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tolerability of weekly DCF compared to standard DCF regimen.

23 Evaluation of Alanine Aminotransferase (ALT) and Gamma-glutamyl Transferase (GGT) in Patients with Type 2 Diabetes Mellitus at the Diabetic Clinic, Guyana

R Kurup, C Boston, R Ramdial, T Stuart, S Lewis-Isles

In this study, high prevalence of elevated Alanine Aminotransferase (ALT) and Gamma-glutamyl Transferase (GGT) in type 2 diabetic patients showed a strong association with metformin use and ethnicity, particular those of Indian decent. Family history showed a greater of high ALT followed by current illness.

27 Serum Ghrelin Levels in Patients with Chronic Urticaria and Atopic Dermatitis and Its Relationship with Metabolic Syndrome

B Demir, D Cicek, S Dertlioglu, S Aydin, H Ucak, C Ergin, I Erden

We detected higher serum ghrelin levels in the patients with chronic urticaria and atopic dermatitis than the controls. We think that the high levels of serum ghrelin in the patients with chronic urticaria and atopic dermatitis may be related to the other mechanisms.

32 Prevalence of Gastroesophageal Reflux Disease Symptoms in a Jamaican Population

DS Braham, MO Mills, MG Lee, EF Brown, G Gordon-Strachan

This study showed that the prevalence of Gastroesophageal Reflux Disease (GERD) symptoms in Jamaican subjects within the previous year was 71.1%. Weekly symptoms were present in 18.6% with moderate or greater severity in 11.7%. The most common and bothersome symptom was heartburn. Nocturnal symptoms occurred in 17.8%. In persons with GERD symptoms, 24.2% (P= 0.000) saw a doctor and 38.6% took medication (p<0.0001).

36 Prevalence of Obesity among Adult Surgical Patients in Tobago

AO Amata, VT Mitchell

Obesity is associated with several medical conditions and leads to a reduced quality of life and increased risks of morbidity and mortality. The prevalence of obesity among adult surgical patients in Tobago is high with females having a significantly higher percentage than males.

40 Primary Mediastinal Cysts: A Review of 41 Surgically Managed Cases

E Akar, T Erkinüresin

In this study, we aimed to review the surgical outcomes of primary mediastinal cysts and to determine the differences among various histopathological types. We retrospectively analyzed the medical records of 41 patients with primary mediastinal cysts who underwent surgical excision. We found that despite advanced non-invasive diagnostic techniques, the definitive diagnosis of primary mediastinal cysts can only be made by interventional techniques. Surgery often provides curative therapy with low morbidity and mortality.

46 Incidence and Prediction of Catheter-related Bladder Discomfort after Transurethral Resection of the Bladder Tumour

N Guo, D Su

This study showed that the incidence of catheter-related bladder discomfort (CRBD) was 82.3% at day 1 in patients undergoing transurethral bladder tumour resection (TURB) and reduced to 23.2% at day 3. Being male, and aged < 65 years, were identified as independent factors associated with moderate or severe CRBD.

50 Use of Additional Locking Plate-derived Poller Screws for Treatment of Femoral Non-union after Intramedullary Nailing

YF Zhao, QH Chang, L Han, FL Chu, B Wu, DL Jia, HB Wang, CY Meng, DX Zhang, YM Zha, FH Zhu

In this study, an additional locking plate and the derived Poller screw technique effectively improved local rotation instability and is an effective and simple treatment method for femoral non-union after intramedullary nailing.

56 Paediatric Galenic Preparations at the Saint Damien Hospital in Haiti: Formulative Study, Stability and Quality

F Baratta, R Cajuste, PH Saint Jean, E Ambreck, P Brusa

In the A.P.P.A.® galenic lab in Haiti, given the low availability of paediatric medicines, it was

EDITORIAL

Poor Routine Healthcare System Data Quality is a Major Obstacle to Clinical and Epidemiological Surgical Research in Developing Countries

JM East, Honorary Senior Lecturer in Surgery, FMS, UWI

Accurate, routine healthcare system data potentially begets valuable research if imagination, creativity, integrity and expertise are applied. Nonexistent, poorly recorded and inaccurate data foretell missed opportunities to answer critical research questions regarding clinical epidemiology of disease, treatment effectiveness and quality of care, and worse, generate misleading findings and conclusions that could jeopardize the well-being of patients if used to guide practice (1).

Although these truisms apply to retrospective observational clinical research in general, they are of special importance for the surgical research enterprise, in which retrospective analysis of prospectively collected data often provides the highest level of evidence practicable. Attempts have been made to quantify the percentage of surgical practice guided by the highest levels of evidence (well conducted randomized controlled trials (RCTs) and meta-analyses/systematic reviews of RCTs) (2), but such estimates are likely to be imprecise, given the complexity and multi-component nature of the specialty, with its preoperative, operative and postoperative elements. Notwithstanding, it has been estimated in one study, admittedly dated, that only 24% of procedures performed in surgery are based on RCT evidence (3). Even where RCTs are performed in surgery, design flaws have been reportedly identified in 56% (4). This has the potential to seriously mislead, since so much weight is accorded RCT evidence.

Why are RCTs not more commonly performed in Surgical Research?

RCTs serve a very specific and narrow purpose in medical research, namely, to determine the true comparative effectiveness of two or more interventions when doubt exists about which is the better or best option. There are several reasons why RCTs may not be necessary, possible, or constitute the best methodology for supporting evidence-based practice in surgery:

1. When an intervention has a dramatic, curative effect and there are no other treatment options (5). This category is becoming smaller as technology advances. For example, endovascular aneurysm repair is now an acceptable alternative to open aneurysmorrhaphy whereas just a relatively few years ago the latter was the only option.
2. Equipoise between treatment options cannot be established (4). Equipoise describes a condition in which there is doubt that either contending treatment option is superior or inferior to the other. For an RCT to be ethical, equipoise must be objectively established on the basis of existing data. Prospective participant surgeons and patients must also be convinced that equipoise exists between contending treatments, otherwise they will not be willing to participate in a RCT, thereby compromising the randomization process (4).
3. Placebo controlled RCTs are difficult in surgery, because equipoise still needs to be established between the tested intervention and no intervention, and sham surgery is unethical (5).
4. Blinding of participant surgeons and patients is usually impossible, thereby decreasing the bias-reducing effect of this procedure (5). Blinding independent assessors of study outcomes should be instituted whenever possible in surgical RCTs.
5. Standardization of procedures is difficult but critical, as different surgeons tend to practice small variations that may affect outcomes (5). The position of participating surgeons on their learning curve for the intervention must be determined and compensations made for this bias in the analysis (4).
6. Similar compensations must be made for variability in the severity of cases and other factors such as physiological status, that affect outcomes.
7. RCTs are expensive and require special expertise that may not be available in resource-constrained countries.

The Importance of Observational Study Designs, particularly Cohort Studies, as evidence in surgical practice

Well conducted cohort studies constitute the level of evidence below single RCTs (2). This versatile

observational study design is well suited to clinical and epidemiological surgical research, from incidence and outcome studies to survival analysis and comparative research, a much broader repertoire than that of RCTs and the source of cumulative evidence required to establish equipoise between treatments. In addition, cohort studies may be retrospective or prospective or both.

The first of the main weaknesses of comparative cohort studies is that they are subject to error from bias and confounding by known and unknown variables. The effect of known confounders may be interrogated using multivariable regression but accounting for unknown confounders requires randomization. However, a relatively recent statistical technique known as propensity score matching offers the prospects of approaching the effect of randomization. Several encouraging studies have demonstrated no difference between the estimates from RCTs and those derived from using propensity score matching to analyze the same data (6, 7).

A second is the high cost and long time required for completion of prospective cohort studies, both of which could be abrogated if retrospective analysis can be achieved with the same degree of reliability. But is that possible?

The great weakness of retrospective cohort studies is unreliability of the quality of the data. In some, mostly developed countries, this problem has been addressed through continuous, prospective collection and integration of structured, digitized, routine and non-routine (e.g., registry) health systems data. This provides a vast, reliable database for retrospective analysis. Too often results of such studies are inappropriately extrapolated to our populations in developing countries because there are no local statistics.

Improving Routine Data Quality in Developing Countries to drive reliable local research

Following are recommendation for improving routine health systems data collection in developing countries:

1. Transitioning from handwritten, paper- to digital-based recording systems. Handwriting is often undecipherable and paper-based systems subject to data loss from misplaced files and deterioration of the medium; records have to be searched manually to find relevant data. Digital-based systems are searchable electronically and can be backed-up and maintained/renewed indefinitely.
2. Transitioning from narrative- to predominantly structured, synoptic-based reporting of health system encounters with patients and population. Purely narrative-based reporting of patient encounters is notorious for missing important observations, the remedy being data capture via structured forms with limited narrative input.
3. Expansion of the range of variables currently recorded, such as structured data describing surgical operations and patient characteristics, like height and weight.
4. Enable direct input into the database by clinicians and clinical support staff from point-of-care encounters.
5. Upgrade skills, training and remuneration of Health Data Management staff.
6. Establishment of registries for recording of non-routine health systems data. Well run routine data collection systems provide seed information necessary to populate disease-based (e.g., cancer, trauma) or quality control registries. Registries, like routine databases, constitute a rich source of data for surgical research but require additional health system funding and dedicated staff.

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ORIGINAL ARTICLE

Clinical Presentation, Demographics and Outcomes of Cases of Tuberculosis at Princess Margaret Hospital, Nassau, The Bahamas 2014–2016

JH McIntosh[1, 2], KM Moss[1], NM Forbes[1, 2], MA Frankson[2]

ABSTRACT

Objectives: *To determine incidence of cases, demographics, clinical presentation, diagnostic methods and outcomes of cases of tuberculosis (TB) in The Bahamas, 2014–2016.*

Methods: *A retrospective chart review of cases of TB diagnosed at the Princess Margaret Hospital, Nassau, Bahamas. One hundred eighty-nine cases of active TB were diagnosed between 2014 and 2016, and all cases were evaluated for demographics, risk factors, clinical manifestation, method of diagnosis, symptoms and treatment outcomes.*

Results: *Of the 189 cases of notified TB between 2014 and 2016, 46 were reported in 2014, 60 in 2015 and 83 in 2016. The mean age was 37.96 (± 18.20) years old. One hundred sixty-four (86.8%) presented with symptoms, 19 (10.1%) were diagnosed by routine screening and 6 (3.2%) cases were diagnosed by contact tracing. One hundred nine (59.9%) were human immunodeficiency virus (HIV) negative and 73 (40.1%) were HIV positive. One hundred forty-four (76.2%) presented with cough, 84 (44.7%) weight loss, 80 (42.3%) fever, 44 (23.3%) night sweats, 43 (22.8%) chills, 32 (16.9%) fatigue and 25 (13.2%) haemoptysis. One hundred twenty-six (66.7%) completed the full course of antibiotic therapy, 29(15.3%) patients expired before completing treatment and 18 (9.5%) of patients defaulted.*

Conclusion: *Human immunodeficiency virus is a major risk factor for TB in The Bahamas, and it is advised that all patients diagnosed with TB be tested for HIV. We also advise screening HIV positive patients for TB. Screening other high-risk groups such as migrant populations would also reduce the amount of latent TB cases, which may progress to active TB.*

Keywords: Clinical presentation, The Bahamas, treatment outcome, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases known to man, yet the World Health Organization reports that TB is one of the top 10 causes of death worldwide (1). Despite research efforts and numerous initiatives to control the disease worldwide, TB has resulted in the death of 1.8 million people and 10.4 million new cases in 2015 (1). The Bahamas which is considered a country of intermediate burden for TB has not been exempted from this public health issue. According to the World Bank, the incidence of TB in The Bahamas for 2015 was 18 per 100 000 persons and over the past 10 years has fluctuated from 21 per 100 000 in 2006 to 9.9 per 100 000 in 2012 (2). Various factors have made the eradication of TB in The Bahamas difficult, such as high rates of human immunodeficiency virus (HIV) infection and immigration from countries with high TB prevalence. In understanding the epidemiology and risk factors of TB cases in The Bahamas, the development of protocols can improve screening procedures and decrease disease burden.

METHODS

This study was a retrospective chart review conducted at the Princess Margaret Hospital (PMH), Nassau, Bahamas. Ethical approval was given by the local ethics committee. Data was collected from the patients' medical records in the medical records department as well as the admissions log book at the PMH Chest Clinic. Here,

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189 cases of active TB were diagnosed at PMH between 2014 and 2016, and all cases were evaluated. Cases of TB are defined as patients who presented with clinical and/or radiologic signs suggestive of TB for whom the clinical decision was made to treat with standard anti-TB therapy regardless of *Mycobacterium tuberculosis* culture positivity.

Variables extracted for analysis included age, gender, year of diagnosis, nationality, HIV status (including CD4, viral load and medication history of HIV positive patients), street address (for global positioning system (GPS) mapping), risk factors (diabetes mellitus (DM), cigarette smoking, alcohol (ETOH) use, history of travel to an endemic area, contact with TB patients or other sill patients with respiratory symptoms), clinical manifestation, method of diagnosis, symptoms, radiologic findings and tuberculin skin test results.

Data was inputted into a spreadsheet and imported into a current version of the IBM SPSS Statistics application software (Chicago, IL, USA) for descriptive and inferential data analysis.

RESULTS

There were 201 cases of TB reported in The Bahamas between 2014 and 2016, and 189 of these cases were reported at the PMH, Nassau, Bahamas. Eleven other cases were reported at Doctors Hospital, Nassau, Bahamas and RAND Memorial Hospital Freeport, Bahamas. Of note, 46 cases were reported in 2014, then 60 in 2015 and 83 in 2016. Incidence per 100 000 for these years was 12.5, 16.1 and 22.0 cases per 100 000, respectively.

Demographics and risk factors are summarized in Table 1.

Table 1: Socio-demographic profile and risk factors of study's participants

Parameter	n (%)	Parameter	n (%)
Age (years)		**Gender**	
<20	33 (17.5)	Male	106 (56.1)
20–40	65 (34.4)	Female	83 (43.9)
40–60	70 (37.0)	HIV	
>60	21 (11.1)	Status known	182
Mean (SD)	37.96 (± 18.20)	HIV negative	109 (59.9)
Nationality		HIV Positive	73 (40.1)
Bahamian	115 (60.8)	Status not known	7
Foreign born	74 (39.2)	Diabetes mellitus	18 (9.7)
Haitian	67 (35.4)	Cigarette smoker	35 (18.5)
Jamaican	5 (2.6)	ETOH use	24 (12.7)
Other	2 (1.1)	Travel to endemic area	14 (7.4)
		Ill/TB contact	24 (12.7)

HIV = human immunodeficiency virus; TB = tuberculosis; ETOH = ethyl alcohol; SD = standard deviation.

Method of diagnosis

Of the 189 cases in this study, 164 (86.8%) presented with symptoms, 19 (10.1%) were diagnosed by routine screening and 6 (3.2%) by contact tracing.

Clinical presentation

In this study, 182 (96.3%) had pulmonary TB and 7 (3.7%) patients had extra-pulmonary manifestations of TB: four TB lymphadenitis, two TB meningitis and one disseminated TB. Clinical presentation ranged from six symptoms to none of the typical symptoms for TB. Table 2 summarizes the frequency of symptoms as well as the number of presenting symptoms of patients. The median number of symptoms was 2 (interquartile range: 1, 4).

Table 2: Frequency and number of symptoms in the study's participants

Symptom	n (%)	Number of symptoms	n (%)
Cough	144 (76.2)	0	30 (15.9)
Weight loss	84 (44.7)	1	28 (14.8)
Fever	80 (43.3)	2	49 (25.9)
Night sweats	44 (23.3)	3	33 (17.5)
Chills	43 (22.8)	4	21 (11.1)
Fatigue	32 (16.9)	5	25 (13.2)
Haemoptysis	25 (13.2)	6	3 (1.6)

Treatment outcomes

Of the 189 cases studied, 126 (66.7%) completed the full course of antibiotic therapy for TB. In total, 29 (15.3%) patients expired before completing treatment, 18 (9.5%) defaulted from treatment, 4 (2.1%) were reported to have returned to their home country before completing treatment and 1 (0.5%) was still receiving antibiotic therapy due to multidrug resistant TB. Treatment outcome was not found for 11 (5.8%) patients.

Global positioning system mapping of cases

Coordinates of cases from New Providence were plotted on Google map using GPS visualizer website and are seen in the figure.

DISCUSSION

There were 106 (56.1%) males and 83 (43.9%) females in this study. Other studies reviewed also demonstrated a male predominance ranging from 53.8% to 77.3% (3–10). In these studies, it was suggested that males may have a higher rate of TB infection due to increased prevalence of risk factors such as smoking and HIV co-infection. This study showed that it was statistically significant

Figure: GPS mapping of cases of TB 201-42016 in New Providence, The Bahamas. GPS = global positioning system; TB = tuberculosis.

that males were more likely to have a history of cigarette smoking ($p < 0.001$), ETOH abuse ($p < 0.001$) and night sweats ($p = 0.028$).

The mean age of the 189 cases was 37.96 (± 18.20) years old. One hundred thirty-five (71.4%) of the patients in this study were in the young and middle-age-groups (20–59). There were fewer cases seen in the < 20 and > 60 population. Here, 33 (17.5%) cases were < 20 years, and 21 (11.1%) cases were ≥ 60 years of age. In this study, some known risk factors that likely contribute to this include HIV positivity, having DM, having an ill TB contact and a history of consuming > 40 g of ETOH per day with the mean ages being 42.64 (± 1.67), 51.44 (± 2.70), 28.50 (± 4.47) and 45.13 (± 3.02) years, respectively. With respect to nationality, it was shown that 115 (60.8%) patients were Bahamian and 74 (39.2%) were foreign born with the majority of the foreign-born patients being form Haiti. Immigrant populations have been regarded as potential carriers of diseases depending on the prevalence of the disease within the country of origin. In some countries such as Switzerland and United States, it was reported that the majority of patients reported to have TB were foreign born being 74% (9) and 51.2% (11), respectively. On the other hand, in other report which evaluated 58 008 cases of TB in the European union showed that in 2014, 27% of cases of TB were from patients of foreign origin which was a 7% increase from 2005 (12).

In this study, 18 (9.7%) patients were documented to be diabetic. Diabetes mellitus is considered a risk factor for TB due to its suppressive effects on the immune system (13). A population-based study in the United Kingdom found DM to be an independent risk factor for TB with the prevalence of 16.5 in diabetics and 13.5 in non-diabetics per 100 000. In a study by Magee *et al*, the prevalence of DM was 11.6% (of 318 patients) (7).

In this study, HIV status for 182 (96.3%) of patients was documented. This was a significant number considering that in other studies that were reviewed, the HIV testing rate for patients with TB ranged from 38.1%–87.1% (5, 14–16). Seventy-three (40.1%) of the 182 patients tested in this study were HIV positive. Numerous studies assessed TB-HIV coinfection rates, and these percentages varied. Gao *et al* did a systematic review and meta-analysis of 'Prevalence of TB/HIV Co-Infection in Countries Except China'. They evaluated 46 studies with a combined population of 272 466. The estimated prevalence of TB/HIV co-infection ranged from 2.93% to 72.34% (17).

In this study, the most common symptom was cough (76.2%), followed by weight loss (44.7%), fever (42.3%), night sweats (23.3%), chills (22.6%), fatigue (16.9%) and haemoptysis (13.2%). It was noted that most patients presented with three signs/symptoms (25.9%) and 30 (15.9%) of the cases did not present with any of the classic signs/symptoms of TB. In various studies that were reviewed, cough was the most common complaint ranging from 46.7% to 100% (8, 10, 18). In other studies, the frequency of other symptoms were varied, in one study of 372 patients night sweats was seen in 127 (34.1%) and fever was seen in 123 (33%) (10). In another study by Rathman *et al* of 340 patients' frequency of

symptoms reported included cough (100%), weight loss (97.4%), fever (94.4%), night sweats (77.9%) and haemoptysis (35.9%) (8).

In this study, of the 189 cases, 66.7% completed the full course of treatment, 15.3% expired before completing treatment and 9.5% of patients defaulted. It should be noted that for expired patients it was not specified whether the cause of death was due to TB or other causes. Faustini, Hall and Perucci conducted a systematic review of TB cases in Europe to assess outcomes. They reported that of 26 studies reviews 74.4% of cases had a successful outcome, 12.3% of cases had an unsuccessful outcome (treatment failure, defaulters, lost to follow up, transferred-out) and 6.8% of patients died (19). Antoine and Che evaluated outcomes for TB cases in France for 2009. Outcomes were available for 2316 out of 3667 cases; they reported successful treatment for 70% of cases. Of the remaining 30% who did not complete treatment 32% died, 32% were lost to follow-up, 17% transferred out, 14% were still receiving treatment and 5% had treatment stopped prior to completion (20).

CONCLUSION

This study shows that HIV remains a major risk factor for TB in The Bahamas, and it is advised that all patients suspected or diagnosed with TB be tested for HIV as well as screening HIV positive patients for TB. In addition, screening other groups such as migrant populations would also be of benefit to reduce the amount of latent TB cases, which may progress to active TB.

AUTHOR CONTRIBUTIONS

JH McIntosh conceived paper, collected data, conducted data analysis and interpretation, wrote manuscript and approved final version. KM Moss provided oversight to study, participated in study design, data interpretation and revision of manuscript and approved final version. NM Forbes participated in study design, data interpretation and revision of manuscript and approved final version. MA Frankson participated in study design, data analysis and interpretation, critically revised manuscript and approved final version. The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Association between Mannose-Binding Lectin 2 Gene Polymorphism and Liver Fibrosis in Patients with Chronic Viral Hepatitis

AT Eminler[1], M Karkucak[2,7], S Gurel[3], T Ayyildiz[4], T Gulten[5], K Irak[6], T Yakut[5,7]

ABSTRACT

Objective: *Mannose-binding lectin (MBL) has become a popular molecule in investigations on basic and clinical gastroenterology and contributed to new approaches to the understanding of infectious and immune diseases associated with intestine and liver. The aim of the present study was to investigate the association between codon 54 polymorphisms in* MBL2 *gene coding MBL and predisposition to fibrosis in patients with viral hepatitis B and C.*

Methods: *One hundred patients with chronic hepatitis (70 hepatitis B, 30 hepatitis C) who underwent liver biopsy and 100 healthy controls with no known chronic disease were included in the study. Patients in both viral hepatitis groups were divided into two groups according to their fibrosis scores with Ishak scoring system. The polymerase chain reaction–restriction fragment length polymorphism method was applied to determine the MBL2 codon 54 polymorphisms. For the statistical analysis, the level of significance was set at $p < 0.05$.*

Results: *No significant differences in allele frequencies for any polymorphism were observed between patients and controls, although the G allele was more frequent in the patient groups ($p > 0.05$). In the comparison in terms of G and A alleles between two groups, hepatitis B patients in Group-II (group with high fibrosis score) were found to have a significantly higher frequency of A alleles ($p = 0.027$).*

Conclusion: *Although it is accepted that* MBL2 *polymorphism plays a part during hepatitis B virus and hepatitis C virus infections, larger studies investigating the relation between MBL2 polymorphism and disease progression, and treatment are required.*

Keywords: Fibrosis, hepatitis B, hepatitis C, *MBL2* gene, polymorphism.

INTRODUCTION

Mannose-binding lectin (MBL), which is the one of innate immune system pattern recognising molecules, is a C-type serum lectin. It is a molecule that recognizes major soluble patterns and plays a significant role in the innate immunity by activating the complement pathway synthesized by the hepatocytes and phagocytosis (1, 2). Its specific structure consists of the collagenous region and lectin domain (3). Lectin contributes to the elimination of many microorganisms through complement pathway and opsonophagocytosis (4). Due to genomic polymorphisms in *MBL2* gene, there are differences in the serum MBL levels of people (5). Therefore, polymorphisms influencing the serum levels of protein may lead to infections and predisposition to autoimmune diseases.

Mannose-binding lectin is coded by *MBL2* gene located on chromosome 10 and containing four exons. It is the only collection with the ability to activate the complement system (1). Recently, it has become a

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popular subject in investigations on basic and clinical gastroenterology and contributed to new approaches to the understanding of infectious and immune diseases associated with intestine and liver.

For *MBL2* codon 54 polymorphism, normal allele is called G and the variant allele is called A. The aim of the present study was to investigate the association between codon 54 polymorphisms in *MBL2* gene coding MBL and predisposition to fibrosis in patients with viral hepatitis B and C.

SUBJECTS AND METHODS

Study subjects

One hundred patients with chronic hepatitis (70 hepatitis B, 30 hepatitis C) who underwent liver biopsy in Gastroenterology Department of Uludağ University Faculty of Medicine and 100 healthy controls with no known chronic disease were included in the study. Patients in both viral hepatitis groups were divided into two groups according to their fibrosis scores with Ishak scoring system (6). Those with the fibrosis score of 3 or lower were defined as Group-I and those with a score of 4 or over as Group-II. The study was conducted in accordance with the Declaration of Helsinki and Principles for Good Clinical Practice and was approved by the local Ethics Committee (2009-12/96). Prior to the study inclusion, all patients read and signed the informed consent form. After signing an informed consent from each patient, a 2 mL of blood taken into ethylenediamine tetraacetic acid (EDTA) tubes for the *MBL2* gene polymorphisms and were stored at −20°C.

Deoxyribonucleic acid extraction and genotyping

Blood samples from both the patient and the control groups were taken in EDTA tubes. Deoxyribonucleic acid isolation was performed according to the procedures of the Dr. Zeydanlı (DZ) DNA isolation kit, and samples were stored at −20°C until polymerase chain reaction (PCR) assay was done.

Mannose-binding lectin 2 gene codon 54 polymorphism was determined using the (PCR)-restriction fragment polymorphism method. For the *MBL2* gene codon 54 polymorphisms, forward 5′-TAGGACAGAGGGCATGCTC-3′ and reverse 5′-CAGGCAGTTTCCTCTGGAAGG-3′ primers were used (7). To identify the *MBL2* gene codon 54 polymorphisms among the products, the *Ban I* enzyme was used. In the analysis conducted in 2% agarose gel after cutting the enzyme, genotypes were determined as follows: if the 349 bp PCR product from the *MBL2* gene was cut into two distinct products of 260 bp and 89 bp, then the genotype was identified as G/G; if three distinct products were formed as 349 bp, 260 bp and 89 bp, then the genotype was identified as G/A and if the product was 349 bp, then the genotype was identified as A/A.

Statistical analysis

The data were analysed using SPSS 13.0 software (IBM Corp., NY, USA). The data was recorded in ± standard deviations. The Mann–Whitney *U* test used to compare the age between the two groups. The Chi-square (χ^2) test was used to compare genotypes. *p*-values smaller than 0.05 were accepted as being statistically significant.

RESULTS

In this study, among the 100 cases in the patient group (53 males and 47 females), the mean age was 43.98 ± 12.75, and among the 100 cases in the control group (54 males and 46 females), the average age was 43 ± 12.46. There was no difference in the age and gender between the patient and control groups.

Regarding the codon 54 polymorphism of the *MBL2* gene in the study, among the 100 chronic hepatitis B and C patients, 73 were identified with the G/G genotype, 16 with the G/A genotype and 11 with the A/A genotype. Among the 100 individuals in the control group, 63 were identified with the G/G genotype, 19 with genotype G/A and 18 with the A/A genotype. Using the subjects with the G/G homozygote genotype as a reference group, we found no association between the G/A and G/A genotypes and the risk of chronic hepatitis B and C with statistical analysis ($p > 0.05$). No significant differences in allele frequencies for any polymorphism were observed between patients and controls, although the G allele was more frequent in the patient groups ($p > 0.05$) (Table 1).

Table 1: MBL2 gene codon 54 allele frequency and genotype distribution among chronic hepatitis patients and control group

Genotype/Allele	Patient group (Chronic hepatitis B and C) (n = 100)	Control group (n = 100)	*p*-Value
G/G Genotype	73	63	1 (Reference)
G/A Genotype	16	19	0.92
A/A Genotype	11	18	0.59
G allele frequency (%)	81	78	0.59
A allele frequency (%)	19	22	

Chronic hepatitis B

Overall, 70 patients (40 male, 30 female) patients at the mean age of 43.17 ± 11.94 who underwent liver biopsy with the diagnosis of chronic hepatitis B were included in the study. Fifty-four patients were found to have (77.14%) GG genotype, 8 (11.43%) GA genotype, and 8 (11.43%) AA genotype.

In the comparison of presence of A allele and absence of A allele groups, it was determined that patients in Group-II, high fibrosis score group, has a higher frequency of presence A allele with a difference approaching near significance ($p = 0.052$). In parallel to these results, the comparison in terms of G and A alleles between two groups, hepatitis B patients in Group-II, (the group with high fibrosis score), was found to have significantly higher frequency of A alleles ($p = 0.027$) (Table 2).

Table 2: Analysis of genotypes and alleles according to chronic hepatitis B fibrosis groups

Hepatitis B	Group-I (Fibrosis score ≤3)	Group-II (Fibrosis score ≥4)	*p*-Value
GG genotype			
(n) (%)	48 (81%)	6 (54%)	0.11
GA genotype			
(n) (%)	6 (10%)	2 (18%)	
AA genotype			
(n) (%)	5 (9%)	3 (28%)	
Absense A allele (GG)			0.052
(n) (%)	48 (81%)	6 (54%)	
Presense A allele (GA + AA)			
(n) (%)	11 (19%)	5 (46%)	
G allele			
(n) (%)	102 (86%)	14 (64%)	0.027
A allele			
(n) (%)	16 (14%)	8 (36%)	

Chronic hepatitis C

Overall, 30 patients (17 females, 13 males) patients at the mean age of 45.8 ± 14.5 were included in the study. Nineteen patients had (63.3%) GG genotype, 8 (26.6%) GA genotype and 3 (10.1%) AA genotype. In terms of presence of A allele, no significant difference was found between three groups. Also, no difference was found between presence A allele and absence A allele groups also. No significant difference was found between two groups in terms of the frequency of G and A alleles ($p > 0.05$) (Table 3).

Table 3: Analysis of genotypes and alleles according to chronic hepatitis C fibrosis groups

Hepatitis B	Group-I (Fibrosis score ≤3)	Group-II (Fibrosis score ≥4)	*p*-Value
GG genotype			
(n) (%)	16 (64%)	3(60%)	0.60
GA genotype			
(n) (%)	6 (24%)	2 (40%)	
AA genotype			
(n) (%)	3(12%)	0 (0 %)	
Absense A allele (GG)			0.86
(n) (%)	16 (64%)	3 (60%)	
Presense A allele (GA + AA)			
(n) (%)	9 (36%)	2 (40%)	
G allele			
(n) (%)	38 (76%)	8 (80%)	0.78
A allele			
(n) (%)	12 (24%)	2 (20%)	

DISCUSSION

The functions of MBL are complement system activation, regulation of apoptosis and also opsonization and modulation of inflammation (5). Mannose-binding lectin is coded by *MBL2* gene located on Chromosome 10 (10q11.2-q21) and contains four exons. Polymorphisms in exon 1 and promoter regions of *MBL2* gene were reported to be associated with lower MBL serum levels. It is known that structural polymorphisms in the first exon of the gene such as codons 52, 54 and 57 also cause functional deficiency by impairing the oligomerization of protein (8). Therefore, these structural polymorphisms influencing MBL serum levels may lead to the predisposition to viral and bacterial diseases.

Personal factors influencing the development of liver damage in viral hepatitis are as follows: sex, age, ethnic origin, duration of infection, alcohol intake, dual infections with human immunodeficiency virus/hepatitis B virus (HBV)/hepatitis C virus (HCV) and genotype of the virus. In addition, the importance of the immune state of the person is one of the issues recently addressed. In relation to this issue, polymorphisms of genes coding pro-inflammatory cytokines, vitamin D receptor, and human leukocyte antigen types are under consideration.

It is known that primarily Th1 response develops against hepatitis B infection and in cases where response remains inadequate, the disease enters the process of becoming chronic. In various studies, the importance of innate immune response in viral infections was stressed, as in all other infectious conditions (9). It has been suggested that MBL plays a part in the clearance of virus

through direct effect of complement activation in hepatitis B infection and in addition decreases inflammatory damage in liver tissue by reducing the release of pro-inflammatory cytokines (10, 11).

The effect of *MBL2* gene polymorphism and MBL serum levels on the course of chronic hepatitis B has been reported so far in few studies. Despite the differences in cohort characteristics, experimental approaches and in investigated polymorphisms, many of these studies found relation between the degree of disease caused by HBV and polymorphisms in *MBL2* gene and resultant low MBL levels. In various studies, an association was found between *MBL2* polymorphisms and viral persistence, advanced disease, HBV acquisition and survival in fulminant hepatic disease. However, there are also studies suggesting the contrary.

The relation between *MBL2* gene polymorphisms and persistence of HBV was first revealed in 1996 (12). In this study, they showed in Caucasian patients an association of the codon 52 polymorphism of the *MBL2* gene with persistent HBV infection. In a study carried out in 1999, it was maintained that codon 54 polymorphism was influential in the persistence and progression of disease, and it was also shown that the probability of the development of symptomatic cirrhosis and spontaneous bacterial peritonitis was higher in adult HBV patients with codon 54 polymorphism. Thus, it was proposed that if people with polymorphism are identified, follow-up approaches may change, and prophylactic treatment against infections may be beneficial to these patients (13). In a study carried out in Vietnam, codon 54 polymorphism was found to be more frequent in the people who have acute hepatitis B. In the same study, an association was found between codon 54 polymorphism and high viral load and transaminase values, and it was suggested that MBL was directly effective in HBV clearance (14). Another study showed that MBL polymorphisms decreases survival in fulminant hepatitis caused by HBV. It was also thought that serum MBL levels may be used as a predictive factor for survival in these patients (15).

Overall, 527 patients were examined in an interesting study, and it was demonstrated that in people without polymorphism, hepatitis B infection resulted in natural immunity, while in cases with codon 54 polymorphism and low serum MBL levels, persistent disease occurred significantly more common. Investigators suggested that low chronic MBL levels are related to persistence of disease (16). In another study, 320 HBsAg carriers, 199 HBV-related cirrhosis and hepatocellular cancer (HCC) patients and 87 HBV infection patients with undergoing spontaneous seroconversion were compared with respect to *MBL2* gene polymorphisms (17). They did not find any relation between the *MBL2* gene polymorphism and MBL level in those who are HBsAg carriers, who have spontaneous seroconversion and in healthy controls. However, in patients who have MBL genotypes with presence of A allele, the risk of advanced disease (cirrhosis, HCC, *etc*) was found to be increased threefold.

In contrast, some publications resulted otherwise. In 1998, codon 52 and 54 polymorphisms were found to have no association with chronic hepatitis B in a German study (18). Similarly, in a study carried out in 2005 in Korea, no association was seen between codon 54 polymorphism and clearance of hepatitis B infection or progression of chronic hepatitis B infection (19).

In spite of the presence of a few articles arguing for the opposite view, the idea that polymorphisms causing a decrease in serum level of MBL have an adverse effect on the prognosis of hepatitis B infection is becoming more dominant.

In our study, 54 patients were found to have (77.14%) GG genotype, 8 (11.43%) have GA genotype and 8 (11.43%) have AA genotype in the hepatitis B group. High fibrosis, score group, has a higher rate of codon 54 polymorphism with a difference approaching significance ($p = 0.052$). In parallel to these results, in the comparison in terms of G and A alleles between two groups, patients in Group-II, (the group with high fibrosis score), were found to have significantly higher prevalence of A alleles ($p < 0.05$).

The role of MBL in chronic hepatitis C has not been clearly defined in studies performed to date. In a few studies, it was thought that high MBL levels have a positive correlation with pathology and response to treatment. These studies are different in terms of cohort characteristics, classification of cases and the investigated *MBL2* gene polymorphisms.

In a study published in 1998, it was established that homozygote genotype in codon 54 was associated with weak response to interferon treatment in patients with chronic hepatitis C (20). In another study performed in 2000, 52 patients with HCV infection were compared with 50 healthy controls; patients with codon 54 polymorphism were found to have more advanced disease, and it was thought that MBL may be one of the factors influencing the course of HCV infection (21).

In 2006, 100 hepatitis C patients were compared with the control group; codon 54 polymorphism was found to be more frequent in the patient group, and it was

concluded that codon 54 polymorphism may be a risk factor for HCV infection in another study (22). *Mannose-binding lectin 2* gene polymorphism was found to occur at a significantly higher rate in cases with HCV infection than healthy controls in more recent studies, and it was also determined that response to pegylated interferon (Peg-IFN) and ribavirin treatment was lower in cases with polymorphism, even though the difference was not statistically significant (23). Another study published in 2008 stated that polymorphisms in *MBL2* gene exon-1 region were associated with low MBL levels and the progression of HCV infection towards liver inflammation and fibrosis (24). Also, *MBL2* gene polymorphism was suggested to be directly related to progression of chronic hepatitis C and response to Peg-IFN therapy (25). In a most recent study, it was reported that *MBL2* variant alleles and hence low MBL levels increase the predisposition to HCV infection, and that HYO haplotype is associated with the severity of fibrosis (26). In a study comparing MBL/MASP-1 activity in a patient group with HCV infection and a healthy control group, a positive correlation was found between liver fibrosis and MBL serum levels. In addition, a significant relation was found between the MBL/MASP-1 activity and the HCV-related liver fibrosis (9).

In a few studies, no significant association was found between MBL2 gene polymorphisms and accordingly low MBL levels, and predisposition to HCV infection, disease progression and response to treatment (27, 28).

In the present study, among patients in the chronic hepatitis C group, 19 had (63.3%) GG genotype, 8 (26.6%) GA genotype and 3 (10.1%) AA genotype. There was no statistically significant difference between fibrosis groups in terms of the presence of A allele.

It is difficult to determine the role of MBL in viral hepatitis accurately since *MBL2* gene polymorphisms and MBL serum levels vary significantly between and within populations. To our knowledge, there is no study in the literature which investigates the association between *MBL2* gene polymorphisms and liver histopathology directly. In the present study, among chronic hepatitis B patients, fibrosis was found to be higher in presence of the A allele carrying group, suggesting that individuals with these polymorphisms should be examined earlier for liver damage and if necessary for treatment.

Although it is accepted that MBL plays a part in the course of HBV and HCV infections, larger studies investigating the association between MBL levels and disease progression, and treatment are required. It is suggested that other single nucleotide polymorphisms in exon1 and promoter regions of *MBL2* gene should be investigated, and accordingly, it is hoped that with the determination of changing MBL serum levels, information predictive of the course of disease and response to treatment can be obtained.

AUTHORS' NOTE

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Breast Cancer Receptor Profiles in Jamaica: A 6-year Analysis

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ABSTRACT

Objective: *To determine the breast cancer immunohistochemistry (IHC) receptor status for tests performed at the University of the West Indies (UWI) from January 2002 to December 2007 and to investigate for an association between receptor profile and patient age, tumour grade and stage.*

Methods: *The UWI breast cancer IHC receptor database was examined to determine receptor profile, patient age, tumour histology, grade, size and lymph node status.*

Results: *In total, 1383 breast cancer cases were tested for oestrogen receptor (ER) and human epidermal growth factor receptor 2 (HER 2) statuses during the study period; progesterone receptor (PR) testing was not available. Receptor profiles were: ER+/HER2− (50.2%), ER−/HER2− (28.1%), ER+/HER2+ (15.3%) and ER−/HER2+ (6.4%). Across all age groups, ER+/HER2− was the most frequent profile (45–52%) and ER−/HER2− was the second most frequent (27–34%). There was no statistically significant association between the receptor profile and age (*p *= 0.079). Amongst Grade III tumours, ER−/HER2− was the most prevalent profile (44.6%); ER+/HER2− was the most prevalent for Grade I and Grade II tumours (60.7 and 48.8%, respectively). There was a statistically significant association between the receptor profile and tumour grade (*p *= < 0.001). There was no statistically significant association between the receptor profile and tumour stage (*p *= 0.359).*

Conclusion: *The prevalence of ER/HER2-negative breast cancer was 28%, in keeping with triple-negative breast cancer (TNBC) prevalence in African-American populations. There was a statistically significant association between the receptor profile and tumour grade (*p *< 0.001) (most Grade III tumours were ER−/HER2−), in keeping with the biologically aggressive behaviour of TNBC.*

Keywords: Breast cancer receptors, immunohistochemistry, Jamaica.

INTRODUCTION

Breast cancer is the most common malignancy among Jamaican women, with an age-standardized incidence rate of 43.1 per 100 000 (1). Breast cancer prognostication has traditionally been derived from tumour-related histopathological variables of size, histological grade and nodal status. The Nottingham Prognostic Index is a well-validated prognostic system that uses these criteria to determine prognosis following surgery for breast cancer (2). While these pathological criteria remain relevant, breast cancer molecular profiling has become increasingly important in prognostication. Biomarkers such as the oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2)-neu oncogene (HER2) are not only important prognostic markers but are also predictive of response to targeted therapies (3). The triad of ER, PR and HER2 status has therefore become a mandatory part of therapeutic decision making in breast cancer management (4), with the predictive value of these biomarkers influencing the choice of systemic therapy.

In Jamaica, breast cancer receptor status testing is centralized to the Immunohistochemistry (IHC) Laboratory in the Department of Pathology, University

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of the West Indies (UWI), which undertakes testing for the entire island. The laboratory receives requests for IHC breast receptor studies from the University Hospital of the West Indies (UHWI), the teaching hospital with which the UWI is affiliated, the National Public Health laboratory (the government laboratory facility), regional pathology laboratories (*eg*, Mandeville Regional Hospital, Cornwall Regional Hospital) and several private pathology laboratories.

The UWI Department of Pathology IHC breast receptor database was established in 2002 and records results of tests performed on tumour tissue from patients with breast cancer. In addition, the database contains records from the histopathology report for each patient, including age, tumour histological type, grade, size and lymph node status (if nodal sampling was performed).

In this report, we will present results from the breast cancer IHC breast receptor database for tests performed during the 6-year period following its establishment. During this period, breast IHC breast receptor testing was limited to ER and HER2 (PR testing was not offered); hence, only results of ER and HER2 are available and will be presented.

SUBJECTS AND METHODS

We reviewed the results of all IHC studies for the assessment of breast cancer expression of ER and HER2, performed at the UWI between January 2002 and December 2007. The biopsy specimens were from patients diagnosed at the UHWI as well as cases referred from other hospitals. The samples included breast core biopsies and mastectomy specimens. The IHC breast receptor database was examined to determine the ER/HER2 receptor profiles for all breast cancer specimens submitted for receptor testing during the study period, to record patient age, tumour histological type and grade, as well as tumour size and lymph node status if nodal sampling was performed.

Protocol for immunostaining for oestrogen receptor and HER-2-Neu

The fixative for all internal and external cases was 10% phosphate-buffered formalin. The most appropriate block for performance of IHC studies was chosen after review of the H&E-stained slides. Paraffin sections were cut at 2–4 μm, mounted on slides coated with poly-L-lysine solution, dried and then deparafinnized in xylene and rehydrated in decreasing strengths of ethanol to deionized water, then rinsed in phosphate-buffered saline. Blocking of non-specific background binding was carried out using Universal Blocking Reagent. Incubations were performed with primary monoclonal antibodies: heat shock protein (HSP) 27 (Biogenex, Cat. No. AM171-5M) and HER-2-neu (Biogenex, Cat. No. AM 134-5M). Antigen/Antibody interaction was developed using anti-mouse immunoglobulin and conjugated streptavidin as a substrate for HSP and diaminobenzidine for HER-2/neu. The primary monoclonal antibody was omitted in the negative control and replaced by phosphate-buffered saline.

Scoring system

Oestrogen receptor

Positivity for ER was scored on a scale of I–III based on the number of cells with bright red cytoplasmic staining as follows: Grade I—few positive cells, Grade II—moderate number of positive cells and Grade III—many positive cells. The absence of any cell staining was interpreted as a negative result.

HER 2-Neu

Human epidermal growth factor receptor -2 receptor status was tested with the HercepTest®, with scores reported on a scale of 0–3+ as follows: Score 0 (no membrane staining, or less than 10%), 1+ (faint membrane staining in more than 10% of tumour cells), 2+ (weak to moderate complete membrane staining in more than 10% of tumour cells) and 3+ (strong complete membrane staining in more than 10% of tumour cells) (5). Human epidermal growth factor receptor 2 protein overexpression was assessed as negative for a score of 0 or 1+, and positive for a score of 3+. Scores of 2+ required confirmatory testing with fluorescent in situ hybridization (FISH) for final determination. A positive result was defined as more than six HER2 gene copies per nucleus or a FISH ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2; a negative result was a FISH result of less than four HER2 gene copies per nucleus, or FISH ratio of less than 1.8 (6).

Univariate analyses with descriptive summary statistics are presented for relevant variables. Chi-square tests for association between different receptor profiles and patient age, tumour grade and tumour stage were done using Stata version 13.0. The UHWI/UWI/Faculty of Medical Sciences Ethics Committee approved this study.

RESULTS

Immunohistochemical studies for the assessment of expression of ER and HER2 were performed on 1383

breast cancer cases during the period 2002–2007. Table 1 summarizes the clinicopathological data for these patients. Median age was 52 years (range 20–94 years, IQR 43–63). Immunohistochemistry receptor profiles were: ER+/HER2− (50.2%), ER−/HER2− (28.1%), ER+/HER2+ (15.3%) and ER−/HER2+ (6.4%) (Table 2). Histopathological staging details, including tumour size and nodal status, were available for 860 patients and are presented in Table 3. The majority of tumours were greater than 2 cm in size (T2 or greater), and 67% had lymph node metastases. The aggregate pTNM stage was derived for the 439 cases with both tumour size and nodal status available and was: Stage I (5%), Stage II (50%) and Stage III (45%).

Table 1: Clinicopathological characteristics of patients with breast cancer IHC testing performed 2002–2007

	Total (n = 1383)
Age	
Range	20–94
Median (IQR)	52 (43–63)
Age group	
40 and under	240 (17.3%)
41–60	730 (52.8%)
61–70	214 (15.5%)
71 and over	199 (14.4%)
Sex	
Female	1377 (99.6%)
Male	6 (0.4%)
Specimen type	
Biopsy	708 (51.2%)
Mastectomy	675 (48.8%)
Histology	
In situ ductal	110 (8.0%)
In situ lobular	4 (0.3%)
Infiltrating ductal	1148 (83.0%)
Infiltrating lobular	108 (7.8%)
Mixed ductal/lobular	13 (0.9%)
Tumour grade (BRS)	
I	208 (15.1%)
II	486 (35.1%)
III	257 (18.6%)
Not graded	432 (31.2%)

IHC = immunohistochemistry; IQR = interquartile range.

Correlative analysis results are presented in Tables 4–6. Across all age groups, ER+/HER2− was the most frequently seen profile (45%–52%), with the ER−/HER2− profile being the second most frequently seen (27%–34%). There was no statistically significant association between the receptor profile and age (Chi-squared test statistic = 15.4453; *df* = 9; *p*-value = 0.079).

Table 2: Receptor profiles results for breast cancer IHC testing during period 2002–2007

Receptor profile	No of patients (%)
ER+/Her2+	212 (15.3%)
ER+/Her2-	694 (50.2%)
ER−/Her2+	88 (6.4%)
ER−/Her2-	389 (28.1%)
Total	1383

IHC = immunohistochemistry; ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2.

Table 3: Histopathological staging for breast cancer cases with IHC testing (2002–2007)

	n = 860
Tumour size, cm	
Range	0–15
Median (IQR)	3 (2–5)
Lymph nodes examined, no	
Range	0–22
Median (IQR)	4 (0–10)
Tumour size (TNM)	
T0	44 (5.1%)
T1	162 (18.8%)
T2	444 (51.6%)
T3	210 (24.4%)
Nodal status (TNM) (n = 458)	
N0	154 (33.6%)
N1	150 (32.8%)
N2	112 (24.5%)
N3	42 (9.2%)
TNM stage (n = 439)	
I	22 (5.0%)
IIA	111 (25.3%)
IIB	110 (25.1%)
IIIA	154 (35.1%)
IIIC	42 (9.6%)

IHC = immunohistochemistry; IQR = interquartile range.

Table 4: Receptor profile according to patient's age

	No of patients within each age range (%)			
Receptor profile	**40 and under**	**41–60**	**61–70**	**71 and over**
ER+/Her2+	23 (17.0%)	70 (15.9%)	14 (9.4%)	16 (11.7%)
ER+/Her2-	61 (45.2%)	202 (46.0%)	77 (51.7%)	61 (44.5%)
ER−/Her2+	14 (10.4%)	35 (8.0%)	8 (5.4%)	5 (3.7%)
ER−/Her2–	37 (27.4%)	132 (30.1%)	50 (33.6%)	55 (40.1%)
Total	135	439	149	137

Chi-squared test statistic = 15.4453; *df* = 9; *p*-value = 0.079; ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2.

Tumour grade was recorded for 658 cases. Amongst Grade III tumours, the most commonly seen receptor profile was ER−/HER2− (44.6%). For both Grade I

Table 5: Receptor profile according to tumour's grade

	No of patients within each tumour grade (%)		
Receptor profile	**I**	**II**	**III**
ER+/Her2+	13 (8.4%)	52 (15.9%)	19 (10.9%)
ER+/Her2-	94 (60.7%)	160 (48.8%)	65 (37.1%)
ER−/Her2+	3 (1.9%)	27 (8.2%)	13 (7.4%)
ER−/Her2-	45 (29.0%)	89 (27.1%)	78 (44.6%)
TOTAL	**155**	**328**	**175**

Chi-squared test statistic = 32.7394; *df* = 6; *p*-value < 0.001 (excluding 'Not Graded'); ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2.

Table 6: Receptor profile according to tumour's stage

	No of patients within each tumour stage (%)		
Receptor profile	**I**	**II**	**III**
ER+/Her2+	2 (9.1%)	22 (9.9%)	31 (15.8%)
ER+/Her2-	11 (50.0%)	101 (45.7%)	77 (39.3%)
ER−/Her2+	0 (–)	17 (7.7%)	18 (9.2%)
ER−/Her2-	9 (40.9%)	81 (36.7%)	70 (35.7%)
Total	**22**	**221**	**196**

Chi-squared test statistic = 6.6; ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2.

and Grade II tumours, the ER+/Her2- profile was most common (60.7 and 48.8%, respectively). There was a statistically significant association between the receptor profile and tumour grade (Chi-squared test statistic = 32.7394; *df* = 6; *p*-value < 0.001).

The majority of ER−/HER2− tumours were Stage II (51%) or Stage III (44%). Most ER+/HER2− tumours were also Stage II (53%) or Stage III (41%). There was no statistically significant association between the receptor profile and tumour stage (Chi-squared test statistic = 6.6078; *df* = 6; *p*-value = 0.359).

DISCUSSION

This 6-year analysis of breast receptor profiles from our IHC database represents the first study of the IHC receptor profiles of breast cancer from multiple centres across Jamaica. Previous studies of patients with breast cancer treated at the UHWI (7, 8) have demonstrated rates similar to that which we found in this larger multi-centre study group, in which cases from the entire island underwent testing at a centralized IHC testing facility. The rate of ER positivity in our study was 66%, compared to 62% (7) and 63% (8) in previous reports, while 22% of cases were HER2 positive, similar to previous reports of 20% HER2 positivity in patients treated at the UHWI (7).

The triple-negative phenotype (ER, PR and HER2 negative) is a surrogate for basal-like breast cancer and is known to be more aggressive and associated with a relatively poorer prognosis (9). In population-based studies, basal-like breast cancer has been shown to have a higher prevalence in African American compared with non-African American patients (10), comprising 26 and 16% of cases in African American and non-African American women, respectively. Stead *et al* showed a 3-fold higher risk of triple-negative breast cancer (TNBC) (95% CI 1.6, 5.5; $p = 0.0001$) in black compared with white women. In their study, the overall rate of TNBC was 20%; 30% of tumours in black women were triple negative, compared with 11%–13% of tumours in other women (11). There have been few studies examining the hormone receptor profile in African patients. In a study of 120 cases out of Kenya, Bird *et al* noted that 24% had ER− positive tumours, 34% were ER-negative and/or PR-positive, 10% were ER-negative but PR-positive and 66% were negative for ER and PR (12).

The Jamaican population is diverse in ethnicity, with a strong Afro-centric background. Based on this, we postulated that the rates of TNBC may be relatively high in our population. As previously noted, PR testing was not done during the study period, limiting our ability to comment on the prevalence of TNBC. Despite this limitation, we found a prevalence of 28% for ER/HER2-negative disease, which is in keeping with TNBC prevalence in the aforementioned North American studies. Despite the African influence on Jamaican ethnic background, it is interesting that our breast cancer hormone expression profile is more similar to that seen in African Americans in North American studies.

Since the period under study, PR testing has been implemented, in keeping with guideline recommendations that determination of tumour ER/PR and HER2 status should be done on all breast cancers (13). As we continue to add to our database with more complete information on PR, we will re-visit these prevalence rates.

We were unable to show a statistically significant association between receptor profile and patient age, which was anticipated based on the known propensity of basal like and TNBC for younger age groups (10). Aggressive tumour features such as high histological grade are characteristic of TNBC, and our study showed a significant association between receptor profile and tumour grade (*p*-value < 0.001).

Human epidermal growth factor receptor 2-overexpressing breast cancer, accounting for 22% of cases in this study, is a disease-subset associated with more aggressive behaviour, with significantly shortened disease-free survival and overall survival (14). Recognition of the

poor prognosis of HER2-positive breast cancer (15) has driven research and development of several anti-HER2 agents. Trastuzumab, a monoclonal antibody targeting the extracellular domain of HER2 protein, was shown to increase the clinical benefit of first-line chemotherapy in metastatic HER2 over-expressing breast cancer (16). This drug was approved by the The United States Food and Drug Administration for the adjuvant treatment of HER2-positive breast cancer based on the significant increases in both disease free and overall survival with its addition to standard adjuvant chemotherapy. In the combined analysis of two landmark Phase III trials, NSABP B-31 and the NCCTG N9831, adding trastuzumab to chemotherapy resulted in a significant improvement in DFS (HR: 0.48; 95% CI: 0.39, 0.59; $p < 0.0001$) and a 33% reduction in the risk of death ($p = 0.015$) (17). Based on our data, trastuzumab therapy may be indicated for up to 22% of our breast cancer patients. In our experience, however, only a limited number of eligible patients are able to receive this treatment primarily due to high drug cost. With such a potential gain in survival, a cost-benefit analysis is encouraged to support implementation of a trastuzumab subsidy by the national insurance scheme (National Health Fund).

CONCLUSION

The prevalence of ER-negative/HER2-negative breast cancer in Jamaica (28%) is in keeping with TNBC prevalence in African American populations in North American studies. Inclusion of PR testing as part of breast IHC receptor testing will help us better define the prevalence of TNBC, and treatment strategies aimed at this aggressive subset of breast cancer must be pursued locally. There was a statistically significant association between the receptor profile and tumour grade ($p = < 0.001$). Over 20% of breast cancers studies over-expressed HER2, and efforts aimed at increasing accessibility of targeted anti-HER2 therapy are encouraged.

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ORIGINAL ARTICLE

A Comparison of Weekly DCF *versus* Standard DCF as First-line Systemic Chemotherapy for Metastatic Gastric Cancer Patients

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ABSTRACT

Objective: *In metastatic gastric cancer (MGC), the goals of treatment are palliation and prolongation of overall survival (OS). Systemic chemotherapy, with docetaxel, cisplatin, infusional 5-fluorouracil (DCF) the most preferred regimen, is the mainstay of the treatment. We evaluated the efficacy and tolerability of weekly DCF (wDCF) compared to standard DCF regimen.*

Methods: *We retrospectively reviewed 49 and 32 MGC patients treated with DCF and wDCF regimens as first-line treatment, respectively. The wDCF protocol included 25 mg/m² docetaxel, 25 mg/m² cisplatin and 750 mg/m² infusional 5-FU on day 1, every week. Each cycle was repeated every 3 weeks.*

Results: *The patients in wDCF arm were significantly older (median 54* vs *72.5) and had poor Eastern Cooperative Oncology Group performance status (ECOG PS) than patients in DCF arm (*p *< 0.001). Progression-free survival was 3 vs 5 months (*p*=0.75) and OS was 7 vs 9 months in wDCF and DCF arms (*p*=0.33), respectively. Overall response rate was observed in 28.5 and 31.2% of the patients in DCF and wDCF arms, respectively (*p *= 0.65). Haematologic toxicities were observed more common in wDCF arm.*

Conclusion: *Weekly DCF is an effective and tolerable regimen and may be an alternative in patients who are elderly and have poor ECOG PS.*

Keywords: Cisplatin, docetaxel, infusional 5-fluorouracil, metastatic gastric cancer.

INTRODUCTION

Gastric cancer is a common type of cancer worldwide and represents an important cause of cancer-related mortality. Gastric cancer is more frequently seen in men than women, in both developed and developing countries (1).

About two-thirds of gastric cancer patients are in metastatic stage at diagnosis. While metastatic gastric cancer (MGC) is a noncurable disease, the goals of treatment are palliation and prolongation of overall survival (OS). Systemic chemotherapy is the most effective treatment modality for these patients (2). In a meta-analysis of three trials comparing chemotherapy results *vs* best supportive care (BSC) in MGC patients, a significant benefit in OS in favour of chemotherapy was reported (hazard ratio 0.37, 95% CI: 0.24, 0.55), which translated into an improvement in median OS from 4.3 to 11 months (3).

Despite large number of randomized trials, there is no consensus on the best chemotherapy regimen for initial chemotherapy of MGC. Although response rates are higher with combination regimens than single agents, only a modest prolongation of disease control and OS can be achieved which measured in weeks to a few months. The ECF (epirubicin, cisplatin, infusional 5-fluorouracil [5-FU]) and DCF (docetaxel, cisplatin, infusional 5-FU) combinations have emerged as standard regimens for first-line treatment in randomized trials (4, 5). In a randomized phase-II trial involving 81 patients, DCF

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was directly compared with ECF in first-line advanced gastric cancer and a trend favouring DCF over ECF was reported (6). In a phase III study comparing DCF *vs* CF (cisplatin, infusional 5-FU), DCF was reported to be significantly better (7). However, in both trials, the incidence of grade 3 or 4 diarrhoea and neutropenia was higher with DCF.

Because of higher toxicity rates reported for DCF, several modifications of standard schedule have been investigated. It was aimed to alter the dose and frequency of the cytotoxic agents to allow less toxicity with no worsening on efficacy. With these adjustments, modified schedules for DCF were shown to have preserved efficacy and improved tolerability (8, 9). Weekly DCF (wDCF) is one of the alternatives of these affords. In a retrospective study, efficacy and tolerability of wDCF in advanced gastric and oesophageal cancer patients, not candidates for standard DCF, was evaluated. It was reported that wDCF demonstrated a modest activity with minimal haematologic toxicity, suggesting that wDCF may be a treatment option in these patients (10).

We performed a retrospective analysis of the efficacy and tolerability of wDCF *vs* DCF in MGC patients in the first-line treatment.

SUBJECTS AND METHODS

We retrospectively reviewed 81 MGC patients who were treated with DCF or wDCF as first-line treatment from April 2007 to July 2014 in Ataturk University, School of Medicine, Department of Medical Oncology.

The DCF protocol included 75 mg/m^2 docetaxel, 75 mg/m^2 cisplatin on day 1 and 750 mg/m^2/daily, days 1–5 infusional 5-FU, repeated every 3 weeks. Primary prophylaxis with white blood cell growth factor support was used in patients who received DCF regimen. The wDCF protocol included 25 mg/m^2 docetaxel, 25 mg/m^2 cisplatin and 750 mg/m^2 infusional 5-FU on day 1, every week. Each cycle was repeated every 3 weeks.

Study inclusion criteria were MGC diagnosed as adenocarcinoma at referral, Eastern Cooperative Oncology Group (ECOG) performance status (PS) to be 0, 1 or 2 at diagnosis, receiving at least two cycles of DCF or wDCF as first-line chemotherapy. Patients who have undergone previous curative operation and received adjuvant treatment were excluded.

Disease and patient characteristics, prognostic factors, treatment response, grade 3–4 haematologic and non-haematologic treatment toxicities, progression-free survival (PFS) and OS were evaluated. Grade 3–4 toxicities were assessed according to National Cancer Institute Common Toxicity Criteria (Version 4.0). Imaging studies were documented by computed tomography and positron emission tomography-computed tomography at baseline for every three cycles. Tumour responses were determined using Response Evaluation Criteria in Solid Tumours (version 1.1) (11).

Statistical analysis

Analyses were performed using the SPSS statistical software program package (SPSS version 20.0 for windows, Chicago, IL, USA). Survival curves were established with The Kaplan–Meier method. Progression-free survival was defined as the time from MGC diagnosis to disease progression or death. Overall survival was defined as the time from MGC diagnosis to death from any cause. Progression-free survival and OS were calculated with the log-rank test. The differences of the clinical characteristics between two groups were analysed by Chi-square test and Mann–Whitney *U* test. Differences were assumed to be significant when *p*-value was less than 0.05.

RESULTS

Patient characteristics

From total of 81 enrolled patients, 49 and 32 patients were treated with DCF and wDCF regimens, respectively. Demographical and clinical characteristics of patients are listed in Table 1.

The patients in wDCF arm were significantly older than patients in DCF arm (median 54 *vs* 72.5, $p < 0.001$). While the percentage of patients over 65 years old in DCF arm was 12.2%, it was 84.4% in wDCF arm. The patients in wDCF arm had significantly poor ECOG PS than DCF arm ($p < 0.001$). The percentage of ECOG PS 2 patients were 59.4% in wDCF arm, whereas 10.2 % in DCF arm. The median follow-up time was 9 (1–60) months and was not significant between two groups ($p = 0.65$).

Efficacy

In DCF arm, patients were received median 6 (2–8) cycles of chemotherapy, whereas in wDCF arm, median 3.5 (2–8) cycles. Progression-free survival was 3 (95% CI: 0.6, 5.4) months in wDCF arm and 5 (95% CI: 4.1, 5.9) months in DCF arm ($p = 0.75$). OS was 7 (95% CI: 5.6, 8.4) months in wDCF arm and 9 (95% CI: 6.9, 11) months in DCF arm ($p = 0.33$). Although both PFS and OS were shorter in wDCF arm, there was no statistically significant difference between two arms (Figure).

Table 1. Demographical and clinical characteristics of patients

	DCF (n = 49)		wDCF (n = 32)		*p*
	n	%	n	%	
Gender					
Men	34	69.4	24	75	0.58
Women	15	30.6	8	25	
ECOG					
0	4	8.2	2	6.3	
1	40	81.6	11	34.4	< 0.001
2	5	10.2	19	59.4	
Age (years)					
<55	26	53.1	2	6.3	
55–64	22	44.9	3	9.4	< 0.001
≥ 65	1	2	27	84.4	
Metastatic site					
Liver	37	75.5	19	59.4	0.12
Lung	2	4.1	7	21.9	0.01
Bone	2	4.1	3	9.4	0.33
Peritoneal	10	20.4	11	34.4	0.16
Chemotherapy cycles					
2–3	7	14.3	16	50	
4–6	40	81.6	15	46.9	0.002
> 6	2	4.1	1	3.1	
Second-line chemotherapy					
Yes	28	57.1	9	28.1	0.01
No	21	42.9	23	71.9	
Last status					
Dead	44	89.8	32	100	
Alive	5	10.2	0		

DCF = docetaxel, cisplatin, infusional 5-fluorouracil; wDCF = weekly DCF.

Overall response rate (ORR) was observed in 28.5 and 31.2% of the patients in DCF and wDCF arms, respectively (p = 0.65). The treatment efficacy and ORR are summarized in Table 2.

Table 2: Treatment efficacy of the patients

	DCF (n = 49)		wDCF (n = 32)		*p*
	n	%	n	%	
Complete response	1	2	1	3.1	0.95
Partial response	13	26.5	9	28.1	
Stable disease	2	4.1	2	6.3	
Progressive disease	33	67.3	20	62.5	

DCF = docetaxel, cisplatin, infusional 5-fluorouracil; wDCF = weekly DCF.

Safety and tolerability

Dose reduction was required in 12 (24.5%) patients in DCF arm and 4 (12.5%) patients in wDCF arm (p = 0.19). Dose was delayed in 12 (24.5%) patients in DCF arm, whereas in 10 (31.3%) patients in wDCF arm (p = 0.5). The haematologic and non-haematologic toxicities are summarized in Table 3. Anaemia, neutropenia and thrombocytopenia were observed more frequently in wDCF arm. No patients died due to chemotherapy toxicity.

Table 3: Grade 3–4 haematologic and non-haematologic toxicities (National Cancer Institute Common Toxicity Criteria, version 4.0)

	No of patients (%)	
	DCF	wDCF
Haematologic toxicities		
Neutropenia	2 (4.1)	5 (15.6)
Anaemia	2 (4.1)	3 (9.4)
Thrombocytopenia	–	2 (6.3)
Non-haematologic toxicities		
Diarrhoea	5 (10.2)	–
Nausea-vomiting	3 (6.1)	1 (3.1)
Thrombosis	4 (8.2)	–
Mucositis	1 (2)	–
Neuropathy	1 (2)	v
Nephrotoxicity	1 (2)	1 (3.1)
Hepatotoxicity	2 (4.1)	1 (3.1)

DCF = docetaxel, cisplatin, infusional 5-fluorouracil; wDCF = weekly DCF.

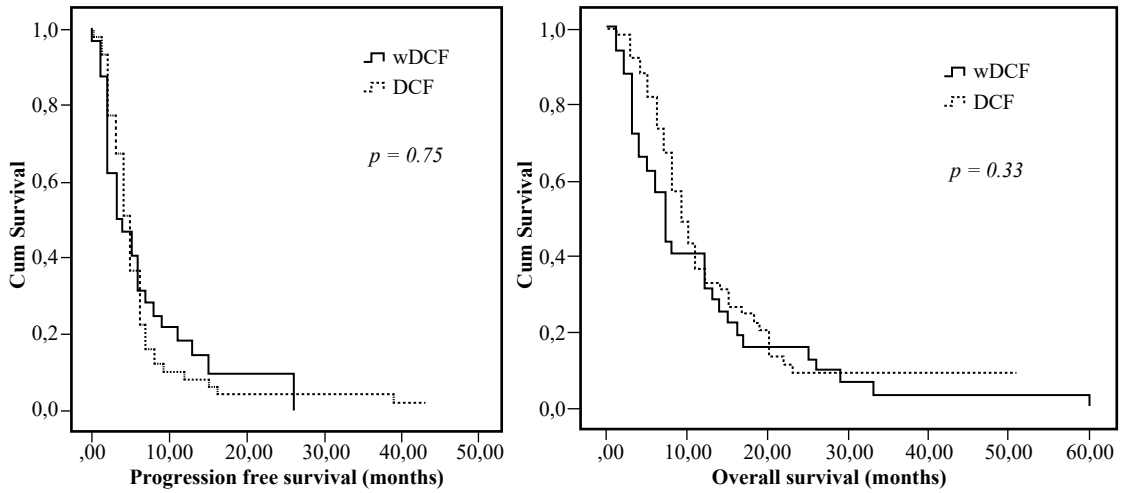

Figure: Progression-free survival and overall survival. DCF = docetaxel, cisplatin, infusional 5-fluorouracil; wDCF = weekly DCF.

DISCUSSION

Gastric cancer is a common form of cancer worldwide. Gastric cancer is a highly mortal malignancy. About two-thirds of gastric cancer patients are in metastatic stage at diagnosis. The 5-year OS for gastric cancer is lower than most of other cancer forms (1). Better OS was demonstrated with chemotherapy compared to BSC in MGC patients. However, the optimal treatment regimen is not clear yet for initial chemotherapy in this population.

Combination regimens seem to be more effective than single-agent treatments (3). DCF is a commonly used regimen in MGC as first-line chemotherapy with a low tolerability rate due to its toxicity. The largest phase 3 randomized study comparing DCF to cisplatin plus 5-FU demonstrated superiority of triplet chemotherapy. Overall response rate (37% *vs* 25%), median time to progression (5.6 *vs* 3.7 months) and median OS (9.2 *vs* 8.6 months) were significantly better with DCF than CF (5). In a retrospective study comparing DCF to modified DCF (mDCF), response rates were similar in both arms. There was no statistically significant difference in terms of complete response (CR) (10.3% *vs* 6.7%), partial response (PR) (35.3% *vs* 40.0%), stable disease (SD) (32.4% *vs* 33.3%), progressive disease (PD) (22.1% *vs* 20.0%) and ORR (45.6% *vs* 46.7%). Progression-free survival was more favourable in DCF arm than mDCF arm, but the difference was not statistically significant (7.4 *vs* 6.5 months); OS was not significantly superior in DCF arm (9.9 *vs* 8.6 months) (9). In another study comparing DCF to mDCF, comparable efficacy was reported with mDCF and CR was 2% *vs* 5.4%, PR was 35% *vs* 21.6%, SD was 30% *vs* 37.9%, PD was 17% *vs* 13.5% and ORR was 37% *vs* 27%, respectively (12).

Overman *et al* retrospectively evaluated the efficacy and tolerability of wDCF in patients with advanced gastric and oesophageal cancer who were not candidates for DCF. In wDCF arm, treatment was administered as 20 mg/m^2 of docetaxel, 20 mg/m^2 of cisplatin and 350 mg/m^2 of 5-FU once weekly for six consecutive weeks followed by a 2-week break. Overall response rate was reported as 34% (95% CI: 24%, 45%), PFS as 4.1 months (95% CI: 3.6, 5.7 months) and OS as 8.9 months (95% CI: 7.7, 10.8 months). The authors suggested that wDCF demonstrated modest activity with minimal haematologic toxicity and wDCF might be a reasonable treatment option for such patients (10).

In our study, PFS was 3 *vs* 5 months and OS was 7 *vs* 9 months in DCF and wDCF arms, respectively. Although both PFS and OS were shorter in wDCF arm, there was no statistically significant difference between two arms. Overall response rate was observed in 28.5 and 31.2% of the patients in DCF and wDCF arms, respectively.

In the V325 study, significant toxicity was reported with DCF despite good ECOG PS (0–1) and young age of the patients (median age 55 years). Grade 3–4 toxicities were reported as neutropenia (82%), anaemia (18%), thrombocytopenia (8%), febrile neutropenia (29%), nausea (14%), vomiting (17%), and diarrhoea (19%) (5). In another study designed retrospectively and compared DCF *vs* mDCF, grade 3–4 toxicities with DCF were determined as neutropenia (48.2%), febrile neutropenia (19%), thrombocytopenia (25.9%), nausea (44.7%) and vomiting (31.8%), while toxicity rates with mDCF were neutropenia (13.6%), febrile neutropenia (4.5%), thrombocytopenia (9.1%), nausea (13.6%) and vomiting (4.5%). Grade 3–4 toxicities were significantly less common in mDCF arm compared to DCF arm (9). Nuriye *et al* reported grade 3–4 toxicities as neutropenia (82% *vs* 8.1%), anaemia (18% *vs* 5.4%), thrombocytopenia (17% *vs* 0%), nausea (14% *vs* 5.4%) and vomiting (14% *vs* 0%) in their study comparing DCF *vs* mDCF, respectively (12). Overman *et al* reported grade 3–4 toxicities with wDCF as neutropenia (4%), neutropenic fever (0%), thrombocytopenia (0%) and anaemia (9%) (10).

In our study, grade 3–4 toxicity rates were as neutropenia (4.1% *vs* 15.6%), anaemia (4.1% *vs* 9.4%), thrombocytopenia (0% *vs* 6.3%) and nausea-vomiting (6.1% *vs* 3.1%) in DCF arm compared to wDCF arm, respectively. Haematologic toxicities were observed more common in wDCF arm in our study. This may be due to higher rates of patients with older age and poor ECOG PS in wDCF arm as 84.4% were 65 years and older and 59.4% had ECOG PS 2 of our patients in wDCF arm.

Limitations of our study are its retrospective design, single-centre experience and low number of patients included.

CONCLUSION

In MGC, DCF is an effective and more tolerable regimen in young patients and patients with good ECOG PS. In our study although PFS and OS were shorter with wDCF compared to DCF, there was no statistically significant difference between two arms. We found that wDCF was an effective and tolerable regimen and may be an alternative in patients who are elderly and have poor ECOG PS. This subject should be investigated in large-numbered prospective randomized trials.

AUTHORS' NOTE

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ORIGINAL ARTICLE

Evaluation of Alanine Aminotransferase (ALT) and Gamma-glutamyl Transferase (GGT) in Patients with Type 2 Diabetes Mellitus at the Diabetic Clinic, Guyana

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ABSTRACT

Objective: *To determine the prevalence of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) abnormalities and contributing factors in adult patients with type 2 diabetes mellitus at the diabetic clinic of public hospital, Guyana.*

Methods: *A prospective study was carried out on 60 randomly selected diabetic patients from the public hospitals' diabetic clinics. Patients were included in the study once they had to get tested for ALT and GGT on their request form from the physician and their informed consent. Analysis was done using SPSS 20.*

Results: *Analysis of ALT and GGT showed mean ALT ± SD as 61.9 ± 28.9 (95% CI: 54.5, 69.4) and mean GGT ± SD as 19.6 ± 9.7 (95% CI: 17.1, 83.3). Family history (RR 2.3 95% CI: 0.6, 8.6) showed a greater risk factor of high ALT followed by current illness (RR 1.3 95% CI: 0.4, 4.7). Correlation analysis between ALT and GGT shows a significant positive correlation.*

Conclusion: *The research showed a prevalence of elevated levels of ALT and GGT in type 2 diabetic patients and a strong association to metformin and ethnicity in particular those of Indian decent. Therefore, further research within a controlled environment should be done in order to evaluate the efficacy of the action of metformin as compared to other diabetic drugs in concert with other contributing factors.*

Keywords: Alanine aminotransferase, alanine aminotransferase, type 2 diabetes mellitus.

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar (1). In 2012, an estimated 1.5 million deaths were directly caused by diabetes and more than 80% of diabetes deaths occur in low- and middle-income countries (2). WHO projects that diabetes will be the 7th leading cause of death in 2030 (3).

Liver plays a major role in maintenance of normal glucose levels during fasting as well as in the post-prandial period. It is also documented that alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) are the common liver enzymes and together make the liver function tests (4). Alanine aminotransferase and AST represent the health status of liver cells and GGT represents the health of biliary tract (5, 6).

Elevated GGT levels among diabetic patients and association between elevated GGT and poor glycaemic state have been studied since years (7, 8). The liver enzymes, AST, ALT, and GGT are very often used in the evaluation of liver function (9). Literature on prospective studies shows associations between concentrations of AST, ALT, GGT and the incidence of type 2 diabetes (10–15).

The purpose of this study is to estimate the associations of serum liver enzyme (ALT and GGT) in patients with type 2 diabetes at the public hospital, Guyana.

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SUBJECT AND METHODS

Description of subjects

This research was carried out at the Georgetown Public Hospital Cooperation (GPHC) Diabetic Clinic in association with the GPHC Medical Laboratory in which type 2 diabetic patients were randomly selected to participate in the study after giving informed consent.

Study design

A prospective, criterion-based study was carried out and the variables were collected simultaneously.

Variables

Independent: Type 2 diabetes mellitus.
Dependent: Levels of ALT and GGT.

Method of measuring variables

Testing was done at the GPHC Medical Laboratory in a Chem-Well Chemistry Analyzer. A proper control procedure was carried out to ensure the validity and reliability of the results. The results were then tabulated and analysed. Participants were asked to answer a questionnaire on related issues and relevant information such as family history and personal activities.

Data analysis

The data obtained were analysed using SPSS version 20 and Microsoft Excel.

Ethical considerations

Approval for the study was granted from the Chief Executive Officer of the Georgetown Public Hospital Cooperation through the Director of Medical and Professional Services and the Director of the Georgetown Public Hospital Cooperation Medical Laboratory. Patient forms were coded to protect the identity of each patient. Patients were also informed that participation was voluntary and they reserve the right to stop participation at any time during the study period.

RESULTS

The present study had 60 participants confirmed with type 2 diabetes. Demographic and clinical characteristics of study participants are shown in Table 1. The majority of the patients were female (71.7%) and most patients were between 51 and 60 age group (31.7%) ($p \leq 0.005$). Mean age among the participants was 62. Indo-Guyanese accounted for 58.3% of the total sample population and as such recorded the highest prevalence. 41.6% of patients were on metformin while 57.0% were on other drugs other than metformin, statin, fibrate and thiazolidinediones. Blood pressure was recorded high in 35% population and 31.7% reported no other medical conditions.

Table 1: Demographic and clinical characteristics of study participants

	n (%)	*p*-value
Gender		
Male	17 (28.3)	
Female	43 (71.7)	< 0.05
Age group		
40–50	17 (28.3)	
51–60	19 (31.7)	
61–70	16 (26.7)	
71–80	8 (13.3)	> 0.05
Ethinicity		
Afro Guyanese	18 (30.0)	
Indo Guyanese	35 (58.3)	
Mixed	7 (11.7)	< 0.05
Drugs		
Metformin	25 (41.6)	
Metformin + other	11 (18.3)	
Other	23 (38.3)	
Statin	1 (1.7)	< 0.05
Medical conditions		
BP	21 (35.0)	
BP + HD	1 (1.7)	
HD	7 (11.7)	
High cholestrol	5 (8.3)	
High cholestrol + BP	4 (6.7)	
High cholestrol + BP + HD	1 (1.7)	
None	19 (31.7)	
Others	1 (1.7)	< 0.05

Analysis of ALT and GGT showed mean ALT ± SD as 61.9 ± 28.9 (95% CI: 54.5, 69.4) and mean GGT ± SD as 19.6 ± 9.7 (95% CI: 17.1, 83.3). Increased ALT level was recorded among 78.3% (95% CI: 73.3, 83.3); however, GGT did not show any significant increase (Table 2).

Table 2: Mean value of ALT and GGT values

	Mean ± SD	95% CI
ALT	61.9 ± 28.9	54.5–69.4
Min	12	
Max	118	
GGT	19.6 ± 9.7	17.1–22.1
Min	3	
Max	35	
	n (%)	
> ALT	47 (78.3)	73.3–83.3
< ALT	13 (21.7)	16.7–26.7

Family history (RR 2.3 95% CI: 0.6, 8.6) showed a greater risk factor of high ALT followed by current illness (RR 1.3 95% CI: 0.4, 4.7), gender (RR 1.1 95% CI: 0.8, 1.4), physical exercise (RR 0.96 95% CI: 0.7, 1.3) and smoking (RR 0.7 95% CI: 0.6, 0.8) (Table 3). Correlation analysis between ALT and GGT shows a significant positive correlation (Figure).

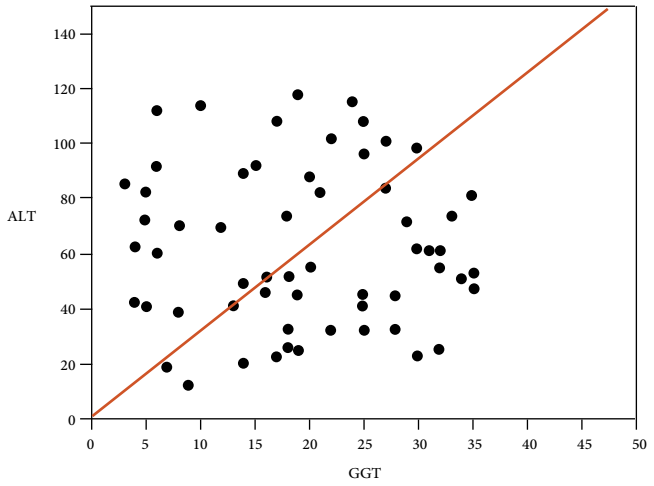

Figure: Bivariate analysis of GGT and ALT among diabetes 2 patients (ALT = 0.5 + 3.1*GGT).

DISCUSSION

Abnormal liver function tests (LFT) is one of the most common findings among type 2 diabetes patients and elevated ALT being the most common abnormality (16). In a case-controlled study in Nigeria showed that ALT and GGT values were significantly higher (52.9 IU/L and 24.3 U/L respectively) in diabetic group compared to the non-diabetic groups (34.4 IU/L and 9.2 IU/L respectively). This study also revealed the most predominant LFT abnormality in diabetic group with elevated GGT (17).

This study also showed metformin as most common drug used among the diabetes population. This could be because of the act that metformin is more readily available and at a lesser cost than most of the other recommended drugs. In addition, in some regards this can be stated as the reason as for a positive correlation with elevated ALT and GGT in patients on that drug. One study has reported that drugs such as statins were a possibility for the increase in ALT and GGT liver function enzyme levels (18). However, in this study it is shown that metformin had a strong association to the elevation of ALT and GGT. The reason for this is unknown and needs to be explored.

Other studies have indicated a link between family history and diabetes; however, this study showed most respondents with no family history of diabetes which firmly places an association to lifestyle and diet. Ethnicity played an important role in the majority of the patients with elevated ALT and GGT being Indo-Guyanese. However, on the account the more than half the sample population was Indo-Guyanese, this can be the causal factor for that outcome. A positive correlation between elevated ALT and GGT was also noted to among patients with duration of 5–10 years type 2 diabetes.

Many studies have proposed different mechanisms to explain this phenomenon, that ALT and GGT liver function enzymes are elevated in people with type 2 diabetes and this leads to a higher incidence of liver enzymes test abnormalities. Aithal *et al* supported the claim that ALT is a specific marker of liver pathology, as it is found primarily in the liver, and is considered to be the marker most closely correlated to liver fat. Although GGT is a less specific marker of liver, higher GGT levels have also been linked with obesity, physical inactivity, hypertension, dyslipidaemia, and hyperinsulinemia, implying

Table 3: Risk factors associated with type 2 diabetes patient at the public hospital, Guyana

		ALT		RR	OR
		Yes	No		
Gender	Female	33 (76.7)	10 (23.3)	1.1 (0.8–1.4)	0.7 (0.2–2.9)
	Male	14 (82.4)	3 (17.6)		
Smoking	Yes	10 (100)	0	0.7 (0.6–0.8)	0
	No	37 (74.4)	13 (26.0)		
Family history	Yes	37 (82.2)	8 (17.8)	2.3 (0.6–8.6)	0.8 (0.6–1.2)
	No	10 (68.7)	5 (33.3)		
Physical exercise	Yes	31 (79.5)	8 (20.5)	0.96 (0.7–1.3)	1.2 (0.3–4.3)
	No	16 (76.2)	5 (23.8)		
Current illness	Yes	32 (80.0)	8 (20.0)	1.3 (0.4–4.7)	0.9 (0.7–1.2)
	No	15 (75.0)	5 (25.0)		

that elevated GGT belongs in the cluster of the metabolic syndrome (19, 20).

In addition, a study by Anderwalt *et al* the elevated ALT and GGT values were significantly higher in men than in women, which is contrary to this study where women showed higher levels of liver function test (21). This study had some major limitations such as Body Mass Index, waist circumference, plasma fasting insulin levels and heavy alcohol consumptions which were not examined as parameters to influence the results, and future studies should take them into consideration. In terms of the severity of elevations recorded in this study, the majority were mild elevations. Since most studies have not reported on the severity of elevated liver enzymes, the exact implications of this finding are not known.

CONCLUSION

In conclusion, the research showed a prevalence of elevated levels of ALT and GGT in type 2 diabetic patients within the Guyanese diabetic population and strong association to metformin and ethnicity in particular those of Indian decent. Therefore, further research within a controlled environment should be done in order to evaluate the efficacy of the action of metformin as compared to other diabetic drugs in concert with other contributing factors.

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ORIGINAL ARTICLE

Serum Ghrelin Levels in Patients with Chronic Urticaria and Atopic Dermatitis and Its Relationship with Metabolic Syndrome

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ABSTRACT

Objective: *Chronic urticaria is a systemic inflammatory disease. Atopic dermatitis is a chronic immunological disease that is characterized by an increase in systemic inflammatory response. In several studies, chronic urticaria and atopic dermatitis were reported to be associated metabolic syndrome (MetS). In this study, we aimed to investigate the serum ghrelin levels in the patients with chronic urticaria and atopic dermatitis.*

Methods: *Thirty patients with chronic urticaria, 30 patients with atopic dermatitis and 30 control subjects participated in this study. Blood fasting glucose and serum lipids, insulin, C-peptide levels and thyroid function tests were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to calculate insulin resistance. Ghrelin levels were determined by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol.*

Results: *The mean serum ghrelin levels in the patients with chronic urticaria (54.13 ± 40.94 pg/mL) and atopic dermatitis (65.33 ± 93.54 pg/mL) were significantly higher than those of the controls (30.36 ± 17.13 pg/mL) (*$p = 0.003$, $p = 0.04$, *respectively).*

Conclusion: *We detected higher serum ghrelin levels in the patients with chronic urticaria and atopic dermatitis than the controls. However, we failed to find any association between serum ghrelin levels and insulin resistance or MetS. We think that the high levels of serum ghrelin in the patients with chronic urticaria and atopic dermatitis may be related to the mechanisms independent of insulin resistance.*

Keywords: Atopic dermatitis, chronic urticaria, ghrelin

INTRODUCTION

Chronic urticaria is defined as urticaria or angioedema or both, persists for longer than six weeks. Numerous mediators such as histamine, leukotrienes, serin proteases, heparin, tryptase and proinflammatory cytokines trigger mast cell degranulation. Urticaria is related with atopic dermatitis and allergic rhinitis, especially in childhood (1). Atopic dermatitis is accepted as a chronic, systemic immunologic disease with a tendency to increase in inflammatory response. In atopic dermatitis, antigenic stimulation of Langerhans cells, mast cells and keratinocytes cause an increase in eosinophils, inflammatory dendritic cells and Th2 response (2).

Ghrelin is a peptide hormone mainly secreted by gastric mucosa (3). Ghrelin has many important functions such as stimulating growth, appetite, lipid storage and gluconeogenesis, controlling gastric motility and gastric acid secretion, regulating pancreatic exocrine and endocrine secretions, proliferation of neoplastic cells and regulating immune system (4, 5). Increased plasma ghrelin levels were observed in obese individuals in a number of studies and high plasma ghrelin level was considered to be a feature of metabolic syndrome (MetS) like hyperinsulinemia and insulin resistance (6, 7).

Recently, there have been some studies reporting the relation between chronic urticaria and MetS (8). Atopic

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dermatitis is considered to be a risk factor for MetS (9). The aim of this study is to evaluate serum ghrelin level and its relationship with MetS in the patients with chronic urticaria and atopic dermatitis.

SUBJECTS AND METHODS

The local ethics committee approved the study (no: 03 of February 21, 2013). An informed consent was obtained from all the participants. A total of 30 patients with chronic urticaria, 30 patients with atopic dermatitis and 30 healthy control subjects were enrolled into the study. The diagnosis of chronic urticaria and atopic dermatitis were based on the clinical findings and Hanifin-rajka diagnostic criteria, respectively (10).

The participants under the age of 18, having systemic diseases, having malignancy or infection, being pregnant or using systemic medications were excluded from the study. The patients with chronic urticaria and atopic dermatitis taking systemic corticosteroids or any immunosuppressive drug for the last three months were also excluded.

Body mass index (BMI) was calculated according to the formula of BMI = weight (kg)/height^2 (m^2): score 0: 18.5–24.9 kg/m^2, score 1: 25.0–29.9 kg/m^2, score 2: 30.0–39.9 kg/m^2, score 3: ≥ 40.0 kg/m^2. Body mass index (BMI) values over 30 were accepted as obese (11).

Serum-fasting glucose level, triglyceride, total cholesterol, LDL, VLDL and HDL cholesterol, insulin, C-peptide and thyroid function tests were measured. The diagnosis of MetS was based on the diagnostic criteria of International Diabetes Federation (IDF). The participants having two or more criteria below were accepted as MetS. The diagnostic criteria of IDF for MetS were: waist circumference ≥ 94 cm (male) or ≥ 80 cm (female), hypertriglyceridemia ≥ 150 mg/dL, HDL cholesterol < 40 mg/dL (male) or < 50 mg/dL (female), arterial blood pressure ≥ 130/85 mmHg, serum-fasting glucose level ≥ 100 mg/dL (12). Homeostasis model assessment for insulin resistance (HOMA-IR) index was used to measure insulin resistance (HOMA-IR = insulin [mU/L] × glucose [mmol/L]/22.5) (13).

Ghrelin is sensitive to proteases because of its peptide structure. Therefore, aprotinin (500 kallikrein unite/mL) was added into the blood collection tubes before the blood samples were collected from the patients to prevent proteolysis. The blood samples were collected between 09:00 am and 10:00 am after an overnight fast of at least 8 hours. The blood samples (5 mL) were centrifuged at 3000 rpm. The serum samples were transferred into microcentrifuge tubes and stored at –80°C. Serum ghrelin levels were determined with an enzyme-linked immunosorbent assay (ELISA kits cat. no: SPI BIO-A05106).

The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 22. Continuous data are expressed as the mean ± SD. Comparisons between the groups were assessed using the student's *t*-test and Mann–Whitney *U* test; *p*-values less than 0.05 were considered statistically significant.

RESULTS

The ages of urticaria patients were between 18 and 67 years, the atopic dermatitis patients were 18–52 years, the controls patients were 18–45 years. The mean age of the urticaria patients, atopic dermatitis patients and the control group patients were 34.43 ± 12.9, 29.70 ± 11.4, 30.90 ± 8.5, respectively. There was no significant difference between the groups in terms of mean age, gender and BMI ($p > 0.05$) (Table 1).

Table 1: Clinical features of the patients and the controls

	Urticaria	Atopic dermatitis	Control	*p*-value
Number	30	30	30	
Gender (M/F)	15/15	15/15	15/15	$p > 0.05$
Age* (year)	34.43 ± 12.9	29.70 ± 11.4	30.90 ± 8.5	$p > 0.05$
BMI* (kg/m^2)	26.08 ± 6.31	24.21 ± 5.50	23.71 ± 3.25	p > 0.05
BMI score*	2.63 ± 1.03	2.48 ± 1.05	2.30 ± 0.79	p > 0.05
Waist circumference *(cm)	76.43 ± 11.76	78.70 ± 21.45	78.90 ± 7.44	p > 0.05

*(mean ± SD).

The mean serum ghrelin levels were significantly higher in the chronic urticaria patients (54.13 ± 40.94, $p = 0.003$) and in the atopic dermatitis patients (65.33 ± 93.54, $p = 0.04$) than in the controls patients (30.36 ± 17.13). There was no significant difference between the mean serum ghrelin levels of the chronic urticaria and atopic dermatitis patients ($p > 0.05$). The laboratory findings of the patient and control groups are shown in Table 2 and Figure 1.

An increased insulin resistance was detected in 11 (36.7%) of the chronic urticaria patients, in six (20%) of the atopic dermatitis patients and in eight (26.7%) of the controls. Although the mean serum ghrelin levels of the chronic urticaria patients with insulin resistance (73.67 ± 52.49 pg/mL) were higher than the chronic urticaria patients without insulin resistance (42.81 ± 28.32 pg/mL), the difference was not statistically significant ($p > 0.05$). The mean serum ghrelin levels of the atopic dermatitis

Table 2: Laboratory results of the patient and the control groups

	Urticaria	Atopic dermatitis	Control	*p*-value
Glucose* (mg/dL)	100.76 ± 32.07	92.73 ± 11.41	89.80 ± 9.71	$p > 0.05$
Triglyceride* (mg/dL)	114.30 ± 50.41	89.76 ± 43.78	115.13 ± 80.08	$p > 0.05$
LDL-cholesterol* (mg/dL)	96.58 ± 36.12	98.44 ± 31.20	92.60 ± 30.42	$p > 0.05$
HDL-cholesterol* (mg/dL)	50.60 ± 13.20	51.80 ± 11.09	49.15 ± 13.06	$p > 0.05$
Total cholesterol* (mg/dL)	167.53 ± 44.04	167.40 ± 37.51	168.90 ± 24.04	$p > 0.05$
Insulin* (µIU/mL)	8.40 ± 3.05	8.90 ± 3.62	10.77 ± 5.74	$p > 0.05$
C-peptide* (ng/mL)	0.90 ± 0.59	1.15 ± 0.58	2.07 ± 0.78	$p > 0.05$
HOMA-IR*	2.17 ± 1.54	2.05 ± 0.92	2.35 ± 1.27	$p > 0.05$
Ghrelin* (pg/mL)	54.13 ± 40.94[a]	65.33 ± 93.54[b]	30.36 ± 17.13[a,b]	**[a]p = 0.003 [b]p = 0.04**

*(mean ± SD).

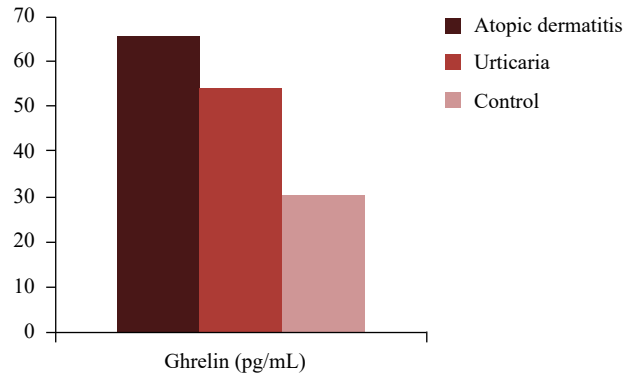

Figure 1: The laboratory findings of the patient and control groups.

patients with insulin resistance (50.47 ± 23.95 pg/mL) were lower than those of the atopic dermatitis patients without insulin resistance (69.04 ± 104.09 pg/mL); however, the difference was not statistically significant ($p > 0.05$). The mean serum ghrelin levels of two patient groups with insulin resistance (65.48 ± 45.07 pg/mL) were higher than those of the patients without insulin resistance (57.45 ± 80.37 pg/mL). The difference was not statistically significant ($p > 0.05$).

Metabolic syndrome (MetS) was found in five (16.7%) of the urticaria patients, in five (16.7%) of the atopic dermatitis patients and in one (3.3%) of the controls. The mean serum ghrelin level of all the patients with MetS (65.23 ± 42.67 pg/mL) and the mean level of the patients without MetS (57.18 ± 82.25 pg/mL) were similar ($p > 0.05$).

DISCUSSION

The secretion of ghrelin increases with fasting and decreases with fullness. It is known that ghrelin has regulatory effects on body weight (14). The blood ghrelin levels were found lower in obese people when compared with non-obese individuals, and it is also reported that weight loss increases blood ghrelin levels. Ghrelin regulates body weight *via* insulin (4). Insulin has regulatory action on ghrelin levels. An increase in insulin levels suppresses ghrelin (15). It is shown that serum ghrelin levels decrease when insulin resistance increases in Type 2 diabetes patients and the patients with insulin resistance and *vice versa* (16).

Recently, in a study that evaluates the relationship between urticaria and MetS, serum glucose, triglyceride levels and the rates of central obesity were found higher significantly in recalcitrant chronic urticaria patients than the controls. The prevalence of MetS was also significantly higher in the urticaria group (8). Another study reported that hypertension, which is a component of MetS, prolongs the duration of the disease in urticaria patients (17).

Atopic dermatitis is accepted as an organ-specific manifestation of atopic complex, which consists of allergic skin changes, allergic rhinoconjunctivitis and asthma. Peripheral eosinophilia and high serum IgE levels may be associated with atopic dermatitis. Immune dysregulation in Th2 pathway and skin barrier dysfunction are considered in the pathogenesis of atopic dermatitis (2). There are studies reporting that atopic dermatitis may be a risk factor for MetS. Silverberg *et al* (9) reported that obesity in adults induced relapses in atopic dermatitis. It was demonstrated that obesity lasting more than two and a half year in early childhood was a risk factor for atopic dermatitis (18). There was a relation between obesity and asthma because pro-inflammatory mediators secreted from adipose tissue had some effect on mast cells. In addition, a positive correlation was demonstrated between the serum levels of total cholesterol, LDL cholesterol and atopy in school-age children (2). Ma *et al* (19) detected that insulin resistance did not induce

asthma attacks in atopic or non-atopic asthma patients and they reported that atopy and asthma had no relation with obesity and insulin resistance.

There is no study evaluating the serum ghrelin levels in chronic urticaria and atopic dermatitis patients in the literature. However, there are some studies reporting the relation between atopy, asthma, obesity and ghrelin. Cobanoglu *et al* (20) declared that there was no difference between the serum ghrelin levels of the children with asthma and the children without asthma. They also reported that there is no correlation between BMI and ghrelin. Okamatsu *et al* (21) reported that there was negative correlation between serum IgE and ghrelin levels in overweight children and there was no correlation between BMI and ghrelin.

The metabolic system and immune system are closely related to each other. Interactions between these systems increase, especially in stress and diseases to keep the balance of the organism (22). Ghrelin is a potent anti-inflammatory hormone. It supresses the production of pro-inflammatory cytokines secreted from activated T-lymphocytes, monocytes, endothelial cells (23). In addition, ghrelin has been found to inhibit the proliferation of anti-CD3-activated T-lymphocytes. Th1 cytokines (IL-1 and IFN-γ) and Th2 cytokines (such as IL-4 and IL-10) which induce IgE synthesis in rodent' spleen were inhibited by ghrelin (24).

Elevated serum ghrelin levels in atopic dermatitis may occur in order to suppress Th2 cell predominance, which is considered to be an important pathogenetic factor of the disease. It is reported that ghrelin induced the release of histamine from rat peritoneal mast cells (25). An increased ghrelin release may induce the secretion of histamine in chronic urticaria patients.

In conclusion, we have found significantly higher serum ghrelin levels in chronic urticaria and atopic dermatitis patients than the controls. We did not find any correlation between ghrelin levels and MetS, BMI and insulin resistance. Therefore, we thought that new studies are needed to disclose if ghrelin plays a role in urticaria pathogenesis.

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ORIGINAL ARTICLE

Prevalence of Gastroesophageal Reflux Disease Symptoms in a Jamaican Population

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ABSTRACT

Objective: *Gastroesophageal reflux disease (GERD) is one of the most common conditions seen in general medical practice, which is associated with an impaired quality of life. This study determined the prevalence of GERD symptoms in a Jamaican population*

Methods: *Eligible individuals between the ages of 18 and 75 years were invited to complete a questionnaire, which included demographic data as well as the frequency and severity of typical GERD symptoms.*

Results: *The questionnaire was completed by 422 subjects with 51% males and 49% females. The prevalence of any GERD symptoms within the previous year was 71.1%. Females were more frequently affected, 75.7%, compared to 67.3% of males. Weekly symptoms were present in 18.6%. Symptoms of moderate or greater severity were reported in 11.7%. The most common and bothersome symptom experienced was heartburn. Nocturnal symptoms that awaken the affected subjects occurred in 17.8%. Gastroesophageal reflux disease was strongly associated with food, supine posture and heavy meals (*$p < 0.0001$*). There was no significant association with GERD and age or body mass index. In persons with GERD symptoms, 24.2% (*$p = 0.000$*) saw a doctor for their symptoms and 38.6% took medication for their symptoms (*$p < 0.0001$*), which included prescription medications in 42.7%, over the counter medications in 36.3% and combination of both in 15.3%.*

Conclusion: *Symptoms of GERD are common and significant problem in the Jamaican population studied. Heartburn was the most bothersome symptom reported. Medications were taken by over one-third of symptomatic persons.*

Keywords: Disease, gastroesophageal, heartburn, Jamaica, reflux.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most common conditions seen in general medical practice and presenting to the gastroenterologist (1). The prevalence of weekly symptoms of GERD in Europe was 10%–20% but lower in Asia at less than 5% (2). The prevalence of GERD symptoms in the Swedish population was 40% with a 15% prevalence of erosive esophagitis in patients undergoing endoscopy (3).

Gastroesophageal reflux disease is a significant contributor to the burden of health care costs and results in a significant loss of productivity (4). It also significantly affects the quality of life in patients in their productive years even with once weekly episodes and without esophagitis (5). Subjects with frequent symptoms experienced greater quality of life impairment than those in whom symptoms were less frequent or absent (5). The diagnosis of GERD is largely clinical and is based on typical symptoms. In fact, a presumptive diagnosis of GERD can be made with the presence of typical symptoms of heartburn and reflux (1). Questionnaires, to elucidate the frequency of symptoms, have been used in several populations to estimate the prevalence of GERD with acceptable specificity and sensitivity (2).

In a previous study, 44% of controls and 29% of HIV patients had reported heartburn in Jamaica (6). However,

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a larger study looking specifically at the prevalence of reflux symptoms is needed to determine if GERD is a significant medical problem in the Jamaican population.

SUBJECTS AND METHODS

Subjects for the study were randomly selected from employees and visitors at the University Hospital of the West Indies (UHWI) in Jamaica. The UHWI is an academic teaching hospital and tertiary referral hospital with approximately 600 beds.

Study collaborators obtained informed consent from all subjects for the study and administered questionnaires to individuals between the ages of 18 and 75 years who were eligible for the study. Subjects included doctors, nurses, clerical staff, ancillary workers, porters and students, as well as persons accompanying or visiting patients at the UHWI. Study participants included persons entering the casualty department, the medicine and surgery clinic waiting area or any of the 20 wards at the UHWI. Every third person was eligible to participate in the study. The participants were approached to participate in the study until 400 questionnaires had been completed.

Data was gathered in the form of a questionnaire. The questionnaire was administered by one of the study collaborators and completed by each individual. The questionnaire was left with the individual to be completed and collected immediately after the person was finished or the study collaborator read through the questionnaire with the individual and assisted him/her to complete it. The instrument captured demographic data as well as the frequency of typical reflux symptoms, investigations performed and medications taken.

Persons admitted to the wards or attending for any service at the hospital were excluded from the study. The study protocol was approved by the Ethics Committee of the University of the West Indies/UHWI. Raosoft software was used to calculate the sample size using a 5% margin of error, 95% confidence interval, population size of 20 000 and response distribution of 40%. The recommended sample size was 363 individuals. The study aimed to complete 400 questionnaires.

The data obtained from the questionnaire was analysed using descriptive univariate analysis (frequency tables and appropriate graphs) as well as a series of cross-tabulations and chi squared (or Fisher's exact) tests. Data was entered and analysed using SPSS version 16.0 (Chicago, IL, USA) for Windows.

RESULTS

There were 429 persons entered into the study; however, there was missing data in seven questionnaires that were excluded, and thus the final study population consisted of 422 subjects with 51% males and 49% females.

One-third (32.9%) of participants were in the 26–35 year old age group and a quarter (25.4%) in the 36–45 year old group. Thirty-nine per cent of persons had a normal body mass index (BMI) and 35% were overweight ($p = 0.214$). The median BMI for persons with GERD symptoms versus asymptomatic respondents was 25.1 and 25.5, respectively.

The prevalence of any GERD symptoms was 71% within the previous year. The highest prevalence was in the 26–35 years old group, followed by 36–45 and 18–25 years old ($p = 0.415$). The prevalence of weekly symptoms of GERD was 18.6%. Moderate to severe symptoms weekly was 11.7%. Females were more frequently affected by symptoms of GERD, 75.7% compared to 67.3% of males who had symptoms within the past year ($p = 0.055$). Nocturnal symptoms that awaken subjects occurred in 17.8% of patients.

The most common symptom experienced was heartburn (burning in the chest retrosternally) with a cumulative percent of 72.2%. Reflux was reported by 68.2% and acid taste in throat in 67.7% ($p < 0.0001$). The least common symptom was dysphagia in 11.7% (Table 1). Symptoms were considered to be mild in 24.7% ($p = 0.011$) who reported burning in the chest retrosternally, 16.4% ($p = 0.014$) who reported reflux, 13.5% ($p = 0.023$) reporting acid in the throat and 7.9% ($p = 0.014$) of persons that reported food reflux (Table 2). Food aggravated GERD symptoms was present in 70.7% ($p < 0.0001$) of cases. Greasy food aggravated GERD symptoms in 48.8% ($p < 0.0001$), followed by meats and/or beans in 18.6% ($p < 0.0001$), alcohol in 14.6% ($p < 0.0001$) and fruits in 11.9% ($p = 0.014$) of persons. Lying down after meal was associated with symptoms in 48.5% ($p < 0.0001$) followed by having a heavy meal in 35.9% ($p < 0.0001$) and lifting in 13.6% ($p = 0.001$) of participants.

Table 1: Symptom frequency

Symptom	GERD %	*p*-value
Burning in chest	72.2	< 0.0001
Acid in throat	67.7	< 0.0001
Burning in stomach	51.5	< 0.0001
Food reflux	42.1	< 0.0001
Awaken at night	25.2	< 0.0001
Dysphagia	11.7	< 0.0001

GERD = gastroesophageal reflux disease.

Table 2: Symptom severity

Symptom	Mild%	Moderate%	Severe%	*p*-Value
Liquid reflux	75.6	20.9	3.4	0.014
Burning in chest	65.8	30.8	3.3	0.011
Burning in stomach	67.4	28.7	3.9	0.003
Acid in throat	78.2	18.2	3.6	0.023
Food reflux	79.7	16.1	4.2	0.168
Dysphagia	73.0	20.3	6.8	0.316
Wake from sleep	64.7	27.5	7.8	0.414

Of persons with GERD symptoms, 24.2% ($p = 0.000$) saw a doctor for their symptoms, whilst 12.1% of persons had investigations done. Blood tests were requested in 69.4%, whilst 16.1% had a barium meal and 4.8% had endoscopy.

In persons with GERD symptoms, 38.6% took medication for their symptoms ($p < 0.0001$). Of subjects taking medications, prescription medications were taken in 42.7%, over the counter medications in 36.3%, combination of both in 15.3% and home remedy in 5.6% ($p = 0.005$). Other oral medication were taken by 27% ($p = 0.022$) of participants, 7.5% ($p = 0.361$) were taking anti-hypertensives, 4.4% ($p = 0.174$) were taking pain medications and 2.4% ($p = 0.943$) aspirin.

There was no significant association between smoking and GERD symptoms, as in those who smoked, 10.6% had symptoms whilst 8.5% were without GERD symptoms ($p = 0.519$).

In persons with symptoms of GERD, 4.4% reported missing work because of their symptoms.

DISCUSSION

The diagnosis and initial management of GERD is based on symptoms, but the frequency of symptoms that constitutes disease is not well defined (1, 4). Most studies on GERD refer to weekly or at least weekly symptoms of gastro-oesophageal reflux (2). The prevalence of GERD symptoms on a weekly basis in this study was 18.6%. Moderate to severe symptoms occurred on a weekly basis in 11.7%. This is comparable to other studies as the prevalence of heartburn and/or acid regurgitation experienced at least weekly was 14% in one study and 19.8% in another (5, 7). In a third study, 9% of responders had experienced heartburn on that day, 15% the preceding week, 21% the previous month and 27% within the year. There was a higher overall prevalence of regurgitation, 5% for the day of response, 15%, within the prior week, 29% prior month and 45% prior year (8). Although GERD appears to be increasing in prevalence in the Western world, in contrast, the reported population prevalence of reflux symptoms in eastern Asia ranged from 2.5% to 6.7% for at least weekly symptoms of heartburn and/or acid regurgitation (9, 10).

In the present study, the most common symptom experienced was heartburn with a frequency of 72.2%. Heartburn is a relatively common symptom in Europe ranging from a prevalence of 38% in Northern Europe to 9% in Italy. Data from the USA suggests an even higher prevalence of 42% overall and weekly in 19.8% (7, 9). However, in China, 3.1% reported weekly heartburn (2). In a previous smaller study in Jamaica, 44% of a control population experienced heartburn symptoms (6). In a study using pH testing, only heartburn (68% vs 48%) and acid regurgitation (60% vs 48%) occurred in more of the patients with GERD than of those with normal pH monitoring (11).

The present study did not demonstrate a significant relationship between BMI and GERD, but other studies have shown an increasing prevalence of GERD was associated with excess body mass/higher BMI (2). In a meta-analysis, obesity was associated with a statistically significant increase in the risk for GERD symptoms, erosive esophagitis, and esophageal adenocarcinoma. The risk for these disorders seems to progressively increase with increasing weight (12). Other studies have found that younger females with lower BMI tend to have non-erosive reflux disease, whilst persons with esophagitis had increased age and BMI and were more likely to have continuous symptoms and require acid suppression (9).

Gastroesophageal reflux disease and nocturnal symptoms are associated with poor quality of life, and the impairment may be comparable with that for heart disease (4, 9). In addition, symptoms severity and nocturnal heartburn are significantly associated with reduced work productivity, particularly when nocturnal heartburn interferes with sleep (4). In one study, over 48% of respondents with severe symptoms reported reduced productivity, compared with 40 and 12% of respondents with moderate and mild symptoms, respectively, and nocturnal GERD symptoms were an important predictor of reduced work productivity (4). Nocturnal symptoms occurred in 17.8% of our patients. This is slightly more than the 10% obtained in a previous study in which quality of life impairment was exacerbated in those who report nocturnal GERD symptoms (13). In addition, meaningful daytime sleepiness was observed in individuals with GERD symptoms (14).

In this study, 24.2% of persons with GERD symptoms actually sought medical attention and 4% reported missing work because of their symptoms. This indicates an

adverse effect on productivity and cost. A previous study on GERD found that only 5% of the population surveyed had consulted their doctor in the previous year because of GI symptoms (5). Most persons with heartburn and reflux symptoms have mild or occasional symptoms and do not seek medical advice but treat themselves with over the counter medications (15). In the present study, over one-third took medications for their symptoms, 42.7% reported taking prescription medication and 36.3% over the counter medication. In addition to an adverse effect on the quality of life, GERD is associated with the added expense of long-term treatment.

Of the 24.2% of persons that sought medical attention, 12.1% had tests done. In an earlier study, medication, most commonly antacids, was used by 16% of symptomatic people and only 5.5% had sought medical advice for symptoms during the past year (8). Based on the American college of Gastroenterology guidelines for the diagnosis and management of GERD, a presumptive diagnosis of GERD can be established in the setting of typical symptoms of heartburn and regurgitation and empiric medical therapy with a proton pump inhibitor is recommended in this setting (1). Endoscopy is recommended in the presence of alarm symptoms, and for screening of patients at high risk for complications, the elderly and non-responders to therapy (1). Esophageal manometry and 24 hour esophageal pH monitoring are only indicated for unclear or refractory cases and to confirm the diagnosis in operative management of GERD.

There were limitations in the present study. This study was a questionnaire-based study and is thus subject to recall bias. The symptoms of GERD (heartburn and acid regurgitation) are of low sensitivity but high specificity when they are the dominant complaint and therefore may not exclude other causes.

In conclusion, this study demonstrated that symptoms of GERD are common and significant problem in our population. Weekly symptoms were present in 18.6%. Symptoms of moderate or greater severity were reported in 11.7%. Nocturnal symptoms occurred in 17.8%. Of persons with GERD symptoms, 24.2% saw a doctor for their symptoms and 38.6% took medication for their symptoms which included prescription medications in 42.7%, over the counter medications in 36.3% and combination of both in 15.3%.

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ORIGINAL ARTICLE

Prevalence of Obesity among Adult Surgical Patients in Tobago

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ABSTRACT

Objective: *To estimate the prevalence of obesity among adult surgical patients in Tobago.*
Methods: *Patients aged 18 years and over who had surgery at the Scarborough General Hospital were studied. Demographic data of age, gender, weight and height were collected from the anaesthesia records. Obesity was assessed using body mass index (BMI) calculated as weight in kilograms divided by the square of height in metres (kg/m^2). The WHO classification was used with obesity defined as BMI ≥ 30 kg/m^2, overweight as BMI ≥ 25–29.9, and normal weight as BMI ≥ 18.5–24.9.*
Results: *Data from 799 patients (214 [26.8%] male and 585 [73.2%] female) were analysed. They had a mean age ±standard deviation (SD) of 44.5 ±15 years, median age of 42 years and a range of 18–97 years. Their mean BMI ± SD was 30.6 ± 8.15 (male 27.6 ±7 and female 31.7 ± 8.3). Two percent were underweight, 21% were normal weight, 32% were overweight and 45% were obese. A greater proportion of females were in the higher BMI categories compared with males. More than half of the females were obese (52%) compared to just over a third of the males (26%).*
Conclusion: *Overweight and obesity are common among adult surgical patients in Tobago and the prevalence is higher in females.*

Keywords: Body mass index, Caribbean, obesity, surgery, surgical patients, Tobago

INTRODUCTION

The World Health Organization (WHO) defines both obesity and overweight as excessive body fat accumulation that may impair health (1, 2). The prevalence of obesity has been increasing rapidly worldwide, with rates more than doubling in the last three decades and it is now considered a major global public health problem (1–3).

Obesity is associated with a number of chronic physical and mental health problems including diabetes, heart disease, hypertension, stroke, gallstones, osteoarthritis, dyslipidaemia, obstructive sleep apnoea, certain types of cancer, and psychological and behavioural problems (4–6). In addition, several surveys and reviews have shown that people who are obese are more likely to be hospitalized and to undergo surgical procedures than those who are not obese (6–9). They are also at higher risks for developing surgical-related complications as well as major adverse cardiovascular events and all-cause mortality (7–12).

Apart from the pathophysiologic changes associated with overweight and obesity, there are technical challenges associated with their perioperative management such as difficulties with venous access and regional anaesthetic blocks, airway management, appropriate monitoring devices, optimal surgical access and requirement for extra personnel and special equipment for positioning, lifting and transporting (6, 9, 11). A worrying concern is that obese persons may be denied necessary surgery because of the perceived fear of increased risks by surgeons and anaesthesiologists (13, 14). Obesity is associated with a reduced quality of life and increased morbidity and mortality and is second only to smoking as the leading cause of preventable deaths worldwide (12, 15, 16).

Obesity is commonly assessed by body mass index (BMI), which approximates a person's body fat percentage. The BMI is a calculated value and is defined as a person's weight in kilograms divided by height in metres squared (BMI = kg/m^2).

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We aimed to study the prevalence of overweight and obesity among surgical patients in Tobago, the smaller and less populated (approximately 63 000 inhabitants) of the twin-island nation of Trinidad and Tobago.

SUBJECTS AND METHODS

In Trinidad and Tobago, healthcare in public health facilities is free of charge and available to all citizens. The Scarborough General Hospital is the largest and main surgical hospital in Tobago where most of the surgical procedures on the island are done. All patients scheduled for a surgical procedure undergo an anaesthetic assessment in the pre-operative anaesthetic clinic where standard demographic data including age, gender, objectively measured weight and height and vital signs are collected and recorded as a part of routine clinical practice. The height, weight, age and gender data of consecutive adult patients aged ≥ 18 years who underwent surgery in 2016 were extracted from the anaesthetic record and inputted into a Microsoft Excel spreadsheet.

Obstetric patients were excluded. The BMI was calculated according to the formula, BMI = weight (kg)/ height $(m)^2$ and patients were grouped according to the WHO obesity classification (1, 2): Underweight is defined as BMI < 18.5 kg/m^2; normal weight is defined as BMI ≥ 18.5–24.9 kg/m^2; overweight is defined as BMI between 25.0 and 29.9 kg/m^2; and obesity is defined as BMI ≥ 30 kg/m^2. The degree of obesity is often subdivided into Class I (BMI: 30–34.9), Class II (BMI: 35–39.9) and Class III (BMI: ≥ 40). For our study and for ease of comparison with other studies and familiarity, we subdivided the obese group into two classes: obesity with BMI 30.0–34.9 (equivalent to class I), and morbid obesity with BMI ≥ 35 (equivalent to class II + III). The data were analysed using descriptive statistics of mean, median, range, standard deviation (SD) and percentages as appropriate. The institution approved the study as clinical audit and informed consent was not required as this was a retrospective registry-based study.

RESULTS

We analysed data from a total of 799 patients; 214 (26.8%) were male and 585 (73.2%) were female and they had a mean age of 44.2 years ±15.3 (SD), median age of 42 years and a range of 18–97 years. The distribution of BMI according to categories is shown in Table 1. The average BMI of all our patients was 30.6 (male: 27.6 and female: 31.7) and more than 3/4th (78%) of them were either overweight or obese (male: 64% and female: 83%). A greater proportion of females were in the higher BMI categories compared to males (Figure) with females being twice as obese as males (52% *vs* 26%). More than half of the females were obese compared to just over a third of the males. The morbid obesity rate (BMI ≥ 35) was 24% overall with a higher prevalence in women (29%) compared with men (9%).

Table 1: Distribution of BMI according to categories

BMI category (kg/m^2)	Male (%)	Female (%)	Total (%)
<18.5 (underweight)	7 (3)	7 (1)	14 (2)
18.5–24.9			
(normal weight)	67 (31)	98 (17)	165 (20)
25–29.9 (overweight)	81 (38)	178 (30)	259 (31)
30–34.9 (obese)	39 (18)	141 (24)	180 (22)
≥35 (morbidly obese)	20 (10)	161 (28)	181 (24)
BMI mean ± SD	27.6 ±7.0	31.7 ± 8.3	30.6 ± 8.2
BMI range	14.5–82.5	15–117	14.6–117
Total	214 (26.8)	585 (73.2)	799

BMI = body mass index; SD = standard deviation.

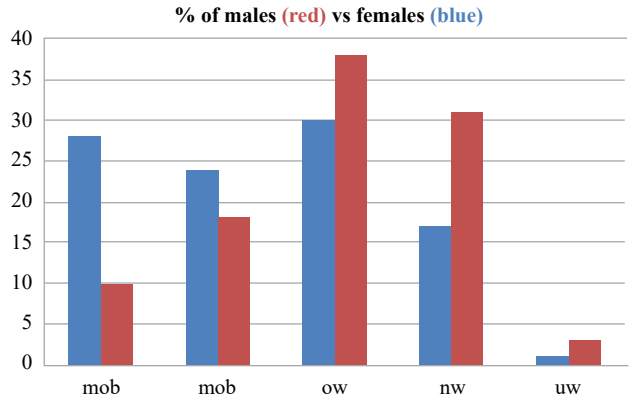

Figure: Comparison of males (red) versus females (blue) in percentages according to BMI categories.

mob = morbidly obese; ob = obese; ow = overweight; nw = normal weight; uw = underweight.

DISCUSSION

The prevalence of overweight and obesity in adult Tobagonians undergoing routine surgery is very high. In fact, it is among the highest documented prevalence worldwide as Table 2 shows. This study is corroborated by the national survey conducted in 2011 that indicated that more than half (55.7%) of the population aged between 15 and 64 years is either overweight or obese and that a quarter (25.7%) is obese (16). As a comparison in 2014, the WHO estimated that globally about 13% of adults aged 18 years and over were obese and 39% were overweight or obese (2). This indicates that the obesity rate of Trinidad and Tobago is twice the global rate.

Table 2: Comparison of the prevalence of obesity among surgical patients in different countries/regions

Country	Netherlands (10)	UK[a] (8)	UK[b] (13)	Benin (20)	Nigeria (21)	USA (18)	Tobago
Obesity BMI ≥ 30 kg/m²	17.3%	34.5%	20.7%	9.7%	11.4%	35.4%	46%

[a]Multi-centre including bariatric centres; [b]single centre; ( ) = reference; BMI = body mass index.

Our figures for BMI, obesity, and overweight and obesity combined, are consistently higher than the corresponding national figures (31.3 kg/m^2, 31%, 78% *vs* 26.5 kg/m^2, 25.7%, 55.7%, respectively). A number of reasons can be offered for this difference. Firstly, the national survey was done in 2011 while this Tobago study was done in 2016, a half decade later. As earlier mentioned, obesity has been noted to be rapidly increasing with time due mainly to dietary and lifestyle changes (3). Secondly, Tobago is an island separate and different from its larger sister island Trinidad and the socio-cultural and economic activities and ethnic mix may be different. Thirdly, our sample is quite different from the national survey. Theirs was a nationally representative population-based survey of individuals in Trinidad and Tobago aged 15–64 years using a stratified randomized cluster sample, although ours was a sample of hospital surgical patients aged ≥ 18 years. They have a relatively younger age group than us. Hospitalized patients have been noted to be more obese than their non-hospitalized counterparts (10, 12).

Similar findings of a difference in BMI between hospitalized persons and non-hospitalized persons within the same population have been observed in other surveys from Europe and North America (8, 10–13, 18). Our findings support these studies that indicate that overweight and obese persons are over-represented in patients who are hospitalized and who require surgery. It must be stated though that the surgical procedures performed at the major surgical hospital in Tobago do not include procedures that target obese patients such as the orthopaedic joint replacement surgery or the bariatric surgery for treatment of morbid obesity.

The majority of Tobagonians (>85%) are of African ancestry, being descendants of slaves mainly from western Africa (19). It was therefore quite instructive and interesting when we compared our BMIs to the BMIs of similar surgical patients in the Republic of Benin and Nigeria, two West African countries that had major slave seaports (20, 21). There were significant similarities and differences. The main and surprising difference was that the percentage of obesity in Tobago was five times greater than that of Benin (46% *vs* 9.67%) and four times greater than that of Nigeria (11.4%) and the main similarity was that women were significantly more obese than men in all three studies. This striking gender disparity among persons of African origin is well recognized (20–22).

Several studies have shown that Africans in the diaspora are generally more overweight and obese compared to their counterparts in the homeland (22–24). This has mainly been attributed to 'westernization and urbanization' leading to the adoption of less healthy diets and lifestyles (3, 22–24). Interestingly, the rate of increase in obesity seems to be related to the level and stage of socio-economic development of the particular region or country. A comparative longitudinal study of BMI of persons of African descent over 5 years was carried out in three countries, representing different levels of development as classified by the World Bank using gross national income (GNI)per capita: Nigeria (low income), Jamaica (middle income) and USA (high income) (25).

As expected, baseline average BMIs were the highest in the USA and the lowest in Nigeria with Jamaica in between; however surprisingly, the annual rate of weight gain was most rapid in Jamaica (1.37 kg/year), the middle-income country compared to the USA (0.52 kg/year) the high-income country and Nigeria (0.31 kg/year) the low-income country (Table 3). The average weight gain/year in Jamaica was about four times that of Nigeria or the USA. This highest increase in weight gain with time has been attributed to 'the accelerating effects of the cultural and behavioural shifts' in these 'transitional societies' (22, 25).

Table 3: Three-country obesity comparison according to the Gross National Income per capita

	Nigeria (low income)	Jamaica (middle income)	USA (high income)
Obesity			
BMI ≥ 30 kg/m²	5%	24%	51%
Weight change (S.E.) kg/year	0.31 (0.05)	1.37 (0.04)	0.52 (0.05)

BMI = body mass index.

Our findings have important clinical and policy implications. Self-perception of overweight and obesity in the Caribbean is often grossly underestimated (26, 27).

Many obese persons are unaware of the severity of their obesity and its health implications and more worrisome, is the fact that greater than 50% of these obese persons state that they have never been told about their weight problem by a clinician even when their weights had been taken during the hospital visit (26). Patients are routinely seen before their surgery by surgeons, anaesthetists and other clinicians, and their data such as weight, height and BMI are usually available in their records. This is a key opportunity to educate the patient about the health risks of obesity and its control. When patients are informed about obesity and its myriad effects, they are more likely to take appropriate preventative and corrective actions.

This study also provides important information to policy makers and public health administrators that our population is becoming less healthy. This implies increased healthcare utilization and costs and decreased productivity (5, 12, 22, 26). Obesity fortunately is a preventable and treatable condition by readily available and proven public health methods (1, 4, 5). In this era of escalating healthcare costs and very limited resources, the importance of preventing and treating obesity cannot be over-emphasized. There is an urgent need for a multidisciplinary and multi-faceted approach to effectively manage this crisis (1, 22).

AUTHORS' NOTE

AO Amata conceived paper, collected data, conducted data analysis, wrote manuscript and approved final version. VT Mitchell participated in the study design, data analysis and interpretation, critically revised manuscript and approved final version.

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ORIGINAL ARTICLE

Primary Mediastinal Cysts: A Review of 41 Surgically Managed Cases

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ABSTRACT

Objective: *To review the surgical outcomes of primary mediastinal cysts (PMCs) and to determine the differences among various histopathological types.*

Methods: *We retrospectively analysed the medical records of 41 patients (19 men, 22 women; mean age 46.5 ± 13.7 years, range 19–78 years) with PMCs who underwent surgical excision betweeen January 2007 and January 2016. Age, sex, indication for surgical intervention, surgical technique, histopathological diagnosis, postoperative complications and duration of hospital stay were recorded for all patients. The differences among the various histological types of PMCs were determined using analysis of variance for continuous variables and Chi-square test for categoric variables. A* p-*value of less than 0.05 was considered statistically significant.*

Results: *A total of 41 patients with PMCs were managed surgically at our clinic over a period of 9 years. A total of 19 men (46.3%) and 22 women (53.7%) were included in the study. The patients had a mean age of 46.5 ± 13.7 years, a mean duration of hospital stay of 4.0 ± 1.0 days and a mean postoperative follow-up duration of 11.2 ± 3.7 months. According to the histopathological analysis, 21 (51.2%) patients were diagnosed as having a pericardial cyst; 16 (39%) had a bronchogenic cyst; 3 (7.3%) had a cystic hygroma and 1 (2.4%) had a thymic cyst. No postoperative mortality or recurrence was observed over a period of 11.2 ± 3.7 months. No statistically significant difference was found among the variables, either.*

Conclusion: *Despite advanced non-invasive diagnostic techniques, the definitive diagnosis of PMCs can only be made by interventional techniques. Surgery often provides curative therapy with low morbidity and mortality.*

Keywords: Bronchogenic cyst, cyst, mediastinum, thoracotomy.

INTRODUCTION

Mediastinal masses may originate from any thoracic organ and display a wide array of pathological findings as malignant and benign lesions (1). Primary mediastinal masses (PMM) constitute approximately 10%–25% of these masses (1, 2). These PMM lesions are congenital or acquired, or formed as a result of the degeneration of a previously existing solid tumour (1). Therefore, cystic lesions may occur in both childhood and adulthood. Primary mediastinal cysts (PMCs) may display a wide array of histological types, such as thymic cysts (TCs), bronchogenic cysts (BCs), pericardial cysts (PCs), and lymphangiomas (cystic hygroma [CH]). Although symptomatic PMCs are unequivocally considered an indication for surgery, the management of asymptomatic PMCs is still controversial. Some surgeons prefer surgical management due to a fear of malignant transformation, cyst infection, progressive growth or spontaneous rupture, while some others advocate that not all PMCs should be removed given the benign nature of these lesions (3–5). Despite this uncertainty, literature data on the surgical experience in PMCs is considerably limited.

The aim of this study was to review the outcomes of the surgical procedures aimed at treating PMCs at our institution in the last 9 years and to report the

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clinicopathological variations with a discussion of the relevant literature.

SUBJECTS AND METHODS

We reviewed the medical data of 41 patients (19 men, 22 women; mean age 46.5 ± 13.7 years (range 19–78 years)) diagnosed with PMC by the histological examination after surgical treatment between January 2007 and January 2016. Patients who were not treated surgically but followed conservatively were excluded. The examined variables included age, sex, indication for surgical treatment, side of surgical treatment, histopathological diagnosis, time to chest tube removal, duration of hospital stay and postoperative complications. The study protocol was approved by the local ethics committee of our hospital. The study was conducted in compliance with the criteria of the Helsinki Declaration.

In the preoperative period, a posteroanterior chest film, thoracic computed tomography (CT) examination with contrast enhancement and/or pulmonary mediastinal magnetic resonance imaging (MRI) taken within the preceding month were reviewed by the surgeon operating on the patients. All patients underwent complete blood count, routine biochemistry tests, respiratory function testing and cardiological examination with electrocardiography as well as echocardiographic examination as needed. All patients were informed about the advantages and disadvantages of the thoracotomy, sternotomy and video-assisted thoracoscopic surgery (VATS) approaches.

Operative technique

All patients were intubated with a double-lumen endotracheal tube following general anaesthesia, except for one patient who was operated on with median sternotomy. Patients to be operated on with thoracotomy were placed in the right or left posterolateral thoracotomy position depending on the lesion's side, and the thoracic cavity was entered through the fifth intercostal space. The thoracoscopic operations were performed using three to four ports with the patients lying in the lateral position. In the TC case, the patient was placed in the supine position following single-lumen intubation. A pillow support was placed in the interscapular area, and median sternotomy was performed (due to our inexperience in endoscopy). Total excision was carried out and extubation was performed at the operating theatre in all patients. All patients had a control chest X-ray at the early postoperative period.

Statistical analysis

The differences between various histological types of PMC were compared with the analysis of variance test for continuous variables and Chi-square test for categoric variables. A p-value of less than 0.05 was considered significant for all tests. All statistical analyses were carried out using SPSS version 21.0 (IBM) (Chicago, IL, USA) software package.

RESULTS

A total of 41 patients with PMC were surgically treated over a period of 9 years. As a total, 19 (46.3%) men and 22 (53.7%) women were enrolled. The age range of the study subjects was 46.5 ± 13.7 years; the postoperative duration of hospital stay was 4.0 ± 1.0 days and the postoperative duration of follow-up was 11.2 ± 3.7 months. Based on the histopathological examination, 21 (51.2%) patients were diagnosed as having a PC, 16 (39%) had a BC, 3 (7.3%) had a CH and 1 (2.4%) had a TC. The operations were right sided in 22 (53.7%) patients, left sided in 18 (43.9%) patients and in supine position in 1 (2.4%) patient. Neither postoperative mortality was observed nor there was any recurrence after a mean of 11.2 ± 3.7 months. Table 1 provides a comparison of clinical characteristics, treatment modalities and follow-up information of 41 patients. No significant difference was found between the groups with respect to studied variables (sex, age, pathological diagnosis).

Bronchogenic cyst

Eight of 16 BC cases were asymptomatic. Six patients had cough and two had dyspnoea. In thoracic CT and MRI examinations, two patients had lesions in anterior mediastinum, nine had lesions in middle mediastinum and five had lesions in posterior mediastinum. Thirteen patients were operated on with thoracotomy and three with the VATS technique. One patient had atelectasis and another one had prolonged air leakage as a postoperative complication.

Pericardial cyst

While 15 of 21 patients with PC were asymptomatic, six had chest pain, cough and dyspnoea. All lesions were located in middle mediastinum. Seventeen patients were operated on with thoracotomy and four with VATS. As a postoperative complication, two patients developed atelectasis, one wound site infection and one transient phrenic nerve paralysis.

Table 1: Demographic properties of patients by histological type

Property	PC (n = 21)	BC (n = 16)	CH (n = 3)	TC (n = 1)
Sex, F/M	10/11	10/6	1/2	1/0
Age, years*	47 ± 1.5	43	49	53
Symptoms	Chest pain, cough, dyspnoea	cough, dyspnoea	Chest pain, dyspnoea	Chest pain, dyspnoea
Mediastinal localization	Middle	Anterior 2, middle 9, posterior 5	Middle	Anterior
Maximum cyst diameter, cm*	4.6 ± 1.3	3.9 ± 1.7	6.9 ± 1.2	4
Thoracic CT	15	13	2	1
Thoracic MR	6	3	1	1
Surgical technique	Thoracotomy 17, VATS 4	Thoracotomy 13, VATS 3	Thoracotomy 3	Sternotomy
Resection width	TE	TE	TE	TE
Postoperative complication	Atelectasi 2 wound inf.1, Phrenic nevre paralysis 1	Atelectasis 1, prolonged air leakage 1	Minimal haemorrhage 1	no
Postoperative duration of hospital stay, days*	4.0 ± 1.0	4.1 ± 1.0	5.0 ± 1.0	5
Postoperative follow-up duration, months*	9.0 ± 3.3	12.9 ± 3.5	11 ± 1.5	17

*Values denote mean ± standard deviation. TC = thymic cyst; BC = bronchogenic cyst; PC = pericardial cyst; CH = cystic hygroma; MRI = magnetic resonance imaging; CT = computerized tomography; VATS = video-assisted thorascoscopic surgery; Min = minute; TE = total excision.

Cystic hygroma

Of three patients with CH, two were asymptomatic, while one patient had chest pain and dyspnoea. The lesions were in middle mediastinum. After showing the relationship of the CH with the adjacent tissues, all three patients were operated on with thoracotomy. The cyst was filled with a serous fluid and enveloped by a thin, transparent membrane. One patient developed minimal postoperative haemorrhage.

Thymic cyst

The patient with the TC had chest pain and dyspnoea. The lesion was located in anterior mediastinum. On thoracic CT, there was a thin-walled cyst filled with a cystic content of fluid density. Lung MRI was used to reveal the borders of the cyst with other adjacent organs. It was observed to have a low signal intensity on T1-weighted images and a high-signal intensity on T2-weighted images. We totally excised the TC with median sternotomy.

DISCUSSION

Primary mediastinal cyst lesions appear as congenital or acquired lesions, or as a result of the degeneration of a previously existing solid tumour. The prevalence of PMCs among mediastinal masses ranges between 10% and 25%. They affect both sexes equally (6).

The prevalence of cysts varies by age groups. While foregut cysts constitute approximately half of congenital mediastinal cysts in adults, 90% of all cysts are foregut cysts (BC, enteric cyst, neurenteric cyst) in the paediatric age group. Pericardial cyst is more common among adults, whereas it is extremely rare in the paediatric age group (1, 2, 6). Depending on cyst size and adjacent organ compression, patients may present with variable symptoms and clinical presentations.

Asymptomatic congenital cysts may not be detected until adulthood. Bronchogenic cysts (mediastinal–bronchogenic) typically arise along the tracheobronchial tree. They are well-circumscribed cystic lesions of bronchopulmonary origin typically located in the middle mediastinum. They constitute approximately half of all mediastinal cysts (7). In a study comprising 86 patients under follow-up for mediastinal and lung BCs, 72.1% of all patients were symptomatic. In the same study, 67% of patients with mediastinal BC were symptomatic, as were 90% of patients having lung BC (8).

The most common symptom of patients with BC was retrosternal pain (61.4%), while those with lung BC mostly had cough, dyspnoea, fever and purulent sputum (8). In a domestic case series containing 22 patients from all age groups, 81% of patients were symptomatic, with cough being the most common symptom (45%) (9). Although BC has been reported as the most common histological variant of PMC, PC was the most common variant in our series, which was responsible for almost half of cases (n = 21; 51.2%) (3, 4). Eight of our BC cases (n = 16) were asymptomatic. Six patients had cough and two had dyspnoea. The cysts were located in anterior mediastinum in two patients, middle mediastinum in nine and posterior mediastinum in five. Signs of BC can be found on plain films in two-thirds of patients. It is

typically seen as a well-circumscribed, round, homogeneous mass lesion of 2–10 cm in diameter. When a cyst opens into a bronchus or is infected, however, an air-fluid level may become evident (1).

Thoracic CT is the most commonly employed diagnostic method in the evaluation of BCs, mainly due to its ability to provide detailed information about a cyst's structure, density, properties of its fluid, calcium content of its wall, its relationship with neighbouring structures and its vascular supply (1, 9). Magnetic resonance imaging provides information depending on a cyst's content. In cysts with fluid content, very-low-density images are obtained on T1-weighted sections and very-high-density images on T2-weighted sections. However, due to low-quality images, it is rarely used in cysts containing a large amount of proteinaceous material (9). Moreover, thoracic CT is superior to MRI for the evaluation of an intraparenchymal BCs since it may more clearly distinguish a cyst from an aerated region (1). Thoracic CT was also the most commonly used diagnostic method in our series (n = 13).

There are some case reports on the use of more invasive histopathological diagnostic methods such as bronchoscopy, endobronchial and oesophageal ultrasonography (EBUS and EUS), mediastinoscopy and thoracoscopy, for the diagnosis of BC (10). Surgical resection is the single method used for confirming the diagnosis (9). If patients are not treated surgically, most of them develop symptoms and complications. Moreover, it has been reported that some patients who are left untreated may develop malignancy originating from cyst mucosa (1, 9, 11). It is particularly recommended that all symptomatic patients be treated surgically (1, 8, 9, 12). Therefore, thoracotomy is usually needed for resection (1, 8, 12). It has been reported that lobectomy is the best surgical choice for intraparenchymal BCs and wedge resection for peripherally located small cysts (13). Mediastinal BCs can also be treated using mediastinoscopy through a small incision (1, 14). It has been reported that VATS can achieve favourable results in the resection of mediastinal cysts, with recurrence rates being not increased (15, 16). Some recent studies have indicated that robotic surgery can be safely used with low morbidity for the treatment of mediastinal tumours and cysts (17, 18). As we were in the beginning of the VATS experience, we performed VATS for three patients and thoracotomy for the remaining 13 patients.

Pericardial cyst is a benign pathological lesion developing as a result of incomplete lacunar fusion during the formation of coelomic cavity during the intrauterine period (19). It is extremely rare in the childhood period (1). Its incidence is 1/100 000 and constitutes 7% of all mediastinal mass lesions (19). The cyst wall is lined by a single layer of mesothelial cells and connective tissue that is rich in elastic and collagen fibres. A cysts' size varies between 1 and 30 cm (19, 20). Cysts can be found in any mediastinal compartment from upper mediastinum to diaphragm (21). They are most commonly located in the lateral basal corner of the pericardium, where it meets diaphragm (cardiophrenic angle), and mostly in the right cardiophrenic angle (51%–70%) (1, 22).

Pericardial cyst is usually asymptomatic (1, 19, 23). It may rarely give rise to compressive signs (right ventricular outflow obstruction, pulmonary stenosis secondary to compression, cyst rupture and sudden death) (23). In our patients with PC, the lesions were located in middle mediastinum, while 15 patients were asymptomatic, 6 had chest pain, cough and dyspnoea.

The cyst appears as a well-circumscribed, round or oval mass in the costophrenic angle on conventional chest films (1). Used for definitive diagnosis, thoracic CT attains a diagnostic accuracy of nearly 100%. Thoracic CT and MRI have no superiority over each other. In our series, the radiological diagnosis of PC was made by thoracic CT in 15 patients and MRI in 6 patients. PCs are benign lesions for which there exists no clear consensus for therapy. Surgery is usually recommended when radiological cyst size changes or when certain signs such as arrhythmia, hemodynamic instability, pericarditis or tamponade emerge (1, 19, 20, 22, 23).

However, malignant transformation has been described in the literature; therefore, surgery is recommended even for asymptomatic cases. A choice between an endoscopic and open surgical approach is influenced by cyst's location, size and relationship with vital organs (1).

Cystic hygroma is a multilocular, thin-walled cystic mass of lymphatic origin. Approximately, 1% of all lymphangiomas are located in the mediastinum. It is a rare tumour that usually develops in the first decade of life. The major reason of its rare occurrence is its asymptomatic nature or very delayed symptoms. Its aetiology commonly involves abnormal dilatation of the lymphatic system (24). Although typically located in anterior mediastinum, it may also be seen in middle mediastinum. Although ultrasonography and thoracic CT are quite helpful for making the diagnosis by detecting CH, definitive diagnosis is made by histopathological examination. On ultrasonography, it appears as having septae

composed of multilocular masses with a dominant cystic appearance.

Thoracic CT and MRI delineate the relationship of CH with the surrounding structures (25). In one of our cases in which mediastinal CH was found, an MRI was taken to clarify the relationship of the lesion with great vessels. Although some authors have recommended a conservative therapy for asymptomatic CH cases, spontaneous regression of these lesions is rare (25). A number of authors have reported favourable outcomes with total surgical excision (25). Riechelmann *et al* (24) did not observe any recurrence after total excision but did so in 56% of cases after subtotal excision and 100% of cases after partial excision or aspiration. We carried out total excision for all of our patients and observed no recurrences after a mean follow-up period of 11.2 ± 3.7 months.

Thymic cyst is a rare lesion located in anterior mediastinum, which may be both congenital and acquired (1, 6). It is believed that congenital cysts develop from remnants of the bronchial sac. Acquired ones are seen after infections, trauma or immune pathologies. It constitutes less than 1% of mediastinal lesions (26).

Symptoms depend on a cyst's localization and relationship with adjacent organs (26). Despite being a benign lesion, it should be differentiated from malignant tumours of anterior mediastinum (such as thymoma, germ cell tumours and lymphoma) (1). It appears as a well-circumscribed mass lesion in anterior and superior mediastinum. The typical sign on thoracic CT is a thin-walled cyst and cyst content of fluid density. Lung MRI clearly demonstrates the relationship of a cyst with adjacent organs (1). Surgical intervention via thoracotomy or thoracoscopy is recommended both for making differential diagnosis and providing a curative therapy (1, 6, 26).

CONCLUSION

Despite advanced noninvasive diagnostic methods, definitive diagnosis of PMC lesions can usually only be achieved by surgical interventions. Surgery is usually a curative option with low morbidity and mortality.

AUTHORS' NOTE

EA conceived paper, oversaw data collection, conducted data analysis, statistical analysis, wrote manuscript and approved final version. TE participated in study design, data analysis and interpretation, critically revised manuscript and approved final version. The authors declare that they have no conflicts of interest. The authors declare that they received no financial funding support for the conduction of their study project and writing of this manuscript.

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ORIGINAL ARTICLE

Incidence and Prediction of Catheter-related Bladder Discomfort after Transurethral Resection of the Bladder Tumour

N Guo, D Su

ABSTRACT

Objective: *Catheter-related bladder discomfort (CRBD) has suggested a large inter- and intra-individual variation in patients after transurethral resection of the bladder tumour (TURB), emphasizing the need for valid predictors and improved treatment. We performed a prospective observational study to determine the incidence and predictors of early postoperative CRBD in patients undergoing TURB.*

Methods: *Consecutive adult patients under general anaesthesia necessitating urinary catheterization were included during a 5 month period. CRBD was assessed with a simple four-step severity scale: no pain, mild pain (revealed only by interviewing the patient), moderate (a spontaneous complaint by the patient) and severe discomfort (agitation, loud complaints and attempt to remove the bladder catheter).*

Results: *Predictors of CRBD were identified by univariate and multivariate analysis. A total of 220 consecutive adult patients were included, of which 82.3% complained of CRBD at day 1 (moderate or severe CRBD: 29.5%) and 23.2% complained of CRBD at day 3 (moderate or severe CRBD: 1.8%). In a multivariate analysis model, age < 65 years (OR = 1.7, 95% CI: 1.2, 4.8,* p *= 0.01) and male gender (OR = 2.7, 95% CI: 1.3, 9.7,* p *= 0.02) were identified as independent predictors of moderate or severe CRBD.*

Conclusion: *Our study identified the incidence and predictive factors of moderate or severe CRBD and suggested that future improved treatment after TURB should focus on < 65 years male patients.*

Keywords: Catheter-related bladder discomfort (CRBD), predictive, Transurethral resection of the bladder tumour (TURB)

INTRODUCTION

Catheter-related bladder discomfort (CRBD) is defined as an urge to void or discomfort in the suprapubic region, observed after operation in patients who are awakening from anaesthesia and have had a urinary catheterization during operation (1). Transurethral resection of the bladder tumour (TURB) is the choice for many bladder cancers (2). Most patients undergoing TURB are of high risk for CRBD. This symptom complex reduces the quality of life postoperatively and increases postoperative pain and agitation (3). It was reported that the preoperative administration of muscarinic receptor antagonists, such as tolterodine and oxybutynin, can be effective in preventing CRBD (1, 4). However, the fact that CRBD is highly variable among patients underlines the need for individualized preoperative drug treatment and predictors of high validity. Besides, the adverse effects of premedication, such as dry mouth, dizziness and facial flushing, cannot be fully avoided. Therefore, we performed a prospective observational study in patients undergoing TURB to characterize CRBD and investigate predictive factors for their possible influence on early postoperative CRBD.

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SUBJECTS AND METHODS

Patients

This prospective observational study was conducted after an approval by the Institute's ethics committee at Sun Yat-sen University Cancer Center. Patients, aged from 18 to 80 years, with an American Society of Anaesthesiologists (ASA) physical status I–II, and scheduled to undergo elective TURB with general anaesthesia, were included from November 2014 to March 2015. Patients with a history of bladder outflow obstruction, overactive bladder, neurogenic bladder, morbid obesity, inability to cooperate with CRBD assessment due to mental disorders, chronic pain and chronic analgesic usage, were excluded from this study.

Data on demographic characteristics, presence of diabetes, counts of urinary white blood cells, history of transurethral catheterization three months prior to the surgery, size of Foley catheter, duration of operation, time to extubation (the time from the end of the procedure to the removal of the airway device), were prospectively collected on preprinted forms.

Anaesthetic and surgical techniques

After admission to the operating room, vital signs and Narcotrend index were monitored. All patients were managed by propofol-based general anaesthesia with a fixed, continuous, intravenous remifentanil at 0.1–0.2 μg/kg/min. Propofol was adjusted to keep the Narcotrend index between D2 and E0 (5). The ventilation was adjusted to keep end-tidal CO_2 between 35 and 45 mmHg. At the end of the procedure, residual neuromuscular blockade was antagonized with neostigmine 0.05 mg/kg and atropine 0.02 mg/kg.

Urinary catheterization was performed by the urological surgeons and its balloon was inflated with 10 ml of distilled water before the end of the operation. The urinary catheter was fixed in the supra-pubic area with adhesive tape, without any traction, and it was always left for free drainage into an urine bag. The bladder was irrigated continuously with 0.9% saline through the urinary catheter. After extubation, patients were transferred to the post-anaesthesia care unit (PACU).

CRBD assessment

The incidence and severity of CRBD were assessed at day 1 (in the PACU) and day 3 (in the ward). The severity of CRBD was recorded as 'none' when patients did not complain of any CRBD even on asking, as 'mild' when reported by patients only on questioning, as 'moderate' when reported by the patients on their own (without questioning and not accompanied by any behavioural responses), and as 'severe' when reported by patients on their own along with behavioural responses (flailing limbs, strong vocal response and attempt to pull out the catheter). Patients were informed before surgery how to distinguish bladder discomfort from postoperative pain.

Statistical analysis

For continuous variables, we calculated the median and range and then evaluated significant differences using the Student *t*-test. For categorical variables, we calculated the number and percent and then evaluated significant differences using the Chi-square test or Fisher's exact tests, depending on the number of subjects in each group. Odds ratios (OR) were calculated by logistic regression. The *p* values less than 0.05 were considered significant. All analyses were per protocol and were performed using SPSS 15.0 software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Clinical data

During the period from November 2014 to March 2015, 231 patients underwent TURB under general anaesthesia. Around 11 patients were excluded from this study, six patients delayed extubation, three patients were unable to communicate or understand the questions in the PACU, and two patients were discharged at the day of operation. Thus, data were available from 220 patients. Clinical data are given in Table 1.

Table 1: Data from 220 patients undergoing TURB

	N = 220
ASA physical class (I:II)	167:53
Age (years)	65 ± 12.3
Male sex (%)	199 (90.1%)
Body mass index (kg/m^2)	25 ± 3.1
Duration of surgery (min)	31.2 ± 16.7
Time to extubation (min)	12.8 ±5.3
Stay in PACU (min)	53 (30–110)

Data are expressed as the mean ± SD, number of patients (%), median (range). ASA = American society of Aneshesiologists; PACU = post-anaesthesia care unit.

Incidence and severity of CRBD

Incidence and severity of CRBD showed an inter- and intra-individual variation throughout this study period, which were highest at day 1 and decreased significantly from day to day (Table 2). A total of 181 patients (82.3%)

had CRBD at day 1, and 65 patients (29.5%) suffered from moderate or severe CRBD. The complaints of patients with CRBD included urination urgency ($n = 87$), urethral burning sensation ($n = 75$) and sensation of urethral foreign body ($n = 19$). No patient was retained in the PACU specifically for bladder discomfort. Fifty-one patients (23.2%) had CRBD at day 3, and only four patients (1.8%) developed moderate or severe CRBD. The complaints of patients with CRBD were urgent urination ($n = 28$), urethral burning sensation ($n = 13$) and sensation of urethral foreign body ($n = 10$).

Table 2: Incidence and severity of catheter-related bladder discomfort

	Day 1	Day 3
CRBD	181 (82.3%)	51 (23.2%)
Severity of CRBD		
Mild	116 (52.7%)	47 (21.4%)
Moderate	61 (27.7%)	4 (1.8%)
Severe	4 (1.8%)	0

Data are expressed as number of patients (%). CRBD = catheter-related bladder discomfort.

Predictors of postoperative CRBD

Results and significant p values from the univariate analysis are given in Table 3. There were significant differences between moderate or severe CRBD and patients' characteristics, such as age, gender, size of Foley catheter. Interestingly, the history of transurethral catheterization 3 months prior to surgery was an independent factor of CRBD. In contrast, no significant associations were revealed between CRBD and the presence of diabetes, counts of urinary white blood cells and duration of surgery.

Multivariate logistic regression analysis showed that the following variables were independently and significantly associated with moderate or severe CRBD: age < 65 years (OR = 1.7, 95% CI: 1.2, 4.8, $p = 0.01$) and male gender (OR = 2.7, 95% CI: 1.3, 9.7, $p = 0.02$).

DISCUSSION

In this prospective observational study, we found that the overall CRBD intensity after TURB showed inter- and intra-individual variation and moderate or severe CRBD was associated with age and gender.

In accordance with our study, others have shown that moderate or severe CRBD intensity is maximal in the PACU at day 1 after urological surgery, with a rapid decline to low median or mean levels during the following 2–3 days (6). Our results, therefore, suggest that future interventional treatment studies should take this large variation in CRBD intensity into consideration, and the issue should be included in the preoperative patient information prior to TURB.

Table 3: Results of univariate analysis for predictive factors of moderate or severe catheter-related bladder discomfort

Variable	N	Odds ratio	95%CI	*p*-value
Age (years)				
< 65	163	3.8	1.3–7.9	0.001
≥ 65	57			
Sex				
Female	21	4.9	1.6–14.6	<0.001
Male	199			
Presence of diabetes				
No	192	0.8	0.3–2.7	NS
Yes	28			
Size of Foley catheter				
< 20 Fr	33	2.2	1.3–6.5	0.001
≥ 20 Fr	187			
Transurethral catheterization 3 months prior surgery				
No	189	1.9	1.4–5.8	0.002
Yes	31			
Urinary white blood cell count				
Normal	193	0.8	0.07–1.3	NS
High	27			
Duration of surgery				
< 30 min	169	0.4	0.1–1.7	NS
≥ 30 min	51			

The incidence of CRBD in our study was comparable to some previous investigations (6–8), while a little higher than other studies (3, 4, 9, 10). It is thought that CRBD is caused by the stimulation to the junction between urethra and bladder and the view is supported by the finding that CRBD was rare in patients who had received suprapubic bladder fistulization (11, 12). Nonetheless, some patients that had no stricture at cysto-urethral junction still reported CRBD. Therefore, we believe that different operative techniques, which cause different wounds and pain, are responsible for the difference in the development of CRBD. In other words, CRBD is not caused by stricture at cysto-urethral junction alone and it might be induced by other contributors such as trauma and pain. Differences in the incidence between studies may be attributed to the fact that our study focused on the patients undergoing TURB which may cause a certain trauma to ureter and bladder.

In our series, the two most common symptoms of CRBD were urgent urination and burning sensation. An observational study involving 116 patients who underwent urological surgery (6) and a study conducted in 164

patients undergoing surgery in two teaching hospitals (10) reported comparable results.

Our univariate analysis revealed that the diameter of the Foley catheter and history of transurethral catheterization 3 months prior to surgery were predictors for moderate or severe CRBD. Similarly, the studies mentioned above also reported these two factors to be significant (6, 10). The link between these two predictors and CRBD might be due to the fact that the large diameter of the catheter-stimulated urethra and the history of urethral catheterization made it more irritable. However, multivariate logistic regression failed to support the link, suggesting that, in this study, they were not independent, predictive factors for moderate or severe CRBD. This inconsistency might be due to the fact that the sample size was small and the study population was in different races, which could diminish the power of the evidence. In our multivariate model, age and male gender were significantly associated with CRBD. The fact that younger patients are more sensitive to external stimuli, and men's urethra is longer than those of women, may explain these findings.

However, there were some limitations in our study. Firstly, this was a single-centre and small sample study that may have decreased the power of the result, particularly for the negative findings. Secondly, the lack of standardized intraoperative and postoperative pain management could induce some bias, which can affect CRBD evaluation.

CONCLUSION

In conclusion, the present study has shown that, overall, CRBD after TURB is usually most intense on the day of operation, but carries a pronounced inter- and intra-individual variation day by day. Age < 65 and male gender are independent risk factors for moderate or severe CRBD after TURB. Our results suggest that future treatment studies after TURB should focus on this population.

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ORIGINAL ARTICLE

Use of Additional Locking Plate-derived Poller Screws for Treatment of Femoral Non-union after Intramedullary Nailing

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ABSTRACT

Objective: *To evaluate the efficacy of using additional locking plate-derived Poller screws to treat post-intramedullary nailing for femoral non-union was investigated.*

Methods: *Between January 2009 and April 2012, six patients who underwent post-intramedullary nailing for femoral non-union were studied. Three, one, and two patients had femoral fractures in the proximal one-third, middle third and distal third, respectively. While the original intramedullary nails were retained, eight-to-nine hole locking plates were used for fixation and two-to-three cortical bone screws were applied to both sides of the fracture to ensure the stability of the intramedullary nail sagittal plane. One-to-two pieces of locking nails were inserted tightly next to the intramedullary nails to ensure a stable coronal plane. Autologous iliac bone grafts were performed around the fractures in all cases.*

Results: *Follow-up evaluations were conducted between 10 and 17 months (mean, 13.8 months). The operative time was 110–160 minutes and the blood loss was 300–500 mL. Bone pain was relieved in 1 month. Continuous callus was observed after 4–6 months (mean, 4.83 months) based on imaging. There were no infections, loosening of internal fixation, or rupture. All patients were able to walk bearing weight within 3 months.*

Conclusion: *An additional locking plate and the derived Poller screw technique effectively improved local rotation instability and is an effective and simple treatment method for femoral non-union after intramedullary nailing.*

Keywords: Blocking screws, femoral fracture, fracture non-union, intramedullary, locking plate

INTRODUCTION

Due to good biomechanical advantages and nearly 99% healing rate, interlocking intramedullary nails are widely used as the gold standard in the treatment of femoral shaft fractures (1); however, there is a report that (2) the long bone fracture non-union rate after intramedullary nailing is 1.8%–7%. For this type of fracture non-union, reaming followed by replacement with a thicker intramedullary nail (3) has become the standard treatment. Indeed, it has been suggested that this method is the ideal treatment (4); however, others have reported (5) that this method only has a 53% success rate. In recent years, supplementing the original intramedullary nailing with a plate fixation technique has been reported (6, 7) to have a high success rate. By improving this technique, we applied locking plate-derived blocking screws (Poller screws) in six such cases. We showed high efficacy as all the fractures had united.

SUBJECTS AND METHODS

Clinical data

This retrospective analysis included six patients with femoral non-union after undergoing intramedullary nailing in our Department between January 2009 and April 2012. The patients were 23–61 years of age (mean, 36.9

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years). Three, one, and two fractures were located in the proximal one-third, middle third and distal third, respectively. Five and one non-union types were atrophic and hypertrophic, respectively. Arbeitsgemeinschaft für Osteosynthesefragen (AO) classification was as follows: type A in three patients; type B in two patients and type C in one patient. Two patients had open fractures (one Gustilo I and one Gustilo II). Both patients with open fractures had non-reamed intramedullary nailing following emergency debridement. The remaining four patients with closed fractures underwent anterograde reamed intramedullary nailing. The fracture non-union time was 8–14 months (average, 10.7 months). All of the patients had C-reactive protein and erythrocyte sedimentation rate testing; both results were in the normal range, thus excluding infectious non-union.

The diagnosis basis of fracture non-union

Eight months after long bone fracture interlocking intramedullary nailing, there were no signs of increased callus formation during the last 3 months. There was pain at the fracture site and local pain worsened after activity or weight-bearing ambulation. Fracture site tenderness or percussion pain was reported, and there were no significant axial abnormalities. An X-ray showed abundant callus in the area of bone non-union and sclerosis; however, there was no continuous callus through the fracture space and no continuous callus on at least three lateral cortices. Fracture end atrophy, bone loss and bone defects were also observed. There were no main nail fractures or fixation failures.

METHODS

Surgical methods

After conventional lateral exposure, the cortical bone stripping technique was used to ring exposed fracture ends and the surrounding 2–3 cm area. Then, periosteal stripper was used to strip soft tissue towards both sides for the steel plate attachment. All non-union cases had rotational instability and axial stability. The fracture site was filled with a large amount of fibrous connective tissue. In the hyperplastic non-union cases, there was considerable callus growth, but the bones were not united between the ends. In the cases with atrophic non-union, no callus growth was noted around the ends, and both fractured ends were absorbed, defective or underwent atrophy. The fibrous connective tissues between the fractured ends were completely removed, and the fractured ends were cut off until the bone began to ooze blood. Both the callus and the original intramedullary nail were retained.

Centring on the fracture ends, rotation deformity was corrected while maintaining normal bone length. Straight locking plates with at least eight holes were selected for placement at the lateral side of the bone. To avoid inserting double cortical screws into intramedullary nails, the locking plate can be placed towards the front or back. The exact location of the plate can be determined by pre-operative standard lateral X-ray (Fig. 1A, 1B) and the position of the exposed intramedullary nails during the procedure. First, two to three pieces of 4.5-mm double cortical bone screws were inserted on both sides (Fig. 2-1). Screws were inserted immediately next to the intramedullary nails to minimize the likelihood of failure. Subsequently, one to two locking screws were inserted on both sides (Fig. 2-2). The key is to assure that the length of the locking screws is 2–4 mm longer than the measured depth. The exact additional length can be determined by pre-operative X-ray and the available space around the intramedullary nails. In the anteroposterior X-ray, the intramedullary nail should be squeezed to one side after fixation (Fig. 2-3). After examining fixation security (mainly rotation stability), autologous iliac bone was cut into a match stick-like shape and grafted between the fracture ends and the 2–3 cm area. If bone deficiency between the fracture ends existed, high-quality three-sided cortical bone filling was used for support first followed by placing the iliac bone sticks.

To prevent fracture of drilling bits intra-operatively when inserting double cortical bone screws and to prevent drilling bits from damaging intramedullary nails, 3.0-mm Kirschner wire was used (8, 9), followed by

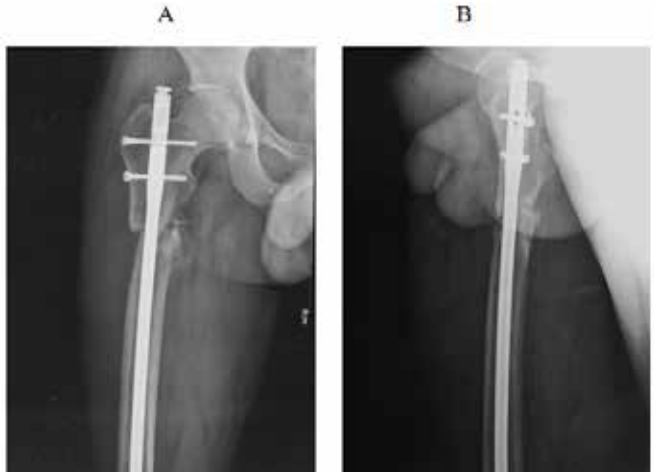

Figure 1: Non-healing X-ray films after intramedullary nail fixation for proximal-segment femoral fracture: positive position (A) and lateral position (B).

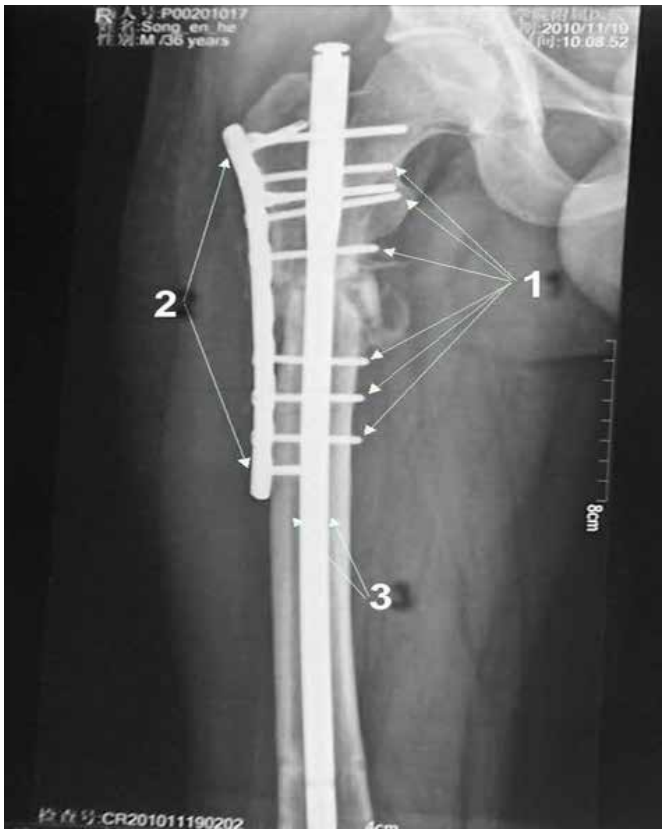

Figure 2: Intramedullary nails were retained, and the side plates were strengthened. Around 2–3 double cortical bone screws inserted on each side of the broken end, coronation block and forming sagittal stabilization (Fig. 2-1); with 1–2 locking nails on both sides, lengthen the locking nails according to the easing of the pulp cavity, and 'squeeze' the intramedullary nails to the opposite side by mechanical force, sagittal block and forming coronal stability (Fig. 2-2); intramedullary nail is pushed to the opposite side (Fig. 2-3); autologous iliac bone grafting.

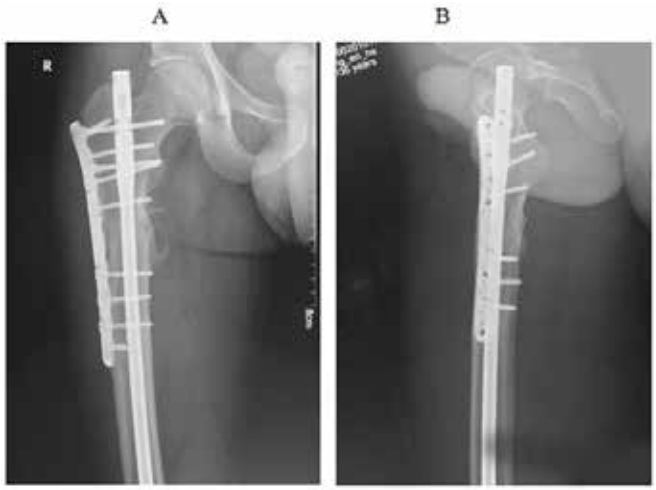

Figure 3: Fracture healing, positive (A) and lateral (B) at 5 months after side plate reinforcement and autologous iliac bone grafting.

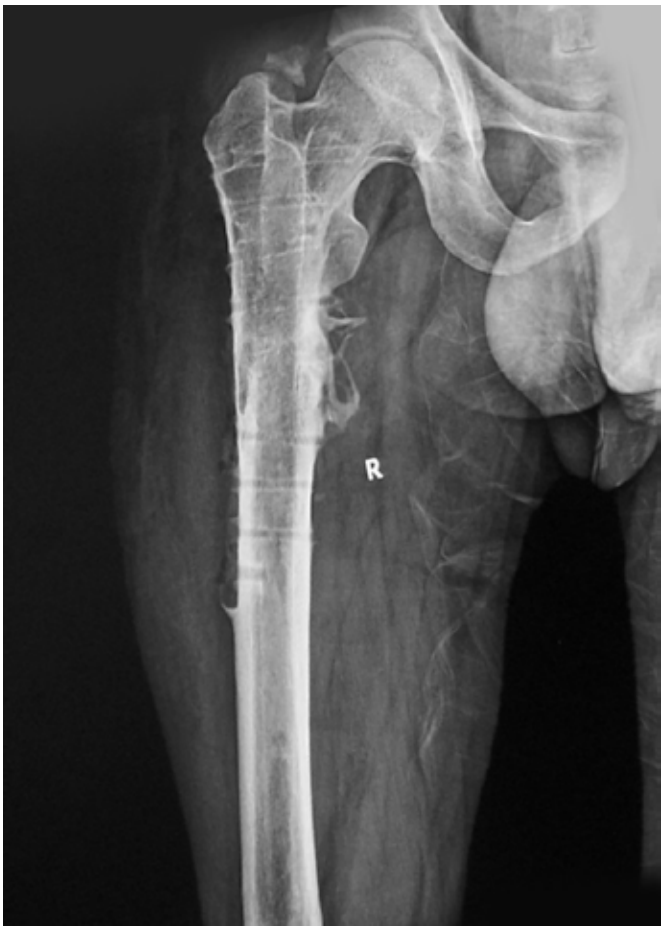

Figure 4: The fracture healed firmly 2.5 years after surgery and X-ray film of positive position after removal of internal fixation.

tapping and inserting cortex bone screws with the appropriate length. The Kirschner wire should be sharp and drilling should be intermittent with water cooling to avoid fixation failure caused by bone burn necrosis.

Post-operative treatment

The negative pressure drainage tube was removed 1–2 days post-operatively. Twelve hours post-operatively, low-molecular-weight heparin calcium was used to prevent deep vein thrombosis. After removal of the drainage tube, patients were encouraged to start limb isometric muscle contraction exercises and adjacent flexion and extension exercises. Within 3 months, patients were gradually transitioned to full weight on the affected limb (10). One, 3, 6, and 12 months post-operatively, fracture healing and limb function recovery was assessed. The fracture healing criteria were as follows: no pain after limb load; no tenderness and no percussion pain at the fracture ends; and formation of continuous callus through fracture and corticalization based on X-ray examination.

RESULTS

The average operative time (exposure, clean-up, bone extraction, bone graft and plate fixation) for the six patients was 110–160 minutes (mean, 131 minutes), and the bleeding volume was 300–500 mL (average, 383 mL). Two patients reported pain at the bone extraction area; the pain was relieved within 1 month. There were no peri-operative complications and the activity of the adjacent joints did not decrease. The follow-up examinations were performed between 10 and 17 months (mean, 13.8 months). Imaging showed continuous callus for 4–6 months (mean, 4.83 months). No other interventions were taken during the follow-up period. No complications, such as infection, loosening of internal fixation or rupture, were observed (Table 1). Typical cases are shown in Figs 1–4.

DISCUSSION

Causes of femoral non-union after intramedullary nailing

After fracture, both biology and biomechanics should be considered to promote healing. In terms of biology, closed reduction of fracture should be attempted first. If closed reduction is difficult, limited open reduction is preferred to reduce destruction of the blood supply. In the current study, all six patients underwent open reduction and internal fixation (not limited open reduction), which greatly damaged the fracture blood supply and was one of the main causes of non-union. In terms of biomechanics, the fracture site should be stabilized as much as possible. Otherwise, non-union is likely to occur. With an intramedullary nail, the diameter of the nail hole is larger than the diameter of the nail, which can lead to a degree of rotation instability after the interlocking intramedullary nail fixed the long bone fracture.

Johnson *et al* (11) confirmed by mechanical testing that there is 10–15 degrees of rotation after intramedullary nail fixation of femoral shaft fracture, especially for comminuted fractures. For long bone distal and proximal third fractures, despite intramedullary nail fixation, because of a large medullary cavity, the frontal plane is unstable and prone to an abnormal force line (8). For most of the patients in this study, fractures occurred in the proximal or distal segment. In two patients, intramedullary nails were too small, which may have also caused instability. In addition to damage to the blood supply and fracture end instability, fracture incomplete contact is an important reason for non-union (5). Two of the patients did not undergo routine procedure to close the gap between fracture ends. Post-operatively, early weight bearing can result in an internal fixation fatigue fracture, suggesting that strain is another important reason for fracture non-union (12).

Treatment of femoral non-union after intramedullary nailing

For femoral non-union after intramedullary nailing, reaming and replacement with a larger intramedullary nail has become the standard treatment (3), achieving a 78%–96% success rate (13). Pihlajamaki *et al* (4) claimed that this method is the best treatment option; however, in 2000, Weresh *et al* (5) applied this procedure to 19 patients with femoral non-union. Distal intramedullary nailing and fracture union occurred in only 10 patients (53%) and a significant number of successful patients required additional treatment. Park *et al* (14) used a similar method on seven patients with non-stenotic femoral non-union after intramedullary nailing, five of whom failed. For humeral non-union after intramedullary nailing, the application of thicker intramedullary nails and even autologous iliac bone graft failed in many cases. The causes of the high failure rate have been summarized as follows: significant comminution of the fracture site; large segmental defect at the fracture site; non-union of a humeral shaft fracture; and small intramedullary nail and large medullary cavity at the fracture ends. All of these factors can lead

Table 1: Six data of non-healing cases after intramedullary nail fixation in femoral shaft fractures

No.	Sex	Age	Part	No healing type	Far-end power	No healing time (month)	Operation time (min)	Blood Loss (mL)	Image healing time (month)	Follow-up time (month)
1.	Male	35	Nearly 1/3	Atrophic	Yes	9	140	400	5	13
2.	Male	61	Far 1/3	Atrophic	No	14	120	400	4	10
3.	Male	34	Nearly 1/3	Atrophic	No	10	110	300	4	16
4.	Female	30	Nearly 1/3	Atrophic	No	14	160	500	5	13
5.	Female	38	Far 1/3	Hypertrophic	No	8	150	300	5	14
6.	Female	23	In 1/3	Atrophic	No	9	110	400	6	17

to unstable rotation of the fracture ends. The existence of an intramedullary nail maintains the normal axis and force line but does not improve rotation stability. Thus, maintaining fracture end rotation stability is essential to bone union (16). More and more literature has shown that reaming and replacement with a thicker needle is suitable for hyperplastic non-union but not for atrophic non-union (17).

In some femoral non-union patients after intramedullary nailing, Bellabarba *et al* (18) removed the original intramedullary nails and fixed with a steel plate instead, and selectively used an autologous iliac bone graft at the fracture ends. Despite a high fracture union rate of 91%, there were still cases which needed re-operation due to a broken plate. Furthermore, this method had the disadvantages of being complicated, requiring a longer operative time, high blood loss and sizable wounds.

In recent years, retaining the original intramedullary nails with additional plate fixation, and if necessary autologous iliac bone grafting, greatly improved the success rate of non-union after intramedullary nailing and the success rate was nearly 100% (6, 9, 10, 14, 19); however, success depended on the stability between fracture ends. To achieve sufficient stability, additional plate nails should be fixed through double cortical fixation, which can be difficult due to the presence of intramedullary nails, especially when non-union occurred in femoral stenosis. Choi and Kim (20) reported a problem with loosening of fixation nails. Some authors have replaced the ordinary plate with a locking plate (7, 17, 21) to better solve the problem. The single-cortical locking feature of locking plate facilitates the implantation of the fixing nails. Together with special angular stability, this change provided a better grip than a non-locking plate in the region where the intramedullary nail is present.

For fracture non-union after intramedullary nailing, Gao *et al* (22) combined the intramedullary nail replacement with Poller screws to improve fracture end stability. Krettek *et al* (8) used an intramedullary nail with Poller screws to correct abnormal force lines and to improve the initial stability of the nail-bone complex. We took full advantage of the original intramedullary nails with an additional locking plate fixation, and the derived Poller screws technique for the treatment of femoral non-union after intramedullary nailing and all six patients recovered. We have described our treatment principles and technical details.

Treatment principles and technical details of the additional locking plate-derived Poller screws technique

With retention of an original intramedullary nail, deformity correction is easy. To increase the stability of fixation, the length of the locking plate should be increased as much as possible with at least eight holes to stay away from fracture ends. As most of the adult femoral shaft diameters are > 30 mm, we only need to insert cortical bone screws into the wide side of the intramedullary nail. Because the work area is usually > 10 mm, inserting double cortical 4.5-mm nails will not be too difficult (Fig. 1). Cortical bone screws should be inserted close to the intramedullary nail, and the frontal plane blocking the stabilized sagittal plane as cortical bone screws play a role in Poller screws. Based on the size of the remaining medullary cavity around the intramedullary nail determined by X-ray, the locking nail length was increased (usually 2–4 mm). Mechanical strength was used to force the intramedullary nail back to one side (Fig. 2). The locking nail blocked the frontal plane, which stabilized the sagittal plane as the locking nail played the role of the Poller screw. This procedure greatly increased the stability of the bone and implant complex. Combined with an autologous iliac bone graft, the treatment ultimately ensured a high fracture union rate.

CONCLUSION

We can confidently conclude that for femoral non-union after intramedullary nailing, retaining the intramedullary nail and using the locking plate-derived Poller screw fixation technique combined with autologous iliac bone grafting is a simple and efficacious treatment option.

AUTHORS' NOTE

Yi-feng Zhao contribute to data analysis and manuscript preparation; Qing-hua Chang and Feng-hua Zhu contribute to definition of intellectual content; and Hai-bin Wang and Dai-liang Jia edited, reviewed and wrote the manuscript. Bin Wu and Chun-yang Meng collected the data. The authors declare no conflicts of interest with this study.

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ORIGINAL ARTICLE

Paediatric Galenic Preparations at the Saint Damien Hospital in Haiti: Formulative Study, Stability and Quality

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ABSTRACT

Objective: *The Aid Progress Pharmacist Agreement (A.P.P.A.®) project, a program of International Health Cooperation, is the result of collaboration between the University of Turin and Community Pharmacists in order to set up galenic laboratories in medical facilities located in developing Countries. In the laboratory established in Haiti at the Saint Damien Hospital, given the low availability of paediatric medicines, it was necessary to study and then introduce several specific galenic formulas.*

Methods: *The main active principles were identified in agreement with local medical doctors taking into account World Health Organization Model Lists of Essential Medicines and cost–benefit relationships. Then, a formulative study was launched preferring liquid pharmaceutical forms, more suitable for children. For each preparation, absorption spectrophotometry in the visible and ultraviolet spectra was applied to test the formulas' quality and stability, respectively, in accordance with the European Pharmacopeia and European Medicines Agency guidelines.*

Results: *All formulations have proved to be stable in 'Refrigerated' conditions (*T = *5°C ± 3°C) and in 'Standard' conditions (*T = *25°C ± 2°C, RH: 60% ± 5%) for 12 months, and in 'Accelerated' conditions (*T = *40°C ± 2°C, RH: 60% ± 5%) for 3 months.*

Conclusion: *The galenics are made at the lab in Haiti according to specific standard procedures and they are used to treat patients. The process is constantly checked.*

Keywords: Developing countries, galenic labs, International Health Cooperation, medicine quality and assurance control

INTRODUCTION

The Aid Progress Pharmacist Agreement (A.P.P.A.®) is a non-profit association (1) whose main activity is the *A.P.P.A.*® project (2). The project began in 2005 and is the result of the cooperation between the University of Turin (Italy) and the Italian Community Pharmacists. The project focuses on Galenic Laboratories (GLs) established in medical facilities located in developing countries (DCs). The project complies with both the European and guest Country's legislation. The project is structured in different steps (Table 1) through which an effective and functional lab can be set up. After 12 years of work, several projects have been established: two in Angola, Cameroon, Chad and Madagascar and one in Haiti (2).

Regarding the *A.P.P.A.*® galenic lab in Haiti, it was established in 2012 at the Saint Damien Paediatric Hospital of 'Nos Petits Frères et Sœurs' in the Tabarre district, one of the poorest neighbourhoods of Port-Au-Prince. In the Haiti lab, it was necessary to introduce

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Table 1: *A.P.P.A.*® project steps

Steps	Description
0—Pharmacoeconomic study	Preliminary pharmacoeconomic study. It implies a trip of the *A.P.P.A.*® staff on site to assess the local situation and recipient areas. Some medicines are purchased in local pharmacies and sent to the laboratory of the University of Turin to determine if these medicinal products reflect the declared characteristics or are counterfeit.
1—Choice of lab location, active ingredients and pharmaceutical forms	Choice of GL location. The Chief MD in charge of the medical centre will highlight local diseases, so appropriate A.P.I.s can be selected and correct pharmaceutical forms can be planned.
2—Students' internship to learn how to prepare galenics	Internship at the galenic *A.P.P.A.*® lab at the University of Turin for Pharmacy students completing a relevant experimental thesis. The internship teaches students how to prepare the required medicinal products.
3—Internship of the local staff to learn how to prepare galenics	A member of the local hospital staff comes to Italy (about one month) to learn the procedures of galenic preparations under the supervision of Italian Pharmacy students. During this period, the material for the GL is sent to the hospital.
4—Training on site	A two-month training period in the DC hospital during which the work of the lab technician, who was in Italy to learn galenic methods and procedures, will be coordinated by the Italian Pharmacy students
5—Quality controls	Quality control of medicinal products routinely prepared in new GL; some samples will be sent to the University of Turin where they will be quality tested.
6—Supervision and introduction of new formulas	Regular 40 days or more internships for students of the University of Turin—during their experimental thesis—these internships are organized each year both to guarantee an ongoing supervision of the medicinal products prepared in the lab and to study new formulations according to the Chief MD's demands which might change over time.

GL = galenic laboratory. MD = medical doctor. A.P.I.s = active pharmaceutical ingredients.

several formulations for paediatric use because the number of young patients is high, and the availability of preparations designed for them is limited.

MATERIALS AND METHODS

Drug selection and formulative study

The main active principles, needed to meet the hospital's needs, were identified in agreement with local medical doctors (MDs) taking into account the World Health Organization Model Lists of Essential Medicines (3, 4) and the related relationship between costs and benefits. Considering the high incidence of heart disease on site (5), the list of the selected active ingredients included a number of preparations for the cardiovascular system. Once this list of active ingredients had been drawn up considering local needs, a formulative study was launched. This took into consideration the fact that the hospital's user base is mostly children. For this reason, it made more sense to provide liquid pharmaceuticals such as syrups, drops, solutions and suspensions. These liquid pharmaceutical forms are easier to administer and allow modulating the dose more simply according to the weight of the patient. The first step of the formulative study was to evaluate the chemical and physical characteristics of each active ingredient with the aim of identifying the most appropriate excipients to use in preparations as well as the most appropriate excipients for paediatric use. Where possible, the active ingredients were dissolved in water, if necessary co-solvents were added, and the pH of the formulation was controlled with a buffer system. In order to try to limit the costs of buying and transporting materials, the same excipients were used for as many formulations as possible.

Stability and quality control

The introduction of a complete list of liquid formulas for paediatric use brought about the need to identify specific ways of measuring the stability of the new formulas, as well as the method for carrying out quality controls on medicines prepared in the GL.

With the intention of analysing the stability of all the studied formulations and evaluating the possibility to use them in the extreme conditions of temperature and humidity like those commonly found in DCs, all formulas were analysed to obtain values that represent the initial condition (T0). These results were used for reference and comparison during the stability study, and for the successive quality controls that were carried out on the formulations prepared on site.

Following this, each medicine was divided into aliquots, and each separate aliquot was conserved in different environmental conditions (Table 2) at different temperatures (T) and relative humidity (RH%) based on the guidelines set by the European Medicine Agency (EMA) (6). The refrigerated conditions (RC) were applied to evaluate the need for this type of storage, the standard conditions (SC) were applied to define the stability of samples under storage conditions accepted by the Eur. Ph.. The stability tests carried out in the accelerated conditions (AC) can be used with two objectives. Firstly, they can reduce the times of analysis, as a

Table 2: Samples storage conditions during the stability tests

Storage condition	T (°C)	RH%	Period covered by data
Standard	25 ± 2	60 ± 5	Analysis at time zero (T0), every 45 days for 3 months (T2 and T3), after 6 and 12 months (T6 and T12)
Refrigerated	5 ± 3	–	Analysis at time zero (T0), every 45 days for 3 months (T2 and T3), after 6 and 12 months (T6 and T12)
Accelerated	40 ± 2	60 ± 5	3 months, analysis at time zero (T0) and every 45 days for 3 months (T2 and T3)

T0 = initial condition; T2 = 45 days; T3 = 3 months; T6 = 6 months; T12 = 12 months.

month of conservation in these conditions is equivalent to four months of conservation in standard conditions (7). Secondly, they reflect the typical climatic conditions of tropical countries and, therefore, they help predict the stability in environments where conservation at controlled temperature and humidity cannot be guaranteed.

Absorption spectrophotometry in the visible (VIS) and ultraviolet (UV) spectra was chosen as the analytic method for the stability tests. The medicines are suitable when the percentage error is ≤ 10% in relation to the absorbance (Abs) obtained at T0. The scientific literature usually proposes high-performance liquid chromatography (HPLC) as a suitable method for medicinal product analysis (8). Taking into account that the Eur. Ph. (9) does not prescribe a specific analytical method to test the active ingredient content of a medicinal product (the Ph. Eur. stating that a 'suitable analytical method' should be applied), we applied an UV–VIS spectrophotometric method. This choice was determined by the lower costs and, therefore, by the opportunity to apply this technique in DCs. In any case, in order to evaluate the equivalence between the two methods, in a past study (10), different samples were analysed using both the HPLC and UV–VIS methods.

Once the stability of each galenic formulation had been verified, the same method of analysis was applied to test the uniformity of content (Eur. Ph., assay 2.9.6) of the medicines prepared at the Haiti lab since 2012. Overall, the quality controls were carried out according to guidelines set out in Eur. Ph. (9). Some of the tests were carried out on site at the hospital, and others were carried out at the University of Turin, depending on the availability of equipment needed to carry out each test.

RESULTS

Drug selection and formulative study

Considering local needs, the chosen active principles were as follows: aluminium hydroxide, ascorbic acid, B vitamins (B_1, B_2, B_3, B_6), captopril, ferrous sulphate, furosemide, ibuprofen, magnesium hydroxide, nifedipine, potassium canrenoate, propranolol, ranitidine and salbutamol.

Based on the need to prepare medicines suitable for paediatric use, according to the different chemical–physical properties of the various active ingredients, 18 formulas were studied.

In general, the active ingredients were dissolved in excipients suitable for paediatric use and in many cases the final volume was reached using a sucrose syrup (11) or a sorbitol solution (50%–70%). Given the low viscosity of sorbitol solutions when compared to sucrose syrup, the desired rheological properties of the final formulation were achieved through the addition of co-solvents, *eg*, glycerol.

For each formula, the optimal pH value was experimentally determined. These values are always checked by local workers at the end of the preparation process.

Following the formulative study, a specific sheet was prepared in the local language for each formula studied in the galenic lab in Haiti. The sheet contained the following information: the components of the formulation and their chemical–physical properties, the method of preparation, the indications for use and information about suitable dosages for paediatric use. All sheets were brought together in a single document, which became the handbook for the GL in the Saint Damien Hospital.

Stability and quality control

In order to evaluate the stability over time of the studied galenic preparations, samples conserved in different conditions were analysed at regular time intervals as described (Table 2). The percentage rate calculated according to T0 during the testing period conformed to what was expected (≤ 10%) from each environmental condition applied.

For each analysed sample, pH was also measured. The pH values collected during the testing period conformed to what was expected from each environmental condition applied.

Regarding quality, since 2012, all the tested medicines satisfied the tests according to the Eur. Ph. and were used to treat the Saint Damien's patients.

DISCUSSION

Regarding the formulative study, sucrose syrup was considered the better choice as it is cheaper, and the sugar is available on site. Considering the high concentrations of sugar in sucrose syrup (66.5%) and the consequent

high osmotic pressure that prevents the growth of bacteria, the use of a conservative would be not necessary. However, it was decided to use a conservative due to two factors. Firstly, during the preparation of galenics, the total concentration of sugar is reduced because other excipients are added. Secondly, the environmental conditions in Haiti meant that it was deemed the best practice to add a conservative. For some of the studied formulations, additional considerations are required.

Ferrous sulphate syrup. Ferrous sulphate was formulated with ascorbic acid to increase intestinal absorption.

Ibuprofen syrup. When preparing ibuprofen syrup, it was necessary to find an appropriate solvent. Propylene glycol was used as a co-solvent, taking into consideration the fact that the maximum dose allowed for paediatric use is 200 mg/kg (12).

Captopril solution. The literature suggests that captopril is particularly sensitive to degradation. The studied formula needed ethylenediaminetetraacetic acid (EDTA), a chelating agent that works to protect captopril which degrades in the presence of metallic ions. EDTA also has weak antimicrobial activity (13).

Furosemide solution. To prepare furosemide solution it was necessary to identify a solvent, other than water, in which the active ingredient is not soluble. Furosemide is, however, soluble in alkaline aqueous solutions. In order to make the active ingredient soluble, while keeping the pH at a suitable level for oral administration, a mix of ethanol and sodium hydroxide solution at 0.4 M was used. The quantity of ethanol used as a co-solvent is compatible with stated levels for paediatric use (14, 15).

After the formulative study, production started but always taking into account that for each medicine prepared in a GL, the quality must be guaranteed, according to the current legislation (9, 11) to ensure its safety and efficacy. With the aim to ensure quality, a key factor is the stability of the medicinal product. As a precautionary measure, it was thought appropriate to give a validity period of three months for the galenic medicines prepared, since at AC they remained stable during this period. The stability tests in AC reflect the environmental conditions of tropical countries, and therefore can help to predict the stability of medicines in environments where ideal storage conditions cannot be ensured.

Following the formulative study and stability tests in June 2012, a specific training was carried out with local operators of the GL and the production of medicines began. The practices of the lab technicians and their ability to adhere strictly to the operating procedures learned during the previous period of training were audited during periodic monitoring missions performed on site by A.P.P.A.® staff members and students of the University of Turin.

The entire process is constantly checked in order to show that it meets the quality stated by the Eur. Ph. The excellent results of the quality controls obtained since 2012 show that the continued adherence to specific operative protocols resulted in galenic preparations that fully meet the requirements of the Eur. Ph..

In addition to this, the stability of these medicines was shown to meet the EMA guidelines. Evaluation of stability according to the EMA guidelines is usually applied to industrially manufactured medicines and not to galenic preparations. In spite of this, the galenic formulas studied for the GL in Haiti were tested according to these guidelines to assess the quality of the preparations in the tropical conditions as those of Haiti. Through testing, we demonstrated that all galenic preparations may be used in total safety at home where, most probably, refrigerators and air conditioning are not present.

The principal aim of the International Cooperation is to create autonomy, and therefore, we hope that as soon as possible it will be possible to introduce on site the necessary equipment for the quality control execution according to Eur. Ph. requirements.

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ORIGINAL ARTICLE

Effects of Endothelin-A Receptor Antagonist BQ-123 on Plasma Leptin Levels in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Objective: *Leptin and endothelin (ET) as important endogenous factors interacting with each other which may contribute to a better understanding of their role in diabetic pathogenesis. We aimed to evaluate the relationship between leptin and ET by investigating the influence of BQ-123, an ET-A receptor (ET_AR) antagonist, on leptin levels in rats with diabetes induced by streptozotocin (STZ).*

Methods: *In this study, 24 male Wistar albino rats were divided into three groups: control, STZ and STZ+BQ-123 groups. Experimental diabetes was induced by delivering a single dose of 60 mg/kg intravenous STZ. The rats in the STZ+BQ-123 group received 4 mg/kg i.v. in total BQ-123 (2 mg/kg +2 mg/kg on the 39th and 40th days). The plasma specimens collected 6 hours after the last BQ-123 delivery were studied for biochemical parameters.*

Results: *At the end of the experiment, the weights of rats in the STZ and STZ+BQ-123 groups were significantly lower compared with the values in the control group. The levels of blood glucose were significantly higher in the STZ and STZ+BQ-123 groups than in the control group. While rats with STZ-induced diabetes demonstrated no changes in leptin, protein carbonyl and K^+ levels, they exhibited reduced NO, Na^+ and Cl^- concentrations. The levels of plasma thiobarbituric acid-reactive substance were significantly higher in the STZ group than in the control and STZ+BQ-123 groups.*

Conclusion: *Although the levels of plasma leptin were not statistically significant different between the groups, BQ-123 groups lead to a further decrease in reduced levels of leptin than in only diabetic group. Our findings have been considered that ET_AR antagonists have positive impacts depending on the dosage in the diabetic rats.*

Keywords: BQ-123, endothelin-1, experimental diabetes, leptin.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine and metabolic disorder of our age, emerging as a result of real or functional lack of insulin, which is characterized by blood, carbohydrate, protein and fat metabolism disorder (1–3).

Today, experimental diabetes models can be formed with various materials and methods. One of the ingredients used to create an experimental diabetes model is streptozotocin (STZ). Diabetes created with STZ is a commonly used model in terms of formation of insulin-dependent diabetes model of human (4).

Endothelins (ETs) are the peptides occurring naturally in many cell types of the body and known as the most potent vasoconstrictor molecules (5, 6). There are ET isopeptides in humans and other mammals, named as

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ET-1, ET-2 and ET-3, which have different structural and pharmacological effects. Endothelin-1 is the primary ET which is synthesized by endothelial cells (7). It shows paracrine and autocrine effects via ET_AR and ET_BR on the endothelial and smooth muscle cells (8). There are many studies that have demonstrated the increase in the level of ET-1 in diabetes (9, 10). Endothelin-1 also leads to the progression of DM (11).

Leptin is a protein hormone, which derives its name from the Greek word leptos, which means thin, produced by ob-gene in fat cells and other tissues and released to plasma (12, 13). Insulin is the most researched leptin-associated hormone and an important regulator of the ob-gene expression (14). Leptin and other adipocytokines were associated with type II DM and insulin resistance (15). It has been demonstrated that leptin deficiency lead to severe insulin resistance in uncontrolled DM (16). Additionally, it has been informed that leptin had an anti-diabetic effect in diabetic rats. Research studies have shown that leptin had powerful anti-diabetic effect in the STZ-induced insulin-dependent diabetic rats and transgenetic mice (17).

There are some studies evaluating the relationship between leptin and ET-1 in different diseases and systems. For example, ET-1 stimulates leptin production with ET_AR in adipocyte cultures (18). In another study conducted by Juan *et al*, leptin increased the levels of ET_AR in vascular smooth muscle cells (19). It has come to light that leptin induced ET-1 in endothelial cells (20). These data suggest that increased ET-1 levels in diabetes due to the relationship between leptin and ET-1 may affect the plasma leptin levels.

Although there are many studies evaluating leptin and ET-1 separately in various diseases and systems, no study that demonstrates how ET-1 and leptin affect each other in diabetes and how ET receptor antagonists affect plasma leptin levels in diabetes can be found. Therefore, we aimed to investigate the effects of ET_AR antagonist BQ-123 on leptin levels in STZ-diabetic rats.

The studies demonstrate that a decrease in leptin levels may occur in STZ-induced diabetic rats. How this decrease affects in diabetic rats given ET_AR antagonist BQ-123 became our scientific curiosity. Therefore, there are some studies that demonstrate an increase in ET-1 and endothelium which caused upregulation of leptin secretion levels in diabetes.

Hence, leptin levels that were already decreased in diabetes may be reduced more with the application of ET receptor antagonist. It is known that a decrease in leptin levels in diabetes caused adverse effects. Therefore, it should be determined how ET_AR antagonist affected the increased levels of leptin in diabetes.

MATERIALS AND METHODS

Before the experiment, the approval was taken from Gaziosmanpasa University Local Ethics Committee for Animal Experiments and animal rights were protected (2010-HADYEK-029). In the experiment, 24 male Wistar albino rats weighing 180–250 g were used. Rats were kept in cages at room temperature 21 ± 2ºC with a 12 hours light/dark cycle, fed with standard rat pellet food and tap water.

Chemicals

Streptozotocin and BQ-123 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Streptozotocin was dissolved in cold phosphate-citrate buffer solution (0.1 M, pH = 4.5). Buffer solution was prepared freshly and protected from light. BQ-123 was dissolved in 0.9 % NaCl.

Procedure for diabetes

Rats were randomly divided into three groups and each group included eight rats (control group, n = 8; STZ group, n = 8; STZ+BQ-123 group, n = 8). Blood glucose levels of each rat, fasted for 12 hours, were measured with a sugar measuring instrument (glucometer) (PlusMED ACCURO pM1300 from Taichung city of Taiwan) and recorded. After that, by measuring the weight of each rat, STZ 60 mg/kg, which was prepared freshly by dissolving in phosphate-citrate buffer (pH = 4.5), was administered *i.p.* to the rats in STZ and STZ+BQ-123 groups. For the control group, phosphate-citrate buffer with the same volume was administered *i.p*. Then, the feeding of rats was released. After 72 hours of STZ administration, fasting blood glucose levels were measured, and the rats with the level of 200 mg/dL or higher were considered as diabetic. In order to complete the formation of diabetic physiopathology, rats were kept under the appropriate conditions for 40 days after the STZ implementation. The diabetes procedure was prepared according to previous studies that have been done before (21, 22).

Treatment procedure

The rats in STZ+BQ-123 and STZ group, 39th and 40th days after the application (2 mg/kg+2 mg/kg), received a total of 4 mg/kgBQ-123 that was administered *i.v*. from tail vein. For the rats in control and STZ group,

instead of BQ-123, same volume of saline was given. Rats were sacrificed by taking 4–5 ml of cardiac blood samples under anaesthesia with ketamine (30 mg/kg) and xylazine (5 mg/kg) 6 hours after the last BQ-123 implementation.

The measurement of plasma leptin

Leptin levels were measured in the plasma obtained after centrifugation of blood by using the method of enzyme-linked immunosorbent assay (ELISA). The Mouse/Rat ELISA Leptin kit (Biovendor, Cat no: RD291001200R, North Carolina, USA) was used for determination of rats' leptin levels by ELISA device (Organ tecnicareade 230 S). The results were read at 450 nm with the help of spectrophotometer (GBC Cintra 10e) and calculated as ng/ml. Leptin ELISA kit is based on the sandwich principle. Microtitre layer is covered with monoclonal antibody that is sensitive against a single antigenic portion of leptin molecule. Patient samples containing large molecules of leptin were incubated in 'rabbit anti-leptin' antibody-coated layer, and the sandwich complex was formed. After incubation, unbound material is washed and in order to determine the bound leptin, 'anti-rabbit' peroxidase is added. Substrate solution is added forming color intensity that is directly proportional to the amount of leptin in patient serum (23).

Measurement of serum thiobarbituric acid-reactive substance levels

Serum thiobarbituric acid-reactive substance (TBARS) levels were measured by the Mihara and Uchiyama method. In this, in TBA test reaction, malondialdehyde (MDA) or MDA-like substances produce a pink colour by entering reaction with TBA and give the maximum absorbance at 532 nm. The reaction was carried out at 90ºC and at pH = 2–3 for 15 minutes. In order to bring down the proteins, the samples were stirred with two-fold volume of 10% cold trichloroacetic acid (w/v). The particles were brought down by centrifugation, and the reaction of liquid part of the supernatant and equal volume of 0.67% of (w/v) TBA was performed in boiling water bath for 10 minutes. After cooling, it was read with the spectrophotometer at 532 nm. The results were calculated as nmol/ml (24).

Measurement of the level of serum protein carbonyl

Detection of protein carbonyl (PC) groups was performed on the basis of the spectrophotometrical measurement of stable hydrazone compounds formed as a result of reaction of PC groups with 2,4-dinitrophenyl hydrazine at 370 nm. During calculations, ε = 22000 M-1 cm^{-1} was accepted as molar absorption coefficient at 370 nm for 2.4 dinitrophenyl hydrazine. Protein carbonyl levels were calculated as nmol/ml (25).

Measurement of serum NO level

Amount of nitrite in the serum was determined by the Griess reaction after deproteinization. Total nitrite (nitrite + nitrate) was evaluated with the modified cadmium reduction method. Nitrate reduction was provided at the end of the 90-minute incubation with copper (Cu)-coated cadmium granules deproteinized sample supernatant in pH 9.7 glycine buffer. Produced nitrites were determined with sulfanilamide and related N-naphtylethylenediamine (NNDA) diazotization. A pink colour occurred as a result of the reaction was read with the help of spectrophotometer at a wavelength of 545 nm. Nitrate concentration was determined by subtracting the resulting concentration from the first concentration (26).

Other biochemical analyses

Plasma Na, K and Cl values were measured with the help of an autoanalyzer (Cobas C 501, Tokyo, Japan) by using indirect Na^+, K^+ and Cl^- kits.

Statistical analysis

Statistical analysis was performed with 'SPSS 19.0 for Windows' (IBM Corp., Armonk, NY, USA). For the comparison of the differences between the groups, 'one-way analysis of variance (ANOVA)' test was used. Since the data did not comply with the normal distribution ($p \leq 0.05$ according to the Levene test), instead of this test, 'Kruskal–Wallis ANOVA' was applied. When the differences between groups were found significant in one-way ANOVA ($p \leq 0.05$), the groups were compared in pairs with 'Tukey honest significant difference' which is one of the post hoc tests. When the differences between groups were found significant in Kruskal–Wallis ANOVA ($p \leq 0.05$), the groups were compared in pairs with 'Mann–Whitney U test (Bonferroni correction)'. Results were expressed as mean ± standard deviation.

RESULTS

Live body weights of rats during the test

The weights of the rats were measured at the beginning, in the middle and at the end of the experiment: for the control group, 184.37 ± 12.37, 193.13 ± 11.63 and 239.38 ± 15.68 g and for STZ group, 208.13 ± 7.53,

200.00 ± 10.35 and 191.25 ± 11.57 g; for STZ+BQ-123 group, 231.25 ± 25.03, 218.75 ± 18.47 and 203.50 ± 29.90 g. The weights were compared at the end of the experiment between the groups; the weights of rats in STZ group ($p = 0.0001$) and STZ+BQ-123 group ($p = 0.015$) were significantly lower than the control group. Diabetic rats lost weight.

Fasting blood glucose levels in rats during the test

The fasting blood glucose levels of the rats were compared on the basis of the groups; no significant difference was found between the groups at the beginning of the experiment ($p = 0.384$). In the measurements performed 72 hours after the application of STZ, the blood glucose levels of rats in control, STZ and STZ+BQ-123 groups were recorded as 127.00 ± 15.32, 491.75 ± 72.65 and 501.13 ± 75.75 mg/dL, respectively. The blood glucose levels of the rats in STZ and STZ+BQ-123 groups were found significantly higher than the levels of the control group (for both $p = 0.0001$). An experimental diabetes model was achieved in rats with STZ.

Plasma levels of leptin, and serum levels of PC, TBARS and NO

The plasma leptin levels of the rats in the experimental groups were in the control 0.92 ± 0.58 ng/ml, in STZ group 0.58 ± 0.34 ng/ml and STZ+BQ-123 group 0.38 ± 0.29 ng/ml. Leptin levels were analysed according to the groups, but no significant difference was found ($p = 0.055$) (Table; Fig. 1).

Thiobarbituric acid reactive substance levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were found as 1.55 ± 0.16 nmol/ml, 1.97 ± 0.24 nmol/ml and 1.65 ± 0.10 nmol/ml, respectively. Thiobarbituric acid reactive substance values of the STZ group were significantly higher than the control ($p = 0.0001$) and STZ+BQ-123 groups ($p = 0.0004$) (Table; Fig. 2).

Protein carbonyl levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were found as 903.88 ± 280.45 nmol/ml, 930.50 ± 252.09 nmol/ml and 1040.50 ± 400.41 nmol/ml, respectively. Protein carbonyl levels were analysed according to the groups; there was no statistically significant difference between the groups ($p = 0.665$) (Table; Fig. 3).

Table: Plasma levels of leptin (ng/ml) and serum levels of TBARS (nmol/ml), PC (nmol/ml) and NO (nmol/ml)

Groups	Leptin (ng/ml)	TBARS (nmol/ml)	PC (nmol/ml)	NO (nmol/ml)
Control	0.93 ± 0.58	1.55 ± 0.16	903.88 ± 280.45	46.32 ± 3.85
STZ	0.58 ± 0.35	1.97 ± 0.24	930.50 ± 252.09	38.52 ± 2.26
STZ+BQ123	0.38 ± 0.29	1.65 ± 0.10	1040.50 ± 400.41	37.48 ± 4.88

PC = protein carbonyl; NO = nitric oxide; STZ = streptozotocin; TBARS = thiobarbituric acid-reactive substance;

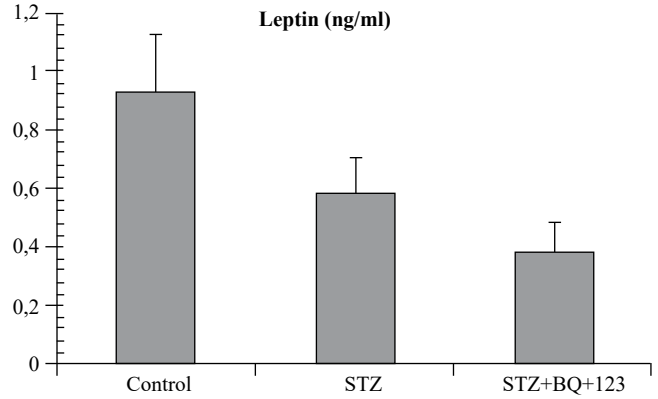

Fig. 1: Leptin levels in the experimental groups (ng/ml): there is no significant difference between the groups. STZ = streptozotocin.

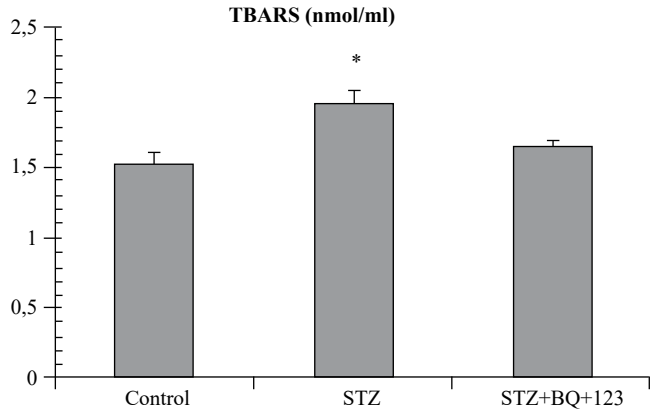

Fig. 2: Thiobarbituric acid-reactive substance (TBARS) levels in the experimental groups: TBARS levels of STZ group were significantly higher than the control (*$p = 0.0001$) and STZ+BQ-123 (*$p = 0.0004$) groups. STZ = streptozotocin.

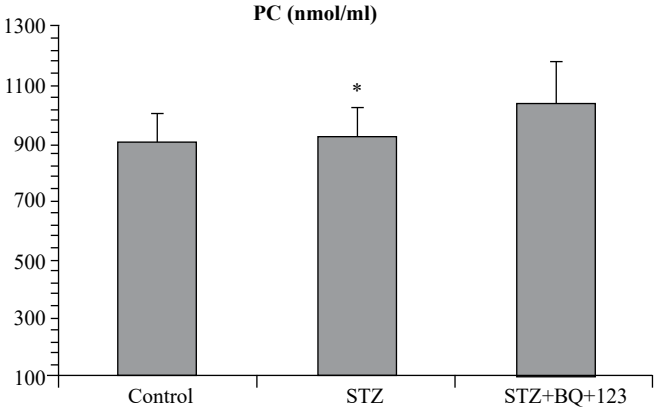

Fig. 3: Protein carbonyl (PC) levels in the experimental groups. There is no significant difference between the groups. STZ = streptozotocin.

Nitric oxide levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were measured as 46.32 ± 3.85 nmol/ml, 38.52 ± 2.26 nmol/ml and 37.48 ± 4.88 nmol/ml, respectively. Nitric oxide levels of STZ ($p = 0.0001$) and STZ+BQ-123 ($p = 0.0001$) groups were statistically significant lower than the values of the control group (Table; Fig. 4).

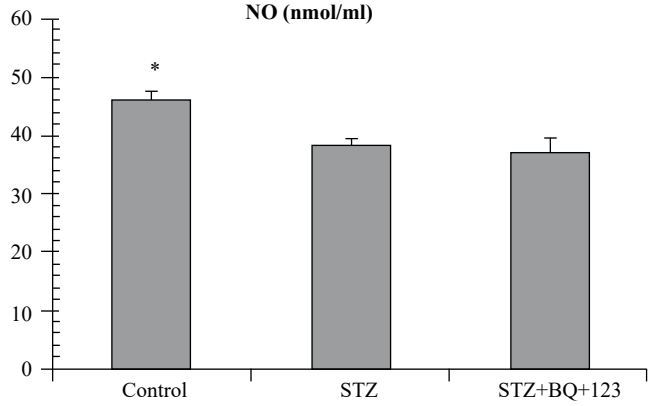

Fig. 4: Nitric oxide (NO) levels in the experimental groups. The other groups were found to be significantly lower than the control group (*$p = 0.0001$). STZ = streptozotocin.

Other biochemical parameters

The serum Na^+ concentration levels were measured in control, STZ and STZ+BQ-123 groups as 137.75 ± 3.01 mmol/L, 126.75 ± 7.57 mmol/L and 128.50 ± 12.34 mmol/L, respectively. Serum Na^+ concentration values of STZ group were significantly lower than the values of the control group ($p = 0.001$). There was no significant difference between the other groups.

Serum K^+ concentrations values were measured in the control, STZ and STZ+BQ-123 groups as 5.45 ± 0.64 mmol/L, 6.03 ± 1.99 mmol/L and 5.08 ± 0.97 mmol/L, respectively. There was no statistically significant difference between the groups in terms of values of serum K^+ concentrations ($p = 0.375$). Serum levels of Cl^- concentration were measured in the control, STZ and STZ+BQ-123 groups as 97.69 ± 0.93 mmol/L, 84.61 ± 5.38 mmol/L and 88.12 ± 9.89 mmol/L, respectively. In terms of Cl^- concentration in serum levels of the groups, the values of STZ group ($p = 0.0001$) and STZ+BQ-123 group ($p = 0.0001$) were significantly lower than the values of the control group.

DISCUSSION

In our study, the blood glucose levels of STZ and STZ+BQ-123 groups measured 72 hours after STZ application were significantly higher than the values of the control group. This finding supports that model of diabetes was successful, and 60 mg/kg dose of STZ was sufficient. In the rats when diabetes was formed after administration of STZ, during the test, for a period of 40 days, polyphagia, polydipsia, polyuria and hyperglycaemia were observed. These findings observed in diabetic rats were consistent with the clinical signs of diabetes.

Hypoleptinaemia accompanies to hyperglycaemia observing due to reduced insulin secretion which occurs due to the destruction of the pancreas with the effect of STZ in this model (27, 28). Previous studies demonstrate that serum leptin levels decreased in diabetes (27, 29). Studies in STZ-diabetic rats have indicated that the levels of serum leptin and leptin mRNA noticeably diminished (28). The levels of plasma leptin also decreased in patients with type II DM (30) and type I DM (31). In our study, although leptin levels in the STZ group were lower than the control group, this was not statistically significant ($p = 0.055$). Despite the decrease in leptin levels in diabetic groups, the reason that we did not find significantly difference may be related with STZ dose and duration.

Biosynthesis of ET-1 increases in diabetes, and therefore this peptide causes vascular complications. There are some studies that have shown an increase in ET-1 levels in diabetes. For example, in a study conducted by Makino and Kamato in STZ-diabetic rats, as well as the formation of hyperglycaemia, ET-1 levels were found to be increased significantly in STZ-diabetic rats (9).

There are some studies evaluating the relationship between leptin and ET-1 on different systems and diseases. For example, in a study conducted by Xiong *et al*, it has been found that ET-1 stimulated the production of leptin adipocytes cultures with ET_AR (18). Also in another study, Quehenberger *et al* found that leptin induced ET-1 in endothelial cells (20). These data also suggest that, due to the relationship between leptin and ET-1 levels, increased levels of ET-1 levels in diabetes may affect plasma leptin levels. In the light of these data, even the decrease in leptin levels in STZ group was not statistically significant; the levels of leptin in BQ-123 decreased more. If so, it may be said that ET_AR antagonist BQ-123 reduced the levels of ET which is increased in diabetes. However, a longer duration of diabetes and administration time of BQ-123 can provide a meaningful detection of reduction in the levels of leptin. The topic may be evaluated better with new studies with different doses and times.

Diabetes mellitus is a chronic metabolic disorder and at the same time as an increased oxidative stress situation (32). Oxidative stress acts as a leading factor

in DM and its complications (33, 34). Several studies inform that free radicals and reactive oxygen species increased in DM rats and patients (34–38). Lipid peroxidation occurring due to free oxygen radicals is one of the most important causes of cell damage. Lipid peroxidation is defined as a chemical event initiated by free radicals and contains the oxidation of unsaturated fatty acids of membrane structure (40). Thiobarbituric acid reactive substances, produced endogenously in the body, is an important indicator of lipid peroxidation (39) and the studies indicate that tissue and plasma TBARS levels increased in diabetes. In our study, in the serum of STZ-diabetic rats, TBARS levels, which are an indicator of lipid peroxidation, were increased significantly compared with control group. This increase in lipid peroxidation in diabetic rats is similar to other studies on diabetic rats and humans. In a study conducted by Turk *et al*, a significant increase of TBARS levels in patients with type II DM has been reported (40). In another study conducted by Ruperez *et al*, it has been detected that plasma TBARS levels in STZ-diabetic rats were significantly higher than the control group (41). In another study conducted by Jeyashanthi *et al*, an increase in serum, liver and kidney tissues levels of TBARS of STZ-diabetic rats was detected (42). Our study is in line with these data.

Researchers have used various agents against DM such as melatonin (43–46). We used BQ-123 that is a selective ET_AR antagonist and decreases ET-1 levels in serum (47). Although the effect of BQ-123 on TBARS levels was investigated, we did not find any study about how BQ-123 affects the increased levels of TBARS in diabetes. However, in renal ischaemia and reperfusion injury, BQ-123 has, as the effects of antioxidant, decreased the increased levels of TBARS (48). In another study conducted by Lund *et al*, it has been determined that BQ-123 reduced cardiac TBARS levels (49). BQ-123 as an ET_AR antagonist inhibited the formation of lipid and protein oxidation products (50). Our results are in line with these studies. In the group treated with BQ-123, levels of TBARS showed a significant decrease compared with STZ group. In our study, it can be said that BQ-123 has a positive effect by reducing the increased levels of TBARS in diabetes.

It has been reported that there is a significant protein oxidation caused by oxidative damage in diabetic patients (22) and diabetic rats (51). In our study, there was no significant difference between the groups in terms of serum PC levels. To keep longer duration of diabetes and examination on tissue samples may help to show the change in the levels of PC. In our study, due to checking only serum PC levels, in 40-day period, protein damage may not be reflected in the serum levels.

Nitric oxide was produced enzymatically by nitric oxide synthase (NOS) from L-arginine (52). It has been reported that, in diabetes, bioactivity of NO, basal NO production and L-arginine which is a NOS substrate decreased, and some researchers indicate that, in diabetes, NO production has been damaged due to decrease in the plasma levels of L-arginine (53).

Decreased levels of NO contribute to vascular damage by facilitating interactions of platelet vascular wall and causing adhesion of circulating monocytes to endothelial surface (54). Therefore, it is important to keep the NO levels in the physiological limits. In our study, in terms of NO levels, there is significant difference between the values of control and STZ groups. A significant reduction in NO levels occurred in diabetic rats. The studies indicate that NO levels decrease in diabetes. In another study conducted by Zhao *et al*, it has been determined that expression of eNOS decreased in the aortas of STZ-diabetic rats (55). In the study conducted by Tessari *et al* on diabetic patients, it has been reported that concentrations of NO significantly decreased in the blood of diabetic patients (56). Our study is in line with these data. In our study, NO levels of rats in STZ and STZ+BQ-123 group decreased significantly compared to control rats, and the effect of BQ-123 that increases the level of NO was not detected.

Na^+/K^+-ATPase ionic pump has an important role for providing low Na^+ and high K^+ in the cell and maintaining the cell haemostasis (57). Deterioration of Na^+/K^+-ATPase enzyme activity in diabetes leads to disruption in the ion balance and decrease of K^+ levels in the cell. In diabetes, due to K^+ ions shift towards the extracellular fluid from inside of the cell, increase of K^+ ions in extracellular fluid is expected. The reason for shifting of K^+ ions towards the extracellular fluid is insulin deficiency or resistance seen in diabetes (58). In our study, although it was not statistically significant, serum K^+ concentration levels in diabetic rats were significantly higher than control. Serum Na^+ concentration levels were significantly lower in the diabetic rats than control. Cl^- concentration levels in serum were lower in diabetic rats than in the control group. Especially, Cl^- is involved in the provision of osmotic pressure in plasma and intracellular liquid. Therefore, it is important to balance the levels of Cl^- in diabetes. Some studies reported that the defect of Na^+/K^+-ATPase activity in experimental diabetes lead to the active transport of cations, which play

an important role in chronic diabetic complications such as neuropathy, nephropathy and retinopathy. Reduced Na^+/K^+-ATPase activity of red blood cell membrane is considered especially as a strong marker for diabetic neuropathy (59). In the study conducted by Raccah *et al*, on a case of type 1 diabetes, decrease in Na^+/K^+-ATPase activity of red blood cell membrane was associated with the formation of peripheral neuropathy (60). Therefore, to control and balance Na^+ and K^+ ions is important in diabetes.

The results of this study demonstrate that leptin levels in STZ-diabetic rats decrease but it is not statistically significant. BQ-123 treatment reduced more leptin levels in diabetes, although it is not statistically significant. This result may be related to the administration time and dose of BQ-123. In the STZ-induced diabetes model, there was no statistically significant difference in levels of PC and K^+, but Na^+ and Cl^- levels decreased significantly. BQ-123 treatment did not have any effect on this decrease. It is known that, in diabetes, oxidative damage increases and a marked increase in the level of TBARS occurred which is an important indicator of oxidative damage. In the group treated with BQ-123, BQ-123 had a BQ-123 by causing a significantly decrease in TBARS levels of the rats. In experimental diabetes, the effects of an ET_AR antagonist, BQ-123 and its possible relationship with leptin are needed to put forward a more precise manner with new studies with different doses and application times. Different ET_AR antagonists can be applied in this context. It is obvious that very complex relationships between autocrine and paracrine factors and their interactions in changing pathophysiological conditions is a topic that needs more investigations. Also, contributions of these changes to homeostasis in pathophysiological conditions is a topic worth exploring and investigating, but it is quite complicated.

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AUTHORS' NOTE

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Safety and Efficacy KollaGen II-xs: A 60-day Clinical Trial

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ABSTRACT

Objective: *To investigate the safety and efficacy of an avian sternal collagen type II hydrolysate, KollaGen II-xs™.*

Methods: *A goniometer was used to measure the range of motion, a pain scale (Borg) was applied to subjectively percept the pain, and a properly calibrated sphygmomanometer was utilized to evaluate muscle strength.*

Results: *The results indicated that the administration of 2000 mg/day of collagen type II hydrolysate for 60 days improved essential symptoms in individuals suffering from joint diseases, including the range of motion, general pain and muscle strength. No adverse effects were observed during the trial.*

Conclusion: *The results support the view that collagen type II hydrolysate may be administered to patients suffering from joint diseases. These data encourage its use for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis and rheumatoid arthritis.*

Keywords: Dietary supplement, efficacy, joint diseases, KollaGen II-xs, safety

INTRODUCTION

KollaGen II-xs™, an avian sternal collagen type II hydrolysate, is a dietary supplement that may be beneficial for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis and rheumatoid arthritis. Its use in the treatment of degenerative joint diseases has increasingly gained support in medical community and among consumers (1).

It has been verified in preclinical studies that orally administered collagen type II hydrolysate is thoroughly absorbed by the intestine and circulated in the blood stream, remaining in the gastrointestinal tract. It was also revealed that a significant amount of collagen type II hydrolysate-derived peptides reached cartilage tissue (2). Additionally, it was exposed that treatment of cultured chondrocytes induced a statistically significant dose-dependent increase in type II collagen synthesis of the chondrocytes in cell culture experiments (3).

Based on the findings that collagen type II hydrolysate is absorbed in its high molecular form, accumulating in cartilage, and is able to stimulate chondrocyte metabolism (4), it might be reasonable to use collagen type II hydrolysate as a nutritional supplement to activate collagen biosynthesis in chondrocytes in humans, especially patients suffering from degenerative joint diseases. Thus, the aim of this single-centre investigation is to extend these earlier findings with KollaGen II-xs™, an avian sternal collagen type II hydrolysate.

SUBJECTS AND METHODS

This single-centre clinical trial was approved by the Ethics Committee of Mortec Scientific, Inc. (Cambridge, ON, Canada) and managed in its Department of Clinical Medicine. According to the study schedule, the consent form was discussed and signed, and a complete physical examination was executed at screening. Activity level,

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diet history, medication/supplement use and medical history were recorded.

Subjects' complaints of joint discomfort were recorded using pre- and post-treatment questionnaires to evidence personal data and issues related to an individual's functional quality. A goniometer was used to measure the range of motion (5), a pain scale (Borg) was applied to subjectively percept the pain (6) and a properly calibrated sphygmomanometer was utilized to evaluate muscle strength (7).

Urine was collected for a pregnancy test for women of childbearing potential. A blood sample was taken for the determination of alanine transaminase, aspartate transaminase, bilirubin, blood urea nitrogen and creatinine. Upon review of blood test results, eligible subjects were instructed to get an X-ray of the affected joints to confirm diagnosis.

A total of 20 subjects were recruited using the inclusion and exclusion criteria outlined in Table 1. At the first visit, selected subjects, properly informed by the consent term approved by the Scientific Committee of the Mortec Scientific, Inc. (Cambridge, ON, Canada), were assigned to receive 2000 mg KollaGen II-xs™ (Certified Nutraceuticals, Inc., San Diego, CA, USA) daily. At the final visit, subjects were required to come to the clinical division for clinical assessment. A subject treatment diary was completed by each patient throughout the study period to determine product compliance, side effects and supplementation use.

For comparing non-parametric values, Wilcoxon's test was used, and for comparing parametric values, the analysis of variance test was performed by GraphPad InStat 3.1. A significance level of 5% was adopted in all comparisons, and statistically significant results were marked with an asterisk (*).

Table 1: Inclusion and exclusion criteria

Inclusion criteria
Males and females of 45–75 years old
Females of childbearing potential must agree to use a medically approved form of birth control and have a negative urine pregnant test result
Disorder of the knee for more than 3 months
Able to walk
Availability for the duration of the study
Subject agrees not to start any new therapies during the course of the study
Able to give informed consent
Exclusion criteria
History of asthma, history of diabetes
Hyperuricaemia
Hypersensitivity to NSAIDs
Abnormal liver or kidney function tests
Abnormal findings on complete blood count
Uncontrolled hypertension
History of allergic reaction to any ingredients in the test product
Hyperkalaemia (potassium > 6.2 mmol/L)
History of cancer as well as gastrointestinal, renal, hepatic, cardiovascular, haematological or neurological disorders
Anticipated problems with product consumption
High alcohol intake (> 2 standard drinks per day)
History of psychiatric disorder that may impair the ability of subjects to provide written informed consent
Use of concomitant prohibited medication (narcotics, NSAIDs)
Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

NSAIDs = non-steroidal anti-inflammatory drugs.

RESULTS

Baseline characteristics of patients are summarized in Table 2. Where applicable, values are expressed as mean ± standard deviation.

Table 2: Baseline characteristics of patients

Characteristics of patients	**Values**
Age (years)	55.9 ± 7.91
Sex (male/female)	10/10
Height (cm)	168.1 ± 8.52
Weight (kg)	81.3 ± 14.6
Systolic blood pressure (mm)	120.5 ± 7.84
Diastolic blood pressure (mm)	80.6 ± 8.33
Heart rate (bpm)	68.9 ± 7.42

The results are presented in Tables 3 and 4, listing values for average and standard deviation for each analysed variable. Statistically significant results are marked with an asterisk (*).

Table 3: Range of motion, pain and muscle strength

	Range of motion[5]		**General pain**[6]		**Muscle strength**[7]	
Treatment	Pre	Post	Pre	Post	Pre	Post
Average	105.22	172.53	8.73	1.92	58.43	104.97
Standard deviation	13.46	10.81	10.54	12.73	10.54	11.73
Standard error	4.22	4.93	4.76	5.48	4.76	5.68

Table 4: Pre- and post-treatment groups

Comparison	***p* value**
Range of motion	0.011*
General pain	0.001*
Muscle strength	0.004*

These results indicate that the administration of 2000 mg/day of collagen type II hydrolysate for 60 days improved essential symptoms in individuals suffering from joint diseases, including the range of motion, general pain and muscle strength. No adverse effects occurred during the 60-day trial period. The treatment was reported to be well tolerated by subjects.

DISCUSSION

Several nutritional supplements, including chondroitin, glucosamine, soybean unsaponifiables and diacerein, have emerged as new treatment options for joint disorders in the last few years (8). The aim of this single-centre investigation is to evaluate the safety and the efficacy of an avian sternal collagen type II hydrolysate, KollaGen II-xs™, which is a complex structural protein that may provide strength and flexibility to connective tissues.

In an observational study, the use of collagen type II hydrolysate as a nutritional supplement to reduce symptoms of joint damage was investigated, with the expectation that this change would reflect improvements in joint health. Individuals were recruited who had not been diagnosed with degenerative joint disease but who complained about joint pain that both the treating physician and the subjects interpreted as being a result of stressful exercising. It was reported that 78% of individuals at the end of the study noticed substantial improvement of their joint symptoms, including the range of motion, pain and muscle strength (9).

The evaluation of muscle strength is an important technique to diagnose the aetiology of the disease and to define rehabilitation strategies. The muscle weakness, which was observed in our study during the pre-treatment assessments, is directly associated with knee joint pain and joint disability (10).

Osteoarthritis results in changes that affect not only intracapsular tissue but also periarticular tissues, such as ligaments, capsules, tendons and muscles. Compared with healthy individuals of the same age, osteoarthritis patients had muscle weakness, reduced knee proprioception, reduced balance and position sense (11).

The presence of joint effusion, even in small amounts, is a potent inhibitory mechanism reflex muscular activity of the joints. A reduced reflex muscular activity causes hypotrophy and weakness early, with the resultant associated mechanical damages, such as the decreased range of motion (12).

Muscle strength declines rapidly during the detention of a member by decreasing the size of the muscle and stress per unit of the muscle cross-sectional area. The largest absolute loss of muscle mass occurs at the beginning of hypotrophy process (13). The pain inhibits reflex muscular activity, causing atrophy and muscle weakness. The painful process is prior to the muscular weakness (14).

This single-centre investigation suggests that the avian sternal collagen type II hydrolysate, KollaGen II-xs™, may be beneficial for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis and rheumatoid arthritis.

CONCLUSION

The purpose of this study was to define whether the administration of 2000 mg of avian sternal collagen type II hydrolysate daily would reduce joint pain in patients suffering from joint diseases. The design of the clinical trial was appropriate to reveal that collagen type II hydrolysate as a nutritional supplement ingested over 60 days was safe and efficacious in reducing symptoms of joint discomfort. The results of the trial provide data supporting the view that collagen type II hydrolysate may be administered to patients suffering from joint diseases. Further research will elucidate additional benefits from collagen type II hydrolysate.

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ORIGINAL ARTICLE

The Effects of Lunar Phases and Zodiac Signs on Recurrent Youth Suicide Attempts—Experience of University Hospital

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ABSTRACT

Objective: *To determine the clinical and demographical features of recurrent youth suicide and identity possible risk factors.*

Methods: *In this study, all patients admitted to our paediatric emergency department with adolescent suicide attempts, from the dates of January 2011 and September 2014, were analysed with the goal to identify the risk factors for reoccurring suicide and clinical outcomes retrospectively.*

Results: *This study included 417 adolescents, 81 men and 336 women with an average age of 15.55 ± 1.86 years. The most common zodiac sign of the patients was Capricorn (48 patients) and Aquarius (44 patients). According to the lunar cycle, 39 (9.4%) attempted suicide during full moon and 34 (8.2%) during the new moon cycle. It has been established that most suicide attempts occurred while being alone (80.2%) and inside the house (90.6%) and the most preferred method is combined drug ingestion (51.0%). The recurrent suicide attempt rate is 13.2%. While determining that attempting the suicide alone is a significant factor in recurrent suicide, psychotropic drug intake was found to have a protective effect. The most diagnosed psychiatric disorder in cases of recurrent suicide and first-time suicide attempts was depression (49.1% and 8.6% respectively).*

Conclusion: *Triggering risk factors such as lunar cycle or zodiac sign do not have an effect on recurrent suicide attempts. A wide participation in clinical studies is necessary to determine the real effect of these risk factors.*

Keywords: Emergency department, lunar cycle, risk factors, suicide, zodiac sign.

INTRODUCTION

One of the major causes of mortality worldwide is suicides in adolescence (1). According to the World Health Organization, suicide attempts between the ages of 10 and 24 rank second as the cause of mortality (2). This situation especially increases along with the age in the paediatric age group and keeps increasing in adolescence. Completed incidences of suicide between the age of 10 and 14 have been reported to be 1/100 000 but at the ages of 15–19 this number increases up to 10 times (3).

Suicides occurring in the adolescent age group tend to be recurrent. The median annual rate of recurrence of attempted suicide is 5-15%. Studies showed that the recurrent risk after 6 months is 10% and rises to 42% after 21 months (4). The finding of recurrent suicide attempts in adolescents are rising which shows that preventative healthcare is needed, and emergency services show a great potential for that. Studies show that the reason behind this is the fact that a suicide is attempted up to 8–25 times before death occurs and 39%–40% of

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these cases have been reported to go to the emergency room at least once a year (5–7).

Most seen risk factors that cause suicide attempts are mostly age, gender, occupation, history of suicide attempts, mental disorder (anxiety, mood), addictions (*eg*, alcohol, tobacco), physical disability, financial stress, personality disorders/impulsivity/aggression, legal problems, lack of religious affiliation, childhood maltreatment, intimate partner violence, suicide in family members, sensational media reporting of suicide, specific cultural factors (*eg*, Native Americans, immigrants, refugees), access to lethal means (guns, pesticides), sexual and physical abuse and mourning process (4, 8–11).

Studies about the possible effects of lunar phases and zodiac signs on personality and behaviour patterns and the emergence of some clinical situations are noteworthy. Differences in available moonlight, barometric pressure (weather conditions), geomagnetic and gravitational variations, solar corpuscular radiation, and other mechanisms are brought forward in these interactions (12). Clinical studies in which lunar phases and zodiac signs play a role are for example cardiopulmonary resuscitations (13), birth rates (14, 15), renal colic (16), postoperative complications (17), survival after therapy (18), and suicide (19, 20). There are only a few studies that research the effect of lunar phases and zodiac signs of recurrent suicide attempts in childhood (1).

The aim of this study was to determine the clinical and demographic characteristics of adolescent suicide cases reported to the children's emergency department and to investigate the effect of lunar phases and zodiac signs in recurrent suicide attempts as possible risk factors.

SUBJECTS AND METHODS

Study design and patient selection

All adolescent cases (age 10–18) of suicide attempts that were reported in the paediatric emergency department of the Ondokuz Mayıs University between January 2011 and September 2014 have been examined retrospectively. Ondokuz Mayıs University, Medical Faculty, Pediatric Emergency Department is a Level III emergency centre that treats 15 000–20 000 patients each year. Ethical approval for this prospective study was obtained from the local ethics committee of Ondokuz Mayıs University in accordance with the Helsinki Declaration. The cases of suicide attempts were analysed upon repetition and the patients were divided into groups based on demographic features (age, gender, distribution of horoscope, season, poisoning time, lunar cycle, type of suicide attempts, place of occurrence, and cause of suicide), clinical and laboratory findings. Intensive care and situations that require emergency interventions and were then analysed with the goal to identify the factors for reoccurring suicide and clinical outcomes. The full and new moon phases were calculated separately for each patient. As stated in literature (21, 22), the time of the full and new moon was accepted as one day after and one day before the corresponding application date and the time of the full and new moon.

Statistical analysis

The data has been given as mean ± SD, median (minimum–maximum) and n (%). All data were analysed with IBM SPSS 21.0 (Chicago, USA). Eligibility was determined by the normal distribution of data with the Kolmogorov Smirnov method. Data fitting the normal distribution were stated as standard deviation and data not fitting the normal distribution were stated as median and minimum–maximum. For the evaluation of categorical data, Pearson's Chi-square test was used. For the analysis of effective factors on recurrent suicide attempts Binary Logistic Regression analysis was performed. To compare the disease rates of recurrent suicide attempts and first-time suicide attempts, a two proportion *t*-test was performed with Minitab 14.0. The value $p < 0.05$ was considered statistically significant.

Results

A total of 417 adolescent patients (ages 10–18) were included in this study period, 81 (19.4%) of which were boys and 336 (80.6%) were girls. The average annual application rate of adolescent suicide in the study period was 0.7% (417/56336). The average age of the patients was 15.55 ± 1.86 year. Examination of the age distribution showed that 76.0% (317 patients) are ≥ 15 years old and 24.0% (100 patients) are between the ages of 10–14. There were more reported cases of suicide attempts in the winter months (160 patients 38.4%) ($p < 0.001$). There was no significant difference in the distribution of cases according to zodiac sign ($p = 0.059$). However it has been seen that the most common zodiac signs were Capricorn (48 patients, 11.5%) and Aquarius (44 patients, 10.6%). According to the lunar cycle, 39 (9.4%) patients attempted suicide during full moon and 34 (8.2%) patients during the new moon cycle. According to the lunar cycle, more cases (263 patients, 63.0%) were reported outside the new and full moon

periods ($p < 0.001$). The examination of cases according to the preferred time frame showed a statistically significant difference ($p < 0.001$). It has been identified that most cases of suicide attempts occurred between the hours of 12:01 AM to 6:00 PM (144 patients, 34.5%) and between 6:01 PM and 00:00 (147 patients, 35.7%). While 94.0% preferred medical poisoning agents in their suicide attempts, 6.0% preferred non-medical poisoning. The most preferred medical poisoning agent in suicide attempts were analgesics (174 patients, 41.7%) and the most preferred non-medical poisoning agent organophosphate poisoning (12 patients, 2.9%) (Table 1).

Table 1: The distribution of poisoning agents used in suicide attempts

Medical poisoning agents	
Analgesics	174 (41.7)
Antidepressant	115 (27.6)
Antipsychotics	58 (13.9)
Antihipertansifler	58 (13.9)
Antihistaminic and decongestants	48 (11.5)
Digestive system drugs	41 (9.8)
Antibiotics	36 (8.6)
Psikostimulant ajanlar	30 (7.2)
Myelorelaksan	27 (6.5)
Antiagregants	16 (3.8)
Antidiabetics	12 (2.9)
Vitamins	12 (2.9)
Iron medications	11 (2.8)
Others	98 (23.5)
Non-medical poisoning agents	
Organophosphate poisoning	12 (2.9)
Narcotic substance intake	8 (1.9)
Other	5 (1.1)

It has been determined that suicide on their own (376 patients, 80.2%) was more common than mass suicide attempts (41 patients, 9.8%) ($p < 0.001$). The frequency of recurrent suicide attempts, however, was 13.2% (55 patients). The most preferred environment for suicide attempts was the home environment for 378 patients (90.6%) ($p < 0.001$). The most preferred kind of suicide is combined drug ingestion (213 patients, 51.0%) ($p < 0.001$). While 357 (85.6%) cases were referred to the hospital, 60 (14.4%) applied from home. of patients. Characteristics of all patients' demographic findings, clinical signs, laboratory findings during the application, and treatment modalities were represented in Tables 2 and 3.

Table 2: The distribution of patients according to demographic findings

	All patients
Age (year (min–max))	16 (10-18)
Gender (n, %)	
Male	81 (19.4)
Female	336 (80.6)
Distribution of horoscope	
Capricom	48 (11.5)
Aquarius	44 (10.6)
Cancer	39 (9.4)
Leo	39 (9.4)
Libra	39 (9.4)
Taurus	37 (8.9)
Gemini	35 (8.4)
Virgo	33 (7.9)
Aries	29 (7.0)
Pisces	26 (6.2)
Scorpio	24 (5.8)
Sagittarius	24 (5.8)
Season distribution (n, %)	
Winter months	160 (38.4)
Spring months	114 (27.3)
Summer months	82 (19.7)
Autumn months	61 (14.6)
Poisoning time (n, %)	
According to moon calendar	
New moon	34 (8.2)
First Quarter	32 (7.7)
Full moon	39 (9.4)
Last Quarter	49 (11.8)
Other time	263 (63.0)
Time zone	
00:01–06:00	39 (9.4)
06:01–12:00	64 (15.3)
12:01–18:00	144 (34.5)
18:01–00:00	149 (35.7)
Unknown	21 (5.0)
Type of suicide attempts (n, %)	
Mass suicide	41 (9.8)
Alone suicide	376 (90.2)
Frequency of suicide attempts (n, %)	
Recurrent suicide	55 (13.2)
First suicide	362 (86.8)
Place of occurrence (n, %)	
Home	378 (90.6)
School	4 (1.0)
Other areas (sea, forest, street, etc)	35 (8.4)
Cause of suicide (n, %)	
Single drug ingestion	179 (42.9)
Combined drug ingestion	213 (51.0)
Organophosphate poisoning	12 (2.9)
Narcotic substance intake	8 (1.9)
Other	5 (1.2)

Self-injuries behaviour (n, %)	
Piercing	5 (1.2)
Forearm laceration	39 (9.4)
Application form (n, %)	
Referred to hospital	357 (85.6)
Applied from home	60 (14.4)
Patient follow-up clinics (n, %)	
Emergency Department	396 (95.0)
Paediatric Intensive Care Unit	21 (5.0)
Patient outcome (n, %)	
Discharged	416 (99.8)
Exitus	1 (0.20)
Total patients (n,%)	**417 (100.0)**

Table 3: Characteristics of all patients' clinical signs, laboratory findings and treatment modalities

	All patients
GCS (median)	15 (7–15)
Clinical signs and symptoms (n,%)	
Miosis	35
Convulsion	4
Vomiting	86
Arrhythmia	3
Hypothermia	3
Hyperthermia	5
Hypertension	15
Hypotension	3
Tachycardia	29
Bradycardia	37
Tachypnoea	24
Cardiopulmonary arrest	1
Laboratory findings (n,%)	
Leucocytosis	25
Neutropenia	33
Thrombocytopenia	4
Hyponatremia	19
Hypernatremia	3
Hypokalaemia	57
Hyperkalaemia	4
Hypoglycaemia	3
Hyperglycaemia	61
Elevated liver enzymes	3
Metabolic acidosis	65
Coagulation disorders	5
Renal function impairment	4
Treatment modalities	
Gastric lavage	353
Activated charcoal	345
Antidote application	57
Fluid therapy	403
Cardiotonic treatment	3
Oxygen therapy	39
Mechanical ventilation	6
Total	417

Only 21 patients (5.0%) required intensive care. The median value of the intensive care follow up period of these cases was 2 days (1–8). Demographic characteristics of the patients in intensive care, clinical and laboratory findings, treatment and follow-up procedures are summarized in Table 4. The majority of patients requiring intensive care (14 patients, 66.6%) were diagnosed with CDI. Only 2 (0.48%) of the patients in intensive care were recurrent suicidal.

While examining the affecting factors of recurrent suicide showed that the effect of type of suicide attempts is higher (OR: 11.911 (95% CI: 2.241, 63.319, $p = 0.004$) psychotropic drug intake was found to have a protective effect (OR: 0.006 (95% CI: 0.002, 0.020), $p < 0.001$) (Table 5).

When examining the underlying psychiatric diseases, the most diagnosed psychiatric disorder in cases of recurrent suicide and first-time suicide attempts was depression (49.1% and 8.6%, respectively). The most consumed medications in both groups were antidepressants and antipsychotics (Table 6).

When examining the medical interventions we see that 353 (84.7%) were given gastric lavage, 345 (82.7%) activated charcoal treatment, 57 (13.7%) antidote application, 403 (96.6%) fluid therapy, 3 (0.7%) cardiotonic treatment, 39 (9.4%) oxygen therapy and 6 (1.4%) mechanical ventilation treatment (Table 2). During the follow-up, only 1 patient (0.2%) had died and 416 patients (99.8%) had been discharged.

Discussion

Adolescent patients admitted to the paediatric emergency department after a suicide attempt were included in the study. As a result, it has been found that most cases of suicide attempt were reported in winter months between the hours of 12:01 PM and 00:00. Especially in cases requiring emergency intervention in the adolescent age group, time of full moon and new moon was observed not to be effective in adolescent attempt. In addition it was found that people with the zodiac sign Capricorn and Aquarius are more likely to attempt suicide but statistically it is of no value. Combined drug ingestion in the home environment has been found to be the preferred method of attempted suicide. While determining that attempting suicide alone is a significant factor in recurrent suicide, psychotropic drug intake was found to have a protective effect. In cases of recurrent adolescent suicide attempt, depression was found to be a common factor.

Table 4: The characteristics of all patients with suicide attempt and followed up in the paediatric intensive care unit

Age	Sex	Cause of suicide	Number of tablet by oral ingestion	GCS	Clinical signs / laboratory findings	Intubation	Fluid therapy/ antidote application	Hospitalization (days)
12	M	CDI	50	15	Not detected	−	+ / + (NAC)	1
12	FM	SDI	20	11	Confusion Tachypnoea	−	+ / −	2
13	FM	CDI	30	15	Not detected	−	+ /−	6
14	FM	OP	–	8	Convulsion, Coma, Hyperglycaemia	+	+ / + (PAM)	4
14	FM	CDI	3	15	Vomiting, Hyponatremia	−	+ / −	3
15	FM	OP	–	10	Confusion Hypertension, Tachypnoea Hypokalaemia, Hyperglycaemia, Leucocytosis	+	+ / + (PAM)	4
15	FM	CDI	44	10	Confusion Bradycardia Hypoglycaemia, Elevated Liver enzymes, Leucocytosis,	+ / CPR	+ / −	4
15	FM	CDI	68	15	Bradycardia, Hypertension, Tachypnoea, Vomiting Hyponatremia	−	+ / −	4
15	FM	CDI	25	15	Tachycardia	−	+ / + (NAC)	1
15	FM	SDI	20	15	Convulsion, Vomiting	−	+ / −	3
15	M	SDI	Unknown	15	Tachypnoea	−	+ / −	3
16	FM	CDI	8	15	Not detected	−	+ / −	2
16	FM	CDI	60	15	Prerenal failure, Leucocytosis	−	+ / −	3
16	M	SDI	50	15	Hyperkalaemia, Prerenal failure, Leucocytosis	−	+ / −	5
17	M	CDI	71	7	Confusion Bradycardia Hyperglycaemia,	+	+ / −	4
17	FM	CSI	–	15	Vomiting	−	+ / −	9
17	FM	CDI	31	13	Confusion Convulsion, Hypokalaemia	−	+ / −	2
17	FM	CDI	39	15	Hypokalaemia	−	+ / −	2
17	M	CDI	21	10	Confusion Convulsion, Bradycardia Hypokalaemia, Hyperglycaemia,	−	+ / −	4
18	FM	CDI	5	15	Vomiting Hypokalaemia, Hyperglycaemia, Neutropenia	−	+ / −	9
18	FM	CDI	Unknown	11	Confusion Tachycardia, Vomiting Neutropenia	−	+ / −	2

GCS = Glaskow Coma Score; M = male; FM = female; CDI = combined drug ingestion; SDI = single drug ingestion; OP = organophosphate poisoning; CSI = corrosive substance ingestion; CPR = cardiopulmonary resuscitation; NAC = *N*-acetylcysteine; PAM = pralidoxime.

Table 5: Analysis of influential factors on recurrent suicide attempts

Parameters	Recurrent suicide attempts n = 55 (13.2%)			First suicide attempts n = 362 (86.8%)	Multivariate analysis			
OR		**95% CI**	**p**					
Male	9 (16.4)			72 (19.9)	1.656		0.568–4.825	0.356
Age (mean ± SD)	15.62 ± 1.87			15.55 ± 1.64	0.892		0.702–1.133	0.348
Distribution of horoscope					1.005	0.883–1.144	0.943	
Capricom	5 (9.1)			43 (11.9)				
Aquarius	8 (14.5)			36 (9.9)				
Cancer	7 (12.7)			32 (8.8)				
Leo	8 (14.5)			31 (8.6)				
Libra	6 (10.9)			33 (9.1)				
Taurus	3 (5.5)			34 (9.4)				
Gemini	3 (5.5)			32 (8.8)				
Virgo	2 (3.6)			31 (8.6)				
Aries	3 (5.5)			26 (7.2)				
Pisces	2 (3.6)			24 (6.6)				
Scorpio	4 (7.3)			20 (5.5)				
Sagittarius	4 (7.3)			20 (5.5)				
Type of suicide attempts					11.911	2.241–63.319	0.004	
Mass suicide	2 (3.6)			39 (10.8)				
Alone suicide	53 (96.4)			323 (89.2)				
Season distribution					1.276	0.833–1.954	0.262	
Winter months	24 (43.6)			136 (37.6)				
Spring months	13 (23.6)			101 (27.9)				
Summer months	9 (16.4)			73 (20.2)				
Autumn months	9 (16.4)			52 (14.4)				
Poisoning time (n, %)								
According to moon calendar					1.012	0.623–1.244	0.910	
New moon	0			34 (9.4)				
First Quarter	5 (9.1)			27 (7.5)				
Full moon	8 (14.5)			31 (8.8)				
Last Quarter	8 (14.5)			41 (11.3)				
Other time	34 (61.8)			229 (63.3)				
Time zone					0.723	0.448–1.168	0.185	
00:01–06:00	6 (10.8)			33 (9.1)				
06:01–12:00	6 (10.8)			58 (16.0)				
12:01–18:00	22 (40.0)			122 (33.7)				
18:01–00:00	20 (36.4)			129 (35.6)				
Unknown	1 (1.8)			20 (5.5)				
Cause of suicide (n, %)								
Single drug ingestion	18 (32.7)			161 (44.5)				
Combined drug ingestion	32 (58.2)			181 (50.0)				
Organophosphate poisoning	3 (5.5)			9 (2.5)				
Narcotic substance intake	1 (1.8)			7 (1.9)				
Other	1 (1.8)			4 (1.1)				
Self-injuries behaviour	9 (16.4)			34 (9.4)	1.007	0.319–3.181	0.991	
Psychotropic drug intake	52 (94.5)			46 (12.7)	0.006	0.002–0.020	< 0.001	
Place of occurrence (n, %)					1.143	0.739–1.767	0.549	
Home	49 (89.1)			329 (90.9)				
School	1 (1.8)			3 (0.8)				
Other areas (sea, forest, street, etc)	5 (9.1)			30 (8.3)				

Table 6: Distribution of psychiatric diagnosis, treatment modalities and outcomes of all patients

	Recurrent-suicide attempts (n, %)	First-suicide attempts (n, %)
Diagnosis		
Depression	27 (49.1)	31 (8.6)
Conduct disorder	8 (14.5)	10 (2.7)
Attention deficit hyperactivity disorder	7 (12.7)	13 (3.6)
Bipolar affective disorder	5 (9.0)	1 (0.2)
Post-traumatic stress disorder	3 (5.4)	1 (0.2)
Generalized anxiety disorder	3 (5.4)	4 (1.1)
Mental retardation	2 (3.6)	-
Conversion disorder	2 (3.6)	-
Prolonged pattern of mourning	1 (1.8)	1 (0.2)
Performance anxiety	1 (1.8)	
Unknown	11 (20.0)	5 (1.4)
Obsessive compulsive disorder	-	2 (0.5)
Drugs used in therapy		
Antidepressant drugs	28 (50.9)	3 (0.8)
Antipsychotic drugs	25 (45.4)	2 (0.5)
Psychostimulant drug	6 (10.8)	–
Antiepileptic drug	2 (3.6)	–
Anxiolytic drug	1 (1.8)	–

Youth suicide is an important problem showing an increase in emergency admissions in childhood. The suicide attempts in this age group have increased four-fold in the recent years (10). According to the Centers for Disease Control and Prevention, we experienced an increase of 8% in the last 15 years (2). Although suicide is rare in childhood, it seemingly increases with the start of adolescence (1) and this increase arrives its peak in late adolescence and the beginning of the twenties. While the prevalence of suicide thoughts in adolescence is approximately 15%–25% (21), the suicide attempt rate of men differs between 1.3% and 3.8% and the rate of women between 1.5% and 10.1% (4).

The first suicide attempt is likely to be a messenger for recurrent suicide attempts in adolescence. The recurrent suicide rate in the adolescent age group increases with the years after the first attempt. In studies, this rate ranges from 10% to 42% (2, 4). While recurrent suicide attempts in adolescence is much higher in women, successful suicide attempts are 30 times higher in men (8, 23). Our study showed a similar rate to the one in literature (13.2%) in recurrent suicide. Although the female–male ratio was 4:1 in our study it had no effect on recurrent suicide attempts.

The annual mortality rate because of suicide attempts is between 0.5% and 1.0% (4). The rate in studies for the necessity of intensive care in youth suicide was determined to be 3.1%–8% (24, 25). A significant portion of these patients requiring intensive care, attempted suicide by multiple drug ingestion. Studies showed that the rate lies between 31% and 45% (24, 25). In our study the rate was 5.0% and 66% of the cases attempted suicide with multiple drug ingestion.

Preferred methods for suicide differ according to geographic and cultural differences (4, 5). More preferred suicide methods in adolescence in developed or developing countries are gunshot wounds and poisonings with medical or non-medical agents (4, 5, 25). Among the most commonly used agents in adolescence for suicide are analgesics, anti-inflammatory agents and anti-psychotic agents (24, 26, 27). Non-medical agents are organophosphates, pesticides, insecticides, organic solvents and household cleaning products (2, 27–29). While 94.0% of patients in our study preferred medical poisoning agents in their suicide attempts, 6.0% preferred non-medical poisoning. The most preferred medical poisoning agents were, like stated in literature, analgesics and antidepressants.

Many risk factors were examined in the studies that are thought to cause suicide attempts occurring in the adolescent age group. Except the risk factors of age and genders, the rest is categorized as affective, cognitive, family and peer factors (8). Nearly 90% of suicidal teenagers are known to have a psychiatric disorder (8). Most commonly psychiatric disorders are depression, bipolarity and drug abuse (8). If we look at the data of 25 emergency rooms of the Pediatric Emergency Care Applied Research Network Core Data Project we can

see that depression is one of the top five diseases accompanying suicide attempts (30). Other studies show that depression accompanies suicide attempts with a rate of 25% (31). Previously attempted suicide, panic attacks, post-traumatic stress disorder, risky behaviour (interpersonal violence, excessive alcohol consumption, tobacco use, illicit drug use, high-risk sexual behaviour), stressful life events, sexual abuse, family conflicts, family history of suicide, self-injuries behaviour and many more are known risk factors of adolescent suicide (5, 6, 8, 9). Protective factors include strong social relationships, legal regulations and psychotropic drug use (4, 8, 32). Our study showed, as stated in literature, that the most distinctive disease accompanying a suicide attempt is depression. However, intake of psychotropic drugs was found to have preventive properties in recurrent suicide attempts.

The lunar cycle and zodiac sign and its effect on human physiology and behaviour are some of the frequently discussed issues in socio-cultural life and scientific fields. Although the topic is very popular, there are no complete scientific ideas about the effects. While some studies support this idea (32, 33) some state that the lunar cycle and zodiac sign are not effective factors (13, 35, 36). Along the associated clinical conditions with lunar phases and zodiac signs are psychosis, depression, anxiety, violent behaviour (37), cardiopulmonary resuscitations (13), birth rates (14, 15), renal colic (16), postoperative complications (17, 38), survival after therapy (18), and suicide (19, 20). Alterations of moonlight, barometric pressure (weather conditions), geomagnetic and gravitational variations, and solar corpuscular radiation are stated to be effective on human behaviour during a lunar cycle (12). These alterations are said to occur to the organs inside the body because of zodiac signs (39). In particular, it has been determined that the Aries sign has an effect on the central nervous system, which is acknowledged to be the centre of human behaviour (39). Our study showed similarities to the literature of Martin and colleagues (39) about suicide and lunar cycle and stated that there is no effect of the lunar cycle on recurrent adolescence suicide. Most commonly seen signs in adolescent suicide were Leo and Aquarius. The Zodiac sign did not have a similar effect of recurrent suicide attempts like the lunar cycle did.

Recurrent suicide attempts in the adolescent age group in developing countries are increasingly seen as an important growing health problem. Taking preventive measures against possible triggers such as depression and conduct disorder and accompanying other factors, is important in this age group with suicide recurring suicide attempts. In our study, we have found no effect on the triggers that include lunar cycle and zodiac signs in the adolescent age group with recurrent suicide attempts which were stated in the results we acquired from one single centre. However, we believe that a broad participation and more clinical studies are needed to determine and evaluate the real impact of risk factors such as lunar cycle and zodiac signs together with other possible risk factors.

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VIEWPOINT

Allocation of Places for Patients to Receive Dialysis in Low- and Middle-income Countries: An Ethical Framework for Distributive Justice

TS Ferguson

ABSTRACT

Access to dialysis and kidney transplantation is limited in low- and middle-income countries, therefore, rationing of dialysis services is usually necessary. Structured rationing systems, however, are often not in place and even when used may result in ethically irrelevant factors determining who gets dialysis. In this paper, I propose a dialysis allocation system, based on a modification of the complete lives system, incorporating the following ethical principles: (a) prognosis (saving the most life-years), (b) saving the most lives, (c) age prioritization (for younger patients) and (d) random selection weighted by waiting time. The application of these principles should result in fair and equitable access to dialysis.

Keywords: Dialysis, distributive justice, equity, low- and middle-income countries, public health ethics

INTRODUCTION

Access to dialysis and kidney transplantation in low- and middle-income countries (LMIC) is severely limited due to lack of resources (1–3). In some countries, kidney transplantation is not feasible, hence, dialysis becomes the only option (4–6). Although private dialysis services may be an option for some, the majority of patients with end-stage kidney disease (ESKD) are unable to afford dialysis and must rely on public dialysis services. The supply of dialysis equipment and trained staff is usually inadequate necessitating some form of rationing (1, 2). Structured rationing systems, however, are often not in place and even when used may result in ethically irrelevant factors determining who gets dialysis. In South Africa, for example, Moosa and Kidd (1) reported that persons most likely to be accepted for dialysis tended to be White, 20–40 years old, employed, non-diabetic and living close to the dialysis centre. In this paper, I will argue that a modified version of the complete lives system proposed by Persad *et al* (7) will serve as an ethically permissible and fair dialysis rationing system for LMIC.

Given that the situation in LMIC is unlikely to improve in the short-term, efforts should be made to develop and implement structured rationing systems. Previously used systems appear to be inadequate and, as seen in South Africa, may result in undesirable outcomes (1). The primary objective of this programme is to minimize morbidity and mortality due to the ESKD in an ethically permissible and fair way. The questions being considered include: How can we arrive at an ethically permissible and fair allocation system? Can we respect each person's liberty and at the same time produce the maximum benefit to the society?

Recommended allocation system

Persad *et al* (7) have recommended the use of a system of allocation for scarce medical interventions called the complete lives system, which uses five individual principles: prioritization of younger patients, prognosis, saving the most lives, lottery and instrumental value of individuals. I propose a modification of this system incorporating the following principles: (a) prognosis (saving the most life-years), (b) saving the most lives, (c) age prioritization (for younger patients) and (d) random selection

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weighted by waiting time (a variation of the lottery principle). Additionally, I would consider patient autonomy, so that the patients could choose whether they want to be considered for dialysis after being fully briefed as to the benefits and risks associated with dialysis.

The allocation system would be administered by an independent committee comprised of physicians, nurses, patients and members of the public. The persons who indicate that they want to be included in the dialysis allocation system would first be assessed by an independent committee who would determine prognosis based on estimated quality-adjusted life-years (QALYs). The system would seek to optimize the number of lives saved by adjusting the optimal QALY to give the maximum balance between QALYs gained and the number persons treated. Recognizing that younger persons have had the least years lived, the system would be adjusted to favour younger persons, giving priority to persons less than 40 years old.

Priority points would be allocated to each person based on the principles outlined above and summed to give a total priority score. After these conditions have been determined, a lottery would be used to randomly select persons for dialysis with the probability of selection weighted by the priority score and waiting time. Ideally, this would use a computer program to select the participants; however, in settings where computer programs are not available, a manual selection could be done with the number of entries determined by the priority score and waiting time. This allocation system would be only for stable patients waiting to be placed on long-term dialysis. Provisions would also be made for persons to receive emergency dialysis when required.

Ethical justification

The ethical theories guiding this allocation system are primarily Rawlsian (egalitarian), but include the utilitarian principles of striving for the maximum benefit to the society. Consistent with the Rawls Liberty principle in an egalitarian society, all citizens should have equal basic liberties, and that the only inequalities allowed are those that benefit the least advantaged (8). In this system, all patients should have an equal opportunity to access dialysis; however, I would prioritize younger patients due to the disadvantage in years of life lived. The selection of younger patients is based on the fair innings principle (9), where individuals are deemed to be entitled to a 'normal lifespan' of about 70–80 years. I have chosen 40 years as the cut-point as this represents about half of the life expectancy in the developed countries and is considered by many as the transition from youth to middle age. While some have advocated a lower priority for the very young, *eg*, children less than 15 years in the complete lives system (7), I would exclude this criterion in this allocation system, given that the proportion of children under 15 years among persons with ESKD is small, and the psychological trauma of denying treatment to children would be difficult to justify to both parents and children. Additionally, these children would be the most disadvantaged from a Rawlsian perspective. The utilitarian view suggests that the morally correct thing to do is that which produces the best state of affairs, or that which maximizes pleasure over the pain (10). In this system, it is expected that maximum benefit can be obtained by prioritizing persons with the best prognosis, maximizing both the number of QALYs gained and the number of lives saved. I have excluded an instrumental value in this allocation system because it is not consistent with the equal opportunity principle and raises the possibility of ethically irrelevant socio-political considerations. It should be noted that Persad *et al* (7) suggest that when considering the ethics of rationing, no single principle recognizes all the morally relevant factors, therefore, it is necessary to form multi-principle allocation systems. This system would satisfy utilitarian reasoning while at the same time upholding the egalitarian principles and the Rawlsian 'difference principle' of prioritization of the worse off. This balancing of equity and utility is generally accepted by the medical community (11).

Counter arguments

Ethical counter arguments for this system would include that it prioritizes young over old and does not include a 'first come first served' and 'sickest first' principles as used in some established allocation systems (7, 11). Although it may initially seem reasonable to treat people equally regardless of age, this does not take into consideration the fact that the youngest have had the least number of lived years, thus prioritization of the young promotes each persons' right to live through all stages of life and the opportunity for a 'fair innings' of life (7, 9). The sickest first principle is counter to the utilitarian view of maximizing utility as the sickest often have the worse prognosis. Additionally, the sickest first policy presumes that scarcity is temporary, which is not the case in LMIC. The first come first served principle while appearing fair initially is subject to manipulation and favours the best-off as persons with a higher social standing and those with a greater knowledge will get

themselves on the list first. As pointed out by Persad *et al* (7) and Zink *et al* (11), both these principles are subject to exploitation. The use of a random selection lottery system overcomes the limitations of the first come first served principle, although weighting the probability of selection by waiting time adjusts for the lottery's lack of sensitivity to the length of time the patient has been on the list.

Another potential limitation of this allocation system is that it is fairly cumbersome to administer, requiring computation of prognosis (QALYs) and sophisticated weighting systems to ensure saving the most lives, younger age prioritization and adjustments for waiting time. Additionally, the system would require a regular meeting of selection committees with its attendant time burden. In order to be properly run the system would need the development of computerized selection protocols and the services of a statistician. These resources would, however, be needed mostly in the start-phase and periodic revision phases. In countries where such expertise is not available, experts from other countries could be asked to help in the initiation phase. Where computer access is limited, prognosis charts could be developed and a manually operated lottery system used. Overall, the system should have a low risk of being corrupted given its reliance on less subjective allocation procedures.

Potential burdens of this allocation system include its need for volunteers to administer the programme and potential for psychological distress among programme officers. The system will also lead to some anxiety among persons waiting for the treatment decisions. These burdens can be minimized by having a revolving system for programme officers, inclusion of multiple stakeholders and providing periodic updates for patients on the waiting list. Fair implementation will be ensured by the periodic review and report of outcomes from the allocation system. This, I believe, will lead to an ethically justifiable and fair distribution of dialysis in LMIC.

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CASE REPORT

Steroid 21-hydroxylase Deficiency in a Newborn Female with Ambiguous Genitalia in Upper Egypt

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ABSTRACT

Congenital adrenal hyperplasia 'CAH' is a group of autosomal recessive disorders, resulting from the deficiency of one of the five enzymes required for the synthesis of cortisol in the adrenal cortex. The most frequent is steroid 21-hydroxylase deficiency, accounting for more than 90% of cases and can have diverse manifestations: from the salt wasting to the non-classical form due to a highly variable genetic mutation. We report a female infant aged two months, from Nag Hammadi, Upper Egypt, whose presentation and laboratory abnormalities were consistent with CAH, the classic 21-hydroxylase deficiency (salt-wasting type). The patient presented with ambiguous genitalia and salt wasting. The karyotyping was that of a normal female. There were elevated levels of serum 17-hydroxy progesterone, adrenocorticotropic hormone, rennin, and rostenedione and testosterone. Also, there were hyperkalaemia and hyponatremia. We conclude that CAH to be keep in mind in the differential diagnosis of any newborn female with ambiguous genitalia with normal karyotyping and we call for dried blood spot 17-hydroxyprogesterone assay to be included in the newborn screening program in Egypt.

Keywords: Ambiguous genitalia, congenital adrenal hyperplasia, salt wasting, steroid 21-hydroxylase, Upper Egypt

INTRODUCTION

Congenital adrenal hyperplasia 'CAH'is a group of autosomal recessive disorders, resulting from the deficiency of one of the five enzymes required for the synthesis of cortisol in the adrenal cortex. The most frequent is steroid 21-hydroxylase deficiency, accounting for more than 90% of cases and can have diverse manifestations: from the salt wasting to the non-classical form due to a highly variable genetic mutation (1, 2).

In CAH, there are various genetic mutations in the enzymes involved in steroidogenesis. Due to this enzymatic defect, cortisol is under-produced and the negative feedback control on ACTH is lost with consequently excess ACTH produced in order to normalize cortisol levels, resulting in over-production and accumulation of steroids precursors prior to the enzyme defect as well as hyperplasia of the adrenal cortex. The clinical manifestation depends on the level of enzyme block in the steroid synthesis (see Fig. 1).

The steroids in the first row are Δ5-steroids, which constitute the preferred pathway to C19 steroids in the human. Not all intermediate steroids, pathways and enzymes are shown. In CAH, due to 17α-hydroxylase deficiency (marked as red X), there is over-production of the precursors/hormones on the left side of the first column with decreased production of the precursors/hormones on the right side of the second and third columns (3).

Congenital adrenal hyperplasia should be considered in infants, children or adolescents with ambiguous genitalia, sexual infantilism, hypogonadism or hypertension, particularly when associated with disturbed water, electrolytes and hydrogen homeostasis. The most common form is 21α-hydroxylase deficiency, which may be diagnosed at birth by the presence of virilization in female infants or by features of salt wasting in both the genders (4). 11β-hydroxylase deficiency is uncommon and 17α-hydroxylase deficiency is a rare form of

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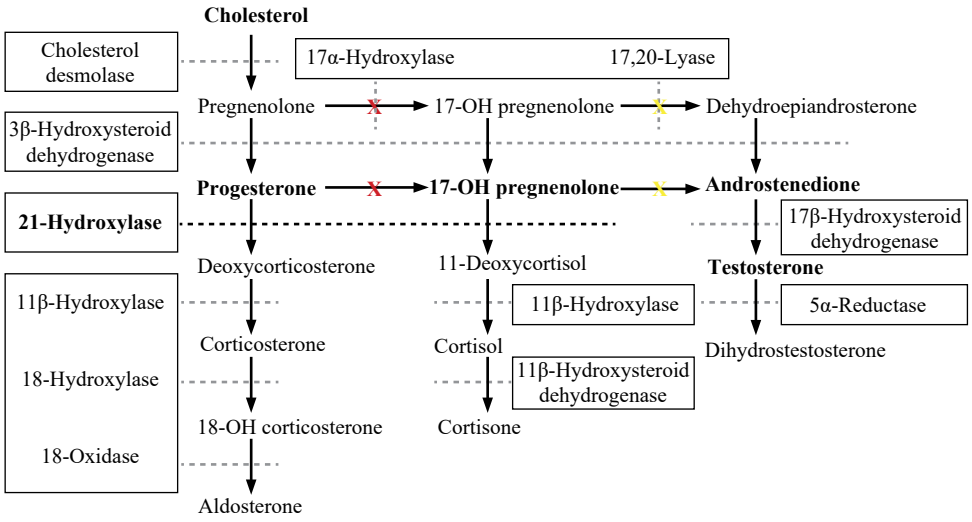

Fig. 1: Major human steroidogenic pathway in the adrenal cortex. Key enzymes are shown as dashed arrows/ shaded boxes indicating the chemical reactions. P450scc (cholesterol desmolase) cleaves cholesterol to pregnenolone, the first intermediate in steroid biosynthesis.

CAH, which may present much later in an adolescence or adulthood (5, 6).

CASE REPORT

A full-term baby aged 5 days (anterior fontanel: 5 cm, head circumference: 36 cm, chest circumference: 35 cm, weight: 3500 g, length: 52 cm) with an abnormal external genitalia was born in a private hospital at Nag Hammadi city, Qena, Upper Egypt. The baby was named Emad by his parents and was registered as a boy. By the 5th day of the baby's life, the parents noticed significant yellow discolouration of the skin and sclera, and they asked medical advice. When the treating physician examines baby's genitalia, he told the parents that the baby has hypospadius and the decision of the physician after doing serum bilirubin (total: 17 mg/dL, direct: 1.2 mg/dL, indirect: 15.8 mg/dL) was to be admitted at the neonatal intensive care unit (NICU) for receiving the phototherapy and after discharge from the NICU, they should refer their baby to a urologist.

At the NICU of Qena University Hospital, South Valley University, we examine the genitalia of the baby which was ambiguous with no palpable testes. An abdominal and pelvic ultrasound was done which revealed a penis-like structure is seen at the perineal region with no definite connection with the urethra, indicating enlarged clitoris . Also no testes could be detected; however, there was a normal-sized uterus and ovaries. The result of the pelvic ultrasound directs us to do peripheral karyotype to exclude chromosomal abnormalities that lead to intersex, which reveals 46 XX (normal female). The mother had never been treated with any drugs during pregnancy or encountered hormonal exposure in utero and she had no signs of androgen excess such as hirsutism, alopecia or clitoral hypertrophy (maternal virilization). There was no family history of infertility, ambiguous genitalia or unexplained neonatal death. The baby's name was changed after discharge from the NICU to Lila 'female name'.

Summary of the results of the biochemical laboratory parameters that have been measured in this case showed in the Table that confirms the diagnosis of CAH—classic 21-hydroxylase deficiency (salt-wasting type) at the age of 2 months. Venous blood (5 ml) was drawn and serum was separated by centrifugation at 3500 rpm for 15 minutes. We assay pH and serum electrolytes 'sodium and potassium' using Easylyte Medica, USA. We measure the serum endocrinal panel required for diagnosis as shown in the Table, using Abbott Architect, USA. The infant is now on the cortisol and aldosterone replacement therapy with a regular follow-up of serum sodium and potassium.

DISCUSSION

Steroid 21-hydroxylase (CYP21, also termed CYP21A2 and P450c21) is a cytochromeP450 enzyme located in the endoplasmic reticulum. It catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol, a precursor of cortisol, and the conversion of progesterone to deoxycorticosterone, a precursor of aldosterone. Owing to this loss of enzyme function, patients with 21-hydroxylase deficiency cannot synthesize cortisol efficiently,

and as a result, the adrenal cortex is stimulated by corticotropin and over-produces cortisol precursors. Some of these precursors are diverted to the biosynthesis of sex hormones, which may cause signs of androgen excess, including ambiguous genitalia in newborn girls and the rapid post-natal growth in both the genders. The concomitant aldosterone deficiency may lead to salt wasting with consequent failure to thrive, hypovolemia and shock (1).

A spectrum of phenotypes is observed. A severe form with a concurrent defect in aldosterone biosynthesis (salt-wasting type) and a form with apparently normal aldosterone biosynthesis (simplevirilizing type) are together termed classic 21-hydroxylase deficiency. There is also a mild, non-classic form that may be asymptomatic or associated with signs of post-natal androgen excess (2).

The present case was diagnosed as a classic 21-hydroxylase deficiency (salt-wasting type), according to the clinical and biochemical findings. Clinically, she had ambiguous genitalia in the form of a large clitoris, rugated and partially fused labia majora and a common urogenital sinus in place of a separate urethra and vagina as shown in Fig. 2. Biochemically, the karyotype is that of a normal female in addition to other biochemical abnormalities summarized in the Table in the form of a high serum level of 17-hydroxyprogesterone, progesterone, oestradiol 'E2', ACTH, rennin, androstenedione, total testosterone, acidosis, low cortisol and aldosterone levels with hyperkalaemia and hyponatremia.

Table: Abnormal biochemical findings in the studied case

Biochemical parameters	Result	Reference range	Interpretation
Cortisol	2.6 µg/dL	3–16.6	Decreased
Aldosterone	4.2 mg/dL	5.8–110	Decreased
ACTH	42 pg/mL	up to 30	Increased
Progesterone	19.3 ng/mL	0.87–3.37	Increased
17-Hydroxy progesterone	5 ng/mL	0.5–2.4	Increased
Oestradiol'E2'	17 pg/mL	< 10	Increased
Sodium	121.8 mEq/L	135–155	Hyponatremia
Potassium	6.5 mEq/L	3.6–5.5	Hyperkalaemia
pH	7.1	7.35–7.45	Acidosis
Testosterone (total)	0.71 ng/mL	0.1–0.56	Increased
Androstenedione	0.9 ng/mL	Up to 0.5	Increased
Renin	6.7 ng/mL/h	2.4–3.7	Increased

Approximately 75% of patients with the classic 21-hydroxylase deficiency have severely impaired 21-hydroxylation of progesterone and thus cannot

Fig. 2: A female newborn with congenital adrenal hyperplasia with ambiguous genitalia: a large clitoris, rugated and partially fused labia majora and a common urogenital sinus in place of a separate urethra and vagina.

adequately synthesize aldosterone. Elevated levels of 21-hydroxylase precursors (progesterone and 17-hydroxyprogesterone) may act as mineralocorticoid antagonists, exacerbating the effects of aldosterone deficiency (7). Since aldosterone regulates sodium homeostasis, renal sodium excretion in untreated patients is excessive and can result in hypovolemia and hyperreninemia. Such patients cannot excrete potassium efficiently and are prone to hyperkalaemia, especially in infancy. The cortisol deficiency in these patients contributes to poor cardiac function, poor vascular response to catecholamines, a decreased glomerular filtration rate and an increased secretion of anti-diuretic hormone (8). Thus, cortisol and aldosterone deficiencies together cause hyponatremic dehydration and shock in inadequately treated patients (9).

Patients with the salt-wasting form are identified through the measurement of serum electrolytes, aldosterone and plasma renin and the finding of expected abnormalities: hyperkalaemia, low levels of aldosterone and hyper-reninemia. Age-specific reference values for renin should be used, since the plasma renin activity is normally higher in neonates than in the older children (10). In the classic forms, epinephrine deficiency occurs because adrenocortical secretion of cortisol is necessary for adrenomedullary organogenesis and the adrenomedullary epinephrine synthesis (11). Both cortisol and epinephrine are counter-regulatory hormones and play an essential role in the regulation of blood glucose, which explains the hypoglycaemia occurring in patients with CAH, especially during stress conditions (12).

The treatment in congenital adrenal hyperplasia should aim to ensure the normal growth in infancy and childhood, the development of puberty at the appropriate age and later, the acquisition of adult reproductive potential (13). The standard treatment for classic CAH due to 21-hydroxylase deficiency is glucocorticoid

(replaces cortisol) and fludrocortisone (replaces aldosterone), given daily. Glucose supplementation is also warranted to prevent hypoglycaemia (12). It is essential for the infant to be seen early by a surgeon experienced in the techniques required for reconstruction of the genitalia. There are basically two structural abnormalities that require surgical treatment: a reduction in the size of the enlarged clitoris and a division of the fused labial folds to exterior rise the vaginal opening (13).

In conclusion, any newborn female infant with ambiguous genitalia should be considered as a medical emergency and should be investigated as soon as possible as this problem causes psychic trauma to the parents and to the infant itself as she is growing up because of the incorrect gender assignment. It is important to keep in mind that CAH is one of the important causes of ambiguous genitalia of the newborn and the commonest cause of congenital adrenal hyperplasia is 21-hydroxylase deficiency. Assay of serum 17OH-progesterone concentration and determination of a peripheral karyotype are essential for diagnosis.

AUTHORS' NOTE

The authors declare that they have no conflicts of interest.

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CASE REPORT

Consecutive, Bilateral Obturator Hernia in a Single Case

HO Aydın, EHA Soy, T Avcı, T Tezcaner, S Yıldırım

ABSTRACT

Obturator hernia (OH) is a rare pelvic hernia. It is difficult to make an early diagnosis due to the absence of a palpable mass, so it has a high morbidity and mortality rate, and there is delay in surgery. Here, in this report, we present a case of bilateral OH diagnosed at different times. Our report is meaningful as it reveals consecutive OH in a single case. An 87-year old, female patient admitted to emergency with intestinal obstruction findings. Right obturator herniation was seen in a computed tomography (CT) scan, then she underwent urgent surgery. After 2 months, she admitted with left thigh pain. With these findings, CT scans confirmed left OH. In an elderly, skinny, female patient with non-specific bowel obstruction symptoms and medial thigh pain, OH should be considered. Early and rapid radiologic evaluation, followed by surgery, is essential for successful management of OH.

Keywords: Acute abdomen, intestinal obstruction, obturator hernia.

INTRODUCTION

Obturator hernia (OH) is a rare pelvic hernia. It is a significant cause of intestinal obstruction and has a high morbidity and mortality due to the difficulty in making an early diagnosis and delay in surgery. In the literature OH is reported mostly as case reports. Here, in this report, we present a case of bilateral OH diagnosed at different times. From this point of view, our report is meaningful as it reveals consecutive OH in a single case.

CASE REPORT

An 87-year old, female patient admitted to emergency with vomitting and abdominal pain for 2 days. Her vital signs were normal. She had mild abdominal distention. In her medical history, she had no co-morbid disease but it was noted that she had right thigh pain for six months. She was skinny with a body mass index (BMI) of 17 kg/m². Physical examination revealed intestinal obstruction findings and it was confirmed with plain abdominal radiography, showing dilated intestinal segments. Her laboratory findings were: creatinine—0.97 mg/dL (normal range 0.5–1.3mg/dL),leucocyte—21.5×10³/mm³(normalrange 4.5–11×10³ /mm³) and C-reactive protein—50 mg/dL (normal range 0–10 mg/dL). For differential diagnosis of intestinal obstruction, we performed computed tomography (CT) scan and we diagnosed right obturator herniation. Computed tomography revealed dilatation of proximal ileal segments and right OH (Figure).

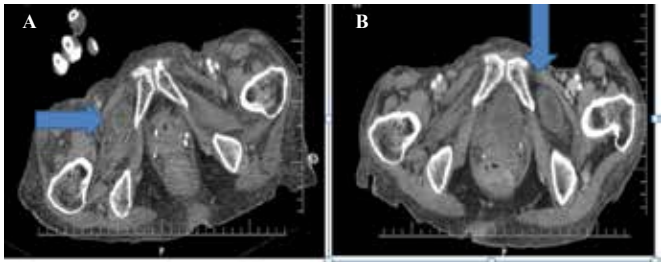

Figure: (A) Abdominal computed tomography (CT) scan, right obturator hernia (OB); (B) Abdominal CT scan, left OB.

We performed immediate laparatomy with low midline incision. After reduction of the herniated segments, the peritoneal layer over the obturator foramen was dissected and retroperitoneal obturator hernia repair was done with prolene mesh. The patient was discharged at the third day of the operation.

After 2 months, she admitted to clinic with left groin and left thigh pain with extension and abduction. Her laboratory findings were: creatinine—0.5 mg/dL

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(normal range 0.5–1.3 mg/dL), leucocyte—5×10³ /mm³ (normal range 4.5–11×10³ /mm³) and C-reactive protein—5 mg/dL (normal range 0–10 mg/dL). With these findings and her medical history, we did CT scans and this time we detected left OH. Laparatomy was performed with low midline incision. After reduction of the herniated segments, retroperitoneal obturator hernia repair was done with prolene mesh. The patient was discharged at the third day of the operation without any complication. We followed-up with her for 15 months without recurrence of the condition.

DISCUSSION

Obturator hernia is a rare pelvic hernia with a reported incidence of 0.05%–1.5% of all hernias (1). Obturator hernia is mostly seen in elderly, skinny, multiparous women (2). Obturator hernia is seen six times more in women due to a wider and more obliquely inclined pelvis. In recent studies, patients with a defective collagen metabolism were reported to be more at risk. Other predisposing factors include conditions which cause increase in intraabdominal pressure, such as chronic constipation, chronic obstructive pulmonary disease, ascites and kyphoscoliosis (3). Although herniation is more common on the right side, bilateral obturator hernia is reported to be seen in 6% of cases (4).

Gray and Skandalakis defined OH in1974 in three stages: the first stage of herniation of preperitoneal fat through the obturator canal, the second stage with the peritoneal dimple neighbouring the obturator foramen and the third stage is the beginning of symptoms with the herniation of intraabdominal organs into the obturator canal (5). The diagnosis is made at this point with intestinal obstruction due to absence of palpable mass. Obturator hernia has non-specific clinical findings like intermittent abdominal pain, and recurrent intestinal obstruction symptoms that resolve without intervention, so diagnosis is mostly delayed (6). The preoperative diagnosis of OH is reported to be 10%–30%. Missed or delayed OH with incarceration has a mortality that rises up to 70% (7). In OH patients, extension and abduction and internal rotation cause pain along the medial part of the leg due to compression of the anterior division of the obturator nerve, and it is called Howship–Romberg sign which is the most evident symptom (8). Howship–Romberg sign is reported to be positive in 65% of OH cases (9). However, this sign is mostly disregarded and mistaken with neuromuscular pain, especially in an elderly patient. As the diagnosis is delayed, the risk of strangulation of an incarcerated intestinal segment increases (10). Early CT imaging provides early diagnosis with reduced morbidity and mortality associated with OH. Computed tomography is found to be more specific and sensitive than plain abdominal radiographs and ultrasonography.

Our patient was an elderly female and the first time she admitted to emergency was with nausea and vomitting due to intestinal obstruction. After CT scanning, obturator hernia of ileal segments was diagnosed. Then, she admitted with left groin pain, reflecting to her medial aspect of left leg and with positive Howship–Romberg sign. Left OH diagnosis was confirmed with urgent CT scans and both OH were repaired without resection of intestinal segments.

Early and rapid radiologic evaluation, followed by surgery, is essential for successful management. Abdominal, inguinal, open or laparascopic surgical approaches are described for OH repair. Mid-line incision is mostly preferred in reports as in the case of intestinal resection and better exposure. Recently obturator hernia repair has been performed by primary repair and placement of synthetic mesh.

In conclusion, OH is rare but may be mortal, due to delayed diagnosis. In an elderly, skinny, female patient with non-specific bowel obstruction symptoms and medial thigh pain, OH should be considered. All hernia orifices should be examined carefully and bilaterally. Immediate CT scanning should be done in a suspicious case for early diagnosis to avoid complications.

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CASE REPORT

An Unusual Presentation of Osteoid Osteoma after Prolonged Foot Pain

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ABSTRACT

Osteoid osteoma is a benign bone tumour that causes significant nocturnal pain, cortical thickness and is relieved by salicylates. When it occurs, typical radiologic and clinical features are diagnostic. Radiography and computed tomography show a cortically based sclerotic lesion with a radiolucent nidus within it. The lesion usually involves long bones and most frequently presents in the diaphysis of the femur and tibia. Rare and unusually located lesions lead to misdiagnosis, even if the characteristic symptoms are present. We report an unusual case of osteoid osteoma in the tarsal navicular bone in which the diagnosis was delayed. This case, in which diagnosis was initially missed and treatment was delayed, resulted in a significant functional loss for the patient. A review of the literature revealed only two reported osteoid osteoma cases in the tarsal navicular bone.

Keywords: Benign foot tumours, navicular bone, osteoid osteoma.

INTRODUCTION

Osteoid osteoma is a rare benign bone tumour. It was first described by Jaffe in 1935 (1) and constituted 1.8% of benign bone tumours. The size (less than 1.5 cm in diameter), characteristic radiographic features and clinical presentation of an osteoid osteoma are diagnostic (2). The lesions usually occur in the extremities, with more than 50% of cases in the femur and tibia (3). Tarsal bone tumours are rare and represent only 1%–2% of all bone tumours (4). The most common lesions of osteoid osteoma occur in the talus and metatarsal bones of the foot (4–7).

Osteoid osteoma is characterized by pain which is continuous, not dependent on exercise, usually worse at night and relieved by non-steroidal anti-inflammatory drugs (NSAIDs) (3). Production of prostaglandins by the tumour causes a chronic inflammatory response (8). This process is responsible for periosteal reaction, synovitis, bone sclerosis and pain (8, 9). After removal of the nidus, chronic inflammation and symptoms regress dramatically. In general, radiographs show a cortically based sclerotic lesion with a radiolucent nidus within it. The nidus is demonstrated in 85% of cases. Computed tomography images improve the detection of the nidus. These images are very useful in the evaluation and differentiation of osteoid osteoma from chronic osteomyelitis (10, 11). Although magnetic resonance imaging (MRI) detects marrow oedema and soft tissue oedema, the small nidus may be overlooked, and it is less sensitive than computed tomography (CT) (6). The aim of treatment is the removal of the nidus by the traditional procedure of en bloc resection, curettage or a variety of other methods (3, 12, 13).

In this study, we report a rare case of osteoid osteoma of the tarsal navicular bone. This unexpected location led to late diagnosis, thus delaying treatment. Despite comprehensive literature, only two documented cases of navicular osteoid osteoma were found (7). Even most large retrospective studies do not consider it (6, 7, 14, 15). Thus, this report is presented as the third case in the literature.

CASE REPORT

A 15-year-old girl presented with a 2-year history of pain in her left foot. During the previous three months, her pain had increased dramatically. The pain occurred

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especially at night and was relieved by analgesic drugs. She usually took acetylsalicylic acid or NSAIDs. There was no history of trauma and no specific medical history. In the month before examination, her pain had become severe during standing and walking. On examination, there was local tenderness at the anterior and medial side of her foot. The mobility of her ankle was normal and painless. Complete blood cell count, sedimentation rate, C-reactive protein, serum electrolytes, alkaline phosphatase and other laboratory studies were normal. Radiologically, plain films demonstrated a well-bordered sclerotic lesion at the navicular bone. Computed tomography scan was then ordered to describe the lesion. The CT scan results confirmed the presence of a 6-mm lucent area and nidus at the navicular bone. (Figs. 1 and 2).

The patient underwent an excisional operation with a 3-cm incision over the navicular bone. After the bone was approached, corticotomy was performed with small osteotomes. The cherry red mass was observed and removed using a curette. The remaining defect was filled with monocortical iliac bone graft. No fixing material was applied after the placement of the bone graft. The specimen was sent to the Department of Pathology. Following the procedure, no cast was placed on the left lower extremity. The patient began partial weight-bearing activity on post-operative day three using crutches. Six weeks following the operation, full weight bearing was initiated. The pathology report confirmed the diagnosis of osteoid osteoma. At the follow-up visit, the patient's pain and other symptoms had regressed dramatically. (Figs. 3 and 4)

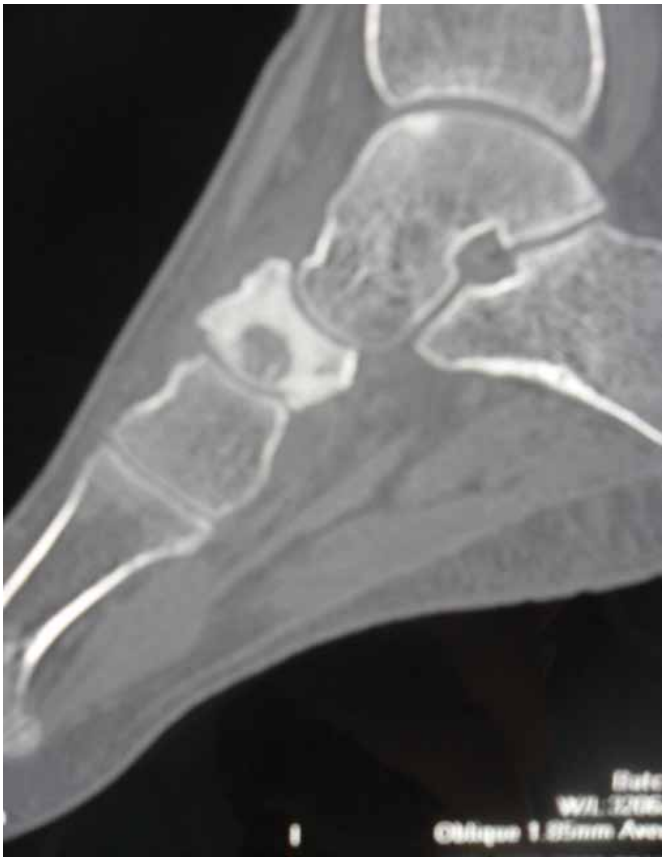

Fig. 1: Pre-operative computed tomography image showing sclerosis and nidus at the navicular bone.

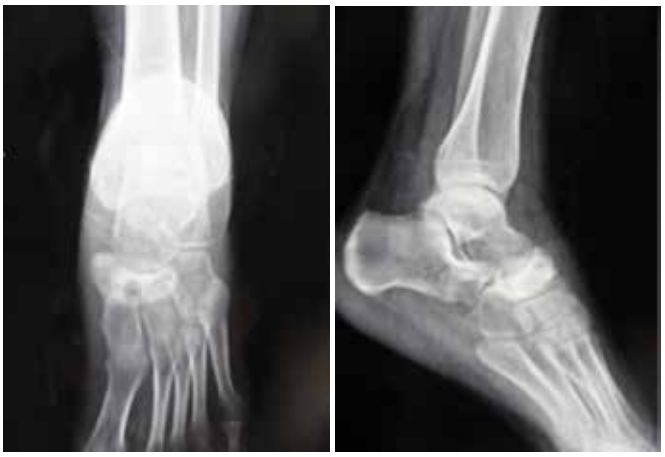

Fig. 2: Plain radiography, anteroposterior and lateral views: circumscribed sclerotic nidus and increased cortical thickness.

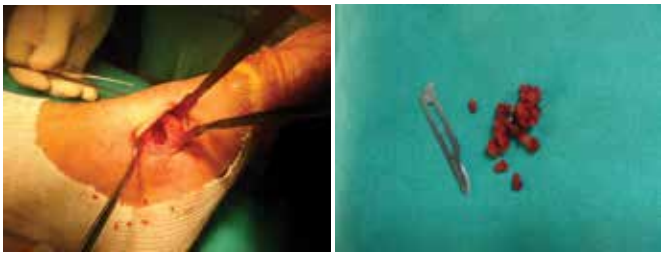

Fig. 3: Perioperative images: the curetted material and remaining defect after excision.

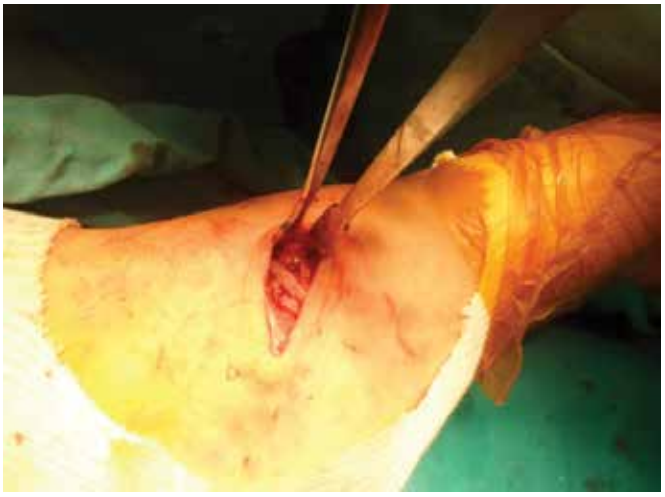

Fig. 4: The remaining gap filled with iliac bone graft.

In the final follow-up examination at 24 months, the patient had normal gait, weight bearing and no pain. On plain radiographs, healed bone was seen clearly with bone graft. The patient was informed that the case could be published. (Figs. 5 and 6)

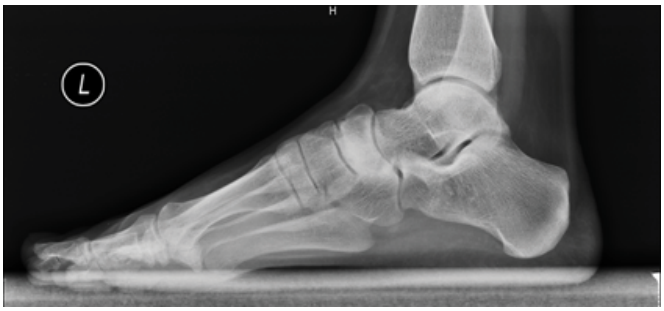

Fig. 5: Post-operative 24-month lateral view.

DISCUSSION

Benign bone tumours of the foot are rare. When they do occur, diagnosis and treatment may be difficult. Osteoid osteoma is a considerably common bone tumour that accounts for approximately 12% of benign bone lesions (16). Osteoid osteoma typically affects young patients in the second or third decades of life.

The diagnosis of osteoid osteoma of the foot is usually difficult because it typically occurs in long bones and rarely in the foot (17). In this case study, the patient's pain was attributed to other pathologies, including plantar fasciitis, stress fracture. When sclerosis became obvious with plain radiography, osteoid osteoma was considered. A definitive radiologic diagnosis was then made when the nidus of the osteoid osteoma was detected on CT scan (18, 19). Magnetic resonance imaging was not necessary because it is equal to CT in demonstrating the nidus (7). Therefore, osteoid osteoma should be considered in young patients with prolonged undiagnosed foot pain.

The treatment of osteoid osteoma is the removal of the nidus. When the nidus is removed, the pain is relieved. There are three main ways to remove the nidus: en bloc resection, excision with curettes and percutaneous CT-guided radiofrequency or laser ablation (14).

Recently, percutaneous radiofrequency ablation has become the preferred treatment option. In a study by Rosenthal (11), 112 of the 126 procedures (89%) completely succeeded clinically. The patients were pain free, did not take medication and did not require additional procedures (14). The percutaneous method is not, however, indicated in most osteoid osteomas of the small bones and the spine. Percutaneous curettage should be used for lesions more than 1 cm in diameter (7, 15). In this case, the navicular nidus was removed by curettage because of the extensive involvement of the navicular bone. After removal of the nidus, bone grafting was used to encourage early bone consolidation and mobilization. Percutaneous CT-guided ablation was not preferred in this case due to the location and diameter of the lesion and the need for bone grafting (5, 11). Excision of the nidus was performed successfully, and the graft that was used accelerated the bone healing.

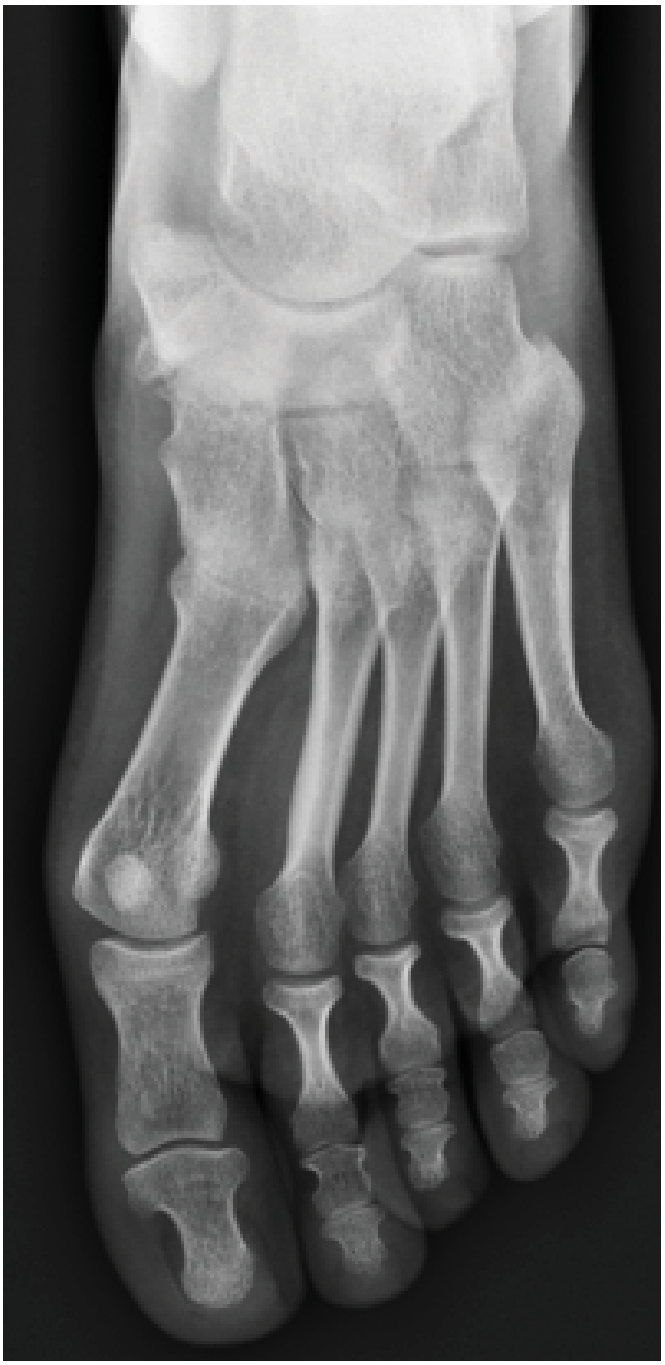

Fig. 6: Post-operative 24-month anteroposterior view.

Bone grafting is involved in most procedures in reconstructive orthopaedic surgery. Autologous bone grafts have excellent biologic and mechanical properties, but donor site morbidity and the limited volume available must be taken into consideration (20).

Osteoid osteoma of the tarsal navicular bone is difficult to diagnose because of its unusual presentation. As a result, it is often misdiagnosed. Navicular osteoid osteoma is responsible for prolonged foot pain and functional loss. Favourable radiologic tests, including plain radiography and CT scan, are necessary to make the diagnosis. The radiologic images and characteristic clinical symptoms have to be evaluated by an experienced radiologist. In this way, functional loss due to misdiagnosis can be prevented.

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CASE REPORT

Atypical Femoral Fracture: Failure to Prevent the Forthcoming

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ABSTRACT

Although biphosphonates showed robust efficacy in fracture prevention, recent data revealed a number of adverse events. Atypical femoral fracture is one of them. Here, a 73-year-old female patient who continued alendronate therapy despite unilateral atypical femoral fracture and developed the second one on the other side 1 year later is presented. The purposes of this manuscript are: emphasizing atypical femoral fracture as an adverse event with increasing incidence, reviewing the knowledge about duration of biphosphonate therapy and drug holiday and highlighting that biphosphonates should be stopped in the presence of an atypical femoral fracture and this should be explained to the patient in a clear way especially if he/she is elderly.

Keywords: Aged, alendronate, communication disorders, femoral fracture, osteoporosis

INTRODUCTION

Biphosphonates, even though the mainstay of osteoporosis treatment, have been found to be associated with an increasing number and frequency of adverse events as the exposure time increases. Atypical femoral fractures (AFFs) are one of these problems (1). Here, an elderly patient who continued alendronate therapy after the first fracture and developed bilateral AFF within a year is presented.

CASE REPORT

A 73-year-old female patient appealed to our outpatient clinic with left thigh pain that has started 1 month ago. The pain was on the antero-lateral part of the thigh and exacerbated during the last week. There was no history of trauma, fall or alcohol intake. The patient was on biphosphpnates for about 15 years and has been receiving alendronate 70 mg once a week continuously for the last 8 years with a diagnosis of postmenopausal osteoporosis. She also had a history of atraumatic spontaneous diaphyseal femoral fracture on the right side 1 year ago. The fracture was treated with intramedullary nailing, and the patient was able to return to weight-bearing activity. She continued taking alendronate therapy after the fracture. Apart from these, she had diabetes mellitus and hypertension, and was taking medication for them.

Clinical examination revealed an antalgic gait. The range of motion of the left hip and knee and muscle strength examinations could not be performed properly due to increased pain. Left femoral shaft was painful on palpation. A prompt plain radiography disclosed beaking and cortical thickening of the lateral femoral diaphysis (Figs. 1 and 2). The patient was diagnosed with AFF of the left femur. Alendronate therapy was discontinued, and the patient was consulted with the orthopaedic department immediately.

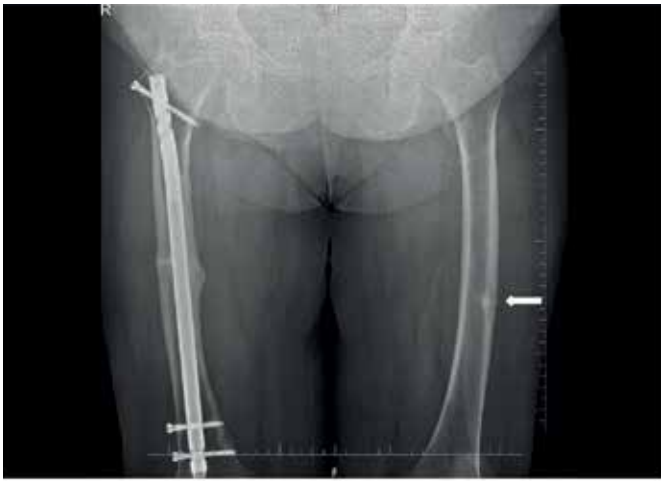

Figure 1: Antero-posterior X-ray showing fractures on both sides and intramedullary nailing of the right femur.

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Figure 2: (a) Antero-posterior X-ray of the left femur, showing lateral cortical thickening and beaking. (b) Lateral X-ray of the left femur showing the fracture line.

DISCUSSION

Biphosphonates supress normal bone turnover by delaying remodelling. As a result, reduced fracture healing and accumulation of microfractures occur. Small decrease in turnover may induce significant accumulation of microdamage. This process results in the reduction of energy absorption capacity and toughness (2, 3). Atypical femoral fractures are defined as atraumatic or low-trauma fractures located in the subtrochanteric femoral region. They originate at the lateral cortical margin and have a transverse or oblique orientation. There is localized periosteal or endosteal thickening of the lateral cortex at the fracture site, which is denominated as beaking or flaring. While incomplete ones involve only the lateral cortex, complete fractures extend through both cortices. AFFs are non-comminuted or minimally comminuted. A dull or aching pain in the groin or thigh may accompany the above mentioned features. The fracture must be located just distal to the lesser trochanter and proximal to the supracondyler flare. Fractures of the femoral neck, intertrochanteric fractures with spiral subtrochanteric extension, periprosthetic fractures, pathological fractures of primary or metastatic bone tumours and miscellaneous bone diseases are not AFFs (4).

Biphosphonate-associated AFFs have an incidence of 1/1000 patients per year. The age adjusted relative risk of AFFs is 55 for women and women have a threefold greater risk compared with men. Alendronate users have a twofold higher age-adjusted risk than risedronate users. The higher risk of developing AFFs is evident only after 1 year of biphosphonate use and increases thereafter. Despite these dramatic numbers, the risk decreases as 70% per year since last use, rapidly after cessation (5–7). Although the risk-benefit ratio is quite favourable at the beginning of biphosphonate treatment in patients with good indication, it seems like prolongation of treatment beyond 5 years does not further reduce the risk, and the risk-benefit ratio is inverted. To date, an optimal duration for biphosphonate treatment has not been determined and decisions to continue or stop treatment should be made on individual basis (1, 6).

Sixty-four per cent of cases with AFFs demonstrate involvement of the contralateral femur so clinicians have the chance to preclude the contralateral femur when first AFF occurs. In this situation, plain radiographies, computed tomography or magnetic resonance imaging of the other side should be performed. On clinical follow-up, biphosphonates should be stopped and better be replaced by a parathormone analogue. In addition, patients should be clearly informed about their clinical condition and the increased risk of contralateral femur fracture so that they will pay close attention to minor abnormalities (2, 3). The cessation of alendronate therapy should also be explained in an explicit way because patients may have difficulty understanding why they stop receiving an anti-osteoporotic drug in the case of a fracture.

In our case, even though we do not have any information whether alendronate therapy had been advised to be stopped or not after the first fracture 1 year ago, it is precluded that she misunderstood and continued taking alendronate. At this point, communication problems in the elderly group come into prominence. For elderly patients and their healthcare providers, communication is the most vital topic. Hearing loss, visual disturbances, memory problems, depression, cognitive impairment, dementia, decrease in speaking volume and fluency, dysarthria affect the communication of elderly. Also, elderly people may have a lack of insight into their illness and difficulties with treatment compliance and following their treatment programme. As a result, clinicians should

make extra effort to make the conversations more understandable. To improve communication, a quiet room, eye contact, simple grammar with pauses underlining the phrases, a clear language and repetitions of important points are important. Written information or pictured documents of what you are telling orally may also help. Baby talk, addressing the patient with endearing or cute names such as 'honey', 'sweetie', 'dear', speaking too loud and speaking very slowly or quickly should be avoided (8).

In conclusion, presenting this case, the authors wanted to emphasize three points. First, patients who have undergone biphosphonate treatment should be under close monitoring. Second, drug holiday should be in mind after some period of regular biphosphonate use. And last but not least, biphosphonates should be stopped in the presence of an AFF, and this should be explained to the patient in a clear way, especially if he/she is elderly.

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CASE REPORT

Historical Perspectives on Medical Professionalism in the Caribbean

D Peters[1], FF Youssef[2]

ABSTRACT

This paper provides a historical overview of the development of perspectives on medical professionalism and the medical profession in the Caribbean. Two historical periods, the colonial and the post-colonial, are explored for continuities and changes in perspectives. Recently, concern over the dilution of medicine's humanistic qualities has caused an increased interest in medical professionalism and ignited a wider movement to reform the profession. Medical education curricula are under renovation as issues of professionalism are now being openly raised among medical practitioners and students to ensure that the medical profession's service to society is un-compromised. In this context, some scholars have felt that an awareness of the history of the medical profession could play a significant role in humanizing medicine and fostering greater professionalism. Much of the emerging discussion on medical professionalism has occurred mainly in the developed countries, namely, Britain and the United States. The wider developing world, including the Caribbean, has fallen behind in this process. There is a lack of historical or contemporary works addressing issues of medical professionalism in the Caribbean. This paper will begin to fill this gap.

Keywords: Caribbean, medical history, professionalism

INTRODUCTION

Medical professionalism is a new but flourishing subject of study. Concern over the dilution of medicine's humanistic qualities has caused an increased interest in the subject and ignited a wider movement to reform the profession (1). Medical education curricula are under renovation as issues of professionalism are now being openly raised among medical practitioners and students to ensure that the medical profession's service to society is uncompromised. In this context, some scholars have felt that awareness of the history of the medical profession could play a significant role in humanizing medicine and fostering greater professionalism (2–3). Medical students need to be reminded of the early foundations of the profession, the Hippocratic tradition and the long journey towards its present state, specific to local contexts. Many historical works have been written, some of which focus on the historical evolution of medical professionalism and these give an overview of the earliest principles and values of the profession that have become its core tenets (4, 5).

The wider developing world, including the Caribbean, has fallen behind in this process. Much of the emerging discussion on medical professionalism has occurred mainly in developed countries, namely, Britain and the United States. In the Caribbean, there are no existing historical or contemporary works, addressing specific issues of medical professionalism. Putting medical professionalism aside, works on the medical profession in the Caribbean are also lacking. To date, for example, there is no comprehensive work on the history of the medical profession, much less medicine, in the Caribbean, especially in the modern era and only few works exploring the history of the profession in individual Caribbean countries. This paper will attempt to give a brief historical overview of the development of perspectives on medical professionalism and the medical profession specific to the Caribbean. Two periods of

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history, the colonial and the post-colonial, are explored for continuities and changes in perspectives.

Medical professionalism, of course, has proved difficult to define, resulting in numerous attempts of definition by scholars (6). Nevertheless, there remains a lack of consensus on what is professionalism and, therefore, no universal definition exists. McDonaugh (7) provides one of the many attempts at a definition:

> Medical professionalism is our behavior as (practitioners). It is how we conduct ourselves as (practitioners) in our interactions with our patients and society. Medical professionalism is a behavior that is predicated on our personal beliefs and our ideas … Medical professionalism is greatly influenced by our contemporaneous social values and norms. Therefore, it remains a flexible concept from age to age, despite maintaining a core set of values.

McDonaugh's definition is useful for this paper in its acknowledgement of the dynamism of professionalism that it is drawn from different sources whether cultural, technical or ethical to name a few but also how it changes over time. Certain expectations, however, were always held of medical practitioners. Here, Cruess and Cruess' (8) definition of medical professionalism as a contract held between medical practitioners and society also proves useful. Medical practitioners have always been subjected to expectations from the society. They were expected to be altruistic, to have the best interests of the individual and the society at large, to do good and to never do harm. As far back as the 4th century BCE, such expectations about medical practitioners were conveyed in what is now popularly known as the Hippocratic oath. This paper will use the term 'medical professionalism' loosely to mean contemporary perceptions of how the service of medical practitioners met the expectations of Caribbean society.

The colonial Caribbean

The pre-emancipation era

Colonialism has been instrumental in shaping the present-day medical profession. Not discrediting the significant history of the pre-colonial Caribbean, the arrival of Europeans by the end of the 15th century led to widespread transformation of Caribbean society. In most territories, the indigenous population rapidly dwindled, thereby decreasing their influence on the colonial Caribbean. Colonization brought a large number of people together and created a number of new health concerns. The disease environment and overall conditions increased the demand for medical practitioners from Europe. Despite a high demand, in the early days, few practitioners migrated to the Caribbean and the few that did catered mostly to the needs of the white elite. By the late 18th century, however, there were concerted efforts to improve the health of the enslaved population influenced by an increase in slave prices and agitation for the abolition of the slave trade (9). The remedy to the situation was to improve the health of existing enslaved persons and, consequently, there was an influx of medical practitioners.

The enslaved community understandably distrusted many of these incoming practitioners. Although this period also saw improvements in their treatment, there was still a fair amount of disingenuous practitioners. There was a general impression that doctors were using the enslaved as guinea pigs to test out their various theories or that they were ignorant to the medical issues in the Caribbean. Fortunately, there were alternatives. Enslaved or 'negro doctors' provided medical care. Higman (10) notes that there was a belief that some 'negro doctors' had comparable medical knowledge to white doctors and also were more successful in gaining cures. For example, many Afro-Caribbean healers treated enslaved persons with the highly contagious 'yaws' with much success and European doctors often borrowed the remedies of these practitioners (11).

The Caribbean medical world was, however, not simply a black or white, European or African one. Many white doctors collaborated with Africans in providing services. In the slave hospitals, for example, the advice and orders of white doctors were implemented by various African or coloured practitioners. Some white doctors also sought to incorporate aspects of African medicine into their professional practice. Both European and African medicine were therefore sought after. Jerome Handler (12) notes that the enslaved persons made the use of services from European practitioners; however, they often relied on the support from traditional healers due to a lack of trust. The issue of trust becomes more significant given the unrestrained, though sometimes criticized, practice of doctors in the Caribbean.

Until the 19th century, medical practitioners in the Caribbean were largely unregulated. There was no practice requirement, and anyone could and did practice if they wished. A wide range of persons therefore served the public. Some came with medical degrees or certification. Others underwent various kinds of apprenticeships. Some had little to no knowledge or experience of medicine. The result of this mixed pot was the development of very negative views towards medical practitioners. Some

settlers simply refused to be treated believing that these medical practitioners were the reason for much death in the Caribbean. In many cases, the presence of diseases like Yellow Fever—which medical practitioners struggled to effectively treat, mostly due to their ignorance of it—made death inevitable at times. Therefore, it could be said that the limitations of the medical sciences rather than misconduct from medical practitioners may have helped to perpetuate negative perceptions. Nevertheless, some practitioners were blatantly irresponsible or dishonest. McNeil (13) notes that people 'had every reason to keep their distance from doctors'. Many of those arriving to practice medicine in the Caribbean were in pursuit of wealth. Some regularly treated illnesses which they knew nothing about (14). The scarcity of medical practitioners in addition to the unregulated Caribbean space ensured that despite shortcomings great profit could be gained.

In the Caribbean, many medical practitioners, like others who travelled to the region in the early modern period, were seeking wealth. Opportunities for the wonton pursuit of wealth were greater in the region, given the lack of regulatory bodies prior to the 19th century. One physician in Jamaica, William Boseley noted in 1789 that while many of the physicians in Jamaica might be men of science and good education, he felt that what plagued the medical profession was that the acquisition of wealth seemed to be the principal goal (9).

By the early 19th century, colonial officials took a greater note of the ills of the medical community and sought to improve it. In 1814, Trinidad Governor, Ralph Woodford issued a proclamation noting that

> 'it has become necessary to prevent the indiscriminate introduction and admission of persons to practice Medicine and Surgery in the said Island, without due proof of their having acquired or being possessed of a competent knowledge in such their profession and practice … (15)'.

He established a medical board to evaluate medical professionals, grant licences and prosecute unlicenced practitioners. Jamaica followed later in 1832 when a college of physicians and surgeons was established to improve the respectability of the profession. These efforts proved largely ineffective in regulating the wide range of persons who practiced. Most importantly, the efforts of colonial authorities focussed solely on competence. Issues of character, morality and ethics, even among those with medical degrees, were therefore left unaddressed. As far as the law was concerned, a good practitioner was a medically competent one.

Post-emancipation

In the post-emancipation period, there was a greater need for healthcare in the Caribbean, especially among the newly freed population. Pemberton (16) argues that emancipation resulted in a diminishing community of European-trained doctors, and therefore, a more expensive and scarce supply of medical services which planters manipulated to secure control over the newly freed labour supply. The establishment of the Barbados General Hospital in 1844 provides a good example of the post-emancipation demand for medical services. Within the first few years, the hospital admitted an average of nearly 300 patients, 72% black. By the end of the 19th century, this number increased to an average of over 3000 patients. Overcrowding became an acute problem, and some began to be turned away for want of vacant beds (17). All the doctors in the Barbados General Hospital were white and mostly recruited from England up to the early 20th century. White, European trained doctors were a premium in the post-emancipation period. For non-elite or less wealthy persons, encounters with these medical doctors were almost exclusively conducted in conditions of dependence. Either they were objects of charity with little autonomy or perhaps they were seen by a doctor paid for by their employers on the plantation.

European-trained doctors, therefore, came into an increasing demand but their services were still scrutinized. Brereton (18) notes that grievances related to medical care were important causes in the post-emancipation riots of the 1870s in the Windward Islands. Deductions were made from the salaries of labourers in Tobago for care from a private physician who practiced in the Windward district. The labourers did not object to the medical care but rather the large deductions. They may still have put up with the expense if the doctor had not been so unpopular, mainly due to a growing reputation for negligence.

In the midst of major concerns about the medical community, colonial states continued to make efforts to improve the medical profession in the post-emancipation era. By the late 19th century, Trinidad had produced four ordinances aimed at regulating the medical profession. The final ordinance in 1898 was described as the most 'stringent medical ordinance or Act enforced in the empire' by a member of the United Kingdom General Medical Council. It went to greater lengths to protect the public from the dangers of persons who were unqualified or unfit to practice. Most interestingly, the ordinance added a criterion for admission based on a person being of good character. Dr Richard Thorne, member of the

council, noted that this added criterion for licencing persons along with an examination was a 'precedent of very great importance (19)'. Colonial officials were no longer simply concerned about competence and also with conduct. It is particularly indicative of what they felt was negatively impacting the medical community.

Not surprisingly, the post-emancipation period saw a slow but steady growth of Caribbean born and also non-white medical practitioners. The majority Caribbean population preferred to be treated by their own. These new practitioners, though local, were trained abroad mainly in Europe and also in the United States (20). They returned with elevated privilege in the society and, for some, it was why they chose to pursue medicine. The ills of colonial society made such motivations rampant, mainly due to massive inequalities between the elite and working classes. Among the working class in particular, there was poverty and social limitations. The desire to rise above these conditions was, therefore, present among most. For many, this dream could be achieved through the education and entrance into a profession. Eric Williams, former Prime Minister of Trinidad and Tobago, describes this colonial society well in his autobiography, *Inward Hunger*. The point is perhaps most evidently made as he describes his showdown with his father over his decision to study history at the Oxford University. Williams writes that his father's 'wish was that (he) should study medicine or law, preferably the former'. He continues 'He wanted me to have 'independence', as he put it (21)'. There was no altruistic consideration in Williams' father's argument; he merely wanted him to better himself. Motivations of social mobility therefore only exacerbated existing problems within the medical community.

Selvon (22) in his historical novel, *A Brighter Sun*, based in the 1930s, gives us a glimpse of these new practitioners. He writes of some interactions with white and non-white doctors in Trinidad in the early 20th century. The protagonist's (Tiger) wife is gravely sick due to the complications with a pregnancy. He ventures out in the middle of the night to seek medical treatment for her. Tiger, of East Indian descent, first seeks out an Indian doctor. His first impulse after telling the doctor that his wife is very sick is to assure the doctor that he will pay him well. Despite Tiger's call for an urgent attention, the doctor brushes him off and says that he will come in the morning. Disappointed, Tiger returns to the taxi that brought him. He is confused at the Indian doctor's lack of concern and asks the taxi whether he was sure that it was an Indian doctor. The driver confirms and further notes 'Is so wen yuh poor and not in society, papa … boy, if I tell yuh about some of de doctors in dis place, yuh dead (22)'. He next goes to a 'creole' doctor but receives a similar response. Tiger eventually receives a positive response from a white doctor who recognizes the situation as severe and agrees to come see his wife immediately. Selvon's historical novel highlights the degree to which there was an expectation that more creole practitioners would lead to a better care for the masses. However, these new practitioners did not always satisfy this expectation. Ultimately, a more systematic change proved necessary. A 1938 West Indian Commission established to investigate the Caribbean riots of the 1930s deemed health as one of the most severe concerns. It highlighted the need for more locals to join the health force and to rise to leadership positions. It also recommended the creation of a local medical school (23).

The establishment of a medical school in the Caribbean was crucial for the medical profession. It was formulated in the age of decolonization along with a high spirit of Caribbean regionalism that filled the air and ultimately culminated in the establishment of the West Indian Federation. The process towards the new medical school began in 1943 and led to the erection of the Medical Department of the University College of the West Indies (UCWI) in Jamaica, which opened its doors in October 1948. Its students, drawn from various parts of the region, were symbolic of the emerging Caribbean nation.

The new school was confronted by negative attitudes among Caribbean people. Colonial subjects had become accustomed to being serviced by the foreign-educated practitioners and as a result, some questioned the school's quality in comparison to those overseas. Special emphasis was therefore placed on the knowledge and competence in the new school. The results of the first examinations were exceptional, a trend that continued in later years. What followed was a shift in attitudes of 'doubt and criticism' to 'pride and confidence' (24). It is not surprising that the early medical students of the UCWI did well. Decolonization in the Caribbean inspired a desire and confidence in governing one's own affairs and influenced a positive work ethic and competitive drive among the Caribbean people.

The mid-20th century was also a watershed time for the global medical profession. It was around this time that the World Medical Association (WMA) was established (1947). One of its first major concerns centred on the general state of medical ethics across the globe. Evidence of medical atrocities by the Nazis, revealed

after the Second World War, indicated the clear need for reform. The WMA then set about working on a charter of medicine, essentially a modern version of the Hippocratic Oath, that doctors around the world would adopt upon earning their medical degree. The result of these efforts was the 'Declaration of Geneva' in 1948, which later gave way to an international code of ethics the following year.

This new global context did not have an immediate formal impact on the UCWI. Although issues of medical ethics and professionalism were not directly discussed in any formal capacity, they would, however, form part of informal consideration. Walsh and Abelson (25) note that in the 1950s 'the concept of professionalism suffused the work of doctors, however, was rarely discussed or addressed'. As a result, an emphasis on medical professionalism, though not explicitly addressed, was evident at the university. One of the committee members of the Irvine Committee noted that the UCWI was established with the intent of encouraging 'the development of a West Indian outlook and … the attainment of the highest ethical and academic standards (26)'. There was, however, no formal effort to foster these ideals nor did the curriculum ever concern itself with such content. It was rather an unspoken understanding. Cotterel (27), a UCWI alumni from the third batch of medical students to graduate, confirms this point of view. Commenting on the issues of medical professionalism during his time at the UCWI, he noted:

> 'You know that (issues of medical ethics and professionalism) did not come up in the curriculum at all. I think it should be in the curriculum, but it did not arise in our time. I think most of us had this sort of grounding at home and the schools with which we went. There was a sort of background awareness to our sort of responsibility to the greater society. It was never taught to us (27)'.

The spirit of decolonization in the Caribbean and the significance of the new medical school ensured that the issues of medical professionalism featured as part of a hidden curriculum. William Pinar describes the hidden curriculum as the 'ideological and subliminal message(s) presented within the overt curriculum, as well as a by-product of what is not offered—the null curriculum (28)'. Dr Cotterel noted, for example, one professor whom he, and he believed many others, felt was a good teacher and was well respected. Dr Cotterel was particularly intrigued by his approach to medicine. He constantly reminded students of their responsibilities to patients, often encouraging them to put themselves in the shoes of their patients.

This approach invoked vigorous debate among his peers, and that while some agreed others did not. Dr Cotterel also shed insight on another faculty member who was not considered a good doctor and whose approach was considered quite the opposite to being patient-centred. He noted an instance when the doctor boldly ventured into the medical care that was known to be well outside of his competence with negative results. For Dr Cotterel, this experience helped him to see an example of the kind of doctor not to become. Essentially, his comments shed some insight into the hidden curriculum at the UCWI and help us to look at how attitudes to medical professionalism developed in the early formation of the Caribbean medical profession.

The establishment of the UCWI saw a concerted effort towards systematic change in the medical community. New medical professionals were now trained in a local environment as part of a new regional nation-building process. There was no distance from the people that these new practitioners would actually serve, unlike the earlier days of colonialism. This was an era where more opportunities were opening up and the people were increasingly responsible for making and managing the society. The accomplishment of the UCWI also challenged some of the medical profession's old concerns. The output of more doctors into the society helped to challenge the power and status of the few practitioners in the Caribbean at the time. Many of the early graduates of the UCWI went on serve at various levels in the medical profession and in the society. The fact also that no need was felt for explicit attention to issues of medical professionalism meant this was not a large concern among students or faculty. Looking ahead, however, it is clear that old concerns for medical professionalism persisted. It may be safe to say then that the UCWI and decolonization may have helped to mask or mitigate some of these concerns.

The post-colonial era

Increasing concerns about medical professionalism and a prevailing negative view of the medical profession continued to mark the post-colonial era. Cruess and Cruess note that in the 1960s and 70s, the medical authority and assumptions of altruism were challenged. They write that '(some) argued that medicine had abused its monopoly to further its own interests, had self-regulated poorly and that its organizations were more interested in serving their members than society (8)'.

These concerns were increasingly present in the Caribbean. Lewis (29) points out that reports on medical

services painted a negative picture. In the Bahamas, for example, a 1960 report noted that 'the temptation of the medical profession to deal more with the wealthy patient had produced a general situation in which some twenty-one inhabited islands had to do with the services of only seven qualified practitioners (29)'. A 1977 study in Trinidad and Tobago revealed that rural areas still used or preferred creole as opposed to doctor medicine. Those interviewed noted a general distrust of doctors as one of the reasons for this preference (30).

A shortage of medical practitioners has also remained a persistent problem in the post-independence era of the Caribbean. The emergence of the brain drain from so-called developing to developed countries has only exacerbated this perennial shortage. The Caribbean remains one of the most affected regions in the world (31). The problem has been most acute in Jamaica. A 2005 study revealed that 41.4% of Jamaica's medical school graduates migrated to the other developed countries, the largest percentage of all countries in the study (32). Many of those migrating do so in pursuit of better opportunities and conditions or because of disenchantment with the local healthcare system. Such reasons are indication of the persistence of old colonial motivations of upward social mobility and self-improvement. These motivations superseded the commitment to serving the nation and the region that first inspired medical students of the UCWI, an issue of medical professionalism in itself.

As a result of these ongoing concerns, Caribbean nations continued to make efforts to improve the medical profession. The post-colonial era brought a new period of transformation. Governments sought to re-shape institutions to acquire national rather than colonial relevance. Independence influenced many attitudes and developments in the Caribbean and, in particular, the management and improvement of the medical profession. Now, independent parliaments created new medical legislation establishing (or re-establishing) medical boards, regulations and institutions. A new Medical Board Act, for example, was passed in Trinidad in 1960 and Jamaica and Barbados followed in 1972. These acts outlined specific activities that amounted to the professional misconduct and displayed the increased effort to regulate and improve the image of the medical profession. Caribbean governments and medical boards have continued to make interventions in recent times. Legislation and codes of conduct or ethics for the medical profession have been produced and renovated. Most recently, for example, Trinidad re-issued its code of ethics in 1990; and Jamaica and Guyana produced a code of conduct in 2008.

In recent times, the global trend has been towards more deliberately raising issues of professionalism among current and upcoming medical professionals. As a result, courses in medical professionalism have been embedded into the medical curricula. Since the creation of the UWI medical school, its medical curriculum has undergone little formal change in updating the curriculum to focus on the issues of medical professionalism. Only, very recently, the Caribbean Accreditation Authority for Education in Medicine and other Health Professions (CAAM-HP) made a specific request for focussed instruction and exposure to the issues in professionalism and ethics. In 2013, the UWI St. Augustine Medical School began a compulsory series of undergraduate courses in professionalism, ethics and communication.

CONCLUSION

Ultimately, this historical overview has revealed the persistence of ethical and moral concerns about medical practitioners in the Caribbean from the colonial era to the present day. Recent efforts to give explicit attention to medical professionalism for medical students and professionals are a progressive step in a long tradition of efforts to curb the concerns. Efforts first focussed on weeding out incompetent or unqualified practitioners and then shifted to describing values of medical professionals should adopt. Most recent efforts seek to raise awareness of and, more importantly, teach these values to the medical profession. This more deliberate intervention goes in tandem with the current perception of an increasing crisis of medical professionalism in the Caribbean and across the globe. Medical practitioners in the Caribbean need to be aware of the evolving state of the profession and how their actions impact the fulfilment of medicine's contract with the society and ultimately regional development. This historical overview provides a brief glimpse at the medical profession's progression thus far and leaves the onus upon the present and upcoming medical practitioners to write the next step.

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LETTER TO THE EDITOR

Hypercalcaemia and Hypervitaminosis A in Chronic Renal Failure

The Editor,

Sir,

Chronic toxicity by vitamin A may occur with long-term ingestion at lower doses in patients with chronic renal failure, but hypercalcaemia is rarely observed in this setting and is probably due to effects of the vitamin A on bone osteoclastic or osteoblastic phenomena (1). Although the first case report was in 1953, this association has been very rarely described (1).

I read the interesting case study by Hammoud *et al* about a 67-year-old woman with arterial hypertension, chronic kidney disease stage IV and hypercalcaemia around 14 mg/dL. Her blood levels of parathyroid hormone (PTH) and of vitamin D were within the normal ranges, and hypotheses for hypercalcaemia including secondary hyperparathyroidism, hypercalcaemic hypocalciuric syndrome, and occult malignancy were ruled out (1). After establishing the diagnosis of hypervitaminosis A, the vitamin supplement was stopped, and vitamin A and serum calcium levels returned to normal few months later (1). The authors pedagogically described the sources and metabolic steps of vitamin A. Risks of toxicity by excessive ingestion were emphasized in people with renal failure because kidneys are the major excretory route of retinol and retinol-binding protein. Caution with vitamin A is highlighted in this scenario (1).

The report by Hammoud *et al* is useful to clear mechanisms of vitamin disorders related or not to chronic renal disease; but I would like to add some Brazilian data (2–4). Santos *et al* reported a 69-year-old woman with neurological disturbances and xanthoderma associated with long-standing excessive ingestion of green vegetables, papaw and tomato (2). Such dietary behaviour yields higher serum levels of vitamin A, carotenoids and metabolites, which produce a cutaneous yellow to orange discolouration, with the mucosa of normal colour. The patient had neither renal failure nor hypercalcaemia, as blood determinations showed (2). Cutaneous discolouration improved in 2 months by reduction of the excessive ingestion (2). Worthy of note, the variable intensity of xanthoderma observed in individuals with chronic kidney disease may be due to the slow conversion of carotenoids to vitamin A in the skin (2).

Costa *et al* (2013) retrospectively studied the relationship between PTH levels and carotid thickness in people with chronic renal failure and secondary hyperparathyroidism (3). The purpose was to evaluate the eventual role of PTH serum levels; hypocalcaemia and hyperphosphatemia; vitamin D and calcitriol deficiency; and traditional cardiovascular risk factors in the carotid changes observed in patients with dialytic chronic kidney failure (3). The authors found a significant correlation between the PTH levels and carotid thickness (3).

Costa *et al* (2014) reviewed epidemiological features of vitamin D including calcium and phosphorus homeostasis, arterial hypertension, PTH levels, and chronic renal failure (4). The 14 evaluated patients were distributed in two groups in accordance with their PTH serum levels: 200 pg/mL, or lower, and above 500 pg/mL. The authors emphasized the role of dialysis, and the normal levels of PTH, calcium and phosphate in the prevention of atherosclerosis in patients undergoing haemodialysis. Furthermore, they concluded that the serum levels of PTH were not predictive to infer the respective serum levels of vitamin D (4).

The commented articles may contribute to enhance the understanding of mechanisms of vitamins, minerals and PTH disturbances associated or not with chronic kidney disease.

Keywords: Calcium, chronic kidney disease, vitamin A, vitamin D.

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Glucose-6 Phosphate Dehydrogenase Deficiency in a Geriatric Patient

The Editor,

Sir,

An otherwise healthy 76-year-old female patient presented to the emergency department with acute fatigue, weakness and jaundice. She stated ingestion of roughly 1 kg of boiled fava beans 2 days prior. She had ingested both fresh and cooked fava beans before without any complaints. Her physical examination was unremarkable apart from visible jaundice. No lymphadenopathy was present, splenomegaly and hepatomegaly were not observed. Laboratory results revealed severe anaemia along with indirect hyperbilirubinemia, reticulocytosis, high aspartate aminotransferase and lactate dehydrogenase levels, haemoglobinuria and low haptoglobin. Peripheral blood smear revealed anisocytosis, poikilocytosis and Heinz bodies (Figure).

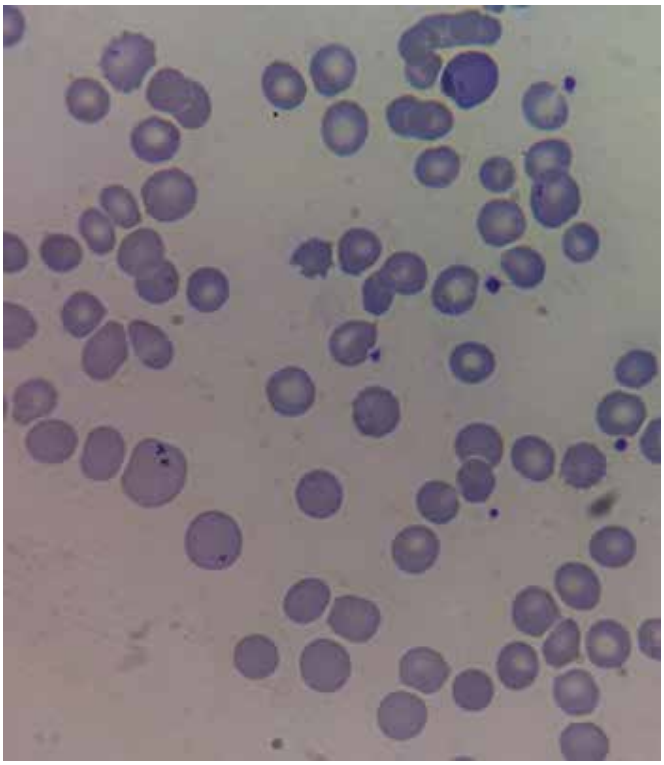

Figure: Peripheral blood smear showed anisocytosis, poikilocytosis and several Heinz bodies.

Direct and indirect Coombs tests were negative. She was diagnosed with non-immune haemolytic anaemia. Etiologic tests revealed a reduced erythrocyte glucose-6 phosphate dehydrogenase (G6PD) level of 1.07 IU/gHb (reference range: 4.1–10.1 IU/gHb). The patient was treated with a presumptive diagnosis of G6PD deficiency. Erythrocyte G6PD level was measured again as 2 IU/gHb after acute haemolytic episode, which confirmed the diagnosis.

Glucose-6 phosphate dehydrogenase is vital for erythrocytes against oxidative stress. Its deficiency is the most common erythrocyte enzyme defect. Deficiency can cause haemolysis episodes triggered by infections, drug use or ingestion of fava beans (1). Herein, we aimed to present a case of G6PD deficiency due to the fava ingestion in a geriatric patient.

The frequency of G6PD deficiency in Turkey varies between 1% and 5%–20% in southwestern Anatolia (2). Most people with G6PD deficiency are not aware of their defect; they are asymptomatic until their first attack. The attack may be triggered due to infections, metabolic conditions, drugs or ingestion of fava beans (3). Favism is generally a paediatric disease and is rarely newly diagnosed in geriatric patients. Favism can develop after ingestion of dried or frozen beans; however, it is mostly associated with fresh beans (4). Not all patients experience favism after ingesting fava beans, and same individual may have different reactions in different times (3).

Glucose-6 phosphate dehydrogenase deficient cells are lysed during acute haemolytic attack, which may cause a normal measurement of erythrocyte G6PD levels; however, G6PD level of patients with favism may be low during acute episode. In the Mediterranean type G6PD deficiency, young erythrocytes are as deficient as older erythrocytes (5). Favism may be more frequently associated with the Mediterranean variant. Glucose-6 phosphate dehydrogenase deficiency is mostly a paediatric disorder; however, in geriatric patients with a history of fava exposure presenting with non-immune haemolytic anaemia, it may be likely and should be included differential diagnosis.

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Keywords: Geriatric, glucose-6 phosphate dehydrogenase, non-immune haemolysis.

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LETTER TO THE EDITOR

A Case of Right Drop Foot Developing Soon after the Diagnosis of Crohn's Disease

The Editor,

Sir,

Nervous system damage may occur in the early stages of Crohn's disease and is one of the most underreported extra-intestinal manifestations despite its significant impact on quality of life and morbidity. For these reasons, early recognition of neurologic symptoms is crucial for treatment (1).

We describe here a case of right drop foot due to radiculopathy developed soon after the diagnosis of Crohn's disease.

A 17-year-old male admitted to our clinic complaining of right drop foot for 2 weeks. The patient was diagnosed Crohn's disease 2 months prior to admission and reported numbness and weakness and ankle sprain in the right foot 6 weeks after diagnosis. Physical examination revealed minimal steppage gait and paresthesia of the right L5 dermatome. According to the manual muscle testing grading system, the score for the right hip abductors, extensors and knee flexors was four while for the right ankle and toe the score was three' should replace 'were three'.

Electroneuromyographic (ENMG) nerve conduction studies were normal. Needle electromyography revealed polyphasia and decreased recruitment of motor unit action potentials in the tibialis anterior and extensor digitorum brevis and gastrocnemius muscles. Fibrillations and positive sharp waves were found in the tibialis anterior muscles. The patient's lumbar magnetic resonance imaging (MRI) was normal. Clinical and ENMG findings were consistent with a right L5 radicular involvement due to Crohn's disease.

The patient was prescribed a home exercise program comprised of progressive muscle strengthening and posture, gait and proprioceptive training. An ankle foot orthotic (AFO) device was applied.

The pathogenesis of neurogenic disorders associated with inflammatory bowel disease (IBD) has not been established and may involve diverse causes. One such neurogenic disorder is monophasic immune radiculoplexus neuropathy which may precede the appearance of digestive symptoms or develop after diagnosis (1–3).

In a population-based study of IBD including 12,476 person-years of follow-up, Figueora *et al* (2) reported that four patients were diagnosed with lumbosacral radiculoplexopathy. This pathology was found to occur late in the course of the disease, mainly during periods of bowel disease inactivity. In the current case, drop foot occurred immediately after the onset of digestive symptoms during the active period of IBD.

Foot drop is a common and distressing problem that can lead to falls and injury (4). It is characterized by the inability or difficulty in moving the ankle and toes in dorsiflexion, foot-slap during loading response and toe-drag during swing (5, 6). Electroneuromyographic, ultrasonography, computerized tomography and MRI are all useful for diagnosis (4). We used ENMG and lumbar MRI for diagnosis in our patient.

The AFO device enables individuals to walk better and more safely and to resolve complications in patients with drop foot (4, 7). Our patient had a history of ankle injury occurring after a fall due to instability. For this reason, we prescribed AFO in addition to the traditional exercise programme.

Keywords: Crohn's disease, drop foot, radiculopathy.

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