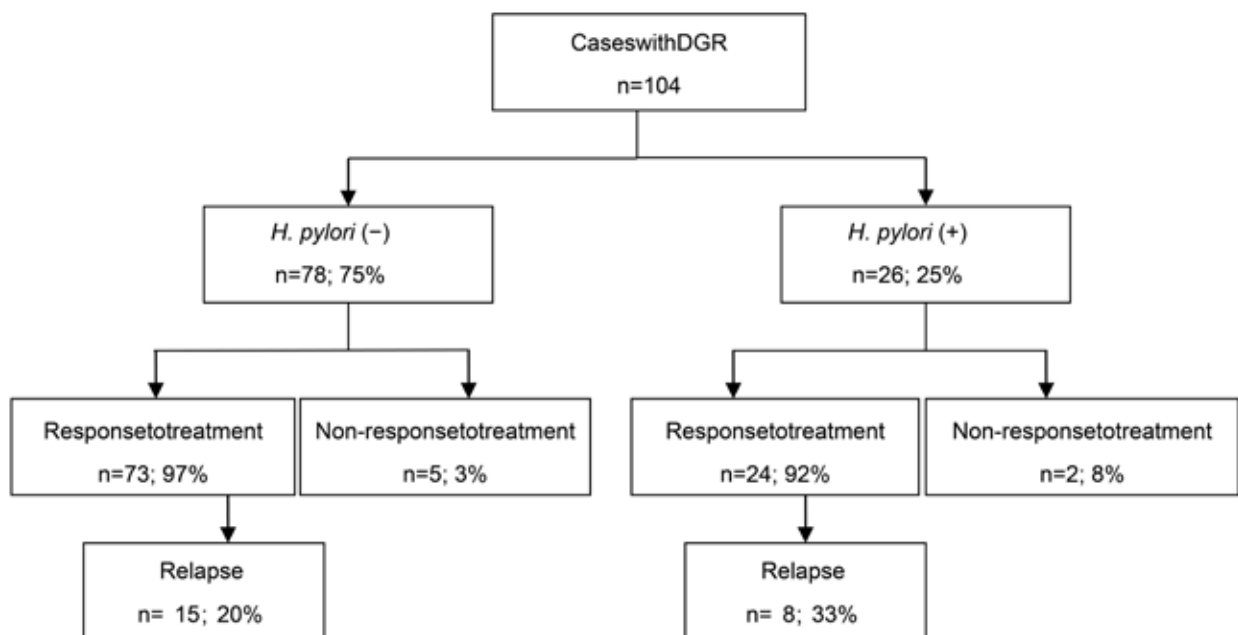


Fig 3: Patients' responses to UDCA treatment according to their *H. pylori* status
DGR: duodenogastric reflux; UDCA: ursodeoxycholic acid

and PPIs. Although most patients respond to these treatments, in some patients, the complaints can continue. Another reason for dyspeptic complaints that do not respond to treatment is DGR. Stomach burns will be more severe if there is bile reflux in DGR in addition to non-response to treatment. Before presenting to our clinic, most of our patients had used many medications and presented to our clinic due to an insufficient response. Again, the average period of time between the onset of symptoms and the patients' presentation to our clinic was 10 months. This period is too long, particularly for patients who have continuous dyspeptic complaints and cannot sufficiently respond to their treatment. We consider that the long delay in presentation was because family physicians and pediatricians do not have sufficient information about childhood DGR. The type of diagnostic methods that should be used, the treatments that should be employed and for how long they should be applied in the treatment of this disease by pediatric gastroenterologists has not been fully covered in the literature. This study was initiated because of a need for answers to these questions and it sought to evaluate the long-term results of UDCA treatment, which has minimal side effects.

Gastric acid and pepsin are the primary gastric agents that form the basis of mucosal damage and symptoms in the esophagus. In previously conducted studies, physiologically, immediately after eating and fasting, a small amount of DGR can occur in the stomach^[15,16]. Gastritis, gastric ulcer and esophageal mucosal damage can develop when this reflux occurs in larger amounts. Fisher *et al* demonstrated that DGR in the stomach is related to gastritis and gastric ulcers in patients in whom dysfunction in the pyloric sphincter is detected^[17]. The patients may have pain in epigastric or retrosternal areas, bile vomiting and pain at night. However, in some studies, symptoms were not found to be related to the amount of alkaline reflux. In addition, people who do not have symptoms may have gastritis^[18].

Today, there is no gold-standard diagnostic method for DGR. There are no specific endoscopic or histopathological findings. Finding bile content in the stomach before the duodenum on endoscopy and detecting gastric inflammation to different degrees during macroscopic and histopathologic examinations can lead the clinician to consider the presence of DGR^[19]. A hemorrhagic, vulnerable stomach wall and greenish stomach fluid indicate bile reflux^[10,20]. A history of gastritis or biliary surgery, absence of any other cause of gastritis and lack of recovery using acid-suppressing treatments supports the diagnosis. Primary duodenogastric bile reflux was considered in our patients because they did not respond to PPI

and anti-acid treatment, and we observed apparent alkaline bile reflux and macroscopic and microscopic gastric inflammation, as well as an absence of a history of using drugs that damage the gastric mucosal barrier and the absence of a history of gastric or biliary surgery.

The presence of bile in the duodenum fluid that refluxes back to the stomach and esophagus may lead to the development of intestinal metaplasia in the stomach and cancer in the esophagus^[2,19]. In this study, gastritis was indicated through both endoscopy and histopathology in all patients. While intestinal metaplasia was not observed in any of the patients, Barrett's esophagus was detected in two.

In most previous studies, any relation between *H. pylori* infection and alkaline bile gastritis was not detected^[21]. Although studies state that *H. pylori* is eradicated by DGR, other studies found that *H. pylori* is observed less frequently in patients with alkaline bile gastritis than in patients with other types of gastritis^[22,23]. This indicates that because DGR increases bacterial flora and pH, the microenvironment *H. pylori* needs to live in are spoiled. In this study, *H. pylori* was detected in the histopathology of 26 patients. *H. pylori* (+) was detected at a lower rate than in other studies that were conducted on patients of similar ages in our country^[24,25].

UDCA, which is a bile acid, occurs because of bacterial oxidation of chenodeoxycholic acid in humans and is synthesized in the liver from 7-ketolithocholic acid. It differs from chenodeoxycholic acid, which is more hydrophobic than UDCA, by equatorial placement of a hydroxyl group. This difference allows UDCA to achieve a more hydrophilic and high-polarity molecule, reduces its potential to create micella and minimizes its toxicity^[26]. Toxic bile acids, *i.e.* colic acid, chenodeoxycholic acid, lithocholic acid and deoxycholic acid cause serious damage by affecting the gastric mucosa. UDCA, which constitutes only 0.1-5% of bile acids in a normal individual, reaches 47-55% depending on the treatment, dosage and duration of administration^[14,26]. With its polar characteristic, UDCA stabilizes the cell membrane and protects it against attacks of cytotoxic micella. In addition, UDCA encircles toxic bile acids and causes the creation of non-toxic micella, which does not harm the cell^[14,27]. UDCA also decreases the levels of cholic acid, chenodeoxycholic acid and deoxycholic acid which occur in the bile in patients with DGR and have direct and damaging effects on the stomach mucosa^[28]. UDCA prevents damage to the gastric mucosa by taurine and conjugated bile acids and provides recovery of clinical and endoscopic findings. The side effects of UDCA are rare and its toxicity is low. In previous studies, treatment was recommended for all adult patients with alkaline reflux gastritis, whether

or not they had a history of surgery. Stefaniwsky *et al* administered UDCA to patients with DGR for one month and reported that their symptoms were significantly decreased^[14]. Pazzi *et al* administered UDCA to patients with DGR and indicated that there was a significant improvement in their symptom score and endoscopic imaging findings^[5]. DGR is considered an important pathogenetic factor of antral gastritis and it is frequently related to peptic ulcer diseases^[29]. The bile acids in the stomach are harmful to the gastric mucosa and cause chronic gastritis^[30]. Ozkaya *et al* applied UDCA treatment to 31 adult patients with DGR for six weeks, and at the end of the treatment, they evaluated the patients using control endoscopy. In gastric and histopathologic findings, they reported full recovery of nine patients and partial recovery of 22^[31]. It was also reported in previous studies that were conducted in later years that the patients with DGR to whom UDCA was given showed significant improvements in symptoms, and in endoscopic and histologic findings^[32-36].

There are no accurate data about the time period of UDCA treatment. In another study, although the symptoms had resolved at the end of the one-month treatment, it was indicated that histopathologic recovery had not occurred. Therefore, treatment should be continued for a longer period of time^[37]. Patients with gastritis that is related to DGR generally cannot be relieved of symptoms with acid suppressor treatments (PPI or H2 receptor blockers). Marshall *et al* indicated that PPIs are not as effective in preventing DGR as they are in treating acid gastroesophageal reflux^[36].

In this study, the patients received PPI, antacid and UDCA treatment together. The antacid treatment was stopped at the end of the first month, while the PPI and UDCA treatments were continued. In the third month of follow-up, we obtained very favorable results, and the number of patients in whom symptoms were fully relieved or reduced by half was high. Five of the seven patients who had not responded to the treatment by the end of the third month responded to it by the end of the sixth month. In four patients who experienced relapse, we found that they did not comply with dietary recommendations. These patients were administered the treatment again and they were found to be recovered at their one-year follow-up examination.

The limiting factor of this study is the lack of a control group. Most of the patients who presented to our clinic used various drugs in other centers due to dyspeptic complaints and still had these complaints. Therefore, all patients were started on UDCA treatment. They were also considered for control

endoscopy, but since the symptoms of most of the patients resolved, we did not find it ethical to conduct control endoscopy. Another limiting factor is that we could not determine the recovery ratio of the patients with *H. pylori* (+) in whom we performed standard eradication treatment. However, we also observed that the existence of *H. pylori* did not change the response to treatment or create any difference that was related to relapse.

CONCLUSIONS

Most patients with DGR and dyspeptic complaints unfortunately cannot obtain symptom relief from given stomach drugs and visit doctors with the same complaints many times. They may have to try many different stomach medications for months. Unnecessarily used stomach medications are not only ineffective and damaging to one's health, but also create an unnecessary burden on the healthcare budget of the country. When the patient cannot benefit from acid suppressor treatment and has dyspeptic complaints, DGR should be considered. There has been no study on the appropriate dose and period of UDCA treatment for children with DGR. In this study, very positive results were obtained by administering UDCA treatment in addition to stomach drugs. Therefore, we believe that this study will contribute immensely to the literature.

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Authorship contribution: Mehmet Agin and Gokhan Tumgor developed the study protocol, screened and enrolled the patients, assessed the outcomes, preliminarily analyzed the data, and wrote the manuscript. Mehmet Agin, Aylin Yucel and Hasan Ali Yuksekkaya developed the study protocol and analytical framework for the study and contributed to the writing of the manuscript. Meltem Gumus screened the patients. Sema Aydogdu supervised the design and execution of the study, performed the final data analyses, and contributed to the writing of the manuscript. All authors have read and approved the final manuscript.

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Original Article

Thrombotic tendency in Sudanese juvenile acute lymphoblastic leukemia patients: 81 cases without leukemia treatment

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ABSTRACT

Objective: To determine the thrombotic tendency in Sudanese juvenile acute lymphoblastic leukemia (ALL) patients, and to evaluate hemostatic mechanism in Sudanese pediatric patients with leukemia

Design: This is a prospective analytical case control study.

Setting: Khartoum Radiation and Isotopes Centre during the period of collection from 2014-2015

Subjects: The population comprises 81 male and female pediatric patients.

Intervention: Citrated blood specimens were centrifuged at 1500 g for 10 minutes at room temperature, and then the plasma was separated into two plain containers.

Main outcome measure(s): Prothrombin time, activated partial thromboplastin time, protein C, platelet count and

fibrinogen in ALL patients

Results: Prothrombin time and international normalized ratio results of control were reciprocally significant with ALL result revealed that there was a correlation between coagulation parameters and ALL; activated partial thromboplastin time results particularly showed strong significant results. Protein C control result presented significant relation with ALL. However, platelet count showed a significant decrease in ALL. Only two patient's results displayed an increase in D-dimer level that were more than normal range.

Conclusion: We conclude that juvenile ALL patients are at high risk of thrombosis but there was no evidence of existing thrombosis.

KEY WORDS: ALL, juvenile, thrombosis

INTRODUCTION

Hypercoagulability is defined as an increased tendency to thrombosis. It may be acquired or inherited, arterial or venous. Venous thromboembolism (VTE) is the most common manifestation of a thrombophilic state and approximately 25% of thrombophilia is detected in over 50% of cases following a first clinical episode of VTE. Inherited hypercoagulable states may be secondary to deficiency of natural clotting inhibitors or elevated procoagulants or decreased fibrinolytic factors. Amongst these, activated protein C resistance is the most common underlying cause and is seen in 20-50% of patients with inherited thrombophilia^[1]. Thrombosis has a significant impact on the morbidity

and mortality of cancer; therefore, it is important to identify which patients may be at higher risk than others, especially before starting chemo-radiotherapy or surgery^[2]. The dramatic improvements seen in the outcome of paediatric patients with acute lymphoblastic leukemia (ALL) have led to increasing incorporation of l-asparaginase in adult treatment protocols. However, its use is associated with a disruption in the physiological balance between haemostatic and anticoagulant pathways, with the predominant clinical manifestation being thrombosis^[3]. VTEs are common among patients with acute leukemia. They are more frequently associated with ALL than with acute myelogenous leukemia, develop within a few months after leukemia diagnosis

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during periods of treatment-related thrombocytopenia, are usually central venous catheter-associated and often recur^[4]. Among the malignant hematologic disorders, the incidence of thrombosis is higher in patients with lymphoma or with acute leukemia. Significant morbidity and high mortality in acute leukemia due to complications of bleeding and infection frequently overshadow thromboembolic events. Case-controlled studies of patients with cancer revealed a fourfold increase in thromboembolic occurrence in acute leukemia, with about the same rate in acute myelogenous leukemia and in ALL. Among patients with acute leukemia, thrombosis has the highest incidence in acute promyelocytic leukemia^[5].

It is also high in those patients with central venous catheters, especially in children with ALL. Most thromboembolic events are venous; the incidence varies from 1.1% to 36.7%, with 50% of these being life-threatening thrombosis in the central nervous system^[6]. There is a complex process of inhibition of blood loss through the combined action of platelets, coagulation factors and blood vessel integrity. A critical balance between clot activating, inhibiting and lysis factors is essential. Thrombosis results when hemostasis occurs at an inappropriate time or place. Disorders leading to thrombosis are those abnormalities that result in an increased tendency to develop thrombin, sometimes called the hypercoagulable or thrombotic risk state^[7]. This study was conducted to determine the thrombotic tendency in Sudanese juvenile ALL patients.

SUBJECTS AND METHODS

This was a prospective case control study conducted in Sudanese pediatric patients with ALL to evaluate the hemostatic mechanism; the sample includes both male and female pediatric patients during the sampling period from 2014-2015. Blood samples were obtained from 81 patients who were admitted to the Khartoum Radiation and Isotope Center between 0-15 years of age.

A total of 81 pediatric individuals were chosen as control group based on the following criteria that matched both sex and age with the group of cases. Samples from apparently healthy individuals attending primary schools, kindergartens and nurseries were collected. Participants in the study were excluded according to the following exclusion criteria: untreated patients, children with known history of coagulation disorders and inadequate, clotted or hemolyzed samples.

A questionnaire was used for collection of demographic and clinical data and observation check list of laboratory investigations.

Sample preparation

Citrated blood specimens were centrifuged at 1500 g for 10 minutes at room temperature and then the plasma was separated into two plain containers. A fresh sample was analyzed for prothrombin time (PT), partial thromboplastin time (PTT) and fibrinogen level. The plasma was frozen at -700 °C for one month, then assayed for protein C.

A fully automated coagulation analyzer (Sysmex CA 500) was used for measurement of PT, PTT and fibrinogen level. The Sysmex XS-1000i gives the counts and sizes of platelets using electronic resistance detection enhanced by hydrodynamic focusing^[8].

Methods

The fibrinogen assay is based on the Claus method. In the presence of a high concentration of thrombin, the time required for clot formation in diluted plasma is inversely proportional to fibrinogen concentration.

The Coagulation-500 employs the photo-optical clot detection methods by using a red light (660 nm) to illuminate the sample plasma/reagent mixture; the CA-500 detects the change in scattered light intensity due to increased turbidity as fibrinogen changes to fibrin. The coagulation curve is drawn by taking the time and scattered light intensity as the X-Y axis respectively. The coagulation time is determined by a percentage detection method. Tests were measured using scatter-light end point detection and the light source and wavelength at 660nm.

Protein C is activated using (commonly) ProtacTM, an extract of the venom of *Akistrodon contortrix* and the concentration of Protein C is determined from the rate of color change in the test sample due to cleavage of a chromogenic substrate.

The D-dimer was a rapid assay used for the qualitative and semi-quantitative measurement of cross-linked fibrin degradation products. During clot formation, these cross-linked products or fibrin degradation products are formed from the conversion of fibrinogen to fibrin by thrombin. Once a clot is formed, it triggers the production of plasmin. Then, plasmin starts to degrade the cross-linked fibrin, forming fragments. D-dimer levels were measured by a quantitative latex assay (STA-LIA test D-DI; Diagnostica - Stago, Asnieres, France) on an STA-R analyzer (Diagnostica-Stago).

Data analysis

Data were analyzed by SPSS program (version: 17.0). All demographic data of the study population were presented in mean and standard deviation in the text, *P*-value ≥ 0.05 was used to detect the power of relationship between the determinant and the outcome and 95% confidence interval was calculated.

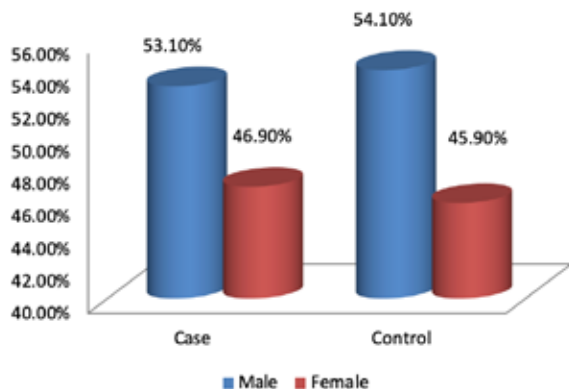


Fig 1: Gender distribution in case and control groups

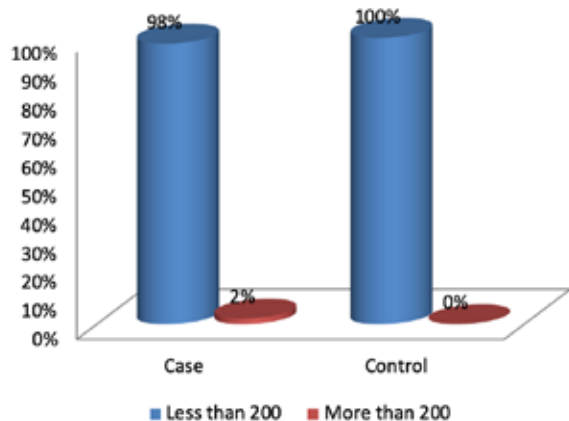


Fig 3: D-dimer distribution in leukemic patients and control group

Ethical clearance

Ethical clearance was obtained from Karary University Ethics Committee (College of Medicine), and informed written consent was obtained from the parents of the participants.

RESULTS

One hundred and sixty-two children were enrolled in the study. Of these, 81 had already been diagnosed with ALL. The absolute frequency of males was 44 (54.3%) and of females 36 (44.4%), as shown in Figure 1. D-timer distribution in leukemic patients less than 200ng/ml constituted 2%, as shown in Figure 2, while ALL was the most common type of leukemia in the study, as seen in Figure 3. Comparison of case and control in different coagulation parameters PT, international normalized ratio, activated PTT, protein C and platelet counts revealed significant variations of *P*-value ≤ 0.05 as shown in Table 1. Correlativity between coagulation parameters and ALL was shown in Table 2, revealing that control PT and international normalized ratio results were reciprocally significant with ALL, particularly with activated PTT results showing strong significance.

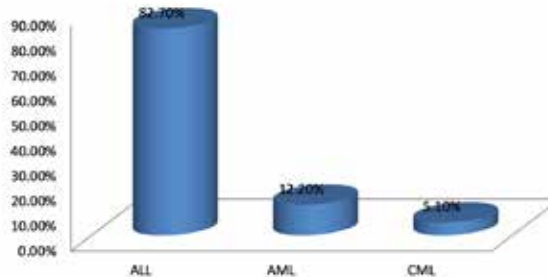


Fig 2: Types of leukemia in study group

Table 1: Comparison between coagulation parameters in case and control

Coagulation parameters	Type	Mean \pm SD N=81	<i>P</i> -value
PT	Control	18.1 \pm 5.8	.042*
	Case	17.7 \pm 0.9	
INR	Control	1.1 \pm 0.4	.033*
	Case	1.0 \pm 0.1	
APTT	Control	36.7 \pm 9.4	.001**
	Case	34.0 \pm 2.4	
Fibrinogen	Control	2.5 \pm 0.5	.110
	Case	2.3 \pm 0.7	
Protein C	Control	0.7 \pm 0.2	.003*
	Case	0.5 \pm 0.01	
Platelets count	Control	257.5 \pm 128.6	.000**
	Case	151.2 \pm 104.1	

P* ≤ 0.05 , *P* ≤ 0.001

SD: standard deviation; PT: prothrombin time; INR: international normalized ratio; APTT: activated partial thromboplastin time

The result of protein C showed significant relationship with ALL. Platelet count, however, showed significant results in ALL, while fibrinogen did not reveal any significant results.

DISCUSSION

Thrombosis is uncommon in children, but it may occur in some pathologic conditions such as ALL. The prevalence and the pathogenesis of thrombosis associated with ALL are obscure. The primary disease

Table 2: Correlation between coagulation parameters in ALL and controls

Coagulation parameters	Control and ALL Mean (\pm SE)	<i>P</i> -value
PT	-3.5 (\pm 0.7)*	.000
INR	-0.3 (\pm 0.05)*	.000
APTT	-14.7 (\pm 1.2)*	.000
Fibrinogen	-0.08 (\pm 0.08)	.323
Protein C	0.1 (\pm 0.04)*	.014
Platelets	61.8 (\pm 20.0)*	.002

**P* ≤ 0.05

SE: standard error; PT: prothrombin time; INR: international normalized ratio; APTT: activated partial thromboplastin time; ALL: acute lymphoblastic leukemia

itself can activate blood coagulation via procoagulant substances or by impairment of fibrinolytic or anticoagulant pathways⁹¹.

In this study, PT, international normalized ratio and activated PTT were significantly higher in the control group than in ALL patients, indicating that the risk of thrombotic tendency is higher. This result is consistent with the study by Omer *et al*¹⁰¹, who concluded in their study of Sudanese patients with hematological and solid malignancy that activated PTT, PT and platelets in the control group were significantly higher than those of patients, and these findings disagreed with Ribeiro and Pui¹¹¹. Clinical and biological causes of coagulopathy were established in a wide range of patients with untreated childhood acute leukemia, and it has been found that patients group have a prolonged PT of greater than 12 seconds, and activated PTT greater than 45 seconds.

As shown in Table 2, the correlation between the measurements of coagulation level in ALL and the control samples is reversed, meaning that the more severe the ALL, less the normal clot time, and also may increase the probability of thrombus formation. The hypercoagulable state may be due to the release of prothrombotic factors produced by leukemic cells like tissue factor and cancer procoagulant. Tissue factor is located mostly on the surface of the cell membrane and normally remains dormant. When cell death or apoptosis occurs, the inner membrane phospholipid, phosphatidylserine, is exteriorized, resulting in the assembly of the earliest coagulation complex, tissue factor-factor VIIa, thus initiating the coagulation cascade. Cancer procoagulant is a cysteine proteinase that activates factor X directly. It has been found in acute lymphoid and myeloid leukemia; however, its role in thrombogenesis in acute leukemia has not been verified clinically¹²¹.

Protein C as a potential coagulation inhibitor was significantly low in our study group of patients. Insufficient protein C may lead to thrombosis and may also increase the hypercoagulability of the blood. This study agrees with Kevin *et al*¹³¹, who found that plasma levels of protein C were significantly lower in patients with active ALL than in controls or patients with remission. In ALL patients with active disease, functional protein C levels were also significantly lower than controls; this result is consistent with the study done by Masanori *et al*¹⁴¹, who reported that the concentrations of coagulation inhibitors (protein C, protein S and plasminogen) significantly decreased in ten patients with ALL and lymphoma.

On the other hand, the mean number of platelets in the patient group is $151.2 \times 10^3/l$, which is within the normal range, but the number of platelets is significantly reduced compared to the control group in

the ALL patients. Our result, in accordance with Hara *et al*¹⁵¹, was that children had a platelet count of more than $150 \times 10^9/l$ when diagnosed.

The present study contradicts with Dixit *et al*¹⁶¹, in which children had bleeding symptoms, thrombocytopenia was present in 57 patients (85%) and 33 (49.3%) had some global coagulation marker abnormality.

Only two patients had D-dimer levels greater than 200ng/ml, while the rest of the control group and the other patient had levels within the normal range (less than 200 ng/ml) This finding agrees with Wei *et al*¹⁷¹, who measured D-dimer levels in different phases of acute leukemia patients and to explore its significance in the progress and curative effect of leukemia. After complete remission, plasma levels of D-dimer had no significant difference in leukemia case group vs control. However, Krzysztof *et al*¹⁸¹ concluded that the level of thrombin antithrombin, D-dimer and plasmin antiplasmin was elevated.

Patients who were recruited in this study were not in treatment protocol according to our exclusion criteria, and demonstrated no evidence of disseminated intravascular coagulation, deep vein thrombosis or pulmonary embolism. This outcome is consistent with Payne and Vora's study¹⁹¹; they found that venous thrombosis is more frequent in patients treated for ALL than other solid malignancies and has distinctive causes, clinical features and remedies. The reported incidence varies from 1% to 36%, depending on the chemotherapy protocol and whether the reported cases are symptomatic or detected on screening radiography. The risk is thought to arise from increased thrombin generation at diagnosis combined with reduced thrombin inhibitory capacity due to depletion of circulating anti-thrombin by asparaginase. Our findings concert with Nowak G²⁰¹ as well, revealing that ALL is the most common VTE-related malignancy in children.

CONCLUSION

We conclude that juvenile ALL patients are at high risk of thrombosis tendency and show no evidence of existing thrombi, and therefore, many precautions should be followed in treatment of ALL to avoid thrombosis formation.

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Original Article

Short-term prognostic importance of ambulatory blood pressure variability in patients with acute ST elevation myocardial infarction

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ABSTRACT

Objective: Blood pressure variability (BPV) is associated with cardiovascular disease and mortality. The aim of this study was to determine whether the effect of the ambulatory BPV on prognosis in acute myocardial infarction (AMI) patients during one month follow-up.

Design: Prospective study

Setting: The study was carried out at Ondokuz Mayıs University, Medical Faculty, Samsun, Turkey

Subjects: Ninety-two consecutive patients with first ST elevation AMI (STEMI).

Intervention: BPV indices were measured as a standard deviation (SD) and coefficient of variation (CV) from the 24-hour ambulatory blood pressure (BP) monitoring.

Main outcome measures: Evaluate BPV as a prognostic factor

Results: 24-hour and daytime systolic SD and daytime CV

values were significantly higher in cardiovascular event group ($P < .0001$ and $P = .001$, respectively). No difference was shown between two groups according to diastolic BP values. After adjustment for covariates including age, sex, rate of hypertension, diabetes mellitus and revascularization treatment, Cox regression analysis showed that cardiovascular events were associated with 24-hours and daytime systolic SD (HR:1.13 for 24-hours, HR:1.18 for daytime) and CV values (HR:1.17 for 24-hours, HR:1.20 for daytime).

Conclusion: In our study, we found that increased systolic BPV indices obtained from 24-hour ambulatory BP monitoring were significantly and independently associated with cardiovascular event after one month follow-up in patients with STEMI.

KEY WORDS: ambulatory blood pressure monitoring, diastolic pressure, myocardial infarction, prognosis, systolic pressure

INTRODUCTION

Epidemiological studies have established a strong association between elevated blood pressure (BP) and coronary artery disease^[1]. However, the impact of BP on outcomes in acute myocardial infarction (AMI) is unclear^[2,3]. BP is not a constant variable, it shows noticeable oscillations during short- and long-term^[4]. Blood pressure variability (BPV) has been shown to be a predictor of cardiovascular disease and mortality^[5-11], although there are discrepant results^[12-14].

There have been a number of reports that day time BPV^[15], night-time BPV^[16,17] and 24-hour BPV^[18,19] were associated with cardiovascular outcomes. Increased systolic BPV has been shown to be a better predictor of all-cause and cardiovascular mortality^[5-7], stroke^[20,21] and cardiac disease^[8-11]. Although the prognostic significance of BPV has been evaluated in different cohorts such as an untreated or treated hypertensive subjects and a general population, it has not been evaluated enough in AMI patients^[22]. Also, it is

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controversial that which time period and which measures of blood pressure component, either alone or combined, is more important in ambulatory BPV measurements^[23]. The aim of this study was to evaluate the one month prognostic value of BP variability estimated with standard deviation (SD) and coefficient of variation (CV) for the day time, night time, 24-hour systolic and diastolic BP in patients with AMI.

SUBJECTS AND METHODS

Patient population

This prospective study was performed in the cardiology department in Ondokuz Mayıs University, Samsun, Turkey. In this prospective study, 116 consecutive patients with their first ST elevation AMI (STEMI) were enrolled. All patients had been admitted to our clinic within 12 hours of STEMI. STEMI was diagnosed according to the presence of ST-segment elevation ≥ 2 mm in adjacent chest leads, ST-segment elevation >1 mm in two or more standard leads, or a new left bundle branch block and positive cardiac markers. Patients with chronic renal failure, prior AMI, inappropriate readings on 24-hour ambulatory BP monitoring and patients who refused to participate were excluded from the study. During the in-hospital period, invasive and medical therapy were performed based on current guidelines^[24]. The study protocol was approved by the ethical committee of Ondokuz Mayıs University, and written informed consent was obtained from all patients.

Finally, 92 patients (74 male, 55 \pm 11 years) were included. The patients were followed up for the development of cardiovascular events (all-cause mortality, recurrent myocardial infarction, recurrence of angina pectoris) one month after AMI. Accordingly, patients were assigned into two groups: patients with cardiovascular events (group 1, n=25) and patients without cardiovascular events (group 2, n=67).

Ambulatory blood pressure monitoring

Ambulatory BP was recorded using the fully automatic monitor (Spacelabs 90207; Spacelabs Medical, Redmond, WA) to take a measurement throughout the 24 hours. It was made in the coronary care unit before the cardiovascular event. Ambulatory BP measurements were not made in the presence of hemodynamic instability requiring inotropic agent or nitroglycerin. An appropriate sized cuff was placed to the nondominant arm for every patient. Monitors were programmed to take a BP recording every 30 minutes during the day time (07 am to 11 pm) and every hour night-time (11 pm to 07 am). If the monitor failed to obtain a BP recording on the first attempt, it would automatically retry the

measurement two minutes later. Three time periods were examined: day time, night-time and the 24-hour period. Average systolic and diastolic BP were calculated for 24-hours, day time and night-time. BP variability was calculated using the SD of the average BPs for every three time periods. The CV of BP (expressed as percent of SD/average BP) was also calculated for every time period.

Echocardiography

Two dimensional echocardiographic examinations were performed with a Vingmed cardiac ultrasound unit using a 2.5 MHz transducer in three (2-4) days after AMI and the results were assessed by two cardiologists. Left ventricular ejection fraction and wall motion score index were measured in all patients by two-dimensional echocardiography. Left ventricular ejection fraction were assessed using the biplane Simpson's method. Wall motion score index were determined according to the 16-segment left ventricle segmentation model by assigning a segmental score (1 = normal or hyperkinetic; 2 = hypokinetic; 3 = akinetic and 4 = dyskinetic) and it was calculated by dividing the sum of all scores by the total number of segments analyzed^[25].

Clinical endpoint

The endpoints were adjudicated by the incidence of cardiovascular events during the one-month follow-up, including all-cause mortality, recurrent myocardial infarction (in-hospital or discharged) and recurrence of angina pectoris (decided according to the expression of the patient after the careful rule-out of several non-cardiac causes of chest pain). When monthly contact was not sufficient, a researcher made phone calls for missing subjects.

Statistical analysis

For continuous variables, the data are reported as mean \pm SD if it shows normal distribution and median (interquartile range) if it is skewed distribution. Categorical variables were expressed in numbers and percentages. Group frequencies were compared by chi-square test. Parametric variables were analyzed by independent sample Student's t-test, and nonparametric variables were analyzed by the Mann-Whitney U test. The cut-off points for each blood pressure variability index to predict cardiovascular event were obtained using receiver operating characteristic (ROC) curve analyses. Thus, values of the area under the curve were also calculated by ROC analysis. The effect of various BPV on survival was evaluated by using the multivariable Cox regression model. The covariates included in the Cox model were age, sex (men/women), hypertension (no/yes), diabetes

Table 1: Baseline clinical and echocardiographic parameters

Variables	Group 1 (n=25)	Group 2 (n=67)	P-value
Age (years)	62.1±11.7	55.4±10.7	.011
Male sex (%)	21 (84%)	58(86%)	.753
BMI (kg/m ²)	25.9±3.3	27.4±4.8	.154
Diabetes mellitus (%)	6(24%)	15(22%)	.870
Hypertension (%)	10(40%)	13(20%)	.042
Smoking (%)	16(64%)	46(68%)	.672
LVEF (%)	44.3±10.0	46.5±8.8	.316
IVS (mm)	12.5(10-13)	12(10-14)	.591
WMSI	1.5(1.2-1.7)	1.3(1.1-1.5)	.386
CK-MB	170(118-391)	245(141-438)	.419
Anterior localisation	15(60%)	35(52%)	.506
Revascularization therapy	24(96%)	66(98.5%)	.463
Medication			
Betablocker	20(80%)	62(92%)	.086
ACE-I	24(96%)	56(83%)	.116
Statin	25(100%)	66(98%)	.539
Acetylsalicylic acid	25(100%)	67(100%)	1.0

Values are mean±SD, median (interquartile range); BMI: body mass index; LVEF: left ventricular ejection fraction; IVS: interventricular septum; WMSI: wall motion score index; CKMB: creatine kinase MB; ACE-I: angiotensin converting enzyme inhibitor

mellitus (no/yes), revascularization (primary angioplasty or thrombolytic treatment and medical treatment). Adjusted hazard ratio and 95% confidence interval for the significant Cox model factors were calculated. The statistical analyses were performed using SPSS 18.0 statistical software (SPSS Inc. Chicago, IL, USA). P-value of less than 0.05 was considered as statistically significant.

RESULTS

During follow-up, a total of twenty-five patients developed a cardiovascular event (group 1), whereas remaining 67 patients did not develop any cardiovascular events (group 2). Cardiovascular events (n=25) included four cases of nonfatal acute myocardial infarction, 15 cases of recurrent angina pectoris and six cases of cardiac death.

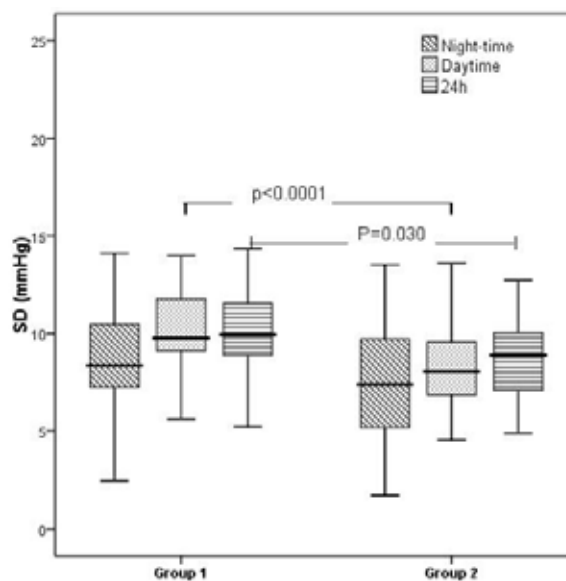
Baseline and clinical characteristics of groups were

Table 2: Ambulatory systolic blood pressure variables for 24-hour mean, day time and night-time blood pressure

Systolic blood pressure	Group 1 (n=25)	Group 2 (n=67)	P-value
24-hour mean (mmHg)	115.4±13.3	112.0±11.2	.220
Day time mean (mmHg)	114.1±12.3	111.3±12.0	.331
Night-time mean (mmHg)	116.8±17.2	112.7±11.9	.305
24-hour SD (mmHg)	9.3(8.0-11.3)	8.8(6.8-10.0)	.030
Day time SD (mmHg)	10.4± 2.9	8.2±1.9	<.0001
Night-time SD (mmHg)	9.6(9.0-11.7)	7.9(6.7-9.5)	.08
24-hour CV (%)	8.2(7.3-10.6)	7.7(6.1-8.6)	.052
Day time CV (%)	9.2±2.7	7.4±1.9	.001
Night-time CV (%)	9.0(7.1-11.1)	7.1(6.2-8.2)	.207

Values are mean±SD, median (interquartile range); SD: standard deviation; CV: coefficient of variation

similar, except for the age and history of hypertension (Table 1). Mean age was significantly higher in group 1 compared to group 2 (61 years vs 55 years, $P=.011$). The rate of hypertension was higher in group 1 (40% vs 20%, $P=.042$). Fifty patients had anterior and forty-two patients had inferior AMI. Clinical endpoints developed in 16% of patients with anterior AMI and 10% of patients with inferior AMI ($P=.506$). Thirty-one patients were treated by primary angioplasty and fifty-nine patients had thrombolytic therapy. There is no difference between the two groups in terms of revascularization therapy ($P=.463$). Revascularization

**Fig 1:** Box plot representation of the mean systolic SD values for 24-hour, day time and night-time in two groups.

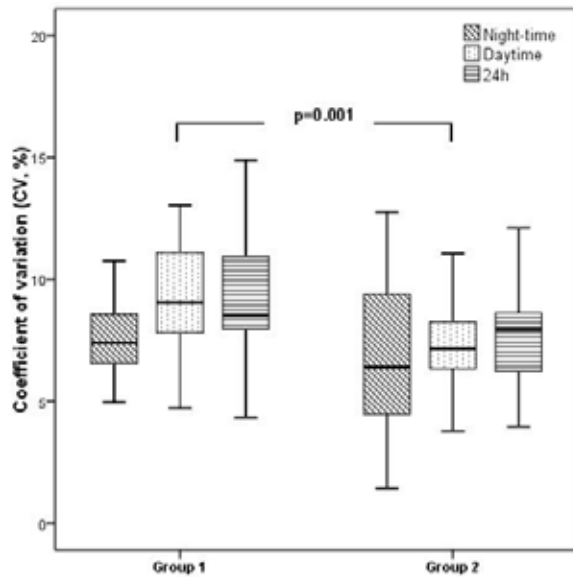


Fig 2: Box plot representation of the mean systolic CV values for 24-hour, day time and night-time in two groups.

therapy was not performed in two patients; they received medical therapy alone.

Ambulatory systolic BP variables for 24-hour, day time and night-time BP are shown Table 2. Twenty four-hour systolic SD and day time systolic SD values were significantly higher in group 1 compared to group 2 ($P=.030$, $P<.0001$, respectively; Figure 1). Also, only day time systolic CV values were significantly higher in group 1 ($P=.001$; Figure 2). Night-time systolic SD and CV values did not differ in the two groups.

Ambulatory diastolic BP variables for 24-hour, day time and night-time BP are shown Table 3. No difference was shown between the two groups according to both day time and night-time diastolic

Table 3: Ambulatory diastolic blood pressure variables for 24-hour mean, day time and night-time blood pressure

Systolic blood pressure	Group 1 (n=25)	Group 2 (n=67)	P-value
24-hour mean (mmHg)	69(64.5-76.5)	69(64-76)	.802
Day time mean (mmHg)	69.7±7.8	69.6±8.8	.959
Night-time mean (mmHg)	71.4±10.7	70.2±8.1	.569
24-hour SD (mmHg)	8.1(6.2)	7.4(6.0-8.6)	.212
Day time SD (mmHg)	8.5±3.4	7.4±1.9	.053
Night-time SD (mmHg)	8.6(6.5-9.1)	7.3(6.0-8.5)	.971
24-hour CV(%)	11.4(8.2-13.7)	10.7(8.6-12.6)	.286
Day time CV(%)	8.1(6.0-11.6)	9.5(6.3-12.6)	.089
Night-time CV (%)	11.3(8.3-14.5)	10.7(8.2-12.7)	.871

Values are mean±SD, median (interquartile range); SD: standard deviation; CV: coefficient of variation

BPV indices. Although the history of hypertension was significantly higher in group 1 (Table 1), the 24-hour, day time and night-time average systolic and diastolic BP levels were similar between the two groups (Tables 2 and 3).

To determine the cut-off values of BP variables for predicting cardiovascular event, ROC analyses were performed. The cut-off values of 9.15 mmHg with 69% sensitivity and 67% specificity (area under the curve: 0.737, 95% CI: 0.618-0.857, $P=.001$) for day time systolic SD (Figure 3a) and cut-off values of 7.8% with 69% sensitivity and 68% specificity (area under the curve: 0.718, 95% CI: 0.580-0.812, $P=.003$) for day time systolic CV (Figure 3b) were found to be highly sensitive and specific for predicting early-term cardiovascular event after STEMI.

Day time and 24-hour systolic BP variability were independently associated with cardiovascular events in the multivariable Cox regression analysis (Table 4). In multivariable Cox regression analyses, hazard ratio

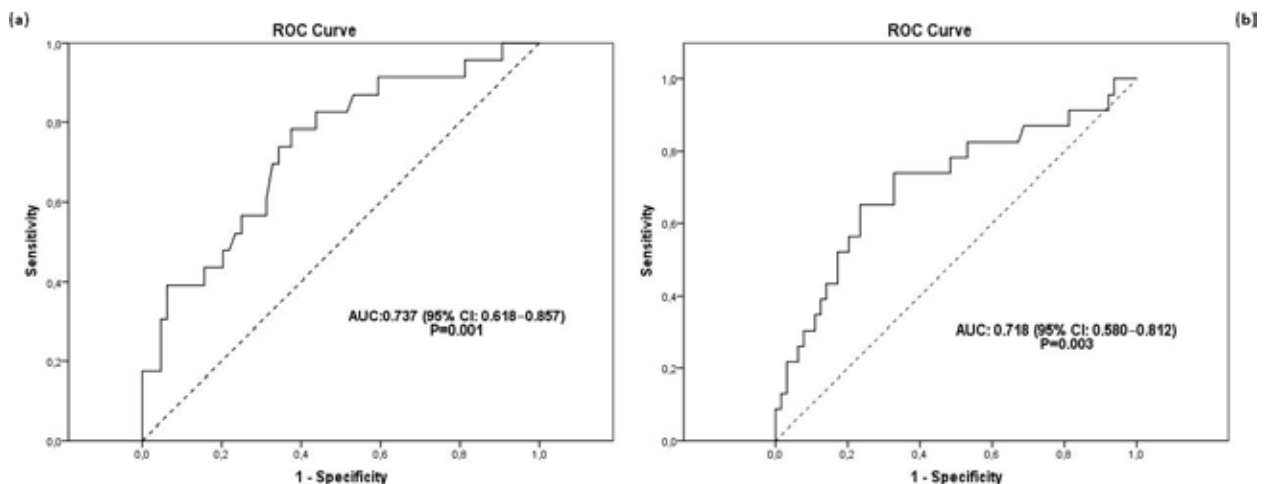


Fig 3: The receiver operating characteristic (ROC) curve for the day time systolic SD (a) and day time systolic CV (b) to predict cardiovascular event.

Table 4: Multivariable Cox regression analyses of systolic and diastolic blood pressure variability for cardiovascular events

Blood pressure variability	Systolic blood pressure		Diastolic blood pressure	
	HR (95% CI)	P-value	HR (95% CI)	P-value
24-hour SD	1.13 (1.008-1.286)	.037	1.06 (0.94-1.21)	.318
Night-time SD	1.06 (0.93-1.20)	.332	0.98 (0.85-1.12)	.795
Day time SD	1.18 (1.04-1.34)	.008	1.09 (0.97-1.22)	.137
24-hour CV(%)	1.17 (1.006-1.374)	.042	1.03 (0.95-1.13)	.373
Night-time CV(%)	1.06 (0.91-1.23)	.425	0.98 (0.89-1.08)	.748
Day time CV (%)	1.20 (1.04-1.39)	.012	1.04 (0.97-1.11)	.202

*Cox models adjusted for age, gender, hypertension, diabetes mellitus and revascularization therapy, SD: standard deviation; CV: coefficient of variation; HR: hazard ratio; CI: confidence interval

(95% CI) of the SD of 24-hour systolic BP was 1.13 (1.008-1.286; $P=.037$), SD of day time systolic BP was 1.18 (1.04-1.34; $P=.008$), CV of day time systolic BP was 1.20 (1.04-1.39; $P=.012$) and CV of 24-hour systolic BP was 1.17 (1.006-1.374; $P=.042$). The SD and CV of diastolic BPV were not associated with cardiovascular events.

DISCUSSION

In our study, we found that ambulatory systolic BPV during day time and 24-hours was significantly associated with increased risk of cardiovascular events among patients with AMI after the adjustment for age, sex, history of hypertension, diabetes mellitus and revascularization therapy in one month follow-up. Also, day time, night-time and 24-hour ambulatory diastolic BPV indices and night-time systolic BPV indices were not associated with cardiovascular events.

The clinical significance of BPV is still controversial. In the studies reporting association between BPV and cardiovascular risk, inconsistent results have been obtained. Although some studies have reported associations between BPV and clinical endpoints^[5-11], some studies have reported no association^[12-14]. Additionally, some studies related to BPV were conducted under non-acute cardiovascular situations and most of the previous studies focused on clinical outcomes in hypertensive patients. After all, we conducted this study to determine whether there is an effect of the blood pressure variability on prognosis in AMI patients.

Generally, increased systolic BP is the more accepted variable than diastolic BP for predicting coronary heart disease, stroke and heart failure^[26-28]. Also, increased systolic BPV has been shown to be a better predictor of all-cause and cardiovascular mortality^[5-7]. In our study, 24-hour and day time systolic BPV indices estimated as an SD and CV were independently associated with cardiovascular events. In a study by Sander *et al*, raised day time systolic

BPV is associated with an increased relative risk of the development of early atherosclerosis and of cardiovascular events during 3.3 years of follow-up^[19]. Their prospective study showed that day time systolic BPV is a strong predictor of carotid artery wall thickness, a marker for early atherosclerosis, progression independent from established cardiovascular risk factors^[19]. In the Ohasama study, increased ambulatory systolic BPV in 1542 subjects was found to be independent predictors of cardiovascular mortality^[29]. Kikuya *et al* have found that in subjects with a day time systolic SD ≥ 16 mmHg, the rate of cardiovascular mortality was significantly higher than in subjects with a systolic SD < 16 mmHg^[30]. In the present study, according to ROC analysis, day time systolic SD > 9.15 mmHg and day time systolic CV $> 7.8\%$ were found to be highly sensitive and specific for predicting early term cardiovascular event after STEMI.

In our study, diastolic BPV were not found to be associated with cardiovascular events for each time period. In the PAMELA study, the increased risk of cardiovascular mortality related with an increased BPV was more evident in diastolic BPV^[31]. Researchers had explained that their result may be related with a relatively high number of young and middle-age patients based on the prognostic significance of increased systolic BP associated with aging^[31]. Although the age of the patients in our study was similar to the PAMELA study, there was no difference between the two groups according to diastolic BPV indices.

Factors affecting BPV have not been fully explained. There is some evidence to suggest that BPV is influenced by a number of neural, humoral, behavioral and structural factors. It has been suggested that arterial stiffness and baroreflex regulation of blood pressure may be factors leading to increased BPV^[5,20]. Also, day time BPV is influenced by BP fluctuations during daily activities or daily stressors. During sleep, BP is less affected by

occasional triggers^[32]. In our study, the effects of daily activities on the BPV were excluded due to the fact that our patients were in the coronary intensive care unit. In the substudy of the Syst-Eur trial, Pringle *et al* found that increased night-time systolic BPV was a risk factor for stroke, even after adjusting for BP level and other confounding variables^[20]. However, in our study, the BPV during night-time was not associated with prognosis. This may be related to timing of ambulatory BP measurement. The timing and frequency of BP measurement are important. The evaluation of the 24-hour BP SD can be inaccurate when the interval of BP readings is lower than 15 or 20 minutes^[33]. In the present study, BP was measured every 30 minutes during the day time and every 60 minutes during the night time. Thus, the results of our study should be confirmed by ambulatory BP measurements at a different interval in day time.

The association between increased BPV and cardiac outcome in AMI patients may be partly explained by the inverse relation between BPV and heart rate variability^[34]. Heart rate variability positively correlates baroreflex sensitivity. Diminished baroreflex function is associated with increased stiffness and decreased compliance of the large elastic arteries^[35]. Also, poor outcomes in patients with reduced heart rate variability has been reported in AMI^[36,37]. Our study cannot prove the causal effect of BPV on the cardiac outcomes, but may suggest that it may be a possible prognostic marker.

Although the rate of hypertension was significantly higher in the cardiovascular event group, we found that the 24-hour, day time and night-time average systolic and diastolic BP levels were similar in the two groups. In a study of the relation between ambulatory BPV and restenosis after percutaneous coronary intervention performed by Cay *et al*, they showed that BPV indices are significantly and independently associated with stent restenosis. They supposed that increased risk of restenosis may be related with impairment of endothelial function owing to increased BPV. The patient population in their study consisted of normotensive individuals. For this reason, the result of their work was independent of known hypertension and coronary artery disease association^[38]. In our study, systolic BPV indices were found to be associated with prognosis in multivariate Cox regression analysis after adjustment for hypertension.

BPV is characterized by two different periods; short-term BPV occurring within a 24-hour period (minutes to days) and long-term BPV occurring over more-prolonged periods of time (visit-to-visit, days, weeks, months)^[39]. Data collection and evaluation for

short term BPV indices is easier compared to long-term BPV indices^[40]. Our data were obtained by 24-hour ambulatory BP measurements as a short term BPV.

In our study, mean age was significantly higher in the cardiovascular event group. This finding is consistent with above-mentioned information regarding the relationship between increased BPV and decreased arterial compliance and baroreflex sensitivity, since both baroreceptor sensitivity and arterial compliance decrease with age^[41,42].

There are several study limitations. First, the study included a relatively small number of patients. Second, cardiovascular events related recurrence of angina pectoris was not confirmed by coronary angiography. Also, the data about coronary angiography were not collected (*i.e.* single or multivessel disease, thrombolysis in myocardial infarction flow). Third, the analysis was performed on both hypertensive and normotensive patients, although there were no significant differences in the 24-hour mean systolic BP and 24-hour mean diastolic BP between the two groups. Fourth, our study cannot prove the causal effect of BPV. Fifth, ambulatory BP measurements were made in coronary intensive care units. The relative impacts of day time and night time environment (*i.e.* lighting, noise) in the coronary intensive care unit may be effective on variations. Lastly, the short-term variability was investigated in our study. Visit-to-visit BPV after discharge should also be obtained to evaluate long-term prognosis.

CONCLUSION

We found that the increased systolic BPV indices obtained from 24-hour ambulatory BP monitoring were significantly and independently associated with cardiovascular event, including cardiac death, reinfarction and recurrent angina pectoris in patients with STEMI. Risk stratification of patients with AMI is important in clinical decision regarding subsequent treatment. In addition to previously well-defined risk factors, we may suppose that the ambulatory systolic BPV indices may be helpful in risk stratification of AMI patients. Prospective studies are needed to assess whether there is an association between the BPV and prognosis in patients with AMI.

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Original Article

What is the best cut-off time to prevent worsening prognosis in muscle-invasive bladder cancer?

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ABSTRACT

Objective: To assess the effect and outcomes of the delay interval between transurethral bladder tumor resection (TURBT) and radical cystectomy (RC) on pathologic upstaging and the survival of the patients

Design: Retrospective study

Setting: Department of Urology, Health Sciences University, Bozyaka Training and Research Hospital, Izmir, Turkey

Subjects: We enrolled 145 patients who underwent RC between March 2006 and October 2016 with stage \geq T2 after TURBT in our tertiary reference center.

Interventions: Radical cystectomy

Main outcome measures: Time to RC and the effects of delay in RC on cancer-specific survival (CSS) and overall survival (OS).

Results: A cut-off value for time to RC on pathologic upstaging in receiver operating characteristic curve analyses was found to be 40.5 days. There were 68 patients in group 1 (\leq 40.5 days) and 77 patients in group 2 ($>$ 40.5 days) according to waiting time. Postoperative creatinine at third month after RC, pathological stage and surgical margin positivity was higher in group 2 than group 1 ($P=.003$, $P=.018$, $P=.003$). OS was 61.7 ± 6.2 months and 43.1 ± 5.8 months; CSS was 70.8 ± 6.4 months and 57 ± 6.9 months in group 1 and group 2 respectively ($P=.013$, $P=.044$).

Conclusion: Prolonged surgical wait times resulted in worse overall survival. The suggested optimum waiting time from transurethral bladder tumor resection to radical cystectomy was <6 weeks (40.5 days).

KEY WORDS: muscle-invasive bladder cancer, radical cystectomy, survival, waiting time

INTRODUCTION

Bladder cancer comprises a heterogeneous group of tumors. On average, 70% of bladder tumors present as non-muscle-invasive bladder cancer (NMIBC), the remaining 30% as muscle-invasive cancer (MIBC)^[1]. Diagnostic transurethral bladder tumor resection (TURBT) represents the standard initial, preoperative diagnostic procedure for NMIBC. This is often followed by adjuvant (additional) therapy, which reduces the chances of the cancer recurring. However, despite all treatment efforts, nearly one-third of these tumors progress to invasive disease requiring more radical treatment modalities. Contemporary demand for radical extirpative surgery is driven not only by the 20% of MIBC cases diagnosed de novo each year, but also by the 15-30% of patients with NMIBC who progress to muscle invasion despite intravesical

therapy^[2]. The aggressive nature of the disease requires timely treatment, but this can be challenging for various reasons^[3]. Radical cystectomy (RC) procedure continues to carry serious perioperative morbidity and mortality, which causes long indecision periods for both physicians and patients to choose RC. Most patients may be reluctant to undergo major surgery and postpone the decision to undergo RC or request a second operation^[3].

Delays in therapy could decrease patient chances of survival by enabling further tumor invasion or systemic spread^[4]. A major problem to determine the factors that contribute to delay to RC are discrepancies in the definition of surgical wait times among bladder tumor studies (*i.e.* time between consultation with an attending physician and surgery, time between urologic consult and hospitalisation, time between

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initial diagnosis and hospitalisation)^[5]. Several factors may contribute to delay to RC and are both health care system-related and patient related^[3]. First of all, hematuria is not being cared enough by both physicians and patients. The persistent use of intravesical therapy for high risk non-muscle-invasive disease despite recurrence and/or progression is another reason for delay^[6]. Also, some centers had overzealous desire to preserve the bladder. Bruins *et al* reported advanced age was associated with delayed RC that presumably reflects a higher comorbidity rate requiring extensive and time consuming preoperative medical evaluation^[3]. Whatever the reason for the delay in treatment, the result is poor survival.

In the present study, by acknowledging the fact that the delays in the treatment of bladder cancer would have a negative impact on survival, we aimed to discuss what should be the ideal surgical time for RC in the context of the literature.

SUBJECTS AND METHODS

A total of 178 patients who underwent RC between March 2006 and October 2016 were retrospectively evaluated. The study was planned according to the Declaration of Helsinki. Patients with non-urothelial bladder tumors and NMIBC who underwent RC were excluded from the study. We retrospectively enrolled 145 patients who had complete follow-up data and diagnosed as stage \geq T2 in pathologic and radiologic data after TURBT in our tertiary reference

center. Clinicopathological features, including age, gender, method for obtaining preoperative histological evidence (diagnostic TURBT), tumor stage, the presence of variant histology (glandular differentiation, sarcomatoid differentiation, squamous differentiation), concomitant carcinoma in situ, lymphovascular invasion, lymph node metastasis and prognostic outcomes were collected. All patients received diagnostic TURBT before the eventual RC.

RC with pelvic lymph node dissection was applied as the main therapy for all patients as soon as early after the initial diagnostic TURBT. The final pathological data analyzed in this study were based on post-RC standard pathological procedures. Tumor stage was assessed according to the Union for International Cancer Control TNM classification of malignant tumors 2002. Tumor grade was assessed according to the World Health Organisation classification of 1973.

Follow up schedule includes physical examination, complete blood count and renal and hepatic function tests, electrolytes and urine cytology at every three months, additionally a computed tomography scan (every six months) until the third year, followed by annual imaging thereafter. Local recurrences were defined as those occurring within the soft tissue field of exenteration, which is inside the bony pelvis. Distant recurrences were those occurring outside the pelvis. Pathologic upstaging is defined as upstaging of clinical stage at the final pathology after RC. Time to RC was defined as the period from the date of last

Table 1: Preoperative patient characteristics by time to radical cystectomy

Variables	Time to radical cystectomy \leq 40.5 days (n=68)	Time to radical cystectomy >40.5 days (n=77)	P*
Age	64.2 \pm 9.7	64.5 \pm 8.5	.989
Gender			.091
Female	9	4	
Male	59	73	
Preoperative hydronephrosis			.504
Positive	22	29	
negative	46	48	
Preoperative T stage			.374
T2	67	74	
T3	1	3	
Preoperative tumor grade			.541
Grade 1	1	0	
Grade 2	2	3	
Grade 3	65	74	
CIS			.503
Positive	18	24	
Negative	49	51	
Variant histology			.330
Positive	13	10	
Negative	55	66	
Operation time (hours)	5.9 \pm 1.1	5.6 \pm 1.2	.1
Preoperative creatinine (mg/dl)	1.2 \pm 0.5	1.3 \pm 0.5	.059

*Mann Whitney U test and Pearson Chi-square test

Table 2: Postoperative patient characteristics by time to radical cystectomy

Variables	Time to radical cystectomy ≤ 40.5 days (n=68)	Time to radical cystectomy >40.5 days (n=77)	P*
Postoperative T stage			.018
$\leq T1$	10	8	
T2	40	31	
T3	6	21	
T4	12	17	
Postoperative tumor Grade			.283
1	2	3	
2	4	1	
3	55	69	
Surgical margin positivity			.027
Positive	6	17	
Negative	60	57	
Number of dissected lymph node	12.4 \pm 5.5	11.4 \pm 4.3	.353
Number of positive lymph node	0.5 \pm 1.7	0.7 \pm 1.5	.134
Lymph node metastasis			.167
Positive	55	53	
Negative	11	19	
Invasion of prostate			.603
Positive	9	8	
Negative	55	64	
Invasion of urethra			.261
Positive	3	7	
Negative	62	66	
Lymphovascular invasion			.925
Positive	12	13	
Postoperative 3-month creatinine(mg/dl)	1.2 \pm 0.6	1.6 \pm 0.7	.003
Δ creatinine	0.1 \pm 0.5	0.3 \pm 0.7	.089
Perineural invasion			.836
Positive	9	11	
Negative	57	63	
Early medical complication			.408
Positive	16	18	
Negative	16	56	
Early surgical complication			.244
Positive	24	31	
Negative	41	35	
Hospitalisation time(days)	11.9 \pm 6.6	12.3 \pm 5.5	.240
Upstaging			.005 OR:2.66
Positive	19	40	
Negative	49	37	
Overall survival (months)	61.7 \pm 6.2	43.1 \pm 5.8	.013
Cancer specific survival (months)	70.8 \pm 6.4	57 \pm 6.9	.044

* Mann Whitney U test and Pearson Chi-square test

OR: Odds ratio

TURBT to the date of RC. A cut-off value for time to RC on pathologic up-staging was found and a comparison of different parameters was made according to this cut-off value. Overall survival (OS) was calculated from the date of cystectomy to the date of death. The primary objective was to assess outcomes of time to RC and the effects of delay in RC on cancer-specific survival (CSS) and OS.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences, version 20.0 (SPSS, Chicago, Ill) software program. Kruskal-Wallis test and Pearson

Chi-square test analyses for waiting time were used between upstaging and non-upstaging groups. Receiver operating characteristic curve analysis were used to determine the cut-off value of time to RC (waiting time) on pathologic upstaging and sensitivity and specificity rates of the value. Patients were divided into two groups as below the cut-off (Group 1) and above the cut-off (Group 2) values. Then, to compare waiting time groups, the Mann-Whitney U test was used for statistical analysis. In addition, Kaplan-Maier survival analysis was used for OS and CSS times between the waiting time groups. Data are given as mean \pm SD. However, results of analysis are given as

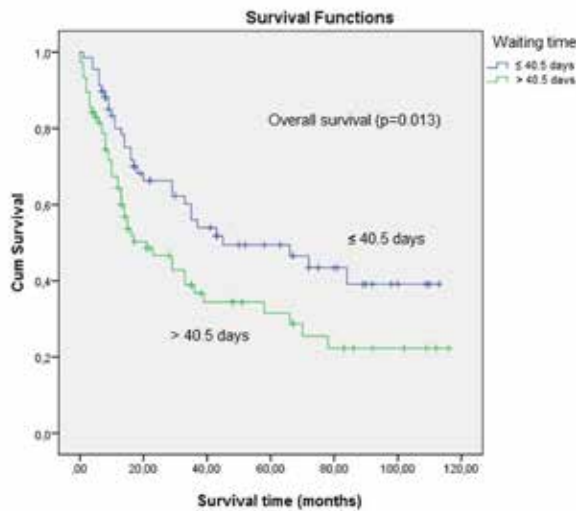


Fig 1: Kaplan-Meier Survival plot for overall survival and analysis result

median data. Statistical significance was defined as $P < .05$.

RESULTS

Preoperative and postoperative patient characteristics by time to RC were shown in Table 1 and Table 2. There were 13 female and 132 male patients with a mean age of 64.4 years (range: 32-83). Mean follow-up time was 31.6 ± 31.8 months (range: 1-116). The mean time to RC was 46.8 ± 17.8 days (range: 15-120). Patients waiting time was 42.6 ± 12.5 days in the non-upstaging group and 53.1 ± 22.1 days in the upstaging group ($P < .001$). A cut-off value for time to RC on pathologic up-staging in receiver operating characteristic curve analyses was found to be 40.5 days (AUC: 0.637, $P = .005$) with a sensitivity of 67.2% and specificity of 56.5%. There were 68 patients in group 1 (≤ 40.5 days) and 77 patients in group 2 (> 40.5 days) according to waiting time.

There was statistically no difference between the two groups in terms of preoperative data (age, sex, stage, grade, presence of carcinoma in situ, hydronephrosis, preoperative creatinine value). There also was statistically no difference between the two groups in terms of postoperative grade, presence of variant histology, number of lymph nodes removed, number of positive lymph nodes, percentage of positive lymph nodes, perineural and lymphovascular invasion and urethral invasion. Postoperative creatinine at third month after RC, pathological stage and surgical margin positivity was higher in group 2 than group 1 ($P = .003$, $P = .018$, $P = .003$).

OS and CSS of all patients are 52.7 ± 4.4 months and 64.3 ± 4.8 months respectively. OS was 61.7 ± 6.2 months and 43.1 ± 5.8 months, and CSS was 70.8 ± 6.4 months

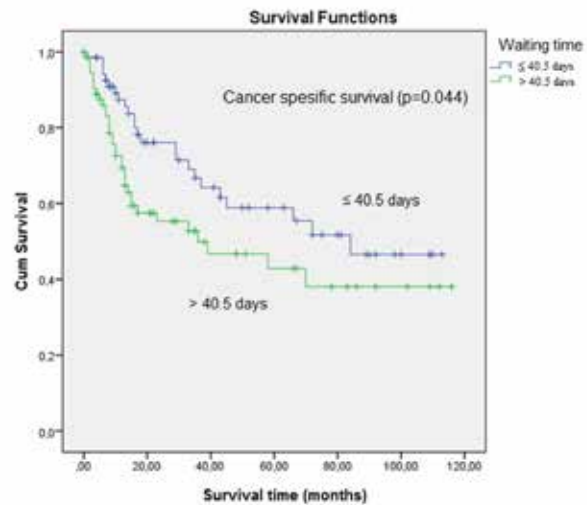


Fig 2: Kaplan-Meier Survival plot for cancer-specific survival and analysis result.

and 57 ± 6.9 months in group 1 and group 2 respectively ($P = .013$, $P = .044$; Figure 1 and Figure 2).

DISCUSSION

RC with pelvic lymph node dissection has been used as the standard therapy for MIBC and NMIBC which progresses to invasive disease, but the five-year survival after RC for clinically localized MIBC is only approximately 50%^[7]. The five-year OS and tumor-specific survival for patients with extravesical stage are only 30% and 37%, respectively^[8,9]. Data from large cystectomy series demonstrate that the majority of deaths occurring within five years of cystectomy are due to bladder cancer^[9]. RC, and the urinary diversion associated with it, can be an overwhelming concept to patients and their caretakers at first presentation^[2]. On the other hand, when the disease becomes metastatic, especially due to delay in the treatment, treatment becomes difficult. Metastatic bladder cancer was the terminal stage of this malignancy and had rather low survival rates after the diagnosis. Despite a favorable initial response to chemotherapy, long-term OS was achieved by very few patients and the median OS of metastatic bladder cancer typically plateaued at 14-15 months^[10]. In the present study, by acknowledging the fact that the delays in the treatment of the disease would have a negative impact on survival, we aimed to discuss what should be the ideal surgical time for treatment in the context of the literature. Our clinical approach is to perform RC as soon as possible after the last TURBT.

Longer wait times are associated with cancer related deaths^[4]. Prolonged wait times increase the risk of micrometastatic disease. Since malignant cells require time to growth, spread systematically and

eventually cause death, the impact on survival would manifest late (18 months) after surgery^[4]. A number of studies from tertiary referral centers have investigated the effect of time from MIBC diagnosis to RC on staging and survival^[3]. There is an ongoing argument about the period from the date of last TURBT to the date of RC. While some authors advocate that delayed RC adversely affects survival, the others suggest that there is no effect of delayed RC to survival. Sanchez-Ortiz *et al* reported a higher pathologic upstaging rate and decreased OS in patients in whom RC was delayed for >12 weeks^[11]. Chang *et al* reported higher extravesical disease rates in patients in whom RC was delayed for >3 months^[12]. A population-based study from the USA SEER database analysed patients who underwent a cystectomy between 1992 and 2001, also concluded that a delay of more than twelve weeks has a negative impact on outcome and should be avoided^[13]. In another study, Lee *et al* concluded that a cystectomy delay of 3.1 months undermines patient survival, likely through the development of micrometastases, since local stage progression is not apparent at this point. Most delays are avoidable and should be minimized^[14]. Nielsen *et al* reported that a delay of RC >3 months in three American centres was not associated with a worse clinical outcome^[15]. Liedberg *et al* found that treatment delay did not influence disease specific survival in their study with 141 patients who underwent RC due to locally advanced bladder cancer^[16]. Furthermore, treatment delay was not significantly longer in cases that progressed compared to those with equal or lower pathological stage in the cystectomy specimen. Ayres *et al* investigated whether a delay >3 months would have the same effect in the United Kingdom^[17]. Initially they found, in agreement with Nielsen *et al*, that cystectomy within 90 days of diagnosis had no effect on OS for MIBC (n=955). However, analysis of T2 tumours showed a statistically significant survival benefit if patients had surgery within 90 days of diagnosis (n=543; HR: 1.40; 95% CI: 1.10-1.79).

In the current study, we have found the mean time to RC was 46.8 days (15-120). Our study results revealed that there was statistically significant difference between the groups of non-upstaging and upstaging according to time to RC. Patients waiting time for RC was 42.6±12.5 days vs 53.1±22.1 days in the non-upstaging and upstaging groups, respectively. The delays in the treatment due to waiting for cystectomy appear to result in upstaging of cancer. Our results suggest that an ideal wait time is 40.5 days. When we compared the two groups with a threshold value of 40.5 days for time to RC, the pathologic stage was found to be significantly different between the patients who underwent RC ≤40.5 days and >40.5 days. The

upstaging was especially prevalent in T3 and T4 stages. While there were pathologically 18 patients with T3-T4 stage underwent RC in ≤40.5 days, 38 patients had T3-T4 stage underwent RC in >40.5 days. Renal function is more affected in the delayed group which may cause the difficulty for possible adjuvant therapies such as chemotherapy. Also, there were significantly higher surgical margin positivity in the delayed group. These findings suggest that delay in RC causes declining results in organ-confined disease which lead to adjuvant therapies to be considered to reach increased CSS. Our findings are compatible with other studies. In a similar study, Kulkarni *et al* suggested that a 40-day window between TURBT and cystectomy is an ideal maximum wait time, and the risk of death from bladder cancer increased significantly after 40 days^[4].

Primary goals in all cancer treatments are to improve CSS and OS. Sanchez-Ortiz *et al* reported that a more than 3-month delay after the diagnosis of muscle invasion may be correlated with advanced pathologic stage and decreased survival^[11]. In another study, Gore *et al* offered an early cystectomy which performed within three months after the muscle-invasive diagnosis was confirmed to improve survival^[13].

A generally accepted opinion in many studies is that any period to RC exceeding 12 weeks has been associated with more advanced stages and poor survival^[11-14,18,19]. Our results also revealed that delay in RC worsened CSS (70.8±6.4 vs 57±6.9 months) and OS (61.7±6.2 vs 43.1±5.8 months).

The present study had several limitations with its retrospective design and the lack of information regarding the time of initial symptomatic presentation and the reasons for the delay in performing RC. We are limited somewhat by the unknown selection criteria and small numbers of patients.

CONCLUSION

Our data suggest that optimum wait time for patients undergoing cystectomy is <6 weeks (40.5 days). It should be remembered that undesirable delays in the cancer therapy will have a negative impact on the survival of the patient in the future period. In order to minimize delay to cystectomy and improve survival, true modifications in health system and surgical approaches should be done.

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Author's contribution:

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Data collection: E Sefik, I Basmaci

Analysis: S Celik, T Degirmenci

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Original Article

Mapping the intensive care unit environment and health care workers for methicillin-resistant *Staphylococcus aureus* with *mecA* gene confirmation and antibacterial resistance pattern identification in a district hospital in Amman

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ABSTRACT

Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a big challenge to the health system by causing hospital-acquired infection. It showed variation in epidemiology and anti-bacterial sensitivity with time and place. This study aimed to determine the distribution and rate of MRSA contamination of surfaces and the health care workers (HCWs) hands in the intensive care unit (ICU) and sensitivity pattern to antibiotics.

Design: Cross-sectional study

Setting: Faculty of Pharmacy, Al-Zaytoonah University and Jamil Totanji hospital

Subjects: Samples were taken from 129 surfaces and HCWs hands.

Intervention: Samples were taken with cotton swabs streaked on Mannitol Salt Agar, then on blood agar to isolate *Staphylococcus aureus*. *Staphylococcus aureus* were stained with gram stain and catalase and coagulase tests were performed.

Disk diffusion and E-test were used to determine MRSA and resistance pattern. Polymerase chain reaction was used to confirm the presence of *mecA* gene.

Main outcome measures: Prevalence and distribution of MRSA in ICU environment and HCWs hands and antibacterial susceptibility pattern

Results: The prevalence of MRSA was 20% to the total number of samples and 30% of *S. aureus* samples. MRSA contamination of surfaces was determined. Among the 14 HCWs samples, the rate of MRSA isolates were 28%. No vancomycin-resistant MRSA could be isolated. Resistance to clindamycin and co-trimoxazole were 81% (including inducible resistance) and 29% respectively. All tested MRSA samples were positive for *mecA* gene.

Conclusion: MRSA is prevalent significantly in the ICU and HCWs hands to represent a potential source of hospital-acquired infections in the ICU.

KEY WORDS: anti-bacterial sensitivity, Methicillin-resistant *Staphylococcus aureus*, surveillance

INTRODUCTION

Since its discovery in 1961 by British scientists, Methicillin-resistant *Staphylococcus aureus* (MRSA) has presented a challenge to health in terms of increasing prevalence and seriousness of infections that it causes^[1]. It is now endemic in most hospitals and communities worldwide^[2-4]. The incidence of MRSA infection in intensive care units (ICUs) is high and of

particular importance due to the presence of critically ill patients^[5]. Health care workers (HCWs) and the hospital environment including surfaces and patient-care equipments have been implicated as sources of infections^[6]. Surveillance of the ICU settings and personnel for MRSA is important for pointing to the potential sources of infection^[7,8]. Improving environment by decontamination of ICU settings can

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reduce the incidence of MRSA morbidity and mortality with decrease in medical cost^[8]. Many studies have been conducted to determine the distribution and incidence of MRSA in health-care personnel, patients and healthy carriage^[4,9,10]. The prevalence of MRSA was also investigated in the ICU environment in some hospitals in other countries as well^[9,11].

Staphylococcus aureus has developed resistance early after the discovery of penicillin by acquiring the capability for producing penicillinase^[12]. Moreover, the bacteria soon developed resistance to methicillin and other anti-staphylococcal penicillins after their introduction in 1959^[13]. This resistance covered most of the available beta-lactams as well. This resistance is mainly due to the acquisition of *mecA* gene that decreases the binding of beta-lactam to penicillin binding protein in the bacteria^[14]. Although MRSA is highly susceptible to vancomycin, there are some reports of vancomycin resistant MRSA^[15]. MRSA has changed its pattern of resistance with time^[16,17]. In addition, hospital acquired MRSA has a different pattern of resistance from community acquired MRSA as the latter is more susceptible to clindamycin^[18] and ciprofloxacin in some reports^[19]. This pattern of type, place and time dependent change in the epidemiology of resistance of MRSA necessitates periodic check of MRSA susceptibility to antibacterial drugs.

MATERIALS AND METHODS

Materials

Mannitol salt agar, blood agar base, Muller Hinton agar, nutrient broth, coagulase test, gram stain and antibiotic disks were purchased from (Liofilchem, Italy). Catalase test and sheep blood were purchased from Arcomex, Jordan. E-test minimum inhibitory concentration (MIC) for vancomycin was purchased from OXOID, United Kingdom. Primers were purchased from IDT, USA. Agarose and Tris/Borate/EDTA buffer were purchased from (Bio Basic Canada). Polymerase chain reaction (PCR) master mix was purchased from iNtRON Biotechnology, South Korea. Green Go Tag reaction buffer stain was purchased from Promega, USA and one hundred bp DNA ladder was purchased from Promega, USA.

Design and setting

Samples were collected from Jamil Totanji Hospital. It is one of the public health sector hospitals in Jordan. It was established in 2000 and located in the city of Sahab, south of the capital Amman. The hospital has 120 beds with six beds in the ICU. The area of the ICU department is 200m². The population of the area served by the hospital is more than one

million people and patients from areas outside the capital Amman who are transferred to the hospital.

Sampling, isolation and identification of *S. aureus*

Samples were collected from the ICU environment and HCWs according to standard protocols^[20,21]. For environmental samples, we took swabs from 21 contact surfaces. Sterile cotton swabs moistened with normal saline were used to take samples from HCWs and ICU environment. Dry swabs were used to get samples from wet surfaces. Representative areas of sampling of surfaces were about 10 cm*10 cm^[22]. The samples were labeled appropriately and placed in a suitable cool box in an upright position and transported to the laboratory as soon as possible^[23]. Swabs were streaked on mannitol salt agar and incubated at 37 °C for 72 hours. Suspected *S. aureus* colonies were transferred to blood agar base and incubated for 24-48 hours^[24] and confirmed by gram stain. Biochemical identification was performed by using catalase test and slide coagulase test. For slide coagulase-negative results, conformation was done by tube coagulase test method.

Antimicrobial susceptibility testing

Tests were done according to the Clinical and Laboratory Standards Institute guidelines^[25]. The tests were done by disk diffusion method for seven antibiotics which are ceftioxin, oxacillin, ciprofloxacin, trimethoprim-sulfamethoxazole (TMP-SMX), erythromycin, clindamycin and teicoplanin. MIC was used for vancomycin.

Molecular identification of MRSA isolates

Extraction of DNA was performed using i-genomic BYF DNA Extraction Mini Kit (Intronbio, South Korea) according to the manufacturer's protocol. Concentration and purity of DNA samples were determined by Quawell DNA/protein analyzer, Japan. DNA samples were then stored at -20 °C until analysis. DNA samples from MRSA isolates were tested using PCR for *mecA* gene amplification. PCR reactions were conducted to a final volume of 20 µl using TTGCCAATACAGGAACAGCA as forward primer and reverse primer GGTGGATAGCAGTACCTGAGC. PCR primers were designed using Primer-Blast^[26]. PCR conditions were initial denaturation at 94 °C for four minutes for one cycle, denaturation at 94 °C for 30 seconds followed by annealing at 57.8 °C for one minute and extension at 72 °C for one minute. The cycling conditions were repeated for 30 cycles followed by final extension at 72 °C for seven minutes. PCR products were electrophoresed on 2% agarose gels and images were captured using UV-transilluminator (BioDoc-It, UK).

Table 1: The prevalence of MRSA isolates from samples collected from HCWs and ICU environment

Samples	No. of Samples	MSSA	MRSA	Percentage of MRSA in the samples
HCW's hands	14	10	4	28
ICU environment	115	76	22	19

HCW: health care workers; ICU: intensive care unit; MSSA: methicillin-susceptible *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*

Ethical approval

This study was approved by the Ethics Committee of ministry of health in Al-Basher hospital. The Committee decided unanimously to approve the study (CODE: MOH REC 160051).

RESULTS

The prevalence of MRSA contamination among HCWs and ICU environment

During our study, of the 129 samples collected from ICU environments and HCWs, 86% were positive for *S. aureus* and 20% for MRSA. Isolates of *S. aureus* were recovered from all of the 14 HCWs samples (six doctors and eight nurses), 28% of them were MRSA (one from a doctor and three from nurses). Among the 115 ICU environment samples, 98 were positive for *S. aureus* with 22 of those samples containing MRSA (Table 1).

Distribution of ICU environment samples is shown in Table 2. The contamination with MRSA was found in 14 of the 21 surfaces investigated. Samples (n=7)

Table 2: The distribution and prevalence of MRSA contamination of the ICU environments

Place	No. of samples	MRSA
Bed head	9	2
Bed side	18	3
Locker	9	1
Food table	9	2
Ventilators	9	2
Patients files	9	2
Doors handle	5	1
Counters	4	2
Oxygen masks	3	2
Suction tube	3	1
IV Stand	9	1
Laryngoscope	4	1
Electronic IV pump	6	1
Telephone	2	1
Medication box	2	0
Chair	3	0
ECG machine	2	0
D.C shock	2	0
Table	3	0
Monitor	3	0
ABG machine	1	0
Total	115	22

ICU: intensive care unit; MRSA: methicillin-resistant *Staphylococcus aureus*; ECG: electrocardiogram; DC: direct current

taken from the bed after patient discharge and cleaning showed no bacterial growth.

Antimicrobial resistance patterns

S. aureus isolates were considered as MRSA when the zone of inhibition was ≤ 21 mm for cefoxitin. The tests were done in triplicates. Resistance rates for ciprofloxacin (inhibition zone ≤ 15), erythromycin (inhibition zone ≤ 13) and clindamycin (inhibition zone ≤ 14) were high, and the least was for TMP-SMX (inhibition zone ≤ 10). The results of the *in vitro* susceptibility testing are shown in Table 3. All of the MRSA isolates were susceptible to vancomycin using E-test with MIC ranged from 0.5-2 $\mu\text{g/mL}$ (MIC breakpoints for sensitivities are: sensitive ≤ 2 $\mu\text{g/mL}$; intermediate sensitivity 4-8 $\mu\text{g/mL}$; resistant ≥ 16 $\mu\text{g/mL}$)^[25].

Amplification of *mecA* gene

Amplification of *mecA* gene from 39 samples of *S. aureus* were analyzed using PCR in comparison with positive strain (*S. aureus* ATCC 43300). PCR was performed on 26 samples that were resistant to cefoxitin and 13 random samples from 86 samples that were sensitive to cefoxitin.

All 26 samples had amplified *mecA* gene, while the 13 samples did not show any band on the gel which indicates absence of *mecA* gene as seen in Figure 1.

DISCUSSION

Surveillance is one of the principle tools in providing the essential data for effective control of infection^[27,28]. The most common cause of hospital

Table 3: Antimicrobial resistance profile of the 22 MRSA isolates of the study

Antimicrobial	Resistance Percentage
Cefoxitin	100
Oxacillin	100
Ciprofloxacin	62
TMP-SMX	29
Erythromycin	81
Clindamycin	58+23 inducible (D test)
Teicoplanin	0%
Vancomycin	0%

TMP-SMX: trimethoprim-sulfamethoxazole; MRSA: methicillin-resistant *Staphylococcus aureus*



Fig. 1: Agarose gel electrophoresis of PCR products amplified *mecA* gene.

Lane M: DNA ladder (100bp); Lane +ve: positive control (*S. aureus* ATCC 43300), Lane -ve: Negative control (*S. aureus* ATCC 25923); Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 13: MRSA samples with positive *mecA* showing 571 bp gene product. Lanes 10, 11, 12 and 14: MSSA with no *mecA* gene amplification.

acquired infections in the ICU settings is *Staphylococcus aureus*, with a majority of them being MRSA^[5,29], which explains the importance of screening for these bacteria. This was the first study in Jamil Totanji Hospital that may represent essential data for any plan to reduce nosocomial infections.

The HCWs may act as vectors or victims. As vectors, they represent an important source for transmission of MRSA infection to patients with high prevalence^[30]. In addition to the risk of disseminating infection to patients, HCWs may themselves get MRSA infections. The prevalence of positive MRSA carriage of HCWs in ICU varies greatly in different studies and ranged from 0% up to 59% with an average of 4.7%^[30,31]. In this study, the prevalence was 28% which is clearly above the average. This may necessitate taking measures to control contamination.

The ICU environment may act as a reservoir for acquisition and transmission of pathogenic bacteria to patients, particularly MRSA, because it has the ability to survive for a long time in the environment^[6]. In this study, sampling was not confined to high-touch surfaces^[32] to give better picture of distribution of the contamination for better planning for decontamination. Regarding the rate of MRSA detection, it can be said that figures in this study are comparable with figures in other studies^[31,33].

MRSA should be recognized depending on classical methods of susceptibility test to methicillin or ceftioxin as an alternative^[25]. For confirmatory purposes, molecular studies to identify *mecA* gene were used by some investigators^[10]. Anyhow, *mecA*-negative MRSA have been identified^[34], which undermine the importance of the presence of *mecA* gene as the sole

method for identification of MRSA. However, in our study, all tested MRSA were positive for *mecA* gene.

One of the concerns regarding the treatment of MRSA infections is the ability of the bacteria to change pattern of susceptibility to antibacterial drugs^[17]. Hence, it is imperative that their resistance to antibiotics is evaluated periodically.

Although there are some reports of vancomycin-resistant MRSA, vancomycin is still the drug of choice for the treatment of severe MRSA infections (pneumonia, bacteremia, infective endocarditis and difficult abscesses)^[35]. Fortunately, we did not observe any resistance in our isolates of MRSA for vancomycin and its classmate teicoplanin. Clindamycin may be indicated in some MRSA infections such as complicated skin infection and pneumonia^[36]. Our results demonstrated high resistance rate for clindamycin. We recommend that clindamycin be used only pending susceptibility results, including the use of D-test for evaluation of inducible resistance. Combined antibacterial TMP-SMX may be recommended in skin, soft tissue, joint and bone MRSA infections^[35]. However, the use of TMP-SMX should be reconsidered according to our results of 29% of resistance. Many investigators addressed MRSA susceptibility to ciprofloxacin. Resistance prevalence ranged between very low to very high^[36-37]. Due to the high resistance rate of our MRSA isolates, ciprofloxacin should only be used guided by sensitivity tests.

CONCLUSION

In conclusion, our results identify high prevalence of MRSA in the ICU environment surfaces and the HCWs hands. These data indicated no alarming

antibiotic resistance for vancomycin and teicoplanin, which are the main therapy for MRSA infection in the ICU. The highest contamination was in the ICU environment. The doctor's hands were less contaminated compared to nurse's hands. In addition, a study after application of the recommendations for lowering MRSA contamination is important to show the effectiveness of these measures.

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Conflict of Interest: None

Ethical approval: This study was approved by the Ethics Committee of ministry of health. The Committee decided unanimously to approve the study (CODE: MOH REC 160051).

Authors' contribution: Luay Al-Essa managed supervision with designing of the study and writing the manuscript; Mohammad Hasan Abunaja performed the technical work; Lama Hamadneh shared in supervision of the molecular part; Mohammad Abu-Sini shared in supervision of the microbiological part.

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Original Article

The effect of disease activity on cardiac autonomic functions in inflammatory bowel disease

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) has effects on neural cardiovascular control mechanisms. The aim of this study was to evaluate the effect of IBD status on cardiovascular autonomic functions by measuring heart rate variability (HRV) parameters with a 24-hour holter electrocardiogram recording.

Design: A prospective analytical case control study

Setting: Yildirim Beyazit University Medical Faculty and Ataturk Education and Research Hospital Ankara, Turkey

Subjects: Sixty-seven patients with IBD and 51 matched control subjects were included in the study.

Intervention: All participants underwent a 24-hour Holter recording to assess HRV parameters.

Main outcome measures: The study population was separated into three groups (active disease, remission and control group) to analyze the effect of disease activity status

on the HRV parameters.

Results: No difference was determined between the IBD and control groups in respect of any HRV parameters. Significant differences were determined between the active IBD patients and the remission group in terms of the HRV measurements of SDNN5, triangular index, AVG, SDVLF and SDdef. The measurements of SDNN and PNN50 were found to be significantly different between the active IBD patients and the control group.

Conclusions: The results of this study demonstrated for the first time that the active phase of IBD is associated with cardiac autonomic abnormalities compared to both control group and IBD patients in remission. Patients with IBD should be followed up closely for cardiovascular events as they appear to be at risk for cardiovascular diseases and arrhythmia, particularly during the active phase of the disease.

KEY WORDS: autonomic nervous system, chronic inflammation, heart rate variability, inflammatory bowel diseases

INTRODUCTION

Inflammatory bowel disease (IBD), with the two main forms of Crohn's disease (CD) and ulcerative colitis (UC), is an autoimmune disease characterized by exacerbation and remission periods of inflammation in the gastrointestinal tract. Currently, the etiology of IBD remains unknown. Previous studies have shown that IBD patients are at an increased risk of myocardial infarction (MI), atrial fibrillation, stroke, heart failure, hospitalization and cardiovascular death^[1-5].

Heart rate variability (HRV) is the fluctuation in the time intervals between adjacent heartbeats. Assessment of HRV is based on analysis of consecutive normal R-R intervals (normal to normal, NN) and may provide quantitative information on the modulation of cardiac vagal and sympathetic nerve input. This is a non-invasive, practical and reproducible test which can be used to assess the autonomic nervous system (ANS) modulation functions under physiological and pathological conditions^[6-9]. Changes in HRV

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patterns provide a sensible and advanced indicator of health involvement. A higher HRV is a signal of good adaptation and characterizes a healthy person with efficient autonomic mechanisms. Conversely, a decrease in HRV is thought to reflect inability or attenuation of the ANS and changes in the sinoatrial node response, which may indicate the presence of physiological malfunction in the patient and require further investigations to establish a specific diagnosis. Moreover, severe cardiovascular disease may also be related to a reduced HRV^[10].

The aim of this study was to examine cardiac autonomic functions in a large patient cohort of IBD, including both those in active and remission periods of the disease, using HRV measurements. To date, the association of HRV and IBD activity status has not been evaluated using 24-hour Holter electrocardiogram (ECG) recording. Thus, it was planned to investigate whether HRV parameters are impaired in IBD patients compared to a control group and to evaluate the relationship between IBD disease activity status and HRV parameters.

SUBJECTS AND METHODS

Patients

This prospective study included a total of 67 consecutive patients with IBD (53 patients with UC, 14 patients with CD, aged 18-50 years) and a healthy control group of 51 subjects. Patients were recruited from the Department of Gastroenterology, Ataturk Education and Research Hospital, Ankara, between December 2013 and October 2015. The IBD diagnosis was confirmed with established criteria of clinical,

radiological, endoscopic and histological findings. A detailed medical history was taken, including disease duration and medications, and all patients underwent a routine physical and echocardiographic examination. Surrogate markers of disease activity were defined as hospitalizations with IBD as the primary diagnosis, initiation of biological anti-tumor necrosis factor treatment and claimed prescription of glucocorticoids. With the combined use of these markers and disease activity scores using the Crohn's Disease activity index and the Mayo index^[11-13], the disease stages of remission and flare-up were defined.

Exclusion criteria were age <18 years, structural heart disease, overt cardiovascular disease on the basis of abnormal echocardiographic findings, diabetes mellitus, hypo- or hyperthyroidism, pulmonary disease and neoplastic or chronic systemic diseases, previous gastrointestinal surgery, or the use of medications such as beta-blockers that could interfere with HRV, anti-arrhythmic drugs, digitalis or central sympatholytic agents, antihistaminic agents, benzodiazepines or antidepressants. The control group was formed of healthy individuals with no complaints of organic or functional disease and who were not taking any medications at the time of evaluation.

Echocardiography

All the echocardiographic evaluations were made by a single, experienced cardiologist, blinded to patient data. The echocardiographic examinations were applied using a Vingmed System 7 (Vivid 7, GE, Horten, Norway) with a 2.5- to 3.5-MHz transducer. The left ventricular systolic and diastolic functions

Table 1: Selected time domain measures of heart rate variability for the study

Variable	Units	Description
Statistical Measures		
SDNN	ms	Standard deviation of all NN intervals Clinical importance: It consists of parts from the sympathetic and parasympathetic nervous systems. The SDNN can be described as an overall variability or total power
SDANN5	ms	Standard deviation of the mean values of the NN intervals in the 5-minute intervals within the defined period Clinical importance: Higher values indicate increased parasympathetic activity
RMSSD	ms	The root of the average sum of squares of two differences of successive RR intervals within the defined period Clinical importance: Higher values indicate increased parasympathetic activity
SDNN5	ms	Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording
pNN50	Percentage	Percentage of the NN intervals within the defined period that differ from the previous NN interval by more than 50 mS Clinical importance: Higher values indicate increased parasympathetic activity
HRV triangular index		Total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7.8125 ms (1/128 seconds)

SDNN: standard deviation of all NN intervals during a 24-hour period; SDANN: standard deviation of the 5-minute average of NN intervals; RMSSD: root mean of squared successive differences; pNN50: proportion of differences between successive intervals >50ms; HRV: heart rate variability; NN: interval between two heartbeats (emphasis on "normal" heartbeats); RR: interval between two heartbeats (R spikes in the QRS complex/ECG)

Table 2: Selected frequency domain measures of heart rate variability for the study

Variable	Units	Description
AVG		Average value of all RR intervals
SDTF	ms ²	Standard deviation of TF over 24 hours
SDVLF	ms ²	Standard deviation of VLF over 24 hours. Clinical importance: The energy in LF and VLF are due to both sympathetic and parasympathetic systems
SDHF	ms ²	Standard deviation of HF over 24 hours.
SDdef	ms ²	Clinical importance: The energy in HF is vagal mediated Standard deviation in the predefined frequency range
LF%	Percentage	Percentage of LF in TF

AVG: average value of all RR intervals; SDTF: standard deviation of TF over 24 hours; SDVLF: standard deviation of very low frequency over 24 hours; SDHF: standard deviation of high frequency over 24 hours; SDdef: standard deviation in the predefined frequency range; LF: low frequency band 0.04-0.15 Hz; RR: interval between two heartbeats (R spikes in the QRS complex/ECG); TF: total frequency 0.01-1.00 Hz; VLF: very low frequency band 0.003-0.04 Hz; HF: high frequency band 0.15-0.4 Hz

were analyzed using standard two-dimensional echocardiography, M-mode echocardiography and pulsed-wave echocardiography according to the latest guidelines^[14]. The LV ejection fraction was calculated with the biplane modified Simpson method.

Heart rate variability measurement

A 24-hour Holter recording was applied to all study participants to evaluate the HRV parameters. Holter ECG was performed using a 3-channel digitized recorder (Custo flash 500, Custo Med, Ottobrunn, Germany). Reviewing and editing of the data was performed by an experienced physician blinded to the study population. To be acceptable for the study, evaluation data suitable for analysis were required from a period of 23 hours. Recordings were repeated in cases where these criteria were not met. HRV analysis was performed on measurements, which were standardised and had been validated for the evaluation of autonomic control of the heart^[15].

There are three methods for quantifying HRV. These are time domain analysis, frequency domain analysis and the geometric method. In time domain analysis, the intervals between adjacent normal R waves (NN intervals) are measured over the period of recording. In the time domain method, the standard deviation of all NN intervals during a 24-hour period (SDNN) is the most commonly used time domain measurement of HRV. The standard deviation of the 5-minute average of NN intervals (SDANN5) measures long-term fluctuations. The most common variables calculated as differences between normal R-R intervals are the square root of the mean squared differences of successive NN intervals (RMSSD) and proportion of differences between successive intervals >50 ms (PNN50). RMSSD is an estimate of high-frequency variations in short-term RR recordings and, therefore,

reflects parasympathetic regulation of the heart. The time domain HRV indices measured in this study were SDNN, the mean of the deviation of 5-minute NN intervals over the entire recording (SDNN5), SDANN5, RMSSD, PNN50 and the HRV triangular index.

Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands. Frequency domain parameters include total power, very low frequency, low frequency and high frequency. High frequency reflects the parasympathetic outflow and total power reflects overall autonomic activity, although the physiological explanation of the very low frequency component is less defined. The low frequency power is modulated by both sympathetic and parasympathetic outflows as well as by other factors, including baroreceptor activity. In the frequency domain analysis of this study, examination was made of average value of all RR intervals (AVG), standard deviation of total frequency over 24 hours (SDTF), standard deviation of very low frequency over 24 hours (SDVLF), standard deviation of high frequency over 24 hours and standard deviation in the predefined frequency range.

The descriptions and clinical meanings of the HRV parameters calculated in this study are presented in Table 1 and Table 2.

Clinical and laboratory assessments

Venous blood samples were taken from all participants after a 12-hour fast. High-sensitive C-reactive protein (hs-CRP) was calculated using a nephelometric method. All laboratory analyses were performed using autoanalyzers. Blood pressure measurements were taken three times with the patient seated and following a five-minute rest period and the average measurement was used in the analyses. Hypertension was defined as blood pressure >140/90

Table 3: Baseline characteristics of the study population

Variables	IBD (n=67)	Controls (n=51)	P
Age (years)	42.67±12.8	43.2±15.3	.83
Male (%)	64.2	54.9	.31
Smoking (%)	6	7.8	.28
Hypertension (%)	6	7.8	.28
BMI (kg/m ²)	23.8±2.1	24.5±3.0	.10
Fasting blood glucose (mg/dL)	93.2±9.9	95.4±11.1	.26
HDL cholesterol (mg/dL)	46.8±19.1	52.08±13.1	.31
LDL cholesterol (mg/dL)	100.6±34.8	89.8±32.2	.13
Triglyceride (mg/dL)	125±73.6	83.1±41.2	.02
	102 (71.25-163.75)	75 (58-102)	
AST	19.5±8.1	20.1±5.7	.77
ALT	18.9±10.6	17.4±7.1	.54
ESR (mm/h)	20.8±24.3 11 (5-26)	12.9±5.06 12 (10-15.75)	.61
C-reactive protein (mg/L)	1.35±2.49 0.42 (0.29-1.12)	0.6±1.14 0.3 (0.06-0.47)	.01
Hgb (g/dL)	13.4±1.7	13.1±1.6	.37
PLT (mL/mm ³)	299.4±93.5	258.04±58.05	.59
WBC (mL/mm ³)	7.53±2.01	6.51±1.36	.38
Creatinine (mg/dL)	0.73±0.16	0.81±0.15	.53
Medications (%)			
Steroid	10.4		
Azathiopurine	11.9		
5 ASA	94		
Active disease (%)	42.1		
Disease duration (yr)	4.57±3.43		

BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ESR: erythrocyte sedimentation rate; Hgb: hemoglobin; PLT: platelets; WBC: white blood cells; 5-ASA: 5 aminosalicylic acid

mm Hg or the use of antihypertensive agents. Diabetes mellitus was defined as a fasting plasma glucose level >126 mg/dL or glucose level >200 mg/dL at any time of measurement, or the use of antidiabetic drugs. Dyslipidemia was defined as total cholesterol level of 260 mg/dL or low-density lipoprotein level of 160 mg/dL or the use of lipid-lowering agents.

Statistical analysis and ethics

All statistical analyses were made using SPSS statistical software (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA). Continuous variables were stated as mean±standard deviation (SD) and categorical variables as number (n) and percentage (%). The normality of the distribution of continuous variables was analyzed using the Shapiro-Wilk test. The significance of the differences in the measurements obtained for the control and patient groups was analyzed using the Student's t-test or the Mann-Whitney U test. ANOVA was applied for multiple comparisons between groups. For quantitative values, the Kruskal Wallis test was used for the comparison of patients with active disease, remission and the healthy control group. To identify pairs of groups with significant differences

in quantitative parameters, Bonferroni adjustment for multiple comparisons was used. The Pearson's correlation analysis was performed for variables with normal distribution and Spearman's rank correlation was used for variables with non-normal distribution. Tests of significance were two-tailed and a value of *P* <.05 was accepted as statistically significant.

The study was conducted in compliance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from each study participant. Approval for the study protocol was granted by the local Ethics Board of Ankara Atatürk Education and Research hospital.

RESULTS

Demographic characteristics

The study included a total of 118 participants, comprising 67 patients with IBD and 51 healthy control subjects. The baseline characteristics of the study population are presented in Table 3. No significant differences were found between the patients and the control group regarding demographic features (*P* >.05). There were 43 male (51.7%) and 24 female patients in the IBD group. The mean age was 42.67±12.8 years in the patient group and 43.2 ± 15.3 years in the

control group. No statistically significant difference was determined between the groups in respect of age, gender, body mass index, fasting blood glucose, alanine aminotransferase, aspartate aminotransferase, creatinine, hemoglobin, white blood cell or platelets. There were six hypertensive patients and six smokers in both groups. Triglyceride levels were significantly higher in the patient group, but no difference was seen between the groups in terms of high or low density lipoprotein cholesterol. In the evaluation of inflammatory markers, erythrocyte sedimentation rate (ESR) was higher in the IBD group, but did not reach a statistically significant level. The hs-CRP level was determined to be significantly higher in the IBD group than in the control group ($P=.01$).

Disease duration was 4.57 ± 3.43 years. Active disease was determined in 42.1% of the patient group. Of the patients with IBD, 20.9% had CD, 9% had proctitis, 37.3% had left-side colitis and 31.3% had pancolitis. All patients used medications for IBD. The majority (94%) of the patients were using 5-aminosalicylic acid and a few patients were taking immunosuppressive drugs such as azathiopurine (11.9%) and steroids (10.4%)

Heart rate variability parameters

The comparisons of HRV parameters between the IBD patients and the healthy control group are shown in Table 4. No differences were determined between

the groups with respect to time domain measures and frequency domain measures. No differences were determined between the groups in the basic parameters of minimum/maximum/mean heart rate (HR), supraventricular extrasystole and ventricular extrasystole count.

To analyze the effects of disease activity on the HRV parameters, the study population was separated into three groups of active disease, remission and control. The baseline characteristics of the three groups are presented in Table 5. No significant difference was determined between the groups in terms of age, gender, body mass index, smoking status and hypertension. The LDL, HDL, total cholesterol and creatinine levels were observed to be similar in all the groups. The hs-CRP, ESR and platelet values were significantly higher in the active disease group compared to the other two groups (Table 5). White blood cell was significantly higher and the hemoglobin level was lower in patients with active disease compared with the control group and patients in remission, respectively. Triglyceride and ESR levels were higher in IBD patients in remission than in the control group. No significant difference was observed with respect to hs-CRP, white blood cell, platelets and hemoglobin between IBD patients in remission and the control group.

Comparison of HRV measures according to disease activity status are shown in Table 6. SDANN5,

Table 4: Differences in heart rate variability measures between the study population

Variables	IBD (n=67)	Controls (n=51)	P
Time domain measures			
SDNN (ms)	137±43.1	156.8±58	.06
SDANN5 (ms)	125.2±39.7	125.6±45.6	.99
RMSSD (ms)	47.7 (30.7-68.9)	56.2 (36.3-89.7)	.21
SDNN5 (ms)	63.8 (51.3-77.5)	71.4 (53-93.4)	.09
pNN50 (%)	9.55 (5.07-15.8)	13.2 (5.2-25.3)	.06
HRV triangular index	35.1 (27.8-43.9)	38 (30.6-52.1)	.24
Frequency domain measures			
AVG	1599.2±275	1686±266	.27
SDTF	45.3 (40.6-49.02)	45.7 (42.2-48.7)	.78
SDVLF	212.9±38.4	220.5±33.7	.43
SDHF	0.76 (0.4-1.33)	0.78 (0.48-1.32)	.74
SDdef LF%	0.39 (0.2-0.76)	0.45 (0.29-0.74)	.052
LF%	75.6 (65.2-84.4)	72.7 (61.6-81.3)	.4
Basic parameters			
Mean HR	75.8±9.3	73.7±11.2	.3
Max HR	126.6±16.9	129.6±18.1	.36
Min HR	55.7±9.1	54.6±11.1	.56
SVES (n)	4.5 (2-14)	5 (1-11)	.95
VES (n)	1 (0-10)	2 (0-8)	.66

The values are presented as median (interquartile range); SVES: supraventricular extrasystole; VES: ventricular extrasystole; HR: heart rate; SDNN: standard deviation of all NN intervals during a 24-hour period; SDANN: standard deviation of the 5-minute average of NN intervals; RMSSD: root mean of squared successive differences; pNN50: proportion of differences between successive intervals >50ms; HRV: heart rate variability; AVG: average value of all RR intervals; SDTF: standard deviation of TF over 24 hours; SDVLF: standard deviation of very low frequency over 24 hours; SDHF: standard deviation of high frequency over 24 hours; SDdef: standard deviation in the predefined frequency range; LF: low frequency band 0.04-0.15 Hz

Table 5: Comparison of the baseline characteristics according to disease activity status

Variables	Active disease (n=24)	Remission (n=43)	Controls (n=51)	P
Age (years)	45.08±13.5	41.3±12.4	43.2±15.3	.55
Male (%)	70.8	60.5	54.9	.42
Smoking (%)	4.2	7	7.8	.78
Hypertension (%)	12.5	9.3	7.8	.22
BMI (kg/m ²)	24.9±5.46	25.3±3.6	24.5±3.0	.84
Fasting blood glucose (mg/dL)			95.4±11.1	
HDL cholesterol (mg/dL)	44.7±18.4	48.02±19.6	52.08±13.1	.27
LDL cholesterol (mg/dL)	91.6±37.2	105.7±32.7	89.8±32.2	.09
Triglyceride (mg/dL)	87 (72-135)	109 (70.5-170) ^c	83.1±41.2	.005
			75 (58-102)	
AST	17.1±7.5	20.9±8.2	20.1±5.7	.14
ALT	16.7±12.4	20.2±9.4	17.4±7.1	.31
ESR (mm/h)	33	9 (3-14) ^c	12.9±5.06	.0001
	(10.2-76.2) ^{ab}		12 (10-15.75)	
C-reactive protein (mg/L)	1.44	0.35	0.6±1.14	.0001
	(0.37-3.2) ^{ab}	(0.2-0.65)	0.3 (0.06-0.47)	
Hgb (g/dL)	12.6±1.76 ^b	13.9±1.6	13.1±1.6	.008
PLT (mL/mm ³)	342.4±98.5 ^{ab}	275.3±82.3	258.04±58.05	.001
WBC (mL/mm ³)	8.15±2.59 ^a	7.18±1.52	6.51±1.36	.015
Creatinine (mg/dL)	0.74±0.2	0.72±0.1	0.81±0.15	.14

BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ESR: erythrocyte sedimentation rate; Hgb: hemoglobin; PLT: platelets; WBC: white blood cells

^a was significant between active disease and control groups

^b was significant between active disease and remission groups

^c was significant between remission and control groups

significance $P < .05$

Table 6: Comparison of heart rate variability measures according to disease activity status

Variables	Active disease (n=24)	Remission (n=43)	Controls (n=51)	P
Time domain measures				
SDNN (ms)	127.5 ± 9.3 ^a	146.03 ± 6.7	156.8 ± 58	.02
SDANN5 (ms)	115.5 ± 8.94	131.5 ± 6.03	125.6 ± 45.6	.36
RMSSD (ms)	45 (27.2 - 58.5)	51.7 (34.5 - 77.7)	56.2 (36.3 - 89.7)	.11
SDNN5 (ms)	55 (33.8 - 75.7) ^{ab}	64.8 (53.3 - 80.7)	71.4 (53 - 93.4)	.013
pNN50 (%)	9.1 (1.2 - 18) ^a	10.2 (6.2 - 18.3)	13.2 (5.2 - 25.3)	.027
HRV triangular index	32.3 (21.7 - 40.8) ^{ab}	39.9 (32.3 - 47)	38 (30.6 - 52.1)	.009
Frequency domain measures				
AVG (ms ²)	1545.4 ± 39.1 ^{ab}	1684.7 ± 41.2	1686 ± 37	.001
SDTF (ms ²)	42.9 (37.2 - 47.6)	45.6 (41.4 - 51)	45.7 (42.2 - 48.7)	.12
SDVLF (ms ²)	202.7 ± 5 ^{ab}	222.6 ± 6.4	220.5 ± 33.7	.01
SDHF (ms ²)	0.47 (0.26 - 1.36)	0.8 (0.51 - 1.33)	0.78 (0.48 - 1.32)	.23
SDdef (ms ²)	0.24 (0.07 - 0.76) ^{ab}	0.47 (0.23 - 0.81)	0.45 (0.29 - 0.74)	.02
LF (%)	76.1 (64.5 - 87.2)	75.6 (65.9 - 82.08)	72.7 (61.6 - 81.3)	.39
Mean HR	78.6 ± 2.16	74.1 ± 1.33	73.7 ± 11.2	.13
Max HR	126.9 ± 3.03	126.4 ± 2.9	129.6 ± 18.1	.65
Min HR	58.9 ± 2.2	53.8 ± 1.18	54.6 ± 11.1	.14
SVES(n)	4 (2 - 14)	5 (1 - 15)	5 (1 - 11)	.9
VES (n)	2 (0 - 13)	1 (0 - 10)	2 (0 - 8)	.25

The values are presented as median (interquartile range)

^a was significant between active disease and control groups

^b was significant between active disease and remission groups

^c was significant between remission and control groups

significance $P < .05$

SDNN: standard deviation of all NN intervals during a 24-hour period; SDANN: standard deviation of the 5-minute average of NN intervals; RMSSD: root mean of squared successive differences; pNN50: proportion of differences between successive intervals >50ms; HRV: heart rate variability; AVG: average value of all RR intervals; SDTF: standard deviation of TF over 24 hours; SDVLF: standard deviation of very low frequency over 24 hours; SDHF: standard deviation of high frequency over 24 hours; SDdef: standard deviation in the predefined frequency range; LF: low frequency band 0.04-0.15 Hz; HR: heart rate

Table 7: Correlation between heart rate variability parameters and laboratory and clinical parameters in all study group (Pearson's test r values and their significance)

	Age	Mayo score	Albumin	Leukocyte	Hemoglobin	ESR	CRP
Max HR	-0.456 <i>P</i> < .001	NS	NS	NS	-0.231 <i>P</i> = .024	NS	NS
MinHR	NS	0.362 <i>P</i> = .011	-0.283 <i>P</i> = .026	NS	-0.264 <i>P</i> = .009	0.268 <i>P</i> = .006	0.387 <i>P</i> < .001
Mean HR	NS	0.374 <i>P</i> = .008	-0.312 <i>P</i> = .014	NS	-0.319 <i>P</i> = .002	0.269 <i>P</i> = .006	0.353 <i>P</i> < .001
SDNN	-0.193 <i>P</i> = .047	NS	0.312 <i>P</i> = .014	NS	NS	-0.213 <i>P</i> = .031	-0.263 <i>P</i> = .019
SDANN5	-0.369 <i>P</i> < .001	NS	0.272 <i>P</i> = .032	NS	0.287 <i>P</i> = .005	NS	-0.302 <i>P</i> = .007
SDNN5	NS	-0.369 <i>P</i> = .009	0.314 <i>P</i> = .013	NS	NS	NS	NS
AVG	NS	-0.291 <i>P</i> = .042	0.283 <i>P</i> = .026	NS	0.313 <i>P</i> = .002	-0.229 <i>P</i> = .021	-0.291 <i>P</i> = .009
SDTF	NS	NS	NS	NS	0.296 <i>P</i> = .003	NS	-0.242 <i>P</i> = .031
SDVLF	NS	NS	0.259 <i>P</i> = .042	NS	0.310 <i>P</i> = .002	-0.213 <i>P</i> = .032	-0.267 <i>P</i> = .016

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HR: heart rate; SDNN: standard deviation of all NN intervals during a 24-hour period; SDANN: standard deviation of the 5-minute average of NN intervals; AVG: average value of all RR intervals; SDTF: standard deviation of TF over 24 hours; SDVLF: standard deviation of very low frequency over 24 hours
r: correlation coefficient, NS: Non-significant *P* > .05,

RMSSD, SDTF, standard deviation of high frequency over 24 hours and low frequency values were lower in the active disease group than in the control and remission groups, with no statistically significant difference observed. No significant differences were determined between the groups in terms of maximum/minimum/mean HR, supraventricular extrasystole and ventricular extrasystole count. The active IBD patients differed significantly from both the control and remission groups in terms of SDNN5, triangular index, AVG, SDVLF and standard deviation in the predefined frequency range. Significant differences were determined between active patients and the control group in respect of SDNN and PNN50. No significant differences were found between IBD patients in remission and the control group in respect of any HRV parameters.

Correlation analyses between inflammatory markers and HRV parameters are shown in Table 7. In all the study groups, CRP was positively correlated with mean HR and minimum HR, and negatively correlated with SDTF, SDANN5, AVG, SDNN and SDVLF. There was a significantly negative correlation of ESR levels with SDVLF, SDNN, AVG and a positive correlation with minimum/mean HR. Age was determined to be negatively correlated with maximum HR, SDNN and SDANN5. There was a positive correlation between SDNN, SDANN5, SDNN5, AVG, SDVLF and albumin levels. Hemoglobin levels were negatively correlated with HR and positively correlated with SDANN5, AVG, SDTF and SDVLF.

In the evaluation of the effects of disease activity scores such as the Mayo score on HRV parameters, minimum HR and mean HR were positively correlated and SDNN5, AVG were negatively correlated. Correlation analysis with Crohn's Disease activity index could not be performed since the number of patients in the CD group was insufficient for analysis. When the CD and UC active status patients were analyzed together in the active group, CRP was determined to be positively correlated with minimum/mean/maximum HR and negatively correlated with SDVLF, AVG and SDTF. In the active disease group, the same interaction was observed with ESR level. In the remission and control groups, CRP and ESR levels were not significantly correlated with most of the HRV parameters (Table 8).

DISCUSSION

IBD, including UC and CD is estimated to affect 2.2 million individuals in Europe. Although the etiology of IBD is not fully known, the process is thought to be triggered by a combination of environmental, genetic and immunological factors in genetically predisposed individuals, leading to a normal endothelial cell immune response causing intestinal microvascular damage resulting in chronic intestinal inflammation^[16].

In some studies, it has been suggested that patients with IBD are at increased risk of cardiovascular events and atherosclerosis^[17], but the cause of increased cardiovascular risk has not yet been fully explained. A chronic inflammatory process may

Table 8: Correlation analysis of inflammatory parameters and heart rate variability measures in active, remission and control groups

Group	Lab. parameters	Min HR	MeanHR	Max HR	SDNN	SDANN5	SDVLF	SDTF	AVG	Triangular index
Active	CRP	0.44 ^{0.03}	0.47 ^{0.02}	0.58 ^{0.003}	NS	NS	-0.47 ^{0.02}	-0.47 ^{0.02}	-0.46 ^{0.02}	NS
	ESR	NS	0.44 ^{0.032}	0.48 ^{0.019}	NS	NS	-0.45 ^{0.03}	-0.53 ^{0.01}	-0.45 ^{0.02}	NS
Remission	CRP	0.34 ^{0.03}	0.37 ^{0.02}	NS	NS	NS	NS	NS	-0.32 ^{0.04}	NS
	ESR	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control	CRP	0.54 ^{0.02}	NS	NS	-0.57 ^{0.01}	NS	-0.47 ^{0.04}	-0.49 ^{0.03}	NS	NS
	ESR	NS	NS	NS	NS	NS	NS	NS	NS	NS

r: correlation coefficient, NS: Non-significant $P > .05$, HR: heart rate; SDNN: standard deviation of all NN intervals during a 24-hour period; SDANN: standard deviation of the 5-minute average of NN intervals; AVG: average value of all RR intervals; SDTF: standard deviation of TF over 24 hours; SDVLF: standard deviation of very low frequency over 24 hours; CRP: C-reactive protein, ESR: erythrocyte sedimentation rate

lead to atherosclerosis. During the course of IBD, the typical elevation of proinflammatory cytokines such as CRP, tumor necrosis factor alpha and interleukin has been shown to be associated with subclinical atherosclerosis^[18]. Inflammation is known to have a role in all stages of atherosclerosis, from initiation to eventual plaque rupture and thrombosis.

IBD is typically seen with periods of exacerbations in which the disease is active and asymptomatic remission periods. Inflammation plays a critical role in the pathogenesis of IBD and proinflammatory cytokines have been shown to contribute to the process of the disease^[19]. However, there is conflicting evidence in respect of a significantly increased risk of MI, stroke and cardiovascular mortality for IBD patients. Two registry-based studies of approximately 40,000 IBD patients and a meta-analysis of 11 studies with a total of almost 14,000 patients have reported increased risk of MI and cardiovascular mortality in IBD compared to matched control subjects without IBD^[20,21].

In the last decade, new evidence has emerged that the risk of MI, stroke and cardiovascular mortality is significantly increased in IBD patients, especially during periods of IBD activity. Kristensen *et al* recently published very important data of a Danish registry study of approximately 20,000 patients. A statistically significant increased risk of MI, stroke and cardiovascular death was reported for patients with active IBD periods when the relative risk of MI increased nearly two-fold (1.49 (CI: 1.16-1.93) for flare and 2.05-fold (CI: 1.58-2.65) for persistent IBD activity^[2]. The same study group also demonstrated that IBD was associated with a greater risk of hospitalization for heart failure and that this risk was strongly related to periods of active disease^[3]. In a study of 86,790 Danish patients with first-time MI between 2002 and 2011, the effect of active IBD was evaluated on major adverse cardiovascular outcomes after MI. It was determined that IBD was associated with hazard ratios of 1.21 (95% CI: 0.99-1.49) for recurrent MI, 1.14 (95% CI: 1.01-1.28) for all-cause mortality and 1.17 (95% CI: 1.03-1.34) for

the composite end point. In comparison with the non-IBD group, IBD flare-ups were strongly associated with an increased risk of recurrent MI and all-cause mortality, whereas during remission, no increased risk was detected^[22]. The latest and most comprehensive study by Panhwar *et al* evaluated over 250,000 patients with IBD from a database and compared these with well-matched patients without IBD. Both CD and UC were reported to have an increased risk of MI and highest risk was seen in younger patients. It was concluded that aggressive risk factor modification for MI is essential for patients with IBD^[23].

In the current study group, inflammatory markers such as hs-CRP and ESR were higher, especially in the active disease group, which were expected results. In accordance with recently published data, it was demonstrated that HRV deteriorated only during active IBD compared to both the control group and the remission group, which suggested a role of shared pathophysiological inflammatory mechanisms. Clinical interpretation of these findings can be summarized as HRV parameters, which are predominantly modified by parasympathetic ANS, were decreased with higher levels of positive phase reactants, a higher Mayo score and active disease status, and increased with higher levels of albumin and hemoglobin. The opposite of this statement is also true.

There seems to be considerable potential for HRV in the assessment of the role of ANS fluctuations in normal healthy individuals and in patients with various cardiovascular and non-cardiovascular disorders. The HRV Taskforce guidelines recommend four measures for time domain HRV assessment: (1) SDNN (estimate of overall HRV); (2) HRV triangular index (estimate of overall HRV); (3) SDANN (estimate of long-term components of HRV); and (4) RMSSD (estimate of short-term components of HRV)^[15].

In addition, the marked relationship between ANS, IBD and cardiovascular disease over inflammatory pathways was seen with similar but less elusive interaction between IBD and HRV through the

enteric nervous system. There is an increasing body of evidence suggesting that the ANS and the immune system have a complex relationship in the pathogenesis of IBD^[24]. ANS have several roles in the gastrointestinal tract, including the modulation of motility and secretion functions, and regulation of mucosal immune and inflammatory responses^[25]. In IBD patients, functional changes in colonic mucosa result in abnormal colonic motility and transit functions. These motor disturbances are suggestive of alterations in colonic neuromuscular components including enteric neurons^[26]. Focal destruction of ANS axons is also present in inflamed and non-inflamed CD patients' small bowel tissue. However, it is not very clear if the inflammation of enteric nervous system triggers the etiopathogenetic cascade or if enteric nervous system plays a minor role, which is only affected by the process.

It has been thought that ANS dysfunction might have a role in the pathogenesis of IBD^[27]. Some studies have suggested that increased sympathetic activity may be responsible for the augmentation of bowel inflammation^[28,29]. Both hypofunction (autonomic neuropathy) and hyperfunction (autonomic hyperreflexia) have been described in IBD. In previous studies, autonomic neuropathy has been detected at a high prevalence in IBD, ranging from 40% to 50%, although in further studies, cardiovascular autonomic neuropathy has been found to be rare in IBD, with prevalence rates of approximately 5%^[30].

The association of altered HRV and IBD has been examined in several studies. Although some observational studies have focused on impaired HRV in patients with IBD, conflicting results have emerged from studies of the autonomic functions in patients with both UC and CD. This could be attributed to the use of different protocols in the recording of HRV, small sample sizes and different disease activity status of the patients. Using time domain and spectral HRV from a 24-hour ambulatory ECG, Mouzas *et al* showed an increase in vagal functions in IBD patients with no distinction between UC and CD subgroups^[31]. With the use of another technique (20-minute HRV record), Coruzzi *et al* concluded that compared to both CD patients and the control group, UC patients had decreased parasympathetic tone and increased sympathetic tone^[32]. Sharma *et al*^[33] reported lower cardiovascular autonomic functions in patients with IBD in clinical remission. It was also indicated that UC patients in particular had significantly lower parasympathetic function compared to those with CD and healthy control subjects. In a recent study by Sarli *et al*, it was demonstrated that IBD was associated with an abnormal heart rate reduction following a treadmill exercise test, but the parameters were not compared

between patients with active disease and those in remission. Unlike other studies, the heart rate recovery index was used to investigate autonomic function rather than HRV^[34].

Although there are conflicting results from studies that have not taken disease activity into consideration, there seems to be increasing evidence that active and persistent periods of disease are a risk factor for cardiovascular disease and arrhythmia. In contrast to previous studies, the current study shows that the HRV parameters were not affected in IBD patients with clinical remission. It was also shown for the first time with the use of 24-hour ambulatory ECG that most of the HRV indices were impaired in active IBD patients.

Several limitations need to be mentioned. This study was cross-sectional in nature and therefore, the findings cannot be generalized. Another important limitation of the study was the low number of patients, which may also prevent the generalization of these results to all IBD patients. Therefore, large scale studies are needed to confirm these results. As inflammation was successfully suppressed with anti-inflammatory therapy in the patients, the majority of IBD patients in the study were in remission, and the number of patients with active IBD was low. Another important limitation was the significant difference between the patient group (active and remission) and the control group in respect of the triglyceride, hematological and inflammatory marker levels. Moreover, that the patients could not be followed up prospectively for future major adverse cardiac events is the most important limitation of the study.

CONCLUSION

The results of this study provided clear evidence that the active phase of IBD is associated with ANS abnormalities, with significant impairment of cardiac vagal modulation compared to both the control group and IBD patients in remission. It can be speculated that in active periods of disease, "an enhanced inflammatory load" is likely to worsen HRV. These results indicate that a treatment strategy aiming to reduce the duration and number of flare-ups in patients with IBD may be warranted to be able to decrease the cardiovascular risk, especially for patients with prolonged or recurrent disease activity. Further studies are needed to explore the predictive role of diminished HRV in the development of future cardiovascular complications in patients with active IBD.

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Author contribution statement: Cenk Sari and Sevil Ozcer Sari conceived the presented idea. Sevil Ozcer Sari, Serkan Sivri, Cenk Sari and Oyku T Yurekli developed the theory and performed the computations. Huseyin Koseoglu, Cenk Sari and Sevil Ozcer Sari verified the analytical methods. Osman Ersoy supervised the findings of this work. All authors discussed the results and contributed to the final manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Original Article

The value of integrated pulmonary index monitoring in pediatric endoscopic interventions under sedation

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ABSTRACT

Objective: To evaluate the diagnostic and predictive role of Integrated Pulmonary Index (IPI) in pediatric patients undergoing sedation during endoscopic intervention

Design: Prospective

Setting: Department of Anesthesiology and Reanimation, Kartal Dr. Lutfi Kırdar Training and Research Hospital, Istanbul, Turkey

Subjects: Sixty children between the ages of 1 and 12 years scheduled to undergo diagnostic gastroscopy or colonoscopy under sedation

Interventions: Demographic variables, the duration and type of procedure, drugs used for sedation (propofol, midazolam, and/or opioid), and the total quantity of drugs used were recorded. Heart rate, systolic arterial pressure, diastolic arterial pressure, mean arterial pressure, respiratory rate, peripheral oxygen saturation (SpO₂), end-tidal carbon dioxide (ETCO₂) values and the respiratory score calculated by IPI monitor were

recorded. Hypoxia, mask ventilation indications related to respiratory failure and the requirement for a chin-lift movement were recorded.

Main outcome measures: Clinical safety and efficacy of IPI monitor during procedural sedation in pediatric patients undergoing gastroscopy or colonoscopy

Results: The IPI and ETCO₂ values demonstrated a similar tendency. Significantly higher mean baseline IPI scores were recorded in patients over 8 years of age. The changes in SpO₂ did not correlate with these variables. However, SpO₂ values were found to be within a normal range, while the IPI value decreased in patients requiring mask ventilation.

Conclusion: Patients of all age groups require procedural sedation. In cases where apnea and hypoventilation are commonly encountered, IPI can provide an early warning system, especially in the pediatric patient population, to prevent hypoxia.

KEY WORDS: apnea, hypoventilation, microstream capnography, respiratory monitoring, sedation

INTRODUCTION

A gastrointestinal endoscopic procedure may be necessary to diagnose and treat gastrointestinal diseases. Although it is a safe procedure, it may cause some complications and it may be necessary to give intravenous sedation and/or general anesthesia to allow children to tolerate unpleasant procedures requiring immobility and anesthetic measures. In both applications, hemodynamics and oxygenation should be monitored closely^[1].

Monitorization of the respiratory rate (RR) and peripheral oxygen saturation (SpO₂) alone is not sufficient to assess pulmonary function; measurement of end-tidal carbon dioxide (ETCO₂) using capnography is a significant contribution to the evaluation of

pulmonary function^[2]. Early warning systems that support the recognition of critical respiratory events may be valuable. The Integrated Pulmonary Index (IPI) offers a single value for quick and easy assessment and monitoring of ventilation and oxygenation.

The capnograph device (Capnostream 20p/ Covidien, Origen Medical, Medtronic, Minneapolis, MN, USA) monitors both ETCO₂ and SpO₂ (Figure 1). It displays each of the ETCO₂, RR, heart rate (HR), and SpO₂ measurements on the monitor screen separately. Furthermore, it combines all of these measurements and reduces them to a single numerical value, the IPI, which comprises a mathematical analysis of all of these values and indicates the defined respiratory status. This device repeats real-time measurements every

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Table 1: Integrated Pulmonary Index scores and patient status

Color Scale	IPI Score	Patient status
Green zone	10	Normal (optimal)
Green zone	8-9	Within normal range
Green zone	7	Close to normal range; requires attention
Yellow zone	5-6	Requires attention and may require intervention
Yellow zone	3-4	Requires intervention
Red zone	1-2	Requires immediate intervention

IPI: Integrated Pulmonary Index

second and creates an IPI algorithm from these measurements and displays them both graphically and numerically on the monitor. The IPI algorithm evaluates the patient's respiratory function using four parameters: $ETCO_2$, RR, HR and SpO_2 [3].

The IPI score is divided into six categories, and the score in each category ranges from 1 to 10 (Table 1). The IPI score can be a marker over time aside from the measured parameters and can be generated instantly at desired time intervals. This means that any change in respiratory state can immediately be displayed. The IPI evaluates the patient's respiratory status quickly and can guide the physician to determine the requirement for an intervention. The capnographic features are valuable; however, the IPI score is notable for use in the clinic and contributes precision to the evaluation.

Since the normal vital values in the pediatric age group are age-dependent, three groups have been identified for these patients as 1-3 years, 3-6 years and 6-12 years. The use of the IPI for newborns and children under one year of age is not yet valid [4]. The aim of this study was to evaluate the diagnostic and predictive role of IPI in pediatric patients who underwent sedation for an endoscopic intervention.

SUBJECTS AND METHODS

After receiving the approval of the ethics committee of our hospital (decision no: 2018/514/123/1; date: 14.02.2018), 60 children between the ages of 1 and 12



Fig 1: The Integrated Pulmonary Index monitor (Capnostream 20p; Medtronic, Inc., Minneapolis, MN, USA) demonstrating the respiratory score (A) calculated based on end-tidal carbon dioxide (B), pulse rate (C), peripheral oxygen saturation (D) and respiratory rate (E).

years who were to undergo diagnostic gastroscopy or colonoscopy were included in the study. In addition to demographic characteristics of the patients such as age, gender, weight and height, American Society of Anesthesiologists (ASA) values were also recorded. The research was conducted in compliance with the principles of the Declaration of Helsinki.

Nasal probe and finger probe data from the capnograph monitor were used in patients who were monitored for noninvasive blood pressure. SpO_2 and HR were monitored via finger probe, and monitorization of $ETCO_2$ volume and RR were performed with a nasal probe. The respiratory score derived by the device based on these values was integrated into the IPI.

A combination of propofol+midazolam or propofol+midazolam+fentanyl was used to provide sedation, according to the preference of the anesthesiologist. Intravenous doses of propofol (1-2 mg/kg), midazolam (0.03 mg/kg) and fentanyl (0.5-1 mcg/kg) were used.

Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), $ETCO_2$,

Table 2: Descriptive statistical data and average values of other data

Variables	Number of patients	Minimum value	Maximum value	Mean \pm SD
Age (years)		1	11	8.2 \pm 2.8
Weight (kg)		7	51	26.9 \pm 9.1
Sex (male/female)	60			33/27
Height (cm)		70	155	129.2 \pm 17.1
Propofol (mg)		20	300	140.9 \pm 61.9
Opioid (mcg)	60	0	50	6.7 \pm 14.5
Midazolam (mg)		0.5	2	1.2 \pm 0.4
Duration of operation (min)		10	60	27.4 \pm 12.9

Table 3: Relationship between age and Integrated Pulmonary Index

Time (minutes)	Number of patients	Age (years)	Mean IPI score	Mean difference	P-value
Baseline	25	≤8	9.84±0.09	0.16±0.08	.0491*
	35	>8	10±0		
1	25	≤8	6.52±0.59	0.31±0.75	.6827
	35	>8	6.829±0.48		
3	25	≤8	6.44±0.513	-0.13±0.79	.8736
	35	>8	6.31±0.554		
5	25	≤8	7.28±0.534	0.01±0.68	.9933
	35	>8	7.29±0.43		
10	25	≤8	7.32±0.573	0.25±0.69	.7167
	35	>8	7.57±0.42		
15	22	≤8	8.27±0.42	-0.42±0.62	.4992
	34	>8	7.85±0.414		
20	18	≤8	8.11±0.46	0.39±0.69	.5733
	30	>8	8.50±0.45		
25	10	≤8	8.40±0.54	0.378±0.61	.5389
	18	>8	8.78±0.34		
30	9	≤8	7.78±0.662	0.28±1.00	.7805
	17	>8	8.06±0.63		
35	5	≤8	8.40±0.51	-0.09±0.87	.917
	13	>8	8.31±0.50		
40	4	≤8	8±0	0.6±0.8436	.4905
	10	>8	8.6±0.5207		
45	3	≤8	8±0	0.25±0.8406	.4761
	8	>8	8.625±0.4978		
50	2	≤8	8.50±1.50	0.30±1.56	.8547
	5	>8	8.20±0.80		

* $P < .05$ was considered statistically significant.

IPI: Integrated Pulmonary Index

RR, SpO₂, HR and IPI values were recorded and saved before initiation of the procedure.

The same parameters were then measured and recorded at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes during the procedure, as well as events of hypoxia and the need for mask ventilation due to respiratory failure. In cases of respiratory insufficiency, the chin-lift movement was performed first and this was recorded. In cases of apnea lasting more than 20 seconds that did not respond to a chin lift, mask ventilation was performed. At the end of the procedure, the type and total quantity of drugs used for sedation were also recorded.

GraphPad Prism version 7.00 for Windows, (GraphPad Software, La Jolla, CA, USA) was used to perform the statistical analysis of the study data. The analysis of study data included descriptive statistical parameters (mean, standard deviation, minimum and maximum value) and for comparisons between groups, Student's t-test, one-way analysis of variance, and the Tukey post-test were used, as appropriate. The levels of statistical significance were * $P < .05$, ** $P < .01$, *** $P < .001$ and **** $P < .0001$.

RESULTS

We have measured and recorded the parameters

for 60 minutes. The number of patients recorded beyond 50 minutes was insufficient to perform the statistical analysis. When the descriptive statistical data were examined, the minimum, maximum and mean age of the patients was determined to be 1, 11, and 8.2 years, respectively. The minimum, maximum, and mean body weight of the patients was 7, 51 and 26.9 kg, respectively. The minimum, maximum and mean height of the patients was 70, 155 and 129.2 cm, respectively. The study population consisted of 33 male (55%) and 27 female (45%) patients (Table 2).

The minimum, maximum and mean dose of propofol was 20, 300 and 140.9 mg, respectively. The lowest, highest and mean midazolam dose was 0.5, 2 and 1.2 mg, respectively. The lowest, highest, and mean opioid dose was 0, 50 and 6.7 mcg, respectively.

Two procedures, gastroscopy or colonoscopy, were performed in 60 patients. Gastroscopy was performed in 46 (76.6%) and colonoscopy in 14 (23.4%) cases. The lowest, highest and mean length of the procedure was 10, 60 and 27.4 minutes, respectively (Table 2).

Relationship between age and IPI score

The IPI score was investigated in terms of any determinative role with respect to the age of the patients. The patients aged ≤8 and >8 years were

Table 4: Comparison between mask ventilation status, IPI and SpO₂

Time (minutes)	Number of patients	Mask ventilation	Mean (±SD) IPI score	Mean (±SD) SpO ₂ (%)	P-value for IPI	P-value for SpO ₂
Baseline	44	NO	9.91±0.05	99.91±0.04		
	16	YES	10±0	99.88±0.12	.322	.744
1	44	NO	6.93±0.41	99.61±0.16		
	16	YES	6.06±0.78	99.56±0.20	.299	.859
3	44	NO	6.66±0.45	99.32±0.18		
	16	YES	5.56±0.72	99.25±0.30	.210	.843
5	44	NO	7.41±0.37	99.16±0.19		
	16	YES	6.94±0.72	99.13±0.30	.535	.927
10	44	NO	7.77±0.35	99.36±0.13		
	16	YES	6.62±0.80	98.81±0.29	.133	.047*
15	41	NO	8.22±0.34	99.15±0.16		
	15	YES	7.47±0.60	99.4±0.25	.270	.415
20	33	NO	8.24±0.44	99.18±0.16		
	15	YES	8.60±0.44	99.13±0.34	.620	.884
25	17	NO	8.35±0.39	99.18±0.18		
	11	YES	9.091±0.3921	99.18±0.32	.215	.987
30	17	NO	8.56±0.46	99.25±0.28		
	9	YES	7.11±1.02	98.89±0.35	.148	.439
35	10	NO	9±0.33	99.40±0.30		
	8	YES	7.71±0.71	98.57±0.68	.091	.238
40	9	NO	8.67±0.47	99.67±0.17		
	5	YES	8±0.63	99±0.63	.414	.214
45	7	NO	9±0.38	99±0.44		
	4	YES	7.50±0.50	99±0.58	.040*	>.999
50	4	NO	9.25±0.75	99.50±0.50		
	3	YES	7.92±0.31	99.19±0.14	.2582	.5665

*P <.05 was considered statistically significant.

IPI: Integrated Pulmonary Index; SpO₂: peripheral oxygen saturation

considered in two groups. The IPI values differed significantly between age groups only in the baseline value (borderline significance); the IPI value did not differ significantly in measurements taken at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 minutes. Accordingly, the mean baseline IPI of the group aged >8 years was higher; however, the mean IPI value of both groups was within the normal optimal range (Table 3).

Relationship between gender and IPI score

The effect of gender on IPI score was investigated, and in this study population of 33 male and 27 female children, there was no statistically significant difference between genders in the IPI values obtained at baseline, 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 minutes.

Relationship between ASA class and IPI score

A potential relationship between IPI and ASA classification was examined. In this study, 43 (71.6%) patients were evaluated as ASA I, while 17 (28.3%) patients were classified in the ASA II category. Among the IPI values obtained at baseline, 1, 3, 5, 10, 15, 20, 25,

30, 35, 40, 45 and 50 minutes, only the 30-minute IPI value was statistically significant for the ASA score. That is, the mean IPI value of ASA class I patients at 30 minutes was significantly higher than the mean IPI value of ASA II patients. The mean IPI value of the patients in ASA class I was 8.94 points, *i.e.*, within normal limits, while the mean IPI of ASA II patients was 6.44 points, indicating that these patients require attention and may require intervention.

Relationship between drug type and IPI score

In order to investigate any determinative role of IPI score according to the drug type used, the patients who received propofol and midazolam and those treated with propofol, midazolam and an opioid (fentanyl) were considered in separate groups. In 12 of 60 patients (20%), an opioid (fentanyl) was used in addition to propofol and midazolam. Although the IPI values in the propofol, midazolam and opioid groups were lower, in the statistical evaluation, the IPI value obtained at baseline, 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 minutes was not statistically significant with respect to the drug type used.

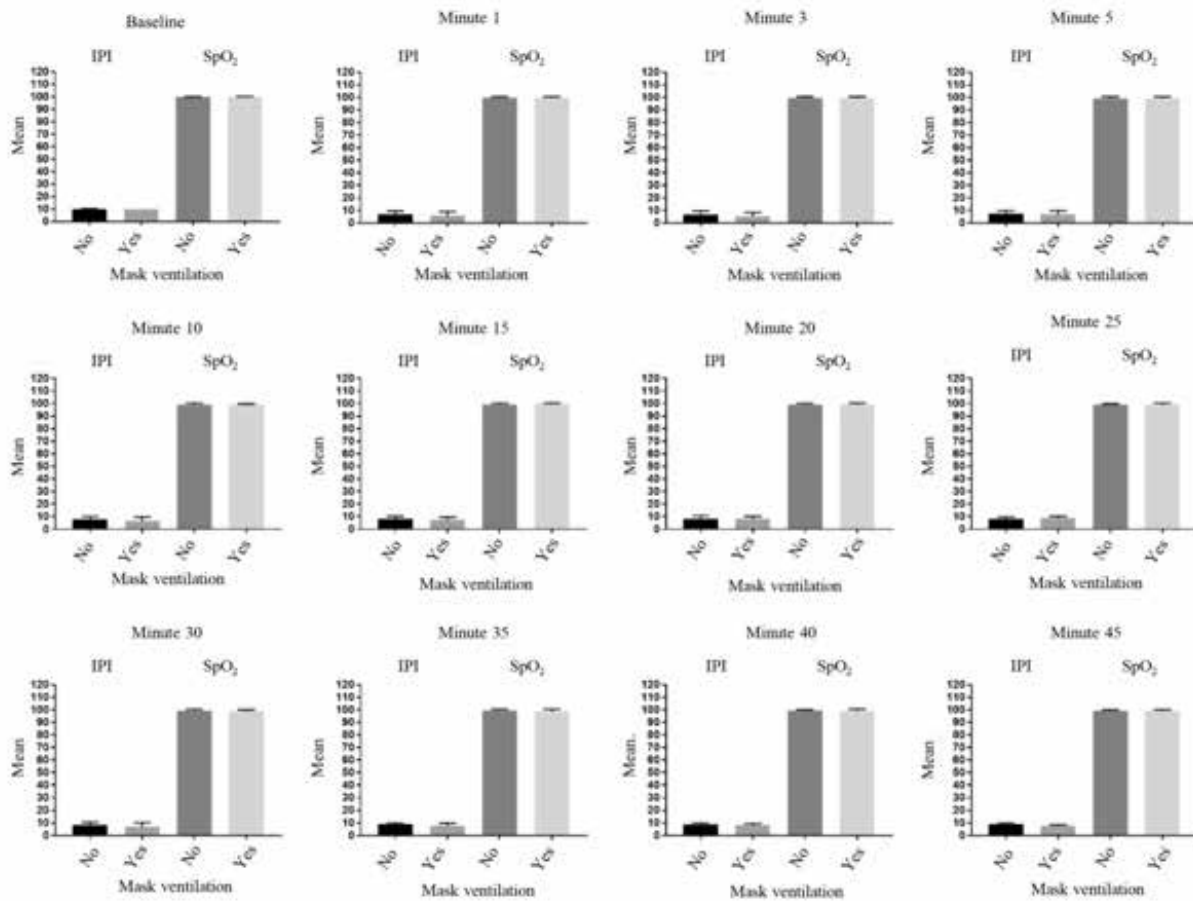


Fig 2: Comparison between the application of mask ventilation and Integrated Pulmonary Index and peripheral oxygen saturation values.

Comparison of apnea-IPI-SpO₂

The effect of SpO₂ values measured and expressed as IPI scores were also evaluated regarding the presence of apnea. In the analyses performed, the IPI and SpO₂ values at baseline, 1, 3, 5, 10, 15 and 20 minutes were not found to be statistically significant for the presence or absence of apnea.

However, when we compared mean IPI and SpO₂ value at different time points in terms of the presence of apnea, the IPI scores of the patients with apnea obtained at one and three minutes were classified in the "attention required, intervention may be required" category, while IPI scores obtained at five and 10 minutes were considered to be "close to normal range, and requires attention." The mean SpO₂ value at these time points was determined to be between 99.07% and 99.58%.

Comparison of mask ventilation, IPI and SpO₂ values

In this study, the effects of SpO₂ values expressed as IPI scores were also compared in terms of the use of mask ventilation. The IPI and SpO₂ values measured at baseline, 1, 3, 5, 15, 20, 25, 30, 35 and 40 minutes were not found to be statistically significant whether or not

masked ventilation was applied. On the other hand, SpO₂ values obtained at 10 minutes and IPI values measured at the 45th minute were found to be statistically significant. Accordingly, the mean SpO₂ value of the patients without apnea at the 10th minute was statistically higher than that of the patients with apnea. The IPI score of the patients without apnea at the 45th minute was found to be within normal limits, while the IPI score of the patients with apnea was interpreted as "close to normal range but requires attention," with a statistically significant intergroup difference (Table 4, Figure 2).

However, when we compared the mean IPI and SpO₂ scores obtained at different time points with respect to the application of mask ventilation, the IPI score at one and three minutes was considered to be in the "attention is required, intervention may be required" category, while the IPI scores recorded at 5, 10, 15, 30 and 45 minutes were evaluated as pertaining to the "close to normal range, but may require intervention" category. The mean SpO₂ value recorded at these time points exceeded 99%, excluding the intervals at 10 and 30 minutes (98.8%-98.9%, respectively).

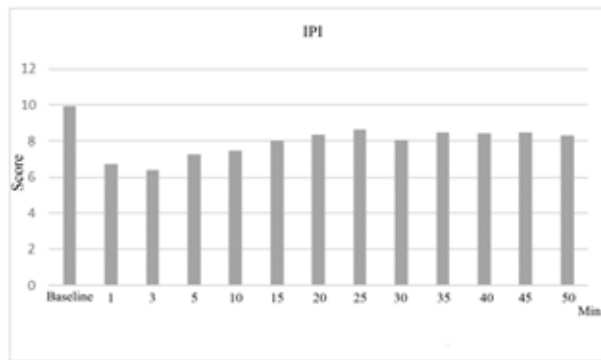


Fig 3: Mean values of time-dependent Integrated Pulmonary Index scores.

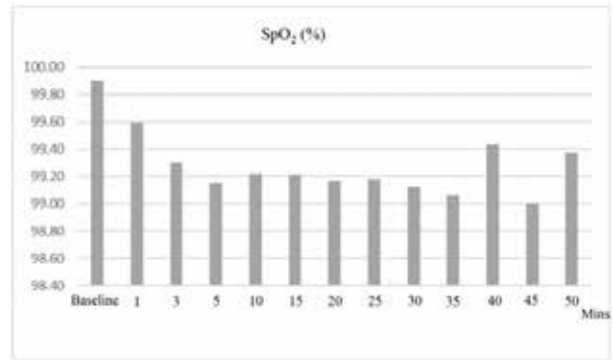


Fig 4: Mean values of peripheral oxygen saturation values over time.

Comparison between type of procedure, IPI and SpO₂ value

The SpO₂ value measured according to the IPI score was also examined with respect to the type of procedure applied to the patients. In this study, a gastroscopy or a colonoscopy was performed and it was determined that while the IPI and SpO₂ values were not statistically significant at baseline, 1, 3, 5, 15, 20, 30 or 35 minutes in terms of gastroscopy or colonoscopy, the mean SpO₂ value of the patients who underwent a colonoscopy was higher at 25 minutes when compared with that of the gastroscopy patients.

The mean IPI and SpO₂ values at different time points were compared with regard to the procedure applied, and IPI scores recorded at one and three minutes in patients who underwent gastroscopy were included in the "attention required, intervention may be required" category, while IPI scores calculated at five and 10 minutes were evaluated as in the "close to normal range, but requires attention" category. IPI scores of patients who underwent a colonoscopy were determined to be in the "close to normal range but requires attention" at 1, 3, 5, 15 and 30 minutes. SpO₂ mean were above 99% at these points.

Time-dependent changes in IPI, SpO₂ and EtCO₂ data

In this study, the IPI, SpO₂ and EtCO₂ data of each patient were recorded at different time periods after a baseline measurement and the mean IPI, SpO₂ and EtCO₂ values were calculated and evaluated.

When the IPI scores of the patients were examined, the highest mean IPI values were detected in baseline measurements, the maximum value was 9.93 points, which was considered to be "within the normal (optimal) range." However, at the time points of one and three minutes, the patients' IPI scores demonstrated a significant decrease. At the first minute, the mean IPI score was 6.7 points ("close to the normal range but may need attention"). At the third

minute, this value decreased further to 6.37 points ("requires attention, may require intervention"). At subsequent time points, the mean IPI value increased again, and at the 5th and 10th minutes was evaluated as "close to normal range." IPI values measured at subsequent time points were evaluated as "within the normal range" (Figure 3).

When the mean SpO₂ value of the patients was assessed, a mean of 99% was determined to be within normal intervals (95% and above) at all time periods. The highest mean, 99.9%, was determined in the base measurement, while the lowest SpO₂ mean was 99%, observed in the 45th minute. No significant difference was seen between time periods (Figure 4).

The mean EtCO₂ value measured at all time points was within the normal range (30-45 mmHg). The lowest mean EtCO₂ was 31.45 mmHg at five minutes, and the highest mean value was 36.18 mmHg at 45 minutes (Figure 5).

However, when the time-dependent changes of the values of IPI, SpO₂ and EtCO₂ values were compared, it was seen that IPI and EtCO₂ have a similar tendency beginning from the baseline. Both values decreased after a baseline point but increased later again. SpO₂ levels decreased for a long time beginning from the baseline. On the other hand, it was observed that IPI

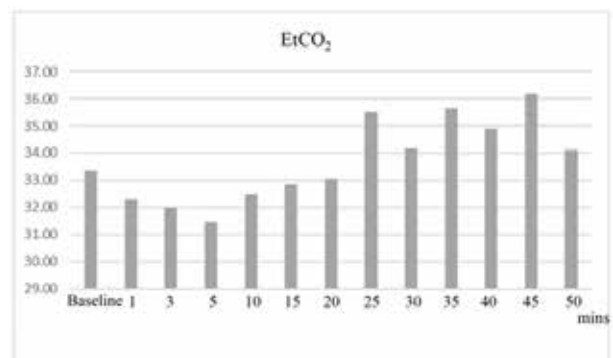


Fig 5: Mean values of end-tidal carbon dioxide over time.

Table 5: Average data based on mask ventilation status

Time (minutes)	Number of patients	Mask Ventilation	HR (minutes)	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	RR (minutes)	IPI	SpO ₂ (%)	EtCO ₂ (mmHg)
Baseline	44	NO	99.9	113.8	69.9	86.7	22.5	9.9	99.9	34.0
	16	YES	102.7	121.4	67.9	86.1	22.9	10.0	99.9	31.6
1	44	NO	96.2	101.1	61.8	74.4	21.2	6.9	99.6	33.3
	16	YES	101.4	103.2	63	78.4	16.5	6.1	99.6	29.4
3	44	NO	95.0	91.6	54.2	67.1	23.0	6.7	99.3	31.5
	16	YES	95.2	94.5	55.6	70.3	20.9	5.6	99.3	33.4
5	44	NO	93.7	90.7	52.4	65.7	23.7	7.4	99.2	32.1
	16	YES	93.4	90.1	52.4	66.2	19.4	6.9	99.1	29.7
10	44	NO	92.5	87.5	50.1	61.5	24.8	7.8	99.4	33.5
	16	YES	92.4	84.9	46.3	54	19.3	6.6	98.8	29.6
15	41	NO	92	86.6	48.7	60.5	22.5	8.2	99.1	33.2
	15	YES	86.9	89.3	48.4	63.9	22.2	7.5	99.4	31.9
20	33	NO	89.5	89.0	48.5	62.8	23.1	8.2	99.2	32.2
	15	YES	90.2	87.6	48.3	63.4	23.8	8.6	99.1	34.8
25	17	NO	91.9	91.6	50	61.6	23.2	8.4	99.2	36.1
	11	YES	89.2	86	46.7	62.5	20.0	9.1	99.2	34.6
30	17	NO	91.1	91.6	50.9	65	23.2	8.6	99.3	35.3
	9	YES	90.6	84.8	41.1	55.8	20.6	7.1	98.9	32.2
35	10	NO	88.5	91.5	52.6	66.8	23.7	9.0	99.4	37.5
	8	YES	86.3	85.7	45.4	64.6	22.9	7.7	98.6	33.0
40	9	NO	92.2	91.3	48.8	68.7	24.7	8.7	99.7	36.2
	5	YES	99.4	95.2	46	60.6	25.4	8.0	99.0	32.0
45	7	NO	83.6	91.9	51.3	66.3	25.0	9.0	99.0	38.7
	4	YES	98.8	90.5	57.3	69.5	26.8	7.5	99.0	31.8
50	4	NO	79.2	93.8	56.6	69.6	24.4	9.0	99.2	34.6
	3	YES	106	91.3	55.7	69.7	24.7	6.5	99.7	33.3

HR: heart rate; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; RR: respiratory rate; IPI: Integrated Pulmonary Index; SpO₂: peripheral oxygen saturation; EtCO₂: end-tidal carbon dioxide

and SpO₂ remained below the baseline level in all measurements, but EtCO₂ levels were above the baseline level after a certain point of time, which was 25 minutes.

Average data based on mask ventilation status

The mean HR, SAP, DAP, MAP, RR, IPI, SpO₂ and EtCO₂ values were evaluated based on the mask ventilation status of the patients.

The highest HR was determined to be 106 beat per minutes (bpm) at the 50th minute, observed in patients with mask ventilation, while the lowest rate was 79.2 bpm at the 50th minute in patients without mask ventilation. When the mean SAP values were considered, the highest mean SAP value was 121.4 mmHg at baseline in patients with mask ventilation and the lowest mean SAP was 84.8 mmHg at the 30th minute in patients with mask ventilation.

The highest mean DAP value (69.9 mmHg) was measured in patients without mask ventilation at baseline while the lowest mean DAP (41.1 mmHg) was measured at the 30th minute in patients with mask ventilation.

The highest mean MAP value was measured as 86.7 mmHg at baseline in patients without mask ventilation and the lowest mean MAP value was 54 mmHg measured at 10 minutes in patients with mask

ventilation. The highest RR seen in patients with mask ventilation was 26.8/minute at 45 minutes, while the lowest RR detected in patients with mask ventilation was 16.5/minute at the one-minute measurement. In patients with mask ventilation, the highest IPI (10.0 points) was found at baseline and the lowest IPI (6.1 points) was seen at the one-minute assessment. The highest mean SpO₂ (99.9%) was measured at baseline, while the lowest mean value (98.6%) was detected at 35 minutes in patients with mask ventilation. Finally, in patients without mask ventilation, the highest mean EtCO₂ level was 38.7 mmHg detected at 45 minutes, while the lowest mean EtCO₂ was 29.4 mmHg, recorded at one minute (Table 5).

DISCUSSION

During sedation procedures, monitoring patients with devices that measure both visual and physiological parameters is necessary to prevent complications^[5,6]. The combined use of capnographs and pulse oximetry is recommended because it is difficult to recognize alveolar hypoventilation in time with only pulse oximetry^[7-10].

The first study of the IPI algorithm was performed by Taft *et al.* Four parameters (ETCO₂, RR, SpO₂ and HR) measured in 85 patients were evaluated by a team of 18 medical professionals including nurses,

physiologists, anesthesiologists and respiratory therapists^[11].

Garah *et al*^[12] divided the patients studied into three groups in their study of the value of IPI monitoring during pediatric endoscopic interventions. Group 1 consisted of five patients who received propofol, Group 2 comprised of 89 patients who received propofol and midazolam, and 15 patients who were given propofol, midazolam and fentanyl constituted Group 3. The IPI values were found to be significantly higher in Groups 2 and 3 than in Group 1, and the IPI values were significantly lower in the age group of 4-6 years compared with the age group of 7-12 years. The sensitivity and specificity of the IPI measurement was 97% and 98% respectively, in a total of 109 children who underwent sedation for an endoscopic intervention in the evaluation of respiratory problems, such as apnea and hypoxia^[12].

In our study, propofol and midazolam was used for sedation in 48 (80%) patients. In 12 of 60 patients (20%), propofol and an opioid (fentanyl) were used in addition to midazolam.

Contrary to the findings of Garah *et al*^[12], lower IPI values were seen in the propofol, midazolam and opioid group when compared with the other groups; however, this difference was not statistically significant.

In our study, only mean baseline IPI score of the group age >8 years was found to be significantly higher than ≤8 years. However, in a study by Garah *et al*^[12], the authors obtained lower IPI scores in the younger age group.

Since a pulse oximeter showing arterial oxygenation cannot demonstrate alveolar hypoventilation in time, in order to detect respiratory problems that may occur during sedation, combined use of a capnograph has been recommended to evaluate ventilation^[3,13-14]. IPI provides a single value by combining pulse oximetry and capnograph values with physiological parameters (RR and HR). The US Food and Drug Administration has stated that IPI monitoring can be used to follow up changes in respiratory parameters in patients undergoing sedation.

Berkenstadt *et al*^[15] examined the results of sedation used for colonoscopy patients and did not find any significant difference in RR, HR or SpO₂ in groups with low, medium and high IPI values, while the EtCO₂ value increased in correlation with IPI.

In our study, a similar tendency was demonstrated in terms of increases and decreases in IPI and EtCO₂ values. However, the changes in SpO₂ did not correlate with these two parameters.

Sabbatani *et al*^[16] reported that IPI monitoring in 45 patients during cardioversion proved to be safe and effective in demonstrating respiratory problems. In addition, IPI was found to have advantages over monitoring EtCO₂ alone. Riphahaus *et al*^[17] investigated

the clinical value of IPI in their study of sedation during interventional upper gastrointestinal system endoscopy and found that IPI was more effective in reducing the incidence of apnea attacks.

In our study, we found that IPI values provided an earlier warning than SpO₂ in case of apnea. In cases where mask ventilation was required, the IPI value decreased while the SpO₂ value remained within normal limits. Gozal *et al*^[18] investigated IPI in a mixed group of adult and pediatric patients and found that IPI reliably interpreted the respiratory state during sedation. The average IPI score of the patients with an ASA I classification was higher than that of ASA II classification patients.

CONCLUSION

IPI monitoring is a noninvasive, dynamic, real-time and continuous measurement method that is noteworthy because it reflects respiratory status with high specificity and sensitivity and can detect problems using a single numerical value at an early stage.

The IPI score can be more effective than SpO₂ measurements to determine whether a patient requires an intervention. However, in order to obtain more accurate and precise data, studies with a larger number of patients are required.

The IPI is a useful tool for simplifying the monitoring of breathing. In the light of all these results, we think that IPI monitoring will be useful in monitoring the respiratory status of patients undergoing sedation.

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Conflict of interest: None

Authorship Criteria

Ozlem Sezen and Banu Cevik: substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Original Article

Genetic studies on glucose-6-phosphate dehydrogenase deficiency in neonates suffering from hemolytic anemia

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ABSTRACT

Objective: To evaluate the association between glucose-6-phosphate dehydrogenase (G6PD) mutations including Mediterranean mutation (exon 6 C563T), Mahidol mutation (exon 6 G487A), Chatham mutation (G1003A) and hemolytic anemia incidence in Saudi population

Design: Cross-section study

Setting: Department of Hematology at the Maternity and Children's Hospital located in Jeddah, Saudi Arabia

Subjects: One hundred blood samples (50 males and 50 females) were collected from neonates.

Intervention: Neonates aged from newborn to 12 months old

Main outcome measures: Cases were asked to answer a questionnaire. Quantitative evaluation of G6PD enzyme activity was performed using spectrophotometric method. Genotypes and allele frequencies were studied by polymerase chain reaction and restriction fragment length polymorphism for the three single nucleotide polymorphisms. Descriptive data were calculated as the

mean±standard deviation. Association between clusters of genotypes was evaluated using Mann-Whitney and Chi-square tests.

Results: The Mediterranean mutation was the most common mutation detected in the male and female groups, whereas Chatham and Mahidol mutations were found in a fewer number of male samples. In total, out of 50 newborns, 22 males and 25 female subjects (44%) were with Mediterranean mutation; one male was Chatham with Mediterranean mutations (2%), one male had only Chatham mutation (2%) and other had Mahidol mutation (2%). Three females (heterozygous CT) belongs to class II G6PD variant and suffered from severe hemolytic anemia. The nucleotide 563T was the most frequent polymorphism observed followed by nucleotide 1003A and 487A.

Conclusion: Our finding strengthens the evidence for the association between G6PD deficiency and genetic variation of G6PD genes in the etiology of hemolytic anemia.

KEY WORDS: Chatham mutations, G6PD deficiency, Jeddah, Mediterranean mutations, Mahidol mutations hemolytic anemia

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked, inherited enzymatic disorder. It is triggered by the mutations in the G6PD gene and results in protein variants with different levels of enzyme activity and are correlated with a varied range of biochemical and clinical phenotypes. The G6PD enzyme deficiency can be produced by a reduction in the number of enzyme molecules, a structural difference in the enzyme producing a qualitative change, or both. However, until now, their precise molecular basis has remained unknown. Data from

several studies suggested that about a third of all male newborns with neonatal jaundice have a G6PD deficiency in them; however, the deficiency is less frequent in female neonates^[1-3]. Most individuals suffering from G6PD deficiency are asymptomatic throughout their lifespan. The most common clinical manifestations are fatigue, back pain, neonatal jaundice and acute hemolysis anemia. In most of the patients, it is activated by an exogenous agent or when red blood cells are subjected to oxidative stress prompted by agents such as drugs, infection or the ingestion of fava beans^[4,5]. G6PD deficiency does not appear to influence

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life expectancy, life quality or affected individuals activity^[6,7].

World Health Organization grouped variants of G6PD deficiency in five different classes^[8]. Variants can also be classified as sporadic or polymorphic^[5]. Class I variants have severe enzyme deficiency and have chronic hemolytic anemia. Class II variants also have a severe enzyme deficiency, but there is usually only intermittent hemolysis. Class III variants have moderate enzyme deficiency with intermittent hemolysis, usually associated with infection or drugs. Class IV variants have no enzyme deficiency or hemolysis. Class V variants have increased enzyme activity. Classes IV and V are of no clinical significance.

Deficient G6PD alleles are distributed worldwide, a conservative estimate is that at least 400 million people carry a mutation in the G6PD gene causing a deficiency. High prevalence is reported from Africa, Southern Europe, the Middle East, Southeast Asia, and the central and southern Pacific islands; however, because of fairly recent migration, deficient alleles are nowadays quite prevalent in North and South America and in parts of North Europe^[9].

The present study was designed to determine the frequency and spectrum of G6PD mutations in newborn patients in Jeddah, Saudi Arabia. The study will help to understand the relationship between G6PD deficiency and variation in the genes that code for G6PD to determine the role of inheritance and the mechanism of its operation in the etiology of hemolytic anemia.

MATERIALS AND METHODS

Sample collection and study subjects

The present study recruited a cohort of 100 participants with 50 hemolytic anemic patients (25 male and 25 female) and 50 normal/control participants (25 male, 25 female). The participants' age was in the range of 0-12 months old. The blood samples of participants were collected in the Department of Hematology at the Maternity and Children's Hospital located in Jeddah, Saudi Arabia and stored at 4 °C until further use. Written informed consent was obtained from all of the participants enrolled in the study. The study was approved by the Ethical Committee (Unit of Biomedical Ethics) (No. H-02-J-002) from Directorate of Health Affairs, Jeddah. The research has been

conducted at the postgraduate studies laboratories, Biological Science Department, Faculty of Science, King Abdul Aziz University, Jeddah.

Determination of enzyme activity in G6PD

The activity of the G6PD enzyme was determined by using G6PD assay kit (Sigma – Aldrich, USA) according to the manufacturer's instructions. The final activity was determined by plotting a standard curve obtained by taking optical density at A450.

Extraction of DNA from blood sample and determination of the concentration

DNA was extracted from entire blood by using QIAamp DNA Blood Mini Kit (Qiagen Inc., USA) according to instructions given in the manual. The DNA concentration was detected by measuring the optical density. The DNA samples were diluted 1:200 in nuclease-free water in 1.5 ml microcentrifuge tube. The mixture was then transferred to 1 ml quartz cuvette and was quantified using spectrophotometric analysis using 6800 UV/ Vis Spectro-photometer (Jenway, UK). The purity of DNA sample was determined by calculating the ratio of absorbance at A260/ A280. Pure DNA sample should have absorbance ratio between 1.7-1.9.

PCR amplification and purification of amplicons Amplification of exon

Polymerase chain reaction (PCR) amplification of exon 6 Mediterranean mutation, exon 6 of Mahidol mutation and exon 9 Chatham mutation gene was done by using the forward and the reverse primers given in Table 1. Briefly, DNA was amplified in a 50 µl volume reaction containing 2 µl (0.2 µg/µl) genomic DNA, 25 µl Hot Start Green Master Mix, 20.5µl nuclease-free water and 1.25 µl of each respective primer. PCR amplification of DNA was set up with cycling conditions as described earlier by Jarullah *et al*, Sulaiman *et al* and Gandomani *et al* for exon 6 Mediterranean mutation, exon 6 of Mahidol mutation and exon 9 Chatham mutation gene respectively^[10-12].

The final amplicon was electrophoresed on 3% agarose gel in a horizontal electrophoresis system (SCIE-PLAS, UK) and visualized in a gel documentation system. The amplicons were visualized under UV light and photographed using gel documentation system (Syngene, USA).

Table 1: Sets of primers, exons, restriction enzymes and PCR fragment size included in the study

Exon	Enzyme	Forward 5'-3'	Reverse 5'-3'	Fragment size(bp)
6	<i>Mbo</i> II	GCAGCTGTGATCCTCACTCC	GCAAGGTGGAGGAAGTCACTGACC	547 bp
6	<i>Hind</i> III	GCGTCTGAATGATGCAGCTCTGAT	CTCCACGATGATCGGTTCAAGC	104 bp
9	<i>Bst</i> XI	CAAGGAGCCCCATTCTCTCCCTT	TTCTCCACATAGAGGACGACGGCTGCCAAAGT	208 bp

Purification and visualization of amplicons

Amplicons or PCR products were purified using QIAquick gel extraction Kit (Qiagen Inc., USA) according to manual's instruction. The final purified products were electrophoresed on 3% agarose and visualized under UV light and photographed using gel documentation system (Syngene, USA).

Genotyping of exon mutation

The genotypes for these single nucleotide polymorphisms (SNPs) were determined by restriction fragment length polymorphism procedure using different restriction enzyme for each exon mutation *i.e.*, 6 C/T Mediterranean mutation, 9 G/A Chatham mutation and 9 G/A Chatham mutation.

(a) exon 6 C/T Mediterranean mutation

The genotype of resulting amplicon of 547 bp was determined using the reaction of 15 μ l of PCR product, 13 μ l of sterile deionized water and 2 μ l of 10X Buffer Tango were added in an Eppendorf tube and mixed thoroughly by pipetting. In the end 2 μ l of restriction enzyme, *Mbo* II was added to the reaction mixture. The tube was incubated for 24 hours at 37 °C followed by heat inactivation for 20 minutes at 65 °C. The genotypes were resolved on a 3% agarose gel electrophoresis.

(b) exon 6 G/A Mahidol mutation and exon 9 G/A Chatham mutation

The genotype of resulting amplicon of 104 bp and 208 bp of exon 6 G/A Mahidol mutation and exon 9 G/A Chatham mutation respectively was determined using the reaction of 10 μ l of PCR product, 18 μ l of sterile deionized water and 2 μ l of 10X Buffer Tango added in an Eppendorf tube and mixed thoroughly by pipetting. In the end, 2 μ l of restriction enzyme, *Hin* III and *Bst*XI was added to the reaction mixture for exon 6 G/A Mahidol mutation and exon 9 G/A Chatham mutation respectively. The tube was incubated for four hours at 37 °C followed by heat inactivation for 20 minutes at 65 °C. The genotypes were resolved on a 3% agarose gel electrophoresis.

Statistical analysis

The final data was analyzed statistically using Statistical Package for Social Science v.16 (SPSS, Inc., Chicago, IL, U.S.A). Descriptive data were calculated as the mean \pm standard deviation (SD). Association between clusters of genotype was evaluated using Mann-Whitney and Chi-square test (χ^2 test). 2-by-3 and 2-by-2 were applied to determine the association between genotypes and clinical groups and alleles in clinical groups respectively. To evaluate the relative risk and strength of association for the various genotypes or their combinations, contingency analysis

was used to calculate the odds ratio and risk ratio at 95% confidence interval. The *P*-value <.05 was considered as statistical significance. To compare the observed and expected genotypes frequencies among the two group of participants (patient and normal) Hardy-Weinberg Equilibrium was applied. It was done by goodness-of-fit χ^2 test, with a degree of freedom value as one. Linkage disequilibrium^[8] resulting from the association between the genotypes of exon 6 C/T and exon 9 G/A polymorphisms was assessed by the χ^2 test. The linkage disequilibrium coefficient was calculated using an online server, Haplotype calculation online: (<http://www.oege.org/software/cubex/>)

RESULTS

Demographic data and characteristics of the subjects

The study subjects were categorized according to their gender and as a patient (affected) and normal (control) participants. Fifty normal participants (control) with 25 males and 25 females and 50 patient participants with 25 males and 25 females were recruited for the study. The mean age for patient group was 3.001 months (SD \pm 3.45) and 5.64 months (SD \pm 3.215) for normal group. The *P*-value was 0.49; therefore, there was no statistically significant difference between patient and normal groups. The mean age for male participants was 5.3 months (SD \pm 2.76) and 5.48 months (SD \pm 3.47) for the patient and normal months respectively. The mean age for female participants 6.96 months (SD \pm 3.91) and 5.8 months (SD \pm 2.99) for the patient and normal months respectively. There was no significant difference between the two groups in case of both male and female (Table 2). The hospital stays for the participants was 2-8 days with mean hospital stay days for 4.4 days (SD \pm 1.2 days). The mean bilirubin level for all participants was 18.3 mg/dl (SD \pm 2.8). The treatment used for icteric patients was phototherapy in 30 neonates (68%) and exchange transfusions in 16 cases (72%). The mean bilirubin levels and hospital stay days showed no significant difference in the groups for different types of mutation.

Table 2: Mean and standard deviation of the subjects depending on age factor in both groups (patient and normal) for male and female gender

Gender	Variable	Group		P-value
		Normal	Patient	
Male n=50	N	25	25	1
	Age (Months)	5.48 \pm 3.47	5.3 \pm 2.76	
Female n=50	N	25	25	.25
	Age (Months)	5.8 \pm 2.99	6.96 \pm 3.91	

Further, the G6PD deficiency was observed in five cases (50%) by the screening test and G6PD Mediterranean mutation in 40 cases (80%). The total frequency of G6PD deficiency among neonates with prolonged or high jaundice was 17.9%.

Classes of G6PD deficiency

The enzyme activity in blood samples was determined using G6PD assay kit. World Health Organization classified G6PD variants on the basis of the extent of the enzyme deficiency and the severity of hemolysis^[8,13]. In the present study, the G6PD deficiency was determined for all the 100 participants. Twenty-six percent of participants were found to be with normal G6PD activity (Class IV), 24% suffered from increased enzyme activity (Class V) and 50% had enzyme deficiency (less than 10% - 60%) (Class I, II and III patients). Among the 50 G6PD deficient participants, 30 (60%) had a mild enzyme deficiency, 10 (20%) had a moderate deficiency and 10 (20%) had a distinct enzyme deficiency. As an expected class I, G6PD variant was found in male participants (10%) only and no female participants were found to be suffering from severe enzyme deficiency and have chronic hemolytic anemia. The class II, III, IV and V G6PD variants were found in both male and female participants (Table 3).

Mutations and detection of nucleotides Mutations

The most common mutation observed in male and female groups was Mediterranean mutation 563 C>T. The Mediterranean with G6PD Chatham mutation, G6PD Chatham 1003 G >A and Mahidol mutations 487 G>A were found mostly female and in some of the male participants. Out of 50 newborns, Mediterranean mutation 563 C>T was found in 22 (44%) males and 25 (50%) females participants, the 2

Table 3: Blood samples (n=100) tested and categorized according to G6PD enzyme activity and the severity of hemolysis

Group	Residual enzyme activity (%)	Number of male samples	Number of female samples
Patient	Class I Severely deficient, chronic hemolytic anemia	10	0
	Class II 1-10% residual activity	6	10
	Class III 10-60% residual activity	9	15
Normal	Class IV 60-150%; normal activity	12	13
	Class V 150%; increased activity	12	12

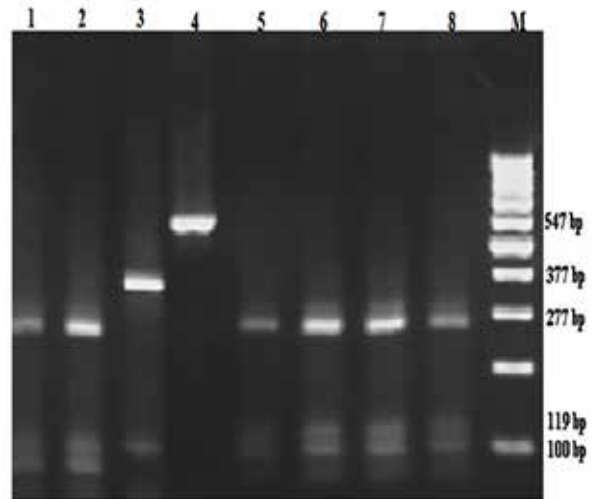


Fig 1: Agarose gel of digested products of 547 base pair fragment containing Mediterranean mutation 563 C>T. Lane 4 showed uncut PCR product. Lanes 1, 2, 5, 6, 7 and 8 showed Mediterranean mutation pattern (pieces of 277, 119, 100 and 60 bp resulted from cutting 547 base pairs fragment of samples with defective G6PD). Lane M indicated 100bp DNA marker. Lane 3 showed the pieces result from cutting 547 base pairs fragment of normal G6PD. In this lane, pieces of 377, 119 and 60 bp can be respectively seen.

(4%) male participants with Chatham mutation G1003A and only one (2%) male participants with Mahidol mutation 487G>A.

Detection of nucleotide G6PD Med 563 C>T polymorphism

PCR amplification of G6PD gene was performed for all the 50 of each patient and controls samples. The G6PD-Med mutation 563 C>T at base site 563 produces a *Mbo* II site in exon 6 of the G6PD gene. A single 547 bp characteristic band for Med. mutation 563 C>T was observed. The control samples showed 60, 119 and 377 bp bands and the mutant samples showed 60, 100, 119 and 277 bp bands. In heterozygote samples, a 60, 100, 119, 277 and 377 bp bands were observed (Figure 1). The G6PD Mediterranean genotype constituted 40 cases (80%) out of the total 50 deficient participants.

Detection of nucleotides G6PD Mahidol variant (487G>A) polymorphism

The samples (PCR product) contain a restriction site for the *Hind* III restriction enzyme; therefore, it was digested with the enzyme. Two bands of low molecular weight, 22bp and 82bp were observed indicating the presence of allele A (the flanking fragment of 22 bp was too small to detect on the gel). A single band of 104 bp was also observed indicating the presence of allele G which lacks the restriction site for *Hind* III enzyme (Figure 2).

Table 4: The association between the presence of G6PD Med. 563C/T polymorphism and the incidence of hemolytic anemia

SNP	Group	Mediterranean Genotype distribution			Allele frequency	HWE	P- value ^a	P- value ^b	P- value ^c
		CC	CT	TT					
563 C>T	Normal	(n=49) 98%	(n=1) 2%	(n=0)	C: 99 (99%) T: 1 (1%)	0.943 P:.33	.05*	.001**	.00**
	Patient	(n=0)	(n= 40) 80%	(n=10) 20%	C: 40 (40%) T: 60 (60%)	0.000 P:.94			

n: number of the sample; HWE: Hardy- Weinberg equation; a: expressed as frequencies and were compared by Mann-Whitney test; b: corresponds to genotype distribution using 2-by-3 Chi-Square test; c: corresponds to allele frequency using 2-by-2 Chi-Square test; ** Highly significant ($P < .05$).

Detection of nucleotides G6PD Chatham variant (1003G>A) polymorphism

The samples were digested with restriction enzyme *Bst* XI. Three bands of 30 bp, 78 bp and 100 bp were observed. That showed the presence of allele A as the sample contains two sites for the restriction enzyme *Bst* XI (The flanking fragment of 23 bp was too small to detect on the gel). Two other bands of 78 and 130 bp were also observed indicating the presence of allele G, as it contains only one site for restriction enzyme *Bst* XI. A single band of 208 bp of G6PD exon 9 Chatham variant (1003G>A) polymorphism was obtained (Figure 3).

Characteristics of subjects according to their G6PD Med. 563 C/T genotype

The frequency of each genotype of the G6PD Med. 563 C>T in patient and normal participants was

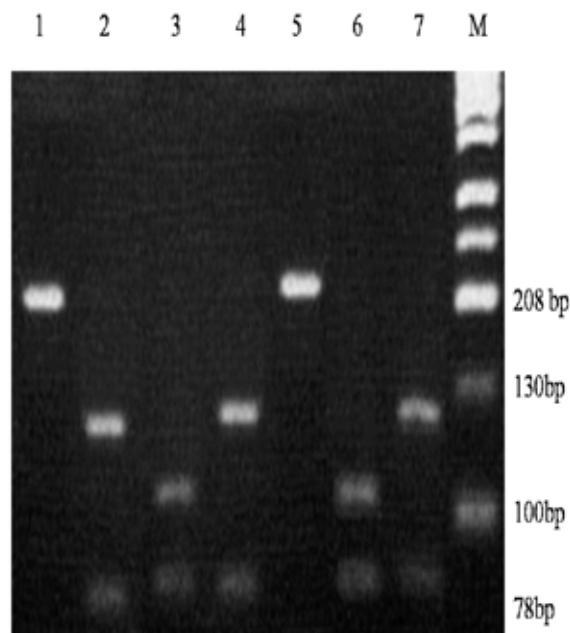


Fig 2: Agarose gel showing hemizygote patient for Mahidol mutation exon 6 variant of G6PD deficiency. Lane M: 100 bp DNA marker; Lanes 1, 4, 6: Mahidol homozygote (82 bp); Lane 3, 4: G6PD normal (Uncut PCR fragment: 104 bp); Lane 5: G6PD normal/ Mahidol heterozygote (pieces of 104 and 82 bp).

determined. In case of normal participants, it was observed that 98% (n=49) were homozygous with CC genotype, 2% (n=1) were heterozygous with CT genotype and 0.00% (n=0) was homozygous with TT genotype. While in case of patient participants, it was observed that 0.00% (n=0) were homozygous with CC genotype, 80% (n= 40) were heterozygous with CT genotype and 20% (n=10) were hemizygous TT genotype males. The P -value was 0.001, which showed there is a highly significant difference in alleles frequencies between patient and normal groups. The distribution of genotype was in the Hardy-Weinberg equilibrium. The stability of homozygotes and heterozygotes observed was as predicted by the Hardy-Weinberg equation from the allele frequencies, Goodness of fit $X^2 = 0.943$, $df = 1$, $P = .33$ and Goodness of fit $X^2 = 0.00$, $df = 1$, $P = .94$ in normal and patient subjects, respectively

The variation between groups among genotypes in Med 563 C/T SNP is shown in Table 4. The P -value was

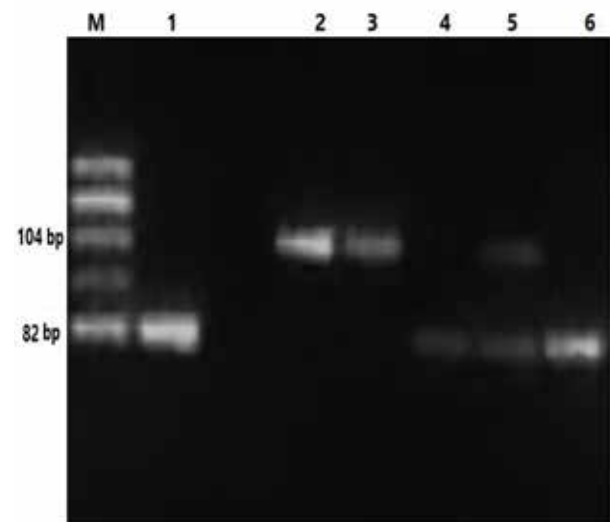


Fig 3: Agarose gel showing restriction digestion of PCR products related to G6PD Chatham mutation with *Bst*XI enzyme. Lane M, DNA marker (50 bp). Lane 1 and 5 PCR product (208bp). Lane 3 and 6 the samples have Chatham mutation. Lane 2, 4 and 7 without Chatham mutation.

Table 5: Frequency of genotypes for both alleles in both groups (patient and normal) for G6PD Med. 563 C/T

Group	Class	Genotype	N	Frequency (%)	χ^2	Alleles	N	Frequency (%)	P- value
Patient	Class I								
	Male	T	10	20.0	4.66	C	139	69.5	.010**
	Female	TT	0	0.0	5.55	T	61	30.5	
	Class II & III								
	Male	C	16	32.0					
Female	CT	25	50.0						
Normal	Class IV & V								
	Male	C	24	48.0					
	Female	CC	25	50.0					
	Total								
	Male		50	100%			200	100%	
	Female		50	100%					

Data were presented as a number of samples with frequency.

χ^2 indicated a significant value in genotype data $P=.03$ and $P=.018$ in male and female, respectively.

0.05, which is statistically high, and therefore there is a significant difference in variation between patient and normal groups among genotypes. The association between genotype distribution and allele frequencies of the polymorphisms in the two groups (patient and normal) was observed and found to be highly significant ($P=.00$). It implies that majority of the patient group tend to be of CT genotype, and most of the normal group tend to be of CC genotype. The odds ratio and risk ratio were 0.013 (93.5% CI: 0.20-1.00), indicating no association among them. Therefore, a risk ratio cannot be calculated. Instead, the study identified the exposure status of a sample of cases and another of controls. The odds ratio calculated from a case-control study can approximate a relative risk, but only when the disease is rare (say, up to around 5% in the sample, as is the case for many chronic conditions).

The Mediterranean variations between the groups among genotype showed a highly significant difference with P -value 0.03 and 0.018 between classes of G6PD variants and healthy controls groups in male and female respectively. Twenty percent ($n=10$) of the patients suffering from chronic hemolytic anemic (class I variant) was male with hemizygote T and no female patient with homozygote TT was found. Further, 32% of the male patient of class II variant was hemizygote C, whereas 50% of the female patient was

heterozygote CT. All normal group participant was homozygote CC (Table 5).

Association between G6PD Med. 563 C/T polymorphism and incidence of hemolytic anemia (a) Male participants

In patient group the Med. 563 C/T SNP, the distributions of genotype were 0% ($n=0$) in case of hemizygous C, 60% ($n=6$) hemizygous class II C and 40% ($n=2$) hemizygous class I T. In normal group, 96% were ($n=24$) hemizygous C, 4% ($n=1$) hemizygous class II C and 0% ($n=0$) hemizygous recessive T. The equilibrium of hemizygote C and T was found to be as predicated by the Hardy-Weinberg equation from these allele frequencies in Med. 563 T/C SNP in both patient ($\chi^2=4.65$, $df=1$, $P=.032$) and normal ($\chi^2=0.001$, $df=1$, $p=0.918$), in tandem. The variation between groups among genotype in both SNPs is shown in Table 6. The association between genotype distribution and allele frequencies of the Med. 563 C/T polymorphism in the two inpatient and the normal group is shown in Table 6. Also, there is a highly significant association between groups and genotype ($P=.001$) in Med. 563 T/C.

The Mediterranean variations between groups among genotype showed a highly significant difference ($P=.00$) in variation between classes of

Table 6: Association between the presence of G6PD Med. 563 C/T polymorphism and incidence of hemolytic anemia in male

SNP	Group	Mediterranean Genotype distribution			Allele frequency	HWE	P- value ^a	P- value ^b	P- value ^c
		CC	CT Class II	T Class I					
563 C>T	Normal N=25	($n=24$) 96%	($n=1$) 4%	($n=0$)	C: 49 (98%) T: 1 (2%)	0.918	.50	.001**	.00**
	Patient N=25	($n=0$)	($n=15$) 60%	($n=10$) 40%	C: 15 (60%) T: 10 (40%)	0.032			

n: number of the sample; HWE: Hardy-Weinberg expectations; a: expressed as frequencies and were compared by Mann-Whitney test; b: corresponds to genotype distribution using 2-by-3 Chi-Square test; c: corresponds to allele frequency using 2-by-2 Chi-Square test; **highly significant ($P < .05$).

Table 7: The association between the presence of G6PD Med. 563 C/T polymorphism and incidence of hemolytic anemia in females

SNP	Group	Mediterranean Genotype distribution			Allele frequency	HWE	P- value ^a	P- value ^b	P- value ^c
		CC	CT	TT					
563 C>T	Normal N=25	(n= 25) 100%	(n= 0)	(n= 0)	C: 50 (100%) T: 0 (0%)	Null	.50	Null	.00**
	Patient N=25	(n= 0)	(n= 25) 100%	(n= 0)	C: 25 (50%) T: 25 (50%)	0.000			

n: number of the sample; HWE: Hardy- Weinberg expectations; a: expressed as frequencies and were compared by Mann-Whitney test; b: corresponds to genotype distribution using 2-by-3 Chi-Square test; c: corresponds to allele frequency using 2-by-2 Chi-Square test; **highly significance ($P < .05$)

G6PD variants and healthy controls groups in male groups. Twenty percent of the male patient was suffering from chronic hemolytic anemic (class I variant) with hemizygote T, and 32% were in class II variant were hemizygote C. All normal male group participants were hemizygote C. In Med. 563 C/T SNP, the frequency of the C and T alleles for the patient group were 60% and 40% respectively. The frequency in the normal group they were 98% and 2% respectively (Table 6).

(a) Female participants

In case of patient group, 0% (n= 0) were homozygous CC and 100% (n=25) heterozygous CT. In normal group, 100% (n=25) were homozygous CC and 0% (n=0) were heterozygous CT. The balance of homozygote and heterozygote observed was as predicted by the Hardy-Weinberg equation from the allele frequencies, $X^2= 25$, $df = 1$, $p= 0.000$ and $X^2= \text{null}$, $df = 1$, $p= \text{null}$ inpatient and normal group respectively. It implies that the population is at Hardy-Weinberg equilibrium. The variation between groups among genotype was shown in Table 7. There was no significant difference in variation between the two groups among genotype ($P=.55$). The association between genotype distribution and allele frequencies in the two female's groups were shown in Table 7. The frequency for the patient group of the C and T alleles were 50% and 50% respectively. In normal group, the frequency of the C and T alleles were 100% and 0%, respectively. There was a strong significant difference in alleles frequencies between patient and normal groups ($P=.000$).

None (n=0) of the patient group participants were in class I variant with homozygote TT. All female patient group participants were in class II variant representing 100% (n=25) with heterozygote CT. In contrast, all (n=25) of normal female group participants were homozygote CC (Table 7). In Med. 563 C/T SNP, the frequency of the C and T alleles for the patient group were 50% and 50%, respectively and for normal group, the frequency was 100% and 0% respectively.

DISCUSSION

G6PD deficiency is a genetic disorder that results in missing or defective enzymes (enzymopathy) affecting millions worldwide. It is also closely related to neonatal jaundice, favism and food or drug-induced acute hemolytic anemia^[14]. Mutations in the G6PD gene may diminish functionality and/or stability of the G6PD enzyme leading to different levels of enzymatic activity and a wide range of biochemical and clinical manifestations. Since it is an X- linked gene, the hemizygous males suffer from more severe hemolytic crises than heterozygous females who have variable proportions of G6PD normal and deficient erythrocytes^[15]. The present study was thus conceptualized to estimate the prevalence of G6PD enzyme deficiency in newborn male and female participants and the molecular defects of the G6PD gene was characterized in deficient patients. Also, the association between G6PD mutations including Mediterranean mutation (exon 6 C563T), Mahidol mutation (exon 6 G487A) and Chatham mutation (G1003A) polymorphisms and incidence of hemolytic anemia.

We identified four different G6PD deficiency variants namely G6PD Mediterranean, G6PD Mediterranean with Chatham, G6PD and G6PD Chatham. We found that about 94% of the participants were suffering from the G6PD Mediterranean mutation. The G6PD Mediterranean, having C to T transition at nucleotide 563 of exon 6 with most prevalent allele, has been reported from Mediterranean Middle East and India as well^[16]. By 1988, around 400 variants of G6PD was documented. According to a study, G6PD Mediterranean mutation is the most common G6PD deficient variant in West Africa, Mediterranean, Middle East and Southeast Asia. Black people often have a mild deficiency, Orientals are more deficient and the Mediterranean is the most severe^[17].

In 2011, another study from Jeddah done by Al-Jaouni *et al* reported high prevalence (89.1%) of G6PD Mediterranean mutation, which is much closer to our study^[18]. The data from the study of Al-Ali *et al* showed

84% of the participants had the G6PD Mediterranean and is the most common type of mutation in the Eastern Province of Saudi^[19]. Our data were consistent with the results obtained by Al-Ali *et al*, suggesting geographical location can be a determinant factor for the similarity. Various other studies from Saudi Arabia also reported G6PD Mediterranean mutation among G6PD deficient patients with a lower percentage. Gari *et al* and Al Jaouni *et al* reported 51.1% and 38.1% G6PD Mediterranean mutation among G6PD deficient patients respectively^[18,20]. Another study of Al-Hassa and Al-Qatif areas of Saudi Arabia's Eastern province also showed G6PD Mediterranean as the most frequent variant, with a prevalence of 45.9% and 36.5% for these two areas^[21]. Karadsheh *et al* reported 53.3% samples with the Mediterranean mutation in Jordan^[22].

Further, the mutations observed in the male and female newborns with G6PD deficiency was not significantly different, with Mediterranean mutation being the most common mutation in all population. High prevalence of Chatham mutation was observed in male than in female, which indicates that the frequency G6PD mutations is lower in female than in the male group. The females because of unequal X-chromosome inactivation are functionally G6PD deficient^[23]. In the present study, 10 (40%) females of class II and 15 (60%) females of class III were affected; one tested positive for G6PD Med mutation (heterozygous) and the other had prolonged decoloration time. Similarly, high frequency (24.5%) of affected females was reported from Iran^[24]. A study from UAE reported 5% of affected females^[25].

We also compared the relative frequencies of the class I mutations to the total number of G6PD mutations. The class I mutations affected the exon 6 (44%) and 9 (2%) more frequently, which encodes the regions that bind the enzyme substrate. This result was in agreement with that study conducted by Minucci *et al* in 2012^[26]. They reported that class I mutations affected exon 6, 10 and 13 which encodes the regions that bind the enzyme substrate, dimer interface and NADP+ structural site, respectively^[26]. It is interesting to note that class I mutations result in the more severe phenotype of G6PD deficiency such as chronic non-spherocytic hemolytic anemia. These mutations appear to be the most easily distinguishable in the wide-ranging^[26]. Further, our findings showed that the percent of exchange transfusion was higher in Mediterranean mutation as compared to other mutations; however, the difference was not statistically noteworthy. In Chatham mutation, the need of exchange transfusion and the clinical phenotype was milder. These findings support a

common ancestry of the population and the theory of genetic drift throughout Mediterranean regions.

The results of screening test showed that there were 10 cases (40%) of G6PD Mediterranean deficiency type II and 15 cases (60%) of G6PD Mediterranean deficiency type III; hence, the overall frequency of G6PD deficiency among the neonates with prolonged or high jaundice was 17.9%. Our results were in agreement with the similar study conducted by El-Menshay and Pao *et al*^[27,28]. Other regions of the world also reported G6PD deficiency among jaundiced neonates^[29-31]. Also, the mean serum bilirubin level in all patients was not statistically significant for different types of mutation. Similarly, in an investigation done by Ainoon *et al*, no difference was found between the incidence of neonatal icter, the mean serum bilirubin level and the percent of newborns in need of phototherapy and duration of phototherapy between the two most common types of G6PD gene mutations^[32].

The association of G6PD enzyme and its genetic variations in the gene, and thus human body, plays an important role in susceptibility to hemolytic anemia. Therefore, it is recommended to perform the further investigation with large sample size and geographical area. Further evaluation of genetic variation in the G6PD regulatory region may fully explain the relationship between G6PD expression and susceptibility to hemolytic anemia.

Finally, we can conclude that molecular analysis of G6PD deficiency conducted in Jeddah revealed a higher prevalence of G6PD Mediterranean (94%), Mahidol (2%) and Chatham (4%) compared with other studies performed in Saudi Arabia. The presence of two mutations Med.563 C/T and Chatham 1003 G/A was detected in one patient causes class I of G6Pd deficiency. The nucleotide 563T is a most frequent polymorphism observed in Saudi Arabia followed by nucleotide 1003A and 487 A.

CONCLUSION

Finally, we can conclude that molecular analysis of G6PD deficiency conducted in Jeddah revealed a higher prevalence of G6PD Mediterranean (94%), Mahidol (2%) and Chatham (4%) compared with other studies performed in Saudi Arabia. The presence of two mutations Med.563 C/T and Chatham 1003 G/A was detected in one patient causes class I of G6Pd deficiency. The nucleotide 563T is a most frequent polymorphism observed in Saudi Arabia followed by nucleotide 1003A and 487 A.

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Original Article

Impact of dialyzer membrane's flow characteristics on parathormone levels and its association with anemia in maintenance hemodialysis patients

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ABSTRACT

Objective: Secondary hyperparathyroidism and renal anemia are cardinal complications of end-stage kidney disease and often occur among patients on maintenance hemodialysis. It is unclear whether a high-flux membrane can lower a middle molecular weight toxin-parathormone and contribute to correction of renal anemia. We aimed to reveal the impact of high-flux membranes on serum intact parathormone levels and its potential association with renal anemia.

Design: Single center, prospective, self-control

Setting: Hemodialysis unit of Harran University between the year 2012-2013

Subjects: Forty-two newly diagnosed end-stage renal disease patients who have been undergoing maintenance hemodialysis treatment for at least 3 months

Intervention: All hemodialysis patients were treated with low-flux membranes for three months and then with high-flux membranes for three months.

Main outcome measures: Pre-dialysis blood tests including urea, creatinine, hemoglobin, intact parathormone, calcium,

phosphorus, alkaline phosphatase, albumin and post-dialysis urea and intact parathormone were performed under hemodialysis treatment using low-flux membrane, and the process was repeated at the second step with high-flux membranes.

Results: Twenty-eight (66.6%) patients completed the study. Both in low-flux and high-flux groups, post-hemodialysis intact parathormone levels significantly decreased compared with pre-dialysis (472.17 ± 347.96 pg/ml vs 371.57 ± 277.38 pg/ml; $P < .05$ and 311.25 ± 180.13 pg/ml vs 233.02 ± 154.80 pg/ml; $P < .05$ respectively), at the end of third month. Switching to high-flux membranes resulted in lower intact parathormone levels, and also post-dialysis intact parathormone decrement (clearance) was more distinct in the high-flux group than low-flux ($P < .05$ for both). High-flux group achieved target hemoglobin levels (11.27 ± 1.23 g/dL).

Conclusion: Our study has demonstrated that high-flux dialyzer membranes can efficiently lower high intact parathormone levels and may contribute to achieving target hemoglobin levels in hemodialysis patients.

KEY WORDS: anemia, hemodialysis, hemodialysis membranes, high-flux membrane, parathormone

INTRODUCTION

Secondary hyperparathyroidism (sHPT) and renal anemia (RA) has been associated with morbidity and mortality in maintenance hemodialysis patients^[1-4].

Despite innovations and too many attempts including new phosphorus-binding agents, cinacalcet, erythropoietin stimulating agents and more frequent and longer hemodialysis sessions, sHPT and RA are still the persisting challenges for nephrologists. It is also attributed that elevated serum parathyroid

hormone (PTH) levels inhibits erythropoiesis via bone marrow fibrosis and have some adverse effects on erythropoietic progenitor cells^[5-6].

High-flux dialyzer membranes are capable of removing middle-heavy molecular weight uremic toxins and have been associated with better outcome in those who are diabetic and hypoalbuminemic, and who have longer than 3.7 years of hemodialysis history, compared to low-flux dialyzers^[7-8]. It is unclear whether a high-flux membrane can contribute to

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improving clearance of the middle molecular weight toxin-PTH and thus to correction of RA. We aimed to reveal the impact of high-flux membranes on serum intact PTH (iPTH) levels and its potential association with RA.

SUBJECTS AND METHODS

This prospective, self-controlled designed study was conducted in the Department of Nephrology, Harran University Faculty of Medicine with forty-two patients who had started maintenance hemodialysis treatment in last three months. The protocol was approved by the Institutional Human Research Ethics Committee and informed consent was obtained from all patients.

Patients older than 18 years, receiving hemodialysis for four hours and three times per week, and with good compliance to both hemodialysis sessions and medications were enrolled in the study. Patients with serious acute or chronic infection/inflammation, blood products transfusion requiring recent bleeding, hematologic disorders, parathyroidectomy and short life expectancy were ruled out. In order to achieve target levels of calcium, phosphorus, iPTH, hemoglobin and ferritin, recommendation and suggestion of the KDIGO 2012 Guideline for Chronic Kidney Disease-Mineral and Bone Disorders^[9] was considered over the course of the treatment and the treatment was edited as needed.

Hemodialysis adequacy was assessed with standard Kt/V and hemodialysis dosing was adjusted once a month to obtain the minimum value of 1.2 for standard Kt/V.

The study was conducted in two steps. At the first step, patients received hemodialysis treatment for three months with low flux dialyzer membranes and blood samples were drawn and centrifuged for laboratory assessment at a prespecified day. Then, all patients were switched to high-flux membranes and the process was repeated at the end of the 3rd month. Blood samples were performed on Cobas e651 autoanalyzer (Roche Diagnostic, Basel, Swiss) by immunoradiometric testing for iPTH; Cobas c501 autoanalyzer (Roche Diagnostic, Basel, Swiss) by photometric testing for sodium, potassium, phosphorus, calcium, albumin, ferritin and alkaline phosphatase (ALP); and Beckman Coulter LH 750 (Abbot, Miami FL, USA) hematology analyzer for whole blood count, both before and after hemodialysis (entry-exit samples).

Gambro AK 95-F volume controlled hemodialysis machines were used for hemodialysis, dry weight was assessed with clinical evaluation and ultrafiltration volume was adjusted as needed.

Rexeed GL (low-flux) and GA (high-flux) series

hemodialyzer membranes made of polysulphone by ASAHI Kasei Medical Co, Ltd. were used as dialyzer membrane. Further technical information about hemodialyzer membranes can be achieved on the company website; <http://www.asahi-kasei.co.jp/medical/en/dialysis/product/rexeed-series/>.

Hemodialysis adequacy was calculated by an online formula exhibited on <http://www.davita.com/ktvcalculator/> which was based on Daugirdas Formulas.

Target levels were determined as 150-300 pg/ml for iPTH and 11-12 g/dL for hemoglobin according to KDIGO 2012 guidelines^[9-10].

Statistical analyzes

The study data was analyzed by using SPSS software, version 11.5 for Windows (Chicago, IL, USA). Shapiro-Wilk test was used for qualification of distribution of variables. The descriptive statistics are presented as the means (\pm standard deviation) for parametric and median-interquartile range (minimum-maximum) for non-parametric variables. Categorical variables among independent groups was assessed by using Chi-square test. Independent sample t-test and Mann-Whitney U test were performed for comparison of parametric and non-parametric variables between the groups. Pre- and post-treatment variables were assessed by paired sample t-test and Wilcoxon Signed-rank test for parametric and non-parametric, respectively. Pearson and Spearman correlation tests were used for correlation analysis of parametric and non-parametric variables. A linear regression analysis model was employed to indicate whether there is an effect of the dependent variables on independent variables. $P < .05$ was assumed as significant.

RESULTS

Forty-two patients were enrolled in the study, but two patients died in consequence of the cardiovascular events, one patient switched to another hemodialysis center, one patient referred to kidney transplantation surgery and 10 patients were excluded because of hospitalization requiring problems (catheter dysfunction, infections) and lack of data. Totally 28 patients (16 men and 12 women) completed the study. Diabetes mellitus and hypertension were the leading causes of end-stage kidney disease. Demographic and characteristic features of the patients who completed the study and comparison of the low-flux and high-flux groups are exhibited in Table 1.

Values of mean iPTH in low and high-flux groups were 472.17 ± 347.96 and 311.25 ± 180.13 pg/ml respectively after three months of treatment periods ($P < .05$). High-flux group came relatively closer to reaching KDIGO target levels for iPTH (150-300 pg/mL).

Table 1: Demographic and characteristic features of the patients and comparison of two groups

Parameters	Low-Flux (n=28)	High-flux (n=28)	P
Age (year)	53.85±12.10	53.85±12.10	
Sex (male/female)	12/16	12/16	
Dry weight (kg)	69.41±16.10	65.55±17.64	.282
PTH1 (pg/ml)	472.17±347.96*	311.25±180.13**	<.05
PTH2 (pg/ml)	371.57±277.38	233.02±154.80	<.05
Δ PTH (%)	18.67±0.39	24.33±0.30	<.05
Calcium (mg/dL)	8.29±1.34	9.21±0.93	<.001
Phosphorus (mg/dL)	5.12±1.81	4.92±1.49	.518
ALP (U/L)	106.50 (36-207)	124(36-595)	.374
Hgb (g/L)	10.81±1.24	11.27±1.23	.57
Ferritin (ng/mL)	794.85±492.05	1117.56±434.04	<.05
Epoietin-zeta (IU)	124.64±74.31	96.42±87.77	.105
Albumine (g/dL)	4.05±0.37	4.28±0.27	<.05
CRP (mg/dL)	0.47 (0.01-6.70)	0.36(0.01-7.48)	.198
UF (L)	2519.64±1137.43	3267.85±1054.90	<.05
sKt/V	1.30±0.34	1.18±0.22	.85
sTA (mmHg)	138.21±18.01	127.85±13.90	<.05
dTA (mm/Hg)	83.39±10.09	78.57±8.37	<.01

PTH1: pre-dialysis serum intact parathyroid hormone level; PTH2: post-dialysis serum intact parathyroid hormone level; Δ iPTH: (PTH1-PTH2)/PTH1 × 100; ALP: alkaline phosphatase; Hgb: hemoglobin; UF: ultrafiltration; sKt/V: standard Kt/V; sTA: systolic tension; dTA: diastolic tension

*PTH1 vs PTH2: P <.05 for low flux group

**PTH1 vs PTH2: P <.05 for high flux group

Switching to high flux membranes from low flux revealed a statistically insignificant hemoglobin increment (11.27±1.23 vs 10.81±1.24, and $P=.57$). However, a conflictive situation has arisen as this statistically insignificant increment in hemoglobin values led the high-flux group to reach target levels for hemoglobin according to KDIGO guidelines.

The number of individuals who achieved target levels of serum iPTH and hemoglobin was similar in the two groups (Table 2). However, larger numbers of individuals who have iPTH >300 pg/mL were in the low-flux group compared to the high-flux group (Table 2). On the other hand, the doses of erythropoietin used to keep the hemoglobin values within target range were similar in both group ($P=.105$). Univariate

Table 2: Number of individuals who achieved target ranges in both groups for iPTH and hemoglobin

Parameters	Low-Flux (n=28)	High-flux (n=28)	P
iPTH (150-300 pg/mL)	6/28*	8/28*	.771
iPTH >300 pg/mL	18/28**	15/28**	<.05
HGB <11 g/dL	14/28***	12/28***	.127

iPTH: intact parathyroid hormone; HGB: hemoglobin

*number of individuals who achieved target range for iPTH

**number of individuals who have serum iPTH value >300 pg/mL

***number of individuals who have hemoglobin value <11 g/dL, low-flux vs high-flux

linear regression analysis revealed no impact of lower iPTH levels on hemoglobin levels in high-flux groups ($P=.687$).

Although high flux dialyzer membranes are capable of removing larger volume, ultrafiltration volume has no effect on hemoglobin level.

Serum ALP levels were similar in both groups ($P=.374$). Besides, a positive correlation existed between iPTH and ALP levels in the low-flux group.

The low-flux group had lower levels of serum calcium ($P <.001$); however, serum phosphorus levels were similar in the two groups ($P=.518$). Linear regression analysis found no impact of lower serum calcium on higher iPTH levels in low flux group ($P=.776$). Besides, serum calcium levels had no impact on lower iPTH levels in high flux group ($P=.998$).

Systolic and diastolic blood pressure were higher in high flux compared to low flux group. A positive correlation was found between erythropoietin dosage and systolic blood pressure. Regression analysis revealed erythropoietin dosage as the only factor for higher systolic blood pressure ($P <.05$). However, erythropoietin dosage has no impact on diastolic blood pressure ($P=.117$).

Standart Kt/V, the indicator of hemodialysis adequacy, was found similar in both groups after three months of treatment periods. Furthermore, no correlation was found between eKt/V and iPTH ($P=.117$ and $P=.775$ for low and high-flux groups respectively).

DISCUSSION

Our prospective, self-controlled study has revealed that high flux dialyzer membranes can lower high levels of iPTH in maintenance hemodialysis patients compared to low-flux. Also, high flux membranes can facilitate to achieve target hemoglobin levels but the decrement of iPTH levels have no effect on hemoglobin rise. We suggest favoring to use the high-flux dialyzer membranes in hemodialysis patients, especially in those with a high level of iPTH.

High levels of iPTH (sHPT) is a common problem which contributes to morbidity and mortality among chronic hemodialysis patients^[1-2]. In DOPPS study, higher mortality risk was found when iPTH levels were above 600 pg/ml and the least risk was with iPTH levels between 101-300 pg/ml and also the CORES study revealed similar results^[2,11]. Hence, reaching target levels is an essential treatment goal in the hemodialysis population. Despite many deleterious outcomes of high levels of iPTH, only 15-20% of patients remain on the target range for iPTH. In our cohort, 25% of patients were in the target range for iPTH.

End-stage kidney disease culminates in an

accumulation of small (<300 Dalton), mid (300-15000 Dalton) and heavy (>15000 Dalton) molecular weight toxins in the body. During the hemodialysis process, urea-like small-molecular-weight toxins can easily pass across the small pores of the low flux dialyzer membranes. Nonetheless, mid and heavy molecular weight toxins (beta2-microglobulin, advanced glycation end products, homocysteine, leptin, erythropoiesis inhibitors) require bigger pores to obtain sufficient clearance. Parathormone is a mid-molecular weight toxin with 9225 Dalton and is theoretically expected to have better clearance using high-flux membranes. However, there is a limited number of studies in the literature that points to whether high flux membranes can contribute to obtaining lower levels of iPTH. Ayli *et al* have randomized 48 hemodialysis patients to either low or high-flux membranes and found similar iPTH levels at the end of six months^[12]. In contrast, a recent study conducted with 30 hemodialysis patients revealed that lower levels of pre- and post-dialysis iPTH are associated with high-flux membranes use^[13]. El Arbagy *et al* found that using high flux dialysis membranes is more efficient in removal of iPTH than low flux membranes^[14]. Makar *et al* similarly found significant decrement in pre- and post-dialysis iPTH with high-flux membranes in a cohort study of 44 pediatric hemodialysis patients^[15]. Due to their differing study designs, it is quite difficult to conclude those studies as a whole. We also obtained distinct iPTH decrement in the low flux group (probably due to good phosphorus control after initiating hemodialysis), but more prominent decrement in iPTH levels occurred after switching to high flux membranes. Moreover, a fewer number of individuals with iPTH >300 pg/ml were in the high flux group.

Clinical trials which aimed to reveal the impact of varies type of hemodialyzer membranes on clearance of iPTH during hemodialysis sessions has indicated that high-flux polysulphone and polyacrylonitrile (PAN-69) type of dialyzer membranes provides more iPTH clearance compared to cuprophane membranes^[16-17]. However, these old studies were short-dated cohort studies with small sample sizes. Hence, prospective large sample sized studies are needed to confirm whether lowering iPTH levels during hemodialysis sessions by high flux membranes is a permanent effect and provide clinical benefits or not. Our study supports high flux membranes capable of maintaining low iPTH levels in the long-term.

However, it is unknown how high flux membranes cause iPTH decrement during hemodialysis. Two mechanisms have been suggested; i) absorption of iPTH by polysulphone form dialyzer membranes; and ii) pass through large pores of high flux membranes to

the dialysate. We also used both low flux and high flux membranes in polysulphone form. So, significant decrement occurred in both groups after hemodialysis sessions. However, the sustained effect of high flux membranes on iPTH might depend on its better phosphorus clearance. Besides, we evidenced that ultrafiltration volume has no impact on iPTH decrement.

High levels of ALP, as a consequence of sHPT, have been associated with morbidity (poor bone mineral density and increased coronary artery calcification score) and mortality. A cut-off value for ALP has been described for poor outcome in hemodialysis patients as >120 U/l^[18-19]. Similarly, our data revealed high levels of iPTH correlates with high levels of ALP in the low flux group (probably due to high bone turnover osteodystrophy). We did not establish a similar relation in the high flux group (commented as normal and also near to normal ranges of iPTH levels sufficient to maintain bone hemostasis).

Renal anemia is one of the cardinal consequences of chronic kidney disease and despite administration of the optimum amount of iron and erythropoietin stimulating agents, about 80% of patients remain anemic^[20-21]. It is assumed that sHPT causes anemia via bone marrow fibrosis and/or direct toxicity on erythropoietic cells^[5]. Treatment of sHPT with vitamin D analogs and parathyroidectomy improve bone marrow fibrosis^[22].

It is controversial whether high flux membranes have favourable effects on RA. Despite existing clinical evidence which emphasizes that high flux membranes have some favourable effects^[13], a recent prospective, multicenter, controlled study did not confirm this suggestion^[23]. Also, Richardson *et al* compared a mid-flux cellulose triacetate and high-flux polysulphone dialyzer membranes and found no significant epoetin-sparing effect through the use of the high flux membrane over the modified cellulose triacetate SF170E membrane^[24]. Besides, PTH clearance and its potential association with anemia have not been investigated in those studies that compared different types of dialyser membrane. High flux membranes provided an increment in hemoglobin levels which were statistically not significant; however, our data revealed high flux membranes may facilitate to achieve target levels of hemoglobin (if target is kept as 11-12 gr/dL) and this effect was independent of iPTH decrement.

CONCLUSION

Our clinical trial revealed that switching from low flux to high flux dialyzer membrane may lower high levels of iPTH, compared to low-flux. After switching to high-flux membranes, the number of individuals

who have optimum iPTH levels (150-300 pg/mL) may increase. We also found that high-flux membranes facilitate to achieve target hemoglobin levels (11-12 gr/dL).

We suggest considering to use high-flux membranes in hemodialysis patients, especially in those who have high levels of iPTH despite optimum dietary phosphorus restriction and taking phosphorus-binding agents, vitamin D analogs and calcimimetics.

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Original Article

External validation of a new nomogram for prediction of cancer specific and all-cause mortality in bladder cancer patients undergoing radical cystectomy

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ABSTRACT

Objective: To externally validate a new nomogram (Williams SB, Huo J, Chu Y, *et al.* Mortality in bladder cancer patients undergoing radical cystectomy: Development and validation of a nomogram for treatment decision-making. *Urology* 2017; 110:76-83) in our patient cohort

Design: Retrospective study

Setting: Department of Urology, Health Sciences University, Bozyaka Training and Research Hospital, Izmir, Turkey

Subjects: A total of 146 patients who were diagnosed with muscle invasive bladder cancer between March 2006 and October 2016 were enrolled in this study.

Interventions: Radical cystectomy

Main outcome measures: The predicted cancer specific survival (CSS) and overall survival (OS) by the recent nomogram were compared with the actual CSS and OS of

the patients in our single center sample.

Results: Mean overall survival of the patients was 54.5±4.4 months; the 3-year and 5-year OS rates were found to be 46.2% and 42.3%. Mean CSS of the patients was 66.5±4.8 months and 3-year and 5-year CSS rates were found to be 59.2% and 53%. Three-year and 5-year OS and CSS results according to the nomogram were found as 54.8% (±12.6), 38.6% (±10.8) and 75.3% (±11.6), 62.8% (±12), respectively. Statistically significant difference was found between the actual values and nomogram results for 3-year and 5-year OS and CSS rates.

Conclusion: The recent nomogram failed to estimate both OS and CSS in our group of patients that have more likely underwent cystectomy and have relatively homogenous age distribution.

KEY WORDS: bladder cancer, cancer specific survival, nomogram, overall survival

INTRODUCTION

Radical cystectomy (RC) with extended pelvic lymphadenectomy is considered the standard of care for muscle invasive and refractory high-grade non-muscle invasive urothelial cancer of the bladder^[1]. Despite significant improvements in surgical technique, the morbidity and mortality rates are still high regardless of the type of urinary diversion^[2,3]. In a large single-centre series, early complications (within three months of surgery) were seen in 58% of patients^[2]. The peri-operative mortality was reported as 1.2-3.2% at 30 days and 2.3-8% at 90 days^[2,4,5]. According to a multi-institutional database of 888 patients undergoing RC for bladder cancer, the five-year recurrence-free

survival was 58% and cancer specific survival (CSS) was 66%^[6]. For predicting individual outcomes and proper pre-operative counseling, nomograms are essential tools. A new nomogram was developed by Kamat *et al* that incorporate all-cause mortality and cancer specific mortality using SEER database^[7]. Our aim is to externally validate this nomogram in a different patient population using our clinical data.

SUBJECTS AND METHODS

Between March 2006 and October 2016, a total of 197 patients who were diagnosed with muscle invasive bladder cancer (MIBC) were enrolled in this study. Data were obtained from electronic record of patients.

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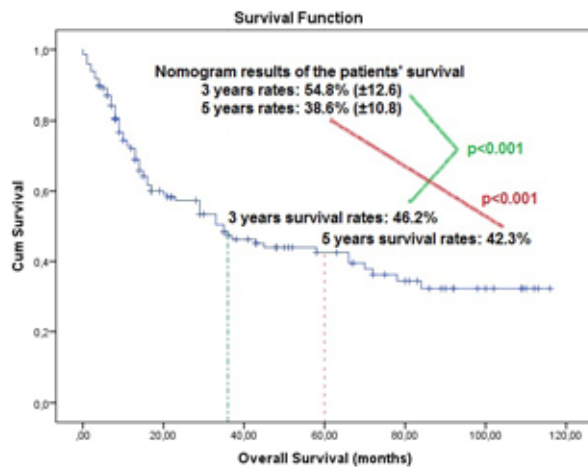


Fig 1: Kaplan-Meier curves for overall survival

Patients with missing data and inadequate follow-up were excluded. Patients with clinical stage T4b and/or node positive and metastatic disease were excluded from the study to be well-matched with the original study and finally, 146 patients were included in the recent study. Clinical and pathological data were analyzed for demographics, preoperative disease characteristics, treatment modality, pathological characteristics and postoperative data (recurrence, survival; Table 1). Comorbidity was assessed using Klabunde modification of the Charlson index^[8] during the year before cancer diagnosis as consistent with the original study. Tumor grade and pathological staging was performed based on TNM and World Health Organization / International Society of Urological Pathology classifications. All patients underwent surgery according to the criteria consistent with current guideline recommendations^[1]. The predicted CSS and overall survival (OS) by the recent nomogram were compared with the actual CSS and OS of the

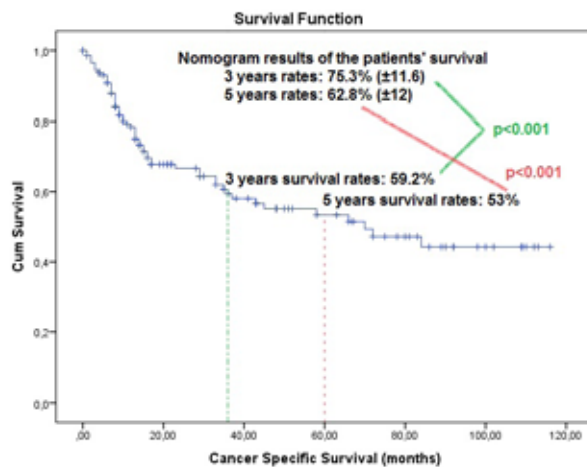


Fig 2: Kaplan-Meier curves for cancer specific survival

Table 1: Patients' characteristics and demographical data

Data	Values (n=146)
Age (year), mean±SD (range)	64.9±9.4 (32-90)
Follow-up (months), mean±SD (range)	31.8±31.8 (1-116)
Gender, n (%)	
Male	133 (91.1)
Hydronephrosis, n (%)	
Yes	48 (32.9)
Radical Cystectomy, n (%)	
Yes	140 (95.9)
Lymphovascular invasion, n (%)	
Yes	30 (20.5)
Neoadjuvant chemotherapy, n (%)	
Yes	1 (0.7)
Clinical T stage, n (%)	
T2	140 (95.9)
T3	6 (4.1)
Preoperative tumor grade, n (%)	
Low	1 (0.7)
High	145 (99.3)
Carcinoma in situ presence, n (%)	
Yes	42 (28.8)
Pathological T stage, n (%)	
≤T1	20 (13.7)
T2	71 (48.6)
T3	27 (18.5)
T4	22 (15.1)
Postoperative tumor grade, n (%)	
Low	10 (6.8)
High	118 (80.8)
Overall mortality, n (%)	
Yes	77 (52.7)
Cancer specific mortality, n (%)	
Yes	56 (38.4)

patients in our single center sample. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences, version 20.0 (SPSS, Chicago, Ill) software program. To observe the effect of the nomogram's variables on overall and cancer specific mortality, Pearson chi-square test was used. The COX regression model was used for the nomogram to our patients for suitability of the nomogram. To detect the survival for our cohort, Kaplan-Meier survival analysis was used for OS and CSS for detecting survival times and three- and five-year survival rates. The nomogram was applied to all patients predicting the three- and five-year OS and CSS rates. One sample T test was used for three- and five-year OS and CSS results of the nomogram according to three- and five-year OS and CSS rates detected in Kaplan-Meier survival analysis.

Table 2: Analysis of the data of our patients according to the nomogram

Data	Overall mortality (n=77), n (%)	P	Cancer specific mortality (n=56), n (%)	P
Radical cystectomy		0.485		0.485
Yes	73 (52.1)		52 (37.1)	
No	4 (66.7)		4 (66.7)	
Lymph node dissection		0.310		0.065
Yes	72 (51.8)		51 (36.7)	
No	5 (71.4)		5 (71.4)	
Neoadjuvant chemotherapy		0.289		0.429
Yes	0 (0)		0 (0)	
No	77 (53.1)		56 (38.6)	
Age group (years)		0.933		0.853
65-69	50 (51.5)		36 (37.1)	
70-74	14 (70)		12 (44.4)	
75-89	9 (60)		6 (40)	
80+	4 (57.1)		2 (28.6)	
Marital status		0.289		0.429
Single	0 (0)		0 (0)	
Married	77 (53.1)		56 (38.6)	
Gender		0.506		0.229
Male	69 (51.8)		49 (36.8)	
Female	8 (61.5)		7 (53.8)	
T stage		0.485		0.145
T2	73 (52.1)		52 (37.1)	
T3	4 (66.7)		4 (66.7)	
T4	-		-	
Hydronephrosis		0.098		0.096
Yes	30 (62.5)		23 (47.9)	
No	47 (48)		33 (33.7)	
Grade		0.289		0.429
Low	0 (0)		0 (0)	
High	77 (53.1)		56 (38.6)	
Charlson comorbidity index		0.274		0.471
0	1 (33.3)		1 (33.3)	
1	5 (83.3)		4 (66.6)	
2	12 (42.9)		9 (32.1)	
3+	59 (54.1)		42 (38.5)	

Data are given as mean±SD. Statistical significance was defined as $P < .05$. However, when detecting the statistical difference, the analysis results interpreted that the nomogram is not suitable for our cohort. However, when detecting statistical indifference, the analysis results interpreted that the nomogram is suitable for our cohort.

RESULTS

Among 197 patients who were diagnosed with MIBC between March 2006 and October 2016, 146 patients who had demographic, pathologic and oncologic follow-up data were evaluated. Fifty-one patients were excluded from the study because of missing data. Patients' overall mortality and cancer specific mortality data were given in Tables 1 and 2 according to the nomogram variables (radical cystectomy data, lymph node dissection data, neoadjuvant chemotherapy (NAC) data, age groups, marital status, gender, T stage, hydronephrosis presence, tumor grade and Charlson comorbidity

index). In univariate analysis, none of the data was statistically significant on overall and cancer specific mortality. To measure meaningfulness of the nomogram, COX regression model was created. The models of OS and CSS were not suitable for our patients ($P=.181$ for OS and $P=.218$ for CSS). In multivariate analysis, only presence of hydronephrosis and Charlson comorbidity index were significant for overall mortality ($P=.05$, HR:0.6, CI:0.35-0.99 and $P=.034$, HR:3.26, CI:1.1-9.7, respectively) (Table 3). In Kaplan-Meier survival analysis, mean OS of the patients was 54.5±4.4 months and 3-year and 5-year OS rates were found to be 46.2% and 42.3%. Mean CSS of the patients was 66.5±4.8 months and 3-year and 5-year CSS rates were found to be 59.2% and 53%. Three-year and five-year OS and CSS results according to the nomogram were found as 54.8% (±12.6), 38.6% (±10.8) and 75.3% (±11.6), 62.8% (±12), respectively (Figure 1, 2). Statistically significant difference was found between the actual values and nomogram results for 3-year and 5-year OS and CSS rates.

DISCUSSION

Nomograms on CSS and OS following RC have been developed and externally validated for proper counselling of patients. However, EAU guidelines still do not recommend their wider use until further data becomes available^[9]. Despite technical improvements and strict perioperative care, RC still has a non-negligible risk of morbidity and mortality^[2,3]. As life expectancy is increasing in developing and developed countries, we are facing more elderly patients in our oncological practice. It is important to make a smart decision to balance benefits versus risks in elderly and co-morbid patients since these patients have significant mortality, re-intervention and re-admission rates^[9]. Reporting of OS outcomes is so important besides CSS for a nomogram, especially in this group of patients. In the recent nomogram, authors aimed to develop a nomogram to assess CSS and OS outcomes in patients with bladder cancer according to the use of RC.

Several nomograms have been established to predict prognosis and to provide individualized risk assessment for patients after RC^[10-12]. Although most nomograms undergo some type of internal validation, to gain widespread acceptance, external validation in different patient cohorts is an essential step. We aimed to externally validate a recent nomogram in our patient cohort of 146 cases with 77 deaths at a mean follow-up of 31.8 months.

In the original study, the nomogram was created based on SEER database, and they also intended to validate the nomogram using the database of Texas Cancer Registry^[7]. Interestingly, only 25.9% of the total 5325 patients with MIBC underwent RC in study group. This rate is too low for a treatment modality that was strongly recommended by all guidelines based on high quality data with improved survival outcomes in this group of patients. In our single center series, 95.9% of patients who were diagnosed as T2-T4a bladder cancer underwent radical cystectomy.

Table 3: COX regression model of the original study and our study for overall survival and cancer specific survival according to the nomogram

Data	Overall survival						Cancer specific survival					
	Original study			Our study			Original study			Our study		
	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI
Radical Cystectomy	.06		0.78-1.01	-	-	-	.02		0.7-0.97	-	-	-
No		1						1				
Yes		0.89						0.83				
Lymph node dissection	<.001		0.45-0.58	-	-	-	<.001		0.43-0.59	.248	0.22	0.02-2.8
No		1						1				
Yes		0.51						0.51				
Neoadjuvant chemotherapy	.75		0.83-1.15	-	-	-	.58		0.77-1.16	-	-	-
No		1						1				
Yes		0.97						0.94				
Age group (years)				-	-	-				-	-	-
65-69	.37	1	0.94-1.19				.64	1	0.89-1.2			
70-74	<.001	1.05	1.11-1.38				.01	1.04	1.04-1.38			
75-89	<.001	1.24	1.47-1.81				<.001	1.2	1.36-1.77			
80+		1.63						1.55				
Marital status	<.001		0.7-0.83	-	-	-	<.001		0.68-0.85	-	-	-
Single		1						1				
Married		0.76						0.76				
Gender	.25		0.97-1.12	-	-	-	<.001		1.12-1.34	-	-	-
Male		1						1				
Female		1.04						1.22				
T stage				.987	0.98	0.12-7.7				.681	0.642	0.08-4.96
T2	<.001	1	1.21-1.45				<.001	1	1.19-1.52			
T3	<.001	1.33	2.62-3.04				<.001	1.35	3.15-3.77			
T4		2.82						3.45				
Hydronephrosis	<.001		1.37-1.64	.05	0.6	0.35-0.99	<.001		1.34-1.68	.077	0.57	0.31-1.06
No		1						1				
Yes		1.5						1.5				
Grade	<.001		1.32-1.81	.480	0.65	0.2-2.15	<.001		1.38-2.12	.923	0.94	0.3-3.2
Low		1						1				
High		1.55						1.72				
Charlson comorbidity index				.034	3.26	1.1-9.7				.068	3.27	0.91-11.7
0		1						1				
1	<.001	1.16	1.08-1.25				.12	1.08	0.98-1.19			
2	<.001	1.34	1.22-1.48				.01	1.18	1.04-1.34			
3+	<.001	1.68	1.53-1.85				<.001	1.34	1.17-1.52			

The patients included in the original study were older, that is 66.6% were >75y and 44% were >80y. This situation may cause a difficulty in assessment of CSS and OS. In our series, patients were younger and have relatively homogenous age distribution. The mean age of the patients in our study was 64.9 years and 7.8% were >80 years whereas 15.1% were ≥ 75 years.

Our series consist of relatively homogenous age distribution, all married, no racial difference and male predominant group. The failure of validation of the recent nomogram in our group of patients may be attributed to the performance of higher rate of surgery and potential difference in demographical data.

Our current study included only one patient who had NAC. This is mostly due to patients' preferences. The patients general approach is undesiring to chemotherapy because of the negative perception that chemotherapy has in the community, although the survival advantages were well discussed with the patient.

The results of the studies about NAC showed an absolute improvement in survival of 5-8% at five years^[1]. SWOG-8710 and Nordic cystectomy trials demonstrated that the largest benefit of NAC is in patients with locally advanced disease (cT3-4a)^[13,14]. Despite the proven OS benefit of cisplatin-based NAC prior to cystectomy, NAC utilization rates for patients with MIBC remain low and are thought to be approximately 20%. The main concerns raised by physicians about recommending NAC use for patients with MIBC include questions regarding treatment toxicity and potential delay of curative RC^[15]. We share the same drawbacks in common, but parallel to global increase in utilization of NAC, number of patients getting NAC has also increased in our clinic, especially in the last two years.

The limitations of our study were as follows: limited number of patients and under-utilization of neo-adjuvant chemotherapy.

The strengths of our study were as follows: single center data, the high rate of cystectomy for MIBC, relatively younger patients that potentially provides better assessment of predictors for both OS and CSS and reasonable follow-up period.

CONCLUSION

The recent nomogram failed to estimate both overall survival and cancer specific survival in our group of patients that are more likely to undergo cystectomy and have relatively homogenous age distribution.

ACKNOWLEDGMENTS

Conflict of interest : None

Author's contribution: Ertugrul Sefik was involved with study design, data collection and manuscript writing and editing; Ibrahim Halil Bozkurt wrote and edited the manuscript; Serdar Celik and Tansu Degirmenci did the study analysis; Ismail Basmaci and Serkan Yarimoglu took part in data collection and analysis.

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Original Article

Prevalence of vitamin D deficiency among patients attending orthopedic clinic in Taif, Saudi Arabia: A single center study

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ABSTRACT

Objective: To assess the prevalence of vitamin D deficiency among patients attending orthopedic clinics in King Abdul-Aziz specialist hospital in Taif city, Saudi Arabia

Design: Retrospective study

Setting: King Abdul-Aziz specialist hospital, Taif city, Saudi Arabia

Subjects: During the period from 18 July to 12 September 2017, data of the patients who were treated between June 2016 and June 2017 (N=1475) was collected to be included in the study. Vitamin D, calcium, phosphorus, parathyroid hormone and alkaline phosphatase levels of the patients were collected from the laboratory system, as well as radiological findings from the radiology system. Socio-demographic data (age, gender, and nationality) were also collected.

Intervention: Patients were classified according to vitamin D

level as deficient, insufficient, normal and high.

Main outcome measure: Vitamin D level and its correlation to different variables was measured using the SPSS program version 21.

Results: Among 1475 patients, 78% were females and 22% were males. Out of all patients, 53.1% had vitamin D deficiency, 27.8% had insufficiency, 15.4% had a normal level and 3.7% had a high level. A higher prevalence of deficiency was found among males (60.3%) than female patients (51%; $P=0.09$). All age groups showed a high prevalence of deficiency and the highest was observed among ≤ 19 years age group (67.9%; $P=0.28$).

Conclusion: High prevalence of vitamin D deficiency is significant among all orthopedic patients. It is most common among male and adolescent patients.

KEY WORDS: orthopedic outpatients, osteoporosis, Saudi Arabia, vitamin D deficiency

INTRODUCTION

Vitamin D is one of the most important hormones in the regulation of bone metabolism and turnover. It also has an important relation to bone mineral density as it is clearly affected by its deficiency, as well as its relation to bone fractures and osteoporosis^[1].

Vitamin D has an important role in the absorption and regulation of phosphorus, parathyroid hormone (PTH) and calcium, and thus has a major role in bone mineralization and normal bone architecture. A

deficiency of vitamin D is associated with osteoporosis and osteoporotic fractures, as well as muscle weakness^[2]. A high prevalence of vitamin D deficiency is a worldwide health issue, especially among Middle East countries, and it affects all age groups. The variety of gender, sunlight exposure, special habits and cultural practices as clothing and dietary habits are all contributing factors to its deficiency^[3-7]. Studies in Saudi Arabia indicate that vitamin D deficiency is a major health problem with a high prevalence among

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Saudi population in all regions and among different age groups^[8-11]. The overall prevalence of deficiency was 60% and around 80% among female patients^[10,11]. Among orthopedic and trauma patients, many studies worldwide found a significant deficiency^[12-15]. An Indian single-center study found that around 91% of male and female patients had a deficiency^[12]. Another study carried out in Germany found that the mean of vitamin D level among 1119 orthopedic patients males and females was deficient^[13]. In another study by Maier *et al*, around 84% of orthopedic patients had insufficiency, out of them, 60% had a deficiency^[14]. In the United States, 71% and 62% of patients had insufficient or deficient vitamin D levels during the winter and the summer, respectively^[15]. In Saudi Arabia, a recent study was carried out in Al-Qassim region among male patients attending rheumatology clinic and found that 70% had vitamin D insufficiency^[16].

In Saudi Arabia, there is a shortage of studies assessing vitamin D deficiency among orthopedic patients. The aim of this study is to assess the prevalence of vitamin D deficiency among orthopedic patients attending orthopedic clinics in King Abdul-Aziz specialist hospital in Taif city, Saudi Arabia.

SUBJECTS AND METHODS

Study design

This retrospective study aims to assess the prevalence of vitamin D deficiency among patients attending orthopedic clinics in King Abdul-Aziz specialist hospital at Taif city from June 2016 to June 2017. Taif city is located in Makkah Province with an overall population of 987,914 (2010 census).

Table 1: General characteristics

Variable	Count	Percentage
Nationality		
Saudi	1290	87.5
Non-Saudi	185	12.5
Age (years)		
≤19	81	5.5
20-40	390	26.4
41-60	694	47.1
61-80	310	21.0
Gender		
Male	325	22.0
Female	1150	78.0
Diagnosis		
Anterior cruciate ligament injury	13	2.3
Disc disease	113	20.3
Femur fracture	18	3.2
Normal bone mineral density	25	4.5
Osteopenia	75	13.5
Osteoporosis	31	5.6
Spondyloarthropathy	15	2.7
Others	267	47.9

Table 2: Laboratory results

Variable	Count	Percentage
Vitamin D category		
Deficiency	783	53.1
Insufficiency	410	27.8
Normal	227	15.4
High	55	3.7
Parathyroid hormone		
Low	2	0.9
Normal	137	64.3
High	74	34.7
Phosphorus		
Low	18	6.5
Normal	247	88.5
High	14	5.0
Alkaline phosphatase test		
Low	35	2.8
Normal	1055	84.6
High	157	12.6
Calcium		
Low	141	12.6
Normal	959	85.9
High	16	1.4

Data collection methods and procedure

Data was collected during the period from 18 July to 12 September 2017. We obtained the data by using patients' medical record numbers and accessing laboratory system to collect vitamin D level, calcium, phosphorus, PTH and alkaline phosphatase (ALP). Also, by accessing radiological system to collect any associated radiological findings such as osteoporosis, osteopenia, fractures, *etc.* that were confirmed by computed tomography, magnetic resonance imaging or dual-energy x-ray absorptiometry. In addition, socio-demographic data (age, gender and nationality) were collected. We classified the patients according to 25-hydroxy vitamin D (25 OHD) levels to normal, deficient, insufficient and high as per King Abdul-Aziz specialist hospital references lab ranges:

- Normal: 25 OHD=30-49.9 ng/ml
- Insufficient: 25 OHD=20-29 ng/ml
- Deficient: 25 OHD=below 20 ng/ml
- High: 25 OHD=50 ng/ml or above
- Vitamin D inadequacy (insufficiency + deficiency): 25 OHD <30ng/ml
- Vitamin D sufficiency (normal +high): 25 OHD >30ng/ml

Inclusion criteria

All patients (males and females) attending orthopedic clinics at King Abdul-Aziz specialist hospital were included. The patients must have at least one laboratory result of vitamin D during the mentioned period to be included in our study.

Table 3: Relation of Vitamin D to general characteristics

Variable	Vitamin D category (n(%))				Chi-square	P-value
	Deficiency	Insufficiency	Normal	High		
Nationality					1.366	.714
Saudi	690 (53.5)	352 (27.3)	200 (15.5)	48 (3.7)		
Non-Saudi	93 (50.3)	58 (31.4)	27 (14.6)	7 (3.8)		
Age						
≤19	55 (67.9)	19 (23.5)	6 (7.4)	1 (1.2)	9.104	.028*
20-40	260 (66.7)	94 (24.1)	33 (8.5)	3 (0.8)	50.017	.000*
41-60	332 (47.8)	205 (29.5)	121 (17.4)	36 (5.2)	19.267	.000*
61-80	136 (43.9)	92 (29.7)	67 (21.6)	15 (4.8)	17.949	.000*
Gender					11.555	.009*
Male	196 (60.3)	85 (26.2)	34 (10.5)	10 (3.1)		
Female	587 (51)	325 (28.3)	193 (16.8)	45 (3.9)		

Exclusion criteria

We excluded patients less than 10 years and older than 80 years, patients not attending orthopedic clinics, patients with no available vitamin D results on the system, and results before or after the mentioned period of study.

Ethical considerations

This study was approved by the Research Ethics Committee of Taif University and Institutional Review Board. The collected data were kept in confidentiality and patients medical record numbers were replaced with ordinal numbers for further confidentiality.

Data analysis

Statistical analysis was carried out using the statistical package for the social sciences program (SPSS 21). Descriptive analysis was carried out to detect prevalence, mean and quantitative variables. Chi-square and t-test were used to assess the correlation between vitamin D deficiency and other variables.

RESULTS

Our study included 1475 patients (78% females and 22% males) with a mean age of 47.7±15.8 years. The maximum age was 80 years while the minimum age was 11 years. Most of the patients were Saudi (87.5%), as shown in Table 1. The mean vitamin D level was 21.3 ng/ml and the lowest level was 1.7 ng/ml. Out of

all participants, 53.1% had vitamin D deficiency, 27.8% had insufficiency, 15.4% had a normal level and only 3.7% had a high level. Regarding other laboratory results, out of all the participants, only 213 (14.4%) had available PTH results and 279 (18.8%) had available phosphorus results. Out of them, 64.3% and 88.5% had normal PTH and phosphorus levels respectively, while 84.5% and 75.7% of participants had available ALP and calcium results respectively. Out of them, 84.6% had normal ALP levels and 85.9% had serum calcium within normal range as shown in Table 2. Out of all the participants, 62.2% had no radiologically confirmed diagnosis. Among diagnosed individuals, the most frequent diagnosis was disc disease (disc prolapse and degenerative disc, 20.3%), followed by osteopenia (13.5%) and the least frequent was spondyloarthropathy (2.7%). Other diagnoses include fracture, osteoarthritis, joint effusion and joint replacement (47.9%) as shown in Table 1.

Out of all female patients, 51% had a deficiency, while among male patients, 60.3% had a deficiency. Only 16.8% of females and 10.5% of males had normal levels of vitamin D as shown in Table 3. The highest prevalence of deficiency was observed among patients with anterior cruciate ligament injury (76.9%) followed by femur fracture (66.7%), and the lowest prevalence was observed among patients with spondyloarthropathy (26.7%), as shown in Table 4. Regarding ALP and calcium levels, 69.4% of patients

Table 4: Relation of vitamin D to different diagnoses

Variable	Vitamin D category (n(%))				Chi-square	P-value
	Deficiency	Insufficiency	Normal	High		
Anterior cruciate ligament injury	10 (76.9)	3 (23.1)			24.562	.137
Disc disease	60 (53.1)	32 (28.3)	18 (15.9)	3 (2.7)		
Femur fracture	12 (66.7)	5 (27.8)	1 (5.6)			
Normal bone mineral density	13 (52)	6 (24)	5 (20)	1 (4)		
Osteopenia	34 (45.3)	17 (22.7)	16 (21.3)	8 (10.7)		
Osteoporosis	12 (38.7)	12 (38.7)	6 (19.4)	1 (3.2)		
Spondyloarthropathy	4 (26.7)	4 (26.7)	5 (33.3)	2 (13.3)		

Table 5: Relation of Vitamin D to laboratory results

Variable	Vitamin D category (n(%))								Chi-square	P-value
	Deficiency		Insufficiency		Normal		High			
Parathyroid hormone									8.750	.188
Low					2	100				
Normal	66	48.2	34	24.8	28	20.4	9	6.6		
High	40	54.1	17	23	14	18.9	3	4.1	4.246	.643
Phosphorus										
Low	10	55.6	5	27.8	2	11.1	1	5.6		
Normal	137	55.5	58	23.5	41	16.6	11	4.5	27.306	.000*
High	11	78.6	1	7.1	1	7.1	1	7.1		
Alkaline phosphatase test										
Low	16	45.7	14	40	5	14.3			17.155	.009*
Normal	524	49.7	304	28.8	183	17.3	44	4.2		
High	109	69.4	34	21.7	12	7.6	2	1.3		
Calcium										
Low	83	58.9	31	22	26	18.4	1	7		
Normal	498	51.9	266	27.7	151	15.7	44	4.6		
High	5	31.3	4	25	7	43.8				

with a high ALP level and 58.9% with a low calcium level had a deficiency ($P=.000$ and $.009$) respectively, as shown in Table 5.

DISCUSSION

Vitamin D deficiency is a common health issue with a high prevalence worldwide and in Saudi Arabia, as reported by many studies^[3,11]. However, no previous studies among orthopedic patients in Saudi Arabia have been reported, while many studies worldwide indicate the significance and the high prevalence of deficiency. Our study found that out of 1475 patients, 80.9% had inadequacy out of them, 53.1% had a deficiency, while 19.1% had a sufficient level. These findings are consistent with many studies^[12,14,15,17-19]. A higher prevalence of deficiency was reported in India out of 1132 orthopedic patients; 91% had inadequacy among them, 61% had deficiency and only 8.7% had a sufficient level of vitamin D^[12]. Another study showed an even higher prevalence among Indian patients who presented with orthopedic trauma; 95% had inadequate level, 82% of them had deficiency, while only 5% had a sufficient level, which exhibits the highest prevalence of deficiency^[17]. A study conducted among 889 orthopedic trauma patients showed that 77% had an inadequate level^[18]. Also, among 1119 German orthopedic patients, 84% were having an insufficient level and 60% had a deficient level^[14]. Among orthopedic trauma patients in the United States, 71% of the patients had a deficiency during summer and 62% of patients had a deficiency in winter^[15]. Also, among 723 patients scheduled for orthopedic surgery in New York, 43% were having insufficiency, and among them, 40% were vitamin D deficient^[19]. Even among rheumatology patients, 90% out of 60 Saudi male patients had vitamin D inadequacy, 70% had a deficiency and only 10% had a sufficient level^[16]. This

may imply a correlation between some diseases and vitamin D deficiency. However, a single study reported a low prevalence of deficiency among 488 healthy Saudi adults as only 29% had a deficiency and 22% had insufficiency^[9], which disagrees with all previously mentioned studies. However, this may be owing to the variation of prevalence between healthy individuals and orthopedic patients, which need further investigations and evaluation. Many risk factors are known to contribute to vitamin D deficiency such as sunlight exposure, skin color, *etc*^[2,3,20], but some factors are still controversial. Many studies stated that gender is not a significant risk factor for vitamin D deficiency among orthopedic patients^[12,14,17,18]. However, we found that deficiency was observed more among male patients, which was statistically significant ($P=.009$). These findings are consistent with other studies^[19], while others indicate that male gender is a probable risk for vitamin D deficiency^[21]. While regarding the age as a risk factor, our study found that vitamin D was deficient among all age groups which was statistically significant, with the highest prevalence among adolescent patients ($P=.028$), which is consistent with other studies^[12,19]. However, these findings conflict with other studies as it reported older age as a potential risk factor ($P \leq .10$)^[21], or it is not significant at all^[18]. Regarding lab results, our study found that P -value was significant for calcium and ALP levels, which is consistent with the normal physiology^[2].

Limitations

Although our study has achieved its aim, there were some limitations. The limitations of this study were that some required data such as diagnosis and laboratory results were not available on the hospital system and considered as missing data. Also, some

diagnoses were not radiologically confirmed (computed tomography, magnetic resonance imaging or dual-energy x-ray absorptiometry) or without report as osteoarthritis, joint effusion, strain, joint replacement and fractures were collected under the category (others). Another limitation was that insufficient sample of patients with a specific diagnosis might affect the prevalence accuracy and the relation to vitamin D level.

CONCLUSION

There is a high prevalence of vitamin D deficiency among all orthopedic patients among all age groups with statistically significant values. A higher prevalence among males and adolescent patients reflects that age and gender are risk factors that need further study and investigations, as well as treatment.

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Original Article

Anesthesia and postoperative complications in sleeve gastrectomy operations performed in morbid obesity surgery

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ABSTRACT

Objectives: Sleeve gastrectomy is one of the most popular bariatric procedures today. Following laparoscopic interventions, common complications such as bleeding, organ damage, respiratory problems and emboli development are observed. The objective of this study was to evaluate our experiences with anesthesia and postoperative complications in sleeve gastrectomy operations performed in morbid obesity surgery.

Design: Prospective study

Setting: Operation room

Subjects: Sixty adult patients who provided informed consent and who underwent laparoscopic sleeve gastrectomy from November 2015 to January 2019 (N=60).

Intervention: Data collection, sleeve gastrectomy operation

Main outcome measures: Postoperative complications were evaluated under headings: bleeding, respiratory

problems, prolonged mechanical ventilation, prolonged hospital stay, emboli development and mortality.

Results: None of the patients had mortality. Mean age was 36.2 (range: 18-59) years. Of the 60 morbid obese patients, 86.6% were female. Mean preoperative body mass index was 48.7 (range: 37-60) kg/m². The integrity of the anastomosis was controlled with a 36 French orogastric tube. The mean operating time was 43 minutes (range: 34-72) Two patients (3.3%) were re-intubated due to hypercarbia. Only two patients had bleeding on the postoperative 1st day. Mean length of hospital stay was 4.8 days.

Conclusions: We believe there are fewer risks in the laparoscopic sleeve gastrectomy technique if there is careful preoperative patient preparation and evaluation with a multidisciplinary approach, appropriate perioperative anesthesia management, successful coordination with the surgical team and postoperative care.

KEY WORDS: anesthesia, laparoscopic sleeve gastrectomy, postoperative complications

INTRODUCTION

Obesity is a serious public health problem that is becoming increasingly common in many parts of the world. Research suggests that obesity affects one in three Americans and approximately 300,000 deaths per year in the USA can be linked to obesity^[1].

Clinically, morbid obesity is defined as body mass index (BMI) ≥ 40 kg/m² (stage 3) or as BMI between 35 and 39.9 kg/m² (stage 2) with an accompanying disease^[1]. The overall classifications for obesity are presented in Table 1.

Morbidly obese individuals have greater risk for

diabetes mellitus, hypertension, sleep-apnea syndrome, esophageal reflux, osteoarthritis, cardiac diseases and some forms of cancer (*i.e.*, endometrial, cervix, uterine, colorectal and prostate)^[2]. Persons may be candidates for obesity surgery if they are defined as morbidly obese (*e.g.*, namely those with a BMI of 40 or more or those with a BMI of 35 or more with serious health conditions).

Studies have shown that it is almost impossible to completely cure morbid obesity using dietary, medical and psychosocial therapies. For that matter, in many countries, bariatric restrictive and malabsorptive

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Table 1: Classifications for obesity

Classification	BMI (kg/m ²)
Normal	18.5-24.9
Overweight	25-29.9
Obesity Stage 1	30-34.9
Obesity Stage 2	35-39.9
Obesity Stage 3	40 or greater

operations are now being performed laparoscopically. Currently, there is no bariatric surgical technique regarded as the 'gold standard.' However, laparoscopic sleeve gastrectomy (LSG) is becoming increasingly common as a primary bariatric surgery and is among the most popular bariatric procedures^[3-6].

Sleeve gastrectomy (SG) is a volume-restricting procedure and one of the most popular bariatric procedures today. However, this procedure, which has been used for more than 10 years, has not been completely standardized. Following laparoscopic interventions, common complications such as bleeding, organ damage, respiratory problems and emboli development are observed at very low rates^[7,8]. LSG is generally performed laparoscopically, permanently removes approximately 85% (greater curvature) of the stomach, leaving a small new stomach that is cylindrical in shape^[9].

As morbid obesity surgery has become increasingly common in recent years, the preoperative evaluation, perioperative anesthesia management, surgical success and postoperative follow-up have gained great importance. In the current study, we aimed to evaluate our experiences with anesthesia and postoperative complications in SG operations performed in morbid obesity surgery.

SUBJECTS AND METHODS

This prospective and observational study included a total of 60 adult patients who provided informed consent and who underwent LSG between November 2015 and January 2019. The study was carried out in bariatric surgery unit of Bozok University Medical Faculty after obtaining permission from the Hospital Education Planning and Ethics Committee (Date: November 19, 2015, No: 30/05). The following data from each patient were evaluated prospectively: age, sex, BMI, American Society of Anesthesiology level, Mallampati score, accompanying diseases,

Table 2: Demographic features of the patients

Patients	Median	Range	Ratio
Age (years)	36.2	18-59	-
Sex (F/M)	52/8	-	6.5
BMI*(kg/m2)	48.7	37-60	-

*Body Mass Index

preoperative evaluation and operation preparations, operation time, perioperative anesthesia management, postoperative complications and hospital stay length. Patients who underwent gastric bypass surgery, which is less frequently performed in our center, were not included in this study.

Statistical methods

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc, Chicago, IL, USA). Data were presented as the number of patients. For all analyses, two-sided P-value of <0.05 was considered significant.

RESULTS

Sixty patients who underwent SG were included in this study. Other procedures performed for morbid obesity in our center were excluded. The demographic data of the included patients are presented in Table 2, while Table 3 shows the prevalence of comorbidities.

Table 3: Prevalence of comorbidities

Comorbidities	Number	Percentage
Hypertension	36	60
Diabetes mellitus type II	30	50
OSAS	9	15
Bronchial asthma	2	3,3
Ischemic heart disease	2	3,3
Hypothyroidism	1	0,6

OSAS: obstructive sleep apnea syndrome

Pre-anesthetic evaluation

Prior to the operation, all patients were informed about the risks of the surgical procedure, its advantages, benefit-harm ratio and possible complications and side effects associated with the surgical procedure and anesthesia that may occur before or after the operation.

Perioperative anesthesia management

Patients were carefully transported and positioned on the operating table. Further, all patients were given anti-emboli stockings, which they continued to wear during the postoperative period. Careful attention was paid to make sure that there was no nerve injury related to the compression. During the procedure, all patients were monitored with electrocardiography, pulse oximetry and non-invasive brachial artery pressure measurement (using a large cuff). Invasive arterial cannulation was applied for those patients requiring close monitoring of arterial blood pressure. Central venous catheter was not routinely applied; instead, it was placed in patients for whom a peripheral i.v. line could not be placed.

Preoperatively, patients were administered benzodiazepine (Dormicum I.V. 0.03 mg/ kg) for anxiolytic purposes. During induction of anesthesia, patients were administered propofol (3 mg/kg according to their ideal body weight, since it is lipophilic), fentanyl (1 mcg/kg according to their total body weight since it is lipid-soluble), rokuronyum (0.6 mg/kg as muscle relaxant) and sevoflurane (has low lipid-solubility), which was used as a volatile agent for the maintenance of anesthesia. Although desflurane is 10 times less soluble in fat compared to sevoflurane and can result in very fast postoperative recovery, we preferred to use sevoflurane because desflurane was not available in our institution.

Modified lithotomy (Lloyd-Davis, abduction in hip joint and flexion in knee joint) and reverse Trendelenburg position were the perioperative positions applied to the patients. Special attention was paid so as not to cause nerve-compression related to the positioning. In addition, all patients underwent pressure-controlled ventilation. In order to correct oxygenation, 35-40 cm H₂O peak inspiratory pressure was manually applied intraoperatively every 5-10 minutes for a five second duration, as suggested in the literature^[10].

A nasogastric tube was placed and gastric air content was emptied. For all patients, SG was performed laparoscopically and no patients required open surgery. Before proceeding to the resection step, a 36F (French) orogastric tube was placed in order to prevent development of stricture. Resection was performed by removing the greater curvature that constitutes nearly 85% of the stomach, leaving a small tube-like portion of the stomach. The integrity of the anastomosis was checked via the 36F (French) orogastric tube. First, air was injected into the tube, followed by the addition of 50 ml methylene blue. The 36F orogastric tube was removed after confirming that there was no leakage during the test.

Table 4: Some problems observed in the patients

Complications	Number	Percentage
Difficult intubation	4	6.6
Dental trauma	1	1.6
Vomiting	15	25
Bronchospasm	4	6.6
Hypertension	6	10
Fever	2	3.3
Bleeding	2	3.3
Pneumonia	1	1.6
Pulmonary Emboli	-	-
Re-intubation	2	2.6
Allergic Reactions	1	1.6
Diarrhea	1	1.6
Myocardial Infarction	-	-
Sepsis	-	-

Follow-up in the postoperative phase

Routinely, all patients were given postoperative patient-controlled analgesia. For embolic prophylaxis, patients were administered low molecular weight heparin (Clexane 0.6 1x1 S.C) at the postoperative 8th hour. Table 4 presents some of the problems observed in the patients.

Table 5: Patient outcomes

Patient outcomes	Mean	Range
Operation time	43 min	34-72
Length of hospital stay	4.8 days	
Mortality	-	-

Post-operative follow-up

Patients were monitored in a supine position at 45 degrees. Two patients (3.3%) were re-intubated due to hypercarbia; they were extubated on post-operative day 1 after the hypercarbia was corrected. Three patients (5%) were given a non-invasive ventilation mask in order to correct hypercarbia. None of the patients developed pulmonary emboli or sepsis. Only two patients had bleeding on the postoperative 1st day; one of them received two units of erythrocyte suspension and two units of fresh frozen plasma, while the other received four units of erythrocyte suspension and three units of fresh frozen plasma transfusion. These patients did not require surgical intervention. All patients underwent a leakage test using methylene blue on postoperative day 1 (after 24 hours) – none of the patients had leakage. A short summary of patient outcomes is presented in Table 5.

DISCUSSION

We believe that these types of interventions are highly beneficial to patients, as they provide successful weight loss in almost all morbidly obese patients, and they reduce the severity of accompanying diseases to more controllable levels. Nonetheless, all professionals agree that patients should be carefully chosen when making the decision to perform one of these interventions. Traditional bariatric surgery guidelines were developed in 2013 by the American Association of Clinical Endocrinologists, the Obesity Society, and the American Society for Metabolic and Bariatric Surgery^[2]. LSG was performed on severely obese patients in several different clinical trials; results revealed that this procedure had considerable clinical advantages when compared to laparoscopic gastric bypass^[11]. According to one report from a center in Japan, LSG was successfully performed in a 40-year-old female patient with a BMI of 90.6 kg/m² and a weight of 228 kg; this surgery was performed without any problems (aside from difficult intubation) and

without the development of any complications (such as thromboembolism, etc.)^[12]. A Swedish study reported excellent preliminary results on 23 patients who underwent SG and were followed-up with for a mean of six months. Their associated metabolic and cardiovascular comorbidities showed considerable remission or resolution^[13]. In a study from Rome University Bariatric Surgery Department, Cerci *et al*^[11] followed-up with patients after SG for a mean of 12.4 months. They reported an average six point reduction in BMI, with a value of 27.2 kg/m² over that time period.

A recent study on aged rats^[14] revealed that SG not only led to weight loss as a result of calorie restriction, but there was also a reduction in insulin resistance and a remarkable decline in blood pressure through endocrine changes, even in aged rats. Another recent study on rats by Moncada *et al*^[15] reported that SG had beneficial effects on glucose metabolism and metabolic profile, in addition to weight loss. Another recent study from a center in Asia conducted on elderly Taiwanese patients^[16] showed that SG led to weight loss and the improvement of accompanying diseases.

It is important to note that we did not encounter any mortality in our current study, which included 60 patients. In a recent study, Moszkowicz *et al*^[17] indicated that careful patient selection, safer alternative procedures and close follow-up in the postoperative period might help diminish the risk of serious complications. A study from Spain by De la Matta-Martin *et al*^[18] reported the rate of bariatric surgery-related mortality as 2.36%. The same study reported the rate of pulmonary complications as 2.3%. That study also found that body mass index ≥ 50 is also related to higher mortality. A recent review of bariatric surgery, which included more than 100,000 surgical interventions between 1998 and 2006, reported a global mortality prevalence of 4.6%. That study also defined independent risk factors for mortality as older age (>65 years), male gender and surgeons who are less experienced in bariatric surgery^[19].

Pulmonary embolism and its complication and deep vein thrombosis are common causes of morbidity and mortality after bariatric surgery. The prevalence of venous thromboembolism has been reported to range between 0.2 and 1.3% at 30 days, and is 0.42% at 90 days during the postoperative period^[20]. It should be emphasized that even though the percentage of patients experiencing thromboemboli is low, it is still the most frequent cause of mortality following bariatric surgery. Further, mortality is also associated with the presence of comorbid disorders such as cardiovascular diseases and coronary heart disease^[21].

In patients undergoing bariatric surgery due to morbid obesity, it is recommended that these patients

use low-molecular weight heparin as a prophylactic^[18]. None of the patients in our current study developed pulmonary emboli. We aimed to prevent deep vein thrombosis and its complication, pulmonary emboli, by administering low-molecular weight heparin prophylaxis (Clexane 0.6 1x1 S.C) together with the use of anti-embolic stockings or pressure devices during the postoperative period. A recent study indicated that the administration of high doses of thromboprophylaxis decreased the rate of venous thromboembolism by 50% in morbidly obese patients^[22]. However, in our current study, we used routine doses of thromboprophylaxis (not high doses).

The most interesting demographic property of patients who underwent SG in our center was that 52 patients were female and only 8 were male. In another recent study from an institute in Paris, France, by Badaoui *et al*^[23], 94.1% of the 68 patients who underwent SG were female, which is consistent with the demographics of our study. The mean age of the patients in our series was 36.2 years. The youngest patient was an 18-year-old adolescent girl and the oldest patient was a 59-year-old man. The mean BMI of our patients was 48.7 kg/m² (range: 37-60). The most frequent diseases accompanying morbid obesity among our patients were hypertension (60%), type-2 diabetes mellitus (50%), obstructive sleep apnea syndrome (15%) and ischemic heart disease (3.3%). The mean length of the surgery was 43 minutes (range: 34-72 minutes). In the French study by Badaoui *et al*^[23], the mean age was 34.4 years (range: 22-55) and the mean preoperative BMI was 42.6 kg/m². The mean operating time in that study was 60 minutes (range: 45-95). The mean time in the recovery room in the study by Badaoui *et al*^[23] was 86.5 minutes (range: 35-240).

In our current study, 14 patients had Mallampati-III, two patients had Mallampati-IV and four patients had difficult intubation. One patient had dental trauma during intubation and that patient initiated a lawsuit. In a large meta-analysis, the overall incidence of difficult intubation in obese patients was reported to be 15.8%, compared to 5.8% in the general population^[24]. Brodsky *et al* reported that neither obesity nor BMI could be a predictive value, and that obstructive sleep apnea is not a risk factor for difficult intubation^[25]. As indicated by Marrel *et al*, the use of a video laryngoscope substantially improves the visualization of the tracheal aperture in morbidly obese patients^[26].

For induction of anesthesia before the SG operation, we preferred to use propofol as an i.v. anesthetic agent and sevoflurane as a perioperative inhaler anesthetic agent. The study conducted by Servin *et al*^[27] revealed that the elimination half-life of propofol was similar in obese (29.1 minutes) and non-obese patients (24.2

minutes). In a related manner, recovery room duration and Aldrete scores were similar in patients given propofol or thiopental. A study from India by Rajan *et al*^[28] concluded that sevoflurane has a better recovery profile than isoflurane in morbidly obese patients undergoing laparoscopic sleeve gastrectomy; these conclusions were based on eye opening, obeying commands, time for extubation and orientation. Furthermore, a randomized studies demonstrated that sevoflurane was associated with hemodynamic stability, quick recovery and low incidence of nausea and vomiting, and was superior to isoflurane in sleeve gastrectomy surgery^[29]. Desflurane is 10 times less soluble in fat compared to sevoflurane and can result in very fast postoperative recovery. However, we preferred to use sevoflurane because desflurane was not available in our institution.

When compared to open surgery, laparoscopic interventions are known to cause fewer disturbances in pulmonary functions^[30]. For patients with BMI >35 kg/m², pressure-controlled ventilation corrects the intraoperative ventilation/perfusion rate better than volume-controlled ventilation in laparoscopic surgery^[31]. Respiratory mechanics in these patients are influenced by obesity and pneumoperitoneum, but not by the patient's position during surgery^[32]. Oxygenation during the surgery may be corrected by applying 40 cm H₂O peak inspiratory pressure intraoperatively every 5-10 minutes manually for five seconds^[10]. In our current study, positive end-expiratory pressure was maintained at a level of 5-8 cm H₂O and no complications were observed at this positive end-expiratory pressure value.

Some published studies report that air aspiration during continuous positive air pressure therapy can force the recently created anastomosis line, which can lead to leakage in morbid obese patients with obstructive sleep apnea syndrome; however, other large-scale studies do not support this finding^[33,34]. Especially following laparotomy, respiration can be suppressed due to pain. A thoracic epidural catheter can aid in pain management, leading to improvement in respiratory functions functions^[35]. Since epidural analgesia is technically difficult and can delay mobilization we applied epidural catheters for the purpose of postoperative analgesia only in a few of patients who underwent SG.

Generally, post-op pain after LSG is not as severe as with any open procedure. Opioids with paracetamol or non-steroid anti-inflammatory drugs as part of a multimodal regimen are preferred for postoperative analgesia in morbidly obese patients^[36].

All surgical interventions may have complications and unexpected results. Twenty-five percent of the

patients in the current study experienced nausea and four developed bronchospasm. One patient initiated a lawsuit because of dental trauma. A total of five patients suffered from hypercarbia. Two patients were re-intubated due to hypercarbia and were extubated on the 1st day postoperative after resolution of hypercarbia. In three patients, hypercarbia was corrected by applying non-invasive ventilation (continuous positive airway pressure mask). None of our patients developed pulmonary emboli or sepsis. Only two patients had bleeding on the postoperative 1st day and neither required re-operation; replacement therapy was sufficient. The study by Shiga *et al*^[24] recorded five surgical complications (of a total of 280 patients), including two cases of gastric fistula, one case of gastric stenosis, one case of scar dehiscence and one case of splenic upper pole ischemia.

In a recent study^[11], out of 100 patients, there were two major postoperative bleeding episodes and an anastomotic leak in the upper portion of the gastric tube observed postoperatively. These episodes required a stenting procedure.

Like any other laparoscopic surgery, carbon dioxide embolism can also occur during LSG. There is a report of an 18-year-old morbidly obese female patient who developed carbon dioxide embolism during LSG^[37]. During the initial intra-peritoneal insufflation with CO₂ at high flows through the upper left quadrant of the abdomen, she had a precipitous fall of end-tidal CO₂ and SaO₂% accompanied by tachycardia. Early suspicion led to stoppage of further insufflation.

We believe that there is still not enough data regarding SG in the literature, and that as the number of reports with favorable outcomes about this intervention accumulate from all over the world, the significance and necessity of SG for morbidly obese patients will be accepted by a larger community of both professionals and patients.

CONCLUSIONS

We believe that LSG technique in morbid obesity surgery may be safely performed by a surgical team that has adequate skill and experience. There are fewer risks if there is appropriate patient selection, careful preoperative patient preparation and evaluation with a multidisciplinary approach, appropriate perioperative anesthesia management, appropriate ventilation strategies, successful coordination with the surgical team and postoperative to prevent and treat possible complications. Our findings indicate that these types of interventions require an experienced surgical team, a successful anesthetist who plays a key role in the appropriate perioperative anesthesia management, prevention and treatment of

complications.

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Case Report

Treatment of acute postpartum uterine inversion case with a different compression suture technique

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ABSTRACT

Uterine inversion is a very rare cause of postpartum hemorrhage that can be fatal for the patient. Primary treatment is the reduction of a collapsed uterus fundus. In this case, we have used Johnson maneuver to correct the collapsed fundus, but we failed. When this rare condition

is encountered, an alternative treatment should always be available. We used compression suture that is used very often for uterine atony and placental adhesion anomalies. The patient's bleeding was stopped and the uterus was prevented from collapsing again.

KEY WORDS: atony, compression suture, postpartum hemorrhage, uterine inversion

INTRODUCTION

Uterine inversion is the disruption of the uterine fundus into the endometrial cavity. In many cases, uterine tubes are accompanied additionally. In very severe cases, the uterus is evident from the vaginal opening through the endometrial surface. Incidence is 3737 births^[1]. The maternal mortality due to uterine inversion was reported as high as 15% in the old series in the literature^[2]. The most important risk factor is uncontrolled traction applied to the cord^[3]. Therapeutic models such as correction of manual uterine inversion and surgical fixation are available. Johnson maneuver is applied for manual correction. Surgical correction is performed in advanced inversion cases^[4]. Risk of bleeding and early inversion relapse always continues in inversion cases. In these cases, compression sutures which are frequently used in uterine atony and placental adhesion anomalies can be used^[5]. We used a different compression suture technique to prevent early recurrence of acute postpartum uterine inversion.

CASE REPORT

A 34-year-old woman delivered in our clinic. At birth, the placenta was taken by the midwife. It was stated that the placenta was subjected to traction. With the release of the placenta, it was said that there

was an explosion-type bleeding. When abdominal examination was performed for fundal massage, the uterine peak was not palpable. Hemorrhagic fragile tissue at the top of the vagina was seen during vaginal examination. It was decided to reposition the inverted uterine part with Johnson maneuver for the patient, who was diagnosed with third degree uterine inversion. Uterotonic agents were cut off before maneuver. Nifedipine was administered at a dose of 20 mg in total at a sublingual repeat dose of 10 mg. The uterine inversion could not be corrected with the pharmacological agent applied and manual thrust. The patient was then sedated with the general anesthetic agent propofol. The maneuver was applied again but manual correction was not successful after general anesthetic agent. There was a blood loss of about 2500 ml. Blood pressure was arterial 60/40 mmHg, pulse was 136 /min. A complete blood count was taken. Hemoglobin was 5.9 mg/dl, hematocrit was 19%, platelets was 156x103/ml and fibrinogen was 210 mg/dl. The emergency laparotomy was made because the patient's clinic worsened very rapidly. In the exploration, a portion of the tubal uterine, utero ovarian ligament and round ligament together with the uterine fundus were inverted as shown in Figure 1.

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Fig 1: Abdominal exploration that showed collapse in tubal uterine, ovaries and round ligaments with uterine fundus.



Fig 2: The appearance of the uterus involved with the Huntington procedure: Collapsed part of uterine fundus were kept with two ring forceps, were subjected to slow and gradual traction.

The correction of the inverted uterine structures was attempted by stroking from the isthmus zone. Huntington's procedure was applied when there was no improvement with stroking. Inverted portions were held with two ring forceps, and a stepwise slow traction was applied and corrected as shown in Figure 2. After correction, there was widespread muscle tonus loss in all uterine segments. 10 IU/2 mL dosage oxytocin was administered intravenously. 30 IU oxytocin was infused in 500 cc isotonic, methylergonovine maleat 0.2 mg/ml intramuscular two doses with 15 minutes intervals and Misoprostol 800 µgr rectal was applied. No uterine muscle tone developed despite uterotonic cure. Hysterectomy was planned but the treatment alternatives for the uterus protective surgery approach were considered because the patient's partner insisted on the need for fertility protection. Severe atonia of the uterus was a major risk for recurrence of bleeding and acute inversion. We performed compression suture to prevent both uterine atony and acute puerperal inversion recurrence. Hemorrhage stopped after



Fig 3: Hayman-like suture procedure: two longitudinal sutures were performed on both sides to the uterus and two short sutures were performed between to the long sutures on both sides of the uterus. The compressed uterine tissue became "S" shaped.

suture and the uterus fundus did not collapse again (Figure 3). The schematic drawing of the compression suture applied to the patient is shown in Figure 4. No uterine artery or hypogastric artery ligation was required. Acute necrosis was not observed. A total of four units of erythrocyte suspension and four units of fresh frozen plasma were infused intraoperatively. Preoperative hematocrit and hemoglobin were 35% and 11.9 mg/dl respectively and after transfusion, hematocrit, hemoglobin and platelets was 26%,

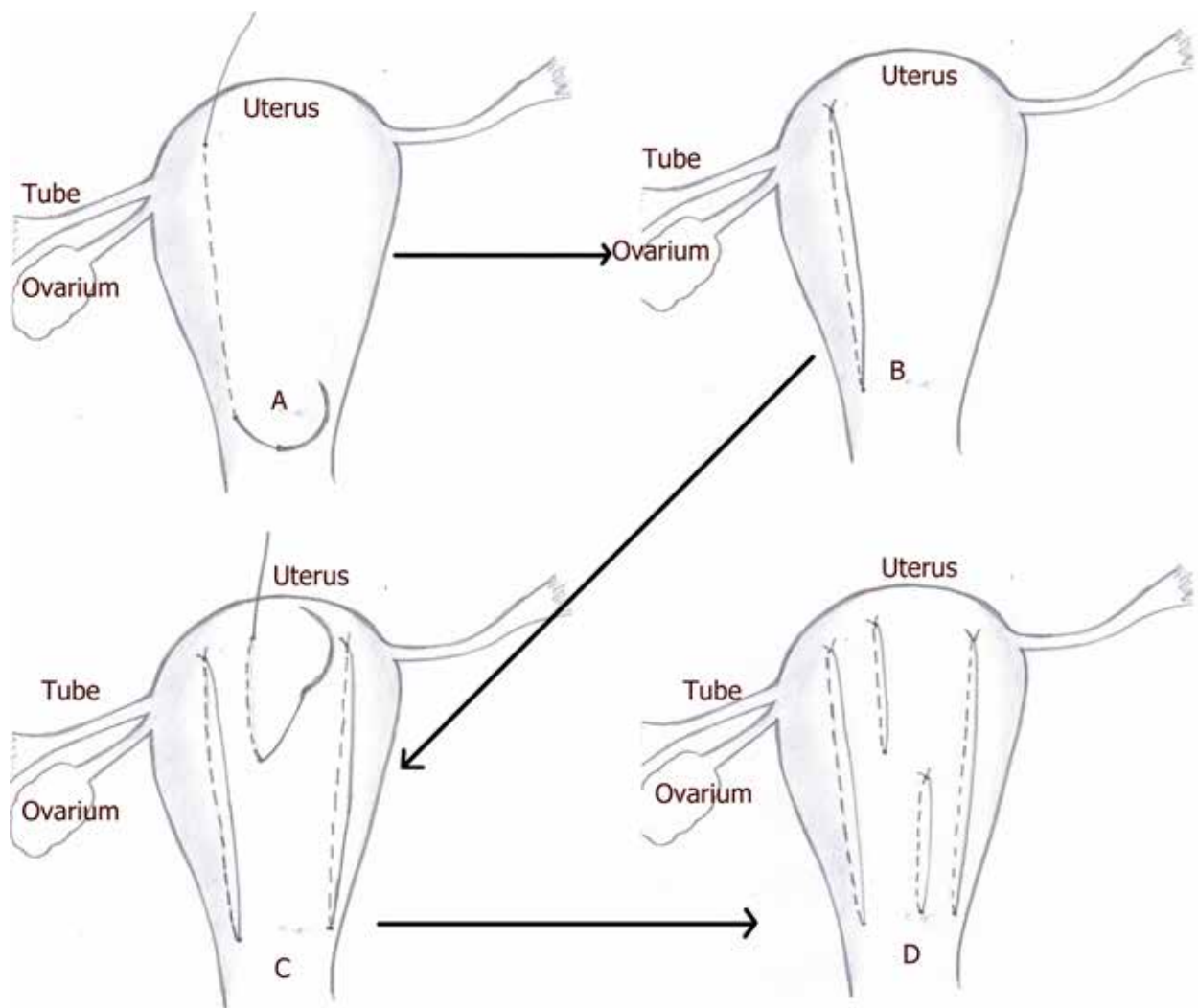


Fig 4: Schematic view of the compression sutures of our case: the uterus was perforated eight times and ligated four knots.1-0 vicryl was used at the compression suture.

8.3 mg/dl and 133000/ml respectively. The patient was followed up for one day in intensive care unit. Due to the risk of uterine necrosis, abdominal pain, systemic fever, white blood cell count and C-reactive protein follow up were performed. There were no signs of uterine necrosis. On the third postoperative day, ultrasonographic examination was performed. Uterine involution was found to be consistent with postpartum third day (Figure 5). Uterine artery color doppler ultrasonography showed normal blood flow (Figure 6). The patient was healed and discharged on postoperative 5th day.

DISCUSSION

Several treatment modalities have been applied in the treatment of uterine inversion. Choosing the treatment that the surgeon is fastest and most experienced in will reduce patient mortality^[6]. Marshall *et al* reported that the patient had cardiac arrest nine minutes after birth due to massive bleeding during an

inversion^[7]. In their case report, Ihama *et al* reported that the patient died 15 minutes after the birth of the baby^[8]. In non-surgical treatment, bakri postpartum balloon can also be used. In the literature, there is a bakri balloon application after inversion correction. Ida *et al* applied a bakri balloon to control recurrence and bleeding after correction of uterine inversion and they achieved treatment success^[9].

The bakri balloon can be applied in cases where the inversion degree is as low as 1-2 and the uterus can be easily reduced. In cases where the inversion cannot be corrected, the bakri balloon cannot be used^[10]. In our case with massive hemorrhage, we had to make a very quick decision at each step, so the laparotomy decision was made when manual correction had no response. A surgical procedure includes preserving of uterus or removal of uterus^[5]. We applied a compression suture as a uterine conservative treatment model.

Compression sutures are surgical procedures applied to protect the uterus in cases of uterine atony



Fig 5: Uterine involution was found to be consistent with postpartum third day. Uterine necrosis was not detected.

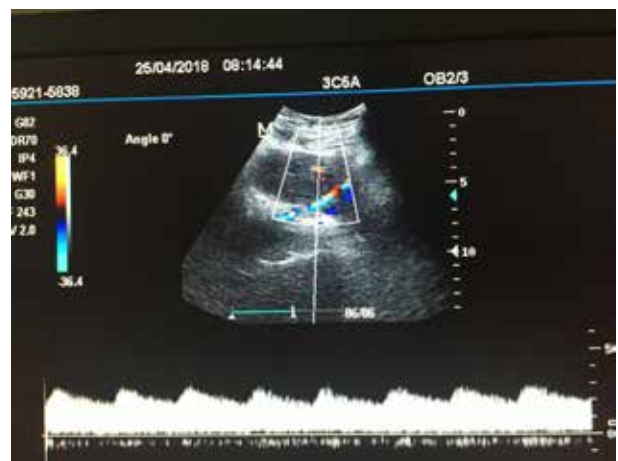


Fig 6: Uterine artery color doppler ultrasonography showed normal blood flow in third day.

and placenta accreta^[11,12]. Matsubara *et al* performed compression sutures to prevent acute recurrence at the time of uterine inversion and they were successful. The compression suture technique they use is matsubara-yano technique. Five suture nodes were attached in this compression suture and the uterus was perforated ten times^[13]. We used a different technique as compression suture in our case. We had fewer knots and fewer uterine perforations. We applied four sutures and the uterus was drilled eight times. In addition, in the technique we applied, the compressed uterine tissue became "S" shaped. Necrosis-protective tissue was maintained (Figure 6). It is necessary to act in proportion to the compressive sutures and vein connections of the uterus. Too many thrown sutures may disrupt uterine blood supply, resulting in uterine necrosis. Cho type uterus compression sutures were applied at postpartum hemorrhage resulting from uterine atony by Benkiranea *et al*. On the third postoperative day, uterine necrosis findings starting with abdominal pain and fever were detected in the patient. The patient had developed sepsis. The necrotized uterus was removed by hysterectomy with relaparotomy^[14]. In addition, increased compression suture number increases the risk of formation of the uterine synechias. Ibrahim *et al* reported that the applied compression suture increased the risk of intrauterine synechias^[15]. Laparotomy should not be avoided in advanced (grade 3-4) uterine inversions since reduction is difficult. Compression sutures should be remembered in cases of laparotomy and uterus-preserving approaches. Compression sutures prevent acute recurrence of the inversion, as well as uterine atony. The compression suture type to be applied depends on the uterine inversion (atonia / placenta accreta) reason and the experience of the surgeon. The aim is to stop the bleeding with lesser

tissue compression and uterus puncture. Compression suture application in uterine inversion cases is available in the literature in a limited number.

CONCLUSION

Compression suture technique in the presented case prevented acute inversion relapse and complications did not occur. A greater number of cases or case series are needed to determine the efficacy of this treatment.

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Talip Karacor: surgical team; Mehmet Can Nacar: manuscript writing; Mehmet Bulbul: manuscript design

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Case Report

Serous cystadenoma of fallopian tube: A case report of a rare pathology presented as an isolated tubal torsion

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ABSTRACT

Serous cystadenoma of the fallopian tube is an extremely rare benign tumour. Here, we report a case of acute abdomen due to an isolated tubal torsion where the tube was hugely dilated and filled with serous fluid. Laparoscopic drainage of

tubal content followed by de-torsion and salpingectomy was performed. Literature search revealed only six cases of this rare pathology.

KEY WORDS: fallopian tube diseases, torsion abnormality

INTRODUCTION

Tumours of fallopian tubes are rare. The World Health Organization classification identified different histological patterns including epithelial, mixed epithelial – mesenchymal, soft tissue, mesothelial, germ cell, trophoblastic, lymphoid and hematopoietic and secondary tumours. These tumours can be benign, borderline and malignant^[1].

The clinical picture of each specific pattern is still not clear. Where most of the benign tumours tend to be asymptomatic, it can rarely present as isolated tubal torsion^[2] Here, we report a case of tubal serous cystadenoma presented with isolated tubal torsion.

CASE REPORT

A 25-year-old nulligravid presented to the emergency department with sudden onset of severe left flank pain associated with nausea. There was no vomiting, diarrhoea, fever or any urinary symptoms. The patient was previously healthy with regular menses without any significant history of dysmenorrhoea. She did not have any sexual activity (virgin). Her vital signs at time of examination were stable. Clinical examination showed left lower

quadrant tenderness on palpation without any costo-vertebral angle tenderness. Blood investigations showed normal white cell count and C-reactive protein. She had normal urine analysis. Urgent computed tomography was performed considering the possibility of having renal calculi. No ureteral calculi were found but instead a left cystic adnexal mass was identified.

Abdominal ultrasound showed 11*9*9 cm simple cyst anterior to the uterus and superior to the bladder. The cyst is arising from left adnexa towards midline. It shows no solid component. The uterus and right ovary looks normal. Colour doppler shows presence of blood flow; however, it is decreased on the left side compared to the right (Figure 1).

Clinical picture was compatible with adnexal torsion. Urgent laparoscopy was performed and showed huge dilated and torted left fallopian tube. An isolated tubal torsion was identified with bluish discoloration. Both ovaries and right fallopian tube appeared normal and healthy looking.

De-torsion was not feasible before drainage of the fluid within the left fallopian tube, so veres needle was inserted suprapubic and around 400 ml of clear fluid

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Fig 1: Sagittal ultrasound view with colored Doppler shows a large midline cystic formation located superior to the urinary bladder. The right ovary is seen and shows regular internal vascularity. The left ovary is compressed by this adnexal cystic structure.

was drained and sent for cytology (Figure 2). Left salpingectomy was performed and t tube was placed in an endobag and removed without spillage. The pathology revealed tubal serous cystadenoma without any evidence of malignancy (Figure 3). The intratubal fluid was acellular and negative for malignancy.

DISCUSSION

Isolated tubal torsion is a rare cause of lower abdominal pain that was firstly described by Bland Sutton in 1890^[3] with a reported incidence of 1 in 1.5 million women in reproductive age group^[4]. The identified aetiologies of this rare entity include intrinsic tubal factors such as congenital anomalies, excessive length of the tube, hydrosalpinx, hemosalpinx and neoplasm. Other extrinsic factors include adhesions, pregnancy, changes in neighbouring organs and pelvic congestion. Also, normal tube was noticed in many cases of isolated tubal torsion^[3]. Adnexal torsion is mainly a clinical diagnosis where acute onset of one-sided severe pain is a key for diagnosis. Imaging can guide the diagnosis especially in case of presence of adnexal masses more than 5 cm (odds ratio 10.6, 95% CI: 2.9-38.8)^[5]. Addition of Doppler flow to look for the characteristic whirlpool sign or the absence of blood flow would be helpful to establish the diagnosis; however, these signs are not always present in torsion cases^[6].

In our case, the tube was largely dilated and it mimics an ovarian cyst with positive Doppler flow. The clinical picture was culprit to diagnose torsion. Preoperatively, tubal disease was not suspected, especially since our patient is virgin and not sexually

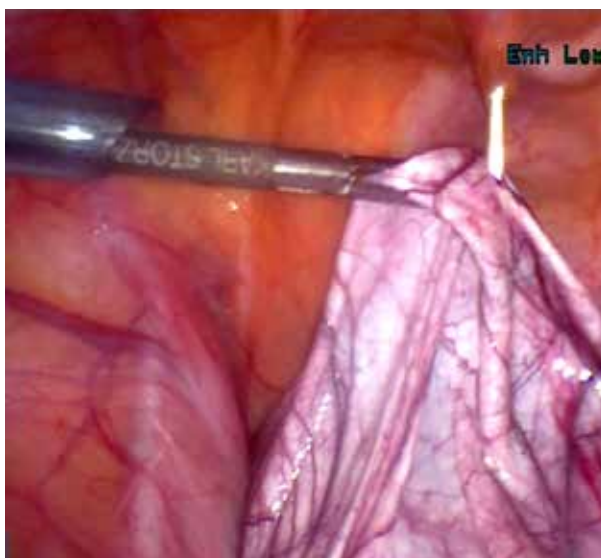


Fig 2: Laparoscopic images show the diseased tube held by grasper and veres needle is inserted inside the tube to drain its fluid content

active at all. So, pelvic inflammatory disease and tubo-ovarian abscess were excluded. The final pathology was serous cystadenoma of the fallopian tube which is an extremely rarely documented pathology presented as a huge adnexal mass.

Medline search revealed six cases of serous cystadenoma in the fallopian tube documented in five case reports (Table 1) and one case identified in

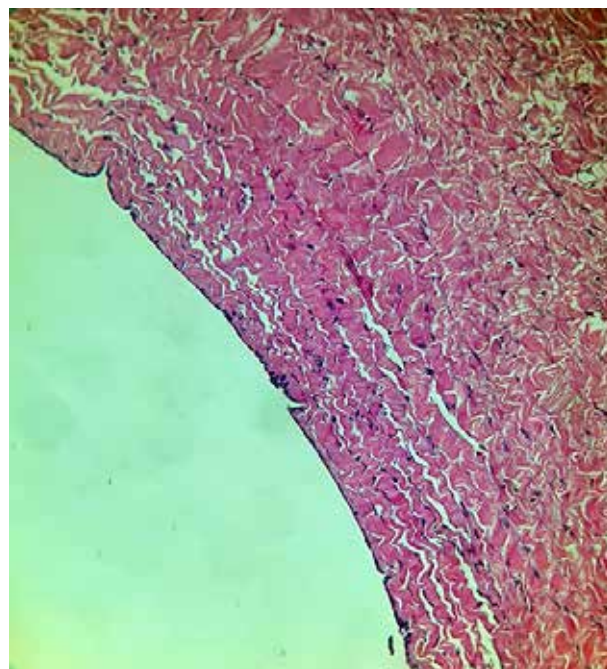


Fig 3: 100X magnification (H&E) Cyst wall lined by a low cuboidal epithelium.

Table 1: Summary of published cases reports.

Author	Year of publication	Age (years)	Clinical findings	Treatment	Site and size
Nagendran <i>et al</i> ^[8]	2010	14	Abdominal pain	Excision of fimbrial mass	25x35x25mm arising from the fimbrial of the right fallopian tube
Werlin <i>et al</i> ^[9]	2010	30	Ruptured ectopic	Laparoscopic salpingectomy	2cm serous cystadenoma
Bika <i>et al</i> ^[10]	2009	39	Asymptomatic	Excision of ampullary cyst	20*30 mm ampullary cyst, well circumscribed and simple looking.
Lee <i>et al</i> ^[11]	2001	28	Abdominal pain	Laparoscopic salpingectomy	Tubal torsion and necrosis
Janovski <i>et al</i> ^[12]	1963	42	Asymptomatic	Bilateral salpingo-oophorectomy (history of breast cancer)	3.8 * 2cm cystic structure at mid portion of right tube.

analysing series of 20 cases of tumours of the fimbriated end of the fallopian tube^[7]. In our case, the full tube was dilated mimicking the appearance of a large ovarian cyst of 11 cm. In previous cases, the tubal cystadenoma was <5 cm in size. This hugely dilated tube elicited the acute pain upon torsion. Our patient denied any episodes of previous pelvic heaviness or pain.

Tubal diseases are rare entities that should be considered preoperatively while counselling the patient where salpingectomy would be the definitive treatment as in our case.

CONCLUSION

Isolated tubal torsion on top of rare pathology is a rare cause of acute abdomen that should be considered preoperatively where salpingectomy is ultimate.

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Case Report

Langerhans cell histiocytosis: diagnosis on thyroid aspirate and effective treatment with systemic chemotherapy

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ABSTRACT

Langerhans cell histiocytosis (LCH) is a disease with monoclonal proliferation and infiltration of organs by Langerhans cells. LCH is commonly seen in the skeletal system and skin, but it may also involve parenchymal organs. Thyroid involvement in LCH is an unusual presentation. The treatment of LCH of the thyroid remains unclear because of

insufficient data. Here, we present a patient with LCH who had thyroid involvement. She had a lesion in the thyroid, which was detected coincidentally. The diagnosis was made by thyroid aspiration. The patient was treated with systemic chemotherapy and after therapy, the lesion disappeared completely and the patient became euthyroid.

KEY WORDS: chemotherapy, langerhans cell histiocytosis, thyroid, thyroid aspiration

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a disease characterized by monoclonal proliferation and infiltration of organs by Langerhans cells, which involves commonly the skeletal system and skin, but may also affect parenchymal organs^[1,2]. It may involve a single system or may present as a multisystem disease^[1]. It is mostly seen in children and the incidence of LCH is reported to be 3-5 per million^[3]. Thyroid involvement is extremely rare and management of such a rare situation is controversial^[4]. Surgery, chemotherapy and radiotherapy are treatment options for LCH involving the thyroid, but the definitive management is still a controversy because of the lack of prospective randomized studies and the low number of patients^[5,6]. Here, we present a case of LCH with lung and thyroid involvement, whose diagnosis was made with thyroid aspiration biopsy and whose lesion of the thyroid completely disappeared after chemotherapy.

CASE REPORT

A 26-year-old woman who had LCH of the lung and pituitary gland for four years was referred to our clinic for thyroid nodules detected on thorax computerized tomography. The thorax computerized tomography showed numerous cystic lesions in both lungs and thyroid nodules in the left lobe of the thyroid. At admission, she had no symptoms including symptoms of hypo or hyperthyroidism. On physical examination, no goiter or other pathological findings were detected. Thyroid function showed subclinical hypothyroidism based on the following hormone levels: free triiodothyronine: 2.4 pg/mL; free thyroxine: 0.97 ng/dL and thyroid-stimulating hormone: 12.30 mIU/L; antithyroid antibodies were negative. Complete hemogram, liver and renal parameters were normal. On thyroid ultrasonography (USG), the right lobe measured 11x10x40 mm and the left lobe measured 10.7x13x40 mm. On the left lobe, a 5x6x14 mm hypoechoic area was detected (Fig 1). Fine needle

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Fig 1: Transverse (Fig 1a) and longitudinal (Fig 1b) gray-scale ultrasound demonstrates a hypoechoic lesion in the left lobe of the thyroid.

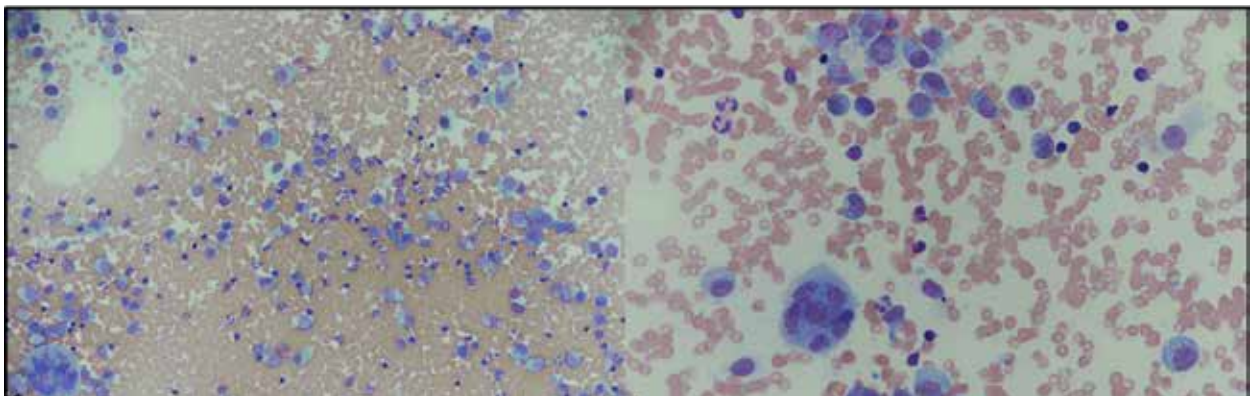


Fig 2: Mononuclear and multinuclear cells with scattered eosinophils. [MGG stained air-dried smear, x200 (Fig 2a) and MGG stained air-dried smear, x400 (Fig 2b)]

aspiration was performed from this area. For cytological examination, four air-dried smears were prepared from the aspiration material. The May-Grunwald-Giemsa-stained smear slides showed mononuclear and multinuclear cells with bean shaped nucleus with nuclear grooves and pale eosinophilic cytoplasm (Fig 2). The background consisted of scattered eosinophils, but no colloid or thyroid follicular cells were seen (Fig 2). Additional slides were

prepared from material by ThinPrep® 2000 Automated Slide Technique. Although it was hypocellular, the Pap stained ThinPrep slide showed some mononuclear and multinuclear cells. On the other slides prepared for immunohistochemical staining, the cells showed positive staining with S-100, CD68 and CD1a (Fig 3). With these findings, the precise diagnosis was LCH involvement of the thyroid. Abdominal USG, whole-body bone scan and bone marrow aspiration were

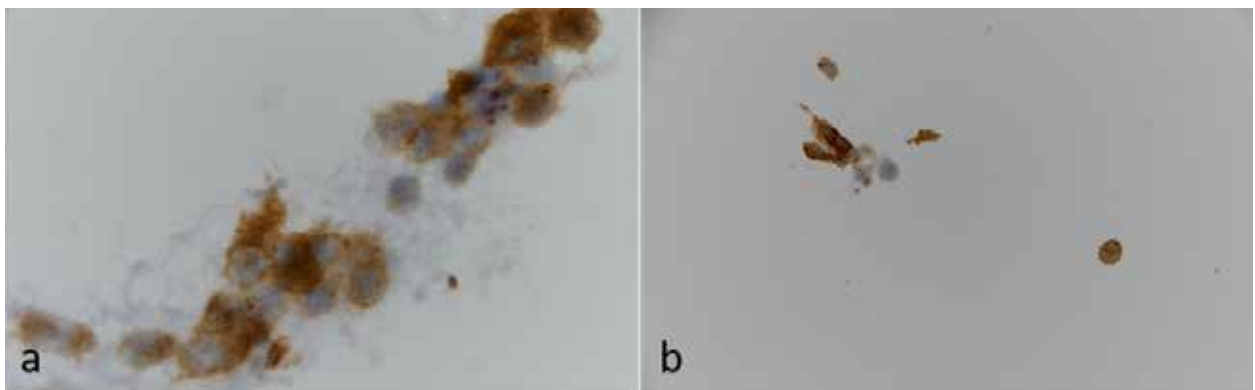


Fig 3: Cells showing positive immunoreactivity for S100 (Fig 3a: Thin-Prep, x1000) and CD1a (Fig 3b: Thin-Prep, x400).

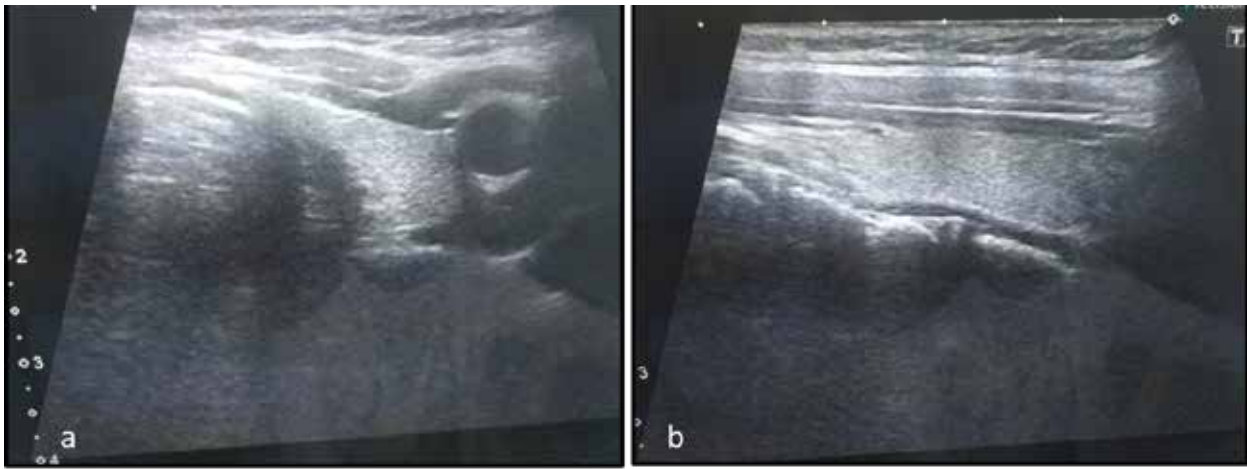


Fig 4: Transverse (Fig 1a) and longitudinal (Fig 1b) gray-scale ultrasound images of the left lobe of the thyroid after chemotherapy. No lesion was detected on both images.

within normal findings. Levothyroxine was prescribed and the thyroid function returned to normal. The patient was discussed in the tumor board and chemotherapy with vinblastine and 6-mercaptopurine, and oral prednisolone were administered to the patient. After six months of chemotherapy, a second thyroid USG was performed and it was detected that the aforementioned hypoechoic area had disappeared (Fig 4). After we have found that the lesion disappeared, the thyroid replacement therapy was stopped and at follow-up the patient remained euthyroid.

DISCUSSION

LCH is a rare neoplastic disorder which is mostly seen in children^[1,3]. The symptoms and clinical findings depend on the involved organs and systems. LCH involvement of the thyroid is seen very rarely and just several case reports and retrospective studies are present in the literature^[4-9]. Patten *et al* showed in their review that 75 cases were present in the literature^[4]. Among these patients, fine needle aspiration was sufficient for the diagnosis in six patients, while the other patients needed core biopsy or thyroidectomy^[10-15]. Most of the cases with thyroid involvement of LCH have goitre and are euthyroid^[4], whereas our case had no goitre but presented with thyroid lesion, and she had subclinical hypothyroidism at presentation. After this aforementioned review, several case reports were reported, and in our English literature review, 29 cases in 25 articles were found^[5-9,16-35] (Table 1). Among these cases, six patients' diagnosis was made with only fine needle aspiration^[17-21]. In most cases, fine needle aspiration was not sufficient for diagnosis, but may be helpful in accurately diagnosing LCH in a minority of patients. The diagnosis of LCH of the thyroid is challenging, and it could be misinterpreted as a benign disorder or as carcinoma or lymphoma^[5,7]. Another reason for difficult diagnosis is the rarity of the disease.

The diagnosis of our case was made with thyroid fine needle aspiration and immunohistochemical investigation. The advantage of our diagnosis was that our patient was formerly under control for LCH with lung and pituitary gland involvement.

Surgery, chemotherapy and radiotherapy are treatment options for LCH of the thyroid^[4]. Most of the patients reported in the literature received thyroidectomy, but chemotherapy alone is an alternative therapy^[4,9,17,34]. For patients with multi-system LCH, chemotherapy may be the treatment of choice^[9]. With this knowledge, we chose to give systemic chemotherapy to our patient. The effect of chemotherapy on thyroid lesion of LCH is not well documented before. It was demonstrated that chemotherapy may shrink the thyroid in a patient with goitre^[9]. In our case, we showed with USG that chemotherapy may completely remove the lesion from the thyroid. Furthermore, after chemotherapy, the requirement of thyroid replacement therapy also disappeared. With these findings, we can advise systemic chemotherapy to patients with LCH of the thyroid to preserve the thyroid functions. In our opinion, patients with thyroid involvement of the LCH, who have no evidence of Hashimoto's thyroiditis with no overt hypothyroidism, may be appropriate candidates for systemic chemotherapy because normal thyroid function may be achieved in these patients and thyroidectomy may be an excessive therapy modality.

CONCLUSION

In this report, we presented a case with multisystemic LCH with thyroid involvement. The diagnosis was made by thyroid aspiration. She received systemic chemotherapy and with treatment, the lesion in the thyroid resolved and the thyroid functions returned to normal. Our case showed that systemic chemotherapy is an effective treatment for patients

Table 1: Recently reported cases of Langerhans cell histiocytosis of the thyroid gland in the English literature from 2011 to date.

Age/gender	Clinical features	Diagnosis	Treatment	Author
40/M	NM	Surgery	Surgery and adjuvant chemotherapy	Wu <i>et al</i> ^[5]
8/M	Euthyroid	Biopsy	Chemotherapy alone	Attakkil <i>et al</i> ^[6]
45/M	Subclinical hypothyroidism	Surgery	Surgery	Pandayaraj <i>et al</i> ^[7]
27/M	Euthyroid	Thyroid FNAC and vertebra biopsy	Chemotherapy and autologous bone marrow stem cell transplantation	Cai <i>et al</i> ^[8]
18/M	Hypothyroidism	Neck lymph node biopsy	Chemotherapy alone	Xia <i>et al</i> ^[9]
45/F	NM	18 F-FDG PET/CT-guided bone biopsy	Chemotherapy (subtotal thyroidectomy was performed before the definite diagnosis)	Malik <i>et al</i> ^[16]
6/F	Euthyroid	FNAC and biopsy	Prednisolone	Oza <i>et al</i> ^[17]
38/M	Euthyroid	FNAC alone	Chemotherapy	
9/M	Euthyroid	FNAC and biopsy	Prednisolone	
33/M	Hypothyroidism	FNAC	Chemotherapy	Marupidi <i>et al</i> ^[18]
54/F	Primary and secondary hypothyroidism	FNAC	Chemotherapy	Sangtian <i>et al</i> ^[19]
27/M	Euthyroid	FNAC	Surgery	Chen <i>et al</i> ^[20]
38/F	Euthyroid	FNAC	Surgery	
13/M	Euthyroid	FNAC	Chemotherapy	Roy <i>et al</i> ^[21]
41/M	Euthyroid	Surgery	Surgery, radiotherapy and interleukin-2 therapy	Xie <i>et al</i> ^[22]
36/M	Euthyroid	Surgery	Surgery	Gul <i>et al</i> ^[23]
27/F	Hypothyroidism	Surgery	Surgery, adjuvant chemotherapy and radiotherapy	AlZahrani <i>et al</i> ^[24]
22/F	NM	Re-evaluation of thyroidectomy material	Prednisolone	Gordon <i>et al</i> ^[25]
39/F	Central hypothyroidism	Surgery	Surgery	Skowronska-Jozwiak <i>et al</i> ^[26]
35/M	Euthyroid	Needle biopsy	NM	Long <i>et al</i> ^[27]
26/f	Hyperthyroidism	Surgery	NM	Chrisoulidou <i>et al</i> ^[28]
35/F	Central hypothyroidism	FNAC and incisional biopsy	Chemotherapy	Saqi <i>et al</i> ^[29]
37/M	NM	Surgery	Surgery	Ceyran <i>et al</i> ^[30]
52/F	NM	Surgery	Surgery, adjuvant chemotherapy and radiotherapy	Bucan <i>et al</i> ^[31]
25/F	Euthyroid	Surgery	Surgery and chemotherapy	Pusztaszeri <i>et al</i> ^[32]
38/F	Euthyroid	Surgery	NM	
3/M	Euthyroid	Surgery	Surgery and chemotherapy	Tajik <i>et al</i> ^[33]
15/F	Central hypothyroidism	Thyroid biopsy	Chemotherapy	Baş <i>et al</i> ^[34]
37/M	NM	Bone surgery, thyroid FNAC re-evaluation after bone surgery	The patient denied therapy	Mohd Ariff <i>et al</i> ^[35]

F: female, FNAC: fine needle aspiration cytology, M: male, NM: not mentioned

with LCH with thyroid involvement and may be preferred to thyroidectomy in selected patient.

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Author Contributions: Derya Köseoğlu did the conception, data collection and literature review, and took responsibility in writing the manuscript. Behice Hande Erenler did data collection and took responsibility in writing the manuscript. Ferit Kerim Küçükler took responsibility in design, literature search and reviewed the article before submission.

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Case Report

An unexpected sign on mammography: Air hole

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ABSTRACT

Air density is not a documented entity in the pertinent literature on mammograms. As a general rule, air on X-ray which is superposed on soft tissues is always associated with penetrated injuries of the involved skin, with laceration of the underlying soft tissues or gas caused

by infections with anaerobic organisms. We present an unusual patient with an air hole on superior aspect of the left breast on mediolateral oblique graph as a sign of deep skin ulceration, who is addicted to heroin and injected the substance on her breast.

KEY WORDS: air, breast, heroin, mammography

INTRODUCTION

Air density superposed on breast tissue on mammography has never been reported before. As a well-known X-ray rule, intensity of radiographic air is usually a cause of exogenous air or infection of soft tissues. We report a case with air density on mammogram as a sign of a skin ulceration of a young woman who is addicted to heroin and chooses her left breast to inject the illicit drug.

CASE REPORT

A 39-year-old woman presented with painful skin ulceration on her superolateral breast. Detailed anamnesis showed that this lesion has been noted for two weeks after injection of heroin. Lesion was aggravated in the last three days prior to admission. She was an intravenous drug abuser for the previous 15 years. Hepatitis profile revealed a positive anti-HCV antibody. Physical examination of breast showed no apparent abnormality other than a 4x3 cm size indurated skin ulceration with purulent material into the cavity (Fig 1). The ulcer was about 1.5 cm deep from the skin surface. Skin surrounding the wound was red and painful. Mammogram of the left breast

demonstrated a 17x10 mm air density with irregular contours on superior aspect of the breast (Fig. 2). Breast ultrasound showed no additional pathology under the ulcerated skin, but axillary ultrasound demonstrated multiple hyper vascular lymphadenopathies (Fig. 3). Patient was directed for further clinical evaluation and treatment to the general surgery department, but she left the hospital. Subsequently, she was difficult to contact.

DISCUSSION

For heroin users, the first injections often begin in the arm for intravenous access. After many years of addiction, they shift to using other sites and many authors mentioned that they can try lots of different body regions for injection^[1-4] and high rates of local complications occurs including tissue damage and infections^[5,6]. Alvi *et al*^[7] has reported breast injection of heroin for the first time in the literature in a pregnant woman who developed skin ulceration. While other reports showed some kind of breast problems such as gynecomastia^[8] and sterile abscess^[9], a skin ulceration is the second time reported on the literature by our report. In our opinion, this ulcer cavity resulted

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Fig 1: Partially triangular shaped ulcer on the left breast at radius 12 with dimensions 4x3 cm, demarcated from the surroundings breast skin with irregular margins, peri ulcerous erythema. The bed of the ulcer appears deep and purulent.

because of subcutaneous injection of the drug other than venous access or an unknown substance that is mixed with the heroin.

On mammogram, air density is not a specific finding and not easy to identify for the first time when an imaging expert or clinician sees it. As a general rule, air on X-ray which is superposed on soft tissue is always associated with injuries in which the skin is penetrated, with laceration of the underlying soft tissues or gas caused by infections with anaerobic organisms. Our patient has a deep ulceration which destroyed the skin and caused this air hole on mammogram. On ultrasound, there was no other pathologic imaging finding under the ulceration or the rest of the breast. Axillary lymphadenopathy was a cause of drainage of the infected tissue that an hypervascularization led us to think like this.

CONCLUSION

Injection of heroin on breast which is finalized with infected skin ulceration is a rare phenomenon. In our opinion, non-arm injection is intriguing because it is associated with high rates of local complications, including tissue damage and infections. Through this case report, we call for the attention of clinicians and radiologists to be aware of uncommon injection sites

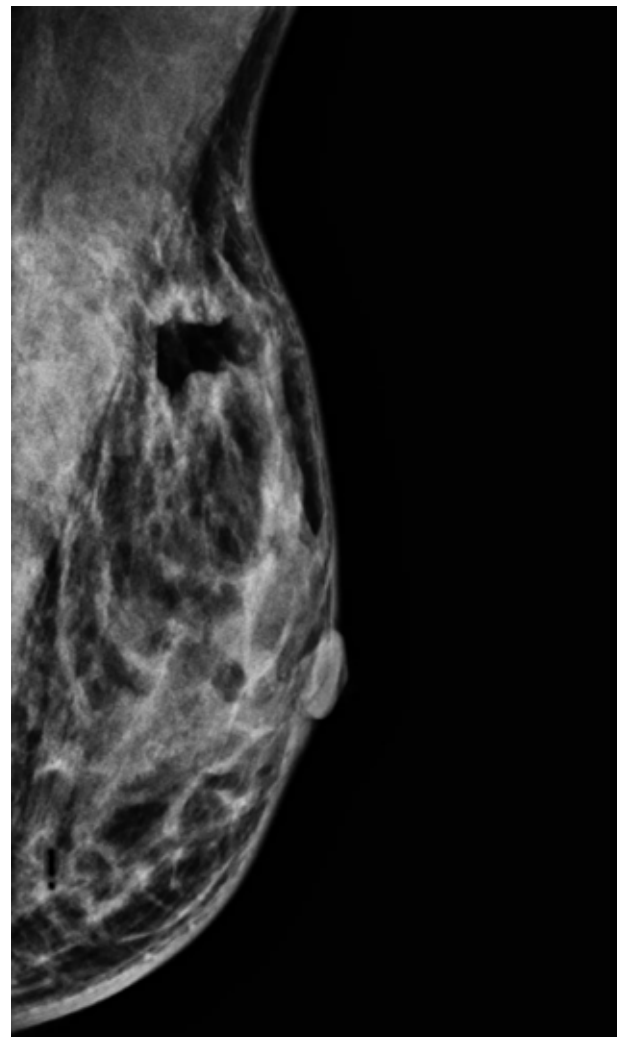


Fig 2: Mediolateral oblique mammogram. An air density in the left superior I quadrant is seen (black arrows).

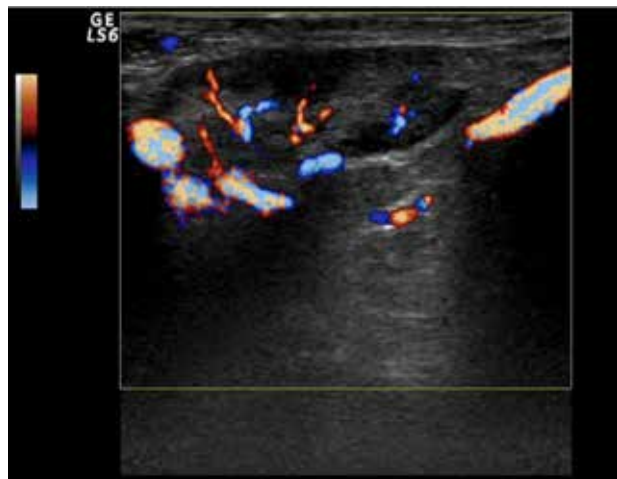


Fig 3: Longitudinal sonogram of inflamed axillary lymph node. The color Doppler ultrasound examination shows vessel segments distributed around the lymph node and in the hilum area.

to make clear the diagnosis, as some patients would be prone to hide their addiction.

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Case Report

Pseudohyperkalemia in a neonate with hyperleukocytosis due to transient myeloproliferative disorder

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ABSTRACT

Pseudohyperkalemia (PHK) needs to be considered in cases where hyperkalemia is associated with normal renal function and normal electrocardiogram. We describe PHK in a twelve-day-old neonate with hyperleukocytosis due to transient myeloproliferative disorder. Serum biochemical test showed high potassium of 10.1 mmol/L with normal renal function. A 12-lead electrocardiogram was normal.

Potassium level was high in serum but was normal or low in plasma and whole blood samples. Pediatricians who manage patients with hemato-oncological diseases manifesting with extreme leukocytosis or thrombocytosis should measure potassium in plasma (lithium heparin tube) and whole blood samples.

KEY WORDS: hyperkalemia, hyperleukocytosis, pseudohyperkalemia

INTRODUCTION

Hyperkalemia is one of the most alarming electrolyte abnormalities because of the potential for lethal arrhythmias and it requires immediate intervention. When a patient does not manifest signs of hyperkalemia such as abnormal electrocardiogram (ECG) and has normal renal function, pseudohyperkalemia (PHK) should be suspected, particularly when the patient has thrombocytosis and/or hyperleukocytosis. PHK, defined as marked elevation of serum potassium concentration (>0.4 mmol/L) compared to plasma, is common in children and it results from inappropriate blood collection techniques, hemolysis, thrombocytosis, leukocytosis and/or hematological malignancies^[1]. Early recognition of PHK is important to prevent unnecessary treatment that may lead to hypokalemia. This case report of a twelve-day-old neonate with hyperleukocytosis emphasizes early recognition of PHK.

CASE REPORT

A twelve-day-old baby girl, fourth born to a 39-year-old mother, with average birth weight, normal APGAR score and with phenotype of Down's syndrome was

admitted in our Pediatric Hemato-Oncology unit as a probable case of transient myeloproliferative disorder. Complete blood count showed white blood cell count (WBC) of $156 \times 10^9/L$, 70% blasts, hemoglobin 77g/L and platelet count $560 \times 10^9/L$. Ultrasound abdomen showed hepatosplenomegaly. Bone marrow examination was reported as acute myeloid leukemia. Pediatric hematologist considered the diagnosis of transient myeloproliferative disorder and planned to start low dose cytarabine. As baby developed respiratory distress, baby was transferred to our Pediatric Intensive Care Unit (PICU).

On admission to PICU, baby was awake, fairly active, well hydrated, hemodynamically stable, temperature 37.1 °C, with mild respiratory distress maintaining good oxygen saturation. Complete blood count showed WBC $154.49 \times 10^9/L$, blasts 74%, hemoglobin 72 g/L and platelet $699 \times 10^9/L$. Serum biochemical profile done in PICU showed normal renal function test (blood urea: 1.5 mmol/L, creatinine: 35 μ mol/L), sodium: 139 mmol/L, potassium: 10.1 mmol/L, normal phosphorus: 1.06 mmol/L and normal uric acid: 123 μ mol/L. Sample was taken by messenger, which is the usual mode of transport of samples in our hospital. ECG was normal.

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Table 1: Potassium results of specimen handled by different methods

Sample Day	Specimen	Bottle	Potassium Level (mmol/L)
On admission	Arterial	Heparinised	4.4
On admission	Arterial	Serum	10.1
Day 1	Venous (Peripheral)	Heparinised	3.1
Simultaneous samples	Venous (Peripheral)	Serum	9.2
Day 2	Arterial	Heparinised	3.2
Simultaneous samples	Arterial	Serum	8.0
Day 2	Venous (Central Line)	Heparinised	2.6
Simultaneous samples	Venous (Central Line)	Plasma	2.7
	Venous (Central Line)	Serum	6.5

Urine output was normal. As tumour lysis syndrome was expected with hyperleukocytosis, baby received hyperkalemia treatment with 10% calcium gluconate and dextrose plus insulin infusion. Arterial blood gas analysis (BGA) was done using whole blood in blood gas machine with potassium electrode (ISTAT), in which potassium was 4.4 mmol/L. Serum biochemical profile was repeated in venous sample which was taken with precautions to avoid squeezing or tourniquet application. Sample was taken immediately by messenger to lab and lab was requested to process the sample immediately. Using the same venous sample, BGA was done in whole blood in blood gas machine with potassium electrode (ISTAT). Serum potassium was 9.2 mmol/L, whereas potassium in BGA was only 3.1 mmol/L. Repeated serum biochemical profile showed normal renal function test. ECG repeated was normal. Maintenance intravenous fluid was free of potassium. After insertion of central venous line, central venous blood sample was collected in two tubes: 1. plain tube for serum; and 2. lithium heparin tube for plasma. Sample was taken immediately by messenger to lab and lab was requested to process the sample immediately. Using the same venous sample, BGA was done using whole blood in blood gas machine with potassium electrode (ISTAT). Serum potassium was 6.5 mmol/L, plasma potassium was 2.7 mmol/L and potassium measured in whole blood using BGA machine was 2.6 mmol/L. The results are summarized in the Table 1. We came to a conclusion that it was PHK in serum due to hyperleukocytosis. With added potassium in intravenous fluid, plasma potassium normalized. Until baby was discharged, plasma samples were used for blood biochemical analysis.

DISCUSSION

Severe hyperkalemia is a potentially life-threatening condition requiring immediate medical intervention. PHK is very common in children because of difficulties in obtaining blood specimen. It is suspected when the laboratory value of the measured potassium is high, but the patient doesn't manifest signs of hyperkalemia, such as abnormal ECG. Potassium is predominantly

intracellular due to the active Na^+/K^+ ATP pump in cell membrane. Potassium is usually measured in serum or plasma (lithium heparin) or heparin anticoagulated whole blood. The serum potassium is normally 0.36 ± 0.18 mmol/L higher than the plasma and whole blood value, secondary to potassium release from cells during clotting.

PHK has been defined by some authors as a difference between serum and plasma potassium concentrations of more than 0.4 mmol/L, when samples remain at room temperature and are tested within an hour of collection^[1]. The need for rapid accurate measurement of potassium in critically ill patients has led to the use of blood gas analyzers and point of care testing using whole blood. Some have found stat measurements using whole blood to be comparable to laboratory analyzers using serum/plasma^[2,3].

Constituents of blood (red blood cells, WBC, platelets) and skeletal muscle release intracellular potassium either due to faulty collection techniques or disease states^[4].

Factors that lead to PHK can simply be grouped as those that operate during collection of blood sample or after collection until analysis in laboratory. Preexisting pathological conditions of the patient are to be considered before collection of samples. They include thrombocytosis, leukocytosis, post-splenectomy state and rarely, familial PHK. Thrombocytosis results in increased release of potassium during clotting process. Thrombocytopenia related false elevation of potassium was studied systematically by Graber *et al* and Thurlow *et al* in in-patient cohorts of 444 and 300, respectively^[5,6]. Both groups concluded that blood platelet counts of $>500 \times 10^9$ cell/L significantly altered the measured serum, but not plasma potassium concentrates. For every 100,000/cu.mm increase in platelet count, the serum potassium level rose by approximately 0.15mEq/L^[7].

This phenomenon also occurs with marked WBC count elevations sometimes seen with leukemia. Elevated WBC counts, typically $>200,000$ /cu.mm, can cause a dramatic elevation in serum potassium concentration^[7].

Ranjitkar *et al* conducted a retrospective analysis to derive leukocyte and platelet thresholds of which patients are at high risk for PHK. They concluded a platelet of $>500 \times 10^9$ and a leukocyte count of $50 \times 10^9/L$ are appropriate for indicating a high risk of PHK^[8].

At high levels of leukocytosis, there is increased consumption and thereby exhaustion of metabolites that fuel the sodium-potassium ATP pump and it leads to leakage of potassium into serum.

Post splenectomy thrombocytosis has also been reported to be associated with PHK^[9,10]. Familial PHK is a rare autosomal dominant genetic condition, associated with excessive leakage of potassium across red blood cell membranes^[11].

During collection of blood sample, conditions that induce in vitro hemolysis include tourniquet application for prolonged periods of more than one minute, fist clenching during phlebotomy, traumatic venipuncture or probing and excessive force with syringe draws either during aspiration or transfer^[12,13].

Ethanol containing antiseptics, if not allowed to dry before venipuncture, can enter the blood stream and disrupt cell membranes, causing PHK^[12].

PHK seen in WBC neoplasms are most probably due to increased membrane fragility and little reserve capacity for withstanding mechanical agitation or by leakage into the serum^[14].

The mode of transport of blood sample to the lab also plays a role in PHK, particularly in children with preexisting pathological conditions mentioned above.

The abnormal fragility of neoplastic leukocytes makes them susceptible to mechanical stress. Kellerman and Thornberry reported PHK due to pneumatic tube transport in a leukaemic patient. Ku *et al* reported PHK caused by pneumatic tube transport of blood specimen from a 10-year-old boy with acute leukemia^[15].

Dickinson H *et al* reported PHK caused by pneumatic tube transport of blood specimen from a 12-year-old boy with acute leukaemia^[16].

Dastyh *et al* studied the effect of tube type used for primary sample collection and the manner of transport prior to assessment on the degree of PHK in leukemic patients. They concluded that manual transport of non-coagulable blood (plasma L-Heparin without separator gel) to the laboratory results in the least possible artificial increase in potassium concentration in the sample^[17].

A delay in separation of cells from serum or plasma eventually causes a misleading increase in potassium. Cold storage of whole blood samples before separation will inhibit glycolysis and the energy dependent Na^+ , K^+ , ATPase will not maintain the transcellular potassium gradient leading to false increase in potassium. Recommended temperature for specimen storage prior to testing is 15-25 °C.

In our patient, the possibility of PHK was suspected as the ECG did not show signs of hyperkalemia and all the values of potassium measured in whole blood using the blood gas analyzer with potassium electrode were normal or below normal, contrary to the very high paired serum samples. Treatment was commenced initially as the patient was at risk of tumour lysis syndrome and as soon as PHK was confirmed, treatment of hyperkalemia was discontinued.

PHK was confirmed when the paired serum, plasma and whole blood samples showed elevated potassium in serum and normal/ lower than normal level of potassium in plasma and whole blood samples. So, in our case, the PHK masked the actual hypokalemia and addition of potassium in maintenance intravenous fluids normalized the plasma potassium level.

CONCLUSION

In conclusion, the clinician managing patients with acute leukemia should be aware of the potential causes of PHK and care should be taken in collecting and handling blood samples. We recommend that in patients with acute leukemia / hyperleukocytosis / thrombocytosis, potassium measurement should be made on a lithium heparin plasma sample, which should immediately be transported manually to the laboratory and the laboratory should be notified to process the samples immediately. In services where point of care/blood gas analyzer with potassium electrode is available in the unit, the same should be utilized to measure potassium in whole blood.

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Author contribution: Sankararaman Nagarajan was the major contributor in writing and reviewing the manuscript, literature review, data collection and was involved with the case. Mona Al Ajmi and Najeeb Al Othman were involved in supervision and reviewing the manuscript. All the authors take the responsibility to approve the final version of the article before publication.

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Case Report

First report of t(1;15)(q21;q11.2) and t(1;21)(q21;q11.2) anomalies in Burkitt Lymphoma

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ABSTRACT

Burkitt lymphoma (BL) is a highly aggressive B cell neoplasm characterized by t(8;14)(q24;q32) which involves the MYC gene. Sometimes, patients have additional cytogenetic anomalies beside t(8;14)(q24;q32) and these anomalies usually lead to more aggressive phenotype. The aim of this paper is to report two novel karyotypic abnormalities which give rise to tetrasomy 1q with an aggressive clinical course of BL.

We present a 41-year-old woman with BL. In this patient, the t(1;15)(q21;q11) and t(1;21)(q21;q11.2) were found in the complex karyotype with the translocation of t(8;14)(q24;q32), which is a characteristic of BL. The t(1;15)(q21;q11.2) and

t(1;21)(q21;q11.2) anomalies were reported for the first time according to the databases that we have investigated. In our case, the result of t(1;15)(q21;q11.2) and t(1;21)(q21;q11.2) was tetrasomy of chromosomes of 1q. Structural anomalies of chromosomes 1q could be seen in BL patients as additional cytogenetic anomalies and gain of chromosome of 1q usually associated with disease recurrence and poor prognosis. In our case, the patient died approximately 8 months after diagnosis, so her prognosis was poor, which was consistent with the literature. The candidate genes on chromosomes 1q which could be involved in tumorigenesis remain to be identified.

KEY WORDS: cytogenetic, rare chromosomal anomalies, rare translocations

INTRODUCTION

Burkitt lymphoma (BL) is a highly aggressive B cell neoplasm characterized by t(8;14)(q24;q32) which involves the MYC gene^[1].

Sometimes, patients have additional cytogenetic anomalies beside t(8;14)(q24;q32), and these anomalies usually lead to more aggressive phenotype^[2]. The aim of this paper is to report two novel karyotypic abnormalities, which give rise to tetrasomy 1q with an aggressive clinical course of BL.

CASE REPORT

A 41-year-old woman who was a hepatitis B carrier had applied to outer center due to weakness, inappetence, fatigue, arm, leg and back swelter, night sweats and fever complications. Patient had been suggested to apply to a superior service because of the abnormal blood parameters and peripheral smear. The patient then applied to our clinic. Blood parameters

were as follows: hemoglobin 14.2 gr/dL, leukocyte $16.2 \times 10^3/\mu\text{L}$, lymphocyte $4.9 \times 10^3/\mu\text{L}$, neutrophil $6.9 \times 10^3/\mu\text{L}$ and thrombocyte $19 \times 10^3/\mu\text{L}$. Blast ratio was 30% in peripheral smear. LDH and beta-2 microglobulin of the patient were 8255 U/L and 2191 mg/dL, respectively. She was hospitalized. The patient's bone marrow aspiration in which cellularity was 90% and with diffuse atypic lymphoid cell infiltration was consistent with BL. Cytogenetic analysis was done from bone marrow sample and the karyotype of the patient was found as; 46,XX,der(15)t(1;15)(q21;q11.2),der(21)t(1;21)(q21;q11.2),t(8;14)(q24;q32) [20] (Figure 1).

After one dose of R-EPOCH chemotherapy, control bone marrow was determined as normocellular. Since blastic cells were present in the lumbar puncture of the patient, intrathecal methotrexate and radiotherapy towards central nervous system were given. While cytological examination of cerebrospinal fluid was

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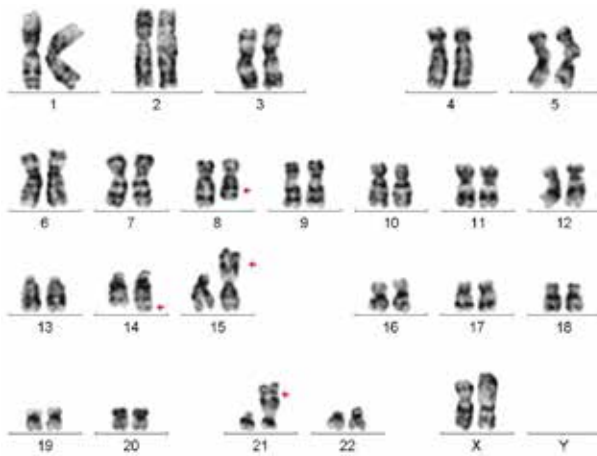


Fig 1: 46,XX,der(15)t(1;15)(q21;q11.2),der(21)t(1;21)(q21;q11.2),t(8;14)(q24;q32)

clear until the 6th dose of chemotherapy and blastic cells were seen in the lumbar puncture before the 7th dose of chemotherapy. Intrathecal treatment was continued. The patient who received 7 doses of chemotherapy in total was planned to receive allogeneic bone marrow transplantation and hospitalized in bone marrow transplantation service. Due to impaired renal function, colymycin dose was decreased to 2x100 mg, and then to 2x75mg. Rituximab thiotepa busulfan cyclophosphamide-allogeneic KIT protocol and defibrotide were initiated. Allogeneic stem cell transplantation was done from her daughter with 100% HLA compatibility. Patient consulted for cardiology due to tachycardia and chest pain and dose reduction of Beloc and albumin replacement for hypervolemia were suggested. Teikoplanin was started with the suggestion of the infection department. The patient was then dialyzed due to hypervolemia. The next morning, the blurring of consciousness developed. The patient whose pH was 7.23 and CO₂ of 43 was intubated. The patient, who was hypotensive, was taken to the intensive care unit due to the preliminary diagnosis of sepsis. Two units of erythrocyte suspension was given to her for support and two units of apheresis were done to maintain thrombocyte level above 40000 to the patient who had fever and was intubated. Clinical and vital findings of patient worsened progressively, pH decreased to 7.05 and continuous renal replacement therapy was initiated. Despite adrenalin and noradrenalin, mean arterial blood pressure was about 20 and she died due to cardiac arrest.

DISCUSSION

In this case, the t(1;15)(q21;q11) and t(1;21)(q21;q11.2) were found in the complex karyotype with the translocation of t(8;14)(q24;q32) which is a characteristic of BL. The t(1;15)(q21;q11.2) and t(1;21)

(q21;q11.2) anomalies were reported for the first time, according to the databases that we have investigated^[3,4]. Translocation between chromosomes 1 and 21 were reported in only one BL patient, but the breakpoint of this translocations were different from the translocation which we identified^[4]. In our case, the result of t(1;15)(q21;q11.2) and t(1;21)(q21;q11.2) was tetrasomy of chromosomes of 1q. Structural anomalies of chromosomes 1q could be seen in BL patients as additional cytogenetic anomalies and gain of chromosome of 1q is usually associated with disease recurrence and poor prognosis^[5]. Siddiqi *et al* reported that additional partial tetrasomy 1q seen with t(8;14)(q24;q32) as seen in our case could be a very aggressive clinical sign^[6]. The patient died approximately eight months after diagnosis, so her prognosis was poor, which was consistent with the literature.

CONCLUSION

We report two novel translocations in one patient with BL. The result of t(1;15)(q21;q11.2) and t(1;21)(q21;q11.2) was tetrasomy of chromosomes of 1q which is usually associated with disease recurrence and poor prognosis. The candidate genes on chromosomes 1q which could be involved in tumorigenesis remain to be identified.

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Authors' contribution to the manuscript:

Sureyya Bozkurt: data analysis and writing; Mufide Okay: data analysis; Celalettin Ibrahim Haznedaroglu: manuscript reviewing.

The authors declare no conflict of interest.

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Brief Communication

Recovery and rehabilitation of patients with COVID-19 and post-COVID-19 syndrome

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ABSTRACT

The therapy of “post-COVID-19 syndrome” requires a multidisciplinary team, an individualized approach and long-term rehabilitation. Recovery and rehabilitation after COVID-19 is aimed specifically at overcoming the consequences of the disease by strengthening the respiratory, nervous and cardiovascular systems through treatment and rehabilitation procedures. The

complex program includes pulmonary rehabilitation to increase lung capacity and overall metabolic effects. Complex rehabilitation is part of the whole treatment plan and includes many physical and rehabilitation activities, health care, healthy behavior, training and prevention of complications of patients after infection.

KEY WORDS: coronavirus infection, treatment plan

REPORT

During the COVID-19 pandemic, of increasing interest to the medical community are residual symptoms, structural and functional changes in various organs and systems, which last for weeks and months after illness and require comprehensive medical care. Symptoms of COVID-19 and “post-COVID-19 syndrome” remain long after the acute phase in many patients^[1]. For “post-COVID-19”, we consider the duration of symptoms to be more than 12 weeks after the onset of the disease, which cannot be explained by another disease and whose duration is not yet definitively known^[2]. Knowledge of its clinical characteristics will help the general and specialized practitioners to recognize its manifestations, and the health care system to prepare for its treatment and rehabilitation in the future. More attention is needed on the somatic, mental and emotional characteristics of patients who have undergone COVID-19. The most reported symptoms during and after COVID-19 are shortness of breath, fatigue and heart complaints^[1].

Commonly reported symptoms are also muscle and joint pain, loss of taste and smell^[3]. This shows that in the period after illness, there is only a partial recovery of symptoms and health status of patients and few patients have full recovery months after the acute phase of the disease. Residual symptoms have a significant dependence on the age, hospital stay, severe course and presence of shortness of breath at the beginning of the disease^[3]. The development of symptoms within after acute and post-COVID-19 is not directly dependent on the severity of acute COVID-19. Such symptoms can have both severe and critical cases that have survived the infection, as well as milder cases that have passed through the infection with short-term general symptoms. Organ symptoms after COVID-19 are mainly controlled by the respiratory system, heart and nervous system, but although less commonly, affected are also the kidneys, liver, pancreas, spleen, immunological imbalance, cytokine storm and blood clotting disorders with subsequent thrombosis in the most severe cases.

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The therapy of “post-COVID-19 syndrome” requires a multidisciplinary team, an individualized approach and long-term rehabilitation. For patients after severe COVID-19, after treatment in the intensive care unit, hospital and post-hospital rehabilitation is a necessity. It should start as early as possible, continuing in the form of outpatient rehabilitation in units for pulmonary rehabilitation and sanatorium treatment. After a coronavirus infection, the potential of patients to undergo rehabilitation measures requires the condition of the respiratory system and blood circulation to be stable without the risk of worsening the existing complaints. Patients with COVID-19 and significant residual symptoms characteristic of “post-COVID-19” who have had a complicated course with severe respiratory failure, respiratory arrest, chronic lung disease or involvement of the respiratory muscles, require early respiratory rehabilitation and assessment of the patient’s oxygen needs at rest and after exercise.

It is important to anticipate early rehabilitation after the acute phase to limit the severity of complications and to promote rapid functional recovery. Physiotherapy occupies a significant place in the treatment plan with targeted physical activity and rehabilitation interventions in patients who have experienced critical conditions associated with COVID-19. Early neurological and neurosurgical rehabilitation should be performed “post-COVID-19” with severe central and/or peripheral nervous system damages. In the presence of secondary emotional disturbances, psychiatric/psychotherapeutic treatment should be initiated, given that fear and depressive disorders are more common in patients after a more severe course of the disease^[4]. The return to normal physical activity or exercise is desirable after a minimum of seven days without symptoms. A thorough examination of the heart (echocardiography) and lung (functional assessment) is required for patients with persistent systemic symptoms before engaging in physical activity. The need for rehabilitation in those who have undergone the disease is an essential stage of treatment, as these patients continue to suffer from residual symptoms, combined with respiratory dysfunction, decreased diffusion capacity and various pathological changes. Early and adequate rehabilitation care can significantly reduce the severity and duration of complaints and improve the quality of life^[5-6]. Patients need regular follow-up and treatment plan after coronavirus infection.

The management of treatment and the late effects of the new coronavirus place significant demands on health resources around the world^[7]. Complex rehabilitation is part of the whole treatment plan and includes many physical and rehabilitation activities, health care, healthy behavior, training and prevention of complications of patients after infection. The optimal use of rehabilitation methods and forms will significantly improve pulmonary ventilation, will contribute to a more complete physical, psychosocial, functional recovery of patients and their quality of life.

ACKNOWLEDGMENT

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Conflict of interest: The authors declare that there is no conflict of interest.

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6. Qin W, Chen S, Zhang Y, Dong F, Zhang Z, Hu B, *et al.* Diffusion capacity abnormalities for carbon monoxide in patients with COVID-19 at three-month follow-up. *Eur Respir J* 2021; 2003677.
7. Polastri M, Nava S, Clini E, Vitacca M, Gosselink R. COVID-19 and pulmonary rehabilitation: preparing for phase three. *Eur Respir J* 2020; 55(6):2001822.

Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Kuwait Medical Journal 2021; 53 (3): 348 - 350

Detection of mutations in *NOD2/CARD15* gene in Arab patients with Crohn's disease

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Saudi J Gastroenterol. 2021 Aug 3. doi: 10.4103/sjg.sjg_582_20. Online ahead of print.

BACKGROUND

Mutations in *NOD2/CARD15* gene have been linked to an increased risk of Crohn's disease (CD). The objective of this study is to determine *NOD2/CARD15* gene mutations, and their association with the risk of CD in Arabs in Kuwait.

METHODS

Four *NOD2* gene mutations, including Pro268Ser (SNP5), Arg702Trp (SNP8), Gly908Arg (SNP12), and Leu1007FsinC (SNP13) were examined in Arab CD patients (n = 103) and control subjects (n = 100). The genomic DNA was isolated and used in polymerase chain reaction (PCR) with four sets of specific primers. The PCR-amplified DNA fragments were sequenced and analyzed for the *NOD2* mutations. Logistic regression was used to estimate the adjusted odds ratios (aOR) and 95% confidence intervals (CI).

RESULTS

Of the four genotyped variants, the Arg702Trp (SNP8) and Leu1007FsinC (SNP13) variants were not informative in our study sample due to minor allele frequency of <1%. The Pro268Ser (SNP5) mutation was detected in 17 (16.5%) CD patients and 32 (32.0%) controls. The Gly908Arg (SNP12) mutation was observed in 24 (23.3%) patients and 10 (10.0%) controls. In the dominant genetic risk model (i.e. carrying at least one minor allele), CD patients compared to controls were less likely to carry either the "CT" or "TT" genotype of variant Pro268Ser (SNP5; aOR = 0.43, 95% CI: 0.22-0.84). In contrast, CD patients compared to controls were more likely to carry the homozygous for the minor allele or the heterozygous genotypes of variant Gly908Arg (SNP12; aOR = 2.67, 95% CI: 1.19-5.97).

CONCLUSIONS

In this Arab population, carrying at least one copy of the minor allele of Gly908Arg (SNP12) mutation in *NOD2* gene was associated with increased susceptibility to CD, while having the heterozygous or homozygous for the minor allele genotype of the Pro268Ser (SNP5) mutation provided protection against CD. Mutations in Arg702Trp (SNP8) and Leu1007FsinC (SNP13) were not detected in this sample of the Arab population in Kuwait.

A torque induced iatrogenic fracture of the humeral shaft in proximal humeral fracture plating - A case report and biomechanical perspectives

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Ann Med Surg (Lond). 2021 Jul 27;68:102615. doi: 10.1016/j.amsu.2021.102615. eCollection 2021 Aug.

INTRODUCTION

Proximal humerus fractures are common amongst the elderly and osteoporotic cohorts. Common treatment methods include proximal locking plates. In this case, we describe an iatrogenic fracture of the proximal humeral shaft during screw insertion under power. Similar cases have not been described previously in open reduction and internal fixation of a proximal humerus fracture. Further, we focus particularly on precautionary measures that aim to avoid such complications that may lead to considerable morbidity.

CASE PRESENTATION

We describe a case of a 65 year old osteoporotic female who underwent open reduction internal fixation of a proximal humerus fracture complicated by an unusual iatrogenic humeral fracture at the level of insertion of the distal screw, likely secondary to inserting the proximal locking screws under power.

CONCLUSION

In this case, we explore the possible factors leading to the fracture and precautionary measures to avoid them. The rate of iatrogenic intraoperative fractures are likely underreported and have not been described in open reduction and internal fixation of an existing fracture. The underlying factors that may predispose to such complications have not been previously described in similar cases. This case serves as a warning of an unanticipated complication and describes the potential biomechanical factors involved.

Association of CNS demyelination and COVID-19 infection: an updated systematic review

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J Neurol. 2021 Aug 12;1-36. doi: 10.1007/s00415-021-10752-x. Online ahead of print.

BACKGROUND

Since the declaration of COVID-19 pandemic, several case reports of demyelination of both peripheral and central nervous systems have been published. The association between CNS demyelination and viral infection has long been documented, and this link was recently reported following SARS-CoV-2 infection as well.

OBJECTIVES

In this systematic review, we aim to investigate the existing literature on CNS demyelination associated with SARS-CoV-2, and the proposed pathophysiological mechanisms.

METHODS

We conducted a systematic review of articles in PubMed, SCOPUS, EMBASE, Cochrane, Google Scholar and Ovid databases, from 1 January 2020 until June 15, 2021. The following keywords were used: "COVID-19", "SARS-CoV-2", "demyelination", "demyelinating disease", "multiple sclerosis", "neuromyelitis optica", and "transverse myelitis".

RESULTS

A total of 60 articles were included in the final analysis of this systematic review and included 102 patients: 52 (51%) men and 50 (49%) women, with a median age of 46.5 years. The demyelination mimicked a variety of conditions with a picture of encephalitis/encephalomyelitis being the most common. At the same time other patterns were less frequently reported such as MS, NMOSD and even MOGAD. Longitudinally extensive transverse myelitis (LETM) was the most frequently reported pattern of spinal cord involvement.

CONCLUSION

A growing body of literature has shown an association between SARS-CoV-2 infection and the development of different types of CNS demyelination. Although causality cannot readily be inferred, this review may suggest a probable causal relationship, through a para-infectious or post-infectious immune-mediated etiology in COVID-19 patients. This relationship needs to be clarified in future research.

Forthcoming Conferences and Meetings

Compiled and edited by
Vineetha Elizabeth Mammen

Kuwait Medical Journal 2021; 53 (3): 351 - 355

SAGES - Society of American Gastrointestinal and Endoscopic Surgeons

Aug 31 - Sep 03, 2021

United States, Las Vegas

Contact: 310-437-0544x158

Email: michelle@sages.org

Event website: <http://www.sages.org>

4th International Conference on Plastic Surgery and Aesthetic Practices

Sep 06-07, 2021

Czech Republic, Prague

Contact: 0044-2033180199

Email: plasticsurgery@sciencesummits.com

6th International Conference on Digestive and Metabolic Diseases

Sep 06-07, 2021

Germany, Berlin

Theme: Exploring Future Perspectives and Shaping Trends in Gastroenterology

Contact: 0044-2033180199

Email: digestivediseases@speakersconclave.com

World Congress on Skin care, Dermatology and Allergic Diseases

Sep 06-07, 2021

Czech Republic, Prague

Exploring the new therapeutic techniques in dermatology

Contact: 0044-2033180199

Email: globaldermatology@europeconferences.com

International Conference and Expo on Heart Ailments

Sep 07-08, 2021

Webinar

Contact: 0044-2033180199

Email: cardiologycongress@expert-meetings.com

17th World Congress on Endocrinology & Diabetes

Sep 08-09, 2021

Czech Republic, Prague

Contact: 0044-2033180199

Email: endocrinology@europemeet.com

37th International Conference on Neonatology

Sep 10-11, 2021

France, Paris

Contact: 0044-2033180199

neonatology@meetingsnexpo.com

20th Asia Pacific Ophthalmologists Annual Meeting

Sep 13-14, 2021

Webinar

Contact: 0044-2033180199

Email: contact@asiameets.com

29th International Conference on Cardiology and Cardiovascular Diseases

Sep 15-16, 2021

United Kingdom, London

Contact: 0044-2033180199

Email: cardiovascularmedicine@lifescienceconferences.org

4th World Congress on Paediatric Neurology & Paediatric Surgery

Sep 16-17, 2021

Australia, Sydney

Contact: 0044-2033180199

Email: neuropediatrics@asia-meetings.com

4th World Congress on Nutrition and Obesity Prevention

Sep 16-17, 2021

Singapore, Singapore City

Contact: 0044-2033180199

Email: meevents@memeetings.com

4th International Conference on Obesity and Chronic Diseases

Sep 20, 2021

Webinar

Advanced Concepts in Treatment and Prevention of Obesity

Contact: 0044-2033180199

Email: meevents@memeetings.com

7th World Congress on Paediatric Disease, Care & Management

Sep 20-21, 2021

Webinar

Contact: 0044-2033180199

Email: pediatricdisease@asiameets.com

4th International Conference on Advances in Neonatal and Pediatric Nutrition

Sep 20-21, 2021

United Kingdom, London

Contact: 0044-2033180199

Email: pediatricnutrition@europemeets.com

3rd Annual Conference on Vascular Medicine

Sep 25, 2021

Webinar

Contact: 0044-2033180199

Email: meeevents@memeetings.com

31st International Congress on Vision Science and Eye

Sep 27-28, 2021

Ireland, Dublin

Contact: 0044-2033180199

Email: events@conferenceseries.com

10th International Conference on Nephrology & Therapeutics

Sep 27-28, 2021

Webinar

Contact: 0044-2033180199

Email: nephrology@eventqueries.com

27th International Conference & Exhibition on Cardiovascular and Thoracic Surgery

Sep 28-29, 2021

Japan, Osaka

Contact: 0044-2033180199

Email: cardiovascular@asia-meetings.com

4th World Congress on Surgeons

Sep 28, 2021

Webinar

Contact: 0044-2033180199

Email: surgeons@memeetings.com

16th International Conference on Endocrinology and Metabolic Disorders

Oct 04-05, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: endocrine@europeanannualconference.com

30th International Conference on Paediatrics & Adolescent health

Oct 04-05, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: pediatrics@brainstormingmeetings.com

8th International Conference on ENT Surgery

Oct 04-05, 2021

United Kingdom, London

Contact: 0044-2033180199

Email: ent-surgery@globalannualmeet.com

Update in Hospital Medicine 2021

Oct 04-07, 2021

United States, Live Streaming

Contact: 617-384-8600

Email: CEPrograms@hms.harvard.edu

Event website: <https://hospitalmedicine.hmscme.com/>**3rd International Conference on Physical Education, Sports Medicine and Doping Studies**

Oct 05-06, 2021

Webinar

Contact: 0044-2033180199

Email: sportsmedicine@asia-meetings.com

International Conference on Pulmonology & Interdisciplinary Medicine

Oct 12-13, 2021

Australia, Perth

Contact: 0044-2033180199

Email: pulmonologycong@asia-meetings.com

Global Congress on Endocrinology and Gynecology

Oct 14-15, 2021

Australia, Sydney

Contact: 0044-2033180199

Email: hypertension@asia-meetings.com

6th Annual World Congress on Paediatric Nutrition, Gastroenterology and Child Development

Oct 15-16, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: pediatricnutrition@annualamericacongress.com

Principles of Medical Education: Maximizing your Teaching Skills

Oct 18 - 20, 2021

United States, Live Streaming

Contact: 617-384-8600

Email: CEPrograms@hms.harvard.edu

Event website: <https://medicaleducators.hmscme.com/>

2nd World Congress on Neonatal, Paediatric Nutrition & Baby Food

Oct 18-19, 2021

Japan, Tokyo

Contact: 0044-2033180199

Email: pediatricnutrition@globalconferences.net

2nd International Congress & Expo on Pulmonology

Oct 19-20, 2021

Philippines, Manila

Contact: 0044-2033180199

Email: neuro@asia-meetings.com

3rd International Conference on Paediatrics, Primary Care and Healthcare

Oct 21-22, 2021

Netherlands, Amsterdam

Contact: 0044-2033180199

Email: meevents@memeetings.com

5th World Paediatrics Conference

Oct 22-23, 2021

Japan, Tokyo

Contact: 0044-2033180199

Email: worldpediatrics@europemeet.com

37th International Conference on Paediatric and Nutritional Research

Oct 22-23, 2021

Spain, Madrid

Contact: 0044-2033180199

Email: nutritionalpediatric@meetingsnexpo.com

5th World Pediatrics Conference

Oct 22-23, 2021

Japan, Tokyo

Contact: 0044-2033180199

Email: worldpediatrics@europemeet.com

33rd European Pediatrics Congress

Oct 25-26, 2021

France, Paris

Contact: 0044-2033180199

Email: europediatrics@europemeet.com

15th World Paediatric Congress

Oct 25-26, 2021

Netherlands, Amsterdam

Contact: 0044-2033180199

Email: meevents@memeetings.com

25th World Congress on Pediatrics, Neonatology & Primary Care

Oct 25-26, 2021

United Kingdom, London

Contact: 0044-2033180199

Email: pediatrics@speakermeeting.com

Otolaryngology and ENT Surgery

Oct 25-26, 2021

Italy, Rome

Contact: 0044-2033180199

Email: ent@theexpertsmeet.com

35th European Ophthalmology Congress

Oct 27-28, 2021

Switzerland, Zurich

Contact: 0044-2033180199

Email: contactus@euroannualmeetings.com

4th Head and Neck Conference: The Multidisciplinary Approach

Oct 28-29, 2021

Netherlands, Amsterdam

Contact: 0044-2033180199

Email: meevents@memeetings.com

2nd International Conference on Orthodontics

Oct 28-29, 2021

Netherlands, Amsterdam

Contact: 0044-2033180199

Email: meevents@memeetings.com

36th International Conference on Advanced Paediatrics and Neonatology

Nov 01-02, 2021

France, Paris

Contact: 0044-2033180199

Email: Adv.Pediatrics@brainstormingmeetings.com

32nd International Congress on Prevention of Diabetes and Complications

Nov 01-02, 2021

France, Paris

Contact: 0044-2033180199

Email: diabetes@speakersconclave.com

Global Meet on Skin Care and Plastic Aesthetics

Nov 02-03, 2021

Australia, Sydney

Contact: 0044-2033180199

Email: skincaremeet@asia-meetings.com

Diabetes and its Complications

Nov 04-06, 2021

United States, Boston

Contact 617-384-8600

Email: ceprograms@hms.harvard.edu

Event website: <https://hmsdiabetescourse.com/>**16th World Conference on Infectious Diseases, Prevention and Control**

Nov 08-09, 2021

Indonesia, Bali

Contact: 0044-2033180199

Email: meevents@memeetings.com

International Congress and Expo on Diabetic Care

Nov 09-10, 2021

Thailand, Bangkok

Contact: 0044-2033180199

Email: cardiodiabetes@asiapacificmeets.com

Neurological Emergencies

Nov 11-13, 2021

United States, Live Streaming

Phone: 617-384-8600

Contact: CEPrograms@hms.harvard.edu

Event website: <https://neuroemergencies.hmscme.com>**4th World Cosmetic and Dermatology Congress**

Nov 11-12, 2021

Indonesia, Bali

Contact: 0044-2033180199

Email: meevents@memeetings.com

19th World Congress on Clinical Pediatrics

Nov 12-13, 2021

Austria, Vienna

Contact: 0044-2033180199

Email: clinicalpediatric@lifescienceconferences.org

International Conference on Diabetes & Heart Diseases

Nov 15-16, 2021

New Zealand, Auckland

Contact: 0044-2033180199

Email: cardiodiabeticcare@asia-meetings.com

20th International Conference on Pharmacology and Toxicology

Nov 18-19, 2021

Austria, Vienna

Contact: 0044-2033180199

Email: remind@scholargatherings.com

7th International Conference on Otolaryngology and Laryngology

Nov 18-19, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: meevents@memeetings.com

12th European Conference on Surgery & Cosmetology

Nov 22-23, 2021

United Arab United, Dubai

Contact: 0044-2033180199

Email: eurosurgery@lifescienceconferences.org

9th Annual Congress on Pulmonary and Critical Care

Nov 22-23, 2021

Webinar

Contact: 0044-2033180199

Email: pulmonary@asiameets.com

3rd World Congress on Advancements in Tuberculosis and Lung Diseases

Nov 22-23, 2021

Webinar

Contact: 0044-2033180199

Email: contact@asiameets.com

World Congress and Expo on Nephrology

Nov 23-24, 2021

Vietnam, Hanoi

Contact: 0044-2033180199

Email: nephrologysummit@asia-meetings.com

23rd Global Nephrologists Annual Meeting

Nov 25-26, 2021

France, Paris

Contact: 0044-2033180199

Email: nephrologists@theexpertsmeet.com

9th Annual Congress on Dental Medicine and Orthodontics

Nov 25-26, 2021

Italy, Rome

Contact: 0044-2033180199

Email: meevents@memeetings.com

3rd World Congress on Vaccine and Immunology

Nov 29-30, 2021

Singapore, Singapore

Contact: 0044-2033180199

Email: vaccineimmunology@meetingsemail.com

3rd Annual Meeting on Cosmetic Dentistry & Orofacial Myology

Nov 29-30, 2021

Canada, Vancouver

Contact: 0044-2033180199

Email: cosmeticdentistry@annualamericacongress.com

27th Global Dentists and Paediatric Dentistry Annual Meeting

Nov 29-30, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: dentists@brainstormingmeetings.com

7th European Otolaryngology-ENT Surgery Conference

Dec 01-02, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: eye@speakersconclave.com

13th International Conference on Chronic Obstructive Pulmonary Disease Conference

Dec 01-02, 2021

Spain, Barcelona

Contact: 0044-2033180199

Email: COPD@speakersconclave.com

41st Global Summit and Expo on Vaccines & Immunology

Dec 01-02, 2021

Germany, Frankfurt

Contact: 0044-2033180199

Email: vaccinescongress@meetingsnexpo.com

Update in Internal Medicine 2021 | Livestream

Dec 05-11, 2021

United States, Boston

Contact: 617-384-8600

Email: ceprograms@hms.harvard.edu

Event website: <https://updateinternalmedicine.com/>**13th World Congress on Endocrinology and Metabolic Disorders**

Dec 07-08, 2021

New Zealand, Auckland

Contact: 0044-2033180199

Email: metabolomics@asia-meetings.com

2nd International Congress on Gastroenterology and Liver Transplant

Dec 07-08, 2021

New Zealand, Auckland

Contact: 0044-2033180199

Email: contact@asiapacificmeets.com

17th International Conference on Cosmetic Dermatology Trichology and Plastic Surgery

Dec 13-14, 2021

United Arab Emirates, Dubai

Contact: 0044-2033180199

Email: queries@conferenceseries.com

42nd European Forum on Dental Practice & Oral Health

Dec 13-14, 2021

United Arab Emirates, Abu Dhabi

Contact: 0044-2033180199

Email: dentalforum@theexpertsmeet.com

24th Global Summit on Paediatrics, Neonatology and Primary Care

Dec 13-14, 2021

United Arab Emirates, Dubai

Contact: 0044-2033180199

Email: meeevents@memeetings.com

13th World Conference on Gynaecology, Obstetrics and Women Health

Jan 26-27, 2022

Singapore, Singapore City

Contact: 0044-2033180199

Email: gynecology@globalannualsummit.com

2nd World Congress on Haematology and Genetic Blood Disorders

Jan 24-25, 2022

Czech Republic, Prague

Contact: 0044-2033180199

Email: contact@globalconferencemeet.com

11th International Conference on Tropical Medicine and Infectious Diseases

Feb 07-08, 2022

United Kingdom, London

Contact: 0044-2033180199

Email: tropicaldiseases@europemeets.com

32nd International Conference on Neurology and Cognitive Neuroscience

Feb 09-10, 2022

United Kingdom, London

Contact: 0044-2033180199

Email: neurologycongress@europemeets.com

2nd World Summit on Diabetes

Feb 21-22, 2022

Czech Republic, Prague

Contact: 0044-2033180199

Email: diabetes@europeconferences.com

International Congress on Traditional and Natural Medicines

Feb 25-26, 2022

Singapore, Singapore City

Contact: 0044-2033180199

Email: queries@conferenceseries.com

WHO-Facts Sheet

1. Childhood cancer
2. Ebola virus disease
3. Headache disorders
4. Marburg virus disease
5. Obesity and overweight

Compiled and edited by
Vineetha E Mammen

Kuwait Medical Journal 2021; 53 (3): 356 - 368

1. CHILDHOOD CANCER

KEY FACTS

- Each year, approximately 400 000 children and adolescents of 0-19 years old are diagnosed with cancer. ^[1]
- The most common types of childhood cancers include leukemias, brain cancers, lymphomas and solid tumours, such as neuroblastoma and Wilms tumours. ^[2]
- In high-income countries, where comprehensive services are generally accessible, more than 80% of children with cancer are cured. In low- and middle-income countries (LMICs), an estimated 15-45% are cured. ^[3]
- Childhood cancer cannot generally be prevented or identified through screening.
- Most childhood cancers can be cured with generic medicines and other forms of treatment, including surgery and radiotherapy. Treatment of childhood cancer can be cost-effective in all income settings. ^[4]
- Avoidable deaths from childhood cancers in LMICs result from lack of diagnosis, misdiagnosis or delayed diagnosis, obstacles to accessing care, abandonment of treatment, death from toxicity, and higher rates of relapse. ^[3]
- Childhood cancer data systems are needed to drive continuous improvements in the quality of care, and to inform policy decisions.

The problem

Cancer is a leading cause of death for children and adolescents, particularly in high-income countries. The likelihood of surviving a diagnosis of childhood cancer depends on the country in which the child lives: in high-income countries, more than 80% of children

with cancer are cured, but in many LMICs only 15-45% are cured ^[3].

The reasons for lower survival rates in LMICs include: delay in diagnosis and advanced disease, an inability to obtain an accurate diagnosis, inaccessible therapy, abandonment of treatment, death from toxicity (side effects), and avoidable relapse. Improving access to childhood cancer care, including to essential medicines and technologies, is highly cost effective, feasible and can improve survival in all settings ^[4].

What causes cancer in children?

Cancer occurs in people of all ages and can affect any part of the body. It begins with genetic change in single cells, that then grow into a mass (or tumour), invades other parts of the body and causes harm and death if left untreated. Unlike cancer in adults, the vast majority of childhood cancers do not have a known cause. Many studies have sought to identify the causes of childhood cancer, but very few cancers in children are caused by environmental exposure or lifestyle factors. Cancer prevention efforts in children should focus on behaviours that will prevent the child from developing preventable cancer as an adult.

Some chronic infections, such as HIV, Epstein-Barr virus and malaria, are risk factors for childhood cancer. They are particularly relevant in LMICs. Other infections can increase a child's risk of developing cancer as an adult, so it is important to be vaccinated (against hepatitis B to help prevent liver cancer and against human papillomavirus to help prevent cervical cancer) and to other pursue other methods such as early detection and treatment of chronic infections that can lead to cancer.

Current data suggest that approximately 10% of all children with cancer have a predisposition because of

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genetic factors^[5]. Further research is needed to identify factors impacting cancer development in children.

Improving outcomes of childhood cancer

Because it is generally not possible to prevent cancer in children, the most effective strategy to reduce the burden of cancer in children and improve outcomes is to focus on a prompt, correct diagnosis followed by effective, evidence-based therapy with tailored supportive care.

Early diagnosis

When identified early, cancer is more likely to respond to effective treatment and result in a greater probability of survival, less suffering, and often less expensive and less intensive treatment. Significant improvements can be made in the lives of children with cancer by detecting cancer early and avoiding delays in care. A correct diagnosis is essential to treat children with cancer because each cancer requires a specific treatment regimen that may include surgery, radiotherapy, and chemotherapy.

Early diagnosis consists of 3 components^[6]:

- awareness of symptoms by families and primary care providers;
- accurate and timely clinical evaluation, diagnosis, and staging (determining the extent to which a cancer has spread); and
- access to prompt treatment.

Early diagnosis is relevant in all settings and improves survival for many cancers. Programmes to promote early and correct diagnosis have been successfully implemented in countries of all income levels, often through the collaborative efforts of governments, civil society and nongovernmental organizations, with vital roles played by parent groups. Childhood cancer is associated with a range of warning symptoms that can be detected by families and by trained primary health-care providers.

Screening is generally not helpful for childhood cancers. In some select cases, it can be considered in high-risk populations. For example, some eye cancers in children can be caused by a mutation that is inherited, so if that mutation or disease is identified in the family of a child with retinoblastoma, genetic counselling can be offered and siblings monitored with regular eye examinations early in life. Genetic causes of childhood cancers are relevant in only a handful of children with cancer. There is no high-quality evidence to support population-based screening programmes in children.

Treatment

A correct diagnosis is essential to prescribe appropriate therapy for the type and extent of the disease. Standard therapies include chemotherapy,

surgery and/or radiotherapy. Children also need special attention to their continued physical and cognitive growth and nutritional status, which requires a dedicated, multi-disciplinary team. Access to effective diagnosis, essential medicines, pathology, blood products, radiation therapy, technology and psychosocial and supportive care are variable and inequitable around the world.

However, cure is possible for more than 80% of children with cancer when childhood cancer services are accessible. Pharmacological treatment, for example, includes inexpensive generic medications included on the WHO List of Essential Medicines for Children (27 cytotoxic agents, 5 targeted therapies and 4 hormone treatments for childhood cancer). Children who complete treatment require ongoing care to monitor for cancer recurrence and to manage any possible long-term impact of treatment.

Palliative care

Palliative care relieves symptoms caused by cancer and improves the quality of life of patients and their families. Not all children with cancer can be cured, but relief of suffering is possible for everyone. Paediatric palliative care is considered as a core component of comprehensive care, starting when the illness is diagnosed and continuing throughout treatment and care, regardless of whether or not a child receives treatment with curative intent.

Palliative care programmes can be delivered through community and home-based care, providing pain relief and psychosocial support to patients and their families. Adequate access to oral morphine and other pain should be provided for the treatment of moderate to severe cancer pain, which affects more than 80% of cancer patients in the terminal phase.

WHO response

In 2018, WHO launched, together with partners, the Global Initiative for Childhood Cancer, to provide leadership and technical assistance to governments to support them in building and sustaining high-quality childhood cancer programmes^[4]. The goal is to achieve at least 60% survival for all children with cancer and reduce suffering, globally, by 2030. This represents an approximate doubling of the current cure rate and will save an additional one million lives over the next decade. The objectives of the Initiative are:

- to increase capacity of countries to deliver best practices in childhood cancer care; and
- to Increase prioritization of childhood cancer at the global, regional and national levels

The **CureAll** framework and its accompanying technical package are used have been developed to support implementation of the Initiative. The package

is intended to help countries assess current capacity, set priorities, generate investment cases, develop evidence-based standards of care and monitor progress. An information-sharing portal has been created to facilitate sharing of expertise between countries and partners.

WHO and the International Agency for Research on Cancer (IARC) collaborate with the International Atomic Energy Agency (IAEA) and other UN organizations and partners, to:

- increase political commitment for childhood cancer diagnosis and treatment;
- support governments to develop high-quality cancer centres and regional satellites to ensure early and accurate diagnosis and effective treatment for children with cancer;
- develop standards and tools to guide the planning and implementation of interventions for early diagnosis, treatment and palliative and survivorship care, all of which take account of the specificities of childhood cancer;
- improve access to affordable and essential medicines and technologies; and
- support governments to safeguard families of children with cancer from financial ruin and social isolation as a result of cancer care.

The Global Initiative for Childhood Cancer is part of the response to the World Health Assembly resolution Cancer Prevention and Control through an Integrated Approach (WHA70.12), which urges governments and WHO to accelerate action toward the achievement of the targets specified in the Global Action Plan for the Prevention and Control of Noncommunicable Diseases (NCDs) and 2030 UN Agenda for Sustainable Development, including the reduction of premature mortality from NCDs and the achievement of universal health coverage.

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2. EBOLA VIRUS DISEASE

KEY FACTS

- Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever, is a rare but severe, often fatal illness in humans.
- The virus is transmitted to people from wild animals and spreads in the human population through human-to-human transmission.
- The average EVD case fatality rate is around 50%. Case fatality rates have varied from 25% to 90% in past outbreaks.
- Community engagement is key to successfully controlling outbreaks.
- Good outbreak control relies on applying a package of interventions, namely case management, infection prevention and control practices, surveillance and contact tracing, a good laboratory service, safe and dignified burials and social mobilisation.
- Vaccines to protect against Ebola have been developed and have been used to help control the spread of Ebola outbreaks in Guinea and in the Democratic Republic of the Congo (DRC).
- Early supportive care with rehydration, symptomatic treatment improves survival. Two monoclonal antibodies (Inmazeb and Ebanga) were approved for the treatment of Zaire ebolavirus (Ebolavirus) infection in adults and children by the US Food and Drug Administration in late 2020.
- Pregnant and breastfeeding women with Ebola should be offered early supportive care. Likewise vaccine prevention and experimental treatment should be offered under the same conditions as for non-pregnant population.

The Ebola virus causes an acute, serious illness which is often fatal if untreated. EVD first appeared in 1976 in 2 simultaneous outbreaks, one in what is now Nzara, South Sudan, and the other in Yambuku, DRC. The latter occurred in a village near the Ebola River, from which the disease takes its name.

The 2014–2016 outbreak in West Africa was the largest Ebola outbreak since the virus was first

discovered in 1976. The outbreak started in Guinea and then moved across land borders to Sierra Leone and Liberia.

The virus family Filoviridae includes three genera: Cuevavirus, Marburgvirus, and Ebolavirus. Within the genus Ebolavirus, six species have been identified: Zaire, Bundibugyo, Sudan, Tai Forest, Reston and Bombali.

Transmission

It is thought that fruit bats of the Pteropodidae family are natural Ebola virus hosts. Ebola is introduced into the human population through close contact with the blood, secretions, organs or other bodily fluids of infected animals such as fruit bats, chimpanzees, gorillas, monkeys, forest antelope or porcupines found ill or dead or in the rainforest.

Ebola then spreads through human-to-human transmission via direct contact (through broken skin or mucous membranes) with:

- Blood or body fluids of a person who is sick with or has died from Ebola
- Objects that have been contaminated with body fluids (like blood, feces, vomit) from a person sick with Ebola or the body of a person who died from Ebola

Health-care workers have frequently been infected while treating patients with suspected or confirmed EVD. This occurs through close contact with patients when infection control precautions are not strictly practiced.

Burial ceremonies that involve direct contact with the body of the deceased can also contribute in the transmission of Ebola. People remain infectious as long as their blood contains the virus.

Pregnant women who get acute Ebola and recover from the disease may still carry the virus in breastmilk, or in pregnancy related fluids and tissues. This poses a risk of transmission to the baby they carry, and to others. Women who become pregnant after surviving Ebola disease are not at risk of carrying the virus.

If a breastfeeding woman who is recovering from Ebola wishes to continue breastfeeding, she should be supported to do so. Her breast milk needs to be tested for Ebola before she can start.

Symptoms

The incubation period, that is, the time interval from infection with the virus to onset of symptoms, is from 2 to 21 days. A person infected with Ebola cannot spread the disease until they develop symptoms.

Symptoms of EVD can be sudden and include:

- Fever
- Fatigue

- Muscle pain
- Headache
- Sore throat

This is followed by:

- Vomiting
- Diarrhoea
- Rash
- Symptoms of impaired kidney and liver function
- In some cases, both internal and external bleeding (for example, oozing from the gums, or blood in the stools).
- Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes.

Diagnosis

It can be difficult to clinically distinguish EVD from other infectious diseases such as malaria, typhoid fever and meningitis. Many symptoms of pregnancy and Ebola disease are also quite similar. Because of risks to the pregnancy, pregnant women should ideally be tested rapidly if Ebola is suspected.

Confirmation that symptoms are caused by Ebola virus infection are made using the following diagnostic methods:

- antibody-capture enzyme-linked immunosorbent assay (ELISA)
- antigen-capture detection tests
- serum neutralization test
- reverse transcriptase polymerase chain reaction (RT-PCR) assay
- electron microscopy
- virus isolation by cell culture.

Careful consideration should be given to the selection of diagnostic tests, which take into account technical specifications, disease incidence and prevalence, and social and medical implications of test results. It is strongly recommended that diagnostic tests, which have undergone an independent and international evaluation, be considered for use.

Current WHO recommended tests include:

- Automated or semi-automated nucleic acid tests (NAT) for routine diagnostic management.
- Rapid antigen detection tests for use in remote settings where NATs are not readily available. These tests are recommended for screening purposes as part of surveillance activities, however reactive tests should be confirmed with NATs.

The preferred specimens for diagnosis include:

- Wholeblood collected in ethylenediaminetetraacetic acid (EDTA) from live patients exhibiting symptoms.
- Oral fluid specimen stored in universal transport medium collected from deceased patients or when blood collection is not possible.

Samples collected from patients are an extreme biohazard risk; laboratory testing on non-inactivated samples should be conducted under maximum biological containment conditions. All biological specimens should be packaged using the triple packaging system when transported nationally and internationally.

Treatment

Supportive care - rehydration with oral or intravenous fluids - and treatment of specific symptoms improves survival. A range of potential treatments including blood products, immune therapies and drug therapies are currently being evaluated.

In the 2018-2020 Ebola outbreak in DRC, the first-ever multi-drug randomized control trial was conducted to evaluate the effectiveness and safety of drugs used in the treatment of Ebola patients under an ethical framework developed in consultation with experts in the field and the DRC.

Two monoclonal antibodies (Inmazeb and Ebanga) were approved for the treatment of Zaire ebolavirus (Ebola virus) infection in adults and children by the US Food and Drug Administration in late 2020.

Vaccines

The Ervebo vaccine has been shown to be effective in protecting people from the species Zaire ebolavirus, and is recommended by the Strategic Advisory Group of Experts on Immunization as part of a broader set of Ebola outbreak response tools. In December 2020, the vaccine was approved by the US Food and Drug Administration and prequalified by WHO for use in individuals 18 years of age and older (except for pregnant and breastfeeding women) for protection against Ebola virus disease caused by Zaire Ebola virus.

The vaccine had been administered to more than 350 000 people in Guinea and in the 2018-2020 Ebola virus disease outbreaks in the Democratic Republic of the Congo under "compassionate use" protocol. The vaccine has shown to be safe and effective against the species Zaire ebolavirus. A global stockpile of the Ervebo vaccine has become available starting January 2021.

In May 2020, the European Medicines Agency recommended granting marketing authorization for a 2-component vaccine called Zabdeno-and-Mvabea for individuals 1 year and older.

The vaccine is delivered in 2 doses: Zabdeno is administered first and Mvabea is given approximately 8 weeks later as a second dose. This prophylactic 2-dose regimen is therefore not suitable for an outbreak response where immediate protection is necessary.

Prevention and control

Good outbreak control relies on applying a package of interventions, including case management, surveillance and contact tracing, a good laboratory service, safe burials and social mobilisation. Community engagement is key to successfully controlling outbreaks. Raising awareness of risk factors for Ebola infection and protective measures (including vaccination) that individuals can take is an effective way to reduce human transmission. Risk reduction messaging should focus on several factors:

- **Reducing the risk of wildlife-to-human transmission** from contact with infected fruit bats, monkeys, apes, forest antelope or porcupines and the consumption of their raw meat. Animals should be handled with gloves and other appropriate protective clothing. Animal products (blood and meat) should be thoroughly cooked before consumption.
- **Reducing the risk of human-to-human transmission** from direct or close contact with people with Ebola symptoms, particularly with their bodily fluids. Gloves and appropriate personal protective equipment should be worn when taking care of ill patients. Regular hand washing is required after visiting patients in hospital, as well as after taking care of patients at home.
- **Outbreak containment measures**, including safe and dignified burial of the dead, identifying people who may have been in contact with someone infected with Ebola and monitoring their health for 21 days, the importance of separating the healthy from the sick to prevent further spread, and the importance of good hygiene and maintaining a clean environment.
- **Reducing the risk of possible sexual transmission**, based on further analysis of ongoing research and consideration by the WHO Advisory Group on the Ebola Virus Disease Response, WHO recommends that male survivors of EVD practice safer sex and hygiene for 12 months from onset of symptoms or until their semen tests negative twice for Ebola virus. Contact with body fluids should be avoided and washing with soap and water is recommended. WHO does not recommend isolation of male or female convalescent patients whose blood has been tested negative for Ebola virus.
- **Reducing the risk of transmission from pregnancy related fluids and tissue**, Pregnant women who have survived Ebola disease need community support to enable them to attend

frequent antenatal care (ANC) visits, to handle any pregnancy complications and meet their need for sexual and reproductive care and delivery in a safe way. This should be planned together with the Ebola and Obstetric health care expertise. Pregnant women should always be respected in the sexual and reproductive health choices they make.

Controlling infection in health-care settings

Health-care workers should always take standard precautions when caring for patients, regardless of their presumed diagnosis. These include basic hand hygiene, respiratory hygiene, use of personal protective equipment (to block splashes or other contact with infected materials), safe injection practices and safe burial practices.

Health-care workers caring for patients with suspected or confirmed Ebola virus should apply extra infection control measures to prevent contact with the patient's blood and body fluids and contaminated surfaces or materials such as clothing and bedding. When in close contact (within 1 metre) of patients with EVD, health-care workers should wear face protection (a face shield or a medical mask and goggles), a clean, non-sterile long-sleeved gown, and gloves (sterile gloves for some procedures).

Healthcare staff working with ANC or obstetric care should be informed about risks of persisting virus in pregnancy related fluids and encouraged to follow protocol for their own safety and the safety of the women they are caring for.

Laboratory workers are also at risk. Samples taken from humans and animals for investigation of Ebola infection should be handled by trained staff and processed in suitably equipped laboratories.

Care for people who recovered from EVD

A number of medical complications have been reported in people who recovered from Ebola, including mental health issues. Ebola virus may persist in some body fluids, including semen, pregnancy-related fluids and breast milk.

Ebola survivors need comprehensive support for the medical and psychosocial challenges they face and also to minimize the risk of continued Ebola virus transmission. To address these needs, a dedicated programme can be set up for care for people who recovered from Ebola.

Ebola virus is known to persist in immune-privileged sites in some people who have recovered from Ebola virus disease. These sites include the testicles, the inside of the eye, and the central nervous system. In women who have been infected while pregnant, the virus persists in the placenta, amniotic fluid and fetus. In women who have been

infected while breastfeeding, the virus may persist in breast milk.

Relapse-symptomatic illness in someone who has recovered from EVD due to increased replication of the virus in a specific site is a rare event, but has been documented. Reasons for this phenomenon are not yet fully understood.

Studies of viral persistence indicate that in a small percentage of survivors, some body fluids may test positive on reverse transcriptase polymerase chain reaction (RT-PCR) testing for Ebola virus for longer than 9 months.

More surveillance data and research are needed on the risks of sexual transmission, and particularly on the prevalence of viable and transmissible virus in semen over time. In the interim, and based on present evidence, WHO recommends that:

- All Ebola survivors and their sexual partners should receive counselling to ensure safer sexual practices until their semen has twice tested negative. Survivors should be provided with condoms.
- Male Ebola survivors should be offered semen testing at 3 months after onset of disease, and then, for those who test positive, every month thereafter until their semen tests negative for virus twice by RT-PCR, with an interval of one week between tests.
- Ebola survivors and their sexual partners should either:
 - abstain from all types of sex, or
 - observe safer sex through correct and consistent condom use until their semen has twice tested negative.
- Having tested negative, survivors can safely resume normal sexual practices without fear of Ebola virus transmission.
- Based on further analysis of ongoing research and consideration by the WHO Advisory Group on the Ebola Virus Disease Response, WHO recommends that male survivors of Ebola virus disease practice safe sex and hygiene for 12 months from onset of symptoms or until their semen tests negative twice for Ebola virus.
- Until such time as their semen has twice tested negative for Ebola, survivors should practice good hand and personal hygiene by immediately and thoroughly washing with soap and water after any physical contact with semen, including after masturbation. During this period, used condoms should be handled safely, and safely disposed of, so as to prevent contact with seminal fluids.
- All survivors, their partners and families should be shown respect, dignity and compassion.

3. HEADACHE DISORDERS

KEY FACTS

- Headache disorders are among the most common disorders of the nervous system.
- It has been estimated that almost half of the adult population have had a headache at least once within the last year.
- Headache disorders, which are characterized by recurrent headache, are associated with personal and societal burdens of pain, disability, damaged quality of life, and financial cost.
- Worldwide, a minority of people with headache disorders are diagnosed appropriately by a health-care provider.
- Headache has been underestimated, under-recognized and under-treated throughout the world.

What are headache disorders?

Headache disorders, characterized by recurrent headache, are among the most common disorders of the nervous system. Headache itself is a painful and disabling feature of a small number of primary headache disorders, namely migraine, tension-type headache, and cluster headache. Headache can also be caused by or occur secondarily to a long list of other conditions, the most common of which is medication-overuse headache.

How common are headache disorders?

Globally, it has been estimated that prevalence among adults of current headache disorder (symptomatic at least once within the last year) is about 50%. Half to three quarters of adults aged 18–65 years in the world have had headache in the last year and, among those individuals, 30% or more have reported migraine. Headache on 15 or more days every month affects 1.7–4% of the world's adult population. Despite regional variations, headache disorders are a worldwide problem, affecting people of all ages, races, income levels and geographical areas.

What is the burden due to headache disorders?

Not only is headache painful, but it is also disabling. In the Global Burden of Disease Study, updated in 2013, migraine on its own was found to be the sixth highest cause worldwide of years lost due to disability (YLD). Headache disorders collectively were third highest.

Headache disorders impose a recognizable burden on sufferers including sometimes substantial personal suffering, impaired quality of life and financial cost. Repeated headache attacks, and often the constant fear of the next one, damage family life, social life and

employment. The long-term effort of coping with a chronic headache disorder may also predispose the individual to other illnesses. For example, anxiety and depression are significantly more common in people with migraine than in healthy individuals.

Types of headache disorders

Migraine, tension-type headache and medication-overuse headache are of public health importance since they are responsible for high population levels of disability and ill-health.

Migraine

- A primary headache disorder.
- Migraine most often begins at puberty and most affects those aged between 35 and 45 years.
- It is more common in women, usually by a factor of about 2:1, because of hormonal influences.
- It is caused by the activation of a mechanism deep in the brain that leads to release of pain-producing inflammatory substances around the nerves and blood vessels of the head.
- Migraine is recurrent, often life-long, and characterized by recurring attacks.
- Attacks typically include:
 - headache, which is:
 - of moderate or severe intensity
 - one-sided
 - pulsating in quality
 - aggravated by routine physical activity
 - with duration of hours to 2-3 days
 - nausea (the most characteristic associated feature);
 - attack frequency is anywhere between once a year and once a week; and
 - in children, attacks tend to be of shorter duration and abdominal symptoms more prominent.

Tension-type headache (TTH)

- TTH is the most common primary headache disorder.
- Episodic TTH, occurring on fewer than 15 days per month, is reported by more than 70% of some populations.
- Chronic TTH, occurring on more than 15 days per month, affects 1-3% of adults.
- TTH often begins during the teenage years, affecting three women to every two men.
- Its mechanism may be stress-related or associated with musculoskeletal problems in the neck.
- Episodic TTH attacks usually last a few hours, but can persist for several days.
- Chronic TTH can be unremitting and is much more disabling than episodic TTH.
- This headache is described as pressure or tightness,

often like a band around the head, sometimes spreading into or from the neck.

Cluster Headache (CH)

- A primary headache disorder.
- CH is relatively uncommon affecting fewer than 1 in 1000 adults, affecting six men to each woman.
- Most people developing CH are in their 20s or older.
- It is characterized by frequently recurring (up to several times a day), brief but extremely severe headache, usually focused in or around one eye, with tearing and redness of the eye, the nose runs or is blocked on the affected side and the eyelid may droop.
- CH has episodic and chronic forms.

Medication-overuse headache (MOH)

- MOH is caused by chronic and excessive use of medication to treat headache.
- MOH is the most common secondary headache disorder.
- It may affect up to 5% of some populations, women more than men.
- MOH occurs by definition on more days than not, is oppressive, persistent and often at its worst on awakening.

Social and economic burden of headache

Headache disorders are a public-health concern given the associated disability and financial costs to society. As headache disorders are most troublesome in the productive years (late teens to 50s), estimates of their financial cost to society – principally from lost working hours and reduced productivity – are massive. In the United Kingdom, for example, some 25 million working- or school-days are lost every year because of migraine alone; this financial cost may be matched by TTH and MOH combined. Headache is high among causes of consulting medical practitioners: one-third of all neurological consultations were for headache, in one survey.

Yet, many of those troubled by headache do not receive effective care. For example, in the United States of America and the United Kingdom, only half of those identified with migraine had seen a doctor for headache-related reasons in the previous 12 months, and only two-thirds had been correctly diagnosed. Most were solely reliant on over-the-counter medications.

Treatment

Appropriate treatment of headache disorders requires training of health professionals, accurate diagnosis and recognition of the conditions,

appropriate treatment with cost-effective medications, simple lifestyle modifications, and patient education. The main classes of drugs to treat headache disorders include: analgesics, anti-emetics, specific anti-migraine medications, and prophylactic medications.

Barriers to effective care

Lack of knowledge among health-care providers is the principal clinical barrier. Worldwide, on average, only 4 hours of undergraduate medical education are dedicated to instruction on headache disorders. A large number of people with headache disorders are not diagnosed and treated: worldwide only 40% of those with migraine or TTH are professionally diagnosed, and only 10% of those with MOH.

Poor awareness extends to the general public. Headache disorders are not perceived by the public as serious since they are mostly episodic, do not cause death, and are not contagious. The low consultation rates in developed countries may indicate that many affected people are unaware that effective treatments exist. Half of people with headache disorders are estimated to be self-treating.

Many governments, seeking to constrain health-care costs, do not acknowledge the substantial burden of headache on society. They might not recognize that the direct costs of treating headache are small in comparison with the huge indirect-cost savings that might be made (eg, by reducing lost working days) if resources were allocated to treat headache disorders appropriately.

WHO response

These evident burdens call for action. WHO recognizes this, and is a partner, with the non-governmental organization Lifting The Burden, in the Global Campaign against Headache. This initiative commenced in 2004 and aims not only to raise awareness of headache disorders but also to improve the quality of headache care and access to it worldwide. WHO published the Atlas of headache disorders in 2011, describing the burden due to headache disorders and resources available to reduce them.

4. MARBURG VIRUS DISEASE

KEY FACTS

- Marburg virus disease (MVD), formerly known as Marburg haemorrhagic fever, is a severe, often fatal illness in humans.
- The virus causes severe viral haemorrhagic fever in humans.
- The average MVD case fatality rate is around 50%. Case fatality rates have varied from 24% to 88% in

past outbreaks depending on virus strain and case management.

- Early supportive care with rehydration, and symptomatic treatment improves survival. There is as yet no licensed treatment proven to neutralize the virus, but a range of blood products, immune therapies and drug therapies are currently under development.
- *Rousettus aegyptiacus*, fruit bats of the Pteropodidae family, are considered to be natural hosts of Marburg virus. The Marburg virus is transmitted to people from fruit bats and spreads among humans through human-to-human transmission.
- Community engagement is key to successfully controlling outbreaks.

Marburg virus is the causative agent of Marburg virus disease (MVD), a disease with a case fatality ratio of up to 88%, but can be much lower with good patient care. Marburg virus disease was initially detected in 1967 after simultaneous outbreaks in Marburg and Frankfurt in Germany; and in Belgrade, Serbia.

Marburg and Ebola viruses are both members of the Filoviridae family (filovirus). Though caused by different viruses, the two diseases are clinically similar. Both diseases are rare and have the capacity to cause outbreaks with high fatality rates.

Two large outbreaks that occurred simultaneously in Marburg and Frankfurt in Germany, and in Belgrade, Serbia, in 1967, led to the initial recognition of the disease. The outbreak was associated with laboratory work using African green monkeys (*Cercopithecus aethiops*) imported from Uganda. Subsequently, outbreaks and sporadic cases have been reported in Angola, the Democratic Republic of the Congo, Kenya, South Africa (in a person with recent travel history to Zimbabwe) and Uganda. In 2008, two independent cases were reported in travellers who had visited a cave inhabited by *Rousettus* bat colonies in Uganda.

Transmission

Initially, human MVD infection results from prolonged exposure to mines or caves inhabited by *Rousettus* bat colonies.

Marburg spreads through human-to-human transmission via direct contact (through broken skin or mucous membranes) with the blood, secretions, organs or other bodily fluids of infected people, and with surfaces and materials (e.g. bedding, clothing) contaminated with these fluids.

Health-care workers have frequently been infected while treating patients with suspected or confirmed MVD. This has occurred through close contact with patients when infection control precautions are not strictly practiced. Transmission via contaminated injection equipment or through needle-stick injuries

is associated with more severe disease, rapid deterioration, and, possibly, a higher fatality rate.

Burial ceremonies that involve direct contact with the body of the deceased can also contribute in the transmission of Marburg. People remain infectious as long as their blood contains the virus.

Symptoms of Marburg virus disease

The incubation period (interval from infection to onset of symptoms) varies from 2 to 21 days.

Illness caused by Marburg virus begins abruptly, with high fever, severe headache and severe malaise. Muscle aches and pains are a common feature. Severe watery diarrhoea, abdominal pain and cramping, nausea and vomiting can begin on the third day. Diarrhoea can persist for a week. The appearance of patients at this phase has been described as showing "ghost-like" drawn features, deep-set eyes, expressionless faces, and extreme lethargy. In the 1967 European outbreak, non-itchy rash was a feature noted in most patients between 2 and 7 days after onset of symptoms.

Many patients develop severe haemorrhagic manifestations between 5 and 7 days, and fatal cases usually have some form of bleeding, often from multiple areas. Fresh blood in vomitus and faeces is often accompanied by bleeding from the nose, gums, and vagina. Spontaneous bleeding at venepuncture sites (where intravenous access is obtained to give fluids or obtain blood samples) can be particularly troublesome. During the severe phase of illness, patients have sustained high fevers. Involvement of the central nervous system can result in confusion, irritability, and aggression. Orchitis (inflammation of one or both testicles) has been reported occasionally in the late phase of disease (15 days).

In fatal cases, death occurs most often between 8 and 9 days after symptom onset, usually preceded by severe blood loss and shock.

Diagnosis

It can be difficult to clinically distinguish MVD from other infectious diseases such as malaria, typhoid fever, shigellosis, meningitis and other viral haemorrhagic fevers. Confirmation that symptoms are caused by Marburg virus infection are made using the following diagnostic methods:

- antibody-capture enzyme-linked immunosorbent assay (ELISA)
- antigen-capture detection tests
- serum neutralization test
- reverse transcriptase polymerase chain reaction (RT-PCR) assay
- electron microscopy
- virus isolation by cell culture.

Samples collected from patients are an extreme biohazard risk; laboratory testing on non-inactivated samples should be conducted under maximum biological containment conditions. All biological specimens should be packaged using the triple packaging system when transported nationally and internationally.

Treatment and vaccines

Currently there are no vaccines or antiviral treatments approved for MVD. However, supportive care – rehydration with oral or intravenous fluids – and treatment of specific symptoms, improves survival.

There are monoclonal antibodies (mAbs) under development and antivirals e.g. Remdesivir and Favipiravir that have been used in clinical studies for Ebola Virus Disease (EVD) that could also be tested for MVD or used under compassionate use/expanded access.

In May 2020, the EMA granted a marketing authorisation to Zabdeno (Ad26.ZEBOV) and Mvabea (MVA-BN-Filo). against EVD. The Mvabea contains a virus known as Vaccinia Ankara Bavarian Nordic (MVA) which has been modified to produce 4 proteins from Zaire ebolavirus and three other viruses of the same group (filoviridae). The vaccine could potentially protect against MVD, but its efficacy has not been proven in clinical trials.

Marburg virus in animals

Rousettus aegyptiacus bats are considered natural hosts for Marburg virus. There is no apparent disease in the fruit bats. As a result, the geographic distribution of Marburg virus may overlap with the range of *Rousettus* bats.

African green monkeys (*Cercopithecus aethiops*) imported from Uganda were the source of infection for humans during the first Marburg outbreak.

Experimental inoculations in pigs with different Ebola viruses have been reported and show that pigs are susceptible to filovirus infection and shed the virus. Therefore, pigs should be considered as a potential amplifier host during MVD outbreaks. Although no other domestic animals have yet been confirmed as having an association with filovirus outbreaks, as a precautionary measure they should be considered as potential amplifier hosts until proven otherwise.

Precautionary measures are needed in pig farms in Africa to avoid pigs becoming infected through contact with fruit bats. Such infection could potentially amplify the virus and cause or contribute to MVD outbreaks.

Prevention and control

Good outbreak control relies on using a range of interventions, namely case management, surveillance and contact tracing, a good laboratory service, safe and dignified burials, and social mobilization. Community engagement is key to successfully controlling outbreaks. Raising awareness of risk factors for Marburg infection and protective measures that individuals can take is an effective way to reduce human transmission.

Risk reduction messaging should focus on several factors:

- **Reducing the risk of bat-to-human transmission** arising from prolonged exposure to mines or caves inhabited by fruit bat colonies. During work or research activities or tourist visits in mines or caves inhabited by fruit bat colonies, people should wear gloves and other appropriate protective clothing (including masks). During outbreaks all animal products (blood and meat) should be thoroughly cooked before consumption.
- **Reducing the risk of human-to-human transmission in the community** arising from direct or close contact with infected patients, particularly with their body fluids. Close physical contact with Marburg patients should be avoided. Gloves and appropriate personal protective equipment should be worn when taking care of ill patients at home. Regular hand washing should be performed after visiting sick relatives in hospital, as well as after taking care of ill patients at home.
- **Communities affected by Marburg** should make efforts to ensure that the population is well informed, both about the nature of the disease itself and about necessary outbreak containment measures.
- **Outbreak containment measures** include prompt, safe and dignified burial of the deceased, identifying people who may have been in contact with someone infected with Marburg and monitoring their health for 21 days, separating the healthy from the sick to prevent further spread and providing care to confirmed patient and maintaining good hygiene and a clean environment need to be observed.
- **Reducing the risk of possible sexual transmission.** Based on further analysis of ongoing research, WHO recommends that male survivors of Marburg virus disease practice safer sex and hygiene for 12 months from onset of symptoms or until their semen twice tests negative for Marburg virus. Contact with body fluids should be avoided and washing with soap and water is recommended. WHO does not recommend isolation of male or female convalescent patients whose blood has been tested negative for Marburg virus.

Controlling infection in healthcare settings

Healthcare workers should always take standard precautions when caring for patients, regardless of their presumed diagnosis. These include basic hand hygiene, respiratory hygiene, use of personal protective equipment (to block splashes or other contact with infected materials), safe injection practices and safe and dignified burial practices.

Healthcare workers caring for patients with suspected or confirmed Marburg virus should apply extra infection control measures to prevent contact with the patient's blood and body fluids and contaminated surfaces or materials such as clothing and bedding. When in close contact (within 1 metre) of patients with MVD, health-care workers should wear face protection (a face shield or a medical mask and goggles), a clean, non-sterile long-sleeved gown, and gloves (sterile gloves for some procedures).

Laboratory workers are also at risk. Samples taken from humans and animals for investigation of Marburg infection should be handled by trained staff and processed in suitably equipped laboratories.

Marburg viral persistence in people recovering from Marburg virus disease

Marburg virus is known to persist in immune-privileged sites in some people who have recovered from Marburg virus disease. These sites include the testicles and the inside of the eye.

- In women who have been infected while pregnant, the virus persists in the placenta, amniotic fluid and foetus.
- In women who have been infected while breastfeeding, the virus may persist in breast milk.

Relapse-symptomatic illness in the absence of re-infection in someone who has recovered from MVD is a rare event, but has been documented. Reasons for this phenomenon are not yet fully understood.

Marburg virus transmission via infected semen has been documented up to seven weeks after clinical recovery. More surveillance data and research are needed on the risks of sexual transmission, and particularly on the prevalence of viable and transmissible virus in semen over time. In the interim, and based on present evidence, WHO recommends that:

- Male Marburg survivors should be enrolled in semen testing programmes when discharged (starting with counselling) and offered semen testing when mentally and physically ready, within three months of disease onset. Semen testing should be offered upon obtention of two consecutive negative test results.
- All Marburg survivors and their sexual partners should receive counselling to ensure safer sexual practices until their semen has twice tested negative

for Marburg virus.

- Survivors should be provided with condoms.
- Marburg survivors and their sexual partners should either:
 - abstain from all sexual practices, or
 - observe safer sexual practices through correct and consistent condom use until their semen has twice tested undetected (negative) for Marburg virus.
- Having tested undetected (negative), survivors can safely resume normal sexual practices with minimized risk of Marburg virus transmission.
- Male survivors of Marburg virus disease should practice safer sexual practices and hygiene for 12 months from onset of symptoms or until their semen twice tests undetected (negative) for Marburg virus.
- Until such time as their semen has twice tested undetected (negative) for Marburg, survivors should practice good hand and personal hygiene by immediately and thoroughly washing with soap and water after any physical contact with semen, including after masturbation. During this period used condoms should be handled safely, and safely disposed of, so as to prevent contact with seminal fluids.
- All survivors, their partners and families should be shown respect, dignity and compassion.

5. OBESITY AND OVERWEIGHT

KEY FACTS

- Worldwide obesity has nearly tripled since 1975.
- In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 650 million were obese.
- 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese.
- Most of the world's population live in countries where overweight and obesity kills more people than underweight.
- 39 million children under the age of 5 were overweight or obese in 2020.
- Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016.
- Obesity is preventable.

What are obesity and overweight

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m²).

Adults

For adults, WHO defines overweight and obesity as follows:

- overweight is a BMI greater than or equal to 25; and
- obesity is a BMI greater than or equal to 30.

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals.

For children, age needs to be considered when defining overweight and obesity.

For children under 5 years of age:

- overweight is weight-for-height greater than 2 standard deviations above WHO Child Growth Standards median; and
- obesity is weight-for-height greater than 3 standard deviations above the WHO Child Growth Standards median.

Children aged between 5–19 years

Overweight and obesity are defined as follows for children aged between 5–19 years:

- overweight is BMI-for-age greater than 1 standard deviation above the WHO Growth Reference median; and
- obesity is greater than 2 standard deviations above the WHO Growth Reference median.

Facts about overweight and obesity

Some recent WHO global estimates follow.

- In 2016, more than 1.9 billion adults aged 18 years and older were overweight. Of these over 650 million adults were obese.
- In 2016, 39% of adults aged 18 years and over (39% of men and 40% of women) were overweight.
- Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2016.
- The worldwide prevalence of obesity nearly tripled between 1975 and 2016.

In 2019, an estimated 38.2 million children under the age of 5 years were overweight or obese. Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries, particularly in urban settings. In Africa, the number of overweight children under 5 has increased by nearly 24% percent since 2000. Almost half of the children under 5 who were overweight or obese in 2019 lived in Asia.

Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016. The prevalence of overweight and obesity among children and

adolescents aged 5-19 has risen dramatically from just 4% in 1975 to just over 18% in 2016. The rise has occurred similarly among both boys and girls: in 2016 18% of girls and 19% of boys were overweight.

While just under 1% of children and adolescents aged 5-19 were obese in 1975, more 124 million children and adolescents (6% of girls and 8% of boys) were obese in 2016.

Overweight and obesity are linked to more deaths worldwide than underweight. Globally there are more people who are obese than underweight – this occurs in every region except parts of sub-Saharan Africa and Asia.

What causes obesity and overweight?

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been:

- an increased intake of energy-dense foods that are high in fat and sugars; and
- an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing, and education.

What are common health consequences of overweight and obesity?

Raised BMI is a major risk factor for noncommunicable diseases such as:

- cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2012;
- diabetes;
- musculoskeletal disorders (especially osteoarthritis – a highly disabling degenerative disease of the joints);
- some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon).

The risk for these noncommunicable diseases increases, with increases in BMI.

Childhood obesity is associated with a higher chance of obesity, premature death and disability in adulthood. But in addition to increased future risks, obese children experience breathing difficulties, increased risk of fractures, hypertension, early markers of cardiovascular disease, insulin resistance and psychological effects.

Facing a double burden of malnutrition

Many low- and middle-income countries are now facing a “double burden” of malnutrition.

- While these countries continue to deal with the problems of infectious diseases and undernutrition, they are also experiencing a rapid upsurge in noncommunicable disease risk factors such as obesity and overweight, particularly in urban settings.
- It is not uncommon to find undernutrition and obesity co-existing within the same country, the same community and the same household.

Children in low- and middle-income countries are more vulnerable to inadequate pre-natal, infant, and young child nutrition. At the same time, these children are exposed to high-fat, high-sugar, high-salt, energy-dense, and micronutrient-poor foods, which tend to be lower in cost but also lower in nutrient quality. These dietary patterns, in conjunction with lower levels of physical activity, result in sharp increases in childhood obesity while undernutrition issues remain unsolved.

How can overweight and obesity be reduced?

Overweight and obesity, as well as their related noncommunicable diseases, are largely preventable. Supportive environments and communities are fundamental in shaping people’s choices, by making the choice of healthier foods and regular physical activity the easiest choice (the choice that is the most accessible, available and affordable), and therefore preventing overweight and obesity.

At the individual level, people can:

- limit energy intake from total fats and sugars;
- increase consumption of fruit and vegetables, as well as legumes, whole grains and nuts; and
- engage in regular physical activity (60 minutes a day for children and 150 minutes spread through the week for adults).

Individual responsibility can only have its full effect where people have access to a healthy lifestyle. Therefore, at the societal level it is important to support individuals in following the recommendations above, through sustained implementation of evidence based and population based policies that make regular physical activity and healthier dietary choices available, affordable and easily accessible to everyone, particularly to the poorest individuals. An example of

such a policy is a tax on sugar sweetened beverages.

The food industry can play a significant role in promoting healthy diets by:

- reducing the fat, sugar and salt content of processed foods;
- ensuring that healthy and nutritious choices are available and affordable to all consumers;
- restricting marketing of foods high in sugars, salt and fats, especially those foods aimed at children and teenagers; and
- ensuring the availability of healthy food choices and supporting regular physical activity practice in the workplace.

WHO response

Adopted by the World Health Assembly in 2004 and recognized again in a 2011 political declaration on noncommunicable disease (NCDs), the “*WHO Global Strategy on Diet, Physical Activity and Health*” describes the actions needed to support healthy diets and regular physical activity. The Strategy calls upon all stakeholders to take action at global, regional and local levels to improve diets and physical activity patterns at the population level.

The 2030 Agenda for Sustainable Development recognizes NCDs as a major challenge for sustainable development. As part of the Agenda, Heads of State and Government committed to develop ambitious national responses, by 2030, to reduce by one-third premature mortality from NCDs through prevention and treatment (SDG target 3.4).

The “*Global action plan on physical activity 2018–2030: more active people for a healthier world*” provides effective and feasible policy actions to increase physical activity globally. WHO published ACTIVE a technical package to assist countries in planning and delivery of their responses. New WHO guidelines on physical activity, sedentary behavior and sleep in children under five years of age were launched in 2019.

The World Health Assembly welcomed the report of the Commission on Ending Childhood Obesity (2016) and its 6 recommendations to address the obesogenic environment and critical periods in the life course to tackle childhood obesity. The implementation plan to guide countries in taking action to implement the recommendations of the Commission was welcomed by the World Health Assembly in 2017.