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EDITORIAL

OMICRON: A CONCERN OR HOPE?

Professor Dr. Khan Golam Mostafa

The new variant of SARS-CoV-2 is named after the 15th letter of Greek alphabet (o). It is now a days the biggest global concern. Reported so far, the new variant of SARS-CoV-2 has spread over 77 nations by now.

The highly mutated variant was first came to light by an article by Ewen Calloway [1] in Nature on November 25, 2021. Subsequently, on November 26, 2021, the WHO's Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE) designated the B.1.1.529 strain detected in South Africa as OMICRON under the Variants of Concern (VOC) category. Within a week, the OMICRON got global recognition and concern.

The novel OMICRON variant was first detected in South Africa's Gauteng province in mid-November 2021 [3]. The South African advanced genomic sequencing infrastructure facilitates early detection and reporting of a new variant to the world. As of 15 December 2021, the OMICRON variant has already popped up in around 77 countries with the majority of the cases from the United Kingdom, South Africa, and the United States [4, 5]. India has also observed OMICRON positive cases. United Kingdom reported the first death with the new COVID-19 variant [6].

The rising concern on OMICRON is because, RNA viruses are known to mutate fast and evolve to adapt and survive in changing environments. The most concerning characteristic of the OMICRON variant is the constellation of more than 50 mutations, of them about 30 mutations are in the spike protein. The more worrisome are the 15 mutated sites in the receptor-binding domain (RBD) that interacts with human cells before cell entry, therefore possibly enhancing the transmissibility [7].

As per World Health Organization (WHO) so far no studies indicated the more severity of the OMICRON variant. Concerns over the high transmissibility, virulence, increased risk of reinfection, and decrease in the effectiveness of available diagnostics, vaccines, and therapeutics are still unresolved. In the lack of OMICRON variant-specific vaccine, the already approved (FDA/EUA) vaccines are not giving hope to counter the infectivity and transmissibility.

The spike protein gene is the target of most of the currently available vaccines, therefore this variant may have a greater potential to escape prior immunity than the previous delta variant. The prime concern over the current vaccines is their decreasing effectiveness against COVID-19 over a period. The efficacy dropped almost to half for the Pfizer vaccine (from 86% to 43%), Moderna vaccine (from 89% to 58%), and J&J vaccine (from 86% to 13%) [8].

Since December 2019, the whole world is dealing with different variants of SARS-CoV-2 and experienced 1st and 2nd waves of the COVID-19 pandemic. However, few nations are still struggling with 3rd wave due to the Delta variant. Amid this, the emergence of a new OMICRON variant might impart a negative effect on the life and livelihood of humankind.

Therefore, the concern is that we are not getting much protection against OMICRON infection but the hope is that the variant is not much virulent. Prevention remains the mainstay to avoid the risk of being infected with COVID-19. So, WHO advised the countries to enhance the surveillance and sequencing capacity for a better understanding of the circulating SARS-CoV-2 variants.

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Original Article

Phyllodes Tumor of Breast: Recurrence and Surgical Aspect

Md. Showkat Ali[1] , Professor Md. Ruhul Quddus[2], Md. Shariful Islam[3], Md. Atiqul Islam[4], SM Golam Azam[5], Md. Hasanuzzaman[6], Asadullahil Galib[7]

ABSTRACT

Background: Phyllodes tumors are fibroepithelial breast tumors capable of a diverse range of biological behavior. Also termed phylloides tumors or cystosarcoma phyllodes, these lesions are similar to benign fibroadenomas in their least aggressive form, albeit with a propensity for local recurrence following excision. These benign tumours, previously sometimes known as serocystic disease of Brodie or cystosarcoma phyllodes, usually occur in women over the age of 40 years but can appear in younger women. **Aims & objectives:** Here in this research, our main aim was to assess the incidence of recurrence and surgical options in a tertiary level hospital, in a statistical way. **Methodology:** This research was conducted as a cross-sectional study. Study population of this research was 42 patients of Phyllodes tumor of breast, in Khulna Medical College Hospital (KMCH), from January 2014 to January 2020. Convenient purposive sampling was the method of selecting population. **Results:** In this study, most of the patients (42.9%) were in 40-49 years of age group. Mean±SD of age of the patients was 45.6±1.3 years. Approximately 38.1% patients had recurrent disease. 43.8% cases with local recurrence had previous history of lumpectomy. Most of the local recurrence was observed in between 06 months to 1 year (43.8%). Mastectomy was found to be most acceptable and optimal treatment option. No case with lymph node involvement or systemic metastasis was observed. **Conclusion:** Local recurrence after excision of Phyllodes tumor of breast is a common problem. And mastectomy is the reliable treatment option to combat with the problem, except in certain situations where wide local excision can be also considered. Early recurrence is most commonly due to incompletely and inadequately resection in first surgery.

Keywords: Phyllodes tumors, phylloides, cystosarcoma phyllodes, serocystic disease of Brodie

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INTRODUCTION

The original term cystosarcoma phylloides coined by Johannes Muller in 1838 was used to describe the tumor's grossly fleshy physical appearance, it was not intended to indicate metastatic potential as is typically implied by the term sarcoma [1]. Phyllodes tumors in their most aggressive form, however, can recur with distant metastases, histologically degenerating into a sarcomatous lesion lack

ing an epithelial component [2]. This metastatic variant is uncommon, however, with fewer than 5% of phyllodes tumors ever developing distant metastases [3]. The first such case of a metastatic phyllodes tumor, in fact, was not reported until 1931 [4]. Given a lack of uniformity in nomenclature, the World Health Organization recommended in 1982 that all such lesions be referred to as phyllodes tumors, which has now been widely accepted [5].

Phyllodes tumours are rare fibro-epithelial lesions. The role of the pathologist in thepreoperative diagnosis of phyllodes tumours of the breast is critical to appropriate surgical planning. Widelocal excision or mastectomy with adequate margin remains the treatment of choice. Local recurrenceoccurs in approximately 10-16.1% of patients [6] and distant metastases occurs in 6.3–31% of patients with malignant phyllodes tumours [6, 7] but only in 4% of all phyllodes tumours [8]. Emphasis should be given in early diagnosisand intervention to decrease morbidity and mortality.

METHODOLOGY

A total 42 patients of Phyllodes tumor of breast, underwent surgery in Khulna Medical College Hospital (KMCH), from January 2014 to January 2020 were included in this study. This research was conducted as a cross-sectional study. All data was collected and analyzed prospectively- such as patient details history, clinical & investigative findings, findings on surgery, operative photograph etc. Convenient purposive sampling was used as a method of selecting sample on the basis of inclusion and exclusion criteria. In this clinical study, both manual and computer based statistical analysis of the data were done. Data were analyzed manually and then rechecked with SPSS (Statistic package for social science) computer package programmer. The survey data were usually be analyzed using both analytic as well as descriptive statistic; such as; mean, SD, percentage etc. Ethical clearance was taken individually from patient and from the ethical review committee of Khulna Medical College Hospital, Khulna, Bangladesh.

RESULTS

Out of these 42 patients, age distribution is shown in table 1. Most of the patients of Phyllodes tumor were in 40-49 years of age group (18 patients, 42.9%), followed by 28.6% (12 patients) were in 50-60 years of age group. Phyllodes tumor is extreme rare below the age of 30 years (01 patient, 2.4%).

Table 1: Age distribution of study population

Age group (years)	Frequency n (%)	Mean ± SD
<30	1 (2.4)	45.6 ± 1.3 years
30-39	6 (14.3%)	
40-49	18 (42.9%)	
50-60	12 (28.6%)	
>60	5 (11.9%)	

In this study, a significant portion of patients presented with recurrent Phyllodes tumor of breast and had a history of previous surgery. Among the total 42 cases 38.1% (16 patients) had recurrence (Figure 1).

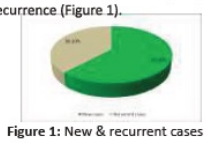
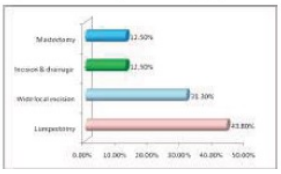


Figure 1: New & recurrent cases

In case of these 16 patients of recurrence, approximately in 43.8% (07) patients previously lumpectomy was done, followed by in 31.3% (05) cases wide local excision was done. In 02 (12.5%) patients, incision & drainage was done, mistaking it as an abscess. Mastectomy was performed in 12.5% (02) cases (Figure 2).

Figure 2: Details of previous surgery



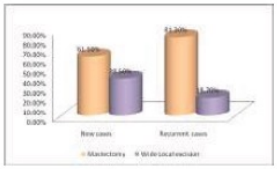
In these 16 recurrence cases, most of the recurrence was observed in 43.8% (07) cases in between 06 months to 1 year. 25% (04) recurrence was found in between 01 to 02 years. Recurrence after 02 years was observed in 18.7% (03) cases, whereas, in 2 (12.5%) patients recurrence was found after incision & drainage operation (Figure 2).

Table 2: Period between surgery and recurrence

Interval	Frequency n (%)	Mean ± SD
Within 6 months	2 (12.5)	7 ± 1.8 months
6 months to 1 year	7 (43.8%)	
1 year to 2 years	4 (25%)	
After 2 years	3 (18.7%)	

After admission in KMCH, all the patients were assessed carefully. Among the 26 new cases, mastectomy was required in 61.5% (16) patients, whereas wide local excision was done in 38.5% (10) patients. On the other hand, 81.3% (13) & 18.7% (3) patients required mastectomy and wide local excision respectively, in case of recurrent patients (Figure 3). In this study, no case with lymph node involvement or systemic metastasis was found.

Figure 3: Surgery done in all cases (mastectomy vs. wide local excision)



Preoperative and postoperative images of a recurrent case in KMCH are shown in figure 4A-F.

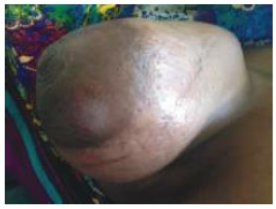


Figure 4A: Recurrent Phyllodes tumor of breast (before surgery)

Figure 4B: Recurrent Phyllodes tumor (Operated)

Figure 4E: Recurrent Phyllodes tumor (Operated)

Figure 4C: Recurrent Phyllodes tumor (Operated)

Figure 4F: Recurrent Phyllodes tumor (Operated)

Figure 4D: Recurrent Phyllodes tumor (Operated)

A case of Phyllodes tumor (confirmed by histopathology of specimen postoperatively) inadvertently drained, mimicking it as an abscess (figure 5 A-B).

Figure 5B: Specimen sent for histopathology

DISCUSSION

The results of this study suggest that the disease is more common in 40-49 years of age group (18 patients, 42.9%). Mean ± SD of the study cases was 45.6±1.3 years, indicating that Phyllodes tumor of breast is a disease of middle aged women. Approximately 42 cases (38.1%, 16 patients) had recurrent disease and previous history of surgery. 43.8% (07) patients with local recurrence had previous history of lumpectomy. This finding is highly suggestive of that possibly local recurrence of Phyllodes tumor of breast is close related with inadequate removal or incomplete excision with residual disease. Most of the local recurrence has been observed in between 06 months to 1 year (43.8%, 07 cases). Mastectomy was found to be most acceptable and optimal treatment option in case of both new patients (61.5%, 16) and recurrent cases (81.3%, 13). No case with lymph node involvement or systemic metastasis was observed in this research.

Phyllodes tumors are rare fibroepithelial lesions. They make up 0.3 to 0.5% of female breast tumors [9] and have an incidence of about 2.1 per million, the peak of which occurs in women aged 45 to 49 years [10, 11]. The tumor is rarely found in adolescents and the elderly. They have been described as early as 1774, as a giant type of fibroadenoma [12]. Chelius [13] in 1827 first described this tumor. Johannes Muller (1838) was the first person to use the term cystosarcoma phyllodes. It was believed to be benign until 1943, when Cooper and Ackerman reported on the malignant biological potential of this tumor. In 1981 [14] the World Health Organization adopted the term phyllodes tumor and as described by Rosen [15] sub-classified them histologically as benign, borderline, or malignant according to the features such as tumor margins, stromal overgrowth, tumor necrosis, cellular atypia, and number of mitosis per high power field. The majority of phyllodes tumors have been described as benign (35% to 64%), with the remainder divided between the borderline and malignant subtypes [15].

Accurate preoperative pathological diagnosis allows correct surgical planning and avoidance of reoperation, either to achieve wider excision or for subsequent tumor recurrence [16-18]. At one extreme, malignant phyllodes tumors, if inadequately treated, have a propensity for rapid growth and metastatic spread. Treatment can be either wide local excision or mastectomy provided histologically clear specimen margins are ensured [19].

CONCLUSION

Mastectomy is the most resilient surgical option for Phyllodes tumor of breast, especially in recurrent cases. Wide local excision can be considered in some certain situations. In contrast, lumpectomy has limited role. Early recurrence within 06 to 12 months is more common, especially due to incompletely and inadequately removal in lumpectomy.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Original Article

Pattern of Lipid Profile and Frequency of Dyslipidemia among Apparently Healthy Adult Individuals of Bangladesh

Ahmed Al Maaruf[1], Prof. Md. Mozammel Hoque[2], Prof. Chowdhury Meshkat Ahmed[3], Prof. Sunil Krishna Baul[4], Shaikh Nazmus Saqueeb[5], Mokerroma Ferdous[6], Md. Asaduzzaman[7], Manosh Kumar Mondal[8]

ABSTRUCT

Introduction: Dyslipidemia is a major independent risk factor for coronary artery disease leading to morbidity and mortality. South Asians have a less favorable lipid profile than Europeans. High risk ethnicities for cardiovascular events and other atherosclerotic disorders include South Asian ancestry. Therefore, among the apparently healthy adult individuals, it is necessary to know the frequency and pattern of dyslipidemia for appropriate implementation of preventive measures. **Methods:** It was a cross sectional study done between the periods of August 2019 to July 2020 in department of Biochemistry and Molecular Biology, BSMMU. 643 apparently healthy adult individuals over 20 years were enrolled for this study. Anthropometric measurements and Lipid biomarkers were evaluated and Pearson's correlation tests were done among different variables. **Results:** In this study, the mean total cholesterol (TC) level was 191.48 ± 3.65 mg/dl, mean triglyceride (TG) was 182.14 ± 109.86 mg/dl, mean low density lipoprotein cholesterol (LDL-C) was 116.22 ± 36.74 mg/dl, and mean high density lipoprotein cholesterol (HDL-C) was 40.49 ± 0.729. Hyperlipidemia found in 25.59% (28.01% male, 23.31% female), hyper cholesterolemia found in 38.70% (39.74% male, 37.73% female), hyper triglyceridemia found in 49.44% (55.37% male, 43.86% female), atherogenic dyslipidemia found in 3.47% (4.23% male, 2.76% female). The study shows positive correlation between BMI and Total Cholesterol, LDL Cholesterol and Triglyceride, whereas negative correlation between BMI and HDL-C. **Conclusions:** Apparently disease free adults had significant dyslipidemia (25.59% hyperlipidemia, 38.7% hyper cholesterolemia, 49.44% hyper triglyceridemia and 3.47% atherogenic dyslipidemia).

Keywords: Lipid profile, dyslipidemia

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INTRODUCTION

Cardiovascular disease (CVD) is the most prevalent cause of death among non-communicable diseases (NCDs) and major cause of health loss for all regions of the world. It is considered that 23.3 million people could die from cardio vascular events in 2030 [1]. More people die annually from CVDs than from any other cause. An estimated 17.9 million people died from CVDs in 2016, representing 31% of all global deaths. Of these deaths, 85% were due to heart attack and stroke [2].

With rapid socioeconomic development and associated lifestyle changes the prevalence of dyslipidemia and atherosclerotic cardiovascular diseases increased dramatically over the past decade in Bangladesh. Several factors including rapid urbanization, demographic changes, rural-to-urban migration, faulty diets, sedentary lifestyle, and socio-cultural factors along with genetic predisposition have emerged as major contributory factors [3].

At least three quarters of the world's deaths from CVDs occur in low- and middle-income countries that have less access to effective and equitable health care services which respond to their needs. As a result, many people in low- and middle-income countries are detected late in the course of the disease and die younger from CVDs and other NCDs often in their most productive years [4].

National Cholesterol Education Program- Adult treatment Panel III (NCEP-ATP III) classifies dyslipidemia into four groups- (a) Hyperlipidemia: TC>200 mg /dl (>5.17mmol/l), TG >150 mg /dl (>1.69 mmol/l), (b) Hyper Cholesterolemia: TC>200 mg /dl (>5.17mmol/l), (c) Hypertriglyceridemia: TG >150 mg /dl (>1.69 mmol/l) and (d) Atherogenic-dyslipidemia: TG >150 mg /dl, LDL-C > 165 mg/dl (>4.27 mmol/l).

Cholesterol and triglycerides are transported in plasma complexed to specific Apo proteins as lipoproteins. These have been categorized by ultracentrifugation into four major families according to density: chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

The lipid and lipoprotein parameters which are predominantly measured as lipoprotein profile include total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglyceride. Total cholesterol is accepted as the initial entry point as recommended by the National Cholesterol Education Program (NCEP). The major burden of ischemic heart disease is associated with moderately elevated lipid levels. The key initiating event in atherogenesis is the retention of low-density lipoprotein cholesterol (LDL-C) and other cholesterol-rich apolipoprotein (Apo) B containing lipoproteins within the arterial wall. The effect of LDL-C on the risk of ASCVD appears to be determined by both the absolute magnitude and the total duration of exposure to LDL-C. The effect of plasma TGs on ASCVD is mediated by changes in the concentration of TG-rich lipoproteins as estimated by non-HDL-C [5]. The Expert Panel of the National Cholesterol Education Program has identified 10 risk factors for the occurrence of an atherosclerotic event. Dyslipidemia preponderated among the nine major risk factors (smoking, diabetes, hypertension, visceral obesity, psychosocial stress, sedentary life, low fruit and vegetable consumption and alcohol consumption), and alone accounted for more than 50% of population attributable risk [6]. Atherogenic dyslipidemia (AD) is characterized by high concentrations of triglycerides and low concentrations of HDL-cholesterol, frequently associated with elevated apolipoprotein B and small, dense LDL particles. In several observational studies and in placebo group of various clinical trials, AD has been described as a potent predictor of future coronary events [7]. Reduction in atherosclerotic cardiovascular disease (ASCVD) risk is directly and positively correlated with the incrementally achieved absolute LDL-C reduction [8]. In order to reduce the prevalence of dyslipidemia, a multifaceted approach with special attention to controlling identified risk factors is crucial.

MATERIALS AND METHODS

This cross sectional observational study was done between the periods of August 2019 to July 2020.

643 study population were enrolled from the interested apparently healthy adult individuals, both male (322) and female (321), age 20 years and above from employees of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh and their relatives. Individuals with no apparent illness, with no known history of cardiovascular, renal, gastrointestinal, hepatic, psychiatric or endocrine diseases; nonalcoholic, nonmalignant and nonpregnant adults were considered apparently healthy. Data were collected on sociodemographic factors and behavioral risk factors using a pretested questionnaire. Anthropometric measurements and Lipid biomarkers were evaluated.

Continuous variables were presented as mean ± standard deviation (SD) and the statistical significance of the differences in the group means was compared by Student's t-test. Pearson's correlation tests were done among different variables.

RESULTS

Data for 643 participants aged 20 years and above, were included in the current analyses. Age distribution of the participants was shown in table 1. The lipid profile of the participants according to age group and sex are given in the table 2 and table 3. Total cholesterol is higher in male below 40 years and above 60 years, whereas progressive rise is seen in female below sixty years. HDL cholesterol found higher in female in all age group. LDL Cholesterol found high in male above 40 years and serum triglyceride level found higher in male than female in all age group.

Pattern of dyslipidemia in study population: Based on ATP III criteria, dyslipidemia is classified into Hyperlipidemia (TC>/=200 mg/dl & TG> 150 mg/dl), Hypercholesterolemia (TC>/=200 mg/dl), Hypertriglyceridemia (TG> 150 mg/dl) and Atherogenic dyslipidemia (TG> 150 mg/dl & LDL-C >165). Out of 643 apparently healthy adult participants all patterns of dyslipidemia were found significantly higher in male than in female. Table-4 shows 25.59% (n=162) had hyperlipidemia (male 28.01%, female 23.31%); 38.70% (n=245) had hypercholesterolemia (male 39.74%, female 23.31%); 49.44% (n=313) had hypertriglyceridemia (male 55.37%, female 43.86%) and 3.47% (n=22) had atherogenic dyslipidemia (male 4.23%, female 2.76%).

Table 1: Age distribution of the participants

Variables	Categories	Frequency n (%)
Age in years	<40	236 (36.7)
	40-60	342 (53.18)
	>60	65 (10.1)
Gender	Male	321 (49.92)
	Female	322 (50.08)

Figure-1: Bar diagram showing distribution and pattern of dyslipidemia

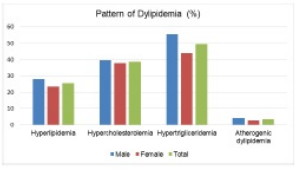

Table 2: Mean (SD) of Total Cholesterol and HDL-C concentrations according to age and sex groups

Age group (years)	Participants		Total Cholesterol			HDL Cholesterol		
			Mean	SD	99% CI	Mean	SD	99% CI
<40	Male	116	191.15	45.91	191.15± 11.01	38.05	8.44	38.05± 1.53
	Female	120	186.63	37.14	186.63 ± 6.75	43.39	13.63	43.39± 2.444
40-60	Male	171	191	40.74	191 ± 6.10	37.47	8.02	37.47 ± 1.20
	Female	171	197	54.68	197 ± 8.19	43.3	9	43.3 ± 1.34
>60	Male	34	183.5	48.03	183.5 ± 16.14	38.29	6.68	38.29 ± 2.24
	Female	31	108.75	49.74	108.75± 17.53	43.70	12.08	43.70 ± 4.25
Total (M+F, n=643)			**191.48**	**47.28**	**191.48 ± 3.65**	**40.49**	**9.43**	**40.49 ± 0.729**

Table 3: Mean (SD) of LDL Cholesterol and Triglyceride concentrations according to sex and age **groups**

Age group (years)	Participants		LDL Cholesterol			Triglyceride		
			Mean	SD	99% CI	Mean	SD	99% CI
<40	Male	116	116.99	18.75	116.99 ± 3.41	193.18	89.42	193.18 ±16.27
	Female	120	117.56	17.61	117.56 ± 3.06	172	111.48	172 ± 19.89
40-60	Male	171	123.89	35.46	123.89 ± 5.31	181.63	86.34	181.63 ±18.94
	Female	171	121.54	41.40	121.54 ± 6.20	179.19	123	179.19 ± 18.44
>60	Male	34	111.42	47.96	111.42±16.12	184.82	120.05	184.82± 40.35
	Female	31	108.80	21.30	108.80 ± 7.50	186.51	103.59	186.51± 36.51
Total (M+F, n=643)			**116.22**	**36.74**	**116.22 ± 2.90**	**182.14**	**109.86**	**182.14 ± 8.48**

Table- 4: Pattern of dyslipidemia among apparently healthy adults

Pattern of dyslipidemia	Male (n=321) n (%)	Female (n=322) n (%)	Total (n=643) n (%)
Hyperlipidemia (TC>/=200 mg/dl, TG> 150 mg/dl)	86 (28.1%)	76 (23%)	162 (25.59%)
Hyper cholesterolemia (TC>/=200 mg/dl)	122 (39.74%)	123 (37.73%)	245 (38.7%)
Hyper triglyceridemia (TG> 150 mg/dl)	170 (55.37%)	143 (43.86%)	323 (49.44%)
Atherogenic dyslipidemia (TG> 150 mg/dl & LDL-C >165)	13 (4.23%)	9 (2.76%)	22 (3.47%)

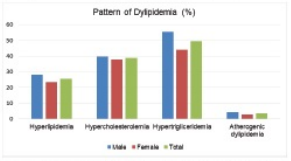

Figure-1: Bar diagram showing distribution and pattern of dyslipidemia

Distribution of lipids in different age groups and gender: Hypercholesterolemia found more in male below 40 years but more in female above 40 years (figure-2).

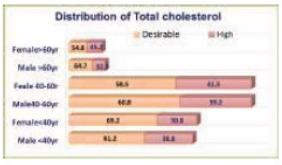

Figure-1: Bar diagram showing distribution and pattern of dyslipidemia

Prevalence of high LDL cholesterol level (150-190mg/dl) is more in all age male than female. Very high LDL cholesterol level (>190mg/dl) is more in female than male in any age group (figure-3).

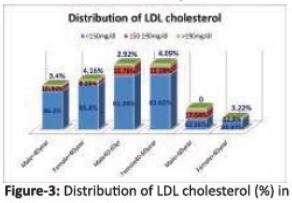

Figure-3: Distribution of LDL cholesterol (%) in different age and sex

Triglyceride level is found higher (>150mg/dl) in male in all age group than female of corresponding ages. Very high levels (>500m g/dl) are found occasionally (1.75% in male and 2.33% in female) among 40-60 years of participants (Figure-4).

Figure-4: Distribution of triglyceride (%) in different age and sex

HDL cholesterol level found very low in all age of female. Low level of HDL also predominant in male in any age group (Figure-5).

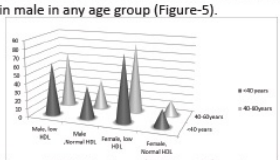

Figure-5: Distribution of HDL in different age & sex

Correlation of lipids with BMI: There were no underweight participants in the study population. 6.99% participants were normal weight, 17.73% were overweight and 75.27% were obese. Pearson's correlation test shows a positive correlation between BMI and Total cholesterol (Figure-6) and a negative Correlation between BMI and HDL Cholesterol (Figure-9). BMI shows a weak positive Correlation with LDL-C (Figure-7) and Triglyceride (Figure-8). Correlation of BMI with Total cholesterol and Triglyceride is statistically significant at the 0.05 level (2 tailed).

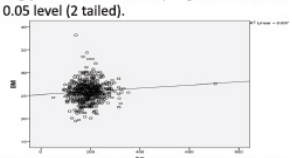

Figure-6: Correlation between BMI and Total cholesterol

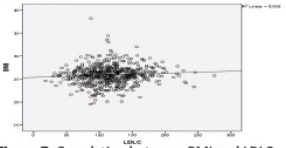

Figure-7: Correlation between BMI and LDLC

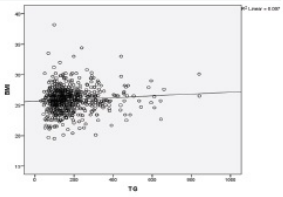

Figure-8: Correlation between BMI and Triglyceride

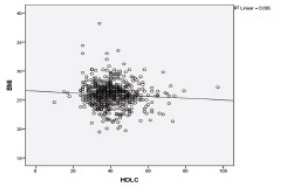

Figure-9: Correlation between BMI and HDL Cholesterol

Discussion

This study was done among apparently healthy adult individuals employed in Bangabandhu Sheikh Mujib Medical University and their relatives. The primary objective was to analyze the pattern of lipid profile and the frequency of dyslipidemia among apparently healthy adult Bangladeshi individuals. In this study, the mean total cholesterol (TC) level was 191.48 ± 3.65 mg/dl, mean triglyceride (TG) was 182.14 ± 109.86 mg/dl, mean low density lipoprotein cholesterol (LDL-C) was 116.22 ± 36.74 mg/dl, and mean high density lipoprotein cholesterol (HDL-C) was 40.49 ± 0.729. These levels were higher from both urban and rural random Bangladeshi population study conducted in 2010 [8] and in 2015 [9]. 200 diagnosed case of dyslipidemia were studied in 2019 and found

TC, TG and LDL-C levels higher than this study [10]. Lipid profile of 286 apparently healthy urban adult Bangladeshi population were studied in 2020 [11] and found TC and TG levels lower and HDL level higher than this study.

This study found a fall in TC, LDL- C in participants aged 60 or more. Cholesterol level tends to decrease with age [12]. This may lead the assumption that the role of cholesterol in determining the risk of cardiovascular disease becomes less relevant in a more aged population. Low cholesterol level increases the risk of functional decline in elderly subjects and has a strong association with malnourishment and frailty [13]. The study shows positive correlation between lipid profile (Total Cholesterol, LDL Cholesterol and Triglyceride) and BMI, but negative correlation with HDL cholesterol.

South Asians (people who have ancestral origins in the Indian subcontinent- the peoples of India, Pakistan, Bangladesh, Sri Lanka, and Nepal) have a high prevalence of coronary heart disease (CHD) and suffer from early-onset CHD compared to other ethnic groups. Conventional risk factors may not fully explain this increased CHD risk in this population. Indeed, South Asians have a unique lipid profile which may predispose them to premature CHD. Dyslipidemia in this patient population seems to be an important contributor to the high incidence of coronary atherosclerosis. The dyslipidemia in South Asians is characterized by elevated levels of triglycerides, low levels of high-density lipoprotein (HDL) cholesterol, elevated lipoprotein(a) levels, and a higher atherogenic particle burden despite comparable low-density lipoprotein cholesterol levels compared with other ethnic subgroups. HDL particles also appear to be smaller, dysfunctional, and pro-atherogenic in South Asians [14]. In this study we found the major types of dyslipidemia were hypertriglyceridemia and low HDL Cholesterol, a finding that shows similarity with studies in South Asian countries.

As shown in the INTERHEART study, although the overall associations between LDL-C and risk for AMI were similar among South Asians and others, South Asians had LDL-C levels that were on average 10 mg/dL lower than other groups. Why South Asians carry a higher CHD risk at a given LDL-C level remains a question. One of the postulated mechanisms is that South Asians carry a higher LDL particle burden at a given LDL-C level. Smaller LDL particles are denser and may be more atherogenic [15].

Out of 643 apparently healthy adult participants, all patterns of dyslipidemia were found significantly higher in male than in female. The study shows hyperlipidemia in 25.59% participants, hypercholesterolemia in 38.70% participants, hypertriglyceridemia in 49.44% participants and atherogenic dyslipidemia in 3.47% participants. Very high levels of LDL-cholesterol and TG found in above 40 female.

The increasing trend of dyslipidemia in apparently healthy adult participants of this study may reflect the recent increase in urbanization, increased family income, and physical inactivity of Bangladeshi population. Although a considerable percentage of South Asians are vegetarians, excess sugars and refined carbohydrates remain problematic for this population. Diet rich in sugar and processed carbohydrates may be a considerable threat to the future health and wellness of the increasingly sedentary South Asian people with their innate genetic predisposition to CHD [14].

Hypercholesterolemia (TC > 200 mg/dL) has

been reported to have a prevalence of up to 35% in men and 36% in women from South Asian countries [16]. Rabeya et al. 2019, found 13.5% hypercholesterolemia [10]. In this study hypercholesterolemia was found in 39.74% of male participants and 37.73% of female participants which were higher than above mentioned studies. Modernization related reduced physical activity among urban populations was associated with higher level of plasma cholesterol than their rural counterparts [9]. Intake of more fish and mustard oil (containing unsaturated omega 3 fatty acids) in rural population were found responsible for low prevalence of LDL than urban population [17].

Conclusion

The burden of dyslipidemia is alarming in Bangladesh regarding morbidity, mortality, and treatment costs. This study shows that apparently disease free adults had significant dyslipidemia (25.59% Hyperlipidemia, 38.7% hyper cholesterolemia, 49.44% hyper triglyceridemia and 3.47% atherogenic dyslipidemia) .The study shows positive correlation with BMI with lipids (TC, LDL-C and TG) but negative correlation with HDL.

All types of dyslipidemia were found higher in male participants than female. In this study the major types of dyslipidemia were hypertriglyceridemia and low HDL cholesterol, a finding that shows similarity with other studies in South Asian countries.

The increasing trend of dyslipidemia in apparently healthy adult participants of this study may reflect the récent increase in urbanization, increase in family income, imbalanced diets, reduced physical activity, long-term sedentary work and other urban stresses.

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Original Article

Expression of AMACR in Prostatic Cancer

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ABSTRACT

Introduction: Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. To diagnose prostate cancer, no specific single histological feature is sufficiently available. It is a challenging task to accurately diagnose small foci of prostate cancer for pathologists and to distinguish cancer from its benign mimickers. A positive diagnostic marker specific for prostatic adenocarcinoma may enhance the ability to detect small foci of cancer and reduce diagnostic difficulties. AMACR (P504S) has been proven to be one of the few biomarkers that can help distinguish cancer from benign cells, with high sensitivity and specificity for prostate cancer. This study focuses on AMACR (P504S) expression in prostate cancer. **Materials and methods:** 21 Prostatic needle biopsies and 16 TURP specimens as cases and 37 TURP specimens as controls were included in this study. Specimens were processed routinely for H and E and IHC using FLEX monoclonal Rabbit anti-human AMACR. Histopathological and IHC results were analyzed. Statistical analysis was done using SPSS version 16. **Results:** Among the 37 cases 35 were diagnosed as prostatic adenocarcinoma and 02 were diagnosed as suspicious for malignancy histopathologically. AMACR was not expressed in any of the 37 benign controls. Among the 35 histopathologically diagnosed prostatic adenocarcinoma cases, 34 showed positive AMACR expression in various intensity and 01 showed negative AMACR expression. Among the 02 histopathologically diagnosed suspicious for malignancy cases, both showed strong AMACR expression. There was statistically significant difference in expression of AMACR between cases and controls, indicated by $p<0.05$. **Conclusion:** Histopathology is the gold standard for diagnosing prostatic cancer. AMACR helps in confirming the diagnosis of carcinoma in the cases of suspicious for malignancy. But only AMACR negativity does not exclude the possibility of cancer. So, strict morphological correlation is essential.

Keywords: Alpha Methyl Acyl CoenzymeA Racemase (AMACR), prostatic cancer

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Introduction

Prostate cancer is the most frequently diagnosed non-cutaneous malignancy in men, and the second leading cause of male cancer-related mortality in the United States [1]. It is responsible for 6.6% of all deaths in men over age of 55 years [2]. In South-East Asian region, the incidence and mortality due to prostatic cancer are 4.7% and 4% respectively [2]. In Bangladesh, the prevalence of prostate cancer is 2.3% in the last 5 years [3].

Prostate-specific antigen (PSA) blood test in addition to digital rectal examination (DRE), have traditionally been the preferred modalities to screen for prostate cancer. PSA has significant limitations because serum PSA is not specific to prostate cancer. One of the major limitations of PSA screening is that serum PSA can be elevated in patients with other common benign conditions, such as benign prostatic hyperplasia (BPH), prostatitis or other minor clinical procedures such as trans- rectal ultrasound [4]. PSA is organ specific but not cancer specific [5].

The diagnosis of prostate cancer is based on a combination of architectural, cytological and ancillary features rather than any single diagnostic feature. Accurate tissue diagnosis can be very challenging due to the presence of either a small focus of cancer or due to the presence of many benign mimickers of malignancy [6].

Now a days, there is widespread use of serum PSA as a mass screening test for prostate cancer. So, there has been an ever increasing number of prostate needle biopsies and the need to give an accurate diagnosis despite the limitations. Under diagnosis of a small focus of prostatic adenocarcinoma might delay early treatment and cause severe adverse consequences for patients [6]. Therefore, a prostate carcinoma specific marker could be of great importance and usefulness as an adjunct to facilitate critical diagnostic decisions with high sensitivity and specificity [1]. Recently, a positive marker for carcinoma prostate, alpha –methyl acyl-coenzyme A racemase (AMACR) has been reported to have sensitivity ranging from 82-100% [6].

AMACR is an enzyme that is consistently over expressed in prostate cancer epithelium;hence it becomes an ideal specific biomarker for cancer cells within the prostate gland [4]. More recently, a study conducted by Jain et al 2017, took total 50 cases including 37 cases of malignant lesions and 13 cases of benign lesions of prostate. They found that AMACR was not expressed in any of the 13 cases of benign lesions and it was expressed in 33 of 37 malignant lesions [5]. Accordingly, evaluation of AMACR as new biomarker of prostatic adenocarcinoma is needed.

So, the study is designed to use AMACR as an immune-histochemical biomarker and its contribution in diagnosis of prostate cancer especially in needle biopsy specimens. In addition, the sensitivity and specificity of AMACR for the detection of prostate cancer were also evaluated.

MATERIALS AND METHODS

This cross-sectional study was carried out in the department of pathology RMC from March 2017 to February 2019. Approval for the research protocol was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi prior to the commencement of the study. Routine haematoxylin and Eosin stains were done in Department of Pathology, Rajshahi Medical College. The immunostaining was done in Department of Pathology, BSMMU. TURP and needle biopsy specimens of prostate that were histopathologically diagnosed as carcinoma or suspicious for malignancy were taken as case. Histopathologically diagnosed BPH patients were taken as control. Poorly fixed samples, inadequate biopsies and samples with marked inflammation were excluded.

In this study, 35 histopathologically diagnosed prostate cancer and 02 histopathologically diagnosed suspicious for malignancy were taken as cases. Subsequently age matched 37

patients with histopathologically diagnosed BPH were selected as control. Tissue samples were obtained from TURP and needle biopsies. A pre-tested questionnaire used to collect data from cases and controls including complete history, physical examination, information on hematological and biochemical investigations.

The specimens were fixed in 10% formalin. Tissue processing and staining were done according to standard protocol followed at Department of Pathology, Rajshahi Medical College. Sections were studied under light microscope and classified into benign, malignant and suspicious for malignancy microscopically. Carcinoma cases were histologically graded according to the Gleason scoring system and 2014 WHO/ISUP consensus conference criteria for grading of prostate cancer. Associated prostatic tissue changes such as tumor invasion, prostatitis and others of any were also analyzed.

Immuno-histochemical staining was carried out using Dako Envision detection technique. 4-micrometer thick sections of formalin fixed, paraffin-embedded tissues were used. The sections were deparaffized in hot air oven, dewaxed in Xyline and rehydrated in descending graded alcohol. Antigen retrived by placing the slides in preheated antigen retrival solution. Blocking endogenous peroxidase and incubated with a rabbit monoclonal antibody AMACR (p504 S, clone no 13H4) in appropriate dilutions. Standard immune-histochemical method was applied for subsequent staining. Tumor cells were scored positive if there was golden brown cytoplasmic or membrane staining in the neoplastic cells. Negative diagnosis was made when no golden brown staining was noted.

Interpretation of Immunohistochemistry: Positive staining for AMACR pertained to dark diffuse or granular, cytoplasmic or luminal, but circumferential. The percentage of positivity were graded from 0+ to 3+ as follows-

- 0% cells (o+, negative)
- 1-10% cells (1+, mild)
- 11-50% cells (2+, moderate)
- >51% cells (3+, strong)

The adjacent benign glands did not show any staining for AMACR. Negative staining pertained to no staining or focal, weak non circumferential fine granular staining.

RESULTS

35 histopathologically diagnosed prostate cancer and 02 histopathologically diagnosed suspicious for malignancy were taken as cases. Subsequently age matched 37 patients with BPH were selected as control. Patients (case) with age ranged from 55 to 87 years (mean age was 67.92±8.5) and control group with age ranged from 51 to 85 years (mean age was 64.76±9.67).The subjects were divided into 04 different age groups: up to 60 years, 61- 70 years, 71-80 years and 81- 90 years. The number and frequency of cases found in different age groups were 09 (24.3%), 19 (51.4%), 07 (18.9%) and 02 (5.4%) respectively (Table-1). 21 (56.8%) needle biopsies and 16 (43.2%) TURP specimens were included as case and 37 (100%) TURP specimens were included as control (Table-2). Among the total 37 cases 35 (94.6%) were diagnosed as prostatic adenocarcinoma and 02 (5.4%) suspicious for malignancy histopathologically (Figure-I). The histopathologically diagnosed prostatic adenocarcinoma cases were graded according to the Gleason scoring system and 2014 WHO/ISUP consensus conference criteria for grading of prostate cancer. Out of 35 adenocarcinoma cases combined Gleason score 6, 7, 8, 9 and 10 were 2 (5.71%), 12 (34.29%), 9 (25.71%), 11 (31.43%) and 1 (2.86%) respectively (Table-2).

According to 2014 WHO/ISUP consensus conference criteria for grading of prostatic cancer, grade group 1, grade group 2, grade group 3, grade group 4 and grade group 5 were 02 (5.7%), 03 (8.6%), 09 (25.7%), 09 (25.7%) and 12 (34.3%) respectively (Figure-II). Immuno-histochemical staining were done in all the 37 cases and 37 controls of prostatic lesions and results were analyzed. In histopathologically diagnosed prostatic adenocarcinoma cases it was expressed in 34 (97.14%) out of 35 cases in various intensity. Among the 02 histopathologically diagnosed suspicious for malignancy cases, all (100%) patients showed strongly positive AMACR expression. On the other hand, among the 37 benign controls, all (100%) patients showed negative AMACR expression (Table-4). There was statistically significant difference in expression of AMACR between cases and controls, indicated by $p<0.05$. Using standard formula for diagnostic accuracy calculation, the sensitivity, specificity and diagnostic accuracy of AMACR for prostatic carcinoma were 97.14%, 100% and 98.61% respectively.

Table 1: Distribution of the patients according to age group (n=74)

Age Group	Case n (%)	Control n (%)
≤60 years	9 (24.3)	17 (45.9)
61-70 years	19 (51.4)	11 (29.7)
71-80 years	7 (18.9)	6 (16.2)
81-90 years	2 (5.4)	3 (8.1)
Total	37 (100)	37 (100)
Age of Cases: Min = 55 years, Max = 87 years, Mean = 67.92 (±8.5)		
Age of Controls: Min = 51years, Max = 85 years; Mean = 64.76 (±9.67)		

Table 2: Distribution of prostatic specimens in cases and controls (n=74)

Type of Specimen	Case n (%)	Control n (%)	Total
Needle Biopsy	21 (56.8)	0	21 (28.4%)
TURP	16 (43.2)	0	53 (71.6%)
Total	37 (100)	37 (100)	74 (100%)

Table3: Frequency of combined Gleason score of histopathologically diagnosed prostatic adenocarcinoma cases (n=35)

Combined Geason Score	Number of Cases	Percentage
6	2	5.71
7	12	34.29
8	9	25.71
9	11	31.43
10	1	2.86
Total	35	100

Table 4: Correlation of AMACR expression with cases and controls (n=74)

	AMACR Expression		Total	p value
	Positive n (%)	**Negative** n (%)		
Case	36 (48.6)	1 (1.4)	37 (50%)	
Control	0	37 (50)	37 (50%)	0.0001
Total	36 (48.6)	38 (51.4)	74 (100%)	

Figure i: Pie chart showing histopathological diagnosis of the cases (n=37)

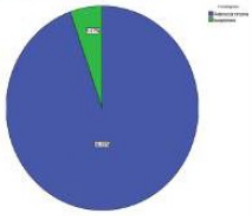
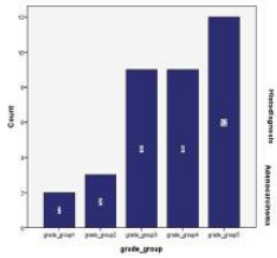


Figure ii: Distribution of the patients of histopathologically diagnosed prostatic adenocarcinoma according to 2014 who/ isup modified Gleason grade group (n=35)



DISCUSSION

Prostate carcinoma is the most common form of cancer in men and the second leading cause of death. The advent of prostate-specific antigen screening has led to a significant increase both in the number of prostate needle biopsies performed and in the number of difficult biopsies with a small foci of adenocarcinoma and atypical glands suggestive but not diagnostic of adenocarcinoma. The diagnosis of prostate cancer is made by use of traditional histological parameters, including architecture, nuclear features and ancillary features (if necessary) rather than any single diagnostic feature. Tissue diagnosis of prostate cancer can be difficult due to the presence of either a small focus of cancer or due to the many benign mimickers of malignancy like adenosis, atrophy, partial atrophy, basal cell hyperplasia, clear cell hyperplasia, post atrophic hyperplasia, nephrogenic adenoma, mesonephric hyperplasia, radiation atypia, seminal vesicle and Cowpers glands. In recent years basal cell markers and prostate biomarker Alpha-Methylacyl- CoA- Racemase (AMACR) have been used as adjuvant to morphology in diagnostically challenging cases with a very high sensitivity and specificity. This has increased the diagnostic accuracy of prostate cancer worldwide [6].

In this study, immune-histochemical staining were done in all the 37 cases and 37 controls of prostatic lesions and results were analyzed. In histopathologically diagnosed prostatic adenocarcinoma cases it was expressed in 34 (97.14%) out of 35 cases in various intensity. Among the 02 histopathologically diagnosed suspicious for malignancy cases, all (100%) patients showed strongly positive AMACR expression. On the other hand, among the 37 benign controls, all (100%) patients showed negative AMACR expression. For accuracy of test to be calculated histopathologically confirmed carcinoma cases and histopathologically confirmed BPH controls were considered as gold standard. Confirmed prostatic carcinoma as evidenced by histopathological findings were 35 (94.6%) out of 37 patients (Figure ⊠). Among the 35 histopathologically confirmed carcinoma cases 34 cases showed positive AMACR expression at different intensity. On the other hand, all the 37 benign controls showed negative AMACR expression. Using standard formula for diagnostic accuracy calculation, the sensitivity, specificity and diagnostic accuracy of AMACR for prostatic carcinoma were 97.14%, 100% and 98.61% respectively (Table-8). These results are almost similar to Rubin, et al. who demonstrated 97% sensitivity of AMACR in the detection of prostate cancer. Shrivastava, et al. reported 100% sensitivity of AMACR in the detection of prostate cancer in their study. Difference in sensitivity of AMACR in different studies including absent staining in prostatic adenocarcinoma can be a result of using different antibodies as polyclonal anti-AMACR is 100% sensitive while the sensitivity of monoclonal anti-AMACR in detecting prostate cancer is only 94%. (7).

This study found that AMACR is over expressed in prostatic adenocarcinoma in both needle biopsy and TURP specimens. However a diagnosis of benignancy should not be made based only on a negative AMACR staining as AMACR can sometimes be negative in adenocarcinoma. Results of AMACR staining should be interpreted only in the context of strict morphologic correlation. Also it is better to combine AMACR with a negative marker of prostatic adenocarcinoma like a basal cell marker as the contrasting staining results for adencarcinoma (positive staining with AMACR and lack of staining with basal cell marker) will not only complement each other but will also increase the diagnostic confidence.

CONCLUSION

Histopathology is still gold standard for diagnosing prostatic cancer. IHC with AMACR/P504S can detect prostatic adenocarcinoma in both needle biopsy and TURP specimens. AMACR helps in confirming the diagnosis of malignancy in histopathologically suspicious cases. AMACR is a highly sensitive marker for prostatic adenocarcinoma. AMACR also showed high specificity for prostatic adenocarcinoma as all the benign controls showed negative AMACR staining. Expression of AMACR is not affected by the Gleason score or histological grading of the prostatic adenocarcinoma. However because of variable sensitivity of AMACR, the diagnosis of prostatic adenocarcinoma should be based on architectural and cytological findings on H&E stain with use of AMACR in suspicious cases containing atypical glands.

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Original Article

Puberty Menorrhagia: a Challenge and Hope

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ABSTRACT

Introduction: Puberty menorrhagia is defined as excessive blood loss in puberty that interfere with woman's physical, social, emotional or material quality of life. Evaluation of an adolescent girl having menorrhagia should include assessment of anemia and endocrine disorder or any bleeding disorder. When an adolescent girl comes with heavy menstrual bleeding, she should be investigated and managed promptly to improve the quality of life. In most of the cases adolescent girls with heavy menstrual bleeding is diagnosed as ovulatory disorder (due to immature hypothalamo-pitutary-ovarian axis). Bleeding disorder or other pathological causes in this age group are less common. Though it is a great challenge to evaluate an adolescent girl with menorrhagia, but it is matter of hope that mostly are responsive to available medical management promptly. **Methods:** This prospective observational study was carried out between the periods of August 2018 to July 2019 in out-patient private chamber. Fifty adolescent girls (age between 10-20 years) with heavy menstrual bleeding were the study subjects. **Results:** Among them 35 were given norethisterone acetate and 15 were given combined oral contraceptive pill (monophasic OCP containing 30-50 µgm ethinyl estradiol). 37 out of 50 responded within 2-5 days. Remaining 13 cases were given tranexamic acid along with OCP/norethisterone and 11 of them responded within 5-15 days. The remaining 2 refractory cases were hospitalized for further evaluation. **Conclusion:** From the study it can be concluded that medical management is an effective option for managing puberty menorrhagia.

Keywords: Puberty menorrhagia

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Introduction

Heavy menstrual bleeding in an adolescent girl is called puberty menorrhagia. Adolescence is a period of enormous physical and psychological change for young girls. As per WHO, adolescence includes the age group of 10–19 years [1]. Puberty is defined as a process of physical changes through which a child's body matures into an adult body capable of sexual reproduction [2]. There are five main physical features of puberty: Breast growth, pubic hair growth, axillary hair growth, increase in height, and menstruation. Although the mechanisms triggering puberty, remain uncertain, certain

factors are influencing the onset include genetic, nutrition, and body weight and most importantly maturation of hypothalamic-pituitary-ovarian axis [3]. The complete maturation of the axis may take up to 2 years. During this time, it is common for adolescents to present with menorrhagia [4]. Abnormal bleeding accounts for approximately 50% of gynecological visits in adolescent girls [5] with complaints ranging from minimal spotting to heavy bleeding. The onset of menstruation does not mean that ovulation has occurred. Initially, cycles are anovulatory and it may take many years before the menstrual cycles are normalized. Without ovulation, estrogen effect is unopposed by progesterone resulting in endometrial proliferation which outgrows its blood supply and architectural supports resulting in partial breakdown and shedding in an irregular manner.

In addition to the erratic ovulation, there may be many structural or functional etiologies for the abnormal uterine bleeding. The International Federation of Gynaecology and Obstetrics working group on menstrual disorders has recently developed a classification system (PALM–COEIN) for causes of the AUB in non-gravid women [8]. There are nine main categories, which are arranged according to the acronym PALM–COEIN: polyp, adenomyosis, leiomyoma, malignancy and hyperplasia, coagulopathy, ovulatory dysfunction, endometrial, iatrogenic and not yet classified.

Puberty menorrhagia is defined as excessive bleeding in amount (>80 ml) or duration (>7 days) between menarche and 19 years of age [5]. The common causes of puberty menorrhagia are anovulatory cycles, hypothyroidism, polycystic ovary syndrome (PCOD), and coagulation disorders [6, 7]. The adolescents with gynecological problems require sensitive handling as dealing with these issues can be embarrassing for them and are still considered taboo in our society even today. Menorrhagia has a significant effect on the adolescent quality of life, daily school activity, and peer relationship. Many adolescents come late to gynecologists making them more vulnerable to complications such as severe anemia and hypoproteinemia. In all cases of puberty menorrhagia, it is important to exclude pregnancy, especially incomplete abortion and ectopic pregnancy.

Human endometrial function is governed by the ovarian steroid hormones. Most research to date has focused on the role of estrogen and progesterone on the endometrium. During the secretory phase of the menstrual cycle, progesterone is the dominant hormone and is a potent anti-inflammatory agent. In the absence of pregnancy, the corpus luteum regresses and progesterone levels sharply decline. It is this marked reduction in ovarian hormones that triggers an influx of inflammatory mediators into the endometrial environment, leading to shedding and menstruation. Maintenance of progesterone exposure limits endometrial inflammation and prevents menstruation. It is therefore unsurprising that the most effective medical treatments available for puberty menorrhagia are hormonal preparations.

Tranexamic acid is an antifibrinolytic medication commonly used to counteract this aberration in women with heavy menstrual bleeding. It has a short half-life, necessitating regular administration of 1 g orally three- to four-times per day during menses. Tranexamic acid is reported to result in approximately 50% reduction in menstrual blood loss [9, 10].

This study was conducted to determine the outcome of medical management of puberty menorrhagia. The hope is that most of the cases of puberty menorrhagia responded to medical management.

Materials and Methods

The study was conducted in an outpatient chamber among 50 adolescent girls came with complaints of heavy menstrual bleeding. After proper history taking and examination, 35 patients were given norethisterone acetate (5-10 mg, 6-8 hourly initially then in tapering dose after acute bleeding arrested) while 15 were given combined oral contraceptive pill (monophasic OCP containing 30-50 µgm ethinyl estradiol) (initially 6-8 hourly until acute bleeding ceased and then tapering the dose). After cessation of acute bleeding they are advised to continue the effective therapy for next 3 cycles at tapered dose. 37 among the 50 patients responded within 2-5 days. But 13 of them failed to respond who were given OCP/ norethisterone acetate with tranexamic acid. 11 of the 13 patients responded within 5-15 days of treatment. The remaining 2 refractory cases were hospitalized for further evaluation.

Results

Age distribution of the patients were given in table 1 which shows the maximum patients were in the age group of 13-15 years. Table 2 shows 70% patients were given combined oral contraceptive pill (OCP) while 30% were given norethisterone acetate. Time of response to acute bleeding to medical treatment were given in table 3 which shows 75% patients responded to the treatment within 1-3 days. Table 4 shows that 80% patients were responsive to given medical treatment. The non-responsive cases with medical treatment were given tranexamic acid in addition to hormonal therapy and 80% of them were responsive to the combined therapy which is shown in table 5.

Table 1: Age distribution of the patients

Age group (years)	Frequency n (%)
10-12	10 (20)
13-15	33 (66)
16-20	7 (14)
Total	50 (100)

Table 2: Treatment given to the patients

Medical Treatment	Frequency n (%)
OCP	15 (30)
Norethisterone Acetate	35 (70)
Total	50 (100)

Table 3: Time of response to arrest acute bleeding

Time of Response	Frequency n (%)
2-5 days	37 (74)
5-15 days	11 (22)
Non-responsive	2 (4)
Total	50 (100)

Table 4: Response to hormonal treatment with OCP or norethisterone acetate (n=50) alone

Medical Treatment	Frequency n (%)
Responsive	37 (74)
Non-responsive	13 (26)
Total	50 (100)

Table 5: Response of hormonal treatment (OCP or norethisterone acetate) in combination with tranexamic acid (n=13)

OCP/ Norethisterone Acetate with Tranexamic Acid	Frequency n (%)
Responsive	11 (84.61)
Non-responsive	2 (15.39)
Total	13 (100)

Discussion

Menarche is a hallmark event in the life of adolescent girls it marks the transition from childhood to puberty. Most common presentation of abnormal uterine bleeding in adolescents is puberty menorrhagia. It is defined as excessive bleeding occurring between menarche and 19 years of age. Anovulation is responsible for 80% of cases of puberty menorrhagia [11]. Mehrotra [12] in their series found 10% of followed their adolescent patients suffering from menorrhagia.

The primary management of anovulatory bleeding is to control symptoms and prevent anemia. In adolescents with mild bleeding, reassurance and prophylactic iron treatment are suitable. Antifibrinolytic like tranexamic acid are a newer form of treatment in puberty menorrhagia. Plasminogen activators are a group of enzymes that cause fibrinolysis. An increase in the levels of plasminogen activators has been found in the endometrium of patients with heavy menstrual bleeding compared to those with normal menstrual loss. Plasminogen activators have been therefore been prompted as a treatment in heavy menstrual bleeding. Hormones either in the form of progesterone or combined OCP constitute the main medical therapy in the treatment of puberty menorrhagia along with hematinics and tranexamic acid. Various studies suggest that these are of value in arresting profuse hemorrhage [14]. Hormonal therapy restores the balance between prostaglandins and thromboxane A2 and stabilizes the menstrual cycle. In our study we treated our patients with norethisterone acetate (70%) and combined monophasic OCP (30%).

Another treatment option to control abnormal bleeding is cyclic medroxyprogesterone acetate 10 mg orally for 10 days from day 15 of each month. There are some reports expressing doubts about the efficacy of medroxyprogesterone, so we have used norethisterone though it has more androgenic effect than medroxyprogesterone [15]. In the present study, we have used oral norethisterone acetate 10 mg three times daily for 14 days for control of heavy bleeding followed by oral norethisterone acetate 5 mg thrice daily or monophasic combined OCP once daily for next 3 cycles.

We have avoided danazole because of its masculinizing side effects in adolescent girls. Majority of patients (80%) in the present study received a combination therapy of tranexamic acid and norethisterone acetate or COP. Our observations are also similar to the study of Roychowdhury et al. [16].

In our study 74% cases responded to our treatment in 2-5 days which was similar to the study of Gleeson et. al. [10]. 2% cases of our study did not responded to a combination therapy of tranexamic acid and norethisterone acetate and they were sent to hospital for admission for further evaluation.

Conclusion

From the study it can be concluded that medical management is an effective option for managing puberty menorrhagia. Majority of patients respond to the medical management with combined oral contraceptive pill or norethisterone acetate. Non responsive cases with combined oral contraceptive pill or norethisterone acetate are mostly responsive if antifibrinolytic (tranexamic acid) is added.

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Original Article

Marital Adjustment among Women in Slum Areas of Dhaka City

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Abstract

Introduction: Marriage is a major turning point of every married person's life. If a person wants to lead a happy life, he or she must try to satisfy his or her marital life and to satisfy ones marital life ones must struggle both of them jointly. **Objective:** The objective of this study was to assess marital adjustment among slum-dwelling women of Dhaka city. **Methods:** This cross sectional stud was conducted among slum-dwelling women over the period of one year from January to December 2018 among 207 women. Respondents were selected by inclusion criteria and then included by cluster sampling; data were collected by face to face interview with a semi-structured questionnaire to determine socio-demographic characteristics and use RDA scale to assess marital adjustment. Data was analyzed by IBM software-SPSS 25 version. **Results:** In this study, majority i.e.88 (42.5%) of the respondents were in the age group 18-29 years. Most of the respondents were illiterate 50(24.2%) and had informal education 63(30.4%) and maximum up to secondary level 55(26.60%). Majority i.e. 169 (81.6%) respondents were from nuclear family and had low 55(26.60%) and middle 134(64.70%) monthly family income. Age at first marriage for most of the slum-dwelling women was between 12-17 years age group 134(64.7%) and the rest 18-23 years age group was 73(35.3%). Association between educational qualification and age at first marriage was statistically significant ($p<0.01$). Most of the slum-dwelling women 146(70.5%) had no marriage registration document and only 61(29.5%) had marriage registration document. Association between educational qualification and marriage registration document was statistically significant ($p<0.01$). Regarding marital adjustment 75(36.2%) women were distressed in marital life. Association between monthly family income group and marital adjustment was statistically significant ($p<0.01$). **Conclusion:** The study findings identified that the sufferings of slum-dwelling women are miserable. There is no collective forum for raising their voices. Effective measures should be taken to prevent this problem.

Keywords: Marital adjustment

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Introduction

Marriage is one milestone of life, where one selects a partner, with a new role and new expectation. They must have been given many interpretations by different persons, religions, philosophers and may be by the various different persons, religions, philosophers and may be by the various interpretations or definitions. The most commonly accepted concept of marriage is that it is a lifelong sacred commitment. It is one of the most important relationships between a man and a woman. Marriage is the term commonly used to refer to a formally-written, verbal, or tradition long-term agreement between a man and a woman for the production of children, food and other commodities in a domestic context. It is changing in its form according to the change in its culture. It is an institution which permits or admits man and women for family life. It is more or less durable condition between male and female, beyond the mere act of propagation. There has been mutual attraction due to certain biological and psychological causes resulting in the establishment of intimate relationship [1]. Marital adjustment is an adjustment of two personalities, which had different socio-cultural backgrounds. Marital adjustment is complex than it may appear. Two persons entering marriage must adjust to each other's sensory, motor, emotional and intellectual capacities [2]. The success of marital life much depends upon the success in marital adjustment by the husband and wife. Marital maladjustment results in conflicts and tensions and many a time divorce. The relationship a man has with a women in marriage is perhaps the highest form of relationship, because it involves physical, emotional, spiritual and social aspects of life. Marital adjustment as the state in which there is an over all feeling in husband and wife of happiness and satisfaction with their marriage and with each other. Marital adjustment is satisfactory relationship between spouses characterized by mutual concern, care, understanding and acceptance. So happiness, satisfaction and fulfillment of expectations are possible only by mutual adjustments that lead to a common concept of marriage. There are six areas of marital adjustment. They are religion, social life, mutual friends, in laws, money and sex [3]. female in the slum becomes an easy victim of violence by her husband mainly because of constant demand of dowry, which the family cannot meet, and also polygamy and economic insecurity. These women in many cases had married without registration. Most of them get married at an early age, which affects their health and marital life. They are not conscious about their legal rights. Most of the women did not have any knowledge that they can take legal steps and can claim dower (Mohr) money, which the husband promised to give to his wife during marriage.Most of them get marriage registration document (Nikah nama) with them, but being illiterate they do not understand what is written there. The 'Kazis' take this opportunity to exploit them. The practice of dowry is higher in the slum [4].

Materials & Methods

This cross sectional study was conducted among 207 slum-dwelling women of Dhaka city during the period of January to December, 2018. By cluster sampling technique, 207 slum-dwelling women were included in this study. After taking informed written consent data were collected by face to face interview by using a pre-tested semi-structured questionnaire .Ethics was maintained strictly at different stages of this study. After data collection data were checked thoroughly for any inconsistency and incompleteness. Then analysis done by using SPSS software.

Results

Regarding age, majority i.e. 88(42.5%) of the respondents were in the age group 18-29 years followed by 69(33.3%) were in the age group 30-39 years and the rest 50(24.2%) were in the age group 40-49 years. Participant's mean (±SD) age was 31.05 (±8.287). Regarding educational qualification of the respondent i.e. 63 (30.4%) respondents had informal educa

tion while 50 (24.2%) respondents had illiterate education, 55 (26.6%) respondents studied up to secondary education level and the rest 39 (18.8%) respondents studied up to primary level. In respect of occupation of the respondents majority of the respondent i.e. 111 (53.6%) were housewife, followed by 73 (35.3%) were serviceholder and the rest 23 (11.1%) were engaged in business. In respect of husband's occupation of the respondents most i.e. 93 (44.9%) were businessman, followed by 62 (30.0%) were serviceholder and the rest 52 (25.1%) were day laborer. In this study, 134 (64.7%) respondent had monthly income within the range of BDT.11000-21000 followed by 55 (26.6%) had income within BDT.6000-10000. Rest 18 (8.7%) had family income within BDT.21000 - 30000 . Mean (±SD) of monthly family income was BDT.14985.51 (±5413.466). Regarding family type, majority i.e. 169 (81.6%) family were nuclear type and the rest 38 (18.4%) were joint type of family. In respect of family members, majority i.e. 126 (60.9%) respondents had 2 – 4 family members and 81 (39.1%) respondents had 5-7 members in the family. Family member's mean (±SD) was 4.15(±1.171) .Regarding the housing condition, majority of the respondent's house i.e.177 (85.5%) were semipucca and Pucca were 30 (14.5%). In respect of age at 1st marriage, most i.e. 134 (64.7%) respondent's age at 1st marriage was in the age group 12-17 years and the rest 73 (35.3%) was in the age group 18-23 years. Respondent's age at 1st marriage mean (±SD) was 16.37(±2.161) . In respect of marriage registration document, i.e. majority 146 (70.5%) women had no marriage registration document and 61 (29.5%) had marriage registration document. Among illiterate group, 45 (90.0%) respondent's age at 1st marriage was between 12-17 years and 5 (10.0%) was in 18-23 years. In case of informal education group, 44 (69.8%) respondent's age at 1st marriage was in 12-17 years and 19 (30.2%) was in18-23 years followed by primary group 23 (59.0%) respondent's age at 1st marriage was in 12-17 years and 16 (41.0) was in 18-23 years. The rest secondary group 22 (40.0%) was between 12-17 years and 33 (60.0) was in18-23 years.There was statistically significant association between educational qualification and age at 1st marriage ($p<0.01$). Among illiterate group, 48 (96.0%) respondent had no marriage registration document and only 2 (4.0%) had. In case of informal education group, 47 (74.6%) respondent had no and 16 (25.4%)) had marriage registration document followed by primary group 26 (66.7%) respondent had no and 13 (33.3%) had marriage registration document.The rest secondary group 25 (45.5%) respondent had no and 30 (54.5%) had marriage registration document. There was statistically significant association between educational qualification and marriage registration document ($p<0.01$). Regarding consensus by RDA scale 143 (69.1%) women lead non-distress adjustment and 64 (30.9%) women lead distress adjustment in marital life. Regarding satisfaction by RDA scale 154 (74.4%) lead non-distress adjustment and 53 (25.6%) lead distress adjustment in marital life.In case of cohesion by RDA scale 199 (96.1%) women lead distress adjustment and 8 (3.9%) lead non-distress adjustment in marital life. Regarding overall score by RDA scale 132 (63.8%) women lead non-distress adjustment and 75 (36.2%) women lead distress adjustment in marital life.

Discussion:

This cross sectional study was conducted among slum-dwelling married women. The objective of this study was to assess marital adjustment and determine socio-demographic condition of slum dwelling women. In this study lowest monthly family income was 6000 BDT and highest family income was 30000 BDT. Majority of the participant's i.e 64.7% were 11000-21000 BDT. , 26.6% were 6000-10000 BDT and 8.7% were 21000-30000 BDT. Another study conducted to assess the socio-economic condition of slum-dweller. Here monthly family income of 19.17% was 1000-5000 BDT and 58.9% was between 6000-10000 BDT while 14.6% and 9.13% was 11000-15000 BDT and 16000-20000 BDT [5]. Possibly this dissimilarity occurs due to that study was conducted on 2015 and this study was conducted on 2018. Regarding educational qualification of the respondent i.e. 63 (30.4%) respondents had informal education while 50 (24.2%) respondents had illiterate education, 55 (26.6%) respondents studied up to secondary education level and the rest 39 (18.8%) respondents studied up to primary level. Another study was conducted among slum-dwelling women and there was found that, 75% respondents had not getting any education. Only 10% were getting secondary education and 15% were getting primary education [6]. Among illiterate group, 48 (96.0%) respondent had no marriage registration document and only 2 (4.0%) had. In case of informal education group, 47 (74.6%) respondent had no and 16 (25.4%)) had marriage registration document followed by primary group 26 (66.7%) respondent had no and 13 (33.3%) had marriage registration document. The rest secondary group 25 (45.5%) respondent had no and 30 (54.5%) had marriage registration document. There was statistically significant association between educational qualification and marriage registration document ($\chi2=30.027$, $p<0.01$). By marriage registration document, the average total score of RDA scale was higher among the women who had marriage registration document (45.75) in comparison to who had no marriage registration document (41.99). The difference was statistically significant ($p<0.05$). In this study regarding marital adjustment 132 (63.8%) women lead non-distress adjustment and 75 (36.2%) women lead distress adjustment in marital life. Another study was also conducted among slum-dwelling women in Bangladesh showed that 38% had happy , 32% had fairly happy and 30% had unhappy married life [7].In current study, marital adjustment was associated with monthly family income and this association was statistically significant ($\chi2=12.670$, $p<0.01$).In another study was conducted among santal people and there also found that marital adjustment was associated with monthly family income and this association was ststistically significant ($p<0.01$) [8].

Table: Distribution of women by marital adjustment (Domain specific)

Domain	Marital Adjustment		Total
	Distress n(%)	Non-distress n(%)	n(%)
Consensus	64 (30.9%)	143 (69.1)	207 (100)
Satisfaction	53 (25.6%)	154 (74.4)	207 (100)
Cohesion	199 (96.1)	8 (3.9)	207 (100)
Overall Score	75 (36.2)	132 (63.8)	207 (100)

Conclusion

Marriage is a social and religious contact. The success of marital life much depends upon the success in marital adjustment by the husband and wife. This adjustment affect not only the person but also the whole family and society in which she belongs. This cross sectional study was conducted among slum-dwelling women to assess adjustment. Most of the slum-dwelling women were illiterate and had informal education and maximum education level up to secondary education. Majority of the respondent' were housewife and their husband were engaged in business. Most of them were from nuclear family and from low monthly family income. Age at first marriage of maximum respondent was below 18 years and child marriage is a violation of human rights. Child brides experience the detrimental physical, psychological and social consequences of child marriage. This is a global phenomenon and a grave cause for concern. On the other hand these women lacked knowledge about conjugal life. In this study showed that age at first marriage, marriage registration document were associated with educational qualification of slum-dwelling women. Among the respondents about more than one-third respondents were distressed in marital life. In distressed couple were belongs in low monthly family income group and monthly family income was associated with marital adjustment. So, the people who live in slum area work very hard but cannot achieve minimum living standards. Due to poor socio-economic condition they lead unstable and fragile marital life. They do not get any respect from their family and society. So, Comprehensive national policies and plans should be developed to ensure their legal rights and promote the benefits and possibilities of extending basic services.

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Original Article

Comparison of Pain Factors in Open versus Laparoscopic Inguinal Mesh Hernioplasty

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ABSTRACT

Introduction: Inguinal hernias are one of the most commonly performed surgery by a general surgeon. There have been numerous open surgical techniques and two laparoscopic techniques described in the literature for thetreatment of inguinal hernias. The treatment outcome of all these surgeries remains the same which is reducing the hernia and preventing recurrence. Our aim was to compare laparoscopic versus open inguinal hernia repair with emphasis on postoperative pain. **Methods:** Sixty patients with unilateral primary inguinal hernias were randomly divided into two groups. Each group included 30 patients. Group one was treated by open Lichtenstein repair, while the second group was treated by laparoscopic transabdominal preperitoneal (TAPP) mesh repair. The two groups were compared to assess the duration of surgery, postoperative pain, duration of hospital stay, return to normal activity, and work. **Results:** Laparoscopic TAPP repair was found to have a longer operative time as compared to Liechtenstein open repair. In terms of other parameters such as postoperative pain, duration of hospital stay, return to normal activity, and work the laparoscopic group was superior. After a one-year follow-up, none of the patients had any chronic pain or evidence of hernia recurrence. **Conclusion:** Laparoscopic TAPP has a clear advantage over the conventional Liechtenstein open surgery especially in terms of reduced early postoperative pain and return to normal activity. Keywords: inguinal hernia, Lichtenstein's repair, postoperative pain, laparoscopic TAPP repair, inguinal hernia repair.

Keywords: Inguinal hernia, Lichtenstein's repair, postoperative pain, laparoscopic TAPP repair, inguinal hernia repair

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Introduction

One of the most common surgeries performed by a general surgeon is inguinal hernia repair. Lichtenstein tension-free hernioplasty was first described in 1989 and is considered as the gold standard treatment for inguinal hernias [1]. Apart from Liechtenstein open hernia repair, the laparoscopic approach has gained significant popularity in many centers throughout the world. The reason for its popularity is a shorter length of hospital stay and quicker resumption of normal activity [2, 3]. Laparoscopic surgery, in general, is associated with less postoperative pain as it involves less surgical trauma in view of smaller incisions as compared to open surgery. Chronic pain is an important factor in the context of hernias as hernia repair involves the placement of a mesh during repair which can contribute to neuralgia. Hence, our study aims to compare laparoscopic transabdominal pre peritoneal (TAPP) hernia repair with open Liechtenstein mesh repair with emphasis on early and late postoperative pain.

Materials & Methods

This prospective randomized study included 60 patients diagnosed with primary inguinal hernia. Patients were randomly divided into two groups. The first group of 30 patients underwent open Lichtenstein tension free hernioplasty while the remaining patients in the second group underwent laparoscopic transabdominal pre peritoneal (TAPP) mesh repair. Inclusion criteria were patients with uncomplicated primary unilateral inguinal hernia aged from 18 to 70 years. Exclusion criteria included patients with bilateral inguinal hernias, complicated inguinal hernias, recurrent hernias, chronic liver or renal disease, unfit for major surgery, and history of chronic groin pain.All the surgeries were performed in Satkhira Medical College Hospital, Sadar Hospital & different private Hospitals in Satkhira. Patients were kept nil by mouth for about six hours prior to surgery and received a single dose of antibiotic prophylaxis half an hour before surgery. The type of anesthesia used was spinal anesthesia for open cases and general anesthesia for laparoscopic TAPP. Epidural anesthesia was not administered in any of the cases. In all patients, a monofilament polypropylene mesh was used and fixed appropriately. During the postoperative period, all patients received intramuscular non-steroidal anti-inflammatory 12thhourly for one day postoperatively. The intramuscular injection was continued for an additional 24 hours if pain was severe [visual analogue scale (VAS) >IV]. Oral non-steroidal anti-inflammatory drugs (NSAIDs)were prescribed as and when required after 48 hours of surgery.All the patients were encouraged for oral feeds after eight hours, initially, the feeds were sips of liquids followed by normal diet after the resolution of postoperative ileus (indicated by passing of flatus and normal bowel sounds on auscultation and return of appetite). The wounds were inspected for any seroma, hematoma or any infection. Patients were discharged after complete ambulation and tolerating normal diet. The pain experienced by the patients in the postoperative period was graded according to the visual analoguescale (VAS) ranging from no pain to the worst possible pain on the scale of 0 to 10. It was recorded on the first and seventh postoperative day. After discharge, patients were encouraged to take normal diet and return to their normal activities as early as possible. After discharge, patients were followed up at one week,one month, three months, and six-month intervals. In the initial follow-up, the patients were evaluated for short-term complications like seroma or hematoma and wound infection. During subsequent visits,

chronic pain at the operated site, return to normal activity were noted. The two groups were compared regarding operative time, postoperative complications, postoperative pain, hospital stay, time to return to normal activity as well as work, and one-year recurrence rate.

Results

The age of patients ranged from 18 to 70 years with the mean being 43 in the open group and 37 in the laparoscopic group. There were more males as compared to females in both the open group and laparoscopic group.

The right inguinal hernia was more common as compared to the left.

Table 1: Patient and hernia characteristics

	Lichtenstein open repair (n = 30)	Laparoscopic TAPP (n = 30)
Mean Age (Y)	43	37
Male sex	26 (28.7%)	28 (93.4%)
Female sex	4 (13.4%)	2 (6.7%)
Hernia in right side	18	16
Hernia in left side	12	14

Laparoscopic TAPP had superior outcomes as compared to open Liechtenstein repair (Table 2). Laparoscopic TAPP has a significant longer operative time; 120 minutes, compared to 55 minutes for open mesh repair. The mean pain score by the visual analogue scale after the first 24 hours was significantly less after laparoscopic TAPP compared to open mesh repair 2.5 compared to 4.

Table 2: Operative outcomes

	Lichtenstein open repair (n = 30)	Laparoscopic TAPP (n = 30)
Operative time (minutes)	55	120
Pain score after 24 hours	4	2.5
Pain score after 7 days	3	1.25
Return to normal activity (days)	7	3
Return to work (days)	21	10

A similar outcome was seen after one week where the mean pain score was 1.25 in the laparoscopic TAPP group, compared to 3 in the open group. Only one patient in the open group had developed a seroma which was treated by aspiration. There were no other wound-related complications.Hospital stay was significantly shorter in the laparoscopic TAPP group compared to open repair 3 days versus 7 days. Lap TAPP group had a significantly faster return to normal activity compared to the open group; 7 days compared to 3 days respectively. Most of our patients that underwent surgery had an active work life and the laparoscopic TAPP group had asignificantly shorter time for return to work compared to the open group 10 versus 21. After a 12-week follow-up for chronic pain assessment, none of the patients had any complaints ofpostoperative pain or neuralgia.

Discussion

The introduction of Lichtenstein tension-free hernioplasty considerably reduced the chances of hernia recurrence to as low as 1-4% making it a gold standard treatment. Since minimally invasive surgery is beingused more commonly

with most surgeries, it has the potential to be the new gold standard if done withexpertise [4]. A total of 60 patients were involved in this study, of which 30 patients underwent laparoscopic TAPP and the remainder underwent Liechtenstein open hernioplasty. There was no statistically significant difference found in terms of age or sex in the study population.

In our study, the operative time for open surgery was significantly shorter as compared to laparoscopic TAPP.This may be due to the fact that setting up laparoscopic equipment takes a longer time as compared to opensurgery. Scheuermann et al. [5] in their meta-analysis found that the operative time is longer in laparoscopicTAPP as compared to open surgery. Utiyama et al. [6] on the other hand found no significant difference. Studies have demonstrated that with specialization and experience, the operative time becomes clinicallyirrelevant [5,7].In terms of postoperative pain and duration of hospital stay, laparoscopic TAPP was at a clear advantage.Since patients undergoing laparoscopic surgery had less postoperative pain, this ensured that they would mobilize early and it resulted in early discharge from the hospital as compared to the open group. One of the reasons for chronic pain following hernia surgeries is entrapment of the sensory nerves. Dividing orpreserving these nerves during surgery has been debatable with no clear advantage of one over the other[8,9]. Laparoscopic TAPP involves a posterior approach, hence significantly reduces the chances of sensory
nerve entrapment. In our study, none of the patients had any postoperative pain or neuralgia after the initial12-week follow-up or after one-year of follow-up. Douek et al. [10], in their study, found that after a five year follow-up 12 out of 242 patients that had undergone Liechtenstein open repair had paraesthesia and no such findings in the laparoscopic group. Patients that underwent laparoscopic surgery in our study returned to normal activity and work quicker ascompared to the open group which were similar to findings by Neumayer et al. [4]In this study, we did not take the cost factor into consideration but since patients in the laparoscopic groupwere sent home early, this may bring down the overall cost of hospitalization but further studies need to bedone to check its statistical significance. One of the disadvantages of our study is that we did not have measures for long-term follow-up to look forrecurrence as well as assess patients for pain after one-year following their surgery.

Conclusions

Laparoscopic TAPP for primary unilateral inguinal hernia have a better outcome than open Lichtensteinrepair, especially in terms of postoperative pain and early resumption of normal activity and work.

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Original Article

Hepatoprotective Effect of *Nigella Sativa* (Black Cumin) in Rats

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ABSTRUCT

Introduction: The liver is the largest exocrine gland in our body. It is the vital organ undertaking wide range of functions, such as detoxification, metabolism, storage of iron and vitamins. It is very much important to protect liver in spite of continuous hepatotoxicity. The present study has been carried out with the aim to investigate the hepatoprotective effect of *Nigella sativa* (black cumin) in paracetamol induced acute toxicity in rat. **Materials and methods:** This study was conducted at the department of Pharmacology, Dhaka Medical College from July 2014 to June 2015. Seven rats were taken as control while 7 rats were given hepatotoxic dose of paracetamol and 7 were given hepatotoxic dose of paracetamol along with *Nigella sativa*. Serum level of bilirubin, ALT and AST were compared to find out any hepatoprotective effect *Nigella sativa*. **Results:** Serum bilirubin level were 0.55 ± 0.09 mg/dl, 3.31 ± 0.53 mg/dl and 0.86 ± 0.17 mg/dl in control group, pretreated paracetamol and pretreated paracetamol with *Nigella sativa* group respectively, serum ALT were 18.51 ± 2.00 U/L, 76.74 ± 17.69 U/L and 28.81 ± 8.26 U/L in control group, pretreated paracetamol and pretreated paracetamol with *Nigella sativa* group respectively and serum AST were 24.75 ± 3.27 U/L, 69.23 ± 15.18 U/L and 33.97 ± 14.07 U/L in control group, pretreated paracetamol and pretreated paracetamol with *Nigella sativa* group respectively which were statistically significant. **Conclusion:** Compiling all results of this study it can be concluded that *Nigella sativa* have hepatoprotective activity. These results provide a rationale for the use of *Nigella sativa* in the development of new herbal medicine, much needed for the treatment of various liver ailments.

Keywords: *Nigella sativa*

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INTRODUCTION

The liver is the largest exocrine gland in our body. It is the vital organ undertaking wide range of functions, such as detoxification, protein-fat-carbohydrate metabolism, storage of iron and vitamins. The liver also plays major role in decomposition of RBCs, hormone production, plasma protein synthesis, glycogen storage and synthesis of urea. The organ liver is inevitable for survival and one cannot live without it for long period [1]. Liver is the key organ for detoxification of toxic substance and disposition of endogenous substances [2]. It is continuously and widely exposed to toxins and chemotherapeutic agents that lead to impairment of its functions. All types of injuries to the liver (e.g. circulatory, traumatic, toxic or microbiological) lead to damage of hepatocytes which results in its malfunctioning [3]. Therefore, the disruption of the integrity of liver function leads to fatal cases or even irreversible organic death. In liver injury, it is supposed that the intervention of free radicals in normal metabolic process is responsible for the pathological changes. Free radicals attack biomolecules and induce lipid peroxidation, enzyme inactivation, and finally cell necrosis [4].

Hepatotoxicity is a growing concern of today's modern society. The World Health Organization (WHO) estimates that 140 million people worldwide suffer from alcohol dependency [5]. The increasing incidence of alcoholism, cigarette smoking, abusing substance and other unhealthy lifestyle options, like eating fatty foods, have contributed to the morbidity and mortality due to liver disease [6]. More than 900 drugs have been implicated in causing liver injury. Chemicals often cause subclinical injury to the liver, which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures [7].

Paracetamol or acetaminophen, chemically named N-acetyl-p-aminophenol (APAP) is generally safe for use at recommended doses [8]. The initial symptoms of overdose are nausea, vomiting, diarrhea and abdominal pain. Acute overdoses of paracetamol can cause potentially fatal kidney, brain and liver damage and, in rare individuals, a normal dose can do the same [9]. In cases of paracetamol overdose, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the cytochrome P450 system to produce NAPQI. As a result, hepatocellular supplies of glutathione become depleted, as the demand for glutathione is higher than its regeneration [10]. NAPQI therefore remains in its toxic form in the liver and reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis [11].

Nigella sativa (NS), an annual Ranun-culaceae herbaceous plant, is commonly known as black seed, belongs to botanical family of Ranunculaceae. It is commonly used as natural food additive [12]. It has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for promotion of good health and treatment of many diseases e.g., asthma. Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, anti-inflammatory and anti-tumour agents [13]. The major active principle of NS is Thymoquinone, which is the bioactive and the most abundant constituent of the volatile oil of this seed which has been shown to possess therapeutic effects, including anti-inflammatory, antimicrobial, anticancer, antihypertensive, antidiabetic and antioxidant agent [14]. In recent year, it has been suggested that oil of NS has protective role against CCl_4 and D-galactosamine-induced hepatic damage in rats [15]. It has been reported that NS oil protects liver against Schistosoma masoni induced liver damage [16]. Pal et al. revealed that NS oil was able to give protection against paracetamol induced liver damage.

So, we have done an experiment in rats with the research question of is there any difference in hepatoprotective effect among *Nigella*

sativa in paracetamol induced acute toxicity in rat? Our aim was to find out the hepatoprotective effect of *Nigella sativa* in animals.

MATERIALS AND METHODS

This study was conducted at the department of Pharmacology, Dhaka Medical College from July 2014 to June 2015. Seven rats were taken as control while 7 rats were given hepatotoxic dose of paracetamol and 7 were given Nigella sativa. Serum level of bilirubin, ALT and AST were compared to find out any hepatoprotective effect *Nigella sativa.*

The experiment was carried out on 21 Long Evan Norwegian rats of either sex weighing between 150-200 grams which were collected from icddr,b, Dhaka. The rats were kept in animal house of the Department of Pharmacology, Dhaka Medical College. Rats of different batches of different groups were kept in different metallic cages. Male and female rats were also kept in different metallic cages and were allowed to feed on standard laboratory diet and to drink ad libitum. These rats were acclimatized five days at room temperature and humidity.

The rats were grouped in-

Group A: This group consisted of 7 rats, were served as control. They received normal pelleted diet with water adlibitum for 15 days. On the 16th day 2cc of distilled water was feed orally to each rat through nasogastric tube.

Group B: This group consisted of 7 rats, were fed on normal diet with water adlibitum for 15 days. On the 16th day all the rats were treated with powdered Paracetamol orally at the dose of 2gm/kg body weight dissolved in 2cc distilled water.

Group C: This group consisted of 7 rats, received normal diet with adlibitum along with Nigella sativa at a dose of 800mg/kg body weight for 15 days. Then on the 16th day, all the rats were treated with powdered paracetamol orally at a dose of 2 gm/kg body weight dissolved in 2cc distilled water.

At the end of the scheduled treatment and after overnight fasting on the 18th day, blood sample was collected from all groups of rat by cardiac puncture and was collected in plain test tube for estimation of serum bilirubin, serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST).

RESULTS

We have taken serum bilirubin, ALT and AST as marker of hepatotoxicity. We have compared these markers with control group (Group A), the group given toxic dose of paracetamol (Group B) and the group given *Nigella sativa* (Group C).

The comparison of serum bilirubin, ALT and AST between group A and group B is shown in table 1 which shows significant hepatotoxic effect of paracetamol on rats.

Table 2 shows the markers of hepatotoxicity is significantly normal after given toxic dose of paracetamol as well as *Nigella sativa.*

DISCUSSION

In this study, hepatotoxicity was induced by single oral administration of paracetamol at the dose of 2gm/kg body weight. Hepatic damage was assessed by significant rise of serum bilirubin, ALT and AST, which were compared to that of the control group. The elevation of the enzyme levels was found by several studies [12, 17].

N. sativa oil was used at a dose of 800mg/kg body weight per day for 15 days. The dose, route of administration and duration was selected according to Pal et al. and El-Dakhakhny et al. [12, 15].

In the present study, comparison of hepato-

Table 1. Comparison of serum bilirubin, ALT and AST between group A and group B

Serum Level	Group	Mean±SD	p value
Serum Bilirubin (mg/dl)	A (Control) B (Paracetamol)	0.55±0.09 3.31±0.53	<0.001[s]
Serum ALT (IU/L)	A (Control) B (Paracetamol)	18.51±2 76.74±17.69	<0.001[s]
Serum AST (IU/L)	A (Control) B (Paracetamol)	24.75±3.27 69.23±15.18	<0.001[s]

s=significant

Table 2. Comparison of serum bilirubin, ALT and AST between group B and group D

Serum Level	Group	Mean±SD	p value
Serum Bilirubin (mg/dl)	B (Paracetamol) D (Pretreated NS)	3.31±0.53 0.86±0.17	<0.001[s]
Serum ALT (IU/L)	B (Paracetamol) D (Pretreated NS)	76.74±17.69 28.81±8.26	<0.001[s]
Serum AST (IU/L)	B (Paracetamol) D (Pretreated NS)	69.23±15.18 33.97±14.07	<0.001[s]

s=significant

protective effects of N. sativa was evaluated by estimating the levels of serum bilirubin, ALT and AST. There was evidence of significant prevention in rise of the biochemical parameters pretreated with *Nigella sativa.* Same observation were found by most of the studies reviewed so far [12, 14 and 17].

Comparing the biochemical findings in different groups of experimental animal, it is obvious that toxic effects in the liver produced by paracetamol can be prevented by pretreated *Nigella sativa.*

CONCLUSION

Compiling all results of this study it can be concluded that *Nigella sativa* have hepatoprotective activity. These results provide a rationale for the use of *Nigella sativa* in the development of new herbal medicine, much needed for the treatment of various liver ailments.

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Original Article

Identification of Different Pseudomonads by Culture, PCR and RFLP

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ABSTRUCT

Introduction: Despite the progress made with respect to infection control and wound management, wound infection still remains a serious and significant clinical challenge particularly in developing countries. The value of microbiology in the prevention and treatment of wound infection cannot be overemphasized, so proactive measures are taken with the knowledge of the prevailing organisms. The prevalence of different bacteria in infected wounds varies and pseudomonads are the most common pathogen associated with wound infection. **Materials and methods:** This cross-sectional study was conducted to isolate and identify different Pseudomonads in clinical samples by culture, PCR and RFLP. Wound swab and ETA were collected from different patients of Dhaka medical college hospital. Organisms were identified by culture. ESBL and MBL producing Pseudomonads were identified by phenotypic method. **Results:** Out of total 310 samples, 238 (76.77%) were gram negative bacteria and 108(72.00%), 45(69.23%, 47(85.45%) and 38(95%) were isolated from surgical, burn, traumatic wound and ETA samples respectively. Of these, 82 (26.45%) isolated organisms were Pseudomonads. Among 82 Pseudomonads, 76 (92.68%) were Pseudomonas aeruginosa, one (1.22%) was Stenotrophomonas maltophilia, one (1.22%) was B. cepacia, 2 (2.44%) were P. fluorescence and 2(2.44%) were P. alcaligenes by culture. Of the 310 samples, 88 Pseudomonads were identified by PCR. Among them, 86 (97.72%) were Pseudomonas aeruginosa, one (1.14%) was Burkholderia cepacia and one (1.14%) was Stenotrophomonas maltophilia. By PCR, 88 samples were identified as Pseudomonads, 76 samples were positive by both culture and PCR whereas two samples that were positive in culture were found negative by PCR. Twelve of the culture negative samples were positive by PCR. Considering culture as gold standard, the sensitivity of PCR was 97.44% and specificity was 94.74%. RFLP analysis was done with 88 Pseudomonads that were identified by PCR. All the Pseudomonas aeruginosa demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp. In case of B. cepacia, there were four bands at approximately 120, 150, 280 and 390 bp and in case of S .maltophilia, there were five bands at approximately 55, 93, 110, 320 and 448 bp. **Conclusion:** The results of this study provided insights into the presence of different Pseudomonads in DMCH. In addition to culture, PCR and RFLP may be suitable method to identify and differentiate species of Pseudomonads and to treat them accordingly.

Keywords: Pseudomonads, PCR, RFLP

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INTRODUCTION

Despite the progress made with respect to infection control and wound management, wound infection still remains a serious and significant clinical challenge particularly in developing countries. In these countries, wound site infections are a major source of post-operative illness, a cause of death among burn patients [1] and accounts for approximately a quarter of all nosocomial infections [2]. These infections have resulted in increased trauma to patient, prolonged hospitalization, increased hospital cost. So the management of infection is a complex and important aspect of wound care. The value of microbiology in the prevention and treatment of wound infection cannot be overemphasized, so proactive measures are taken with the knowledge of the prevailing organisms [3]. The prevalence of different bacteria in infected wounds varies and pseudomonads are the most common pathogen associated with wound infection [4, & 5]. According to the National Nosocomial Infections Surveillance system report from October 2004, Pseudomonads are immensely concerning to the researchers due to its multidrug resistant pattern.

Infections in burn wounds also carry a heavy medical and economic burden not only in the developed world, but also in the developing world, where 70% of burns affect children and mortality is approximately 100% in patients with burns covering 40% total body surface area [6, 7]. Burn infections caused by P. aeruginosa often deteriorate rapidly and lead to systemic spread and death within days or weeks [6]. Japoni, 2009 [8] reported that 22 to 73% Pseudomonas strains were isolated from wound of burn patients.

Healthcare Associated Infections (HAIs) (or nosocomial infections) are the worldwide public health problem causing morbidity and mortality especially in the developing countries. Furthermore, if the causative organism has developed resistance to a number of antimicrobial agents, management of the issue gets harder [9]. Intensive care units (ICUs) are units where healthcare infections seen more often because of commonly critically ill patients and invasive interventions used in these units. In ICUs antimicrobial resistance rates are increasing because of various reasons such as broad spectrum and/or inappropriate antimicrobial usage and prolonged length of stay in hospital [10]. As a result, it increases healthcare infection rates caused by multidrug resistant microorganisms. These infections require more extensive diagnostics and treatment and are associated with additional costs [10, 11].

Pathogens differ greatly between different types of ICU corresponding to the different risk structure of the patients. Different studies have been conducted to highlight the incidence and importance of hospital acquired infections in ICUs, to contribute to empirical treatment methods by determining the causes of common hospital infections and antibiotic resistance rates, to minimize the emergence of resistant microorganisms by preventing unnecessary antibiotic use, and to emphasize the need for protective measures against risk factors that favor hospital infections [12, 13, 14].

Pseudomonads are in the third rank to cause UTIs [15] and dermatitis, otitis, conjunctivitis, GIT, soft tissue, bone, and joint infections are also often caused by these species [16]. In fact Pseudomonads induced invasive infection is observed with growing frequency among immunocompromised hosts and patients with some predisposing conditions, such as malig

nancies, extremes of age, neutropenia, prolonged hospitalization, surgery, trauma, and instrumentation [17]. It is the main causative agent of morbidity and mortality in patients of grown age with cystic fibrosis of the respiratory tract infection [18].

Among the Pseudomonads, P. aeruginosa is more frequently isolated which can colonize the healthy individual as harmless saprophytic and opportunistic pathogen of human [19]. This bacterium represents an opportunistic pathogen with high clinical relevance in intensive care units [20]. P. aeruginosa related infections are frequently life threatening and often difficult to treat due to the intrinsic resistance to many antimicrobial agents. Moreover, the resistance to antipseudomonal agents has become an increasing problem in recent years [21, 22, and 23].

Since determining bacterial isolates relatedness is essential for understanding the transmission routes, different typing methods that can be divided into two major categories, phenotypic and genotypic methods, have been established [38]. Phenotypic methods such as serotyping, pyocin typing and antimicrobial susceptibility typing depend on the environmental factors, therefore, have low discriminatory power [24].

Phenotype based identification is time consuming and may misidentify different Pseudomonads and other non-fermenting gram-negative bacilli due to their close similarity [25]. This may affect patient management, particularly with respect to antimicrobial therapy, patient prognosis and infection control. Genotypic methods have been performed by different typing techniques such as Polymerase Chain Reaction(PCR), Restriction Fragment Length Polymorphism (RFLP), Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR), Variable Number Tandem Repeat (VNTR), DNA Hybridization and Random Amplifed Polymorphic DNA (RAPD) [40-45]. However, PCR-RFLP assay represents an excellent, simple and cheap complement to the conventional microbiological methods for rapid and early diagnosis of Pseudomonas infection.

MATERIALS AND METHODS

It was a cross sectional study done in the department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh between the periods from January, 2016 to December, 2016. Total 310 samples were collected. Among them 150, 65 and 55 were surgical, burn and traumatic wound samples respectively and 40 were endotracheal aspirates. Wound swab sample was taken from clinically diagnosed infected patients who got admitted in surgery, burn, gynecology and orthopedics unit of Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh irrespective of age, sex and antibiotic intake. The procedure of wound swab collection was explained to the patient and written informed consent was taken. Two samples (one for culture and another for PCR and RFLP) were collected aseptically by sterile swab stick avoiding contamination from external sources. Swabs were collected using sterile cotton tipped swab sticks from clinically deep area of wound site prior to any cleansing. In case of collection of samples from dry surfaces, swabs were moistened with sterile normal saline. The specimens were immediately kept in a sterile test tube, capped properly and labeled. Then the samples were transferred to the microbiology laboratory without delay.

The patients who predominantly developed respiratory symptoms (fever with excessive

airway secretion) while using endotracheal tube for more than 48 hours. For endotracheal aspirate (ETA), aseptically a 50 cm 14 Fr size sterile suction catheter was introduced gently through the endotracheal tube (ETT) for a distance of about 25-30 cm. The ETA was obtained by suction without giving saline. The tips of the catheter were brought to the laboratory for sample processing.

Samples were submerged under PBS in sterile test tube and centrifuged at 9500 rpm for 5 minute under 4°C to make pellet. Then, the supernatant was discarded and the deposit was kept at -20°Cas pellet until DNA extraction (Laboratory for Environmental pathogens Research Department of Environmental Science University of Toledo, 2004).

300 µl of sterile distilled water was added to the micro centrifuge tubes having pellets and vortexed until mixed well. Then the mixture was heated at 100ºC for 10 minutes in a heat block. After heating, immediately the micro centrifuge tubes were placed in an ice pack and was kept for 5 minutes and then centrifuged at 13000 rpm for 6 minutes at 4ºC. Finally, the supernatant was taken into another eppendorf tube and was used for PCR. Extracted DNA was preserved at 4ºC for 7-10 days and -20ºC for prolong use.

Amplification in thermal cycler (Gene Atlas, Master Cycler gradient, Japan, model-482) PCR reaction consisted of preheat of preheat at 94ºC for 10 minutes followed by 36 cycles of denaturation at 94ºC for 30 seconds, annealing at 56ºC for 40 seconds, extension at 72ºC for one minute with a final extension at 72ºC for 10 minutes. Annealing temperature varies with GC contents of primers.
RFLP analysis was done with 88 pseudomonads that were identified by PCR.

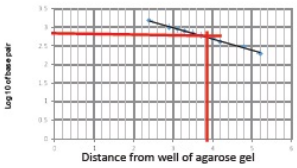

Figure: Log graph for determination of base pair of unknown PCR product comparing to known base pair of DNA ladder.

RESULTS

Table 1. Of the total 310 samples, 150, 65 and 55 were surgical, burn and traumatic wound samples respectively and 40 were endotracheal aspirates. From these 310 samples, 238 (76.77%) gram negative bacteria were isolated.

Table 2 shows the pattern of mixed organisms isolated from samples. Of the 82 isolated Pseudomonads, 20 samples had mixed infections with other organisms (Esch.coli=8, Klebsiella spp=4, Proteus spp. =5, Acinetobacter =3). Distribution was highest in traumatic wound (18.17%), followed by 10% in ETA, 6.08% in burn wound and 1.33% in surgery.

Table 1: Isolated gram negative bacteria from different samples (N=310)

Type of Samples	Number of Samples	Isolated Gram Negative Bacteria n (%)
Surgical Wound	150	108 (72)
Burn Wound	65	45 (69.23)
Traumatic Wound	55	47 (85.45)
ETA	40	38 (95)
Total	310	238 (76.77)

Table 2: Isolation of different organisms from different samples (N=310)

Name of organism	Surgical wound n (%)	Burn wound n (%)	Traumatic wound n (%)	ETA n (%)	Total n (%)
Pseudomonads	34 (41.46)	18 (21.95)	15 (18.29)	15 (18.29)	82 (26.45)
Esch. coli	26 (46.4)	10 (17.86)	15(26.79)	5 (8.93)	56 (18.06)
Klebsiella pneumoniae	15 (46.88)	5 (15.63)	7 (21.88)	5 (15.63)	32 (10.32)
Klebsiella oxytoca	2 (40)	2 (40)	0 (0)	1 (20.00)	5 (1.61)
Proteus vulgaris	10 (66.67)	2 (13.33)	1 (6.67)	2 (13.33)	15 (4.84)
Proteus mirabilis	8 (57.14)	3 (21.43)	1 (7.14)	2 (14.29)	14 (4.52)
Acinetobacter baumannii	6 (33.33)	2 (11.11)	6 (33.33)	4 (22.22)	18 (5.81)
Citrobacter freundii	2 (50)	1 (25)	0 (0)	1 (25)	4 (1.29)
Citrobacter koseri	3 (37.5)	1 (12.5)	2 (25)	2 (25)	8 (2.58)
Enterobacter aerogens	2 (50)	1 (25)	0 (0)	1 (25)	4 (1.29)
No growth	42 (58.38)	20 (27.78)	8 (11.11)	2 (2.78)	72 (23.23)
Total	150 (48.39)	65(20.97)	55 (17.74)	40 (12.9)	310 (100.00)

Table 3 shows different Pseudomonads which were identified by PCR. Of the 88 identified Pseudomonads, 86(97.72%) were P. aeruginosa, 1 (1.14%) was B. cepacia and 1 (1.14%) was S. maltophilia from samples. Of the 82 culture positive Pseudomonads, 74(97.36%) were P. aeruginosa, one (1.32%) was B. cepacia and 1 (1.32%) was S. maltophilia from samples.

Table 3: Isolation of different organisms from different samples (n=310)

Pseudomonads	Samples n (%) (n=310)	Culture n (%) (n=82)
Pseudomonas aeruginosa	86 (97.72)	74 (97.36)
B. cepacia	1 (1.14)	1 (1.32)
S. maltophilia	1 (1.14)	1 (1.32)
Total	88 (100)	76 (100)

Table 4 shows the comparison between the results of PCR and culture of isolated Pseudomonads. Of the 310 samples, PCR identified 88 samples as Pseudomonads, 76 samples were positive by both culture and PCR whereas two P. aeruginosa that were positive in culture were found negative by PCR. Considering culture as gold standard, the sensitivity of PCR was 97.44%. Twelve of the culture negative samples were positive by PCR. The specificity of PCR was 94.74%.

Table 4: Comparison between results of PCR with culture of isolated Pseudomonads (P. aeruginosa, B. cepacia, S. maltophilia) (N=310)

PCR	Culture	
	Positive n (%)	**Negative n (%)**
Positive (n=88)	76 (97.44)	12 (5.26)
Negative (n=218)	2 (2.56)	216 (94.74)
Total	78 (100)	228 (100)

Table 5 shows RFLP analysis of different Pseudomonads. All the Pseudomonas aeruginosa isolates demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp. In case of B. cepacia, there were four bands at approximately 120, 150, 280 and 390 bp and in case of S.maltophilia, there were five bands at approximately 55, 93, 110, 320 and 448 bp.

Table 5: RFLP analysis of different Pseudomonads (n=88)

PCR	Number of Bands	Position of Bands (bp)
P. aeruginosa (n=86)	3	80, 190, 250
B. cepacia (n=1)	4	120, 150, 280, 390
S. maltophilia (n=1)	5	55, 93, 110, 320, 448

DISCUSSION

Pseudomonads are one of the most important gram negative organisms involved in various types of infection. Infections caused by drug-resistant Pseudomonads are associated with significant increase in morbidity, mortality, need for surgical intervention, length of hospital stay and chronic care and overall cost of treating the infection.

In the present study, 238 (76.77%) gram negative bacilli were isolated from 310 wound swab and ETA samples (Table 1). Most of the samples were wound swab. Highest (95%) gram negative bacilli were isolated from ETA.

Out of 238 gram negative bacilli, most of the organisms were Pseudomonads (26.45%) followed by Eschericia coli (19.67%) (Table 2).

The present study showed that among 82 isolated Pseudomonads, 20 samples had mixed infections with other organisms (Esch. coli-8, Acinetobacter-3 Proteus spp.-5, Klebsiella spp.-4) (Table 2). In the present study of the mixed organisms, 18.17% were found from traumatic wound, 6.08% from burn wound, 10% from ICU and 1.33% from surgical wards. Findings of this study were similar to the study of Rahman (2013) at DMCH who observed that 14.28% of the mixed organisms were found from traumatic wound, 8.86% from burn wound, 12.5% from ICU and 0.8% from surgical wards. It is probable that mixed infection occurs in burn and traumatic wound due to wide exposed area of the wound where opportunistic organisms easily colonize. Mixed infection in traumatic wound may also probably be attributed to the contamination of the wound with soil and other environmental microbes as majority of the cases were victims of road traffic accident.

In this study, RFLP analysis was done with 88 Pseudomonads that were identified by PCR (Table 5). The groEs gene of Pseudomonas aeruginosa were amplified and digested with HaeIII enzyme and then RFLP analysis was done. The RFLP analysis of all Pseudomonas aeruginosa demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp. In accordance with the present study, Clarke et al. (2003) also used HaeIII enzyme and reported that all (groEs) gene positive Pseudomonas aeruginosa demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp which was almost similar to the present study. Similarity between these two studies might be due to the amplification of same gene and digestion of genes with similar digestion enzyme in both studies.

In the present study, The recA gene of B. cepacia was amplified and digested with HaeIII enzyme and then RFLP analysis was done. Regarding RFLP analysis of B. cepacia, it was observed that there were four bands at approximately 120, 150, 280 and 390 bp. In accordance with the present study, Mcdowell et al. (2001) amplified the recA gene of B. cepacia and RFLP analysis with HaeIII showed that there were also four bands at approximately 120, 150, 280 and 390 bp which was close to the present study. Similarity between these two studies might be due to the amplification of same gene and digestion of genes with similar digestion enzyme in both studies. According to Rasmussen et al. (2012), the presence or absence of the restriction enzyme recognition site results in the formation of restriction fragments of different sizes.

In the present study, RFLP analysis of S. maltophilia was done with amplification of gyrB gene. Regarding RFLP analysis with HaeIII, it was observed that there were five bands at approximately 55, 93, 110, 320 and 448 bp in S. maltophilia. In accordance with the present study, Coenye et al. (2004) amplified the gyrB gene of S. maltophilia and digested with HaeIII. The resulting RFLP patterns consisted of five to eight bands in the approximate size range of 50-500 bp which was close to the present study. Similarity between these two studies might be due to the amplification of same gene and digestion of genes with similar digestion enzyme in both studies. PCR-RFLP has been applied for the detection of intraspecies as well as interspecies variation (Rasmussen et al., 2012).

CONCLUSION

Pseudomonads were the most common organisms isolated from infected wound and ETA in DMCH and the commonest (92.68%) species was Pseudomonas aeruginosa. PCR-RFLP was a rapid and reliable detection method for Pseudomonads from samples. PCR and RFLP had a detection time less than 12 hours for DNA of Pseudomonads directly from sample whereas culture requires about 72 hours.

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