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Editorial

Evolution of "Superbugs": Are We Heading Towards a Post-Antibiotic Era?

Chowdhury RT

Imagine a scenario of what a world without effective antimicrobials would look like! A minor cut or a sore throat, once treated with ease, could turn into a life threatening bacterial infection. Even though having shelves full of various antimicrobials, doctors will be unable to stop infections and can do nothing but to say sorry to the patients. This is no longer an apocalyptic fantasy rather a very real possibility of near future. Evolution of "Superbugs", the dangerous multidrug-resistant bacteria and other pathogens, are now threatening to roll back a century of medical advances.

Introduction of antibiotics revolutionize healthcare, becoming the bedrock of many of the greatest medical advances of the 20th century. Common yet, frequently deadly illnesses such as pneumonia and tuberculosis could be treated effectively; a small infected cut, dangers of routine surgery and childbirth- no longer had the potential to be fatal and were vastly reduced. More recently, advances in antiviral developments over the past 20 years have transformed HIV from a probable death sentence into a largely manageable lifelong condition.[1] However, fast forward several decades, after an era when miraculous cures became the norm, the war is changing and we are again running out of effective antibiotics as "Superbugs" have been gaining strength, becoming resistant to all currently available antibiotics.

Scientists have known for long time, even with the introduction of penicillin that, bacteria can adapt to resist against the drugs formulated to fight them. Sir Alexander Fleming while accepting his Nobel Prize in 1945, warned about possibility of emergence of antibiotic resistance as a consequence of misuse of the miracle drug Penicillin. Unfortunately, this serious threat is no longer a prediction for the future. It is happening right now in every region of the world and has the potential risk to affect anyone, of any age, in any country.[2] And it is not just a problem confined to bacteria, but all microbes that mutate and render our drugs ineffective.

This crisis is the fruit of several decades of over reliance on antibiotics and careless prescribing practice as well as misuse of the medicines to nurture livestock. Up to 50% of all the antibiotics prescribed are not needed or incorrectly prescribed. Even in United States 47 million unnecessary antibiotic prescriptions are given every year.[3] Moreover, Global consumption of antibiotics in human medicine rose by nearly 40% between 2000 and 2010.[4] U.S. Centers for Disease Control and Prevention has reported that, more than two million people become sick every year due to antibiotic-resistance infection in United States and 23,000 die as a result.[3] Across Europe and U.S. resistance claim at least 50,000 lives each year, while it kills approximately 700,000 people annually worldwide. If this current trend of antibiotic use continues and appropriate measures are not taken immediately, this number is accepted to balloon to 10 million by 2050.[1]

Antibiotic resistance is putting patients in peril in both developing and developed countries, as superbugs evolve resistance to the drugs that once vanquished them. Drugs that were considered previously as a last resort are now becoming the first-line treatment. Gonorrhea, once well treated by antibiotics, is once again a major public health threat due to the emergence of new, resistant strains. Treatment failure gonorrhoea to third generation cephalosporin antibiotics has been confirmed in at least 10 countries like Australia, Austria, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden, the United Kingdom and Northern Ireland. WHO recently updated the treatment guidelines for gonorrhoea that do not recommend quinolones due to widespread high levels of resistance. In addition, treatment guidelines for Chlamydia infections and syphilis were also updated. Resistance in *Klebsiella pneumoniae* to a last resort treatment has spread to all regions of the world as carbapenem antibiotics no longer work in more than half of people treated in some countries. Fluoroquinolones used to treat urinary tract infections have also become ineffective against *E. coli* in more than half of sufferers in many parts of the world. Resistance to first-line drugs to treat infections caused by *Staphylococcus aureus* is widespread. People with MRSA (methicillin-resistant *Staphylococcus aureus*) are estimated to die 64% more than people with a non-resistant form of the infection.[5] Globally, 480 000 people develop multi-drug resistant TB each year among which an estimated 9.7% people have XDR-TB(Extensively Drug Resistant-TB).[6] Moreover, the great strides forward made over the past few decades to manage malaria and HIV are also all under threat since these diseases could be reversed and once again spiraling out of control. Colistin is the last resort treatment for life-threatening carbapenem resistant infections caused by Enterobacteriaceae. Recently colistin resistant *E. coli* infection is reported in several countries and regions.[5] First discovered in Chinese meat products, a resistance to colistin represents a fear among physicians of even our strongest drug becoming ineffective, making infections untreatable.

WHO warns that the situation could have sweeping effects on global medicine, economics and societies unless global actions are taken swiftly. A dearth of effective antibiotics will mean that infected patients will need more extensive care, require longer hospital stays, increased health-care cost and die in greater numbers. Only through concerted commitment and by widespread engagement, especially among leaders in clinical medicine, healthcare leadership,

agriculture, and public health, the world will be able to succeed in averting this threat. Immunization, safe food preparation, hand washing and using antibiotics as directed and only when necessary can prevent infections and further spread of superbugs. Stopping even some of the inappropriate and unnecessary use of antibiotics in people and animals would help greatly in slowing down the spread of resistant bacteria. Moreover, we will need new antibiotics to keep up with resistant bacteria as well as new diagnostic tests to track the development of resistance.[3]

Superbug explosion is a looming global crisis, putting the gains of the Millennium Development Goals at risk and endangers achievement of the Sustainable Development Goals.[5] In a landmark meeting, the United Nations General Assembly voted to take a coordinated approach to antibiotic resistance as a global health crisis. If we don't take immediate initiative to combat resistance then in that bleak future, medicine would descend into a "post antibiotic era," and infectious diseases would once again reign supreme.

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Original Article

Trend of Metabolic Syndrome among the Young Female Doctors in Bangladesh

Khanduker S[a], Hoque M M[b]

Abstract

Background: In recent years there has been striking increase in number of metabolic syndrome (MS) in developing world. Increasing trend of metabolic syndrome among the young female doctors is of serious concern in the context of health professionals and health service delivery in every country.

Objective: To estimate the frequency of metabolic syndrome among the young female doctors of Bangladesh**.**

Methods: This cross sectional study was carried out in various medical college hospitals and BSMMU during the period of January 2010 to December 2011. Through purposive sampling 300 female doctors of aged 30-45 years having minimum MBBS degree were enrolled in this study. According to age we divided them into two groups: Group-I (30-37 years) and Group-II (38-45 years). Metabolic syndrome was evaluated according to NCEP ATP III criteria**.** History, blood pressure and waist circumference (WC) measurements were done. In laboratory, blood sample was analyzed for fasting blood glucose and lipid profile as components of metabolic syndrome. Data analysis was done by using statistical software SPSS version 16.0. In two groups metabolic syndrome were measured and analyzed at 95% CI and comparison was done by Chi-square test.

Results: Among the 300 total study subjects, metabolic syndrome found in 83 (8%) doctors. In Group-I (age 30-37 years), out of 172 doctors and in Group-II (age 38-45 years) out of 128 doctors 30 (23%) and 53 (34%) were diagnosed with metabolic syndrome respectively. The proportion of metabolic syndrome found higher in Group-II or higher age group doctors.

Conclusion: Presence of metabolic syndrome was high among the young female doctors of Bangladesh. Lifestyle modification and periodic screening can play a greater role for its prevention and control.

Keywords: Frequency, Metabolic Syndrome, Female, Doctors.

Introduction:

Metabolic syndrome is characterized by the cluster of physical and biochemical abnormalities in an individual which predisposes a person to increased risk of developing cardiovascular disease and it is also predictive of future diabetes mellitus.[1] It is also a cluster of risk factors including obesity, atherogenic dyslipidemia, hypertension, glucose intolerance and a pro-inflammatory and prothrombotic state that predispose a patient to the risk of developing cardiovascular diseases, type 2 diabetes mellitus, renal failure and stroke.[2]

Previously obesity was confined to the industrialized world, but recently there has been striking increase in number of people with metabolic syndrome. In developing countries the prevalence of the metabolic syndrome varies from 13% to 30%.[3] Recent data show that one fourth to one third of urban population of India has the metabolic syndrome. Data show metabolic syndrome to be higher in South Asians (men 29%, women 32%) than Caucasians (men 18%, women 14%).[4] This commonly observed aggregation has been done by several different names: syndrome X, insulin resistance syndrome, dysmetabolic syndrome and deadly quartet. Among them "Metabolic syndrome" is widely used and broadly accepted. Although it was recognized as early as 1923, the coining of the term "syndrome" was done by Reaven in 1988.[5]

Different definitions have been drawn up by The World Health Organization (WHO), US National Cholesterol Education Programme Adult Treatment Panel III (ATP III) and International Diabetes Federation (IDF).While most agree on the essential components of the metabolic syndrome which are glucose intolerance, obesity, hypertension and dyslipidemia; they differ in the cut off points used for each of these and the combinations of components used to define the syndrome.[6]

Practicing physicians involved with health care are an important segment of public health service providing system. It has been observed that the prevalence of metabolic syndrome is more in peoples used to sedentary life. Nature of profession makes the doctors to lead sedentary life because they are awfully burdened with institutional and private practice. Most studies from developed countries show that doctors, generally do not take care of their health.[7,8] Young Indian physicians have

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high rates of cardio metabolic risk factors and metabolic syndrome. Prevalence of metabolic syndrome among them measured to be 29% according to the ATP III criteria modified to suit Indian population.

Materials and Methods:

This descriptive and cross sectional study was carried out in the Department of Biochemistry, BSMMU, Dhaka during the period from January 2010 to December 2011. Total 300 young female doctors (aging 30-45 years) were selected from various medical college hospitals and BSMMU. They had a minimum of 5 years' experience. This list included all physicians with different levels of medical education ranging from MBBS, post-graduation (MD, MS, M.Phil, FCPS and MRCOG) and specialty training for post-graduation education. Medical history, family history of diabetes, hypertension and treatment details were noted. The study subjects divided into two age groups as Group-I (aged 30-37 years) and Group-II (aged 38-45 years). Along with WC measurements blood pressure, fasting blood glucose and lipid profile were measured and compared between the two groups. Waist circumference was measured in standing position with feet together and arms at the side and abdomen relaxed. A tape was placed around the waist at the level of umbilicus midway between the bottom of the rib and the top of the hipbone. After 10-12 hours of overnight fasting, 5ml of venous blood was collected from median cubital vein of each study subjects by disposable syringe with all aseptic precautions. All the biochemical tests were performed by auto-analyzer in the Department of Biochemistry, BSMMU, Dhaka.

Laboratory Method:

1) Estimation of fasting plasma glucose was done by 'Glucose Oxidase' (GOD-PAP) method.
2) Estimation of fasting serum total cholesterol was done by enzymatic end-point (CHOD-PAP) method.
3) Estimation of fasting serum triglycerides (TG) was done by enzymatic (GPO-PAP) method.
4) Estimation of fasting serum high density lipoprotein (HDL) cholesterol was done by enzymatic end point (CHOD-PAP) method.
5) Estimation of fasting serum Low-density lipoprotein (LDL) cholesterol was calculated by using Friedwald's formula.

According to modified ATP III criteria metabolic syndrome was diagnosed if any three or more of the following were present: waist circumference >90cm for men and > 80 cm for women, SBP =130 mm of Hg and/or DBP = 85 mm of Hg or medical treatment of previously diagnosed hypertension, TG=150 mg/dl, HDL<40 mg/dl (male) and< 50mg/dl for women & fasting glucose =110 mg/dl.[9]

Data were analyzed using the software-SPSS (Statistical Package for Social Sciences) for windows version 16.0. The results were expressed as Mean±SD (Standard deviation) and proportion of metabolic syndrome was measured at 95% CI. Comparisons were done by chi-square test; p- value of <0.05 considered as significant.

Results:

Table 1: Status of the components of MS among different age group doctors (n=300)

Parameters	Age (years)		Total
	Group I (30-37)	Group II (38-45)	
WC (>80 cm)	120 (69.8%)	106 (82.8%)	226 (75.3%)
SBP (>130 mm of Hg)	12 (7.0%)	17 (13.3%)	29 (9.7%)
DBP (>85 mm of Hg)	16 (9.3%)	21 (16.4%)	37 (12.3%)
FSG (>6.1 mmol/L)	14 (8.1%)	9 (7.0%)	23 (7.7%)
TG (>150 mg/dl)	27 (15.7%)	28 (21.9%)	55 (18.3%)
HDL-C (<50 mg/dl)	137 (79.7%)	97 (75.8%)	234 (78.0%)

Among the total study subjects and different groups frequency and prevalence of the components of MS doctors having high WC (>80cm) and HDL-C (<50 mg/dl) found to predominate than other components.

Table 2: Comparison of the components of MS among the two age group doctors

Parameters	Age (years)		p value
	Group I (30-37)	Group II (38-45)	
WC (>80 cm)	120 (69.8%)	106 (82.8%)	0.010
SBP (>130 mm of Hg)	12 (7.0%)	17 (13.3%)	0.068
DBP (>85 mm of Hg)	16 (9.3%)	21 (16.4%)	0.064
FSG (>6.1 mmol/L)	14 (8.1%)	9 (7.0%)	0.721
TG (>150 mg/dl)	27 (15.7%)	28 (21.9%)	0.171
HDL-C (<50 mg/dl)	137 (79.7%)	97 (75.8%)	0.424

Chi-square test was done to measure the level of significance. Comparison of the two group of doctors were done by chi square test to see the level of significance, but no significant difference found between the different components of MS except in waist circumference (p=0.010).

Table 3: Distribution of MS among the study subjects in different age groups

MS	Age (years)		Total
	Group I (30-37)	Group II (38-45)	
Absent	132 (76.7%)	85 (66.4%)	217 (72.3%)
Present	40 (23.3%)	43 (33.6%)	83 (27.7%)
Total	**172 (100.0%)**	**128 (100.0%)**	**300 (100.0%)**

Chi-square test was done to measure the level of significance and found p=0.048

Table 3 shows MS among the total study subjects, Group-I & Group-II doctors which was measured at 95% CI. In the total study subjects 28% (n=83) doctors presented with metabolic syndrome. Among the two groups, MS was found in group-I, 23% (n=40) and in group-II, 34% (n=43). Frequency of MS found higher in Group-II doctors and it was statistically significant (p =0.048).

Discussion:

To provide proper health care service for population at large, a healthy doctor's community is essential. The prevalence of metabolic syndrome is more in people who are used to a sedentary life. Doctors lead sedentary life and they do not have enough time for physical exercise, moreover, they are to bear high stress out of professional intricacy. Everyone expects that a doctor will serve for the community for a reasonable period and certainly nobody expect their premature morbidity and mortality. Metabolic syndrome seems to be increasing among the doctors which is alarming for our health service delivery system, because these risk factors give rise to various life threatening medical problems like diabetes mellitus, cardiovascular disease, stroke, chronic kidney disease, and polycystic ovary syndrome etc. Metabolic syndrome is not only the problem for the affluent class and western countries, it is a new hidden burden for developing countries and for Bangladeshi population too. Urbanization, modern lifestyle, change of food habit cumulatively contribute for development of metabolic syndrome. There is paucity of data on the lifestyle-associated disorders among physicians. A study in medical students of Karachi, Pakistan, showed general neglect of their health and highlighted the urgent need to promote preventive knowledge and practice among them.[10]

Illnesses among doctors include all the categories for the general population at large such as cardiovascular diseases, respiratory disorders, musculo-skeletal disorders, cancer and psychiatric illness.[11]

Most studies from developed countries also showed that doctors, generally do not take good care of their health. Our finding is supported by a study in India. Ramachandran et al conducted a study among 2499 Indian physician of mean age 39.0±9.0 years and found the prevalence of metabolic syndrome 29%, diabetes 13.3%, impaired glucose tolerance 10.7%, hypertension 35.6% and obesity 55.5%. They concluded that in India, doctors had high prevalence of cardio- metabolic risk factors and metabolic syndrome than general population. They also showed that the prevalence of MS was higher among female doctors (25.3%).[12]

In another South Indian study of Madurai, among 1433 physician in the year 2009 aged 35-65 years, 23% of female doctors were hypertensive, 49% had MS with IDF criteria, 39% had MS using ATP III guidelines, 82% had abdominal obesity, 76% exhibited an HDL abnormality & 33% of the female doctors had hypertriglyceridemia. HDL abnormality and abdominal obesity found more in females than males.[13] Another study among Indian physicians in Jaipur in 2001 also showed high prevalence of coronary risk factors. They found prevalence of obesity 51.4%, hypertension 20%, diabetes 12.9%, high total cholesterol 32.3%, high LDL cholesterol 29% and high triglyceride 12.9% in female doctors.[14]

A study among the doctors of Bangladesh done by Baul in 2010 at BSMMU, Dhaka from July 2009 to June 2010 documented high prevalence of Metabolic syndrome and the proportion was higher in female than male doctors (42.8% vs 36.8%).[15] A study among the medical students of Bangladesh done by Nazneen in 2009 in BSMMU, Dhaka from July 2008 to June 2009 documented high prevalence of obesity and atherogenic phenotype positivity among the female students than male (22% vs16%).[16]

Results of our study show that young female doctors have high rates of metabolic syndrome (28%) The study also showed increased prevalence in higher age group (38-45 years) than younger age group. Therefore they need more motivation to follow good health care practices which they advocate to their clients. They have good access to information on disease frequency and determinants. Therefore, knowledge and awareness regarding the health consequences of lifestyle changes are generally expected to be high among physicians. This in turn could influence the prevalence of lifestyle diseases such as diabetes and hypertension among them.

Conclusion:

The prevalence of metabolic syndrome is high among the young female doctors of Bangladesh. They must need more motivation to follow good health care practices for their longevity. This is of serious concern in the context of healthy doctors and health service delivery in our country.

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Original Article

Clinico-Pathological and Outcome Analysis of Male Breast Cancer: a Rare Experience of 10 cases

Tabassum F[a], Hasan SK[b], Masud ZM[c] , Tabassum SK[d], Parvin G[e], Shuchi S[f]

Abstract

Background: Breast cancer treatment has progressed significantly over the past 20 years. However, knowledge regarding male breast cancer is sparse because of its rarity. This study is an investigation of the clinico-pathological features, treatment and clinical outcomes of male breast cancer.

Methods: Clinical records of 10 male breast cancer patients, diagnosed during 2007 to 2016, were reviewed. The cases were collected from Bangladesh Medical College Hospital and Ahmad Medical Centre, Dhaka.

Results: The median age of 10 male breast cancer patients was 66 (26-75 years) years. Eight (80%) patients complained of a palpable breast mass initially. The median duration of symptom was 5 (1-36) months. Mastectomy was performed in 9 patients. The most frequent histology was infiltrating ductal carcinoma. Only 4 patients performed hormone status; among these 3 were estrogen receptor-positive & epidermal growth factor receptor 2 (HER-2) negative. One patient was triple negative. All patients presented with locally advanced breast cancer with lymph node involvement. After curative surgery, 8 patients received adjuvant chemotherapy, 2 received neo-adjuvant chemotherapy and 9 patients received hormone therapy. The median survival duration was 12 (6-75) months.

Conclusion: Almost all the male breast cancer patients were in advance stage with poor treatment outcome. Anti-HER-2 and hormonal therapy, except tamoxifen, have been underutilized in male breast cancer patients compared to female breast cancer.

Keywords: Male breast neoplasm, Clinic-pathologic feature, Survival outcome.

Introduction:

Since the early 2000s, the incidence of female breast cancer (FBC) in the United States has been decreasing, a phenomenon attributed to a decline in the use of hormone replacement therapy among postmenopausal women.[1] In contrast, according to the Surveillance, Epidemiology and End Result (SEER) and the United Kingdom Association of Cancer Registries databases, between the 1970s and 2000s, the incidence of male breast cancer (MBC) increased from 0.1% to 1%.[2,3]

As MBC is rare and behaves similarly to FBC, the general treatment approaches for MBC are based upon those for FBC.[4] Since the development of human epidermal growth factor receptor 2 (HER-2)-targeted agents such as trastuzumab, pertuzumab, trastuzumab-DM1, lapatinib and hormone agents such as aromatase inhibitors, the treatment outcomes for FBC have improved remarkably. However, these treatments have not been fully implemented in MBC patients because of the lack of clinical trials investigating MBC, as well as concerns that the tumor biology may be different between the sexes. Moreover, studies report contradictory treatment outcomes between MBC and FBC patients.[5,6,7]

In the present study, we analyzed data from two institutions, spanning a 10-year period, to evaluate the clinicopathologic characteristics, treatment strategies, and clinical outcomes of MBC patients.

Materials and Methods:

Patients

Among 1500 patients with breast cancer treated between 2007 to 2016 at Bangladesh Medical College Hospital and Ahmad Medical Centre, data for 10 MBC patients were collected for analysis.

The patients' medical records were reviewed in June 2016 to obtain the age at diagnosis, complaints at the time of diagnosis, symptom duration, family history of cancer, marital status, smoking habits, alcohol intake, treatments, and clinical course of the disease. The informed consent was waived because of its retrospective design of the study.

Statistical Analysis

All statistical analyses were performed using software SPSS 19.0 version.

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Descriptive statistics were presented for the baseline characteristics. Overall survival (OS) was defined as the time interval from the date of diagnosis to that of death from any cause or the last follow-up date.

Results:

Table 1: Characteristics of 10 male cancer patients

Characteristics	N=10	% or Range
Median age at diagnosis (Yrs)	66	26-75
Laterality:		
Right	2	20%
Left	8	80%
Marital status:		
Married	10	100%
Unmarried	0	0%
Alcohol	0	0%
Tobacco:		
Current smoker	5	50%
Ex smoker	0	0%
Never smoker	5	50%
Complaint at the time of diagnosis:		
Palpable mass	10	100%
Nipple discharge	2	20%
Back pain	2	20%
Dyspnea	0	0%
Median duration of symptom	**5**	**1-36 months**
Less than 1 month	2	20%
1-6 month	2	20%
6 month 1 year	5	50%
1-2 year	1	10%
Family history of Cancer:		
Yes	2	20%
No	8	80%
Clinical stage:		
II	0	0%
III	6	60%
IV	4	40%

The baseline patient characteristics are summarized in Table-1. The median symptom duration was 5 months (range, 1-36 months). Left-sided tumors accounted for 8 out of 10 cases. The most common complaint at diagnosis was a palpable breast mass. The majority (6/10) of patients had stage III at the time of diagnosis. Among patients (4/10) with initial stage IV disease, 3 demonstrated skin metastases, 01 demonstrated apparent lung and 10 with bone metastasis.

Table 2: Pathologic characteristics of 10 breast cancer patients

Characteristics	N= 10	%
Histology:		
Infiltrating ductal carcinoma	9	90%
Mucinous carcinoma	1	10%
Grade:		
Moderately	6	60%
Poorly	4	40%
TNM:		
II	1	10%
III	6	60%
IV	3	30%
Pathological T stage:		
pT1	2	20%
pT2	6	60%
pT3	2	20%
Pathological N stage:		
pN1	2	20%
pN2	4	40%
pN3	2	20%
NA	2	20%
Lymphatic invasion:		
Positive	7	70%
Negative	3	30%
Estrogen receptor (N=4):		
Positive	3	75%
Negative	1	25%
Progesterone receptor(N=4):		
Positive	3	75%
Negative	1	25%
Her-2 over expression (N=4):		
Positive	0	0%
Negative	4	100%

The histo-pathological features are in Table 2. The most common MBC histological subtype was infiltrating ductal carcinoma. Most tumors showed poor or moderate differentiation. The pathologic (p) T classification data were available and most of them were pT2. Among 4 patients with available data, 3 were positive for estrogen receptor (ER) and progesterone receptor (PR) expression. Of the 4 patients all were HER-2 negative.

Table 3: Treatment profile and survival outcome of 10 male cancer patients

Mode of Treatment	N=10	%
Surgery:		
Sentinel LN biopsy	0	0%
Modified radical mastectomy	3	30%
Total mastectomy	6	60%
Lumpectomy	1	10%
Adjuvant treatment:		
Chemotherapy	8	80%
Hormone therapy	9	90%
Radiotherapy	8	80%
Status at last follow up:		
No evidence of disease	5	50%
Alive with disease	1	10%
Died with the disease	4	40%

Details of the treatments administered are in Table 3. All patients underwent curative surgery, including modified radical mastectomy or total mastectomy. Out of 10 patients, 8 received adjuvant chemotherapy, 9 patients received adjuvant hormone therapy and 8 received radiotherapy. Four patients out of 10 died at the time of analysis. The median overall survival of all study patients were 12 (6-75) months.

Discussion:

In the present study, over a 10-year period, MBC patients were detected in 0.66% of total breast cancer patients in two institutions. The global incidence rates of MBC vary widely by location and ethnicity. In Japan, the rate is reported at 0.49%; in the United States and Europe, it is 1%; and across African nations, it is reported at 5-15%.[2,3,8] As the global population's life expectancy and diagnostic techniques improve, the global incidence rate of MBC continues to rise.

The median age of MBC onset in our study, 66 years, was consistent with the literature (62-69 years), and is much later than that reported for FBC (<50 years). This late onset might be due to lack of awareness of the early signs of MBC, cultural barrier which may lead [6,8] to delayed screening and diagnosis.[6] The duration of symptoms was longer (6 months to 1 year) than the median duration of 5 months in the present study.[7] This prolonged period of symptom duration likely contributes to the differences in stage distribution between MBC and FBC patients and may be the reason metastatic disease is more frequently detected at the time of diagnosis in MBC patients. The rate of disseminated disease in the present study (40%) whereas the rate for FBC patients was lower (5-6%).[6,9,10]

The distinct histologic characteristics and related biology of MBC compared with FBC have not been fully described and therefore, knowledge is still lacking regarding MBC.

HER-2 over expression occurs in approximately 25% of FBC cases, and there are contradictory reports about the rate of HER-2 over expression in MBC.

However, the study has limitations, small number of patients, short duration of study and including the lack of information regarding molecular characteristics such as *BRCA1*/*BRCA2* mutations and the p53 status. In addition, the pathologic data were collected in a retrospective manner and could not be reviewed for each patient.

Conclusion:

Almost all the male breast cancer cases were in advance stage with poor treatment outcome. Anti-HER-2 and hormonal therapy, except tamoxifen, have been underutilized in male breast cancer patients compared to female breast cancer. Personalized treatment should be performed for MBC in concordance with the advances in the treatment of FBC.

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Original Article

Correlation of Serum Lipid Profile with Glycemic Control in Type-2 Diabetes Mellitus

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Abstract

Objective: To determine lipid profile in controlled and uncontrolled type-2 diabetes mellitus and to compare the serum lipid profile between these two groups.

Methods: This cross sectional comparative study was done among 120 patients of type-2 diabetes mellitus. Forty (40) patients having good glycemic control (Gr-1) of HbA1C <6 %; 40 patients having intermediate glycemic control (Gr-2) of HbA1C 6-8% and another 40 patients having poor glycemic control (Gr-3) of HbA1C>8% were selected on the basis of purposive sampling technique. Brachial venous blood samples were collected for fasting plasma glucose, HbA1C and serum lipid profile from all subjects in the morning after twelve (12) hours fast at OPD of BIRDEM. Their dyslipidaemia was compared with reference to glycemic control in type-2 diabetics and analyzed with statistical software SPSS version 20.0.

Results: Out of 120 patients 78 were male and 42 were female. The mean age was 48.78 years in Gr-1, 51.06 years in Gr-2 and 52.75 years in Gr-3; mean age of 120 patients was 52.84±SD7.52 years. Triglyceride level was normal in Gr-1 and Gr-2 (180 ± 78) mg% and (181± 82) mg % respectively whereas it was slightly higher in Gr-3 patients which was (203 ± 89) mg% but there was no significant differences between these three groups (p value= 0.305). Serum total cholesterol level was normal in all the groups. In Gr-1 and Gr-2, it was almost same (168.93± 42.98) mg% and (167.86± 37) mg% respectively but in Gr-3 patients though it was in normal level but was slightly higher than other groups (174.65 ±42.7) mg%, still there was no significant difference between these groups (p>0.05). Again serum LDL level was normal in all 3 groups of patients with highest value in Gr-3 patients (95.6 mg%, 92.05 mg% and 105.9 mg%) respectively having no significant difference between the three groups (p>0.05). HDL level was almost same in all groups and it was below normal level (i.e < 40 mg %).

Conclusion: In type-2 DM patients good glycemic control is not associated with statistically significant differences in total TG, TC, LDL-C and HDL-C.

Keywords: Type-2 DM, Dyslipidaemia, Glycemic Control, Glycosylated Hemoglobin HBA1C

Introduction:

Diabetes Mellitus (DM) is an increasing important medical and public health issue. The worldwide prevalence of type-2 DM has been estimated to rise from 150 million to 25 million by the end of 2010 and to as many as 300 million by 2025.[1-3] The epidemic is particularly acute in the South-East Asia where by Bangladesh will have the highest growth in DM. Type 2 DM is the major problem and will account for over 90% of this case.[2,4] Various studies conducted in Bangladesh have reported 7-11% prevalence of DM.[5] Currently it is 8th in the world according to WHO estimation of prevalence of DM and by the year 2025 is expected to raise 4th position. The prevalence of DM in North-West Frontier Province (NWFP), according to WHO criteria is 11.1%.[6,7]

Dyslipidaemia is an important component of the metabolic syndrome observed in type-2 DM patients and is characterized by moderate hypertriglyceridemia and low levels of HDL-C.[8] Type-2 DM is associated with various pattern of dyslipidaemia that predispose patients to macro vascular complication like CHD. Once clinical disease develops the patients have a poorer prognosis than normoglycemic individuals with normal lipids. Similarly hypertriglyceridemia, low HDL-C, and high LDL-C represent a high rises group for CHD.[9] Morbidity and mortality in type-2 DM elevated serum TG are commonly associated with insulin resistance and represent a valuable clinical marker of metabolic syndrome.[10]

Duration of DM is associated with higher incidence of dyslipidaemia. In newly diagnosed and established DM correlation was found between HbA1c level and carotid intima media thickness.[11-13] The oxidation of Lipoproteins, in particular LDL-C seems to be increased in DM patients, especially those with poor glycaemic control, hypertriglyceridemia, micro-vascular and macro-vascular disease. Oxydation of LDL-C results in a moiety that is

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cytotoxic to vascular endothelial and smooth muscles cells contributing to atherosclerosis.[12,14] For, every one percentage point increase in HbA1c the relative risk for any cardiovascular event was 1.18 (CI-95%, 1.10-1.26).[15] Keeping in view, the large number of type-2 DM and poor knowledge of the subjects, most patients are prone to develop multiple lipid disorder. Very few studies have been conducted in our community to know the impact of glycaemic control and lipid profile in type 2 DM. Our study is focused on Bangladeshi community and give results that are applicable to our patients and the impact of the results will help type-2 DM patients in reducing the irreversible complications associated with this disorder.

Materials and Methods:

This cross sectional comparative study was carried out at BIRDEM on a total of 120 diabetic patients enrolled from OPD by purposive sampling. Detailed history and clinical data were obtained. Informed consent was taken from all the study subjects included in this study. The study subjects were divided in three groups:

Gr.1: n=40, HbA1c= <6% (good glycaemic control)

Gr.2: n=40, HbA1c= 6-8% (Intermediate glycemic control)

Gr.3: n=40, HbA1c=> 8% (poor glycemic control).

Inclusion criteria for this study were:

1. Patients with at least one year passed of type-2 DM with medication.
2. Male and female between 30-70 years of age.

Exclusion criteria:

1. Type-2 DM patients with ischaemic heart disease and hypertension.
2. Terminally ill patients.
3. Family history of hyperlipidaemia.
4. Those patients who were on lipid lowering therapy.

Brachial venous blood samples were collected for fasting plasma glucose, HbA1c and serum lipid profile (TC, TG, HDL-C, and LDL-C) from all study subjects in the morning after 12 hours fast at presentation. Blood samples for lipid profile and fasting blood glucose were analyzed by enzymatic colorimetric technique and HbA1c was determined by HPLC Method at BIRDEM Biochemistry laboratory. All data were compiled in computer on SPSS, version-20 and were analyzed accordingly. For qualitative variables such as sex, educational status, category of glycemic control's frequencies, ratio and percentages were calculated. Chi-square test was used to determine significant differences of descriptive frequencies between the groups.

For quantitative variables such as age, various lipid components (TC, TG, HDL-C, and LDL-C), FBS, HbA1c, duration of type-2 DM and BMI mean and standard deviation were calculated. The student T test was applied to determine the significant differences between means of the groups. p value of less than 0.05 was considered significant.

Operational Definitions:

1. **Good glycemic control**: HbA1c level<6% and fasting blood glucose 90-125 mg/dl or 5.9 mmol/l.
2. **Intermediate glycemic control:** HbA1c level 6-8%, FBS 126-168 mg/dl or 7-9.3 mmol/l.
3. **Poor glycemic control**: HbA1c level >8%, FBS >169 mg/dl or >9.4 mmol/l.

Results:

In this study 120 adult patients with type-2 DM has been selected. Fulfilling inclusion criteria they were divided in to 3 groups. On the basis of glycemic control there were 40 patients in each group as operationalized in methodology.

Table 1: Distribution of study subjects by age & sex (n=120)

Age Group	Male No.	Female No.	Total No. (%)
30-40	15	10	25 (100) (20.8)
41-50	24	12	36 (100) (30)
51-60	23	15	38 (100) (31.6)
61-70	16	05	21 (100) (17.5)
Total	**78 (65%)**	**42 (35%)**	**120 (100%)**

Among 120 patients 78 (65%) were male and 42 (35%) were female. Majority 74(61.6%) were in the age group of 41-60 years as shown in Table-1.

The mean age was 48.78 years in Gr-1, 51.06 years in Gr-2 and 52.75years in Gr-3. The total mean age of 120 patients was 52.84±7.52 years.

Table 2: Distribution of patients by lipid profile and glycaemic control

Parameters in Mean±SD	HbA1c<6% (good glycemic control) N=40	HbA1c= 6-8% (intermediate glycemic control) N=40	HbA1c>8% (poor glycemic control) N=40	p value
TG	180±78.1	181±82	203.21±89.6	0.305
TC	168.93±42.98	167.86±37	174.65±44.27	0.385
LDLC	95.6±44	92.05±32.07	101.92±38.15	0.242
HDLC	37.8±4.55	37.7±6.4	37.92±6.69	0.309

Table-2 shows TG level was normal in Gr-1 and Gr-2 (180±78 and 181±82) mg% respectively, whereas it was slightly higher in Gr-3 patients which was 203±89 mg%. But there was no significant difference between three groups with p value (p=0.305). Serum total cholesterol levels were normal in all three groups. In Gr-1 and Gr-2 patients it was almost same (168.93 ±42.98) mg% and (167.86±37) mg% respectively, but in Gr-3 patients though it was in normal level, was slightly higher than other two groups (174.65±42.7) mg%. Still there was no significant difference between three groups (p>0.05). Again, serum LDL-C level was normal in all three groups of patients with highest value in Gr-3 patients (95.6 mg%, 92.05 mg% and 105.9 mg% respectively, having no significant difference between three groups p>.05. The HDL-C level was almost same in all three groups and it was below normal level (<40 mg%).

Table 3: Educational status of study subjects

Level of education	Gr-1	Gr-2	Gr-3	p value
Illiterate	6.7	11.8	7.3	
Primary	13.3	13.7	10.9	
Secondary	26.7	39.2	43.6	-0.098
Higher secondary	20	11.8	20.0	
Graduate	13.3	13.7	10.9	

Negative correlation: Table- 3 shows a negative relationship between educational status of study subjects and study groups depending on glycemic control (p= - 0.098)

Discussion:

Duration of DM is associate with higher incidence of dyslipidaemia. Type-2 DM is associated with a marked increased in risk of coronary heart disease.[12] Patients with type- 2 DM have a 2-6 fold increased risk of coronary heart disease, peripheral vascular disease and cerebro vascular disease than those without it.[13] Approximately 80% DM patients die of large vessel disease as compared to 50% of the rest of the population.[14] Usual risk factors of CHD accounts for only 25-50% of increased atherosclerosis risk in DM. Other obvious factors are hyperglycemia and dyslipidaemia.[15] Like dyslipidemia in DM have been described in numerous international study trials with consistent findings and few differences. American diabetic association has reported that well control type-2 DM have a mixed hyperlipidemia with high TG, low HDLC and high LDL-C.[11] Our study is relevant with that but the study results were not statistically significant to that study.

Amer A, Zafar S and Mjrooh A, et al. did similar trial and found all lipid fractions deranged in patients with uncontrolled DM[16] in which type-2 DM patients were enrolled on the basis of good glycemic control (HbA1c<8 mg %) and uncontrolled type-2 DM where HbA1c >8 mg% and the outcome was the change in lipid profile of both these group. The mean lipid level in both groups were compared and "p" value <0.005, which was statistically significant. But our recent study reveals that lipid profile parameters TG, TC and LDL-C increases in group 3 (poor glycemic control DM) compared to Gr-1(good glycemic control) but the result was not statistically significant because p>0.05 and HDL was below normal level in all groups.

Conclusion:

Elevated total serum cholesterol, TG, LDLC and low HDLC were observed in type-2 DM with poor glycemic control compared to patients with good and intermediate glycemic control. The glycemic control of the patients has got a strong impact on the serum lipid and dyslipidaemia is frequently encountered in those DM who have got poor glycemic control. We got no significant association between poor glycemic control and lipid profile. We found a negative correlation between education and glycemic control. So patients should be educated about regular monitoring of lipid profile and if found to be abnormal, should control blood sugar and lipids very effectively.

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Original Article

Antimicrobial Resistance Pattern of *Salmonella enterica* serovar Typhi-Isolated in a Tertiary Care Hospital in Dhaka City

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Abstract

Background: *Salmonella enterica* serovar Typhi and Paratyphi are predominantly known to cause enteric fever. We planned to isolate *Salmonella* species responsible for typhoid fever and their antibiotic resistance pattern over a period of 6 months in a tertiary care hospital in Dhaka city.

Methods: A retrospective analysis of *Salmonella* Typhi and Paratyphi, isolated from patients attending Bangladesh Medical College and Hospital during the period of January to July 2015 was carried out. All the isolates were identified by standard microbiological tests and antimicrobial sensitivity profiles of pathogens were determined by disc diffusion method as recommended by the NCCLS guideline.

Results: A total of 123 *Salmonella* Typhi isolates were obtained from 1561 blood culture samples. *Salmonella* Typhi were resistant to amoxicillin 52.84% and co-trimoxazole 26.82%. Resistant to azythromycin was 93.49%. Regarding quinolone groups, all the isolates were 100% resistant to nalidixic acid whereas ciprofloxacin resistance was 8.94%. Among the 123 isolates, ceftriaxone resistance was found 1.62% followed by cefixime 23.57% respectively.

Conclusion: This study suggests regular monitoring of antibiotic sensitivity pattern of pathogenic organisms to reduce the mortality and morbidity of enteric fever patients. Indiscriminate use of ciprofloxacin or ceftriaxone should be strongly discouraged. Proper use of antibiotics and combination therapy may help to decrease or prevent the emergence of resistance.

Keywords: Enteric fever, blood culture, antibiotic resistance, nalidixic acid, ceftriaxone, azythromycin, indiscriminate use.

Introduction:

Salmonella enterica serovar Typhi and Paratyphi are predominantly known to cause enteric fever.[1] Enteric fever continues to be a global health problem with an estimated 12-33 million cases[2] and 6 lakhs deaths occurring annually.[3] Majority of this burden occurs in Asia.[4] Although the incidence of typhoid fever is falling in developed countries, the disease is still endemic in many parts of the world including Bangladesh.[5,6,7,8] In an urban slum in Dhaka, the incidence of *Salmonella typhi* was found to be 390/100,000 population.[3] The emergence of multidrug resistant strains (resistant to chloramphenicol, ampicillin and co-trimoxazole)[9] led to the use of first generation fluoroquinolones especially ciprofloxacin as first line drug for the treatment of enteric fever.[2] With the development of ciprofloxacin resistance among multidrug resistant *Salmonella* typhi, cephalosporin group including ceftriaxone, cefixime, cefepime have been recommended as an alternative.[9]

But there are reports of emergence of multidrug resistant *Salmonella* spp., especially fluoroquinolones and third generation cephalosporin resistant *Salmonella* spp., has been reported worldwide and is considered as a serious problem due to limitations of the effective treatment of human infections.[10,11] Therefore, now it is a prime concern to consider new, effective and alternative choice of drug in forthcoming days to combat against typhoid caused by *Salmonella enterica* serovar Typhi and Paratyphi resistant to traditional antibiotics like ciprofloxacin.[12,13]

Changing trends in antibiotic resistance patterns have been reported from different parts of Bangladesh. So the present study was undertaken to isolate *Salmonella* species responsible for typhoid fever and their antibiotic resistance pattern over a period of 6 months in a tertiary care hospital in Dhaka city.

Materials and Methods:

A retrospective analysis of *Salmonella* Typhi and Paratyphi isolated from patients attending Bangladesh Medical College and Hospital during a period of January to July 2015 was carried out. All the isolates were identified by colony morphology and standard biochemical reactions.[14] The isolates were tested for susceptibility to amoxycillin 10 µg, azythromycin 15 µg, co-trimoxazole 25 µg, nalidixic acid

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30 µg, ciprofloxacin 5µg, ceftriaxone 30 µg, cefixime 5 µg by a disc diffusion method as recommended by the NCCLS guideline. All discs were obtained from Oxoid Ltd.

Results:

Table 1: Frequency of isolated pathogens from suspected blood stream infection (n=174)

Organisms	Number of isolates	Percentage
Salmonella Typhi	123	70.68
Salmonella Paratyphi	27	15.51
Esch. coli	9	5.17
Acinetobacter species	8	4.59
Enterobacter species	4	2.29
Pseudomonas species	3	1.72
Total	**174**	**100%**

Table-1 shows the frequency of isolated pathogens from suspected blood stream infection. A total of 174 isolates were obtained from 1561 blood culture samples of which *Salmonella* typhi was the predominant 123 (70.68%) followed by *Salmonella* paratyphi 27 (15.51%), *Esc. coli* 09(5.17%), *Acinetobacter* 08(4.59%), *Enterobacter* 04 (2.29%) and *Pseudomonas* 03 (1.72%).

Table 2: Rate of isolation of *Salmonella* typhi in relation to age and sex groups (n=123)

Age (in years)	Sex		Total (%)
	Female	Male	
Up to 18	23 (18.70%)	44 (35.77%)	67 (54.47%)
Adult	27 (21.95%)	29 (23.58%)	56 (45.53%)
Total	50 (40.65%)	73 (59.35%)	123 (100%)

Table 2 showed rate of isolation of *Salmonella* typhi in relation to age and sex groups. Maximum organisms were isolated from males which was 73 (59.35%) among the 123 *Salmonella* typhi isolates. Highest (35.77%) rate of isolation was among male of up to 18 years age group.

Table 3: Antibiotic resistant pattern of *Salmonella* typhi (n=123) by disc diffusion method

Antimicrobial agents	No. of isolates	Percentage
Amoxycillin	65	52.84
Azythromycin	115	93.49
Co-trimoxazole	33	26.82
Nalidixic acid	123	100
Ciprofloxacin	11	8.94
Ceftriaxone	2	1.62
Cefixime	29	23.57

Multiple response

Antibiotic resistant patterns of *Salmonella* typhi against amoxycillin, azythromycin, co-trimoxazole, nalidixic acid, ciprofloxacin, ceftriaxone and cefixime were seen as shown in Table- 3. *Salmonella* typhi were resistant to amoxycillin 52.84% and co-trimoxazole 26.82%. Resistant to azythromycin was 93.49%. Regarding quinolone groups, all the isolates were 100% resistant to nalidixic acid whereas ciprofloxacin resistance was 8.94%. Among the 123 isolates, ceftriaxone resistance was found 1.62% followed by cefixime 23.57% respectively.

Discussion:

Typhoid fever remains a major public health problem in many developing countries including Bangladesh due to poor sanitation and infrastructure of health care. Strains of *Salmonella* typhi and *Salmonella* paratyphi resistant to commonly used antibiotics such as amoxycillin, co-trimoxazole, ciprofloxacin are emerging in many parts of the world including Bangladesh.[15] Therefore, isolation of *Salmonella* typhi from blood culture and assessment of antibiotic resistance pattern of those isolates is necessary for selection of drugs to treat such infections.

In this study, out of 1561 blood culture samples 174 isolates were culture positive. *Salmonella* typhi was the predominant serotype 123 (70.68%) followed by *Salmonella* paratyphi 27 (15.51 %), *Esch. coli* 9(5.17%), *Acinetobacter* 8(4.59%), *Enterobacter* 4(2.29%) and *Pseudomonas* 3 (1.72%). Our study was similar with a study done in Bangladesh by Alam et al (2010), who found 66% patients were *Salmonella* spp. positive and one third of the isolated *Salmonella* typhi were mostly resistant to amoxicillin, co-trimoxazole and chloramphenicol.[15] This study does not correlate with a study in India where 8% *Salmonella* typhi were obtained.[16] Another study in BIRDEM hospital, found 385 *Salmonella* species from blood cultures over a period of 6 years, out of them 79% were *Salmonella* typhi and 21% were *Salmonella* paratyphi.[17]

The present study emphasizes the rate of isolation of *Salmonella* typhi as a cause of typhoid fever among different age and sex groups. In this study highest (35.77%) rate of isolation was among pediatric male group. This study correlates with a study where *Salmonella* typhi was noted as higher (17.54%) among pediatric age group when compared to adult group (13.95%).[18]

The overall simultaneous resistance to three first line antibiotics namely ampicillin, chloramphenicol and co-trimoxazole of isolated *Salmonella* typhi during 2004-2009 was 38% (117 out of 304) and the range was between 30-50%.[17] In our study, amoxycillin and co-trimoxazole were 52.84% and 26.82% resistant.

Azythromycin has done well in clinical studies for typhoid,[19] however; there have been sporadic reports of azythromycin resistance.[20] *Salmonella* typhi were 93.49% resistant to

azythromycin in this current study which is similar to another study done by Wadud et al., where 99.58% isolates were azythromycin resistant. This study does not correlate with a study where azythromycin resistance was 5.57%.[21] Very high use of Azythromycin might be the cause of acquiring resistance gene from environment that has resulted dramatic increase of phenotypic resistance. But, it requires further study to find out the real picture about Azythromycin resistance.[22]

The global emergence of resistant strains and reduced susceptibility to fluoroquinolones is of great concern. *Salmonella* Tyhi were 100% resistant to nalidixic acid whereas these isolates were 8.94% resistant to ciprofloxacin in this current study which correlates with another study where nalidixic acid was 100% resistant but ciprofloxacin were 0.27% resistant.[21] Outbreaks of enteric fever due to the infection of *S. enterica* serovar Typhi that are resistant to nalidixic acid and showed reduced susceptibility to the fluoroquinolone antibiotics, viz., ciprofloxacin, have been reported in a number of countries.[23] It has been reported that nalidixic acid resistance is a marker for predicting decreased susceptibility to ciprofloxacin among S. *enterica* serovar Typhi, and also an indicator of treatment failure to ciprofloxacin.[24,25]

In response to the development of ciprofloxacin resistance *Salmonella* typhi, a number of studies have investigated the efficacies of expanded spectrum cephalosporins. Resistance of 37.5% to cefixime and 6.25% to ceftriaxone was noted.[16] Presently, resistance to third generation cephalosporin has also been reported.[26] Cefixime, an orally administered third generation cephalosporin, is a commonly used drug in South Asia for the treatment of enteric fever. Although cefixime is recommended as a drug of choice by the World Health Organization (WHO) for the treatment of enteric fever[27] but the use of cefixime in enteric fever has found failure rates ranging from 4% to 23%. A high overall failure rate associated with cefixime despite all of the strains being fully sensitive in vitro to the drug shows that the mechanism of action of cefixime[28,29] may not be suited to the eradication of *S.* typhi or paratyphi A from the body or blood, and the poor intracellular penetration into macrophages and reticulo endothelial tissues where the typhoid organisms colonize may be the cause of high failure rates.[30]

In our study, we found resistance of 1.62% to ceftriaxone and 23.57% to cefixime. Notable results were found with cefixime and ceftriaxone since no resistance was found against them[20] and in another study where100% ceftriaxone sensitivity was found.[31] So, Ceftriaxone remains a viable parenteral option for treatment of typhoid fever and it should be used only when typhoid fever is non-responsiveness to ciprofloxacin.

Increasing resistance to quinolones is alarming, it should not be used in population like Bangladesh as treatment of typhoid fever by the clinician but gatifloxacin may be used as alternative as it is a relatively inexpensive fluoroquinolone antibiotic in South Asia with orally administered once daily dose,[32] has different binding motif than some other fluoroquinolones. [33] Thus it has been recommended that any isolate showing nalidixic acid resistance with decreased susceptibility to ciprofloxacin should be reported.

Conclusion:

This study suggests regular monitoring of antibiotic sensitivity pattern of pathogenic organisms to reduce the mortality and morbidity of enteric fever patients. Indiscriminate use of ciprofloxacin or ceftriaxone should be strongly discouraged. Proper use of antibiotics and combination therapy may help to decrease or prevent the emergence of resistance.

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Original Article

The Effect of Cigarette Smoking on Semen Parameters among Male Partners of Infertile Couples of Bangladesh

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Abstract

Objective: The objective of this study was to evaluate the sttatus of cigarette smoking and semen profile of the male partners of the infertile couples undergoing infertility workup in Bangladesh.

Methods: This cross secctional study was done between December 2011 and May 2012 in Infertility Management Center, Dhaka. Male partners of 200 infertile couples who were attending for infertility workup were the study subjects for this study. Proper history including age, duration of infertility, socioeconomic status and personal history was taken on a predesigned questionnaire. Each of the male partners was instructed to collect semen within the premises of the laboratory after three days of abstinence. The semen parameters were interpreted according to WHO (1999) semen analysis reference values. Collected data for each individual subject were compiled and analyzed using SPSS.

Results: In this study, majority of the couples had primary infertility (75.5%). Most (78%) of the male partners of the infertile couples were between 30-40 years of age. Most of the couples (41.5%) reported within 2 to 5 years of marriage. About 35.5% of the male partners in this study had habit of cigarette smoking. Out of 200 cases, 162 (81%) semen specimens showed normal sperm concentration (> 20 million/ml) compared to 38 (19%) which showed low or absent sperm in the semen sample. Out of 178 semen samples, 56 (31.5%) showed normal sperm motility (> 50%) and 163(91.6%) had > 30 % normal sperm morphology. This study revealed that all male partners with azoospermia and severe oligospermia had more than 10 years of history of smoking. Those with moderate oligospermia had 5 to 10 years of smoking history. Duration of smoking in those with normzoospermia is variable. The distribution of quantity of cigarettes smoked per day according to sperm count was not statistically significant.

Conclusion: Prolonged cigarette smoking has devastating effects on sperm count hence reducing male fertility. So, the healthcare providers should facilitate smoking cessation by providing education, monitoring and constant support to male partners of the infertile couples.

Keywords: Male infertility, Smoking, Semen analysis

Introduction:

Infertility is defined as one year of unprotected intercourse without pregnancy. It is a significant and common problem, affecting perhaps one couple in six.[1] The reported incidence of male infertility varies widely, and the overall incidence is estimated to be 30 to 50 percent of sub fertile couples.[2] In a multi centric study done by World health Organization (WHO) in 1989 of over 10,000 infertile couples from 33 centers in 25 countries, a possible cause in the male partner was found in the third of the cases, in the female partner in 25 percent and in both partners in 25 percent cases. In the remaining cases, neither partner had a detectable cause for in fertility.[3]

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The current basis for diagnosis of male infertility in men who have erections and who can ejaculate, is semen analysis. It is a relatively inexpensive and simple laboratory test, and can provide valuable information regarding male infertility status if performed correctly. Besides that, introduction of assisted reproductive techniques has moved the interest of many infertility clinics from the man as a whole to his semen and its usefulness in assisted fertilization.

Despite strong anti-smoking campaigns worldwide, it is evident that cigarette smoking is very common specially among young males in developing world. According to the World Health Organization (WHO), 30% of all 15 years and older men, smoke.[4] The highest prevalence of smoking is observed in young adult males during their reproductive period (46% smokers between 20 and 39 years).[5] Although the prevalence of male smokers, based on nationally representative sources from 187 countries, decreased from 41.2% in 1980 to 31.1% in 2012, the actual number of everyday smokers increased from 721 million in 1980 to 967 million in 2012.[6]

Although the effect of smoking on male fertility remains inconclusive, the evidence of adverse effects of smoking on semen parameters suggest that smoking reasonably may be regarded as an infertility risk factor.

Therefore, the objective of this study was to evaluate the relationship of cigarette smoking and semen profile of the male partners of the infertile couples undergoing infertility evaluation to ascertain the impact of smoking on male fertility in Bangladesh.

Methodology:

This cross sectional study was done between December 2011 and May 2012 in Infertility Management Center, Dhaka, a tertiary center for infertility management. Male partners of two hundred (200) infertile couples who were attending for infertility workup in the above center were the selected subjects for this study. Only couples trying for conception for more than one year were included in this study. Those male partners who had a history of vasectomy or has ejaculatory problem were excluded from this study.

Proper history including age, duration of infertility, personal history and socioeconomic status was taken on a predesigned questionnaire.

Each of the male partners was instructed to collect semen by masturbation in a clean, dry, wide mouthed container provided by the laboratory after three days of abstinence. The semen was collected within the premises of the laboratory to minimize collection error.

The sperm concentration was estimated by using the Makler counting chamber. Sperm morphology was assessed under light microscope by using semen smear. The semen parameters were interpreted normal or abnormal according to WHO (1999) semen analysis reference values. In patients with absence of sperm, the semen analysis was repeated again before declaring azoospermia.

Data collected for each individual subject were compiled and analyzed using computer-based software- Statistical Package for Social Science (SPSS) for Windows. A 'p' value < 0.05 was considered as minimum level of significance.

Results:

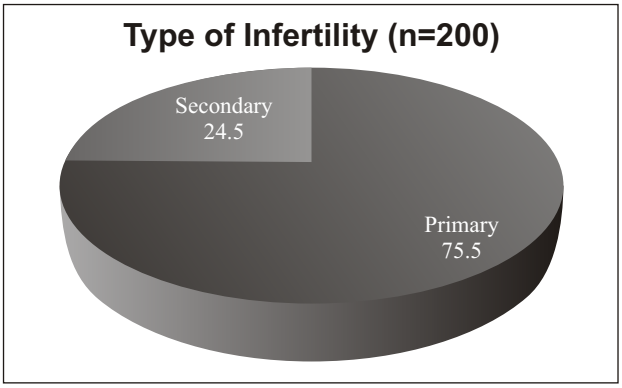

Figure 1: Type of Infertility in the Male Partners

In this study, majority of the couples had primary infertility (75.5%).

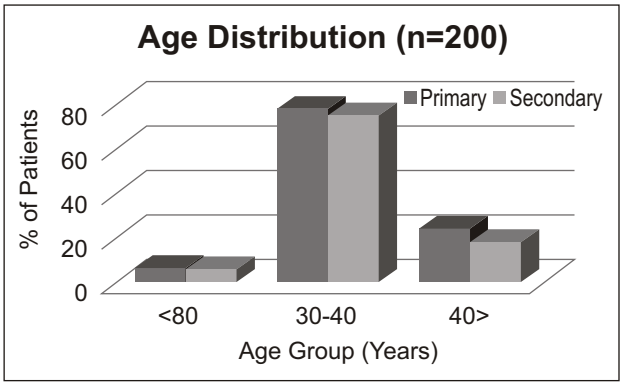

Figure 2: Incidents of Male infertility according to Age distribution

Majority (78%) of the male partners of the infertile couples were between 30 -40 years of age.

Table 1: Socio-economic status of the Male Partners (n = 200)

Monthly income	No. of Male Partners	Percentage
Low Income (< Tk 5,000)	35	17.5
Middle Income (Tk 5,000 -10,000)	53	26.5
High Income (> Tk. 10,000)	112	56

Majority (56%) of the partners belonged to high socioeconomic group.

Table 2: Duration of Infertility (n = 200)

Duration (Years)	No. of Male Partners	Percentage
1 to 2	15	7.5
2+ to 5	83	41.5
5+ to 10	71	35.5
>10	31	15.5

In this study most of the couples (41.5%) reported at the infertility management center within 2 to 5 years and 35.5% within 5 to 10 years of marriage.

Table 3: Smoking Habit of Male Partners (n = 200)

Smokers	No. of Male Partners	Percentage
Yes	71	35.5
No	110	55
Gave Up	19	9.5

In this study, 35.5 percent of the male partners had the habit of cigarette smoking

Table 4: Sperm Concentration of the Male Partners (n = 200)

Sperm Concentration (million/ml)	No. of Male Partners	Percentage
0	22	11
≤ 5	8	4
6 to 20	8	4
>20	162	81

Out of 200 cases, 162 (81%) semen specimens showed normal sperm concentration (> 20 million/ml) compared to 38 (19%) which showed low or absent sperm in the semen sample. Statistically the proportion is highly significant ($p<0.001$)

Table 5: Sperm Count and active motility of sperm (n = 178)

		Actively Motile Sperm		
Sperm Count (million/ml)	Male Partners (%)	< 10%	11 - 50%	P> 50%
≤ 5	8 (4.5)	4 (22.2)	4 (3.8)	0
6 to 20	8 (4.5)	2 (11.1)	6 (5.8)	0
>20	162 (91)	12 (66.7)	94 (90.4)	56 (100)
Total	178 (100)	18 (10.1)	104 (58.4)	56 (31.5)

Chi-sqare (X^2) = 5.719, df = 4
p = 0.001 (Significant as p< 0.001)

Out of 178 semen samples, 56 (31.5%) showed normal sperm motility (> 50%) and the rest 122 (68.5%) had low sperm motility (≤ 50%).The sperm count and motility was found highly significant (p<0.001).

Table 6: Sperm Count and morphology of sperm (n = 178)

Sperm Count (million/ml)	Male Partners (%)	≤10 Normal (%)	10 - 30 Normal (%)	> 30% Normal (%)
≤ 5	8 (4.5)	1 (100)	6 (42.9)	1 (0.6)
6 to 20	8 (4.5)	0	3 (21.4)	5 (3.1)
>20	162 (91)	0	5 (35.7)	157 (96.3)
Total	178 (100)	1 (0.6)	14 (7.9)	163 (91.6)

Chi-square test: $X^2 = 87.435$, df = 4
p = 0.000 (Significant as p < 0.001)

In this study 91.5 percent had > 30 % normal sperm morphology and 0.6 percent semen had <10% normal sperm morphology. The distribution of male partners according to normal sperm morphology to their count is highly significant.

Table 7: Sperm Count and duration of Smoking (n = 71)

		Duration of Smoking (Years)			
Sperm Count (million/ml)	Male Partners (%)	Occasional (%)	<5 (%)	5 - 10 (%)	> 10 (%)
0	6 (8.5)	0	0	0	6 (13)
≤ 5	3 (4.2)	0	0	0	3 (6.5)
6 to 20	8 (11.3)	0	1 (25)	3 (15.8)	4 (8.7)
>20	54 (76.1)	2 (100)	3 (75)	16 (84.2)	33 (71.7)
Total	71 (100)	2 (2.8)	4 (5.6)	19 (26.8)	46 (64.8)

Chi-square test: $X^2 = 2.398$, df = 9, p = 0.001

In this study we see that the male partners with azoospermia and severe oligospermia had more than 10 years of history of smoking. Those with moderate oligospermia had 5 to 10 years of smoking. Sperm count and duration of smoking was found statistically highly significant.

Table 8: Sperm Count and quantity of cigarettes smoked (n = 71)

		Quantity of Cigarette (Sticks/day)			
Sperm Count (million/ml)	Male Partners (%)	Occasional (%)	< 5 (%)	5 - 15%	< 15%
0	6 (8.5)	0	1 (5.3)	2 (5.7)	3 (27.3)
≤ 5	3 (4.2)	0	1 (5.3)	1 (2.9)	1 (9.1)
6 to 20	8 (11.3)	1 (16.7)	2 (10.5)	5 (14.3)	0
>20	54 (76.1)	5 (83.3)	15 (78.9)	27 (77.1)	7 (63.6)
Total	71 (100)	6 (8.5)	19 (26.8)	35 (49.3)	11 (15.5)

Chi-square test: $X^2 = 8.708$, df = 9
p = 0.465 (not significant)

In this study, 8.5 percent men were occasional smokers, 26.8 percent had less than 5 sticks/day, 49.3 percent had 5-15 sticks/day and 15.5 percent had more than 15 sticks/day. The distribution of quantity of cigarettes smoked per day according to sperm count is not statistically significant.

Discussion:

Out of the 200 infertile couples, 75.5 percent had primary infertility and 24.5 percent had secondary infertility. Among the male partners, 78% were between 30-40 years of age.

Majority (56%) of the partners belonged to high socioeconomic group. As the data were collected from a specialized institute for infertility management, probably explains that majority belonged to high socioeconomic status.

In this study most of the couples (41.5%) reported at the infertility management center within 2 to 5 years of marriage. This early reporting may be due to increased awareness among the infertile couples about their fertility status and the newer treatment options available now days. This observation has important implications as Dhaliwal *et al* [7] have shown that age of the male partners of the infertile couples and duration of infertility correlated significantly with the pregnancy rates.

In this study, 35.5 percent of the male partners had the habit of cigarette smoking.

Out of 200 cases, 162 (81%) semen specimens showed normal sperm concentration (>20 million/ml) compared to 38 (19%) which showed low or absent sperm in the semen sample. Eleven percent of the male partners had azoospermia which differs from earlier study done by Chowdhury and Fatema[8] on Bangladeshi population, who observed azoospermia in only 7.5 percent cases. On the other hand, in Nigeria in a similar study, Omoriah[9] found 40.9 percent very severe oligospermia with 16.19 percent azoospermia. Thonneau[10] in France revealed 9 percent azoospermia. This shows that rate of sperm concentration varies in different countries which may be due to variation in prevalence of sexually transmitted diseases.

After carrying out the sperm count, motility of the sperm was noted. (n=178). In majority of the semen samples, 56 (31.5%) showed normal sperm motility (>50%) and the rest 122 (68.5%) had low sperm motility ($\leq$ 50%).The proportion is highly significant ($p<0.001$). In this study 91.6 percent semen specimen had > 30% normal sperm morphology.

In this study we see that the male partners with azoospermia and severe oligospermia had more than 10 years of history of smoking. Those with moderate oligospermia had 5 to 10 years of smoking. Duration of smoking in those with normzoospermia is variable. So it was found that smoking especially of long duration is an important factor in the development of decreased sperm concentration. Zhang *et al* [11] also observed that medium, heavy and long term smoking adversely affected the semen quality in a population of men visiting the infertility clinic in China.

When sperm count was correlated with amount of smoking, it was found out that 8.5 percent men were occasional smokers, 26.8 percent had less than 5 sticks/day, 49.3 percent had 5-15 sticks/day and 15.5 percent had more than 15 sticks/day. The distribution of quantity of cigarettes smoked per day according to sperm count is not statistically significant.

Study done by Chia *et al* [12] in Singapore revealed that the smokers had significant poorer sperm density ($p<0.04$), lower percentage of normal sperm morphology ($p<0.001$) and a higher percentage of spermatozoa with head piece defects. A more recent study in Brazil showed that cigarette smoking may impair sperm motility and decrease the antioxidant activity (negative correlation with superoxide dismutase) in the seminal plasma.[13] In a large cross-sectional study, Ramlau-Hansen *et al* [14] reported an inverse dose-based association between smoking and semen volume, total sperm count, and the percentage of motile spermatozoa. The sperm concentration of heavy smokers was 19% lower than that of non-smokers. Therefore, smoking cessation, or at least a reduction in cigarette use, may be advised as a way of reducing toxin exposure.

This differs from the study done by Holzki *et al* [15] that revealed smoking had no detrimental effect on spermatogenesis. Trummer *et al* [16] also revealed that smoking did not affect conventional semen parameters though it significantly increased round cells and leucocytes in the semen. Furthermore, a study done in Canada in 2000 showed that there is no evidence for an association between smoking and sperm DNA fragmentation.[1]

So, there has been numerous studies in the last twenty years regarding the effect of smoking on semen parameters with contradictory results. The reasons for this considerable discrepancies are still unclear, although difference in methodology has been suggested and variation might exist among different laboratories despite newly changed World Health Organization reference [18] values for human semen characteristics. Additionally, sperm quality can be affected by local environmental elements, including stress, the amount of nicotine in the tobacco that is consumed, and other life-style factors. Moreover, studies reporting an effect of smoking on semen parameters have not clearly demonstrated any effect of smoking on male fertility.[19,20]

This study included a relatively small number of participants undergoing fertility treatment in a tertiary center which may have decreased the power of the study. So the findings of the present study should be interpreted with caution. Thus, a much larger study population with age stratification may be required to achieve sufficient statistical power to adequately assess the relationship between smoking and male semen parameters.

Conclusion:

Smoking cessation should certainly be advised to any male partners of infertile couples as this study shows that smoking of long duration is associated with decreased sperm concentration which can affect his fertility and success in future assisted reproductive technologies. So this study reinforce the need for discouraging cigarette smoking among men, in particular, those suffering from infertility.

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Original Article

Comparative Study of Ziehl - Neelsen Stain v/s Fluorescence Stain for Detection of Acid Fast Bacilli from Tuberculous Lymphadenitis

Akhter H[a], Nahar A[b], Habib ZH [c], Lutfor AB[d]

Abstract

Background: The incidence of tuberculous lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. Cervical adenopathy is most common, but inguinal, axillary, mesenteric, mediastinal and intramammary involvement also have been described.

Objective: To correlate the fluorescence method with the conventional Ziehl-Neelsen method and to assess the efficacy and advantages of using fluorescence microscopy for the detection of Mycobacterium tuberculosis in paucibacillary cases.

Methods: This cross-sectional study was conducted in the laboratory of Microbiology Department, Sir Salimullah Medical College, National Tuberculosis Reference Laboratory (NTRL) and National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka during the period of January 2009 to December 2010. A total of 107 patients of both sexes and different age groups with peripheral lymphadenopathy, clinically suspected to be of tuberculosis origin were included in this study using purposive sampling technique. These patients attended the Pathology department, E.N.T and Medicine outdoor of Sir Salimullah Medical College (SSMC), National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh Medical College Hospital (BMCH), Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU). Microscopic examination of aspirate samples: Microscopic examination of sample was done for identification of Mycobacterium tuberculosis by Ziehl-Neelsen (Z-N) staining and fluorescence stains (FS).

Results: The cervical group of lymph node was involved in 92(85.9%) of cases, while axillary group and inguinal group were 13(12.1%) and 2(1.8 %) respectively. The fluorescence staining positive cases were 49(45.8%), among them 38(35.5%) were Ziehl-Neelsen stained positive and 11(22.42%) were Ziehl-Neelsen stained negative. Fluorescence staining 58 (54.2%) negative cases were also negative in Ziehl-Neelsen staining. The sensitivity of FS and Z-N staining for detection of tuberculous lymphadenitis were 78.6% and 67.9% and specificity of Z-N staining and FS staining were 100% and 90.2% respectively.

Conclusion: Fluorescence staining is 78.6% sensitive & 90.2% specific in comparison to Z-N staining where sensitivity was 67.9% & specificity was 100.0%. Fluorescence staining is a simple, rapid, easy as well as better screening method where work load is high.

Keywords: Acid fast bacilli, Tubercular lymphadenitis, Ziehl- Neelsen stain, Fluorescence stain.

Introduction:

The term extrapulmonary tuberculosis (EPTB) has been used to describe isolated occurrence of tuberculosis at body site other than the lung. Pulmonary tuberculosis is the most common presentation and EPTB is also an important clinical problem. When an extra pulmonary focus is evident in patient with pulmonary tuberculosis, such patients have been categorized under pulmonary tuberculosis as per the guidelines of the World Health Organization (WHO). Common sites of EPTB include lymph nodes, pleura, abdominal organs & osteo-articular areas.[1]

The incidence of tuberculous lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide. Cervical adenopathy is most common, but inguinal, axillary, mesenteric, mediastinal and intramammary involvement also have been described.[2] According to the World Health Organization (WHO) a third of the world's population is infected with *Mycobacterium tuberculosis*. Bangladesh is ranked 6th among 22 High Burden Countries (HBCs) in the world (WHO, 2010).[3] According to fact sheet 2009[4] published by National Tuberculosis Control (NTP), 1,10,601 (73%) new smear positive cases, 22,187 (15%) smear negative tuberculosis cases and 18,361 (12%) EPTB cases were identified in Bangladesh in 2008.

Lymphadenopathy is one of the most common extrapulmonary manifestation accounting for around

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30-40% of TB.[5,6] The clinical findings for the suspicion of TB in lymph nodes are neither specific nor do their absence exclude TB involvement. [7]Although Mycobacterial culture is the gold standard method for diagnosis of TB, it is time consuming and requires specialized facilities in the laboratory. Serological techniques and Molecular techniques are costly and less specific. Fine needle aspiration cytology (FNAC) can be an important tool in diagnosing TB lymphadenitis based on identifying epithelioid granulomas and caseous necrosis. However, conventional Ziehl Neelsen (ZN) method for detection of acid-fast bacilli (AFB) is simple and rapid but lacks sensitivity ranging from 20% to 43%.[8] Hence, fluorescence method for detection of AFB has proven more effective than ZN method in paucibacillary cases.

The purpose of the study was to correlate the fluorescence method with the conventional ZN method and to study the efficacy and advantages of using fluorescent microscopy for the detection of Mycobacterium tuberculosis in paucibacillary cases.

Materials and Methods:

This cross-sectional study was conducted in the laboratory of Microbiology Department, Sir Salimullah Medical College, National Tuberculosis Reference Laboratory (NTRL) and National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka during the period of January 2009-December 2010. A total of 107 patients of both sexes and different age groups with peripheral lymphadenopathy, clinically suspected to be of tuberculosis origin were included in this study using purposive sampling technique. These patients attended the Pathology department, E.N.T and Medicine outdoor of Sir Salimullah Medical College (SSMC), National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh Medical College Hospital (BMCH), Dhaka Medical College Hospital (DMCH) and Bangabandhu Sheikh Mujib Medical University (BSMMU). Selection criteria of patient: a) Inclusion Criteria: Patients with peripheral lymphadenopathy with clinical symptoms suggestive of tuberculous origin were included in this study. b) Exclusion Criteria: i) Clinically suspected or known cases of lymphadenopathy other than tuberculosis such as lymphoma, connective tissue diseases, metastasis were excluded from this study. ii) Patients on anti-tubercular drugs. A total of 107 fine needle aspirate samples were collected from clinically suspected cases of tuberculous lymphadenopathy.

Sample Collection Procedure: Fine needle aspiration was done with all aseptic precaution by using 10 ml sterile disposable plastic syringe. Skin over lymph node was cleaned with 70% ethyl-alcohol. Sample was collected into a sterile test tube and carried to the laboratory as soon as possible. Sample was then transferred to the falcon tube in the laboratory within the Bio-safety cabinet (BSC), and few sterile glass beads were added. Then vortexes until all the materials became homogenized. With the help of sterile wire loop, small amount of sample taken on slide and two smears were prepared for microscopic examination. Aspirate materials were examined in naked eye for identification of the nature of sample. Three types of materials were identified: pus, blood mixed and caseous materials.

Laboratory procedure:

Smear preparation for acid fast bacilli: The homogenized aspirate sample was used for smear preparation. A new, clean, grease free slide was used for smear preparation with patient identification number. A smear size of 3cm×2cm was prepared by slow circular and rotatory movement with a bacteriological loop or wooden stick. Two smears were prepared from each sample. Both smears were dried and fixed with heat by passing the slide over a Bunsen burner flame for 3-5 times. After fixation, slides were stained by Ziehl-Neelsen (Z-N) and Fluorescence staining method.

Examination of smears for acid fast bacilli:

a) **Ziehl-Neelsen staining:** The Ziehl-Neelsen stained smear was examined microscopically under the 100×oil immersion objective. One length and one width of the stained smear were examined under oil immersion which corresponded to about100 microscopic fields.[9]

b) **Fluorescence staining:** Fixed smear covered with auramine-phenol stain was heated for 10 minutes, then rinsed with water and drained. It was decolorized with 1% acid alcohol for 5 minutes. Rinsed with water and drained. The smear was counterstained with potassium permanganate for 2 minutes. Again rinsed with water and drained. The Fluorescence stained smear was examined within 24 hours after staining by using 45X objective and always keeping the slides away from the light and using one positive control smear with each panel of slides examined. The result was reported quantitatively as WHO recommended guideline. All examined smears, irrespective of results, were preserved.

Results:

Table 1: Distribution of location of lymph node swelling (N=107)

Site of sample	Number
Cervical Lymph node	92 (85.9)
Axillary Lymph node	13 (12.1)
Inguinal Lymph node	2 (1.8)
Total	107 (100.0)

Table-1 shows the distribution of location of lymph node swelling. Commonest site of lymphadenopathy was seen in the head-neck region. The cervical group of lymph node was involved in 92(85.9%) of cases, while axillary group and inguinal group were 13(12.1%) and 2(1.8 %) respectively.

Table 2: Lymph node aspirates having varying characteristics (N=107)

Nature of Aspirate	No. (%)
Pus	61 (57.0)
Blood mixed	28 (26.2)
Caseous	18 (16.8)
Total	107 (100)

Table-2 shows lymph node aspirates having varying characteristics. In this study three types of aspirate were obtained by FNA. Among 107 cases, pus 61(57%), blood mixed 28(26.2%) and caseous material 18(16.8%) were found.

Table 3: Comparison of Ziehl-Neelsen stain and Fluorochrome stain among the aspirates of tuberculous lymphadenitis cases

Fluorescence stain	Ziehl-Neelsen stain		No. (%)
	Positive	Negative	
Positive	38 (35.5)	11 (22.4)	49 (45.8)
Negative	0 (0)	58 (54.3)	58 (54.2)
Total	38 (60.7)	69 (64.5)	107 (100.0)

p value=0.001

Chi-square test was done to measure the level of significance

Table -3 compares the finding of AFB between the Fluorescence stain and Ziehl-Neelsen stain. The Fluorescence stained positive cases were 49(45.8%), among them 38(35.5%) were Ziehl-Neelsen stained positive and 11(22.42%) were Ziehl-Neelsen stained negative. Fluorescence stained negative 58 (54.2%) cases were also negative in Ziehl-Neelsen stained. The chi-square test reveals that the difference between two types of stains statistically significant.

Table 4: Sensitivity, Specificity, Positive Predictive Value, Negative predictive value and Accuracy of Ziehl-Neelsen staining and Fluorescence staining

Staining methods	Validity tests				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Z-N stain	67.9	100.0	100.0	73.9	83.2
Fluorescence stain	78.6	90.2	89.8	79.3	84.1

Table-4 shows comparison of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of Ziehl-Neelsen stain and Fluorescence stain. The sensitivity of FS and Z-N staining for detection of tuberculous lymphadenitis were 78.6% and 67.9% respectively, specificity of Z-N staining and FS staining were 100% and 90.2% respectively. Positive predictive value of Z-N staining and FS staining for detection of tuberculous lymphadenitis were 100% and 89.8% respectively, while negative predictive value of FS staining and Z-N staining were 79.3% and 73.9% respectively. Accuracy for detection of tuberculous lymphadenitis by FS staining and Z-N staining was 84.1% and 83.2% respectively.

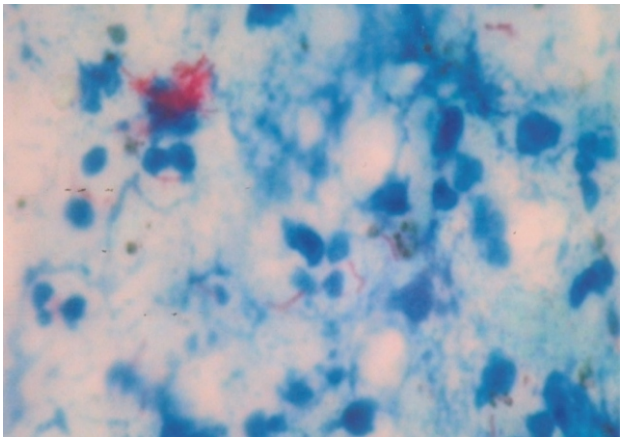

Figure 1: Ziehl-Neelsen staining

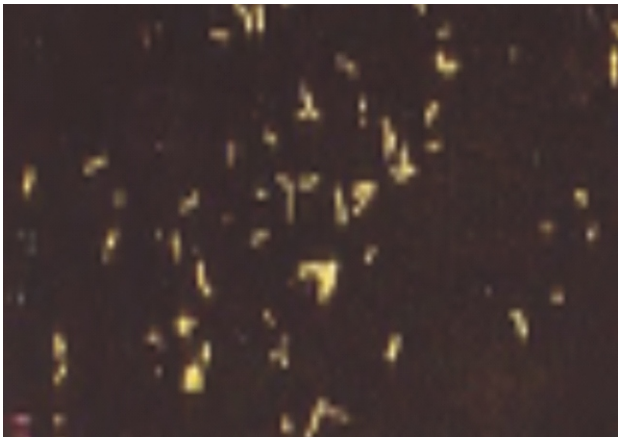

Figure 2: Fluorescence staining

Discussion:

We compared results of Ziehl-Neelsen stain smear by light microscopy with that of auramine phenol stain smear by fluorescent microscopy for detection of AFB. The diagnosis of tuberculous lymphadenitis remains challenging in spite of the availability of various diagnostic tools. Traditionally, the diagnosis of TB-L is established by histopathology and smear microscopy or by Mycobacterial culture from biopsy specimen. Fine needle aspiration cytology (FNAC) has proved valuable in the diagnosis of tuberculous lymphadenitis and also provides an alternative and easy way for collecting materials for bacteriological

examination.[10, 11] However, FNAC has several limitations, especially in the absence of demonstrable acid fast bacilli (AFB).[12] Polymerase chain reaction (PCR) for *M. tuberculosis* has already proven to be a useful tool for the diagnosis of tubercular infection. PCR and its modification are the most recently applied methods in the diagnosis of tuberculous lymphadenitis.[13,14] In the present study, staining techniques were used for identification of causative agent in FNA samples to find a suitable, rapid, less invasive and accurate method for diagnosis of tuberculous lymphadenitis.

Among the 107 patients with lymphadenopathy 92(85.95%) patients had cervical lymphnode enlargement followed by axillary groups 13 (12.4%) and inguinal groups 2 (1.8%) as shown in Table-1. This observation is comparable with findings of other research where 67.5% cervical, 9% axillary and 6.4% inguinal lymphnode group were reported.[15] Another study in Butajira, Ethiopia, revealed that 82.2% cases had cervical involvement (Yassin *et al*., 2003)[16] which correlates with our findings.

In this study three types of aspirate were obtained by FNA from study population, where pus were 61(57%), blood mixed 28(26.2%) and caseous material 18 (16.8%) cases. The findings are not comparable with study done by Masilamani S et al., who found blood mixed aspirate in 84.4%, pus material in 12.3% and caseous material in 3.3% cases.[17] Irrespective of types of aspirate, all 107 samples were tested for Mycobacterium tuberculosis by microscopy (ZN stain and FS stain).

In the current study, Ziehl- Neelsen staining smears revealed 38 (35.5%) positive among the 107 cases. This finding correlates with the findings of Pamra and Mathur reported 35.6% AFB positivity rate.[18] In other studies, the rate of AFB positivity of aspirated smears was 37.4% to 59.4%.[19] FS method of smear revealed 49 (45.8%) positive among the 107 cases (Table 3). Statistically the FS stain was found to be much efficient method than ZN stain. This study showed smear positivity for AFB on conventional ZN method was 38(35.5%) while the positivity increased to 49(45.8%) by fluorescence method. Mohan et al., showed 24% smear positivity for AFB on conventional ZN method while the positivity increased to 38% by fluorescent method in their study.[20] Our findings do not correlate with the study done by Ulukanligil et al., who obtained 85.2% positivity by Fluorescent microscopy and 67.6% by ZN method.[21] Z-N staining method is rapid and cost-effective, but lacks in sensitivity and positive result does not distinguish Mycobacterium tuberculosis from other Mycobacteria. Fluorescence staining is 10% more sensitive than Z-N staining because in fluorescence microscopy a low magnification objective is used to scan smears for detection of AFB. By which a much larger area of the smear can be scanned resulting in more rapid examination and also by the fluorescence staining, bacilli stand out brightly against the dark background (Forbes et al, 2002).[22] As low power is used for examination, large number of specimens can be examined in a given time and colour-blind investigators may also use this method without difficulties. These are additional advantages of fluorescence microscopy.[23]

In this study, the sensitivity of FS stain was 78.6%, which is higher than Z-N staining 67.9% and specificity of FS and ZN staining were 90.2% and 100% respectively (Table 4). Other studies demonstrated that FS stain is more sensitive (81.8%) than that of other conventional methods but showed poor specificity (28.2%).[24]

Conclusion:

We conclude that fluorescence stain is more efficient in detecting acid fast bacilli in extra-pulmonary samples than ZN stain. Fluorescence method improved diagnostic value especially in patients with a low density of bacilli. Fluorescence method is a simple, rapid, easy staining method as well as better screening method where work load is high.

Acknowledgement:

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Review Article

Knowledge about Blood Product Utilization during Massive Transfusions with their Complications and Transfusion Protocols

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Abstract

Haemorrhage remains a major cause of potentially preventable deaths. Rapid transfusion of large volumes of blood products is required in patients with haemorrhagic shock which may lead to a unique set of complications. Recently, protocol based management of these patients using massive transfusion protocol (MTP) has shown improved outcomes. MTP include higher ratios of plasma, platelet and red blood cell transfusions which has improved outcomes which need further clinical investigation. Additionally, tranexamic acid has been shown to decrease the mortality in trauma patients requiring MT. Greater use of cryoprecipitate or fibrinogen concentrate might be beneficial in MT patients from obstetrical causes. Throughout the resuscitation, the patient should be closely monitored and both metabolic and coagulation abnormalities are to be corrected.

Keywords: Massive Transfusion, Massive Transfusion Protocol, Transfusion Management

Introduction:

Acute exsanguination is the leading cause of mortality in trauma patients.[1-3]. Massive blood loss potentially results in the development of the 'lethal triad', comprising hypothermia, acidosis and coagulopathy.[4] Without prompt intervention, including the appropriate administration of blood and blood products, the majority of these patients will demise within 6 hours.[2,4,5,6,7]

Management of patients requiring massive transfusion (MT) is challenging.[7] For optimal management of massively bleeding patients, effective preparation and communication between transfusion and other laboratory services and clinical teams are essential.[8] Timely recognition and efficient management are vital for successful outcomes after major blood loss.[9] A common potential complication of all complex oncological surgeries is massive intra- and postoperative hemorrhage and the subsequent risk for massive blood transfusion.[10]

Massive haemorrhage is one of the main causes of death and intraoperative cardiac arrest in adults as well as children.[11-14] It is usually defined in relation to the volume of blood products transfused over 24h by kilograms of body weight.[15]

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Definition of Massive Transfusion:

MT refers to the transfusion of large volume of blood products over a short period of time to a patient who has severe or uncontrolled haemorrhage including correction of coagulopathy arising from severe haemorrhage.[16-18] The three most common definitions of MT in adult patients are[8,19-22]

1. Transfusion of =10 red blood cell (RBC) units, which approximates the total blood volume (TBV) of an average adult patient, within 24 hrs,
2. Transfusion of >4 RBC units in 1 hr with anticipation of continued need for blood product support, and
3. Replacement of >50% of the TBV by blood products within 3 hrs

Data regarding MBT in the paediatric population is scarce. Definitions of MBT suggested for use in children are: transfusion of >50% TBV in 3 hrs, transfusion >100% TBV in 24 hrs or transfusion support to replace on-going blood loss of >10% TBV/min.[23]

Another author also defined MBT as replacement of >1 blood volume in 24 hours, or >50% of blood volume in 4 hours (adult blood volume is approximately 70 mL/kg), or in children: transfusion of >40 mL/kg (blood volume in children over 1 month old is approximately 80 mL/kg).[24]

Epidemiology of MT:

The need for MT occurs in trauma, obstetrics and major surgery. Trauma-related mortality is the fourth leading cause of death in the USA, and according to the Center for Disease Control and Prevention, unintentional injury accounted for more than 120,000 deaths in 2010.[25] About 40% of trauma-related mortality is due to uncontrolled bleeding. It has been estimated that among the injured patients admitted to trauma centers, up to 10% of military and up to 5% of civilian patients require MT.[26,27] In general, injury severity and transfusion requirement are associated

with mortality. Most (99%) of the patients receiving <10 RBC units within the first 24 hrs survived, whereas only 60% of patients who received >10 RBC units within the first 24 h survived.[28] Obstetrical haemorrhage is another common cause of MT and massive haemorrhage is the most common cause of shock in obstetric patients and is the number one cause of maternal mortality worldwide.[29] Other causes of MT include gastrointestinal haemorrhage and major surgeries, such as cardiac, spinal, and liver surgery, and liver and multivisceral transplantation.[7]

Predicting MT:

Early recognition and prompt treatment results in improved outcomes in massively bleeding patients. Models have been developed using both clinical and laboratory parameters to predict who would need MT in trauma patients.[30-37] However, none of them are a perfect tool.[38] For example, the Trauma Associated Severe Hemorrhage (TASH)-score incorporated seven clinical and laboratory variables (haemoglobin, base excess, systolic arterial pressure, heart rate, presence of free intra-abdominal fluid and/or complex fractures, and gender) into a composite score to predict the need for MT (defined in the study as administration of 10 or more RBC units within 24 hr).[39] Nonetheless, Nunez and colleagues demonstrated that simple and rapidly available parameters, such as the presence of penetrating trauma, systolic arterial pressure <90 mm Hg, heart rate >120 beats/min[40,1], and a positive focused abdominal sonography for trauma, appears to be as good as other more complex scoring systems to predict MT. If any two of the above four parameters are positive, the patient is likely going to require MT.[7]

Massive Transfusion Protocols:

Patients requiring MT is to develop an institutional MTP to facilitate communication between different services (trauma, nursing, transfusion medicine, and other laboratories), avoid delay in clinical care, laboratory testing and blood product transfusion, and nursing care.[41] Use of standardized protocols for massive transfusion may lead directly to a significant reduction in patient mortality. This improvement in outcome has been largely attributed to increased use of FFP[42,43] instead of crystalloid intravenous fluids. The standardized approach to resuscitation as taught by the Advanced Trauma Life Support (ATLS) course for adults with significant bleeding, advocates use of 1-2 litres of isotonic crystalloids for initial volume resuscitation.[44]

Some authors feel that this approach is only appropriate in trauma patients not requiring a massive blood transfusion.[45] Infusion of large of amounts of crystalloids not only precipitates the dilutional coagulopathy, but also has pro-inflammatory effects[34] as well as increasing the risk of subsequent infection.[46,47] Conversely, use of smaller amounts of crystalloids and larger amounts of fresh frozen plasma in the initial resuscitation period is associated with improved 24 hour and 30-day survival.[48-50] Fresh whole blood has been suggested as the ideal resuscitation fluid for trauma patients, and has recently been widely reported within the American Armed Forces.[42] Blood is obtained on demand and immediately transfused.[51] Data from the US Military showed improved survival in these patients,[52] with no significant increase in the rate of transfusion-transmitted infection.[53,54] MTPs can include preparation and administration of blood products based on laboratory test results, predetermined transfusion packages or integration of both.[55]

Rationale for Massive Transfusion Protocol:

Physicians involved in managing war injuries noticed that early administration of fresh frozen plasma (FFP) during massive transfusion decreased coagulopathy and improved survival in patients. Studies have shown improved survival using higher ratio of FFP to RBC transfusion as compared to the conventional approach.[56-58]

Transfusing fresh whole blood would seem ideal but the time required to conduct safety tests on blood is long enough to cause significant depletion of coagulation factors. Therefore, administering RBCs, coagulation factors and platelets together maintains the physiological constitution of blood and prevents deficits of one or more constituents. MTPs have a predefined ratio of RBCs, FFP/cryoprecipitate and platelets units (random donor platelets) in each pack (e.g. 1:1:1 or 2:1:1 ratio) for transfusion.[59,60] Once the patient is in the protocol, the blood bank ensures rapid and timely delivery of all blood components together to facilitate resuscitation.[61]

Complications from MT:

Besides the risk of transfusion reactions that occur with single unit transfusions, patients with MT are at risk of other adverse events due to large transfusion volumes.[4,62]

Citrate toxicity: It manifests as signs of hypocalcaemia.[63] Eighty (80) ml of citrate phosphate dextrose adenine solution present in each blood bag contains approximately 3 g citrate. A healthy adult can metabolize this load in 5 min.[64,65] When circulating volume is well maintained, cardiovascular manifestations occur with infusion rates of 150 ml/70kg/min of citrated blood. However, when there is hypothermia of 31°C, citrate metabolism rates drop by 50% and toxicity may occur with slower infusion rates.[66]

Neonates are at risk of developing heart failure due to hypocalcaemia during transfusion due to citrate toxicity which may be prevented by keeping the transfusion rate below 1ml/kg/min.[12] However, hypoperfusion or hypothermia associated with massive blood loss can cause citrate toxicity. Unmetabolized citrate can lead to hypocalcaemia, hypomagnesemia which may cause

myocardial depression that manifests earlier than hypocalcaemic coagulopathy. Hypotension not responding to fluids should alert the physician to this complication. Calcium supplementation is thus required in most cases of MBT.[67]

Hypocalcaemia: Although it is defined as a total serum calcium concentration of less than 8.5mg/dl (4.5mEq/l, 2.10mmol/L)[68] clinical hypocalcaemia may occur even with normal total calcium values when serum ionized calcium concentrations are lower than 4.5mg/dl. In surgery, the most common causes of hypocalcaemia are hyperventilation and citrated blood infusion at a rate of more than 1.5ml/kg/min.[69]

Hyperkalaemia: It is defined as a serum potassium level greater than 5.5mEq/L. It is usually considered mild up to 6mEq/L, moderate between 6 and 7mEq/L, and severe when greater than 7mEq/L. It may be caused by external or internal balance disruptions.[70-72] The most frequent causes include severe renal failure, iatrogenic injury, the use of angiotensin converting enzyme (ACE) inhibitors[32] and bank blood transfusions.[73,14] Potassium concentrations in PRBCs can range from 7 to 77 mEq/L depending on age of stored blood.[67,74,75] Development of hyperkalaemia will depend on the underlying renal function, severity of tissue injury and rate of transfusion. At transfusion rates exceeding 100-150 ml/min, transient hyperkalaemia is frequently seen.[76]

RBCs contain a CPD (citrate-phosphate-dextrose) or CPDA (citrate-phosphate-dextrose-adenine) solution with a pH of 5.5, which lowers the pH from 7.0 down to 6.6 after 2135 days of storage. The result is an accumulation of potassium, fixed acids and CO2 that may produce myocardial depression when infused in the context of massive bleeding.[77] Potassium concentrations in RBC units increase with radiation and diminish with red blood cell washing.[78] Rapid transfusion through a central venous catheter may deliver higher potassium concentrations into the coronary circulation than when given through a peripheral venous line, and this may contribute to the risk of cardiac arrest.[79,37] Moreover, some pressurized infusion devices may traumatize red blood cells, giving rise to greater potassium outflows from the cell.[80] The new rapid infusion and fluid warming devices do not produce significant cell destruction.[14] Also, acidosis secondary to hypoperfusion may worsen hyperkalaemia. Cardiac effects of hyperkalaemia are accentuated by hypocalcaemia.[67]

Hypothermia: Factors contributing to hypothermia include infusion of cold fluids and blood and blood products, opening of coelomic cavities and decreased heat production. Hypothermia leads to decreased citrate metabolism and drug.[81]

Hypomagnesemia: Citrate also binds to magnesium and can lead to hypomagnesaemia which can further accentuate effects of hypocalcaemia. Infusion of large amounts of magnesium poor fluid can also contribute to hypomagnesemia.[61]

Acidosis: After 2 weeks of storage, PRBCs have a pH below 7.0, and each unit has an acid load of approximately 6 mEq. One of these mEq of acid comes from the fact that PRBCs are made from venous blood with a starting pH of 7.35, a second mEq is acquired in buffering the citric acid in the anticoagulant, and 4 mEqs are generated by glycolysis during PRBC storage.[82] Acidosis directly reduces activity of both extrinsic and intrinsic coagulation pathways. A pH decrease from 7.4 to 7.0 reduces the activity of FVIIa and FVIIa/TF by over 90% and 60% respectively.[83,71]

Late complications: Late complications are Transfusion related acute lung injury (TRALI),SIRS, Sepsis, Thrombotic complications.[61,84]

Practical Tips:

1. Early recognition of massive blood loss and triggering MTP.
2. Collect blood sample for cross match early as colloids may interfere with cross matching (mainly dextrans by coating RBC surface).
3. Inotrope/vasopressor drugs should only be used in a blood loss scenario during severe hypotension to avoid critical hypoperfusion and to buy time for fluid resuscitation. They should be stopped as soon as volume deficits are replaced, and a safe blood pressure is achieved.
4. Do not exceed recommended maximum doses of colloid.[85]

Conclusion:

The literature review suggests that a standardized protocol for massive transfusion not only improves patient outcome, but also leads to a reduction in final volume of blood products utilized for resuscitation of heavily injured patients. This will likely be best achieved as part of a hospital-based protocol, involving all departments participating in trauma resuscitation, rather than within individual departments.[87]

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Case Report

Cutaneous Myiasis Caused by Cordylobia Anthropophaga: A Case Report in Central Africa

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Abstract

Cutaneous myiasis caused by Cordylobia Anthropophaga**,** the tambu fly, involves the infestation of human tissue with fly larvae and is common in tropical Africa. We report a case of a 39 year-old Bangladeshi United Nations Peacekeeper with cutaneous myiasis who deployed temporarily in Central Africa. The epidemiology, clinical presentation, various methods of larval extraction, treatment and prevention on public health aspect, are discussed.

Introduction:

Myiasis, from the Greek *myia* for "fly," has been defined as the infestation of live human or vertebrate animals with larvae of the insect order Diptera (flies), which feed on living or necrotic tissue.[1-3] Myiasis can be accidental, as when fly larvae occasionally find their way into the human body or facultative, when fly larvae enter living tissue opportunistically after feeding on decaying tissue in neglected, malodorous wounds.[4] Myiasis can also be classified according to the site of infestation. Cutaneous myiasis involves the invasion of the skin, with the most common target being a wound, near which an obligatory or facultative parasitic fly will lay eggs.[1,2,4] In "wound myiasis" both healthy and necrotic tissues can be fed on by the larvae, depending on the conditions and species of fly involved. Flies (eg-the human botfly *Dermatobia hominis* in tropical America, the "tumbu fly" *Cordylobia anthropophaga* in tropical Africa, and *Wohlfahrtia vigil* in North America) penetrate healthy skin and produce itchy sores that develop into painful boil-like lesions or furuncles, hence the term "furuncular myiasis".[1,5,6] Creeping myiasis is a type of cutaneous myiasis involving the migration of fly larvae underneath the skin.[1,7] Apart from the skin, the eyes, ears, nose and sinuses represent relatively common sites of attack whereas less common sites are the mouth, throat, urogenital, and gastrointestinal tracts.[1,2,4,8-10] As the modern, rapid international travel increases these myiatic infestations are now encountered outside these endemic regions. The presenting paper is the case report of a cutaneous myiasis caused by the "tumbu fly" Cordylobia anthropophaga.

The eggs of Cordylobia species are deposited on the soil or wet and soiled clothes hung outside for drying. The hatched larvae invade unexposed skin (of the buttocks, trunk, the limbs and penis) in contact with the wet clothes. Mature larvae then emerge from the host and pupate in the soil.[3] Myiasis occurs mainly in tropical and subtropical latitudes and often originates in these areas even when reported in temperate climates.[1] The main contributing factors are probably the higher levels of exposure to myiasis-causing flies due to poorer clothing and hygiene conditions, combined with the increased aggressiveness of myiasis-causing flies in the tropics.[1,3,9] Nevertheless, many cases acquired in temperate parts of Eurasia and North America, including Canada, have been described in the literature.[3,4,10,11] For the temporary employer/visitor in endemic area this disease is very unknown to them, as well as human myiasis remains an unfamiliar illness for most physicians of South Asia deployed in UN peacekeeping mission in Africa; misdiagnosis and inappropriate treatment are common.[4] Awareness of myiasis by health professionals would facilitate recognition and augment the effectiveness and expediency of care.

Case Presentation:

A 39 years old male Soldier presented with multiple small swellings over right forearm, left hand and left thigh associated with stinging sensation for five days. The patient was a Bangladeshi UN peacekeeper deployed in Central African Republic. He came to this country six months back and he never been in this region earlier. All the lesions were very pruritic and the patient believed them to be mosquito bites. The patient said that the lesions continued to grow and eventually began to have area of redness without any exudates. In the days just prior to presentation, the patient said that the lesions became very painful and described the pain was sharp and like "hot needles" going in and out of beneath his skin wound.

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Fig 1: Furuncular Swelling

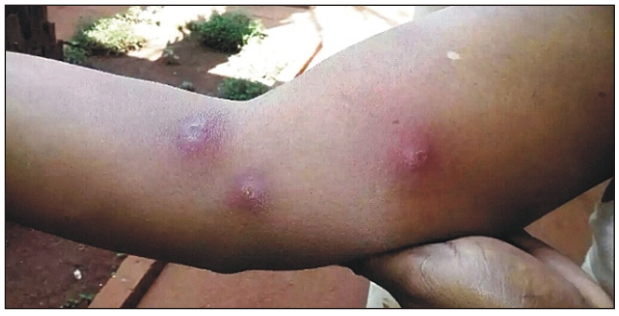


Fig 2: Views of larva immediately after extraction.

Fig 3: Wound immediately after larvae extraction

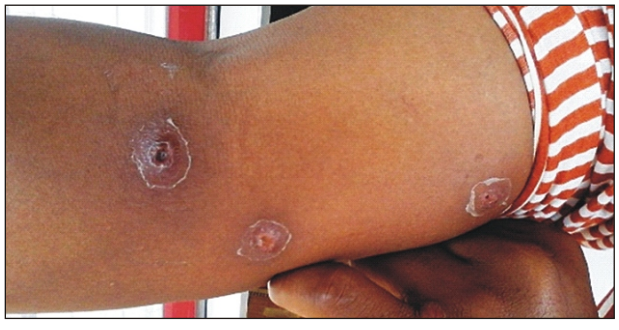
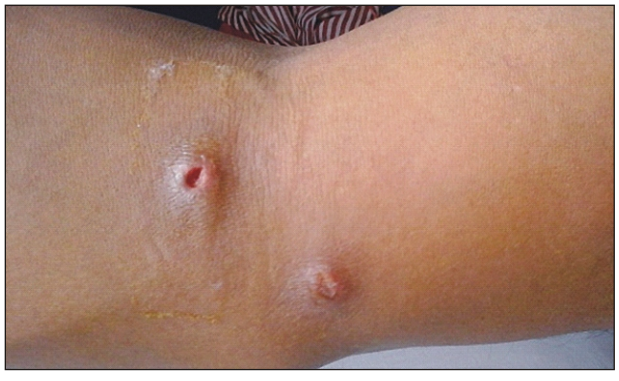


Fig 4: after five days of larvae extractionpatients



Patient was in a distant remote camp and treated by antibiotic and antihistamine. For this complain he evacuated to Bangladesh UN hospital. In the hospital, on examination patient was anxious and afebrile and no lymphadenopathy found. Total six tender furuncular swellings was observed over anterior side of forearm, at the junction of 4th and little finger of left hand and over front of the left thigh, with surrounding erythema and central pore (Fig-1). The average size of the swellings was 1.5cm x 1.5cm. Live motile larvae, one from each lesion were extruded when the swelling was squeezed. Out of six, only one lesion needed small elliptical incision and single larva was expelled from the wound (Fig-2 & Fig-3). All the larvae were more or less 1cm in length. The wound was cleaned with mild antiseptic and bandaged, given analgesia and the patient was observed more five days in the hospital. Patient was asked to report to hospital after two weeks. On his follow up, all the wounds healed without complication and there was a residual fading skin pigmentation. The live larva was sent to an Entomologist and an expert of infectious disease consultant, where it was identified as maggot of the Tumbu fly, Cordylobia anthropophaga.

Discussion:

Three reasons lead us to believe that the parasite involved in the reported case was Cordylobia Anthropophaga. First, the sore had the form of a furuncle, and there was no sign of any wound or necrotic tissue that could have attracted flies before the invasion by the maggot; therefore, facultative parasitic flies that breed typically in decaying organic matter could almost certainly be excluded. Second, the patient was not a resident tropical Africa and he came there only 6 months back and having a typical symptoms of cutaneous myiasis. Thus, the causal agent was most likely an obligatory parasite associated with furuncular myiasis in temperate Africa. Finally, the shape of the larva, pictured after extraction (Fig-2), excludes all remaining candidates except Cordylobia.

The first description of myiasis was by Hope in 1840.[12] The disease is usually uncomplicated and self-limiting, but there have been reported cases of fatal cerebral myiasis in young children resulting in meningitis and death.[12] Clinically, infections with myiatic flies start out as itchy sores that develop into painful boil-like lesions with a central punctum which often ooze.

An intense inflammatory reaction may be seen in the surrounding tissue during a later stage of the infestation.[13] Secondary infection by bacteria is uncommon, because bacteriostatic activity in the gut of the larva seems to prevent undesirable overgrowth of pyogenic bacteria.[14] Symptoms may include mild pruritus, periodic stinging, or intense cutaneous pain. Due to their infrequent occurrence, these lesions are often misdiagnosed as cellulitis, leishmaniasis, furunculosis, staphylococcal boil, insect bite or sebaceous cyst.[2]

Diagnosis:

The diagnosis is mainly clinical and should be suspected in a patient with a non-healing furuncular skin lesion. Ultrasound has been used to aid in diagnosing and

deciding upon a course of treatment for cutaneous myiasis involving mature larvae. Definitive diagnosis is made with demonstration and identification of the larva based on typical morphology. The lesion heals rapidly after the larva is removed or it spontaneously exists.

Treatment:

For furuncular cutaneous myiasis, digital pressure on both sides of the lesion (or using a pair of wooden spatulas) is often sufficient to expulse the larva (e).[15] Pressure can be reinforced with gentle traction with forceps or tweezers. If this method is not satisfactory petroleum jelly, paraffin oil or bees wax can be applied to the opening of the lesion to asphyxiate the larvae and force it out.[6] It may take 24 hours for the larva to come out and it may be necessary to press it out or to grasp it with tweezers while coming out.[6] Surgical excision may be used if the larva is dead or if other methods have failed. To facilitate excision, the larva may be numbed by application of lidocaine gel.[16] In any case, care should be taken not to rupture the maggots because they may cause secondary infections or trigger potentially severe allergic reactions. The presence of additional maggots in the lesion should be considered (especially in wound myiasis). The wound should be disinfected and tetanus prophylaxis to be updated as necessary. Antibiotics should be prescribed for signs of bacterial infection.[4] Complications of cutaneous myiasis include cellulitis, abscess formation, tetanus and osteomyelitis.[17]

Prevention:

On public health aspect this disease can mostly negotiable by taking adequate preventive measure. The female flies of *Cordylobia anthropophaga* lay eggs in shaded ground, especially sand, or on clothing, favouring the subsequent invasion of skin by hatched larvae; avoiding laying on the ground for long, ensuring that no clothes are left outside (especially not in the shade), or ironing clothes when left outdoors to kill eggs or larvae may help reduce the risk of myiasis in areas where *C.anthropophaga* is endemic.[18] Also improvement of sanitation, personal hygiene and exterminating the flies by insecticides are crucial in controlling the disease.[19] Fly breeding habitats should also be reduced by managing food residues and garbage containers properly.[18]

Human cases of cutaneous myiasis are most probably underreported because many remain undiagnosed or unpublished. Also most of the cases are treated by traditional remedies, or passed unnoticed and heal spontaneously. Awareness of myiatic infestation by health professionals would assist animal resources, agriculture and other departments in monitoring the different species of myiatic fly in the region.[19]

The present observations confirm that this calliphorine species infestation is present in Central African Region. Every year United Nations peacekeepers of different countries of the world are coming in this region. Many of them are never experienced this cutaneous infection. We want to emphasise that they should be well aware of this parasitic disease and take adequate preventive measures.

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Case Report

Large Salivary Calculus Causing Sialo-Oral Fistula: A Case Report and Review of Literature

Chowdhury N H[a]

Abstract

Sialolithiasis is the most common salivary gland disease, responsible for more than 50% of the cases. Mostly occur in the submandibular gland and its duct. It has male predilection and seen in adults. Majority of the calculi are less than 10 mm in size. Calculi > 15 mm in size are considered giant. Giant calculi within the salivary glands parenchyma are common, but they are uncommon in the salivary ducts. Here a case of large salivary calculus in the left submandibular gland duct causing sialo-oral fistula in a 45 years male was reported with review of literature since 1990. Etiology, pathogenesis, clinical features, diagnosis and management were also discussed.

Keywords: Calculus, giant, salivary gland, Wharton's duct

Introduction:

Sialolithiasis is a pathological condition caused by the obstruction of a salivary gland or its duct by a calculus.[1] It is the most common salivary gland disease accounting for more than 50% of the cases.[2,3] It has an incidence of about 0.012% in the adult population. It is common in adult.[2] There is a peak incidence in 4th-6th decades.[4] It is uncommon in pediatric population only 3% of all cases.[1] Males are affected more and male: female ratio 5.5:4.[4] Majority of salivary calculi (80%-95%) occur in the submandibular gland, whereas only 5% to 20% are found in the parotid gland. The sublingual gland and minor salivary glands are rarely affected (1%-2%).[5] When minor salivary glands are involved, the most common sites are buccal mucosa or upper lip and it presents as a firm nodule that may mimic tumor.[6]

In general, sialoliths are common in submandibular duct than gland parenchyma.[7] However, giant sialoliths are more common within the parenchyma of the salivary glands. Sialoliths located in the duct are usually elongated, while those situated in the gland or hilus tend to be round or oval.[4] Though bilateral cases have been reported (3%), salivary calculi are usually unilateral and occur equally on right and left sides.[8] Single sialolith is found in majority of cases (70-80%), two in about 20% cases.[4] They are usually yellowish in color and consist of mainly calcium phosphate with small amounts of carbonates in the form of hydroxyapatite, also made up of magnesium, potassium, and ammonia. Submandibular stones are composed of 82% inorganic and 18% organic material, whereas parotid stones are composed of 49% inorganic and 51% organic material.[2] Sialolithiasis typically presents as a painful swelling of the affected gland during meal times.[9,10] However, most salivary stones are asymptomatic,[10] because the stone usually does not block the flow of saliva completely.

Sialoliths commonly measure between 5 and 10 mm in size and stones over 10 mm can be reported as unusual size.[2] Lustman in a study on 245 patients with sialolithiasis found that 78.8% were less than 10 mm in size, 13.6% were between 10-15 mm and only 7.6% were > than 15 mm in size.[4] Calculi > 15 mm are considered giant.[1] Although, giant sialoliths have been frequently reported in the body of the salivary glands, they are rare in the duct of salivary gland.[2] The purpose of this article is to report a case of giant sialolith in the Wharton's duct and to review the related literature. For review, only those articles published from 1990 onwards in English language with the sialolith present in Wharton's duct and having size > 15 mm were selected. From review it is clear that large salivary duct calculus are rare and as far as we could determine only 30 cases have been reported in last 26 years

Case Presentation:

A 45 year-old male reported to the dept. of ENT & HNS of Bangladesh Medical College Hospital with the chief complaint of pain and swelling in the floor of mouth on left side since five months. It started as small swelling which used to increase during meals five months back. It gradually increased over next three months and then it burst leaving a yellowish white mass in left floor of mouth which was noticed by patient. Patient experienced pain, as well as pus discharge from that region. Intraoral examination revealed a well-defined round swelling of approximately 2×1 cm in size in the floor of the mouth in relation to lower right second premolar and first molar. Overlying mucosa was normal in color except in the distal most part of swelling where there was break in continuity exposing the underlying yellowish mass. It was hard in consistency and tender on palpation (Figure-1). A provisional diagnosis of sialolith in the left submandibular gland duct was made.

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Fig 1: Pre-operative view of large salivary calculus.

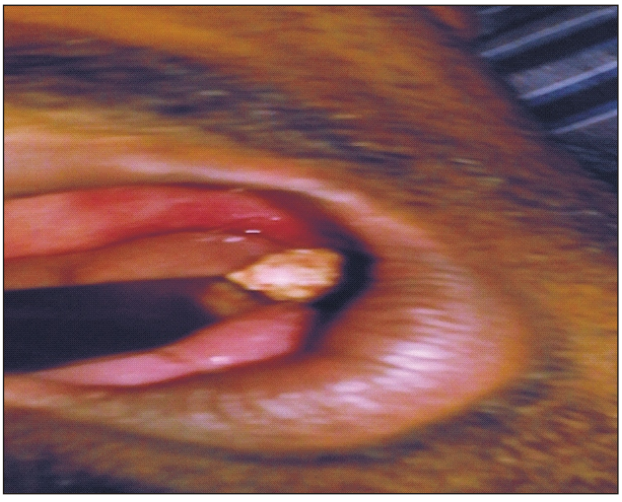
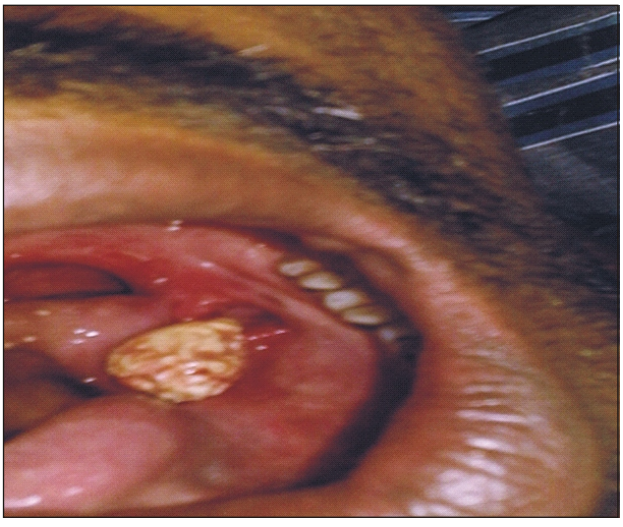


Fig 2: Position of salivary stone and silo-oral fistula.



Radiographic evaluation included cross sectional mandibular occlusal view and orthopantomogram [OPG] which revealed a large well defined oval homogenous radio-opacity in the floor of the mouth on left side_in relation to lower left second premolar and first molar teeth.

Fig 3: Excised giant sialolith, measuring 16 x 11

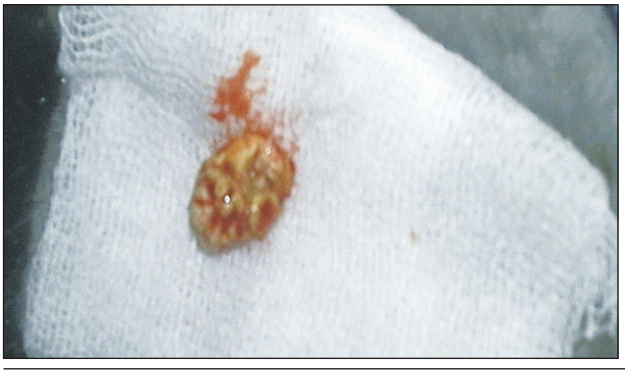


Fig 4: Post-operative photograph of sialo-oral fistula on left side

Sialolith was enucleated under local anesthesia. The preexisting sialoral fistula was extended antero-posterior direction to the required length and blunt dissection was done to enucleate lesion *in toto*.

Discussion:

The exact etiology and pathogenesis of salivary calculi is unknown. However, salivary stagnation, increased alkalinity of saliva, infection, inflammation or physical trauma to salivary duct or gland are predisposing factor to calculus formation.[6,11] Commonly they are thought to occur as a result of deposition of the tricalcic phosphate salts around a nidus of salivary mucin, desquamated epithelial cells.[1] It is suggested that bacterial toxins produce a local environment with pH less than 5.5 leading to tissue damage; and when 7.2 pH is re-established during the healing process, crystallization of salivary ions (especially calcium phosphates) occurs leading to calculus formation.[1] According to another theory an unknown metabolic phenomenon increases the salivary bicarbonate content, altering calcium phosphate solubility and leading to precipitation of calcium and phosphate ions.[6] Another popular theory is substances or bacteria within the oral cavity migrate into the salivary ducts and become the nidus for further calcification.

The age in the cases reviewed ranged from 35 to 70 years with average of 46.7 years. Among the cases reviewed, majority occurred (26/29) in patients over the age of 40 years. None of the giant sialolith was seen in children which is consistent with the literature on sialolith. Among the 30 case reports the incidence is higher in men ($n = 22$) compared with women ($n = 7$) with male to female ratio of 3.1:1. Size of the sialolith in the reported cases ranged from 15mm to 72 mm. The sialolith presented by Rai[2] is perhaps the largest ever reported calculus in Wharton's duct (72 mm). The ability of a calculus to become giant depends mainly on the reaction of the affected duct. If the

duct adjacent to the sialolith is able to dilate allowing nearly normal salivary flow, it might remain asymptomatic for a long period; thus eventually creating a giant calculus.[12] Weight of the giant sialolith was reported in only 6 cases, which varied from as light as 1.34 gm[13] to as heavy as 45.8 gm.[2] Duration of the sialolith was reported in 15 cases which ranged from 1 week to 22 years. It is believed that a calculus may enlarge at the rate of approximately 1 to 1.5 mm per year.[9] Thus, it is possible to explain the long duration of sialolith in few cases. In 16 cases it affected left side whereas right side was affected in 9 cases. Thus, it appears that giant sialolith has affinity for left side. However, in the present case right side was affected. Pain and swelling was the most common symptom in the reviewed cases. Sialolith causes pain and swelling of the involved salivary gland by obstructing the food related surge of salivary secretion.[6] The severity of symptoms depends on the degree of obstruction. In few cases patients were asymptomatic.[6,9,10,13]

The submandibular gland is more susceptible to the development of the salivary calculi because its duct is longer and tortuous, salivary flow is against gravity, salivary pH is more alkaline and its saliva has greater content of mucin, proteins, calcium, as well as phosphates.[3,11] Generally, the most common radiographic techniques to diagnose submandibular sialoliths are panoramic and occlusal views.[14] Giant sialoliths are mostly radiopaque and are easily depicted on panoramic radiographs, probably because their lithogenesis is long enough for calcification to be completed.[5] Investigations like sialography, ultrasonography, and computed tomography may be required to locate small sialoliths (as 20% to 30% are radiolucent).[1,2,10] However, sialoliths smaller than 3 mm may not be detected during ultrasonographic examination, as they will not produce acoustic shadows.[15] Magnetic resonance sialography is a newer diagnostic modality that allows for visualization of the ducts without any radiation or dye injection, but it is limited by its cost and feasibility in claustrophobic patients.[16] Sialoendoscopy is a new, minimally invasive technique developed for direct visualization of intra-ductal stones.[1,9] In this report, as the lesion was observed clearly on occlusal and panoramic radiographs, no further investigations were performed for diagnosis.

Giant calculi may cause various complications. They may perforate the floor of the mouth by ulcerating the duct or may result in a fistula by causing a suppurative infection.[9] Perforation of the floor of the mouth is more likely to occur when calculus is present in anterior part of duct.[13] In our case, calculus had extruded in the floor of mouth causing sialo-oral fistula. Similar finding was reported by El Gehani, Akimoto, Patil, Huber, Shetty; whereas Paul and Chauhan reported sialo-oral as well as sialo- cutaneous fistula caused by sialolith.[12,13.15,17,18,19] Also, long term obstruction in the absence of infection can lead to atrophy of the gland with resultant lack of secretory function and ultimately fibrosis.[6] However, after elimination of the obstruction, the apparent resiliency of the submandibular gland results in no adverse symptoms.[20] Consuegra reported two cases of giant sialolithiasis within the Wharton's duct causing unilateral absence of submandibular gland due to complete acinar atrophy. The submandibular glands were replaced by fat in the computed tomography images.[21] Association of sialolith with systemic diseases is questionable. Lustmann in a study on 245 patients with sialolithiasis found that 10.7% patients had associated nephrolithiasis.[4] Gout is the only systemic diseases known to predispose to salivary stone formation, although in gout the stones are predominantly made up of uric acid.[6] The differential diagnosis of sialolith includes calcified lymph node, embedded tooth, foreign body, phlebolith, and myositis ossificans.[14]

The treatment objective for giant sialoliths, as for the standard-sized stones, is restoration of normal salivary secretion.[11,12] Treatment approach for sialolith depends on its size and location.[6,11,16] Removal of stones through an intraoral approach is recommended whenever stones can be palpated intra-orally.[2,5,10] If the stone is small and sufficiently forward it can be milked and manipulated through the duct orifice.[6,11] However, if the calculus is of a medium or large size, like the giant salivary gland calculi, a salivary colic may occur and the sialolith cannot be expelled spontaneously.[1] Almost half of the submandibular calculi lie in the distal third of the duct and are amenable to simple surgical release through an incision in the floor of the mouth.[6]

Newer minimally invasive treatment modalities such as shock-wave lithotripsy, sialoendoscopy, interventional radiology are effective alternatives to conventional surgical excision for smaller sialoliths (<7 mm).[22] Sialolithotripsy is a non-invasive method of fragmenting salivary stones into smaller portions in order to favor their possible flushing out from the salivary duct system spontaneously or after salivation induced by citric acid or other sialogogues. The shock-waves may be generated extra-corporeally using piezoelectric and electromagnetic techniques or intra-corporeally using electro-hydraulic, pneumatic or laser endoscopic devices.[22] In intra-corporeal lithotripsy, the shock-waves reach the stone surface through a lithotripsy probe placed inside the salivary duct system under endoscopic guidance.

In the early 1990s, salivary duct endoscopy or sialendoscopy emerged.[20] For sialoendoscopy flexible, rigid, and semi-rigid endoscopes have been used with outer diameters ranging from 0.8 to 2.7 mm. All of these sialoendoscopes have a working channel that allows the introduction of graspers, microforceps, Dormia baskets or balloon catheters for the removal of single or multiple stones.[22] The sialendoscopes also have an optic channel that transmits the image using fiberoptic channels and an irrigation channel allows a continuous irrigation to be performed to maintain duct patency for endoscopic

visualization of the salivary duct lumen. However, the major limitation of sialoendoscopy alone is the difficulty in removing stones with a diameter > 4 mm.[22] Intermediate size stones between 5-7 mm may need further fragmentation either using a Holmium laser or lithotripsy prior to endoscopic extraction. Fluoroscopically guided stone retrieval with Dormia baskets and sialolithectomy with carbon dioxide laser are other special methods for removal of the calculus.[7] Retrieval of stones by baskets is usually done for stones less than 5mm.[8] The CO_2 laser is set up in continuous mode at 4-6W with a focusing spot. It has a low incidence of complications and can be readily managed on an out-patient basis.[20]

However, for giant sialoliths, transoral sialolithotomy with sialodochoplasty or sialadenectomy remains the mainstay of management.[2,6,23] Akimoto reported an interesting and only case of a giant sialolith in which the calculus was not extracted surgically, but patient himself removed the calculus. The patient could easily pull it out because it was long, extremely narrow and its tip had perforated floor of the mouth.[13]

In some cases, excision of the entire gland is required. Submandibular gland removal is indicated if- 1) the gland has been damaged by recurrent infection and fibrosis, 2) there is a stone of substantial mass within the gland itself that is not surgically accessible intraorally, 3) there are small stones present in the vertical portion of Wharton's duct from the comma area to the hilum, 4) the size of an intra-glandular stone reaches 12 mm or more as the success of lithotripsy may be less than 20% in such cases.[2,6,24]

Lustmann[4] in a survey on 245 patients with sialolithiasis for 20 years (1968-1988) found a recurrence rate of 8.9% for a follow-up period of 10 years, which is higher than reported in literature. A diet rich in proteins and liquids including acid food and drinks is advisable in order to prevent recurrence.[1]

Table 1: Summary of case reports of large salivary calculus in chronological order

First Author	Year	Age	Sex	Lesion size (mm)	Weight (gm)	Duration	Side	Symptoms
Hubar [17]	1990	65	M	52	17.5	NR	Left	Intermittent swelling and pain after eating
Paul and Chauhan [19]	1995	45	M	45	4.23	8 years	Right	Discharging sinus and painful swelling
Siddiqui [6]	2002	52	F	30	NR	Unaware	Right	Asymptomatic
Goncalves [14]	2002	52	F	22	NR	6 months	Left	Severe pain and swelling on eating
Bodner [5]	2002	45	M	30x20	NR	NR	NR	NR
		46	M	32x16	NR	NR	Left	NR
		61	M	16x13	NR	NR	NR	NR
		25	M	32x20	NR	NR	NR	NR
		50	M	20x12	NR	NR	NR	NR
Akimoto [13]	2004	70	M	45x7	1.34	5 years	Right	Intermittent swelling and pain after eating
Chan [16]	2006	27	M	35	NR	NR	Left	painful swelling
Ledesma-Montes [3]	2007	34	M	36	12	12 years	Right	painful swelling
Alkurt [9]	2009	45	M	28x8	NR	1 week	Right	Asymptomatic
		65	M	31x10	NR	3 years	Right	Dry mouth and painless swelling during mealtimes
Rai [2]	2009	60	M	72	45.8	6 months	Left	Severe pain and swelling
Patil S [15]	2009	50	M	38	NR	1 month	Left	Swelling
El Gehani [12]	2010	41	M	35	NR	8 years	Left	Pain and swelling
		32	F	25	NR	4-5 years	Left	Pain and swelling
Consuegra [21]	2010	70	M	16x20	NR	2 days	Left	Pain and swelling
Boffano P [24]	2010	48	M	22	NR	NR	Right	Pain and swelling during meals
Abdeen [8]	2010	53	M	33x11	NR	NR	Left	Swelling during meals
Shetty [18]	2010	50	M	27x8	NR	1 month	Left	Pain and swelling
Silva-Junior [23]	2010	58	M	35	6.45	NR	Left	Swelling
Cottrell [10]	2011	75	M	30x20	NR	unaware	Left	Asymptomatic
Oteri [1]	2011	40	F	20x6	NR	3 months	Right	Pain and swelling
		51	F	15	NR	NR	Left	Pain and swelling
Omal [7]	2011	62	F	23	NR	22 years	Left	Swelling, difficulty in speaking and swallowing
Leite [11]	2011	54	F	35x7	NR	unaware	Right	Swelling
Iqbal [20]	2012	55	M	35x30	NR	unaware	Left	Asymptomatic
Current case	2011	65	M	22x14	-	8 months	Right	Pain and swelling

NR-Not reported

Conclusion:

The diagnosis of giant sialolith in the Wharton's duct is further simplified when it presents as a hard mass in the floor of mouth causing sialo-oral fistula. Transoral sialolithotomy remains mainstay of the treatment for giant sialolith in the duct of submandibular gland. Also, patients should be followed up regularly as recurrence has been reported in the literature.

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Case Report

Carcinosarcoma: A Rare Malignant Tumor of the Uterus

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Abstract

Carcinosarcoma is a rare malignant tumor of the uterus with a poor prognosis. Here we presented a case of uterine carcinosarcoma in a 65 years old woman suffering from per-vaginal excessive discharge for 3 months, severe lower abdominal pain and generalized weakness for 15 days. Upon histopathology of the uterus following total abdominal hysterectomy with bilateral salpingo-ophorectomy, the tumor was diagnosed as carcinosarcoma. The histopathological examination revealed that the malignant tumor consisted of both malignant epithelial & mesenchymal element. The malignant epithelial element was of endometrioid type, malignant mesenchymal element was chondrosarcoma. The tumor was limited to the uterine corpus. The depth of myometrial invasion of the tumor was <1/2. Furthermore, there was no evidence of tumor in the ovaries or fallopian tubes (pT1A, pN-0, pMx; stage 1A).

After histopathological report, patient was referred to National Institute of Cancer Research & Hospital for chemotherapy. Now patient is getting chemotherapy.

Keywords: uterus, carcinosarcoma, total abdominal hysterectomy, salpingo-oophorectomy, chemotherapy.

Introduction:

Carcinosarcomas of the uterus (malignant mixed Müllerian tumors) are a rare occurrence, accounting for only 25% of all uterine malignancies.[1-4] Carcinosarcomas are, however, highly aggressive and are composed of epithelial and mesenchymal elements.[5] Carcinosarcomas are classified into two histological subtypes based on their sarcomatous component, namely homologous or heterologous;[6] those of the homologous type tend to be fibrosarcomas, endometrial stromal tumors or leiomyosarcomas, while those of the heterologous type consist of a sarcomatous component made up of tissues that are non-native to the uterus and include rhabdomyosarcomas, chondrosarcomas, osteosarcomas and liposarcomas. In both types, the carcinomatous component is mainly composed of endometrioid, serous or clear-cell type adenocarcinoma. Homologous and heterologous carcinosarcomas arise with approximately equal frequencies.[7] We here presented a case of a heterologous carcinosarcoma of the uterus in a 65 years-old woman.

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Case Presentation:

A 65 years-old woman, para 3 & had history of one abortion, was admitted in the International Medical College & Hospital for evaluation of per-vaginal excessive discharge for 3 months, severe lower abdominal pain for 15 days with generalized weakness. The patient was found cachectic with hemoglobin (Hb) concentration 10.1 g/dl. On abdominal examination abdomen was soft but severely tender, uterus was about 18 weeks pregnant uterus size. Per-speculum examination revealed- cervix was flushed and huge amount of clear watery discharge was coming through the os; bimanually uterus was enlarged in size. Ultrasonography of lower abdomen revealed- the uterus was enlarged. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. The enlarged uterus approached 18 weeks gravid uterus size. On cut section of the uterus huge growth filled the uterine cavity. The histopathological examination revealed that the malignant tumor consisted of both malignant epithelial & mesenchymal element. The malignant epithelial element was of endometrioid type, malignant mesenchymal element was chondrosarcoma. The tumor was diagnosed as a carcinosarcoma. The tumor was limited to the uterine corpus. The depth of myometrial invasion of the tumor was <1/2. Furthermore, there was no evidence of tumor in the ovaries or fallopian tubes (pT1A, pN-0, pMx; stage 1A according to the 2008 International Federation of Gynecology and Obstetrics staging criteria). After the histopathological report, patient was referred to National Institute of Cancer Research & Hospital for chemotherapy. Now patient is getting chemotherapy.

Figure 1: Shows macroscopic appearance of the resected uterus, fallopian tubes and ovaries. Macroscopic appearance of the uterine cavity.

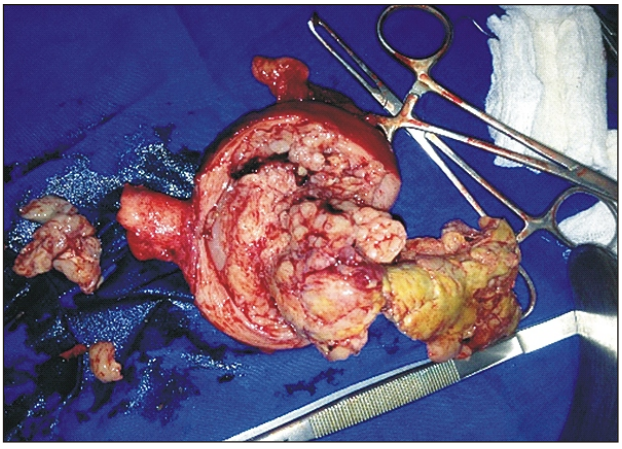
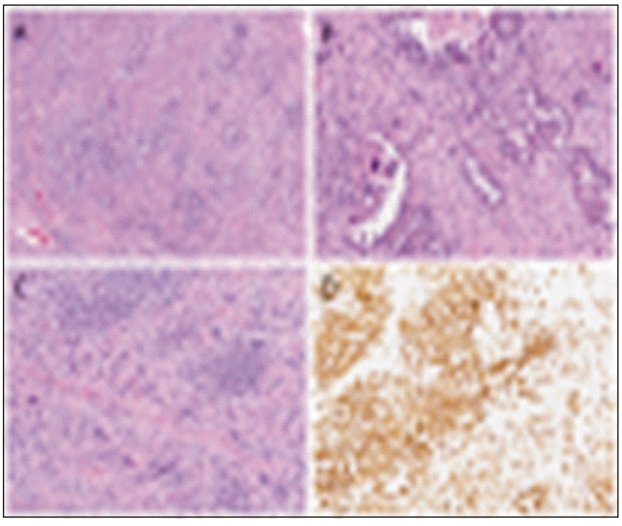


Figure 2: Shows histological findings of the uterine carcinosarcoma.



Discussion:

Carcinosarcomas occur mainly in women 15-17 years after menopause,[4,8,9] with the most common symptoms being genital bleeding and uterine enlargement.[10] Abdominal pain also occurs in a proportion of the cases.[11] In the present case, the carcinosarcoma was diagnosed 20 years after menopause, with the patient complaining of vaginal excessive discharge for 3 months & severe lower abdominal pain for 15 days prior to the consultation. The Hb level in the peripheral blood had decreased to 10.1 g/dl, ESR was 71 mm (1st hour). These symptoms and findings were atypical of uterine carcinosarcoma, as the patient did not complaint of vaginal bleeding. Carcinosarcomas are characterized by an aggressive clinical course and an extremely poor prognosis. It has been previously reported that 70-90% of tumor-related deaths occurred within 18 months after diagnosis.[2,12] However, a recent study reported that the prognosis of uterine carcinosacomas had improved, with an overall median survival of 39 months.[13] Uterine carcinosarcomas are mixed epithelial and stromal tumors, with both components being malignant.[6,11] Homologous carcinosarcomas have a sarcomatous component of fibrosarcoma, endometrial stromal sarcoma and/or leiomyosarcoma. By contrast, the heterologous type includes sarcomatous components that are made up of tissues non-native to the uterus. The carcinomatous component is mainly composed of endometrioid, serous or clear-cell type adenocarcinoma. In our case, there was a dominant epithelial was endometrioid type. However, the mesenchymal element was sarcoma. These results suggest that the carcinosarcoma in our case was a homologous carcinosarcoma. The patient is closely monitored.

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College News

Obituary:

- Prof. Dr. M. I. Chowdhury, 1st Principal of BMC, Founder Member and Academic Director of BMSRI passed away on 02.08.2016 due to old age complications.
- Prof. Dr. M.A. Zaman, Former Principal of BMC and renowned Cardiologist passed away on 19.07.2016 due to cancer.
- Prof. Dr. Md. Shamsuzzoha, Former Professor and Head of the Dept. of Pharmacology, BMC passed away on 13.08.2016 due to old age complications.

Seminar in BMC:

- Celebration of 13th years "World Cancer Day" was held on 4th February 2016, organized by Oncology department of BMC.
- Celebration of "World Hearing Day 2016" was held on 3rd March 2016, organized by ENT department of BMC.
- CME on "Teaching, Learning and Evaluation; Record Keeping; Quality Assurance" was organized by MEU, BMC on 4th April, 2016 for the teachers of clinical depts. of BMC. Resource Persons of the event were Prof. Dr. Sharmeen Yasmeen, Head of the dept. of Community Medicine, BMC and Ms. Nahid Shahana, Associate Professor of Anatomy, BMC and Medical Educationist. The program was coordinated by Prof. M L Kabir, Head of MEU and the dept. of Medicine, BMC.
- A Seminar on "Basic Aspects of Anesthesia" was held on 25th April 2016. Speakers were Prof. Kamal Ibrahim, Head of the dept. Prof. Md. Nurul Amin, Associate Professor Dr. Md. Rafiqul Hasan of Anesthesiology, BMC.
- A Seminar on "Urology in Bangladesh Medical College" was held on 19th May 2016, organized by dept. of Urology, BMC. Speakers were from dept. of Urology, BMC.

Participation in the International Conferences/Seminars/Workshop/ Congress/ Meetings:

- Prof. Dr. M. Touhidul Haque, Professor and Head of the Dept. of Cardiology, BMC attended the Africa PCR Congress held in South Africa from 10-12 March, 2016.
- Prof. Md. Ashraful Islam, Professor and Head of the Dept. of ENT, BMC attended the workshop on Cochlear Implantation held on 5th March 2016 at Bhopal, Modhya Pradesh, India.
- Prof. Dr. A.H.M. Shamsul Alam, Professor and Head of the Dept. of Surgery, BMC attended the Observership Program on Laparoscopic Hernioplasty held in Kolkata, India from 11-13 March,2016.
- Dr. Kamruzzaman, Associate Professor, Dept. of Orthopedics, BMC attended the 19th Asia Pacific Orthopedic Association Congress held from 29 March to 01 April 2016, in Melbourne, Australia.
- Prof. Dr. A K M. Akhtar Murshed, Professor & Head of the Dept. of Orthopedics, BMC attended the 19th Asia Pacific Orthopedic Association Congress held from 29 March to 01 April, 2016 in Melbourne, Australia.
- Dr. A. B. M Mahbubur Rahman, Assistant Professor, Dept. of Surgery, BMC attended the Advanced Laparoscopic Gastrointestinal Surgery Workshop held from 12-13 April 2016, in UK.
- Dr. Md. Tarek Alam, Associate Professor, Dept. of Medicine, BMC attended the CHEST World Congress 2016 held at Shanghai, China from 15-17 April 2016.
- Prof. Dr. M. Touhidul Haque, Professor and Head of the Dept. of Cardiology, BMC attended the 21st Cardiovascular Summit-TCTAP 2016 held from 26-29 April, 2016 in Seoul, South Korea.
- Dr. Md. Tarek Alam, Associate Professor, Dept. of Medicine, BMC, attended the Non-Invasive Ventilation (NIV) Workshop held at Kolkata, India on 29th May 2016.
- Dr. Akhil Chandra Biswas, Associate Professor, Dept. of ENT, BMC, attended the Lateral skull Base Workshop held at Mumbai, India from 3-5 June 2016.
- Prof. Dr. M. Fakhrul Islam, Professor & Head, Dept. of Urology, BMC attended the 27th World Congress on Video Urology & Advances in Clinical Urology held in Thailand from 9-11 June, 2016.
- Prof. Md. Zahid Hassan Bhuiyan, Professor, Dept. of Urology, BMC attended the 14th Urological Association of Asia Congress 2016 held in Singapore from 20-24 July 2016.

New Promotions and Appointments in BMC:

- Prof. AHM Shamsul Alam, Vice-Principal of BMC
- Prof. Md. Rezaur Rahman Talukder, Professor of Surgery
- Prof. Dr. Riaz Uddin Ahmed, Professor of Dermatology
- Prof. Zafor Md. Masud, Professor of Oncology
- Dr. Sultant Jebunnaher, Associate Professor of Gynae & Obstetrics
- Dr. Syed Khalid Hasan, Associate Professor of Surgery
- Dr. Kazi Salma Binte Faruky, Associate Professor of Physiology

- Dr. Farzana Yasmin, Associate Professor of Physiology
- Dr. Mushtaque Ahmed Rana, Associate Professor of Gastroenterology
- Dr. Md. Shahriar Munit, Assistant Professor of Anesthesiology
- Dr. Tanzin Ara Begum, Assistant Professor of Physiology
- Dr. Jannatun Naiem, Assistant Professor of Forensic Medicine.

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