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EDITORIAL

Defeating Dengue: Control, Treatment or Vaccination

Professor Dr. Quazi Arif Ahmed

Dengue infection is one of the commonest mosquito born diseases occurring in tropical and subtropical countries. Every year there are about 100 to 400 million infected cases globally [1]. Dengue is endemic in Bangladesh. But in recent years there has been a significant change in the patterns of disease occurrence in terms of early on set and increase virulence. The usual Dengue surge occurs in our country around late June. According to the press release on 30th December 2023 the total number of infected cases were 3,21,073 from 1st January to 30 December with a male to female ratio of 1:4:1. In the month of September the infection rate was highest (79,598) [2]. And most of the infected cases came from Dhaka city.

Dengue is transmitted primarily by the Aedes mosquito, presents a considerable challenge for endemic countries. With no specific antiviral treatment available, the importance of prevention becomes paramount. Current control efforts primarily focuses on mosquito control measures, which is crucial, but often fall short in the face of rapid urbanization and environmental changes.

At present, the current mainstay of management for most symptomatic dengue patients remains careful observation and prompt but judicious use of intravenous hydration therapy for those with substantial vascular leakage [3]. With no specific antiviral treatment available, prevention becomes paramount. So, dengue vaccination is the ultimate goal for the control of dengue infection.

The development of vaccines had been a great challenge in dengue therapeutics due to the complication of its four antigenically distinct serotypes which can cause infection. A primary infection from one of the DENV serotype will result in long term homotypic protection but with a short term heterotypic protection against infections from other serotypes, thus a person might face disease enhancement during a second heterotypic infection [4]. The production of long-term antibody-secreting plasma cells through the formation of germinal centers (GC) in secondary lymphoid tissues with the aid of follicular helper T cells (Tfh) is important for developing an effective dengue vaccine [5].

Until now, the only authorized vaccine available in worldwide is known as Dengvaxia™ (CYD-TDV) [6], which is a live chimeric, attenuated and tetravalent vaccine consisting a non-structural Yellow fever 17D strain virus backbone with combination of structural pre-membrane (prM) and envelope (E) genes of the four DENV serotypes [7]. However, Dengvaxia™ is only applicable for infected person who are between 9 and 16 years age group [8]. Other vaccine candidates are still on the way of succeeding through clinical trials of different stages form Phase I until Phase III. Dengvaxia™ is available from 2022 for use in children 9 through 16 years old who have laboratory-confirmed previous dengue virus infection and are living in an area where dengue is endemic. The live attenuated dengue vaccine (CYD-TDV) has been licensed in many countries including the United States which is approved by FDA in children of 6-16 years living in dengue endemic areas. The vaccine has 3 doses; 0.5ml subcutaneously at

0, 6 & 12 months.

Apart from mosquito control, as antiviral treatment is not so effective and symptomatic management is problematic and close monitoring during treatment is needed, the main focus is on development of more effective vaccination. As the vaccine is costly the Government should come forward with subsidy to the citizens as Bangladesh is one of the dengue endemic countries. The policy makers should also be more conscious on effective mosquito control for dengue prevention.

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Original Article

Correlation of Plasma MDA with Pulmonary Function Tests in Patients of Bronchial Asthma

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Abstract

Introduction: Bronchial asthma is a chronic inflammatory airway disease associated with strong oxidative stress. Oxidative stress plays an essential role in development and persistent of bronchial asthma. The present study is attempted to assess the level of plasma MDA as a marker of oxidative stress and to evaluate if there is any correlation between this marker and pulmonary function tests in asthmatic patients. **Methods:** This cross-sectional comparative study was carried out in the department of Pharmacology & Therapeutics, Rajshahi Medical College, Rajshahi, Bangladesh from July 2018 to June 2019. Forty patients of bronchial asthma and 20 healthy controls were selected as sample. Pulmonary function tests were done and plasma MDA level was measured. **Results:** The mean plasma MDA found significantly higher in bronchial asthma patients compared to healthy controls ($p < 0.001$). The plasma MDA showed strong inverse correlation with FEV1 & PEFR and moderate inverse correlation with FEV1/FVC (%). **Conclusion:** As oxidative stress marker MDA significantly correlates with pulmonary function test values in asthmatic patients, this marker may be used to assess the prognosis of bronchial asthma. Augmentation of antioxidant defenses to restore the oxidant-antioxidant balance by therapeutic interventions might be beneficial in the management of bronchial asthma.

Keywords: Bronchial asthma, oxidative stress, malondialdehyde (MDA), pulmonary function tests (PFTs).

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Introduction

Bronchial asthma is a global health problem affecting 1%-18% of the population in different countries. About 30 crore people in the world suffer from asthma [1, 2]. It affects all age groups and both sexes. The disease cannot be cured, but proper management can control it. The improvement of asthma treatment is still an issue of modern medicine. There is strong evidence that asthma is associated with a strong oxidative stress. Oxidative stress is implicated in pathogenesis as well as progression of the disease. Bronchial asthma is characterized by airway inflammation and airway hyper-reactivity. Airway inflammation cause repeated episodes of airway obstruction. Reactive oxygen/nitrogen species take part in inflammatory processes. Oxidative stress plays an essential role in development and persistent of bronchial asthma [3, 4, 5].

Oxidants are free radicals & non-radical reactive derivatives of oxygen & nitrogen. Non-radical oxidants can easily produce free radicals. Free radicals are unstable, highly reactive and difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. MDA is a final product of lipid peroxidation and its level is a widely used marker of lipid peroxidation [6, 7, 8].

Plasma MDA is regarded as a marker of Oxidative stress. The present study is attempted to assess the level of plasma MDA in bronchial asthma patients and to evaluate if there is any correlation between this marker and pulmonary function tests in asthmatic patients. If the association of plasma malondialdehyde with pulmonary function tests in asthmatics is explored, it would help better understand the pathogenesis of bronchial asthma. Plasma MDA might be a prognostic tool for the management of bronchial asthma.

Methodology

This cross-sectional comparative study was carried out in the department of Pharmacology & Therapeutics, Rajshahi Medical College (RMC) in collaboration with the asthma clinic and the outpatient department (OPD) of Rajshahi Medical College Hospital (RMCH), Rajshahi, Bangladesh from July 2018 to June 2019. Purposive sampling technique was employed to determine the required sample. Forty patients of bronchial asthma and 20 healthy controls were selected as sample.

Inclusion criteria

1. Patients of bronchial asthma (bronchial asthma was diagnosed by clinical history, relevant physical examination and a positive bronchodilator reversibility test showing an increase in FEV1 by more than 12% and at least more than 200 ml in comparison to base line value).

2. Age group between 12 to 50 years.

3. Both gender were taken for study.

Exclusion criteria

1. Subjects with chronic respiratory illness other than asthma like COPD, bronchiectasis, pulmonary tuberculosis etc.

2. Subjects having recent surgery, trauma.

3. Subjects having cardiovascular, thyroid, hepatic or renal diseases.

4. Subjects with altered antioxidant levels for reasons other than asthma such as sickle cell disease, diabetes mellitus and cancer.

5. Smokers, alcoholics.

Pulmonary function tests were done by computerized spirometer (Spirobank-MIR). FEV1, FEV1/FVC (%) & PEFR was taken for study. Plasma MDA level was measured by Kei Satoh method, 1978 [9].

Permission & ethical consideration

Prior to the commencement, the study was approved by the IRB of Rajshahi Medical College. Permission was also taken from Rajshahi Medical College Hospital authority. Permission from the Ethical Review Committee of Rajshahi Medical College was taken after informing thoroughly regarding study procedures & experiments. The aim and objectives of the study along with its procedures, risks and benefits were explained in easily understandable language to each participant and written consent was taken. They were assured that all information and records would be kept confidential and would be used only for study purpose.

Results

The study included 40 asthmatic patients and 20 normal healthy controls. Among the asthmatic patients 20 were newly diagnosed and 20 were bronchial asthma patients on treatment. The age distribution was from 15 to 50 years.

Table I shows the mean age of the study subjects was around 34 years & the mean BMI was around 24.8 kg/m2. In each group number of male and female were same. There was no significant difference among the three groups.

Table II shows comparison of pulmonary function test values. The mean forced expiratory volume in 1st second (FEV1), mean FEV1/FVC

Table I. Comparison of demographic characteristics (n=60).

Demographic characteristics	Normal healthy controls (n = 20)	Newly diagnosed patients (n = 20)	Patients on treatment (n = 20)	*p* value
Age* (years)	34.3 ± 8.6	34.0 ± 11.0	33.8 ± 8.8	> 0.05
Sex#				
Male	10 (50%)	10 (50%)	10 (50%)	> 0.05
Female	10 (50%)	10 (50%)	10 (50%)	> 0.05
Body mass index (Kg/m²)	24.9 ± 2.5	24.6 ± 2.9	24.9 ± 2.8	> 0.05

* Data were presented as mean ± SD and test of significance done by ANOVA test.

Figures in the parentheses indicate corresponding percentage and test of significance done by Chi-square Test

Table II. Comparison of pulmonary function tests (n=60).

Pulmonary function tests	Normal healthy controls (n = 20)	Newly diagnosed patients (n = 20)	Patients on treatment (n = 20)	*p* value
FEV_1 (L)	2.63 ± 0.64	1.35 ± 0.58	2.05 ± 0.63	< 0.001^s
FEV_1/FVC (%)	88.7 ± 5.7	73.4 ±11.5	86.9 ± 4.8	< 0.001^s
PEFR (L/sec)	6.31 ± 1.77	2.54 ± 1.19	4.12 ± 1.16	< 0.001^s

Data were presented as mean ± SD and test of significance done by ANOVA test.

Table III. Comparison of plasma MDA of the study groups (n=60).

Variable	Normal healthy controls (n = 20)	Newly diagnosed patients (n = 20)	Patients on treatment (n = 20)	*p* value
Plasma MDA (µmol/L)	1.96 ± 0.47	8.17 ± 0.67	4.64 ± 0.69	< 0.001^s

Data were presented as mean ± SD and test of significance done by ANOVA test.

Table IV. Correlation of plasma MDA with pulmonary function tests (n=40).

Variable	Correlation coefficient (r)	*p* value
MDA with FEV_1	-0.834	
MDA with FEV_1/FVC (%)	-0.564	< 0.001^s
MDA with PEFR	-0.871	

Data were analysed using Pearson's correlation coefficient test.

(%) and mean peak expiratory flow rate (PEFR) were significantly lower in bronchial asthma patients compared to healthy controls ($p < 0.001$).

Table III shows the mean plasma malondialdehyde (MDA) was higher in bronchial asthma patients and was lowest in the healthy control group ($p < 0.001$).

Table IV shows strong negative correlation between plasma MDA & FEV1 ($r = -0.834$, $p < 0.001$) and between plasma MDA & PEFR ($r = -0.871$, $p < 0.001$), moderate negative correlation between plasma MDA & FEV1/FVC (%) ($r = -0.564$, $p < 0.001$) in patients of bronchial asthma.

Discussion

Bronchial asthma is a chronic inflammatory airway disease. Oxidative stress is involved in asthma pathogenesis as well as exacerbation of the disease. Oxidative stress has many detrimental effects on airway function such as airway smooth muscle contraction, mucus hyper-secretion, induction of airway hyper-responsiveness. Thus, oxidative stress plays an important role in progression of the disease.

In the present study, out of 60 subjects, 20 were newly diagnosed bronchial asthma patients, 20 were bronchial asthma patients on treatment and 20 were healthy controls. The mean plasma malondialdehyde (MDA) of the three groups were 8.17 ± 0.67 µmol/L, 4.64 ±

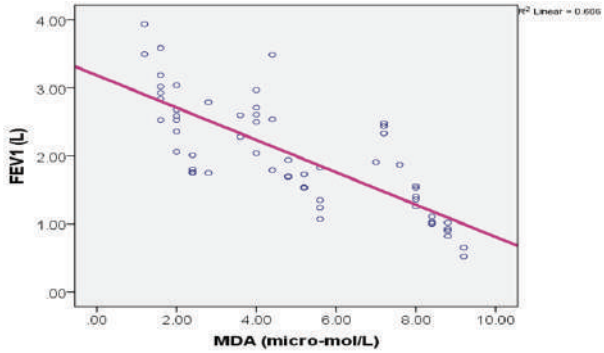

Figure 1: Scatter diagram showing inverse correlation between plasma MDA and FEV1 in patients of bronchial asthma.

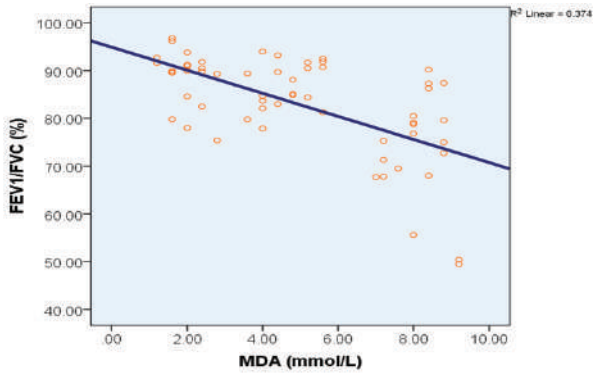

Figure 2: Scatter diagram showing inverse correlation between plasma MDA and FEV1/FVC (%) in patients of bronchial asthma.

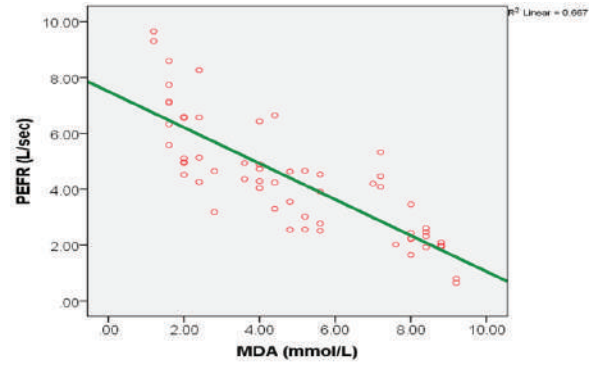

Figure 3: Scatter diagram showing inverse correlation between plasma MDA and PEFR in patients of bronchial asthma.

0.69 µmol/L and 1.96 ± 0.47 µmol/L respectively. The results revealed that the mean plasma MDA was significantly higher in bronchial asthma patients compared to healthy controls and was highest in newly diagnosed group (table III). Similar findings were noted by Ahmad et al. (2012) [10] and Al-Afaleg et al. (2011) [11]. In their studies they reported significantly higher plasma MDA level in asthmatic patients compared to healthy controls which are in agreement to our present study. Yadav & Saini (2016) [12], Ruprai (2011) [8] and Rai & Phadke (2006) [13] also observed significantly higher serum MDA level in asthmatics which supports our present study.

In our present study plasma MDA showed remarkable correlation with pulmonary function test values in asthmatic patients. The plasma MDA showed strong inverse correlation with FEV1 & PEFR and moderate inverse correlation with FEV1/FVC (%) (table IV, figure 1-3). This finding suggest that increased oxidative stress was associated with worsening of pulmonary function in asthmatic patients. Similar findings were observed by Ahmad et al. (2012) [10] and Yadav & Saini (2016) [12].

Ahmad et al. (2012) reported negative association of plasma MDA level with FEV1 ($r = -0.39$, $p < 0.01$) in bronchial asthma patients which are in consistent with the present study [10].

Yadav & Saini (2016) found a remarkable negative correlation of serum MDA level with FEV1 ($r = -0.2$, $p < 0.05$) in asthmatic patients which are in agreement to the present study [12].

Conclusion

The findings of the present study have clinical implications. As plasma MDA, an oxidative stress marker significantly correlates with pulmonary function test values in asthmatic patients, this marker may be used to assess the prognosis of the disease. The oxidative stress in inflammatory process of asthmatic airways causes oxidant-antioxidant imbalance and impairment of normal function of the lungs. So, augmentation of antioxidant defenses to restore the balance by means of therapeutic interventions might be beneficial in the mana-

gement of bronchial asthma.

Recommendations

Based on aforementioned data, we suggest that antioxidant therapy could be practiced in management of bronchial asthma along with conventional treatment. Early management through antioxidant supplementation would help to prevent exacerbation and clinical deterioration. However, further studies are needed before this supplementation could be officially recommended as an adjuvant therapy.

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Original Article

Comparison of Vitals and ECG Waves with Severity of Smoking: A Comparative Study

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Abstract

Introduction: Tobacco consumption is the most common cause of preventable deaths globally. Tobacco is often consumed in the form of cigarettes. Deleterious effects of tobacco are seen in all body systems but most markedly on respiratory and cardiovascular systems. **Methods:** This comparative cross sectional study was carried out in the department of Physiology, Rajshahi Medical College, Rajshahi from January 2018 to December 2018. A total number of 92 apparently healthy adult male smokers were our study subjects and they were categorized into light smokers (1-10 stick/day), moderate smokers (11-20 stick/day), and heavy smokers (>20 stick/day). Vitals and ECG waves (p-wave, the PR interval and the QRS complex. the QTc, the QT interval, the ST segment and the T wave duration) were compared among the groups. ANOVA was done to detect statistical significance. **Results:** There was statistically significant increase in respiratory rate, systolic BP, diastolic BP, p-wave duration and PR interval with severity of smoking. **Conclusion:** This study showed respiratory and ECG abnormalities are significantly associated with severity of smoking. This might be used by physicians as a tool for counseling the smokers to stop smoking as early as possible.

Keywords: Cigarette smoking, vitals, ECG.

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Introduction

Cigarette smoking and its health consequences represents one of the most serious public health problems and also an important health challenge worldwide (Centers for Disease Control and Prevention., 2014), it carries major health risks with the most cause specific mortalities being those of respiratory and cardiovascular diseases [1]. The world Health Organization (WHO) report on the Global Tobacco Epidemic in 2008 highlighted that approximately 5.4 million deaths every year are related to tobacco use, unless urgent attention is taken, more than 80% of tobacco-related deaths will occur in low and middle income countries by 2030 and may kill one billion people during this century [2].

Bangladesh is one of the top 10 countries that make up two-thirds of the world population of smokers [3]. It is estimated that around 13% of cardiovascular disease death are due to tobacco smoking [4]. The prevalence rate of current smoking in our country is 25% on adult population. The prevalence of current smoking of manufactured cigarettes and bidis were reported as 14% and 11% respectively. Current

cigarette smoking has been reported to be very high among males as compared to females [5]. The prevalence of cigarette smoking has peaked among high school students [6].

Tobacco smoke contains more than 4000 chemicals and around 40 carcinogens [7]. These include nicotine, carbon monoxide (CO), oxidative gases, polycyclic aromatic hydrocarbons, carbonyls, butadiene, minerals, carbon disulphide and benzene. Smoking ranks among the top causes of cardiovascular disease, Ischemic stroke, peripheral vascular disease and abdominal aortic aneurysm [8]. It is also associated with an increased risk of certain type of cancer and is a major cause of chronic obstructive pulmonary disease [9].

There has been a growing recognition of the importance of the autonomic nervous system in cardiovascular disease. Various measurement of heart rate variability evaluate changes in beat to beat interval durations using ambulatory ECG shortly after smoking cessation. Changes in heart rate and heart rate variability are also described in association with acute passive smoking or exposure to respirable suspended particles [10].

It is important to identify the effects of smoking on vital parameters and cardiovascular system so that physicians can counsel the patients. The present study was designed to determine the effect of smoking on vitals and ECG changes.

Methods

We did a cross sectional comparative study in the department of Physiology of Rajshahi Medical College, Rajshahi, Bangladesh between the periods of January 2018 to December 2018. A total number of 92 apparently healthy adult male smokers from the randomly selected citizens of Rajshahi city were our study subjects and they were categorized into light smokers (1-10 stick/day), moderate smokers (11-20 stick/day), and heavy smokers (>20 stick/day). Vitals and ECG waves (p-wave, the PR interval and the QRS complex. the QTc, the QT interval, the ST segment and the T wave duration) were compared among the groups. ECG was done in the department of Physiology of Rajshahi Medical College, Rajshahi by ECG machine (model- 96202.18745 by NIHON KOHDEN). All the tests were done between 10:00 to 17:00 hours to avoid possible diurnal variation. Each person was allowed to rest for about two minutes before actual test. ANOVA was done to detect statistical significance. The Study was approved by institutional Review Board (IRB) & Ethical Review Committee (ERC) of Rajshahi Medical College, Rajshahi, Bangladesh.

Results

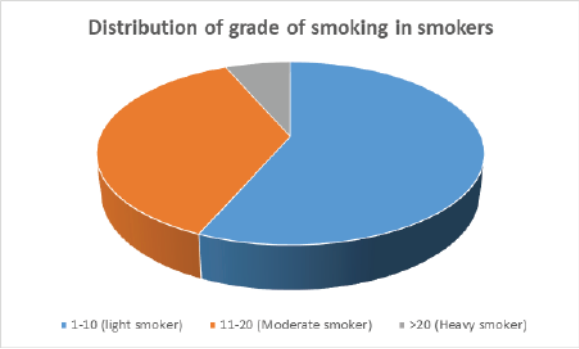

Figure 1: Distribution of grade of smoking in smokers (n=92).

Table 1 shows comparison of vitals and ECG among smokers and non-smokers based on pack years (1-3 pack year, 4-6 pack year and >6 pack year) done. The mean values of respiratory rate, systolic BP, diastolic BP, P wave duration and PR interval were slightly higher with increasing pack years and these differences were statistically significant (p value<0.05) whereas the other parameters like pulse, heart rate, P wave amplitude, QRS interval, QT, QTc, QRS axis and ST segment were not statistically significant (p value >0.05).

Table I. Comparison of ECG waves among smokers and non-smokers based on pack years (n=92).

Parameters	1-3 pack years	4-6 pack years	>6 pack years	*p* value
Pulse	91.16 ± 13.25	89.32 ± 10.04	89.04 ± 11.95	0.818
Heart rate	90.95 ± 13.13	89.21 ± 9.83	89.13 ± 11.93	0.849
Respiratory rate	14.84 ± 1.80	15.57 ± 2.20	17.22 ± 1.56	0.001[s]
Systolic BP	120.79 ± 5.07	124.11 ± 9.23	131.52 ± 11.22	0.001[s]
Diastolic BP	78.84 ± 1.01	82.07 ± 5.17	85.57 ± 5.68	0.001[s]
P wave amplitude	1.04 ± 0.08	1.02 ± 0.06	1.01 ± 0.05	0.459
P wave duration	0.08 ± 0.0	0.08 ± 0.004	0.08 ± 0.006	0.017[s]
PR interval	0.16 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.001[s]
QRS	0.08 ± 0.003	0.08 ± 0.004	0.08 ± 0.005	0.428
QT	0.34 ± 0.029	0.35 ± 0.032	0.35 ± 0.031	0.586
QTc	0.39 ± 0.20	0.40 ± 0.02	0.40 ± 0.02	0.672
QRS axis	38.53 ± 14.78	43.21 ± 15.89	44.78 ± 14.86	0.399
ST segment in duration	0.28 ± 0.013	0.28 ± 0.013	0.2778 ± 0.012	0.646

s=significant.

The test of significance was calculated using ANOVA test.

Discussion

Tobacco is one of the important agents that cause deleterious effects on health. Tobacco smoke contains more than 4000 chemicals and around 40 carcinogens [7]. These include nicotine, carbon monoxide (CO), oxidative gases, polycyclic aromatic hydrocarbons, carbonyls, butadiene, minerals, carbon disulphide and benzene. The worse impact is on respiratory and cardiovascular system and the effects increase with severity of smoking. So, we did a comparative study to see the adverse effect on cardiovascular system with severity of smoking.

This comparative cross sectional study was carried out in the department of Physiology, Rajshahi Medical College, Rajshahi from January 2018 to December 2018. A total number of 92 apparently healthy adult male smokers were our study subjects and they were categorized into light smokers (1-10 stick/day), moderate smokers (11-20 stick/day), and heavy smokers (>20 stick/day). Vitals and ECG waves (p-wave, the PR interval and the QRS complex. the QTc, the QT interval, the ST segment and the T wave duration) were compared among the groups. ANOVA was done to detect statistical significance.

We did not found any statistical significant change of heart rate with severity of smoking but respiratory rate was significantly higher in heavy smokers. Our findings was similar to that of Sharma N K et al.,(2017) and Kharel, S. et al., 2017 [11 & 12]. The increase in heart rate and respiratory rate could be due to stimulation of sympathetic ganglia and discharge of catecholamine's from adrenal medulla.

We found statistical significant increase on respiratory rate, systolic and diastolic blood pressure, p-ware duration and PR interval in heavy smokers than light smokers. The findings are similar to Sharma et al., (2005) [11]. This increase may also be due to stimulation of sympathetic ganglia.

Conclusion

From the study we can conclude that, respiratory and ECG abnormalities are significantly associated with severity of smoking. This might be used by physicians as a tool for counseling the smokers to stop smoking as early as possible.

Recommendations

1. Longitudinal study on cohort should be done on larger sample size to confirm the findings.
2. Multiple age and gender group should be included in the study for better understanding of hazards of smoking.
3. Harmful effects of active as well as passive exposure of smoking should be studied.

Limitation of the study

1. Only male subjects of Rajshahi city were included in the study.

2. Effect of exposure to active and passive smoking were not observed separately.
3. We only considered respiratory rate and ECG changes of a smokers but other health related abnormalities were ignore related with smoking.

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Original Article

Prevalence of Hypothyroidism in Infertile Women of Satkhira, Bangladesh

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Abstract

Background: Hypothyroidism can exert a substantial impact on a woman's ability to conceive by leading to irregular menstrual cycles, luteal phase irregularities, elevated prolactin levels, and imbalances in sex hormones. To detect both potential and manifest cases of hypothyroidism that may be contributing to infertility, it is advisable to conduct thyroid screening for all women experiencing infertility. **Aim of the study:** The study aims to conduct an extensive examination of the occurrence of hypothyroidism in women experiencing infertility in the region of Satkhira, Bangladesh. **Methods:** This research is a prospective observational study conducted at the Department of Satkhira Medical College and Hospital, Satkhira, Bangladesh, from 1st of April 2022 to 31st March 2023. The study focused on 102 infertile women aged between 20 and 40 years who were seeking treatment at the outpatient department. Ultimately, data from 90 eligible patients were enrolled and analyzed in the study. **Results:** In our study, a significant majority of 53 participants (58.89%) belonged to the 21-30 age group, with 24.44% being under 20 years old and 16.67% falling in the 31-40 age range. The population's average age was 24.83 years, with a standard deviation of 5.68. In terms of menstrual cycles, 45.56% experienced irregular menstruation, 44.44% had regular cycles, and 10% dealt with oligomenorrhea. The mean±SD values for the menstrual period and married life duration were 5.6±3.63 days and 6.13±4.96 years, respectively. Among participants, 58.89% had primary infertility, while 41.11% had secondary infertility. In thyroid status, 90% were euthyroid, and 10% were hypothyroid. We had 90 participants, with 81 being euthyroid and nine hypothyroid. In the primary infertility group, comprising 53 individuals, 59.26% were euthyroid, and 55.56% were hypothyroid. In the secondary infertility group, consisting of 37 participants, 40.74% were euthyroid, and 44.44% were hypothyroid. **Conclusion:** Hypothyroidism has gained recognition as a growing contributor to infertility, affecting both individuals trying to conceive for the first time and those facing secondary infertility challenges. As a result, it is recommended that every woman experiencing fertility issues undergo a thyroid profile screening to enhance their chances of achieving successful conception.

Keywords: Prevalence, Hypothyroidism, and Infertility.

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Introduction

In recent years, there has been a growing concern about the potential impact of thyroid dysfunction on reproductive health, especially in the context of female infertility [1]. Thyroid hormones affect cells in nearly all body tissues despite having no specific target organ [2]. The two primary thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3), play vital roles in body growth, sexual development, basal metabolic rate, and reproductive function modulation [3]. Hypothyroidism is the most common endocrine issue affecting women with ovulatory dysfunction, leading to infertility. Subclinical hypothyroidism (SCH), a milder form characterized by slightly elevated thyroid-stimulating hormone (TSH) levels and regular free thyroxine (FT4) levels, can also disrupt reproductive function. Studies have reported SCH prevalence in the 0.7-2.3% range in large groups of unselected infertile women [4]. Undiagnosed and untreated thyroid disorders can be a significant factor in infertility and subfertility, with significant medical, economic, and psychological implications in our society. Infertility is the inability to conceive after a year of regular intercourse without contraception, affecting one in six newlywed couples [5]. It is a global health issue, impacting 8-10% of women worldwide [6]. Infertility is categorized as primary if a couple has not previously achieved a pregnancy and secondary if a couple has conceived before but is currently facing difficulties to be pregnant. In South Asian countries, the prevalence of infertility varies, with estimates of 4% in Bangladesh and up to 15% among women in the 45-49 age group [7], which is notably high compared to other South Asian countries. In a certain percentage of infertile couples, no clear cause can be identified, even when all relevant parameters appear normal. These cases are classified as unexplained infertility [8]. The prevalence of hypothyroidism among women of reproductive age ranges from 2-4% [9]. This concern is particularly relevant in Bangladesh, a South Asian country burdened with a high incidence of thyroid disorders and a significant prevalence of infertility [10]. Understanding the prevalence of hypothyroidism among infertile women in Bangladesh is essential for enhancing reproductive healthcare in the region. This study aims to thoroughly investigate the prevalence of hypothyroidism among infertile women in Satkhira, Bangladesh, offering valuable insights into the relationship between thyroid health and female fertility.

Materials & Methods

This prospective observational study was conducted at a private chamber in Satkhira, Bangladesh. The study spanned one year, starting from 1st April 2022 and concluding on 31st March 2023. Throughout the study, 102 infertile women between the ages of 20 and 40 were excluded from the study due to in educate information. However, 12 patients needed to meet our inclusion criteria and needed more information, leading to their exclusion from the study. Subsequently, the study enrolled and analyzed data from 90 eligible patients.

Inclusion criteria:

- Reproductive age group (20-40 years).
- Both primary and secondary infertile women of the above age group.

Exclusion criteria:

- Women experiencing tubal defects leading to infertility.
- Women with a medical history of hypophysis-hypothalamic disorders affecting fertility.
- Women with chronic systemic conditions like liver, renal, and cardiac diseases that may impact fertility.
- Women with congenital abnormalities in the urogenital tract or evident organic genital lesions affecting fertility.
- Infertility due to male reproductive factors.

In this study involving 90 female participants, 53 were categorized under primary infertility, while the remaining 37 were classified as experiencing secondary infertility. Before gathering any data, the research team ensured that informed consent was obtained from all the participants and that strict measures were in place to safeguard the confidentiality of the information collected. The infertility investigation encompassed a comprehensive approach. It included obtaining detailed clinical histories and gathering information about factors such as age, marital status, menarche, menstrual history, obstetrical history, clinical signs of hypothyroidism, prolactinoma and medical and surgical histories. A thorough gynaecological examination and a general health assessment of the participants were also carried out. Various routine tests and evaluations were conducted, which involved complete blood counts, random blood sugar tests, and the measurement of hormonal profiles, including TSH (Thyroid Stimulating Hormone), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and free testosterone levels. Semen analysis of the participants' husbands and screening for infectious diseases were also performed. In cases where it was deemed necessary, more specific diagnostic procedures such as hysterosalpingography, pelvic ultrasonography, diagnostic laparoscopy, and tuberculosis polymerase chain reaction (TB-PCR) of menstrual blood or endometrial tissue were carried out. The participants' thyroid health was assessed by categorizing them as euthyroid if their TSH levels ranged from 0.45 to 4.49 microIU/mL and hypothyroid if their TSH levels exceeded 4.49 microIU/mL. The collected data was meticulously organized and presented in suitable tables and graphs that reflected their inherent characteristics. Each table and graph were accompanied by a descriptive explanation to facilitate clear comprehension of the information.

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) program on a Windows platform. Continuous variables were expressed as mean values with standard deviations (mean±SD), while categorical variables were presented as frequencies and percentages.

Results

Table 1 provides an overview of the clinical characteristics of the study population. A majority of 53 (58.89%) individuals fell within the 21-30 age group, with 24.44% of participants being under 20 years old, and 16.67% falling within the 31-40 age range. The mean age for the population was 24.83 years, with a standard deviation of 5.68. In terms of menstrual cycle patterns, 41 (45.56%) participants experienced irregular menstruation, 40 (44.44%) had regular menstrual cycles, and 9 (10.00%) were dealing with oligomenorrhea. The mean±SD values for the menstrual period and married life duration were 5.6±3.63 days and 6.13±4.96 years, respectively (Table 1).Among the study population, 58.89% of participants had primary infertility, while 41.11% were experiencing secondary infertility (Figure 1). As indicated in Figure 2, the majority, 90% of participants, had normal thyroid function (euthyroid), with the remaining 10% being hypothyroid. Table 2 presents a comprehensive breakdown of infertility cases categorized by both infertility type and Thyroid status. The study involved 90 participants, with 81 being in the Euthyroid category and 9 in the hypothyroid category. Within the primary infertility group, consisting of 53 individuals, 48 (59.26%) were Euthyroid, while 5 (55.56%) were Hypothyroid. In the secondary infertility group, comprising 37 participants, 33 (40.74%) were Euthyroid, and 4 (44.44%) were Hypothyroid. These statistics reveal the distribution of thyroid status among individuals experiencing primary and secondary infertility. The table

provides a clear overview of how thyroid status relates to different types of infertility, highlighting potential variations in thyroid function between these two groups.

Table I. Clinical characteristics of the study population (N=90).

	Variables	Frequency n (%)
Age range (Years)	<20	22 (24.44)
	21-30	53 (58.89)
	31-40	15 (16.67)
	Mean±SD	24.83±5.68
Menstrual cycle	Regular	40 (44.44)
	Oligomenorrhea	9 (10)
	Irregular	41 (45.56)
	Menstrual period (days)	5.6±3.63
	Married for (years)	6.13±4.96

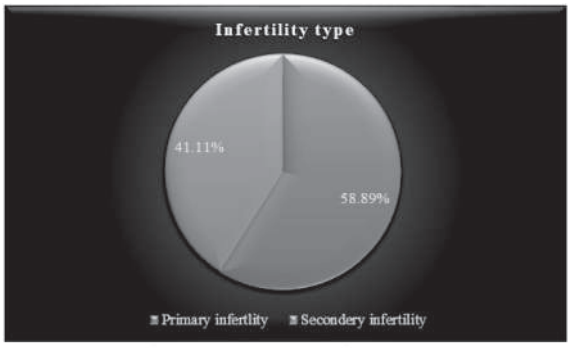

Figure 1: Infertility type of the study population based on parity (N=90).

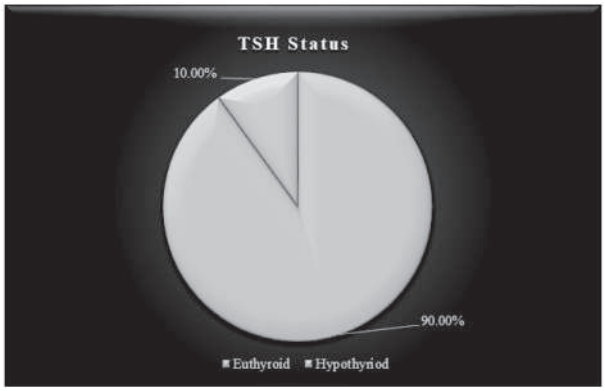

Figure 2: Thyroid status of the study population (N=90).

Table II. Correlation between hypothyroidism and infertility.

Infertility type	Euthyroid (N=81) n (%)	Hypothyroid (N=9) n (%)
Primary infertility (N=53)	48 (59.26)	5 (55.56)
Secondary infertility (N=37)	33 (40.74)	4 (44.44)
Total	81 (100)	9 (100)

Discussion

In this observational study, we analyzed 90 patients. The average age of the patients was 24.83 years, with a standard deviation of 5.68. Among these patients, 53 (58.89%) fell within the 21-30 age group, almost similar to the findings in a study conducted by Bharti G. et al. [11]. As presented in Table 1, 41 (45.45%) of the patients experienced irregular menstrual cycles, with an average menstrual period lasting 5.6 days (±3.63 SD) and an average married life duration of 6.13 years (±4.96 SD). Among the 90 women, 58.59% had primary infertility, while 41.11% had secondary infertility, consistent with the results of a prior Indian study [11]. Notably, most of the study's population (90.00%) had normal thyroid function. In comparison, only 10% of patients were diagnosed with hypothyroidism, aligning with the outcomes of Indu Verma's study, where 76.14% of patients were euthyroid, and 23.86% had hypothyroidism [12]. Within the euthyroid group, comprising 81 patients (90.00%), 48 (59.26%) experienced primary infertility, while 33 (40.74%) had secondary infertility. Among the hypothyroid patients, 5 (55.56%) suffered from primary infertility, and 4 (44.44%) had secondary infertility. Given the unconditional nature of this data, we applied the Pearson chi-square test, resulting in a statistically significant P-value of 0.004, supporting the idea that thyroid dysfunction, particularly hypothyroidism, may contribute to

infertility. Our study found that the mean values of various hormones remained within the normal range, and these levels were comparable in both primary and secondary infertility groups, thereby minimizing the impact of confounding factors. Our findings indicated that the overall prevalence of hypothyroidism among infertile patients seeking care at tertiary centers was 10%, consistent with the study by Verma et al., which reported 23.9% of hypothyroidism in a sample of 394 infertile women [12]. In the primary and secondary infertility groups, the prevalence was 19.2% and 38.6%, respectively. This is in line with the observations of Akhter et al., who reported that 58.59% of their patients had primary infertility, while 41.11% had secondary infertility [13]. Furthermore, our study revealed that 48 (59.26%) primary infertile patients and 33 (40.74%) secondary infertile patients had normal thyroid function. Mohana Priya et al. also conducted a study involving 98 infertile women, and they found that 53.7% of these women suffered from hypothyroidism [14]. Additionally, studies by Raber's W et al. and Bals-Pratsch M et al. detected a high incidence of subclinical hypothyroidism in infertile women, with rates of 34% and 25%, respectively [15,16]. These disparities could be attributed to these studies' relatively small sample sizes. Hypothyroidism appears to be prevalent in both infertile women and the general female population. In India, the estimated prevalence of hypothyroidism among women is 15.86%, according to a study by Unnikrishnan and Menon. However, our study found that the prevalence among infertile women was slightly lower at 10.00% [17]. As a result, we recommend routine thyroid screening as part of the standard infertility workup for women.

Limitations of the study

The study on the prevalence of hypothyroidism in infertile women in Satkhira, Bangladesh, has several limitations. Firstly, the study's cross-sectional design restricts the ability to establish causal relationships between hypothyroidism and infertility. Additionally, the study's reliance on self-reporting of infertility and the lack of access to medical records may introduce recall bias and affect the accuracy of infertility data. Furthermore, the research may not account for potential confounding factors, such as lifestyle and dietary habits, which can influence both hypothyroidism and infertility. Finally, the study's geographical scope may limit the generalizability of its findings beyond the Satkhira region.

Conclusion & Recommendations

In conclusion, our study highlights the significant prevalence of hypothyroidism among infertile women in Satkhira, Bangladesh. The findings underscore the importance of routine thyroid screening for this vulnerable population. Addressing hypothyroidism as a potential contributing factor to infertility can lead to more effective treatment and improved reproductive outcomes. We recommend that healthcare providers incorporate thyroid function tests into their standard infertility workup protocols, ensuring early diagnosis and appropriate management. Furthermore, public health campaigns should raise awareness about the interplay between hypothyroidism and infertility, promoting timely medical intervention and ultimately enhancing the reproductive health of women in Satkhira, Bangladesh.

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Conflict of interest: None declared.

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Original Article

Bacteriological Study of Gall Bladder Bile in Patients with Cholelithiasis: a Study of 100 Cases

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Abstract

Background: Gall bladder disease is a health problem throughout the world. The commonest reason being the gallstones. Apart from surgery prompt administration of appropriate antibiotics to control the biliary infection is important. **Aims:** To find out the frequency of culture positivity of gall bladder bile among adult patients undergoing cholecystectomy due to cholelithiasis. **Methods:** It was cross-sectional study conducted in the department of Surgery of BIRDEM Hospital, Dhaka, Bangladesh. Total 100 cases were included in this study during the period of July 2008 to June 2009. **Results:** In our study mean age was 50.53 ± 7.31 & male female ratio was 1:1.86. Out of 100 patients 62 had multiple stone and 38 had single stone. Out of 100, only 08 had culture positive and rest 92 had no growth in gall bladder bile. Among isolated organisms 05 (62.5%) were Escherichia coli, 02 (25%) were Klebsiella sp. and 01(12.5%) was Enterococcus sp. No anaerobic organisms were isolated. All organisms were most commonly sensitive to imipenem, co-trimoxazole, aminoglycosides and cephalosporines. All the biochemical parameters were within normal range. **Conclusion:** Antibiotics sensitive to Escherichia Coli, Klebsiella sp. and enterococcus sp. should be used during the surgical treatment of cholelithiasis.

Keywords: Bacteriological study, cholelithiasis.

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Introduction

Cholelithiasis is one of the most common surgical disorders. The natural history of gallstone development is unknown. Bacteria are found in high concentration in bile and stone. It is difficult to ascertain whether bacterial infection of the bile arose before stone formation or vice versa [1]. Bacteriological studies have shown that in these patients the bile cultures usually present several kinds of bacteria, especially Gram-negative aerobic bacteria such as E. coli and Klebsiella sp. These diseases may cause severe infection and/or sepsis. In addition to surgical treatments, prompt administration of appropriate antibiotic is important to control the biliary tract infection [2]. The incidence is four times higher in women than in men with high prevalence among the age group of 30-40 years. The natural history of gallstone development is unknown. Bacteria are found in high concentration in bile and stone. Documented risk factors for gallstone disease include obesity, rapid weight loss, high dietary intake of fat, multiple deliveries and congenital hemolytic anemias such as sickle cell disease. In Western countries, gallstones are mainly composed by cholesterol and bacteria have a minor role in their pathogenesis. Bacterial infections of the bile duct might play a critical role in the development of brown gallstones (formed mainly from bilirubin), while stones may format a background for bacterial development in some cases. Prompt administration of the appropriate antibiotics is crucial in the

management of biliary tract infection and antimicrobial treatment is commonly administered pre- or peri-operatively and often inhibits the bacterial growth. Moreover, it is suggested that the recovery of the bacteria in bile cultures is affected by toxicity of bile salts. Thus, traditional culturing methods of bile might miss a large number of underlying bacterial infections that could lead to acute or chronic cholecystitis [3].

Methodology

A total 100 patients of cholelithiasis selected by ultrasonography, underwent surgery in BIRDEM Hospital, Dhaka, Bangladesh between the periods of July 2008 to June 2009 were included in this study. This research was conducted as a cross-sectional study. Purposive sampling technique was used as a method of selecting sample on the basis of inclusion and exclusion criteria. After taking informed written consent, socio-demographic information were collected by face to face interview and other per operative and post operative information were collected from the patient's treatment sheet. Bile was sent for bacteriological culture and sensitivity and report of culture sensitivity were collected form the microbiology department of BIRDEM. In this study, bile samples were obtained per-operatively from the GB from those patients who underwent laparoscopic cholecystectomy. Antibiotic was administered to each patient following dissection of GB from GB bed. This antibiotic was either Ceftriaxone or cefuroxime. As soon as having the gallbladder removed, bile aspirated from GB by 2 sterile syringe. One sample was inoculated in Blood agar and MacConkey agar media for aerobic culture at 370c. The growth of the organisms were observed after 48 hours of inoculation and then antibiotic sensitivity tests were done. Another sample was immediately inoculated in anaerobic culture media. Growth of anaerobic organisms were not observed. Both aerobic & anaerobic culture were done in microbiology laboratory in BIRDEM Hospital. Ethics was maintained strictly at different stages of this study. After data collection, data were checked thoroughly for any inconsistency and incompleteness. Then analysis was done by using SPSS software.

Results

Out of 100 patients 28 (28.0%) were in the age group of less than 45 years, 55 (55.0%) were in the age group of 45 to 55 years and 17 (17.0%) were in the age group of 55 to 65 years. Regarding gender, majority i.e. 65 (65.0%) patients were female and the rest 35 (35.0%) were male. Table 1 shows the distribution of characteristics of GB stones. 62 patients had multiple stone and 38 had single stone. Highest numbers of stone were yellow (48%), followed by whitish (30%), brown (18%) and black (4%). In consistency, maximum number i.e 80% of stone was friable and rests 20% were hard.

Table I. Distribution of characteristics of gall bladder stones (n=100).

Characteristics of stones	Frequency n (%)
Number of GB stone	
Multiple	62 (62)
Single	38 (38)
Colour of stone	
Black	4 (4)
Brown	18 (18)
Yellow	48 (48)
Whitish	30 (30)
Consistency of stone	
Friable	80 (80)
Hard	20 (20)

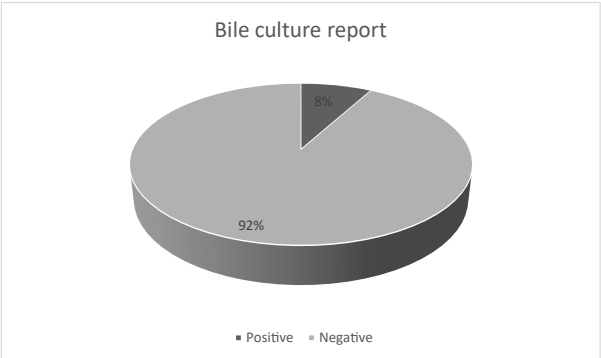

Figure 1: Distribution of bile culture report (n=100).

Table II. Distribution of isolated organisms (n=8).

Organism isolated	Frequency n (%)
E. Coli	5 (62.5)
Klebsiella sp.	2 (25)
Enterococcus sp.	1 (12.5)

Table III. Distribution of antibiotic sensitivity of isolated organisms.

	Antibiotic	Sensitive	Resistance
E. coli (n=5)	Ciprofloxacin	3	2
	Ceftriaxone	4	1
	Cefuroxime	4	1
	Cefixim	4	1
	Amikacin	5	0
	Cotrimoxazole	5	0
	Ceftazidime	4	1
	Imipenem	5	0
	Tetracycline	3	2
	Gentamicin	5	0
Klebsiella sp. (n=2)	Ciprofloxacin	2	0
	Ceftriaxone	2	0
	Cefuroxime	1	1
	Cefixime	2	0
	Amikacin	1	1
	Cotrimoxazole	2	0
	Gentamicin	2	0
	Imipenem	2	0
	Cefotaxime	2	0
	Ceftazidime	2	0
	Netilmicin	2	0
Enterococcus sp. (n=1)	Ciprofloxacin	0	1
	Ceftriaxone	1	0
	Cefuroxime	1	0
	Cefixime	1	0
	Amikacin	0	1
	Cotrimoxazole	1	0
	Gentamicin	0	1
	Imipenem	1	0
	Tetracyclin	0	1
	Ampicillin	1	0
	Netilmicin	1	0

Discussion

In the present study out of 100 patients 28 were in the age group of less than 45 years, 55 were in the age group of 45 to 55 years and 17 were in the age group of 55 to 65 years. Mean ± SD was 50.53 ± 7. Out of 100 patients 65 were female and 35 were male. Male female ratio was 1:1.86. Ballal et al. (2001) studied microbiology of GB bile in uncomplicated symptomatic cholelithiasis [4]. In their study 51 out of 70 patients were female, 19 were male (M:F=1:2.68). The median age was 37 years. In this present study out of 100 cases 8 were bile culture positive, those all (100%) were GB with chronic inflammation.

Among isolated organisms 5 (62.5%) were Escherichia coli, 02 (25%) were Klebsiella sp. and 01 (12.5%) was Enterococcus sp. All isolated organisms were most commonly sensitive to imipenem, co-trimoxazole, amikacin, gentamicin, ceftriaxone, ceftazidime, cefuroxime and cefixime.

Gomes et al. (2006) in a study illustrate the bacteriology of gallstone disease. In their study out of 100 patients 16 were bile culture positive [5]. Escherichia coli 7 (43.75%), Klebsiella spp 6 (37.5%) and Enterobacter spp 3 (18.75%) were the commonest isolates. They recommend obtaining of cultures of bile and gallstones at the time of cholecystectomy so that appropriate antibiotics can be administered in the event of a positive culture to prevent serious complications like gram negative septicaemia. They recommended co–amoxiclav in combination with an aminoglycoside for aerobic bacteria along with metronidazole to cover anaerobic bacteria is an empirical therapy that can be used before the results of bacteriological culture. Cefotaxime or imipenem can be used for aerobic bacteria as an alternative treatment [6]. Malatani et al. (1996) studied one hundred and fifty-two consecutive cases of cholelithiasis. Among

them 120 cases was chronic calculous cholecystitis [7]. Out of 120 cases 32 (26.67%) were bile culture positive. The most common organism cultured was E. coli followed by Klebsiella spp. Abeysuriya et al. (2008) studied microbiology of GB bile in uncomplicated symptomatic cholelithiasis. Bile samples were obtained peroperatively from 70 patients. Positive bile culture was found in 38 (54%) cases. Twenty eight of these 38 bile samples were shown positive only after enrichment in brain heart infusion (BHI) medium. The overall bacterial isolates from bile samples showed E. coli 21 (55.3%), P. aeruginosa 9 (23.7%), Enterococcus spp. 5 (13.1%), Klebsiella spp. 2 (5.3%) and Staphylococcus epidermidis 1 (2.6%). Most organisms were sensitive to imipenem, amikacin and gentamicin [8].

Conclusion

From the above study it can be concluded that, frequency of culture positivity is low. So, prophylactic antibiotics should not be used. If we want to use antibiotics, we can use antibiotics sensitive to Escherichia Coli, as this is the highest isolated organism.

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Original Article

Utility of Mucin Stain (Periodic Acid Schiff and Alcian Blue) in the Categorization of Cervical Cancers

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Abstract

Background: Carcinoma of cervix is the fourth most common cause of cancer in women worldwide and second leading cause of cancer death. But it is potentially treatable if the precursor lesions and lower grade carcinomas can be identified at an earlier stage. Due to the poor prognosis of adenocarcinoma and adenosquamous carcinomas, it will be much beneficial for the patient if these carcinomas can be identified accurately. So, the use of mucin stain by cost effective Periodic Acid Schiff and Alcian Blue may be useful in early detection of carcinomatous process and detecting mucin secreting carcinomas more accurately. **Methods:** This cross-sectional study was carried out in the Department of Pathology, Rajshahi Medical College, Rajshahi, from March 2020 to February 2022, to evaluate the utility of mucin stain in invasive cervical carcinoma. Seventy cases of invasive carcinoma were included in this study. Specimen were processed routinely for Hematoxylin and Eosin stain and combined periodic acid Schiff and Alcian blue stain with diastase treatment. Histopathological results were analyzed, and statistical analysis was done using Statistical Package for Social Science version twenty-eight. **Results:** Among the seventy cases of invasive cervical carcinomas, mucin was positive in 28 cases, acidic mucin was predominant in fifteen cases, mixed mucin in eleven cases and neutral mucin in two cases. Mucin positivity was found in fifteen of grade-I tumors which is significantly higher than grade-II and grade-III cancers. Among seventeen poorly differentiated squamous cell carcinoma, mucin was found in four cases. Hence three of them had to be reclassified as adenosquamous carcinoma and one into adenocarcinoma. This finding was statistically significant ($p < 0.05$). **Conclusion:** Histopathology is the gold standard for diagnosing cervical carcinoma. Mucin stain is helpful in more accurate detection of mucin secreting adenocarcinoma, adenosquamous carcinoma. It is also helpful for predicting the prognosis and planning patient management. So, mucin stain may be done along with routine Hematoxylin and Eosin stain in all cases of invasive cervical carcinomas.

Keywords: Cervical carcinoma, Mucin stain, Combined PAS and Alcian Blue.

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Background

Cervical cancer is the fourth most common cancer in women worldwide with an estimated 604,127 new cases and 341831 deaths in the year 2020 [1]. In Bangladesh, cervical cancer is the 2nd most common cancer in women with 8268 new cases and 4971 deaths in the year of 2020 (Globocan Bangladesh, 2020). Broadly invasive cervical cancers are categorized into squamous cell carcinoma, adenocarcinoma and adenosquamous (mixed) carcinoma as other types are rare [2]. Eighty percent of inva-

sive cervical cancers are squamous cell carcinoma; adenocarcinomas and adenosquamous carcinomas constitute less than 20% of cervical cancers in most developing countries [3]. The lesions which are well differentiated do not produce diagnostic challenges. But in 20-30% of cases, the lesions which are poorly differentiated squamous cell carcinomas diagnosed on Hematoxylin & Eosin (H & E) stain may turn out to be adenocarcinoma or adenosquamous carcinoma when stained with mucin stains [4]. The significance of identifying the mucin secreting cancers lies in the fact that these neoplasms run an unusually aggressive course and are associated with a much worse prognosis than their pure squamous counterparts [1]. Mucin histochemistry of normal endocervical glands show mixture of mucosubstances, both neutral and acidic with a predominance of neutral mucin. But malignant glands show a mixture of both neutral and acidic mucin with predominance of acid mucin. So, there is a shift in the mucin pattern as compared to normal [5]. Periodic Acid Schiff with diastase digestion (PAS-D) stains neutral mucin and Alcian Blue (AB) stains acidic mucin. Combined PAS-D/AB stain is preferable than singly PAS-D or AB due to greater specificity and intensity of staining, and for sharp differentiation between neutral and acidic mucin [6].

Considering above points, this study was planned to evaluate the variations in mucin secretion in different cervical precancerous lesions and invasive cervical cancers by applying cost effective combined PAS/AB stain in the Pathology department of Rajshahi Medical College (RMC), to help in diagnosis, prediction of prognosis and plan of management of patients.

Materials and Methods

This cross-sectional descriptive study was carried out in the department of pathology RMC from March 2020 to February 2022. Approval for the research protocol was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi prior to the commencement of the study. Both Routine Hematoxylin and Eosin stain and combined PAS/AB stain were done in Department of Pathology, Rajshahi Medical College. Histopathologically diagnosed cases of carcinoma of cervix were taken as case. Poorly fixed samples, inadequate biopsies and samples with marked inflammation were excluded. In this study, 70 histopathologically diagnosed cervical carcinoma were taken as cases. Tissue samples were obtained from total abdominal hysterectomy and colposcopic biopsies. A pre-tested questionnaire was used to collect data from cases 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 squamous cell carcinoma, adenocarcinoma, and adenosquamous (mixed) carcinoma microscopically. Carcinoma cases were histologically graded as well differentiated, moderately differentiated and poorly differentiated carcinoma.

Combined PAS-D/AB staining was carried out using standard protocol followed in department of pathology Rajshahi medical college.

Interpretation of Combined PAS-D/AB stain:

Acidic mucin and neutral mucin are stained as blue and pink respectively.

- Strong: >70% of tumor area.
- Moderate: 30-70% of tumor area.
- Mild: 5-30% of tumor area.
- Negative: <5% of tumor area.

For the comparison of mucin content and intensity a control section of combined PAS/AB stained normal endocervical tissue was used.

Categorization of tumor under combined PAS/AB stain [7]:

At first the stained section was screened at low power (10x) to identify the areas of the 'neoplastic cells'. Then mucin content in percentage of total volume of tissue section along with the chemical type of mucin is assessed.

- Tumors with squamous growth pattern, keratin formation, intercellular bridge formation and mucin secretion of <5% of tumor volume were classified as squamous cell carcinoma.
- Tumors with acinar differentiation or mucin secretion >70% of tumor volume were classified as adenocarcinoma.
- Lesions exhibiting both squamous and acinar differentiation with the minor component constituting at least one third of the tumor or showing mucin production of 30-70% of tumor volume were classified as adenosquamous/ mixed carcinoma.
- Tumors with mucin content 5-30% of tumor volume is termed as squamous cell carcinoma with mucin secretion, although as it is no different from adenosquamous carcinoma in biological behavior and prognosis. So, it is included in mixed tumor category.
- Mucin types were assessed according to the predominant PAS or AB stain. Tumors were classified as having predominant acidic, predominantly neutral and mixed type.

Results and observation

Seventy histopathologically diagnosed cervical carcinoma cases were taken. Patients (case) with age ranged from 29 to 74 years (mean age was 51.42 ± 8.75) (Table I). Out of 70 cervical carcinoma cases, 45 (64.3%) cases were invasive cervical squamous cell carcinoma, 15 (21.4%) cases were invasive adenocarcinoma, 10 (14.3%) cases were invasive adenosquamous carcinoma (Figure 1). Out of 70 invasive cervical cancers, most cases were grade-I; 43 (61.43%), followed by 17 (24.29%) grade-III and 10 (14.29%) grade-II tumors (Figure 2). Most grade-I cancers (18) were squamous cell carcinomas. All other grade-II (10 cases) and grade-III (17 cases) invasive cervical cancers were squamous cell carcinoma (Table II). All adenocarcinoma and adenosquamous carcinoma were grade-I (Table II). Out of 70 cases of invasive cervical cancers, 42 (60%) cases were mucin negative and 28 (40%) were mucin positive (Figure 3). Out of 28 mucin positive cases 15 (53.57%) cases show predominantly acidic mucin, 11 (39.29%) cases show predominantly mixed type mucin and 2 (7.14%) show neutral mucin (Figure 4). Twenty four (55.8%) of grade-I invasive cancers showed mucin positivity, 4 (23.5%) of the grade-III invasive cancers showed mucin positivity (Table III). But none of the grade-II tumors showed any mucin positivity. The mean difference was not statistically significant ($p>0.05$) among the study groups. Among the invasive cervical cancers, 15 (21.4%) cases showed predominantly acidic mucin, followed by 11 (15.7%) mixed and 02 (2.9%) neutral mucin (Table IV). In grade-I tumors, 15 (34.9%) were predominantly acidic, 07 (16.3%) predominantly mixed and 02 (4.7%) were predominantly neutral and rest 19 (44.2%) showed no mucin positivity (Table IV). In grade-II squamous cell carcinomas, no mucin positivity was found. In grade-III tumors, 04 (23.5%) cases showed mixed type of mucin, and no mucin was present in 13 (76.5%) of cases (Table IV). The mean difference was statistically significant ($p<0.05$) among the study groups (Table VI). Out of 17 cases of poorly differentiated squamous cell carcinoma, only 01 (5.9%) showed strong mucin expression, 03 (17.6%) cases showed mild mucin expression and rest 13 (76.5%) showed no mucin (Table V). According to the classification system based on the mucin content, only 04 poorly differentiated squamous cell carcinoma needed to be reclassified. Three

(17.65%) cases were reclassified as adenosquamous carcinoma and 01 (5.88%) case was reclassified as poorly differentiated adenocarcinoma. Rest 13 (76.47%) cases remained as poorly differentiated squamous cell carcinoma. The mean difference was statistically significant ($p<0.05$) among the study groups.

Table I. Distribution of the study subjects by their age (n = 70).

Age (years)	Frequency n (%)
<40	5 (7.1)
40-60	52 (74.3)
>60	13 (18.6)
Mean ± SD	51.42 ± 8.75
Range (Min-Max)	29-74

Table V. Mucin expression in poorly differentiated-cervical squamous cell carcinoma (n=17).

Mucin expression	Frequency n (%)
No mucin	13 (76.5)
Mild (5-30%)	3 (17.6)
Moderate (31-70 %)	0 (0)
Strong (>70%)	1 (5.9)
Total	17 (100)

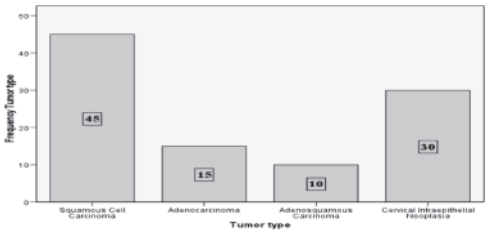

Figure 1: Frequency of tumor types (n=100).

Table II. Distribution of invasive cancers by histological grades (n=70).

Tumor type	Tumor grade of invasive cancers			Total	*p* value
	Grade-I n (%)	Grade-II n (%)	Grade-III n (%)		
Squamous Cell Carcinoma	18 (41.9)	10 (100)	17 (100)	45 (64.3)	<0.001
Adenocarcinoma	15 (34.9)	0 (0)	0 (0)	15 (21.4)	
Adenosquamous Carcinoma	10 (23.3)	0 (0)	0 (0)	10 (14.3)	
Total	43 (100)	10 (100)	17 (100)	70 (100)	

Table III. Association of mucin status with the grades of invasive cervical carcinoma cases (n=70).

Mucin status	Tumor grade of invasive cancers			Total	*p* value
	Grade-I n (%)	Grade-II n (%)	Grade-III n (%)		
Mucin positive	24 (55.8)	0 (0)	4 (23.5)	28 (40)	0.072^{ns}
Mucin negative	19 (44.2)	10 (100)	13 (76.5)	42 (60)	
Total	43 (100)	10 (100)	17 (100)	70 (100)	

Table IV. Association of mucin status with tumor grade (n=43).

Mucin status	Tumor grade of invasive cancers			*p* value
	Grade-I n (%)	Grade-II n (%)	Grade-III n (%)	
Predominantly acidic	15 (34.9)	0 (0)	0 (0)	0.039
Predominantly neutral	2 (4.7)	0 (0)	0 (0)	
Mixed	7 (16.3)	0 (0)	4 (23.5)	
No mucin present	19 (44.2)	10 (100)	13 (76.5)	
Total	43 (100)	10 (100)	17 (100)	

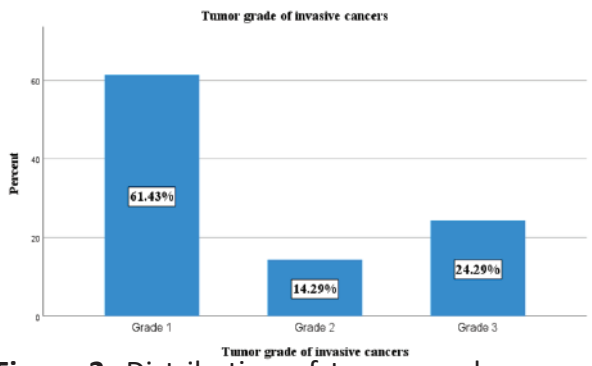

Figure 2: Distribution of tumor grade among the study cases (n=70).

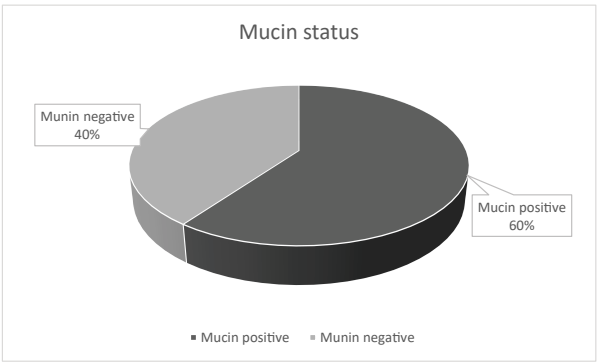

Figure 3: Distribution of mucin positivity among the study cases (n=70).

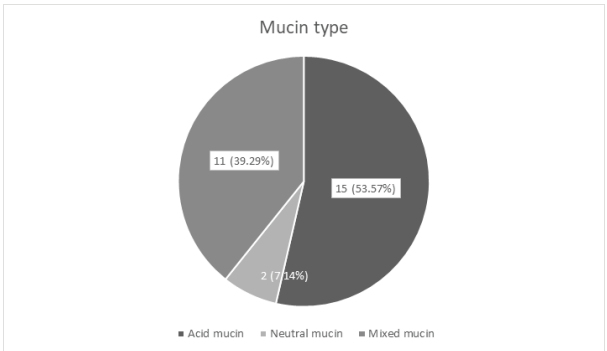

Figure 4: Distribution of mucin type among the mucin positive cases (n=28)

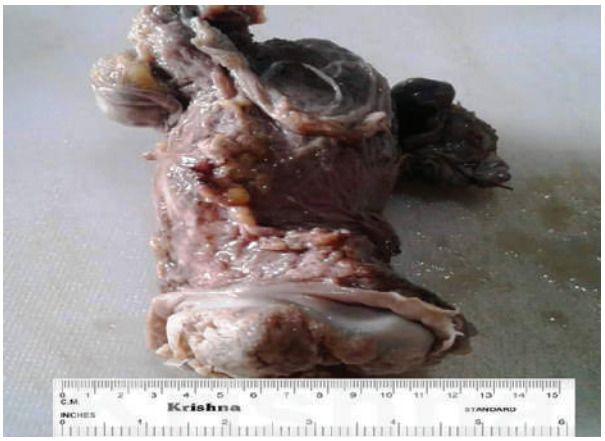

Figure 5: Photograph showing exophytic growth of cervix. *(sample no. 59: invasive squamous cell carcinoma, poorly differentiated)*

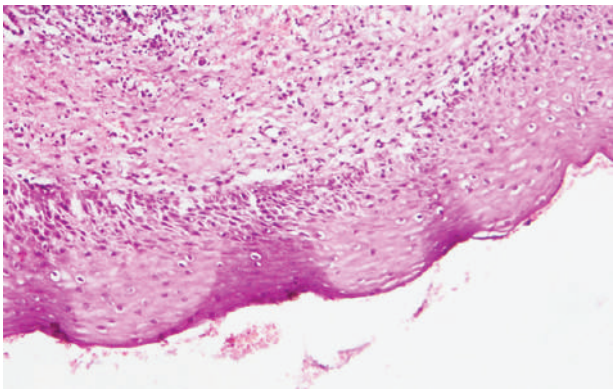

Figure 6: Photomicrograph showing normal cervical epithelium *(case no- 101, h&e, 400x)*.

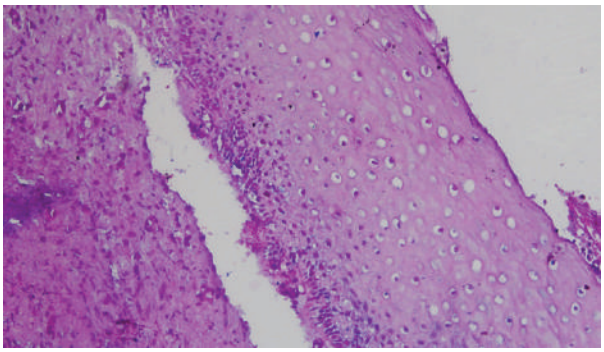

Figure 7: Photomicrograph showing normal cervical epithelium *(case no-101, pas/ab, 400x)*

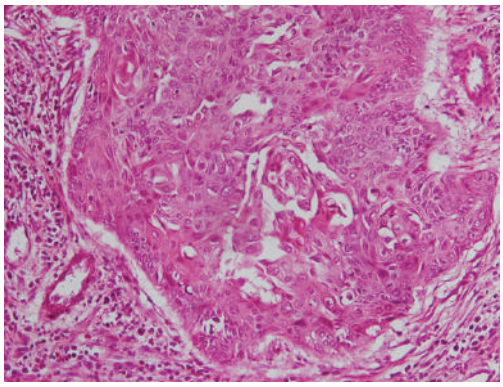

Figure 8: Photomicrograph shows invasive Squamous Cell Carcinoma *(well differentiated) (case-31, H &E, 200x)*

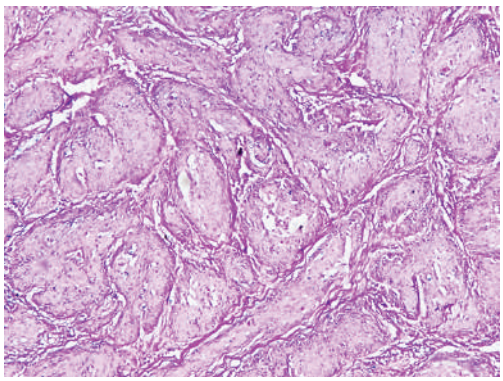

Figure 11: Photomicrograph shows squamous cell carcinoma, mucin negative *(moderately differentiated) (case no-50 pas/ab 100x)*

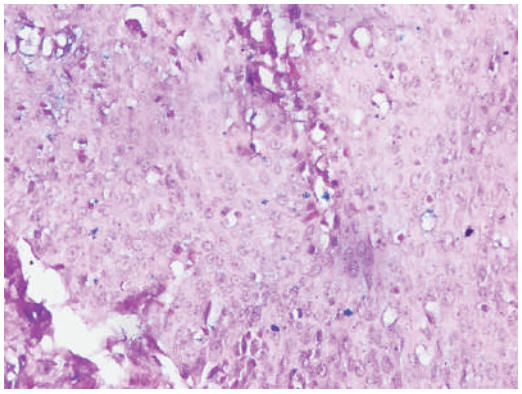

Figure 9: Photomicrograph shows invasive Squamous Cell Carcinoma *(well differentiated) (case-31, H &E, 200x)*

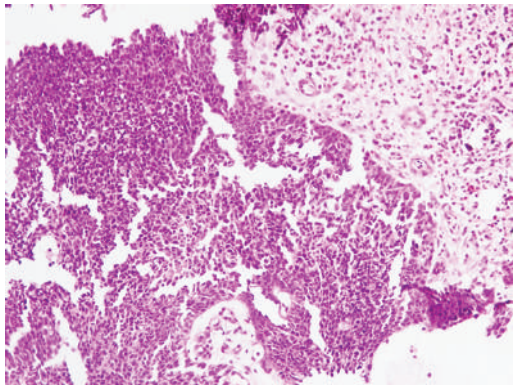

Figure 12: Photomicrograph shows squamous cell carcinoma *(poorly differentiated) (case no-49, H & E, 100x)*

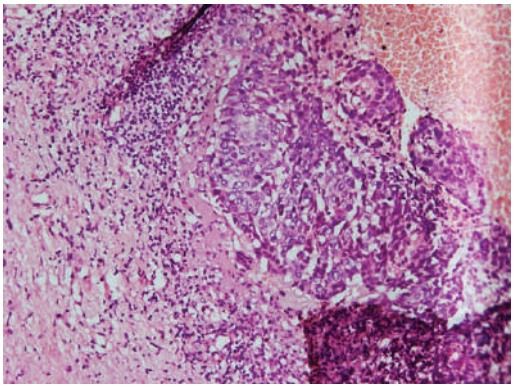

Figure 10: Photomicrograph shows squamous cell carcinoma (moderately differentiated) *(case no-50, H & E, 400x)*

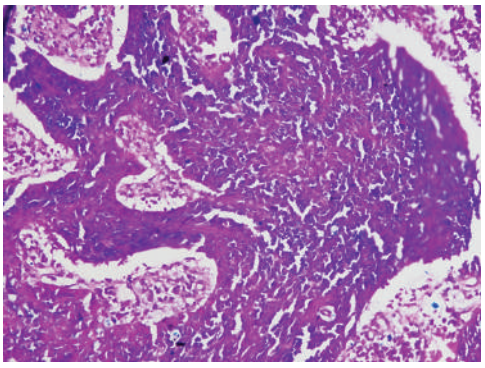

Figure 13: Photomicrograph shows squamous cell carcinoma *(poorly differentiated, mixed mucin) (case no-49, pas/ab, 400x).*

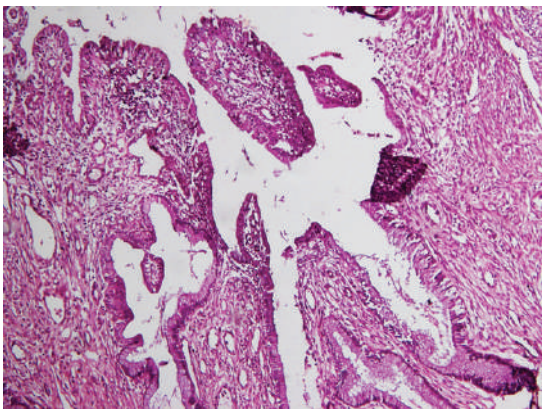

Figure 14: Photomicrograph shows adenocarcinoma *(case no-81, H & E, 200x).*

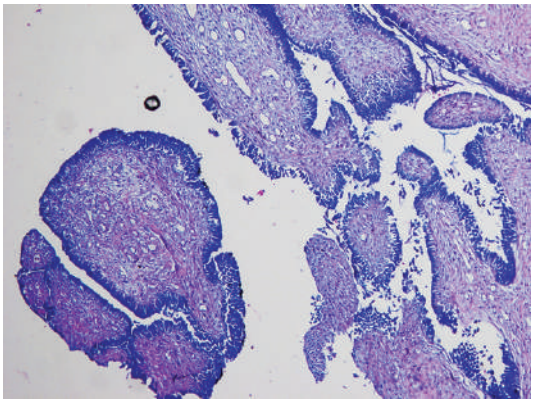

Figure 15: Photomicrograph shows adenocarcinoma, acidic mucin *(case no-81 pas/ab, 400x).*

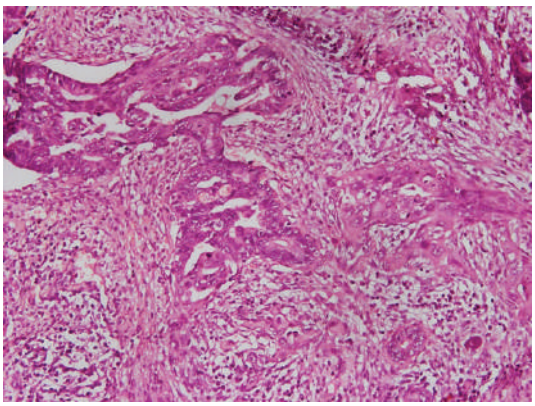

Figure 16: Photomicrograph shows adeno-squamous carcinoma *(case no-92, H & E, 400x).*

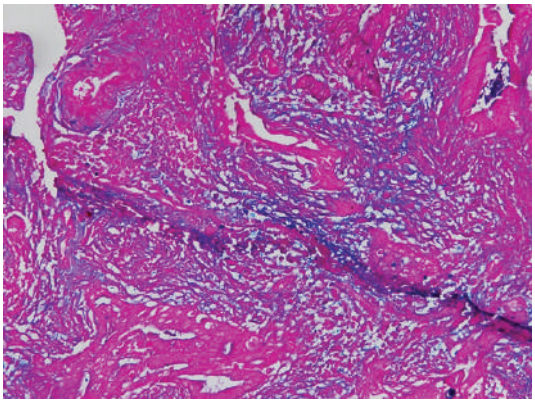

Figure 17: Photomicrograph shows adenosquamous carcinoma, mixed type of mucin *(case no-92, pas/ab, 200x).*

Discussion

This present study included 70 cervical cancer cases. The age group ranged from 29-74 years with a mean age 51.42 ± 8.75. Out of total 70 cases, 45 (64.3%) cases were invasive cervical squamous cell carcinoma with 18 cases grade-I, 10 cases grade-II and 17 cases grade-III. Fifteen (21.4%) cases were invasive adenocarcinoma, 10 (14.3%) cases were invasive adenosquamous carcinoma and all of them were grade-I tumors.

Among the total 70 invasive cancer cases, 28 (40%) of cases were mucin positive and 42 (60%) were mucin negative. Out of 28 mucin positive cases 15 (53.57%) cases showed predominantly acidic mucin, 11 (39.29%) cases showed predominantly mixed type mucin and 2 (7.14%) showed neutral mucin. Ajay et al., (2012) conducted similar study and found that, out of 30 mucin secreting carcinoma 28 showed decrease in mucin. Mucin type was mixed acidic and neutral with predominance of acid mucin in all the cases [8] which is consistent with our study. This present study result is almost similar to the study conducted by Ambali et al. (2015), in which they found that all 10 cervical adenocarcinomas gave strong reaction to Alcian blue stain [9]. In another study conducted by Hayashi et al., (2008) showed that, out of 41 cervical adenocarcinomas 11 (28.82%) showed neutral mucin, 28 (68.29%) showed mixed type mucin and 2 (4.87%) showed predominantly acidic mucin

[10]. Mucin was found positive in 4 (8.9%) cases of squamous cell carcinoma, 15 (100%) cases of adenocarcinoma and 9 (90%) cases of adenosquamous carcinoma. The mean difference was statistically significant ($p<0.05$) among the study groups. So, it is clear that well differentiated adenocarcinoma and adenosquamous carcinoma are almost always mucin positive. This result correlate with the finding of the study conducted by Linda et al., (2018) that 100% of adenocarcinoma and adenosquamous carcinoma cases were mucin positive [6]. Keshav et al., (2016) also mentioned similar result of having 100% cases of adenocarcinoma and adenosquamous carcinoma mucin positive [11]. In our study most of the mucin positive cancers are grade-I 24(55%), followed by grade-III (23.5%) and there was no mucin positive grade-II cancers. Considering the grade of the invasive cancers, grade-I cancers showed predominantly acidic mucin with 15 (34.9%) cases. All the 04 (23.5%) grade-III cancers showed mixed type of mucin. No mucin was present in 44.2% of grade-I tumors, 100% of grade-II tumors and 76.5% of grade-III tumors. Thus, it can be concluded that acidic mucin is more prevalent in grade-I and mixed type mucin in grade-III tumors. So, mucin content is gradually reduced as the grade of tumor increases.

The mucin positive poorly differentiated squamous cell carcinoma cases were reclassified as adenocarcinoma and adenosquamous carcinoma depending on the volume of mucin present. Out of 17 poorly differentiated cases of squamous cell carcinoma, 4 cases were found to be mucin positive. Three (17.65%) cases had mucin content ranging from 5-70% of the total tumor volume. These cases were reclassified as adenosquamous carcinoma. One (5.88%) case showed mucin content >70% of total tumor volume, this case was reclassified as poorly differentiated adenocarcinoma. Other studies also had similar results. Linda L et al., (2018) conducted mucin stain on cervical cancers and had to retype the histological diagnosis of 11 (24.07%) more cases as adenosquamous and 04 (7.4%) more cases as adenocarcinoma, which had been diagnosed as squamous cell carcinoma on H&E stain alone [6]. Mathur et al., (2002) conducted a study on significance of mucin secretion in carcinoma of cervix and he concluded that 16 out of 282 cases (5.6%) had to be reclassified as mixed carcinoma from squamous cell carcinoma [4].

Considering the histological types of invasive cancers well differentiated adenocarcinoma and adenosquamous carcinoma revealed acidic mucin in 60% of cases, conversely squamous cell carcinomas revealed only mixed type mucin in 8.9% of cases. Three (75%) of which were latterly reclassified as adenosquamous carcinoma and 1 (5.88%) as adenocarcinoma. So, it can be said that poorly differentiated carcinomas can present with either acidic or neutral mucin, but the mucin content is considerably decreased.

Conclusion

This study concludes that mucin may be a helpful marker for the early recognition of cancerous transformation, prediction of future prognosis. It can be used as an adjunct to H&E stain in detection of poorly differentiated mucin secreting carcinomas, which are very difficult to diagnose on H&E stain alone. Moreover PAS/AB stain may be useful for identifying intraepithelial mucin which can help clinicians for planning more accurate management considering the increased risk of developing aggressive cancers in future.

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Original Article

Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer : A Clinicopathological Study on Risk Factors

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Abstract

Background: Worldwide, cervical cancer is the fourth most common female cancer. In our country, it is the second leading cause of women cancer death. Bangladesh has a high burden of cervical cancer due to the lack of screening & high prevalence of risk factors like- persistent HPV infection, early marriage, early initiation of sexual activity, multiparity, multiple sexual partners, male partner with multiple previous or current sexual partners, sexually transmitted diseases (STDs) and low socio-economic condition, as well as older age group. **Objective:** To evaluate the clinicopathological aspect of CIN & carcinoma cervix risk factors. **Methods:** This cross-sectional study was performed in Sir Salimullah Medical College, Dhaka (from January 2018 to December 2019) on CIN & cervical cancer cases diagnosed histopathologically. Statistical analyses were carried out as required. **Results:** This study showed increase in risk of CIN and cervical cancer with increasing age and parity, early menarche, early marriage, post- menopausal state and polygamous sex behavior. **Conclusion:** Neoplastic cervical lesions has association with increasing age, early menarche and marital age, parity, menstrual status and polygamy.

Keywords: CIN, Cervical Cancer, Risk Factors.

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Introduction

Cervical cancer is the eighth most commonly occurring cancer overall [1]. Approximately 90% of deaths from cervical cancer occurred in low- and middle-income countries. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, effective screening and treatment programs. There are currently vaccines that protect against common cancer-causing types of human papilloma virus and can significantly reduce the risk of cervical cancer [2].

Human papillomavirus (HPV) is the main cause of cervical cancer. E6 and E7 oncoprotein of HPV-16 & 18 inactivate p53 & RB tumor suppressor gene leading to increased cell proliferation, dysplasia and eventually carcinoma [3]. Persistent infection with high risk Human Papilloma Virus (HPV) is the main cause of squamous intraepithelial lesions which in turn leads to invasive squamous cell carcinoma. About 10% of CIN I usually progress to CIN III, from CIN III only 12% progress to invasive cancer [4]. Cervical cancer is classified histomorphologically into squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, neuroendocrine carcinoma and

others [3].

Cancer incidence and mortality are rapidly growing worldwide. The reasons are complex but reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors, several of which are associated with socioeconomic development [1]. Late onset menarche is inversely associated with cancer of the female reproductive organs [5]. Marriage at early age is an important risk factor for cervical carcinoma. It can be explained as early exposure indicates higher susceptibility of cervical tissue to HPV infection as well as longer period of exposure to carcinogenic factors [6]. High parity is biologically plausible as a higher frequency of unprotected sex results in greater exposure to HPV and increased risk of developing cervical cancer [7]. Repeated trauma to the cervix during child birth, hormonal changes during pregnancy also makes women more susceptible to HPV infection. Parity causes oestrogen-hormonal environment throughout the fertile years of a women and oestradiol induces immortalization of HPV. According to the observation of American Cancer Society (2013) pregnant women might have weaker immune system allowing for HPV infection and cancer growth [8]. Postmenopausal women are more prone to have persistant HPV infection [9]. Because HPV is transmitted sexually, cervical cancer is more common among persons with polygamous sex behavior [8].

Materials & Methods

A total of 86 adult female patients with CIN & cervical cancer diagnosed histopathologically in the Pathology department of Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh between the periods of 1st January 2018 and 31st December 2019 were included in this study. Patients with coexistent malignancy of any other organ in addition to cervix were excluded from the study. Tissue blocks with extensive necrosis or hemorrhage, patient with cervical cancer previously exposed to chemotherapy and radiotherapy, patients with metastatic carcinoma of cervix and patients who refused to be enrolled were excluded from the study. Ethical clearance and permission was taken from the institutional ethical committee of SSMC. Statistical analysis were carried out by using SPSS version 22 for Windows. A descriptive analysis was performed for all data. The mean values were calculated for continuous variables. Quantitative observations were indicated by frequencies and percentages.

Results

Table I. Distribution of the study patients by histological diagnosis (n= 86).

Histological diagnosis	Frequency n (%)
CIN-I	12 (14)
CIN-II	9 (10.5)
CIN-III	7 (8.1)
SCC grade I	10 (11.6)
SCC grade II	28 (32.6)
SCC grade III	5 (5.8)
Adenocarcinoma	11 (12.8)
Adeno-squamous carcinoma	3 (3.5)
Small cell carcinoma	1 (1.2)

Table III. Distribution of the study patients by age of menarche (n= 86).

Age of Menarche (years)	Frequency n (%)
<10	5 (5.8)
10-12	55 (64)
13-15	26 (30.2)
Mean±SD	11.78±1.38
Range (min-max)	9-15

Table VI. Distribution of the study patients by history of exposure (n= 86).

History of exposure	Frequency n (%)
Yes	10 (11.6)
No	67 (77.9)
Not answered	9 (10.5)

Table II. Distribution of histological diagnoses according to age of study patients (n= 86).

Age	CIN-I	CIN-II	CIN-III	SCC grade-I	SCC grade-II	SCC grade-III	ADC	AdSq	SCC	Total
≤40	7	1	1	0	4	0	1	0	1	15
41-50	5	7	2	2	2	1	0	1	0	20
51-60	0	1	4	6	9	0	7	0	0	27
61-70	0	0	0	2	8	2	3	2	0	17
≥71	0	0	0	0	5	2	0	0	0	7
Total	12	9	7	10	28	5	11	3	1	86

CIN = Cervical Intraepithelial Neoplasia, SCC = Squamous Cell Carcinoma, ADC- Adenocarcinoma, Adsq = Adenosquamous Carcinoma)

Table IV. Distribution of the study patients by age at marriage (n= 86).

Age at Marriage (years)	Frequency n (%)
<15	14 (16.3)
15-20	63 (73.3)
>20	9 (10.5)
Mean±SD	17.2±2.64
Range (min-max)	12-23

Table V. Distribution of the study patients by parity (n= 86).

Parity	Frequency n (%)
Para 1	1 (1.2)
Para 2	9 (10.5)
Para 3	27 (31.4)
Para 4	23 (26.7)
Para 5	17 (19.8)
Para 6	8 (9.3)
Para 7	1 (1.2)
Mean±SD	3.86±1.23
Range (min-max)	1-7

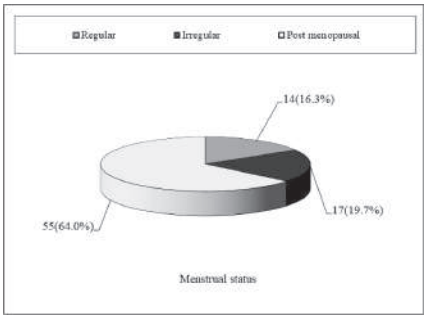

Figure 1: Pie Chart showing menstrual status of the study patients (n= 86).

Discussion

In this current study, most of the CIN III and invasive cancer cases are above 50 years of age. Study done in this institute by Yasmin S. (2017) & Rizwana R. K. (2019) stated similar findings [10, 11]. Manisa et al. (2017) observed that maximum patients representing 51.28% cases belonged to 41-50 years and 21.79% patients belonging to 51-60 years age group [12]. Misra et al. (2009) observed higher frequency of SIL in >40 years age group. The findings of present study were comparable to the above studies [13]. Above discussion might indicate that incidence of age in cervical lesion is influenced by geographic variation, ethnic differences, and their patient's awareness about cervical cancer.

Gong et al. (2015) obtained in their study that There was a 4.0% reduction in cervical cancer risk per 2 years delay in menarcheal age [5]. In this present study, 64.0% patients belonged to age of menarche 10-12 years. The mean age of menarche was 11.78±1.38 years which ranged from 9 to 15 years. Yasmin S. (2017) found more than half of the patients had their age of menarche between 9 to 16 years [10].

In this current study, maximum (73.3%) patients got married between 15-20 years. Similarly, in Bangladesh Karim S. S. (2017) observed the mean marital age was 16.7±2.7 years [14]. Such results were also observed by Yesmin S. (2017) & Rizwana R. K. (2019) [10,

11]. Nessa K. et al. (2014) reported that those who were married at an early age are affected more by cervical intra epithelial lesion and malignancy than those who are married after 18 years of age [15]. All these studies are comparable with the current study.

Regarding the menstrual status, most (64.0%) of the patients were post menopausal. Rizwana R. K. (2019) in her study found similar result [11]. Previous study conducted in this institute by Yasmin S.(2017) found 78.6% of patients with carcinoma of cervix to be postmenopausal [10].

Nessa et al. (2014) found that 55% of her study population with cervical carcinoma had >2 children, which indicate that multiparity may be related risk for pre cancerous lesions of cervix [15]. In Denmark, women who had two or more pregnancies were found to have more risk of CIN compared with nulliparous women [6]. Present study shows that most of the patients were multiparous having 3 children (31.4%) or more (56.9%).

In the present study, only 11.6% patients disclosed positive history of exposure admitted that their husbands had multiple sex partners. Yasmin S. (2017) in her study found relevant result [10].

Conclusion & Recommendation

From the above study it can be concluded that, neoplastic cervical lesions has association with increasing age, early menarche and marital age, parity, menstrual status and polygamy. In an over populated country like Bangladesh, where women usually get married in their teens, have a high parity and unaware of the importance of cervical cancer screening system it is important to improve the awareness level about the possible risk factors.

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Original Article

Experience of Tubeless Percutaneous Nephrolithotomy (PCNL) in Satkhira Medical College Hospital

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Abstract

Aim: To observe the safety and efficacy of tubeless percutaneous nephrolithotomy (PCNL). **Materials and methods:** From January 2023 to December 2023, 40 cases were included in this prospective cross-sectional study in the department of Urology of Satkhira Medical College Hospital, Satkhira, Bangladesh. **Results:** The mean age of the patients were 41.27 ± 14.61 years (21 male and 19 female). Locations of the stones were 4 pelvic stones, 29 solitary calyceal stones, 3 staghorn stones and 4 concomitant kidney and upper ureteric stones. Mean stone size range 1.90+0.93 cm. The number of tracts varied from 1-2, while the size of the tracts varied from 26 to 30 Fr. The duration of the procedure varies from 30-240 minutes and mean operation time 79±47.24 minutes. Stone clearance was rate 95%. Average hospital stay (after operation) was 4.11±1 day (one patient required a longer stay due to post-operative pyrexia). One patient has required blood transfusion.Post-operative pyrexia is noted in one patient. There is no perforation of the pelvicalyceal system, no extravasation of fluid, no pulmonary complications, no urinoma formation, no gut injury. There is no significant post-operative haematuria, no major leak is noted at wound site but in one patient has been required conversion as unable to push back. There is no need of angioembolization. **Conclusion:** PCNL (Percutaneous Nephrolithotomy) is a modern, less invasive surgery for renal stone disease and it has proven to be the most effective procedure for stone clearance with minimum hospital stay and complications. So large volume of cases should be involved which will also enhance the patient's compliance.

Keywords: PCNL (Percutaneous Nephrolithotomy), Tubeless PCNL, percutaneous nephrostomy tube(PNT).

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Introduction

The role of percutaneous nephrostomy tube (PNT) after PCNL has come under consideration in recent years. In standard PCNL, after completion of the procedure a nephrostomy tube is placed for drainage [1]. But in recent years, this procedure has been modified to use of small diameter tubes or 'Tubeless PCNL' in which no nephrostomy tube is given only a Double–J stent is given for internal drainage. Compared to open stone surgery, PCNL has advantages in terms of reduced morbidity and

mortality, quicker recovery, and cost effectiveness [2]. AUA declared PCNL to be the standard of care for patients with complex stones and staghorn stones in 2005 [3]. The standard procedure involved placing a percutaneous nephrostomy tube (PNT) in the pelvicalyceal system's tract. PNT drained urine, acted as a hemostatic tamponade for the tract, and provided easy access in case PCNL needed to be checked again. Many urologists have attempted to demonstrate its non-essential nature since 1997. Several reports have confirmed that PCNL without PNT is a safe procedure in a subset of patients and has a lower analgesic requirement, faster recovery, early discharge, and less morbidity [4-6]. At our institute, tubeless modification for percutaneous renal surgery has been a regular practice since january2023. We compared our observations of the safety and effectiveness of tubeless PCNL in this study in a descriptive manner, assessing variables like post-operative fever, transfusion rate, angioembolization, pulmonary complications and mortality, reexamination, nephrectomy, major leak, sepsis, pain score, and early discharge.

Material & Method

It was a prospective cross sectional study done in the department of Urology of Satkhira Medical College, Satkhira, Bangladesh between the periods of January 2023 and December 2023. Forty cases were included in this study.

Operation Procedure

Under general anesthesia, urologists carried out all PCNL procedures using the standard operating technique. The ureteric catheter was kept in the lithotomy position using a cystoscope with both ends open. If there is a stone in the middle or upper ureter, it can be pushed into the kidney using an ureteroscope, either fragmented or not, and placed in the lithotomy position. In the prone position, the renal access tract was obtained using the flouro Bull's eye/gradual descent/triangulation technique. Depending on the weight of the stone and the structure of the kidney, the tract size ranged from 26F to 30Fr and single to double tracts utilizing a pneumatic lithotripter, the stones were broken up. A 5 Fr double J stent was positioned antegradely for post-operative urinary drainage following stone clearance. Amplatz was eliminated, and a 5-minute hand compression was applied to the tract. There was no use of cautery, hemostatic gel, or foam. Single/double stitches were used. Every patient had a Foley's catheter placed for urinary drainage, and the catheter was taken out the following morning. After 4 weeks, the double J stent was removed. Age, size of the stone, length of surgery, length of stay in the hospital following surgery, fever, rate of transfusions, and other complications were noted and examined.

The diameter of the stones were measured by plain X-ray KUB region 100% film and USG of KUB region. The duration of the operation was measured from the start of the cystoscopy to the Amplatz sheath removal. Stone-free refers to the total elimination of stones observed during fluoroscopy at the operating table. Post-operative fever is defined as a body temperature that is higher than 1010F during hospitalization or readmission. A patient with suspected infection and systemic inflammatory response syndrome was classified as having sepsis.

Statistical analysis

The Microsoft Excel sheet contained all of the patient's details. The tool used for descriptive analysis was Microsoft Excel 2010. Whereas quantitative data were presented as mean with standard deviation, qualitative data were expressed as frequency and percentage.

Results

The patients ranged in age from 18 to 65 years old, with a mean age of 41.27 ± 14.61 years (21 male and 19 female). Locations of the stones are 4 pelvic stones, 29 solitary calyceal stones, 3 staghorn stones, 4 concomitant kidney and upper ureteric stones. Mean stone size range 1.90±0.93 (4.5 cm-0.9 cm). The number of tracts varied from 1-2, while the size of the tracts varied from 26 to 30 Fr. Pyonephrosis, radiolucent stones, non-visualized kidney on IVP, residual stones (real-time flourescence detected) on table, and single kidney all require a PCNL reexamination.

The duration of the procedure varied from 30-240 minutes with mean operation time 79±47.24 minutes. Stone clearance is rate was 95%. Average hospital stay (after operation) was 4.11±1 day (one patient required a longer stay due to post-operative fever). One patient required blood transfusion (rate 2.5%). Post-operative pyrexia is noted in one patient (rate 2.5%). There was no perforation of the pelvicalyceal system, no extravasation of fluid, no pulmonary complications, no urinoma formation, no gut injury. There was no significant post-operative haematuria. No major leak is noted at wound site but in one patient required conversion as unable to push back. There is no need of angioembolization.

Table I. Distribution of the patients according to complications.

Complications	Frequency n (%)
Perforation of pelvicalyceal system	0 (0)
Extravasation of fluid	0 (0)
Urinoma formation	0 (0)
Pulmonary complications	0 (0)
Gut injury	0 (0)
Major leak	0 (0)
Angioembolisation rate	0 (0)
Significant post-operative haematuria	0 (0)
Post-operative pyrexia	1 (2.5%)
Blood transfusion	1 (2.5 %)
Re-exploration or nephrectomy	0 (0)
Haematoma	0 (0)
Unable to push back	Conversion to open
Urosepsis	0 (0)
Mortality	0 (0)

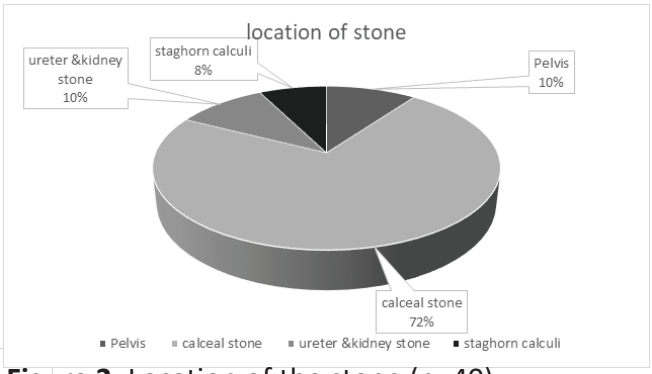

Figure 2: Location of the stone (n=40).

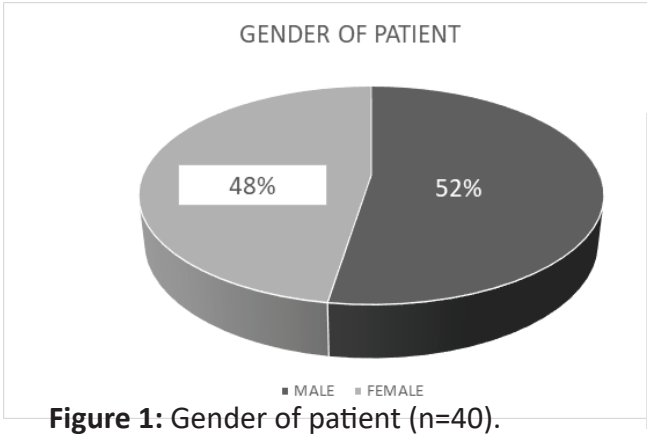

Figure 1: Gender of patient (n=40).

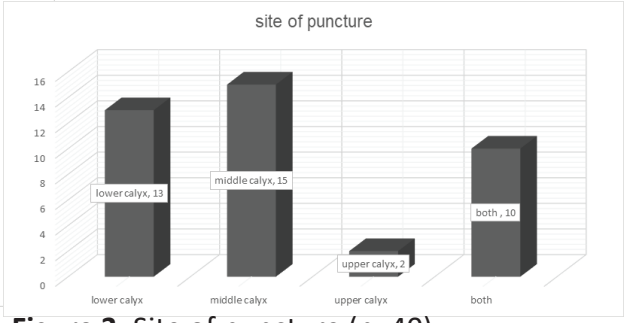

Figure 3: Site of puncture (n=40).

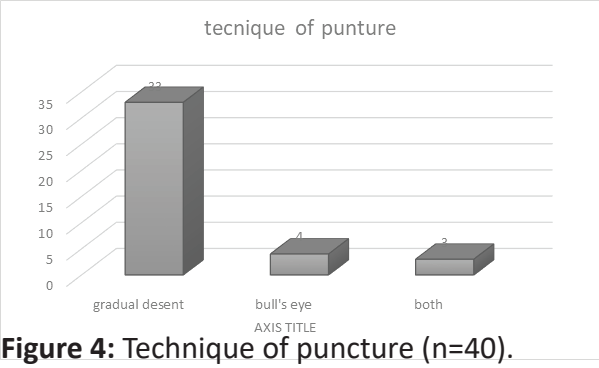

Figure 4: Technique of puncture (n=40).

Discussion

Fareed et al. (2021) reported that 210 consecutive tubeless PCNL procedures were completed; 134 of the patients were male and 76 were female, with an average age of 57 ± 11.8 years, and 7 patients were between the ages of 8 and 12 [1] . In our study from January 2023 to December 2023, 40 cases were included in this prospective cross-sectional study. The patients ranged in age from 18 to 65 years old, with a mean age of 41.27 ± 14.61 years (21 male and 19 female).

It was believed earlier that percutaneous nephrostomy tube (PNT) has tamponade effect on tract and prevent parenchymal bleeding. Many authors tried to observe the relationship with diameter of PNT and its tamponading effect as reasoning was that bigger PNT, better tamponade. But as advancement of instrumentation occurred and experience of urologist increased day by day, many authors tried to prove its non-necessity. Last steps of PCNL include DJ stenting/leaving of ureteric catheter and placement of percutaneous nephrostomy tube (PNT). As in 1997 Bellman [4] and associates showed that without PNT there was less cost, decreased hospital time, was less pain and therefore less analgesia required. Candela et al. [7] compared cost of tubeless procedure with conventional procedure. It was 1638 $ with tubeless and 3750 $ with PNT. Many authors' studies proved safety and low morbidity with tubeless PCNL [8-11]. Earlier studies were had strict case selection. But as PCNL spread widely, and more and more urologist started doing it thus experience increased and fear faded, and they studied its safety and applicability in more complex cases like in 2005 Shah et al. [11] was done in solitary kidney and multiple renal tracts and Wang et al. [12] in 2011 showed its effectiveness in bilateral stag horn stones. In our study we did all the complex stones with multiple tracts. We did not evaluate patient for cost effectiveness but we studied our patients in relation to pain, post-operative pyrexia, transfusion rate, angioembolisation, pulmonary complications, mortality, re exploration, nephrectomy, major leak and urosepsis, compared our results with traditional PCNL results.

Many author used different haemostatic method in tracts like gelatin thrombin sealant [13, 14] , fibrin glue [15-18] oxidized cellulose (surgicel) [19] with mixed results. In our study we did not use any hemostatic agent but after the procedure we compress the wound site for 2-3 min and primary closure was done by single stitch. Many authors cauterised the tract [20, 21] we did not use any maneuver in the tract and did not sutured skin incision also only 5 min hand compression.In 1000 PCNL, Segura et al. [22] reported transfusion rate 3.0%, angioembolisation 0.6% and pnumothorax 0.1%. Jones et al. [23] reviewed 1000 cases and reported mortality 0.7%, blood transfusion 8.4%, angioembolisation 0.6% and 7.5% sepsis. Tefekeli et al. [24] in 2008 retrospectively reviewed 811 PCNL and find his transfusion rate was 10.9%, post-operative fever was 2.8%.

In our study, tubeless PCNL did not show worse complication rate than the traditional PCNL with PNT. Our transfusion rate is 2.5 % which is lower than many studies; angioembolisation is 0 % which is comparable to other studies and post-operative fever 2.5 % which is not very high. We do not have mortality, sepsis, pulmonary complications and major leak.

Berkman DS et al. [25] showed tubeless patients were more likely to be discharged on post-operative day 1 compared to standard patients (96% versus 72% P = 0.02). In Agarwal MS et al. [26] study, the average hospital stay in the tubeless group (21.8 + 3.9 hours) was significantly shorter than standard PCNL group (54.2 + 5 hours) (P < 0.01). Sofikerim M et al. [27]. Mean hospital stay with tube and tubeless were 74.4 hours (3.1 day) and 38.4 hours (1.6 day) respectively (P= 0.003).

Conclusion

From the above study it could be concluded that, PCNL (Percutaneous Nephrolithotomy) is a modern, less invasive surgery for renal stone disease and it has proven to be the most effective procedure for stone clearance with minimum hospital stay and complications. If an initial puncture is good and an excellent tract is made, tubeless PCNL can be done with favorable outcome. So large volume of cases should be involved which will also enhance the patient's compliance.

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Original Article

Effect of Probiotics with Standard Treatment in the Management of Acute Watery Diarrhea among Children Aged 6 Months to 5 Years in Comparison with Standard Treatment Alone: A Randomized Controlled Trial

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Abstract

Objective: Diarrheal disease is a major health problem specially in children. If acute watery diarrhea (AWD) is not treated promptly and effectively it can lead to serious sequelae. Probiotics are very effective in addition to Oral Rehydration Solution (ORS) and zinc. Because probiotics can improve the intestinal microenvironment, promote immunity, and enhance resistance. **Methods:** It was an open clinical randomized controlled trial study which was conducted at the department of Pharmacology in collaboration with the department of Pediatrics in Rajshahi Medical College, Hospital,Rajshahi, Bangladesh over a period of 1 year from July 2022 to June 2023. A total of 134 patients were enrolled according to inclusion and exclusion criteria. Data were collected by a semi-structured questionnaire. The control group (group -A) was treated with ORS and zinc and the intervention group (group-B) was treated with probiotics plus ORS and zinc for 7 days. **Results:** The mean duration of diarrhea in the control group was 94.92±12.13 hours and in the intervention group was 78.08±10.52 hours which was significant. The frequency of diarrhea was 1.46±0.51 times/day in the control group and 1.20±0.40 times/day in the intervention group on day 2 ($p<0.001$) after administering probiotics which was statistically significant. **Conclusion:** Probiotic is effective, safe, well tolerated in patients with acute watery diarrhea. It reduces not only the total duration of diarrhea but also the frequency of diarrhea.

Keywords: Acute watery diarrhea, Zinc, Probiotics.

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Introduction

Diarrheal disease is a major public health concern and second leading cause of death among children under 5 years old. According to the World Health Organization (WHO) [1] and United Nations International Children's Emergency Fund (UNICEF) [1] globally there are nearly 2 billion cases of diarrheal diseases every year. Eighteen % of all the deaths of children under 5 years old means that more than 5000 children are dying everyday as a result of diarrhea. About 1.9 million children younger than 5 years of age perish from diarrhea each year mostly in developing countries. Overall, the prevalence of diarrhea is, 8.39 % in the Philippines and 18.21% in Indonesia respectively [2]. In our country the prevalence rate is 2% of the total population and

in Rajshahi it is about 4.3% [3]. According to the World Health Organization the under-five mortality rate in low-income countries was 73.1 deaths per 1000 live births, nearly 14 times the average rate in high-income countries (i.e., 5.3 deaths per 1000 live births) [4].

According to WHO, diarrhea is defined as passage of three or more loose or watery stools in a 24-hour period. It is a life threating disease in children. It is often accompanied by fever, vomiting, electrolytes and pH imbalance. There are three types of diarrhea:

1. Acute-presence of three or more abnormally loose or watery stools <2 weeks.
2. Persistent -acutely starting episode of diarrhea lasting 2 to 4 weeks.
3. Chronic-diarrhea lasting > 4 weeks.

The most common organism for acute watery diarrhea is enterotoxigenic *Escherichia coli. Vibrio cholerae* is endemic in approximately 50 countries in Asia Africa and Central and South America where predictable seasonal outbreaks occur. *Norovirus, Campylobacter species, non-typhoidal salmonellae, Aeromonas species*, and entero-aggregative *Escherichia coli* that can cause acute watery diarrhea. Wardlaw et al. (2010) have shown that early onset of diarrheal episodes predisposes children to lasting disabilities, stunted growth, and impaired cognition and school performance [5]. Notwithstanding obvious improvements in the case management of diarrhea, including early administration of oral rehydration solutions, continued feeding, oral zinc supplements. According to WHO, the standard treatment of acute watery diarrhea is oral rehydration salts (ORS) solution which is a mixture of clean water, salt and sugar with zinc supplements. ORS is absorbed in the small intestine and replaces the water and electrolytes lost in the feces and zinc supplements reduce the duration of a diarrhea episode by 25% and are associated with a 30% reduction in stool volume. But beside these two, probiotics are often used as an adjuvant therapy to improve intestinal barrier function and immune interactions [1].

Probiotics are nonpathogenic live bacteria that grow in the intestine. They harmlessly regulate the intestinal normal flora like *Lactobacillus, Enterococcus, Bifidobacterium* by colonizing and changing the composition of them in intestine that help in reproduction and growth of beneficial normal flora and enhancing the ability to resist when intestinal environment is disturbed by rotavirus or any other pathogens. Now a days about ten countries use probiotics to treat diarrhea including China, India, Bangladesh, Philippines, South Korea, Taiwan, Indonesia, Thailand, Italy and Malaysia [6]. The name of some probiotics is *Bacillus clausii, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Saccharomyces boulardii, Bifidobacterium lactis, Lactobacillus reuteri* [7].

In recent years, the use of probiotics has gained increased popularity, even if the concept of using probiotics for prevention and treatment of some human illnesses has been around for more than a century [8]. Probiotics also reduce the duration of rotavirus shedding in India. Strategies to reduce fecal shedding of pathogens are important for the billions of people who live without adequate sanitation. Probiotics could also have a role in immunization programs.

Lactobacillus rhamnosus increases the virus specific antibody response in children with acute rotaviral gastroenteritis. So immuno-stimulatory probiotics might help children's immune system increase the memory response to vaccines. But probiotics seem to have a more positive impact on growth in healthy children rather than severely malnourished children. The effect of probiotics on

improving nutritional status might be limited [9]. So, it is thought worthwhile to conduct this study to monitor the effect of probiotics in acute watery diarrhea with nutritionally good children under 5 years.

Materials & Methods

Total 134 children aged 6 months to 5 years admitted in Rajshahi Medical College Hospital (RMCH), Rajshahi, Bangladesh with acute watery diarrhea (AWD) during July 2022 to June 2023 were selected. This study was a hospital based randomized controlled clinical trial (RCT) study.

Patient who had diarrhea that had been treated for >3 (three) days at home, dysentery, severe malnutrition, who has received any type of medication (antibiotics, antiprotozoal etc.) except fluid and zinc, septicemia or any other inflammatory diseases, any cardiac anomaly, immunodeficiency condition were excluded from this study. Immediately after got admitted into hospital with AWD, patients meeting the inclusion criteria were selected for the study. The case was randomized & systematically selected as group A (ORS plus zinc supplements) and group B (probiotics, ORS and zinc supplements). A consent form is signed by the parents before initiating any treatment. In group A received ORS and 20 mg of zinc syrup daily for 10 days. In group B each patient received 20 mg once daily zinc syrup, ORS and 1 tube probiotics one time for 7 days. Daily follow up the number of stool passes was recorded and signs of dehydration were assessed. We did not perform a stool examination because of the high cost required.

Total duration and daily frequency of loose stool were done during hospitalization and after the patient was discharged. Toxicity and side effects relating to zinc and probiotics were also observed (nausea, vomiting, abdominal pain, and sepsis). We defined recovery from diarrhea as stool passed < 3 times. Home monitoring (24 hour) was done by contacting the parents or caregivers over telephone. All data were compiled and edited meticulously by thorough checking and rechecking. All omissions and inconsistencies were corrected and removed methodically. The results were collected, tabulated and statistically analyzed by using a window-based computer software device with a statistical package for social science (SPSS-24). Patient characteristics were reported as means ± standard deviations and n (%) as appropriate. t-test was used for continuous variables.

Results

Mean age of the control group and intervention group were almost similar 10.11 ± 5.69 months and 13.30 ± 8.10 months. In the control group boys 59.70 % & girls 40.20 % and in the intervention group boys 67.10 % & girls 32.80 %. A large number of the patients were boys in both groups (59.70% & 67.10 %). Mean weight of the control group was 7.2 ± 2.2 kg & the intervention group was 7.1 ± 1.7 kg. Mean weight of both groups were almost similar (7.2 ± 2.2 and 7.1 ± 1.7). In the control group, 89.5 % and 10.44 % patients had no sign and some sign of dehydration respectively. On the other hand, in the intervention group 88.05 % and 11.94 % patients showed no sign and some sign of dehydration. Total duration of diarrhea in the intervention group was 78.08 ± 10.52 hours and in the control group was 94.92 ± 12.13 hours ($p<0.001$) which was statistically significant. So, it was evident that in the intervention group the severity of the diarrhea was significantly reduced in comparison to control group. The frequency of diarrhea before intervention and after intervention in both groups was different. Before intervention the mean frequency of diarrhea in the control group was 1.44 ± 0.50 times/day and in the intervention group was 1.50 ± 0.51 times/ day (p 0.233), which was non-significant. But after interven-

Table I: Baseline characteristics of study subject (n = 134).

Variables	Control group (n = 67)	Intervention group (n = 67)
Age (month)		
Mean age ± SD	10.11 ± 5.69	13.30 ± 8.10
Sex		
Boys; n (%)	40 (59.8)	45 (67)
Girls; n (%)	27 (40.2)	22 (33)
Dehydration		
No sign of dehydration; n (%)	60 (89.5)	59 (88.05)
Some sign of dehydration; n (%)	7 (10.44)	8 (11.94)
Mean Weight ± SD (Kg)	7.2 ± 2.2	7.1 ± 1.7
Mean frequency of diarrhea before treatment (times/day)	1.44 ± 0.50	1.50 ± 0.51

Table II: Total duration of diarrhea and mean frequency of diarrhea after intervention among study population (n = 134).

Parameter	Control Group (ORS+Zinc) (n = 67)	Intervention Group (Probiotics+ORS +Zinc) (n = 67)	*p* value
Total duration of diarrhea	94.92 ± 12.13	78.08 ± 10.52	<0.001
Mean frequency of diarrhea after intervention (times/day)	1.46 ± 0.51	01.20 ± 0.40	<0.001

tion, on day 2 it was 1.46 ± 0.51 times/day in the control group and was 1.20 ± 0.40 times/-day in the intervention group which was statistically significant (p<0.001). Complete recovery in the control group was on day 7 and in the intervention group was on day 5 (table 2).

Discussion

About two million deaths are caused by acute watery diarrhea every single calendar day worldwide. One of the main reasons for childhood mortality and morbidity is gastrointestinal disease. Every child develops five to six episodes of diarrhea each year resulting in malnutrition. Acute watery diarrhea is mainly treated by correcting dehydration. One study showed that serine protease (54-kDa) was secreted by probiotics causing inhibition of clostridium to bind with the intestine [10]. When *Bacillus clausii* is added in treating acute watery diarrhea, it reduces the duration of diarrhea. It is also reported that treatment with probiotics are associated with shorter duration of AWD as compared to treatment with fresh yogurt [10]. Similarly, probiotics not only inhibit the overgrowth of pathogens, but also enhance the anti-pathogenic ability against microbiota associated with diarrhea [10].

The mean age of the patients in the control group was 10.11 ± 5.69 months and in the intervention group was 13.30 ± 8.10 months. The majority of patients in the study subjects were within the age range of 6-12 months and 13-24 months. This is likely due to the introduction of possibly contaminated foods and the increased contact with human or animal feces when the infants begin to crawl. Nearly similar findings were found in the study done by Salam MA et al, (2013) [11] in. In the current study, gender distribution of patients revealed that in the control group 59.8% were boys and

40.2 % were girls and in the intervention group 67% were boys and 33% were girls. Boys were predominant in both groups. Similar findings were found in a study done by Salam MA et al., in 2013.In the current study, in the control group, the majority (89.5%) of the patients had no sign and only (10.44%) patients had some sign of dehydration. On the other hand, in the intervention group (88.05%) patients showed no sign and (11.94%) patients showed some sign of dehydration during admission. In an another study done in Philippines, Johnson HL et al. (2012) found that, about 36.36 % had no sign and 63.64 % had some sign in the control group and 30.90 % had no sign and 67.27 % had some sign of dehydration in the intervention and the contradictory finding was that in this study patients with some sign of dehydration were more than the no sign of dehydration [12].

In this study, the total duration of diarrhea in the intervention group was 78.08±10.52 hours while in the control group was 94.92± 12.13 hours which was statistically significant ($p<0.001$). Similar findings were found in a study done by Bhat S et al., 2018 in India where total duration of diarrhea in the control group was 57.65 ± 26.31 hours and in the intervention group was 53.33 ± 16.78 hours ($p<0.001$) [13]. In the present study the frequency of diarrhea before intervention and after intervention in both groups was different. Before intervention the mean frequency of diarrhea in the control group and in the intervention group was non-significant. But after intervention, on day 2 it was 1.46± 0.51 times/day in the control group and was 1.20± 0.40 times/-day in the intervention group which was statistically significant ($p <0.001$). Complete recovery in the control group was on day 7 and in the intervention group was on day 5. Similar findings were found in a study done by Kliegman RM et al., (2010) [14] in Iran.

Limitations of study

We had some limitations in our study. In our study, the sample size was not so. Also, it was a single center study which dealt with the pediatrics population of a specific geographical area. Study was followed for a short time in patients so long-term effects of probiotics were not evaluated, the etiology of diarrhea was unknown.

Conclusion

From the study it could be concluded that, use of probiotics is significantly associated with reduction of total duration and frequency of diarrhea. When probiotics are used with ORS and Zinc supplements in treating acute watery diarrhea, it also results in early recovery.

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