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Bangladesh Medical College Journal

INFORMATION FOR CONTRIBUTORS

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Editorial

COVID- 19 in children- No scope of contentment

Arefin K E

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus SARS-CoV-2.[1] WHO declared this disease as pandemic on 11th March 2020.[2] Across the world, due to the spread of COVID-19, total 11,49,91089 people are infected, among them 25,50192 people died as of February 2021.[3] So far, data suggests that children under the age of 18 years represent about 8.5 % of reported cases, with relatively few deaths compared to other age groups.[4] In United States children represented 12.9 % of all cases.[5] Children are not the face of this pandemic. But children's lives are nonetheless being changed in profound ways. All children, of all ages, and in all countries, are being affected, in particular by the socio-economic impacts and, in some cases, by mitigation measures that may inadvertently do more harm than good. This is a universal crisis and, for some children, the impact will be lifelong.[6]

Infections and transmission among children

The incubation period of SARS-CoV-2 appears to be about the same for children as in adults, at 2-14 days with an average of 6 days.[7] Recent evidence suggests that compared to adults, children likely have similar viral loads in their nasopharynx,[8] similar secondary infections rates, and can spread the virus to others.[9,10] A recent systematic review estimated that 16% of children with SARS-CoV-2 infection are asymptomatic,[11] but evidence suggests that as many as half of paediatric infections may be asymptomatic.[12] There are several potential reasons that children have relatively milder illness. In addition to a fewer outdoor activity, children have a number of characteristics that protect them against SARS-CoV-2 infection. They have a healthier respiratory machinery alongside a different expression of receptors in the lower respiratory tract. Also, children have a less vigorous adaptive system beside a preliminary potent innate response, the constitutional higher level of lymphocyte counts, the trained immunity with cross-reactive neutralizing antibodies, the lack effects of aging, and the interaction between the immune system and respiratory tract might be protecting children against SARS-CoV-2 infection.[13]

The signs and symptoms of COVID-19 in children are similar to those of other infections and noninfectious processes, including influenza, streptococcal pharyngitis, and allergic rhinitis. The lack of specificity of signs or symptoms and the significant proportion of asymptomatic infections make symptom-based screening for identification of SARS-CoV-2 in children particularly challenging.[12]

The most common symptoms of COVID-19 in children are fever or chills, and cough, others are nasal congestion or runny nose, new loss of taste or smell, sore throat, shortness of breath or difficulty breathing, diarrhea, Nausea or vomiting, stomachache, tiredness, headache, muscle or body aches. Poor appetite or poor feeding, especially in babies under 1-year-old.[14]

A study was done to see the clinical and demographic features Covid 19 among Bangladeshi children. Among 236 children, the male-female ratio was 1.7:1. Mostly was 1 year to 2 years (21.13%) and 5 years to 10 years (21.13%). About twenty percent was asymptomatic, 80.28% was symptomatic, coinfections were 29.58%, and co-morbidities were 8.45%. The duration of RT-PCR was positive up to two, four, six, and more than six weeks 49.30%, 30.99%, 16.90%, and 2.82% respectively. Fever (80.28%), cough (45.07%), sore throat (33.80%), runny nose (29.58%), anorexia (28.17%), convulsion (25.35%), respiratory distress & acute diarrhea (15.50%), weakness (14.08%), paralytic ileus, rash and acute abdomen (4.23%).[15]

Severity of illness in children

Babies under 1-year-old and children with certain underlying conditions may be more likely to have severe illness from COVID-19.[14] Regardless of age, with the following underlying medical conditions might also be at increased risk of severe illness compared to other children. Those are asthma or chronic lung disease, diabetes, genetic, neurologic, or metabolic conditions, sickle cell disease, heart disease since birth, obesity, immunosuppression, children with multiple chronic conditions that affect many parts of the body.[14]

Children with severe COVID-19 may develop respiratory failure, myocarditis, shock, acute renal failure, coagulopathy, intussusception or diabetic ketoacidosis and multi-organ failure.[16,17]

Children infected with SARS-CoV-2 are also at risk for developing Multisystem Inflammatory Syndrome in Children (MIS-C).[18] MIS-C is diagnosed when an individual aged <21 years presenting with fever(Fever >38.0°C for≥24 hours), laboratory evidence of inflammation(one or more of the following: an elevated CRP, ESR, fibrinogen, procalcitonin, d-dimer, ferritin, LDH, IL-6, neutrophils, reduced lymphocytes and low albumin) and evidence of clinically severe illness requiring hospitalization, with multisystem (>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND No alternative plausible diagnoses; AND Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms.[19]

Management and prevention

Treatment of COVID-19 remains largely supportive and includes prevention and management of complications.[20] Emphasis should be given more on preventive measures. Make sure child washes their hands often with soap and water for at least 20 seconds. If soap and water are not readily available, make sure that the child uses a hand sanitizer that contains at least 60% alcohol. Practice cough and sneeze etiquette by covering nose and mouth with a tissue when sneezing or coughing, throwing the tissue in the closest garbage can.[21] The CDC and AAP recommend that individuals ≥2 years of age wear a mask when they are in public settings where social distancing may be difficult to achieve while WHO and UNICEF advise against masks for children age 5 years and younger.[22] Correct and consistent use of masks may be challenging for some children. Keep your child at least 6 feet away from others who don't live with them and those who are sick. Limit in-person playtime and connect virtually with other children. While your child may be spending time with other people as they return to childcare or school settings, you should limit your child's interactions with additional children and adults outside of childcare or school to decrease risk. Clean and disinfect frequently touched surfaces daily which includes tables, doorknobs, light switches, remotes, handles, desks, toilets, and sinks. Staying home is the best way to protect your child and others from getting sick.[21] Recently vaccine trial including children from 6 years to 18 years has been started. Result of trial is expected to come in mid-2021.[23]

In the upcoming days vaccination coverage among the adult and elderly people will increase and it might happen that the disease would be more prevalent among the children and can be transmitted to others. Regardless of vulnerabilty and gradient of severity of COVID-19, children should be brought under coverage of vaccination in all countries. Now scientific trials have to be undertaken to determine the lower limit of age for vaccination with assurance of safety and efficacy.

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Original Article

Factors associated with endometriosis among women with infertility in a tertiary care hospital in Dhaka city

Yasmin N[a], Khatun M[b], Chowdhury T A[c]

Abstract

Background: Endometriosis is a cause of acquired dysmenorrhoea, dyspareunia, intermenstrual bleeding and menorrhagia, infertility, and pelvic pain of varying severity and location. Endometriosis is a chronic and recurrent disease characterized by the presence and proliferation of functional endometrial glands and stroma outside the uterine cavity. Around 10% of women of reproductive age are affected by endometriosis, suffer from chronic pelvic pain and contribute to infertility.

Objective: To find out the factors associated with endometriosis among a group of women presenting for infertility treatment in BIRDEM General Hospital.

Methods: It was an analytical cross sectional study. A total of 67 patients with infertility between January 2015 to December 2015 were included in this study. Fertile women with endometriosis were excluded. Endometriosis was diagnosed by laparoscopy. Study population were categorized in two groups which were Group A - infertile patients with endometriosis (32 pts) and Group B - infertile women without endometriosis (35 pts). The risk factors of endometriosis were assessed in both group. Data were presented in tabulated form and analyzed by computer based software SPSS windows 20.0 version. Chi square tests, regression analyses and Odds Ratio were used to find the significance. p values less than 0.05 were considered as statistically significant.

Results: Among 67 respondents, the highest (34.4%) incidence of endometriosis was found in age group 26 – 30 years among the infertile patients. Risk factors of endometriosis are early menarche (46.9% vs 25.7%, $p<0.05$), shorter menstrual cycle length (50.0% vs 48.6%, $p<0.05$), prolonged duration of flow (62.5% vs 40.0%, $p<0.05$) showed significant difference between two groups. Whereas association with body mass index was not statistically significant. Significant relationship between family history and endometriosis was found (18.8% vs 0.0%, $p<0.05$).

Conclusion: From this study it is concluded that there is a consistent risk for endometriosis in patients with earlier menarche, shorter menstrual cycles, prolonged menstrual periods and family history. Endometriosis may have more risk factors that have not been studied.

Keywords: Endometriosis, Dysmenorrhoea, Menarche, Infertility.

Introduction:

Endometriosis is a medical condition in which tissue similar to normal endometrium in structure and function is found in sites outside the uterine cavity and myometrium.[1] About 25- 50 % of infertile women have endometriosis and 30- 50% of women with endometriosis are infertile.[2]

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Endometriosis is a cause of acquired dysmenorrhoea, dyspareunia, intermenstrual bleeding and menorrhagia, infertility, and pelvic pain of varying severity and location. Endometriosis is a chronic and recurrent disease characterized by the presence and proliferation of functional endometrial glands and stroma outside the uterine cavity. Around 10% of women of reproductive age are affected by endometriosis, suffer from chronic pelvic pain and contribute to infertility.[2] Endometriosis not only lowers the quality of life of the affected women; it jeopardizes her conjugal life, hamper wellbeing of other members of the family and ultimately the society is also affected. The original three theories of histogenesis of endometriosis fail to explain all the criteria of histogenesis. The recent molecular explanation for Sampson's theory states that aberrant production of aromatase converts the precursor of steroids to estrogen in endometriotic tissue that plays a key role in the pathophysiology of endometriosis.[3]

Endometriosis run in families. Endometriosis is a complex disorder with a considerable genetic component (heritability = 51%). The mode of inheritance is probably polygenic and multi factorial. Chromosome no 10 q26, 7p13-15, and 20p13 is thought to carry the involved gene.[4] There is some defect in immune response to tissue injury in women with endometriosis that fail to remove refluxed menstrual debris from the extra uterine site. The scavenger activity of peritoneal macrophages is reduced with abnormal T-cell-mediated cytotoxicity, natural-killer-cell activity, B-cell functions and complement deposition. They suffer from different autoimmune diseases, allergic reactions and certain toxins. Growth factors (such as TGF-B, IGF-1, HGF and VEGF) and Cytokines (IL-1, IL-4, IL-6, IL-8, IL-10 and TNF A) may also have some role[5]. Patients with endometriosis may have elevated CA-125.[6]

Infertility is defined as the inability of a couple to conceive within 1 year. Existing definitions of infertility lack uniformity. A couple that has tried unsuccessfully to have a child after a certain period of time (often a short period) is said to be subfertile, meaning less fertile than a typical couple. Both infertility and subfertility are defined as the inability to conceive after a certain period of time (the length of which vary).

Endometriosis is most commonly characterized by numerous painful symptoms and is also associated with numerous other symptoms that can lower the quality of life. The most common symptoms include dysmenorrhea, dyspareunia, chronic pelvic pain which is defined as more than 6 months of non-cyclic pain, abnormal uterine bleeding, and reduced fertility. Less common symptoms include chronic fatigue, painful bowel movements, painful urination or pain in areas other than the pelvis, such as back, legs or shoulders. There are also numerous psychological symptoms, such as depression, that can be associated with endometriosis due to the heavy emotional burden of the disease.

The more Endometriosis is studied, the more it is found. Every new report claims a higher and higher incidence. It is now becoming apparent that most, if not all women probably develop Endometriosis at some time in their life but only a small percentage exhibit the clinical disease which we call Endometriosis with its associated pain or infertility. Supporting this theory is a recent report in which very careful laparoscopic examination of women with no pelvic symptoms found that 50% had peritoneal implants consistent with Endometriosis. It has therefore been suggested that at least in some women, Endometriosis is not truly a disease but perhaps an exaggeration of a normal process in which the endometrial cells shed into the abdominal cavity implant but fail to proliferate or create any significant pain, scar tissue, or interfere with fertility.[7]

Apart from causing personal and a variety of complaints affecting the young age group endometriosis adds a huge economic burden being diagnosed by a surgical procedure and with complications like infertility. It is a source of psychological stress not only on the women with a poor health related quality of life but also on the male partner.[8]

Endometriosis is found in women who have a first degree relative (mother, sister, daughter) with the disease. The heredity aspect of endometriosis is found by studies conducted in twins. These demonstrated that incidence of endometriosis in monozygotic twins was twice that in dizygotic twins. In addition, it has been shown that the severity of endometriosis is higher among patients with a positive family history. Women with first degree relative with endometriosis has a risk of developing endometriosis 4-7 times higher than that of the general population.[9]

There is a lack of knowledge on the natural progression of the disease in women since the severity measurement will require repeated invasive surgery. Currently there is no curative treatment for endometriosis and clinical management of symptoms such as pain is through medical and or surgical measures.[10]

Endometriosis is often called a “career woman's disease”. The longer a woman goes without having a baby the more likely she is to develop endometriosis.[7] Women who are having babies for the first time after the age of 30 are at an increased risk of endometriosis.[11] Women who have had children are somewhat less likely to have endometriosis. Pregnancy may disrupt the course of endometriosis, although not cure it in all patients.[12]

Endometriosis is found more in primary subfertility patients than secondary subfertility.[13] More recent data indicate that the incidence of endometriosis has not increased in the last thirty years and remains 2.37-2.49/1000/Y, which equates to an approximate prevalence of 6–8%.[14] Endometriotic implants can be microscopic, penetrate deeply into tissue or have a subtle appearance making them almost impossible to observe with the naked eye.[15] The aim of this study was to find out the risk factors associated with endometriosis among women with infertility.

Material and Methods:

This cross sectional analytical study was done on 67 infertile patients from CARe hospital and BIRDEM general hospital undergoing laparoscopy and laparotomy in department of Obstetrics and Gynaecology, BIRDEM general hospital, Dhaka from the period of January 2015 to December 2015. The research protocol was approved by the Ethical review committee of DAB (Diabetic Association of Bangladesh). The infertile patients were divided into two groups**.** Group A- infertile women with endometriosis (32 in number). Group B- infertile women without endometriosis (35 in number). Purposive Sampling was done to select the study population based on inclusion criteria who underwent diagnostic laparoscopy and laparotomy for the treatment of infertility at BIRDEM Hospital, Dhaka. The variables which were studied in the

present study were menstrual characteristics, BMI, family history, age and laparoscopic findings and were documented on the pre designed data collection sheet with informed written consent. Data were processed by computer and analyzed by using SPSS (Statistical Package for Social Science) version 20.0. The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Chi-Square test was used to analyze the categorical variables, shown with cross tabulation. p values <0.05 was considered as statistically significant.

Results:

It was an analytical cross sectional study, conducted in the department of Obstetrics and Gynecology of Center for Assisted Reproduction (CARe) and in BIRDEM) during January 2015 to December 2015 to find out the factors associated with endometriosis among a group of women presenting for infertility treatment.

Table 1: Age distribution of the study subjects (n=67)

Age group	Group-A (Infertile women with endometriosis)		Group-B (Infertile women without endometriosis)		p value
	Frequency	Percent	Frequency	Percent	
≤25	8	25.0	9	25.7	0.156
26-30	11	34.4	17	48.6	
31-35	9	28.1	9	25.7	
≥36	4	12.5	0	0.0	
Mean±SD	29.94±4.70		28.31±3.34		
Range (min-max)	24-41 years		24-35 years		

Table 1 shows age range of women with endometriosis (Group A) was 24-41 years. Most (34.4%) were in the age group 26-30 years. In women without endometriosis (Group B), most women (48.6%) were in the age group 26-30 years. There was found no statistical significance.

Table 2: Distribution of menstrual characteristics in relation with risk for patients of endometriosis (n=67)

Variable	Group A (n=32)	Group B (n=35)	OR	p value
Age at Menarche (years)				
≤ 11	4 (12.5%)	10 (28.6%)	1	
12-13	15 (46.9%)	9 (25.7%)	4.16	0.049*
>13	13 (40.6%)	16 (45.7%)	2.03	0.311
Menstrual Cycle Length (days)				
≤ 27	11 (31.2%)	2(5.7%)	13.33	0.016*
28-29	16 (50.0%)	17 (48.6%)	2.51	0.004*
≥ 30	6 (18.8%)	16 (45.7%)	1	0.120
Duration of Flow (days)				
≤ 4	5 (15.6%)	19 (54.3%)	1	0.066
5-6	20 (52.5%)	14 (40.0%)	5.42	0.006*
≥7	7 (21.9%)	2 (5.7%)	13.30	0.008*

Table 2 shows early menarche in the age group 12-13 years was significantly associated with endometriosis ($p<0.05$). Shorter menstrual cycle, ≤ 27days and 28-29 days were significantly associated with endometriosis ($p<0.05$). Duration of flow was significantly associated in endometriotic women who had 5-6 and ≥7days of flow ($p<0.05$).

Table 3: Relationship of BMI and endometriosis(n=67)

BMI ($kg/m^{2)}$	Group A (n=32)	Group B (n=35)	p value
Normal (18.50-24.99)	21 (65.6%)	14 (41.2%)	0.081
Over weight (25.0-29.99)	8 (35.0%)	18 (52.9%)	
Obese (≥ 30.0)	3(9.4%)	2 (5.9%)	

p Value obtained from Fisher's Exact test

Table 3 shows in Group A, 65.5% women with normal BMI had endometriosis as compared to overweight (35%) and obese women (9.4%). There was no significant relationship between BMI and endometriosis.

Table 4: Relationship between family history and endometriosis

Family history of endometrosis	Group A (n=32)	Group B (n=35)	p value
Yes	6 (18.8%)	0 (0.00%)	0.009*
No	26 (81.2%)	35 (100.0%)	

p value obtained by Fisher's Exact test

Table 4 shows there was a significant relationship between family history and endometriosis. The women who had previous family history of endometriosis had a higher probability of developing endometriosis. About 18.8% of patients with endometriosis had family history of endometriosis compared to 100% women without endometriosis who did not have family history of endometriosis.

Discussion:

This cross sectional analytical study was carried out with an aim to find out the risk factors associated with endometriosis in infertile woman. The study was carried out in the department of Obstetrics and Gynaecology of CARe and BIRDEM during January 2015 to December 2015. A total of 67 patients with infertility were selected out of which 32 were diagnosed as endometriotic infertile woman through laparoscopy and laparotomy and 35 were without endometriosis. In this present study findings were discussed and compared with previously published relevant studies.

It was observed that the highest number (34.4%) of women with endometriosis belonged to the age group of 26-30 years, the mean age was found 29.94± 4.70 years and varied from 24 to 41 years (Table-1). In a study, age at diagnosis ranged from 17 to 68 years, with a mean of 35 years±9.26; median of age was 34.6 years. For histologically verified cases, the mean age was 38±9.31 years; median, 37. 5 years. The proportion of cases diagnosed after age 40 years was 27.4% (21.8% of histologically verified cases).[16] Whereas, 48.60% of women with infertility but without endometriosis were in the same age group. Macer ML and Taylor HS[17] found the growth of endometriotic implants is dependent on ovarian produced steroids, it is a disease that most severely affects women of 25–35 years of age. Ashrafi M et al[1], found the mean age of women with endometriosis to be 31.4± 5.2 years. Brosens I and Benagiano G[18] in their study found majority of patients were between 20 and 45 years of age. Mamdouh HM et al.[19] in their study found age 27.9 ± 6.8 years. Khuwaja UB et al[8] in their study found mean age of patients was 29 ± 5.3 years, majority of patients fell between ages 25 and 33 years. Molloy D[12] in his study found that women in their twenties and in their thirties are more likely to develop endometriosis. The difference in age range may be due to geographical variations, racial, ethnic differences, genetic causes and different lifestyles in their study patients.

This study showed that there was a trend for women with endometriosis to have an earlier menarche compared to without endometriosis (Table-2). On further analysis it was found to be statistically significant (p=0.049, OR 4.16) in the age range of 12 – 13 years (46.9%). Mamdouh HM et al.[19] found that there was a significant association in women experiencing early menarche (defined as ≤ 11years) (p<0.05). Matalliotakis IM et al.[20], found that increased association of endometriosis with early menarche ($p <$ 0.024). Sinaii N et al.[21] found in their study 85% of women had their menarche between the age of 11 and 14 years. Variation of menarche may be due to racial and lifestyle differences.

In this series it was observed that there was a significant association between endometriosis and shorter menstrual cycle (p = 0.016; OR = 13). Mamdouh HM et al. [19] found similar association in their study between having menstrual cycle of 27 days or less (p<0.001) and endometriosis. Women with cycle length ≤ 27 days had 6 times and women with cycle of 28 days had 3.5 times more risk of developing endometriosis than women with cycle of 30 days or more. Ashrafi M et al.[1] found in their study that shorter cycle length was associated with endometriosis (p= 0.04; OR= 0.9; CI 95% = 0.9-0.9). Matalliotakis et al.[20] found in their study that shorter cycle length was significant (p<0.001). Similarly, Sinaii N et al.[21] found in their studies 42% reported a 28-day cycle length and 16% with a cycle length of 24 days or less. Darwish AMM et al.[22] in their study of prevalence and risk factors of endometriosis among Egyptian women reported that women with endometriosis had significantly short cycles of 27 days or less.

Vercellini et al[23] found in their study that menstrual flow duration was slightly longer in women with endometriosis (mean difference, 0.33 days). In this study there was significant association between longer menstrual period and endometriosis. (p = 0.006; OR = 5.4).

The regression model showed an association between endometriosis with early menarche, shorter cycle length, prolonged duration of period and dysmenorrhoea.

In this present study it was observed that there was increased risk 65.6% in women with BMI in the range of 18.5 – 24.99 kg/m^2. Whereas there was a less chance of endometriosis in women who were overweight (25.0 – 29.99 kg/m^2). But on further statistical analysis significant association was not detected (p = 0.081). Mamdouh et al.[19] found overweight was associated with approximately 50% decrease in the risk of developing endometriosis (OR = 0.4, CI 95% 0.26 – 0.85). They found obesity was associated with approximately two times increase in risk of developing endometriosis. Peterson CM et al.[2] found in their study that decreased BMI is associated with the diagnosis of endometriosis (p<0.05; OR = 0.95; CI 95% 0.93 – 0.98). Hediger ML et al.[24] found that each unit decrease in BMI (kg/m^2) there was an approximate 12 – 14% decrease in the likelihood of being diagnosed with endometriosis. Normal BMI has a significant association (p=0.045). Shah et al.[25] in their study found that obese infertile women with current BMIs of 35–39.9 kg/m^2 and ≥40 kg/m^2 had a 55% (95% CI 0.30–0.67) lower risk of endometriosis compared with the low-normal BMI referent (18.5–22.4 kg/m^2). The difference with this study in significance of BMI may be due to smaller sample size.

In this series it was observed that women with a family

history of endometriosis has a risk of developing endometriosis (p= 0.009). Similarly, Mamdouh HM et al.[19] found a nearly two fold increased risk of developing endometriosis among women with one or more female relatives with endometriosis (p<0.001). Ashrafi M et al.[1] in their study found family history of endometriosis was a risk factor (p<0.001). Nouri K et al.[9] found first drgree relatives of patients with endometriosis were at a significant risk (p<0.05). Bischoff FZ and Simpson JL[26] found in their studies that endometriosis was present in 6.2% of mothers and 3.8% of sisters of endometriotic patients.

Conclusion:

From this study it is concluded that there is a consistent risk for endometriosis in patients with earlier menarche, shorter menstrual cycles, prolonged menstrual periods, dysmenorrhoea and family history. Finally, endometriosis may have more risk factors than recognized.

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Original Article

The role of Morus alba leaf extract in paracetamol-induced hepatic injury in rats

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Abstract

Background: Liver, the major drug metabolizing and detoxifying organ in the body is prone to injury from a large number of therapeutic and environmental agents. The leaves of *Morus alba* tree contains many anti-oxidant components that are thought to be useful in free radical mediated liver injury.

Objective: This study was carried out to find out evidence of protective effect of *Morus alba* leaf ethanolic extract in liver injury.

Methods: A total number of 24 adult Long Evans rats were divided into four equal groups, each group containing 6 rats. Group A was the control group which was fed normal rat diet. Group B, the paracetamol control group received paracetamol at a dose of 250 mg/kg body weight/day orally for 7 days to induce liver injury. Group C, the ethanol extract of *Morus alba* (EEMA) control group received EEMA at a dose of 800 mg/ body weight/day orally for 7 days, to see if there's any hepatotoxic effect of EEMA itself. Group D was the experimental group which was pre-treated with EEMA at a dose of 800 mg/kg body weight/day, followed by, 3 hours later, hepatic injury induced by paracetamol 250 mg/kg body weight/day orally for 7 days. All of the animals were sacrificed and the levels of AST, ALT, ALP and total bilirubin were measured. Liver was collected for histological examination.

Results: There was significant decrease in serum bilirubin, serum ALT, AST and ALP levels in the EEMA pre-treated group in comparison with the paracetamol control group B. Also on histological examination, no evidence of hepatic injury was found in group D, indicating the possible protective effect of EEMA on hepatocytes.

Conclusion: This study suggests possible protective effect of EEMA exerted on injured hepatic tissue.

Keywords: Paracetamol-induced hepatotoxicity, *Morus alba*, alcohol extract.

Introduction:

Liver is a large organ of human body and it is a site where intense metabolism takes place. It plays many functions including carbohydrate, protein and fat metabolism and detoxification. As it performs multitude of functions, liver is continuously exposed to a variety of possible harmful toxic agents (like drugs, chemicals, microbiological and viral agents) that may in turn interfere with its function and may also damage hepatic tissue causing disorders like hepatitis. Hepatitis due to any cause would jeopardize the function of liver, leading to variety of unwanted consequences. If hepatitis is kept untreated, remains refractory to treatment or if it is allowed to chronicity, a chain of adverse consequences would take place.

Hepatic injury may result from direct toxicity, through hepatic conversion of xenobiotics to active toxin, or by immune mechanism. Drug induced liver injury has global incidence of 1 to 14 per 100,000.[1] On the other hand, approximately one third of the world population is living with hepatitis B virus infection and 400 million people have chronic hepatic infection. Among the affected, global prevalence of chronic hepatitis B infection is highest in Africa, Asia and the Western Pacific. About 170 million people are infected with hepatitis C worldwide and chronicity occurs in 80-90%.[1]

It has been reported that some agents like paracetamol and carbon tetrachloride can mimic almost any naturally occurring acute and chronic hepatic disease in man.[2] Moreover, the histological changes reported in human cases of paracetamol overdose are similar to those observed in the rat and the common changes observed in

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the hepatic tissue were hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity[3] and hence paracetamol is a commonly used model for inducing hepatic injury in experimental animal studies.

From the ancient time, various plants had been used in traditional medicine as efficacious remedies for various diseases including liver disease. Over time, different chemical constituents from plants have been isolated. According to WHO, herbal medicine is curing diseases of an estimated 3.5 billion of the world's population.

In Bangladesh, more than 250 of such medicinal plants are now in common use in the preparation of traditional medicine. It is very important to ensure that these medicinal plants or their products really possess the claimed properties and exert their desired therapeutic effects. However, due to the introduction of large number of plants in traditional medicine based only on their empirical evidence, many of these medicinal plants are now found to be therapeutically useless. So, extensive scientific study must be conducted to find out their therapeutic efficacy.[4]

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver diseases are still increasing. As not many effective treatments are available in modern medicine, efforts are being made to find alternative herbal remedies for hepatitis and cirrhosis.[5]

Morus alba Linn. of *Moraceae* family ('White mulberry' or 'Tut') has long history of use in Chinese oriental medicine and it is claimed that this plant is useful in liver disorders.[6] It has been found that the ethanolic extract of *Morus alba* leaf has free radical scavenging property.[7] This property can be useful in counteracting liver injury as oxidative stress contribute to initiation and progression of liver injury.[8]

The alcoholic extract of *Morus alba* leaf was evaluated for hepatoprotection at a dose of 800mg/kg/day and it exhibited a significant liver protective effect by lowering the serum levels of AST and ALT and resulting in less injury to the rat liver[9] and the ethanolic extract of *Morus alba* leaf has shown more anti-oxidant absorption compared to the aqueous extract in rat intestine.[10]

Therefore, the present study is aimed at investigating the hepatoprotective role of ethanolic extract of *Morus alba* leaf in experimentally induced hepatotoxicity in rats with the aim of developing a natural hepatoprotective drug.

Material and Methods:

***Animals*:** A total number of 24 Long Evans rats of both sex and of different age groups, weighing 90 to 110 grams were selected for the study. They were procured from the animal house of icddr,b, Dhaka.

The rats were kept in standard sized wood and metallic cages in a well-ventilated room in the animal house of the Department of Pharmacology, Dhaka Medical College. Rats of different sex and groups were kept in separate cages and labeled accordingly. They were allowed to feed on standard rat feed (15 grams/rat/day) and allowed to drink water ad libitum. The rats were acclimatized five days at temperature and humidity.

***Plant material*:** The leaves of *Morus alba* (Tut) was collected from Gazipur, Dhaka. It was identified as *Morus alba Linn* (White mulberry or "Tut") by the Department of Botany of University of Dhaka, authenticated by Bangladesh National Herbarium, Dhaka.

About 2 kilograms of leaves were cleaned and washed with water. Then it was dried using hot air oven at 40 degrees celsius temperature. Then the dried leaves were crushed to make powder with a hand grinder. About 280 grams of leaf powder were obtained. The powder was submerged into 2 liters of absolute ethanol for 3 days, then filtered using cotton wool to separate the extract liquid. The filtrate was then concentrated with help of rotator evaporator and then freeze-dried. Finally, 15.7 grams of extract was obtained. This was stored in sterile glass container in the scientific laboratory of Dhaka medical college. The entire extract preparation process was carried out in the department of Chemistry of University of Dhaka.

***Experimental design*:** Total 24 (N - 24) rats were randomly divided in 4 groups, each group having 6 rats. Group A served as a control group, group B served as Paracetamol control group, group C served as EEMA control group and group D served as EEMA and Paracetamol treated experimental group. On day 8 of experiment, the animals were sacrificed and blood was collected by cardiac puncture for estimation of serum bilirubin, serum AST, serum ALT and serum ALP. Liver was dissected out and the specimen was collected in 10% formalin containing bottle for histological examination.

A total of 24 rats were randomly and equally divided in the following four groups:

Group A: Control group, received normal rat diet and distilled water for 7 days.

Group B: Paracetamol control group, received normal rat diet and distilled water. The dose of Paracetamol for inducing hepatic injury was 250 mg/kg body weight/day, administered orally, for 7 days.

Group C: EEMA control group, received EEMA along with normal rat diet and distilled water. 800 mg/kg body weight/day of EEMA was administered orally, for 7 days. This group was designed to observe any injurious effect of EEMA on hepatocytes.

Group D: EEMA pre-treated experimental group, received normal rat diet, distilled water and EEMA 800 mg/kg body weight/day. Three hours following the dose of EEMA, Paracetamol 250 mg/kg body weight was administered per orally, daily, for 7 days to observe the protective effect of EEMA.

All the rats were sacrificed on day 8 to measure serum bilirubin, ALT, AST and ALP levels and liver was collected for histopathological examination.

***Statistical analysis*:** All relevant information for each rat was recorded in a predesigned data collection sheet. Collected data were tabulated and statistical analysis (One-way ANOVA and Unpaired t-test) was done by appropriate significant test using statistical software. Data were considered statistically significant at $p < 0.05$.

Results:

Biochemical findings

Table 1: Comparison of mean serum bilirubin, ALT, AST and ALP among control, paracetamol control, EEMA control and EEMA pre-treated group (n=6)

Biochemical Parameters	Group A (Mean±SD)	Group B (Mean±SD)	Group C (Mean±SD)	Group D (Mean±SD)	f value	p value
Serum bilirubin (mg/dl)	0.12±0.09	0.80±0.24	0.14±0.07	0.15±0.02	36.04	<0.001*
ALT(U/L)	57.0±9.9	125.9±7.8	75.1±26.1	75.4±24.0	14.91	<0.001*
AST (U/L)	98.3±11.3	124.3±3.7	112.8±7.6	105.5±6.6	12.08	<0.001*
ALP (U/L)	74.5±4.73	101.5±10.94	77.3±3.92	86.8±2.93	21.56	<0.001*

Data were expressed as Mean±Standard deviation. Statistical analyses were done by One-way ANOVA test. The test of significance was calculated and p values < 0.05 was accepted as level of significance. *=Significant, n = Number of samples.

Table 1 shows that the highest mean value of serum bilirubin (0.8 mg/dl) was of group B, in which, hepatotoxicity was induced by paracetamol. The lowest value was of the control group A (0.12 mg/dl). In EEMA control group C and EEMA pre-treated group D, the mean levels of serum bilirubin were also considerably lower than the paracetamol control group B (0.14 mg/dl and 0.15 mg/dl respectively). In group D, the serum bilirubin level did not increase as it did in the paracetamol control group B. Statistical significance of difference among the groups was evaluated by using one-way ANOVA test. Highly significant difference [$F(3, 20) = 36.04$; $p<0.001$] was found among the groups, which was further analyzed by using Unpaired t-test (Table 2).

Table 1 also shows the highest mean value of serum ALT (125.9 U/L) was of group B and the lowest value was of the control group A (57.0 U/L). In EEMA control group C (75.1 U/L) and EEMA pre-treated group D (75.4 U/L), the mean levels of serum ALT were also considerably lower than that of paracetamol control group B. **S**erum AST concentration was 98.3 ± 11.3 in group A, while in paracetamol control group B it was 124.3 ± 3.7, in EEMA control group C it was 112.8 ± 7.6 and in EEMA pre-treated group D it was 105.5 ± 6.6. Highly significant difference [$F(3, 20) = 12.08$; $p<0.001$] was found among the groups. The mean value of serum ALP (101.5 U/L) was also highest in group B, whereas the ALP values of the group A, group C and group D were considerably lower than the paracetamol control group B.

Table 2: Comparison of serum bilirubin, ALT, AST and ALP concentrations (mean ± SD) between control, paracetamol control, EEMA control and EEMA pre-treated groups (unpaired t-test)

Groups	Serum Bilirubin	ALT	AST	ALP
Group A vs Group B	<0.001*	<0.001*	<0.001*	<0.001*
Group A vs Group C	0.708^{ns}	0.143^{ns}	0.026*	0.298^{ns}
Group A vs Group D	0.460^{ns}	0.113^{ns}	0.210^{ns}	0.001*
Group B vs Group C	<0.001*	0.001*	<0.008*	<0.001*
Group B vs Group D	<0.001*	<0.001*	<0.001*	0.010*
Group C vs Group D	0.712^{ns}	0.982^{ns}	0.107^{ns}	<0.001*

Table 2 shows comparison of all the biochemical parameters (Serum bilirubin, ALT, AST and ALP) between the groups. There was significant differences in serum bilirubin ($p<0.001$) between control group A and paracetamol control group B; between paracetamol control group B and EEMA control group C and between paracetamol control group B and EEMA pre-treated group D. However, no statistically significant difference was found between group A and group C; group A and group D and between group C and group D.

Serum ALT concentrations were also compared between the groups and significant differences were found between control group A and paracetamol control group B ($p<0.001$); paracetamol control group B and EEMA control group C ($p=0.001$) and between paracetamol control group B and EEMA pre-treated group D ($p<0.001$). However, no statistically significant difference was found between group A and group C; group A and group D and between

group C and group D. Table 2 also shows significant differences in serum AST concentrations between control group A and paracetamol control group B; control group A and EEMA control group C; paracetamol control group B and EEMA control group C; and between paracetamol control group B and EEMA pre-treated group D. Though there was no statistically significant difference was found between group A and group D and between group C and group D. Significant difference in serum ALP concentrations were found between group A and group B; between group A and group D; between group B and group C; between group B and group D; and between group C and group D. However, no significant difference in serum ALP level was found between group A and group C.

Histological findings

Microscopic examination of the sections of the liver from group A showed normal histological pattern. The hepatic lobules were well-defined. In the centre of each hepatic lobule there was a central vein. Radiating from the central vein towards the periphery of the lobule were plates of hepatic cells. Located between the hepatic plates were the hepatic sinusoids. The section of liver from group B (Paracetamol control) showed signs of hepatic tissue necrosis, disarrangement of normal hepatic cells with more eosinophilia and a few lymphocyte infiltrations (figure 1). The section of rat liver from group C (EEMA control group) had no evidence of liver injury showing EEMA as non-injurious to the hepatocytes. The section of rat liver from group D (EEMA pre-treated group) showed normal hepatic architecture with no evidence of necrosis or inflammatory infiltrations suggestive of possible hepatoprotective effect of EEMA (figure 2).

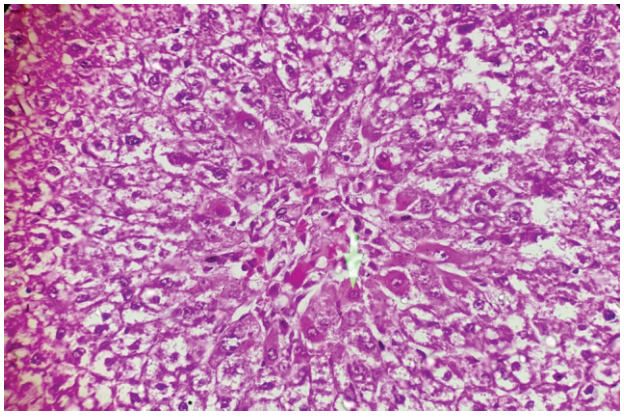

Figure 1: Microscopic picture of section of liver of paracetamol-treated rat Group B) showing necrotic changes with more eosinophilic cells and lymphocyte infiltrations.

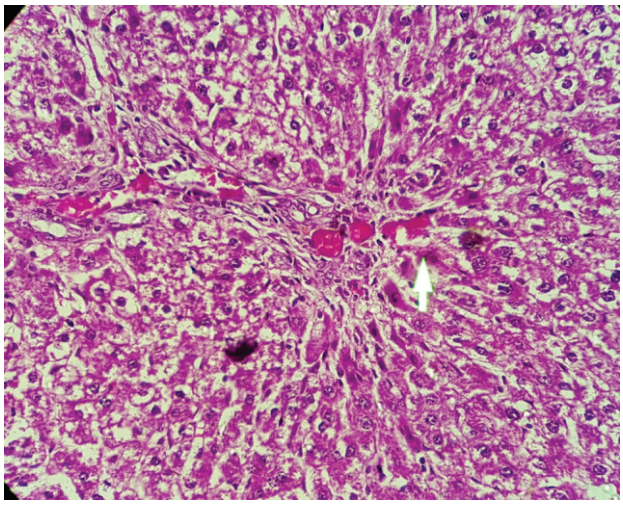

Figure 2: Hepatic section of EEMA pretreated rat (Group D) showing no evidence of hepatic injury

Discussion:

The present study was carried out to evaluate the role of ethanolic extract of *Morus alba* leaf (EEMA) in experimentally induced hepatotoxicity. The hepatotoxicity was induced by administration of paracetamol at a dose of 250 mg/kg body weight/day[11]. In the study, the mentioned dose of paracetamol was administered per orally, once daily, for 7 days to induce hepatotoxicity.[12]

The rise of serum AST and ALT levels had been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after hepatocellular damage.[13] Paracetamol administration caused damage to hepatocytes demonstrated by significantly increased ($p<0.001$) serum ALT, AST and ALP and total bilirubin levels in the paracetamol control group B as compared to control group A. This is indicative of cellular damage and loss of functional integrity of hepatocytes in paracetamol-induced hepatotoxicity.[12,13]

Histological examination of the hepatocytes in the paracetamol control group B showed tissue necrosis, disarrangement of normal hepatic cells with more eosinophilia and a few inflammatory lymphocyte infiltrations, thus indicating paracetamol to be a potent hepatotoxicity inducing model in animal experiments.[5,9,12]

The EEMA control group C received EEMA at a dose of 800 mg/kg body weight orally per day[9], for 7 days. The serum ALT, which is a more specific marker for damage to liver cells[14], also serum ALP and serum total bilirubin levels of this group C were measured after 7 days, which showed no significant difference with the control group A ($p>0.05$), so the levels did not increase significantly. Also, on histological examination, the liver showed no evidence of hepatic injury in this group of animals. This finding indicates that the EEMA at a dose of 800 mg/kg body weight was not

cytotoxic to hepatocytes. According to previously conducted research, the ethanolic extract of *Morus alba* leaves showed no toxicity up to 2000mg/kg body weight dose as there was no alterations of ALT levels, which is a more specific marker for damage to liver cells.[14] Also, the alcoholic extract of *Morus alba* leaves showed no toxic effect up to a dose of 3000 mg/kg body weight.[15]

Thus this study indicates there is no significant toxic effect of EEMA itself on liver at a dose of 800 mg/kg body weight and this result correlates with previous studies.[14,15] So it can be said that EEMA did not exhibit any significant hepatotoxic effect on its own at a dose of 800 mg/kg body weight/day.

In a previous study, hydroalcoholic extract of *Morus alba* L. leaves was administered at doses of 200, 400 and 800 mg/kg body weight in different groups, where the dose 800 mg/kg body weight showed relatively more and statistically significant ($p<0.05$) hepatoprotective effect by lowering the levels of serum ALT and AST and reduction of necrosis, tissue damage and vacuoles in histological study of the liver.[9] The dose of EEMA (800 mg/kg) in my study was selected by keeping in conformity with that[9] and the duration of treatment was selected to be 7 days.[11,12]

In this study, the evaluation of the protective effect of Ethanolic extract of Morus *alba* leaf (EEMA) in liver injury was done by estimating the levels of serum total bilirubin, ALT, AST and ALP and by histological examination of liver specimen. Group D was the EEMA pre-treated experimental group, in which the animals were pre-treated with EEMA at a dose of 800 mg/kg body weight and then three hours later, hepatic injury was induced by administering paracetamol at the same dose as the paracetamol control group B, that is, 250 mg/kg body weight, per orally, for 7 days. On day 8, the animals were sacrificed and the study parameters were measured. The study revealed significant decrease in serum ALT ($p<0.001$), AST ($p<0.001$), ALP ($p<0.05$) and serum total bilirubin ($p<0.001$) levels in the EEMA pre-treated group D in comparison with the paracetamol control group B. This indicates possible hepatoprotective effect of EEMA.

On histological examination, the EEMA pre-treated group D showed preservation of normal hepatic architecture with healthy centrilobular regions. The condition of the hepatic architecture was well preserved in comparison with the paracetamol control group B, where hepatic injury was evidenced by tissue necrosis and disarrangement of hepatic architecture. In the EEMA pre-treated group D, there was no sign of necrosis or inflammatory infiltration observed which can be considered due to the possible protective effect exerted by the EEMA.

By comparing the biochemical parameters and histological findings in the different groups of the experimental animals, it can be concluded that the ethanolic extract of *Morus alba* leaves may be useful in exerting protective effect in hepatic injury, which was evidenced by lowering of the enzyme levels, total bilirubin level and improvement of the inflamed hepatic architecture on histological examination. However, to evaluate the claim, more studies will be necessary.

Conclusion:

In conclusion, it can be speculated that ethanolic extract of *Morus alba* L. leaf showed evidence of hepatoprotective activity. These preliminary data suggest a basis for the use of ethanolic extract of *Morus alba* L. leaves in the development of a new hepato-protective herbal medicine.

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Original Article

Evaluation of visceral adiposity index with dietary patterns in Type-2 Diabetes Mellitus patients in Bangladesh

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Abstract

Background: Diabetes Mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from deficiency of insulin secretion, action, or both. Globally, type 2 diabetes mellitus (T2DM) is considered one of the most common diseases. Dietary habits and sedentary lifestyles are the major factors for the rapidly rising incidence of DM among developing countries.

Objective: This study was carried out with an objective to evaluate the association between visceral adiposity index (VAI) and dietary patterns regarding fast food-taking habits in T2 DM patients in Bangladesh.

Methods: This case-control study was performed from 2015 to 2017 in BIRDEM with 700 study subjects aged 30 to 60 years. Out of which 350 were T2DM and 350 were healthy control. Cases were selected from the outpatient department of BIRDEM, and controls were selected from healthy workers and employees of BIRDEM & IMC. We evaluated waist & hip circumference, BMI, systolic and diastolic blood pressure, fast food taking the history, exercise history using a preset questionnaire. Serum fasting glucose, ABF, TAG, Total cholesterol, HDL-C, LDL-C, HbA1c, and Serum insulin were estimated according to standardized method and by calculation method HOMA IR, HOMA B, Secretory-HOMA & VAI were estimated.

Results: There were significant differences in mean age, BMI, waist circumferences, hip circumferences, waist & hip ratio, systolic and diastolic blood pressure in between case and control**.** The value of Systolic blood pressure was 126 ± 15**.**84 mmHg in diabetes and 117 ± 15**.**46 mmHg in the control group, whereas the diastolic blood pressure was 82 ± 8**.**12 mmHg and 79 ± 8**.**89 mmHg, respectively**.** There were significant differences found in the waist and hip circumference in T2DM & controls, which were in **(**94**.**68±8**.**64**)** cm, **(**103**.**23±7**.**75**)** cm & **(**87**.**75±10**.**67**)** cm, **(**97**.**52±9**.**34**)** cm respectively**.** There were significant increases in mean VAI in T2DM compared to control, where p <0**.**001 & it was 3.93±2.72 in the case and 2.60±2.22 in control respectively. LSD test within One-way ANOVA had done to test the differences of fast food taking habit pattern per week with different glycemic, Lipidemic markers and had shown significant difference with fasting blood glucose, 2 hours after breakfast, HBA1c with fast food taking group where p values were 0.01, 0.004, 0.03 respectively and non -significantly associated with HOMA-IR, HOMA B%, VAI, TAG, Cholesterol & LDL-C level in the study population. Multiple linear regression of VAI with glycemic and Lipidemic parameters and significant (p<0.001) association was found for HBA1c and HDL-C in the study population. Correlation of VAI with anthropometric, glycemic and Lipidemic parameters shows a significant (p <0.001) positive correlation with FBS, ABF, HBA1C, TAG, Cholesterol, LDL-C, HOMAIR and negative correlation with HDLC in T2DM subjects.

Conclusion: VAI was positive and significantly correlated with FBS, ABF, HBA1c, TAG, Cholesterol, LDL-C, HOMA-IR and negatively correlated with HDL-C. VAI was non-significantly associated with fast food taking habit in the study population.

Keywords: Visceral adiposity index, Dietary patterns, Fast food taking habit, Type**-**2 Diabetes Mellitus, HBA1C, Fasting blood sugar, HOMA**-**IR**.** .

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Introduction:

EExcess visceral adipose tissue (VAT) is one of the most deleterious fat depots in the body, with strong links to cardiovascular disease and certain types of cancer.[1,2] Lipid accumulation product (LAP) index, a recently developed biomarker of central fat accumulation, has been recommended as a precise indicator of the risk of insulin resistance, metabolic syndrome, type 2 diabetes, and cardiovascular disease.[3-5] Higher LAP has been associated with abnormal glucose homeostasis and insulin resistance, as well as elevated alanine aminotransferase in healthy individuals.[6] A Chinese study showed that both LAP and visceral adiposity index (VAI) were effective markers for stratifying adults for obesity phenotypes.[7] In addition,

another study reported that LAP was a helpful indicator for the screening for metabolic syndrome.[8]

The VAT seems to be affected by diet and lifestyle modifications.[9,10] Furthermore, it has been suggested that VAT is mainly influenced by the non-caloric qualitative aspects of diet, although evidence on the association between macronutrient composition of diet and VAI is still limited. A recent investigation indicated that consuming energy mainly as carbohydrate or fat for three months did not affect visceral fat and metabolic syndrome in a low-processed, lower-glycemic dietary context.[11] There are contradictory findings regarding the association between different dietary patterns (DPs), LAP, and VAI. The significant association between carbohydrate intake,[12,13] dietary fatty acids[14] including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids(PUFAs) with VAT has not been reported in all investigations.[8] However, it is important to note that foods and nutrients are consumed in combination, and complex combinations of nutrients are likely to be interactive or to have a synergistic effect.[15] The approach of evaluating single nutrients or foods might therefore be limited in terms of potential interactions and high inter-correlations between several food components, which might make it challenging to estimate the general, or independent impacts of different nutrients or foods, perhaps slight and thus untraceable impacts of a single nutrient may be concealed, and the concern of multiple comparisons is also crucial in this area.[16] Therefore, in an effort to overcome these issues, the analysis of DPs has gained importance.[15,17] The mechanisms by which nutrient patterns affect the risk of chronic conditions are not fully understood, and there is good evidence that it is a combination of nutrients, rather than an individual one, that will affect the risk. Therefore, a pattern of nutrients may provide more information about probable underlying mechanisms.[18,19] This study was aimed to evaluate the association between VAI and dietary patterns regarding fast food taking habits in T2 DM patients in Bangladesh.

Material and Methods:

This case-control study, which was carried out on-350 type 2 diabetic subjects & 350 nondiabetic healthy volunteers as a control. The patients were selected from the outpatient department of BIRDEM and the controls were selected from the volunteers and workers of the outpatient department of BIRDEM & employees of the residential hall in Dhaka university campus. Age & sex was matched with the cases. Type 2 diabetes subjects with the duration of diabetes of 2-10 years and age 30-60 years were included as the case in the study population. Non-diabetic healthy volunteers & age 30-60 years were included as healthy control. Exclusion criteria for both case and control were: Presence of any kind of acute infection and systemic disorder, evidence of hepatic dysfunction: ALT (SGPT) or AST (SGOT)>100 units, evidence of renal dysfunction: S creatinine> 1.7mg/dl, presence of malabsorption syndrome, presence of autoimmune disease, pregnant women, a recent history of cardiovascular diseases, cancer were excluded from the study. Anthropometry and baseline characteristics, including sociodemographic data, family history of diabetes, medical history, fast food taking habit, were taken using a preset questionnaire. The research was approved by the Ethical Review Committee of BIRDEM. Data were recorded for baseline study (age, sex, BMI, height, waist-hip ratio, blood pressure, exercise history, fast-food taking history/week, smoking, etc)

Blood sample collection

Three milliliters of venous blood were collected in an EDTA (Ethylene diamine tetra acetic acid) containing vacutainer tube (Vacuette®) from the study group (350 cases & 350 controls) of adult patients with T2DM and non-diabetic controls with informed consent. Separation of serum from a part of whole blood cells. Estimation of serum fasting glucose, ABF (2 hours after breakfast), TAG(Triacylglycerol), Total cholesterol, HDL-C, LDL-C, HbA1c, Serum insulin, were measured according to the standardized method and by calculation method, we measured HOMA IR(homeostatic model assessment of insulin resistance), HOMA B (homeostatic model assessment of pancreatic beta-cell function), Secretory-HOMA & VAI.Glucose was measured by Glucose Oxidase (GOD Glucose Oxidase -PAP Glucose peroxidase) method. Triglyceride by enzymatic colorimetric (GPO-PAP) method. Total cholesterol by enzymatic endpoint method (Cholesterol Oxidase/ Peroxidase method). HDL cholesterol by enzymatic colorimetric (Cholesterol CHOD-PAP) method. LDL-cholesterol was calculated by the Friedewald equation. Insulin by enzyme-linked immunosorbent assay (ELISA) method, Glycosylated Hemoglobin (HbA1c) by HPLC (High performance liquid chromatography) method.[6]

By the calculated method, HOMA-IR, HOMAB% (homeostatic model assessment of pancreatic beta-cell function), Secret HOMA (homeostatic model assessment of pancreatic secretion) & VAI were measured.[16] The triglyceride (TG)-glucose (TyG) index was calculated as the ln(Fasting TG [mg/dL]xGlucose [mg/dL]/2).[16] The anthropometrically predicted VAT (apVAT) was estimated with sex-specific validated equations that included age, body mass index (BMI), and circumferences of the waist (WC) and HIP.[17] The equation for men was: 6 x WC - 4.41 x Proximal hip circumference + 1.19 x Age- 213.65; and the equation for women was: 2.15 x WC - 3.63 x Proximal hip + 1.46 x Age + 6:22 × BMI - 92.713. VAI was calculated using sex specific formulas:

males WC/39.68+[1.88xBMI])x(TGs/1.03)x(1.31/high-density lipoprotein [HDL]); females: (WC/36.58+[1.89xBMI])x(TGs/0.81) x (1.52/HDL), where both TGs and HDL levels are expressed in mmol/L.[17] LPA was calculated as (WC/65)xTG in men, and (WC/58)xTG in women.[17] Statistical Methods-Student t-test (two-tailed, independent) has been used to find the significance of study parameters on a continuous scale between two groups Intergroup analysis) and chi-square test has been used to analyze the data having ordinal variables. Significant figures were analyzed, Suggestive significance (p-value: 0.05<p)

Statistical software The Statistical software, namely EPI Info 7.0, IBM SPSS 20 and Vassar stats (www. vassarstats.net) for the analysis of the data and Microsoft word were used. A p-value of <0.05 was considered significant.

Results:

Table1: Baseline clinical characteristics and laboratory results of the study population

Variables	Healthy Group (Control)	Diabetic Group (case)	p value
	(n=350)	(n=350)	
Male (%)	175(50%)	175(50%)	NS
Female (%)	175(50%)	175(50%)	NS
Age (year)	39.11±9.30	49.49±9.95	<0.001
BMI	25.09±4.07	28.85±3.42	<0.05
Waist circumference	87.75±10.67	94.68±8.64	<0.001
Hip circumference	97.52±9.34	103.23±7.75	<0.001
Waist :Hip ratio	0.89±0.05	0.91±0.04	<0.001
SBP (mmofHg)	117±15.46	126±15.84	<0.001
DBP(mmof Hg)	79±8.89	82±8.12	<0.001
FBG(m.mol/L)	4.82±1.21	8.77±3.0	<0.001
ABF(m.mol/L)	6.9±1.21	12.13±4.05	<0.001
Fasting Insulin (μ unit/L)	16.03±10.79	23.22±15.73	<0.001
HbA1c (%)	5.23±0.74	7.26±1.76	<0.001
HOMA IR	3.39±2.65	9.19±7.62	<0.001
HOMA-B%	309.12±47	101.34±26	<0.001
Secretory HOMA	357±1021	103±583	<0.001
Triglycerides(mg/dL)	142.57±87.28	189.45±106.31	<0.001
Cholesterol(mg/dL)	180.00±110.49	184.92±42.86	>0.05
HDL- Cholesterol (mg/dL)	45.69±17.14	38.20±7.34	<0.001
LDL- Cholesterol (mg/dL)	105.38±79.31	113.42±42.03	>0.05
VAI	2.60±2.22	3.93±2.72	<0.001

p <.05 was the level of significance.

Table 1 showed the baseline characteristics of the study population and significant differences in mean age, BMI, waist circumferences, hip circumferences, waist &hip ratio, systolic and diastolic blood pressure. The mean ± SD age of the diabetes group and control group was 49.49 ± 9.95 years and 39.11 ± 9.30 years, respectively. There was a non-significant difference in sex distribution between the two groups (Male: 50% and Female: 50%). The value of systolic blood pressure was 126 ± 15.84 mmHg in diabetes and 117 ± 15.46 mmHg in the control group, whereas the diastolic blood pressure was 82 ± 8.12 mmHg and 79 ± 8.89 mm Hg, respectively. There were significant differences found in the waist and hip circumference in T2DM & controls, which were in (94.68±8.64) cm, (103.23±7.75) cm & (87.75±10.67) cm, (97.52±9.34) cm, respectively. And also, The Fasting Blood Glucose (FBG) levels were 8.77±3.0(mM/L), and 4.82±1.21 (mM/L), 2 hrs after breakfast (ABF), 12.13±4.05(mM/L), 6.9±1.21(mM/L), and HbA1c% were 7.26±1.76, and 5.23±0.74 in type 2 diabetes and control group respectively. FBG, ABF, and HbA1c% levels of the diabetic group were significantly higher than the control group (p <0.001). To investigate insulinemic status, fasting serum insulin was estimated by standardized method, and beta-cell function (HOMAB%), insulin resistance (HOMA IR), and insulin secretory capacity (Secretory HOMA) was calculated. The Fasting serum insulin level of the case group (23.22±15.73) (μ unit/L) was significantly (p<0.001) higher than control (16.03±10.79) (μ unit/L). On the other hand, the HOMA B% (101.34±26vs309.12±47; p<0.001); HOMA-IR (9.19±7.62vs3.39±2.65; p<0.001) and secretory HOMA (103±583vs 357±1021; p<0.001) were significantly lower in the case group compared to control; whereas insulin was significantly higher (23.22±15.73vs16.03±10.79; p<0.001) in the case group than in control. Table 4 demonstrated the lipidemic status of the enrolled study subjects. The TG level was significantly (p<0.001) higher (189.45±106.31 mg/dL) in the diabetes group than that of the control group (142.57±87.28 mg/dL). Total cholesterol level was184.92±42.86mg/dL and 180.00±110.49 mg/dL in diabetes and control groups, respectively. Although the cholesterol level was lower in case subjects than that of control, the difference was not statistically significant. The HDL level was significantly (p<0.001) lower in case group (38.20±7.34mg/dL) compared to control (45.69±17.14 mg/dL); and LDL level was non significantly higher (p>0.05) in case group (113.42±42.03mg/dL) compared to control group (105.38±79.31mg/dL). No significant differences were found in LDL-C and cholesterol levels between the two groups.

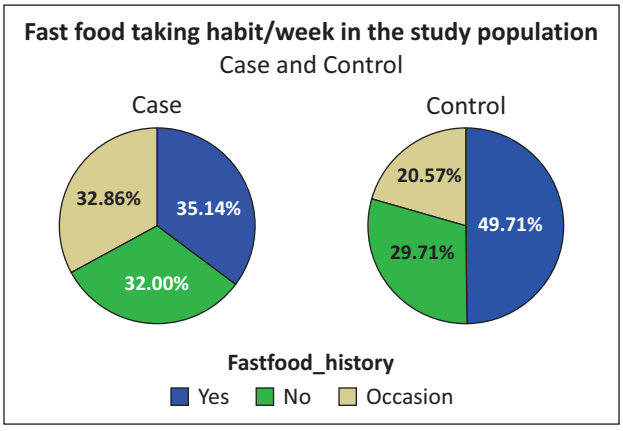

Fig 1: Pie chart of fast food taking habit/week in the study population

Fig 1 showed that positive(yes) fast food taking habit was more in control than T2DM subjects whereas negative (no), and the occasional group were more in T2DM subjects than in the control group in the study subjects.

Table 2: Correlation of VAI with anthropometric, glycemic and lipidemic parameter in the total study population

Parameter	Case (350)		Control (350)	
	r value	p value	r value	p value
BMI	-0.053	0.326	0.139**	0.009
Waist	0.083	0.123	0.257**	0.000
Hip	0.333	0.050	0.271**	0.000
WHR	0.076	0.181	0.144**	0.007
SBP	0.017	0.754	0.076	0.158
DBP	-0.061	0.257	0.103	0.055
FBS	0.247**	0.000	-0.006	0.909
ABF	0.195**	0.000	0.066	0.221
HBA1c	0.259**	0.000	0.098	0.066
TAG	0.890**	0.000	0.689**	0.000
Cholesterol	0.338**	0.000	-0.010	0.854
HDL-C	-0.472**	0.000	-0.528**	0.000
LDL-C	0.113*	0.035	-0.011	0.836
Insulin	0.102	0.057	0.052	0.333
HOMA-IR	0.184**	0.001	-0.081	0.129
HOMAB%	-0.088	0.099	0.098	0.066
Secrt-HOMA	-0.069	0.197	0.098	0.066

$p<.05$ was the level of significance.

Table 2 showed positive significant correlation of VAI with BS, ABF, HBA1c, TAG, Cholesterol, LDL-C, HOMA-IR and negative correlated with HDL-C in T2DM subjects where r values were 0.247**,0.195**,0.259**,0.890**, 0.338**,0.113*,0.184** & 0.472**respectively.

Table 3: Multiple comparisons of VAI with Fast food taking habit/week in the study population

Parameter	Fast food taking habit/week	p-value
FBS	Yes	0.01
	No	0.168
	Occasional	0.270
ABF	Yes	0.004
	No	0.156
	Occasional	0.143
HBA1c	Yes	0.030
	No	0.201
	Occasional	0.001
	Yes No	0.05
VAI		0.874
	Occasional	0.139

$p<.05$ was the level of significance.

Table 3 showed the multiple comparisons of fast food taking habit with different glycemic and Lipidemic-markers in the study population by one-way ANOVA and the LSD test had shown significant differences with fasting blood glucose, 2 hours after breakfast, HBA1c with yes fast food taking group where p values were 0.01, 0.004, 0.03 respectively and non -significantly associated with HOMA-IR, HOMA B%, VAI, TAG, Cholesterol & LDL-C level in the study population & p-value was>0.05.

Table 4: Multiple linear regression of VAI with glycemic and Lipidemicparameters in the study population

Parameter	beta	significance	95% CI lower value	95% CI upper value
FBS	0.042	0.672	-0.130	0.202
ABF	-0.024	0.770	-0.119	0.088
HBA1c	0.250	0.000	0.207	0.552
Insulin	-0.025	0.804	-0.041	0.032
HOMA-IR	0.170	0.162	-0.028	0.165
HOMA B%	0.016	0.668	0.000	0.000
TAG	0.780	0.000	0.019	0.021
Cholesterol	-0.108	0.000	-0.005	-0.002
HDL-C	-0.363	0.000	-0.074	-0.062
LDL-C	0.053	0.065	0.000	0.004

$p<.05$ was the level of significance.

Table 4 showed the multiple linear regression of VAI with glycemic and lipidemic markers in the study population and showed that VAI was positive and significantly associated with HBA1C and negative significantly associated HDL-C in the study population.

Discussion:

Findings from this study revealed that adiposity factors and markers of glucose/insulin homeostasis were positively associated with the diet, which highly consisting of fast food-taking habits like the burger, pizza, sugar and total fat. Moreover, we found a significant negative association between VAI and HDL-C, and FBS, ABF, HBA1c were significantly associated with the positive fast food-taking habit group. However, our study also revealed that fast food taking habits are non-significantly associated with VAI, TAG, and LDL-C & VAI. But VAI was significantly associated with HBA1c and HDL-C. In contrast to our findings, in a prospective study, no relation was detected between SFAs, MUFAs, PUFAs, and 5-year percent change in VAT[14] however, one cross-sectional study revealed a positive association between fat intake and VAT in overweight young adults aged 30 to 45 years diabetics.[14] Our study findings are consistent with this study. An Iranian investigation reported that increasing MUFA by decreasing total protein or PUFA in isoenergetic diets was positively

associated with visceral adiposity index changes.[20] Contrary to our results, some observational studies did not find a significant association between fast food intake and VAI[21]; however, it has been proposed that replacing carbohydrates with total protein was positively associated with VAI in women only.[22] A recent Iranian investigation reported that higher dietary proportions of protein and animal-derived MUFA could be positively associated with VAI; in addition, in isoenergetic diet, replacing carbohydrate, MUFAs, and PUFAs with protein was positively associated with 3-year changes in VAI.[19] However, no significant association was reported between 2-year changes in total protein intake and change in VAT in 85 overweight adolescents aged 11 to 17 years[22] as well total protein intake was also not associated with 5-year percent change in VAT in 1114 black and Hispanic overweight adults in another prospective study[23]. An investigation reported that LAP and VAI were markers of insulin resistance and metabolic-related disturbances in young women with polycystic ovary syndrome.[19] Our study findings were not consistent with that. Recent meta-analysis investigated the effects of saturated fat, polyunsaturated fat, monounsaturated fat, and carbohydrate on glucose-insulin homeostasis.[20] It reported that only energy intake substitution with PUFA was associated with lower fasting glucose, lower HbA1c, improved HOMA-IR, and improved insulin secretion capacity. Furthermore, insulin secretion capacity similarly improved when PUFA replaced MUFA. This study showed multiple linear regression of VAI with glycemic and lipidemic markers in the study population and showed VAI was positively significantly associated with HBA1c and negatively significantly associated with HDL-C in the study population.

Conclusion:

VAI was positive and significantly correlated with FBS, ABF, HBA1c, TAG, Cholesterol, LDLC, and HOMA-IR and negatively correlated with HDL-C. VAI was non - significantly associated with fast food taking habit in the study population.

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Original Article

Plasma Homocysteine, Atherogenic Index and their correlation in newly diagnosed hypothyroid individual

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Abstract

Background: Independent of age, sex and associated disorders, hypothyroidism was found to favor the development of Coronary artery atherosclerosis. Atherogenic index of plasma (AIP) is the new marker of atherogenecity, since the AIP is related directly to the atherosclerosis risk. AIP is the ratio calculated as log (TG/HDL-C). Homocysteine is a sulfur-containing, non-protein amino acid that is formed during catabolism of essential amino acid methionine. Hyperhomocysteinemia in hypothyroid individual increase the risk for cardiovascular disease.

Objective: The main objective of the study was, to evaluate if there is any association between plasma Homocysteine and Atherogenic Index of Plasma in newly diagnosed hypothyroid patient.

Method: This case control study was carried out in the department of biochemistry, BSMMU, Shahbag, Dhaka. One hundred subjects were included in the study and were grouped into case (hypothyroid) and control (euthyroid) on the basis of value of TSH & FT_4. Out of one hundred subjects, 55 newly diagnosed hypothyroid patients were grouped as cases and 45 were grouped as normal controls considering the inclusion and exclusion criteria. cases were selected from OPD of Endocrinology of BSMMU and controls were selected from normal population of BSMMU. Plasma TG, HDL-C and plasma homocysteine were collected from all subjects in fasting state. All data were recorded in a preformed data collection sheet were analyzed by using SPSS version 16.0. Athorogenic index of plasma among the newly diagnosed hypothyroid individual were calculated. The results were expressed as mean ± SD, and t test, chi square test and correlation test were done to evaluate the level of significance at $p < 0.05$.

Results: Mean serum homocysteine was found significantly higher in case group (19.00 ± 7.58 µmol/L) than control group (9.59 ± 1.91µmol/L) with p value 0.001. Mean AIP was found significantly higher in case (0.75 ± 0.27) than control (0.35 ± 0.20) where $p < 0.001$). In this study it was revealed that 96**.4%** cases were within increased risk group of atherogenesis where as in control group it was 71 % which was statistically significant **(**$p < 0.001$**).** This study also revealed that there was positive significant correlation between AIP and serum homocysteine **(**$p = 0.007$**).** Present study also showed serum homocysteine to be positively correlated with serum TG **(**$p = 0.016$**).** A negative correlation had been seen between serum homocysteine and serum HDL**-**C which was statistically significant **(**$p = 0.043$**).**

Conclusion: From this study it may be concluded that hyperhomocysteinemia and high atherogenic index is associated with hypothyroidism.

Keywords: Hypothyroidism, Hyperhomocysteinemia, Atherogenic index of plasma.

Introduction:

Hypothyroidism is defined as a deficiency of thyroid activity, which results from reduced secretion of both T3 and T4 irrespective of the cause.[1] It may be due to primary disease of the thyroid gland itself or lack of pituitary TSH.[2] Biochemically decrease in T4 and T3 concentrations lead to hyper secretion of pituitary TSH and an amplified increase in serum TSH levels.[3] Primary hypothyroidism is a graded phenomenon with different levels of severity, showing a wide inter individual range of clinical and biochemical presentation.[4] The earliest form of hypothyroidism, called subclinical hypothyroidism (SCH) or mild thyroid failure is defined by an increased serum TSH level in the presence of normal concentrations of circulating thyroid hormones.[5]

Subclinical hypothyroidism was defined as a TSH level greater than 4.0 mU/L in the presence of a normal free thyroxin level (11 to 25 pmol/L [0.9 to1.9 ng/dL]). Clinical hypothyroidism was defined as a TSH level greater than 4.0 mU/L and a decreased free thyroxin level (<11 pmol/L [<0.9 ng/dL]).[6] Euthyroidism was defined as a normal TSH level (0.4 to 4.0 mU/L).

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Overt Hypothyroidism is a clinical condition when the TSH levels are more than 20mu/l.[7] Overt thyroid disturbances, characterized by symptoms and/or clinical signs with abnormal serum levels of thyroid hormones, are generally associated with perturbations in the lipid profile.[8]

Hypothyroid patients also have an increased incidence of hypercholesterolemia[9] and hypertriglyceridemia.[10] Hypothyroid patients have an increased LDL, VLDL, HDL-C, apolipoprotein (Apo B) and lipoprotein (a). [11]

Atherogenic index of plasma (AIP) is the new marker of atherogenecity, since the AIP is related directly to the atherosclerosis risk. AIP is the ratio calculated as log (TG/HDL-c).[12] Triglycerides and HDL-cholesterol in AIP reflect the balance between the atherogenic and antiatherogenic lipoproteins respectively.[12] Atherogenic lipoprotein profile of plasma is an important risk factor for coronary heart disease CHD). It is characterized by high low density lipoprotein cholesterol to high density lipoprotein cholesterol ratio and increased level of triglyceride.[13] There is medium risk of cardiovascular disease with increased AIP value in hypothyroid patient.[14] AIP values of less than 0.1 are associated with low risk, 0.1 to 0.24 with medium risk and above 0.24 with high risk of cardiovascular diseases.[15]

Homocysteine is a sulfur-containing, non-protein amino acid that is formed during catabolism of essential amino acid methionine. This by-product of methyl-transfer reactions is important for the DNA synthesis, methylation of proteins, neurotransmitters and phospholipid. [16]

Homocysteine is metabolized by one of two pathways; remethylation and transsulfuration. Thus, Hyperhomocysteinemia may be due to deficiency of enzymes of the remethylation pathway that recycles homocysteine to methionine, or of enzymes of the transsulfuration pathway (cystathionine β-synthetase).[17] Several reports in the literature indicate that hypothyroidism is associated with elevated plasma Homocysteine concentrations.[18] Thyroid status has a profound influence on a variety of biochemical processes, some of which may have secondary effects on the Homocysteine metabolism. Thyroid hormones markedly affect riboflavin metabolism, mainly by stimulating flavokinase and thereby the synthesis of flavin mononucleotide and flavin adenine dinucleotide (FAD).[18-20]

Circulating tHcys concentrations in hypothyroidism can rise through reduced activity of the flavoprotein methylene tetra hydro folate reductase (MTHFR), an enzyme involved in the catalysis of tHcys and its remethylation to methionine. Hypothyroid individuals can be defective in converting riboflavin to the co-enzyme FAD, and consequently, deficient in MTHFR activity.[7] Normal tHcy levels range between 5 and 15 µmol/l (12 mmol/l being the upper reference limit for populations on a folic-acid fortified diet) with elevations of 16 to 30 mmol/l, 31 to 100 mmol/l, and >100mmol/l classified as mild, moderate, and severe hyperhomocysteinemia, respectively.[21] A strong inverse relationship between homocysteine and thyroid hormones confirms the effect of thyroid hormones on homocysteine metabolism.[22]

Hyperhomocysteinemia and hypercholesterolemia could help to explain the increased risk for arteriosclerotic coronary artery disease in hypothyroidism. This study aimed to evaluate any association between plasma Homocysteine and Atherogenic Index of Plasma in newly diagnosed hypothyroid patient.

Material and Methods:

This case control study was carried out from January 2012 to December 2013 in the department of Biochemistry, BSMMU in cooperation of department of Endocrinology, BSMMU. One hundred subjects were included in the study and were grouped into case (hypothyroid) and control (euthyroid) on the basis of value of TSH & FT_4. Out of one hundred subjects, 55 newly diagnosed hypothyroid patients were grouped as cases and 45 were grouped as normal controls. Hypothyroid patients are selected from OPD of Endocrinology of BSMMU and control subject have been selected from normal population of BSMMU. Hypothyroid patient with hypertension, diabetes, mellitus, renal failure, patient with thyroid hormone medication and obesity were excluded from the study by history and relevant laboratory investigation. With all aseptic precautions, after an overnight fast, blood samples were collected from all study subjects to estimate the Plasma TG, HDL-C and plasma homocysteine. All data were recorded in a preformed data collection sheet were analyzed by using SPSS version 16.0. Athorogenic index of plasma among the newly diagnosed hypothyroid individual were calculated. Then find out the relation of AIP and plasma homocysteine level in newly diagnosed hypothyroid individual. .The results were expressed as mean ± SD using t test, chi square test and correlation test. Statistical significance was set at $P < 0.05$.

Results:

This case control, analytical study was conducted on a total of 100 subjects based on predefined enrollment criteria. Among them 55 subjects were grouped into case (hypothyroid) and 45 subjects were grouped into control (euthyroid) on the basis of level of TSH &FT-4. All the variables were compared between these two groups.

Table 1: Comparison of homocysteine & AIP between case and control

Biochemical parameters	Group		p value
	Case	Control	
Homocysteine (µmol/L)	19.00 ± 7.58	9.59 ± 1.91	0.001*
Atherogenic index of plasma	0.75 ± 0.27	0.35 ± 0.20	0.001*

* t test was done to measure the level of significance.

Table1 shows homocysteine & AIP level of study subjects. Mean homocysteine was 19.00 ± 7.58 µmol/L in case group and 9.59 ± 1.91µmol/L in control group. Mean AIP level was 0.75 ± 0.27 in case group and 0.35 ± 0.20 in control group. Regarding biochemical parameter there was statistical significant difference (p< 0.05).

Table 2: Distribution of study subject according to Level of AIP

AIP	Group		p value
	Case	Control	
AIP < 0.21	2 (3.6)	913 (28.9)#	
AIP ≥ 0.21	53 (96.4)	32 (71.1)	
Total	**55 (100.0)**	**45 (100.0)**	0.001*

*Chi square test was done to measure the level of significance.

#Figure within parentheses indicates in percentage.

Table 2 shows, 53 (96.4%) cases were within increased risk group of atherogenesis where as in control group it was 32 (71.1%) which was statistically significant (p < 0.05).

Table 3: Status of homocysteine level in case and control

Hyperhomocysteinemia	Group		p value
	Case	Control	
Normal	16 (29.1)	45 (100.0)	
Mild	35 (63.6)	0 (0.0)	
Moderate	4 (7.3)	0 (0.0)	
Total	**55 (100.0)**	**45 (100.0)**	0.001*

*Chi square test was done to measure the level of significance.

#Figure within parentheses indicates in percentage.

Table 3 showing, status of t-Hcy level in case & control group. In case out of 55 hypothyroid patients, 29.1% represent normal t-Hcy level, 63.6% mild & 7.3% moderate hyperhomocysteinemia level. In control group 100% represent normal t-Hcy level and the difference was statistically significant (p < 0.05).

Table 4: Correlation of plasma homocysteine with TG, HDL-c and AIP in Hypothyroid cases

Parameter	Case	
	r value	p value
TG	0.324	0.016
HDLc	-0.274	0.043
AIP	0.359	0.007

Table 4 shows, Correlation of plasma homocysteine with TG, HDLc and AIP in Hypothyroid cases. Pearson's correlation test showed significant positive correlation (r=0.324) between plasma t-Hcy and plasma TGlevel.In between plasma t-Hcy and plasma HDL-C shows significant negative correlation (r = -0.274) and between plasma t-Hcy and AIP shows significant positive correlation (r = 0.359).

Discussion:

Hypothyroidism is a common metabolic disorder in general population. Hypothyroidism is a commonly encountered health problem in Bangladesh and morbidity and mortality toll due to cardiovascular disease resulting from hypothyroidism is quite high. Hypothyroidism, dyslipidaemia and hyperhomocysteinemia are recognized risk factor for atherosclerosis and cardiovascular disease.

In this study mean serum homocysteine was significantly higher in case group than control group (P=0.001). Mean serum homocysteine was 19.00 ± 7.58 µmol/L in case whereas 9.59 ± 1.91 µmol/L in control group. In a similar study conducted by Ellatif MA and et al[23] found a significant higher total plasma homocysteine concentration in hypothyroid patients 15.4 ± 7.5 µmol/L than in control group 7.9 ± 2.8 µmol/L (P <0.01). Nedrebo B and et al[18] showed that, mean t-Hcy level was higher (16.3) than the control subject (10.5).

A similar study showed mean homocysteine level in case were found to be having 12.73 ± 5.58 µmol/L while that in control was 11.15 ± 9.50 µmol/L.[24] The homocysteine was significantly higher (P=0.001) in hypothyroids as compared to control.

In a study conducted by Rajab TMA[14] showed that, mean atherogenic index was higher in hypothyroid individual (0.20 ± 0.03) compared to euthyroid (0.11 ± 0.02) with p < 0.05.

Here Atherogenic index was calculated by using AIP= log (TG/HDL-C) formula. Shivakrishna G and et al[25] also found that atherogenic index was higher in hypothyroid than euthyroid subjects. In their study, the atherogenic index was 0.27± 0.20 in overt hypothyroid case, 0.17± 0.10 in subclinical hypothyroid case and 0.07± 0.10 in euthyroid control subject (p value <0.01). In present study, the atherogenic index was 0.75 ±0.27 in case group and 0.35 ±0.20 in control group. Here p value is 0.001.

Here Atherogenic index was calculated by using AIP= log (TG/HDL-C) formula. Recent studies have indicates that TG/HDL-C ratio transformed logarithmically can estimate the atherogenic risk better than all others. TG and HDL-C perfectly reflects the balance between atherogenic lipoproteins and protective lipoproteins. Clinical studies revealed that atherogenic index of plasma can estimate the cardiovascular risk. This index is also sensitive to pharmacological treatment, being a barometer of therapeutic success. The reduced risk of cardiovascular events is associated with low AIP level (<0.11); range from 0.11 to 0.21 indicates intermediate risk of CV disease and

values > 0.21 shows a high cardiovascular risk.[26] Present study showed that 53 (96.4%) cases out of 55 were within increased risk group of atherogenesis where as in control group it was 32 (71.1%) out of 45 which was statistically significant ($P < 0.05$).

In present study, there is significant positive correlation (r=0.324, p=0.016) between TG & serum homocysteine and this was found similar with another study.[27] There is significant negative correlation (r = 0.274, p =0.043) between HDL-C & serum homocysteine in this study. Zhou X Q[27] also showed serum homocysteine was negatively correlated with HDL-C (P = 0.01). Syed et al. (2003) showed negative correlation between serum homocysteine and HDL (P = 0.009). This study also found significant positive correlation (r=0.359, p=0.007) between AIP & serum homocysteine which was in agreement with another study.[27] In a study, done by Saini V and et al,[28] there is positive correlation between AIP & TG and negative correlation between AIP & HDL-C in hypothyroid patients. So present study was in agreement with the study above mentioned.

Conclusion

From this study it may be concluded that hyperhomocysteinemia and high atherogenic index is associated with hypothyroidism.

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Original Article

Effects of social media on academic performance of medical students of a selected tertiary medical college

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Abstract

Background: Internet is a very useful medium for quick access to information, especially for students. Social media applications and their use among students have witnessed dramatic increase in the last decade and data on their effect on students' academic performance are inconsistent.

Objective: The objective of this study was to assess the effects of social media on the academic performance of medical students of a selected tertiary medical college in Dhaka city.

Methods: Cross sectional study was done on 3rd 4th and 5th year MBBS students of Bangladesh Medical College. Data were collected from 205 respondents by a self-administered semi structured written questionnaire. Students were selected by purposive sampling. Information was collected from pertinent variable related to social media and academic performance. Data were analyzed by SPSS, virsion 16.

Results: In the study, mean age of the respondents was 22±SD1.32 years;129**(62.9%)** were female and rest 76 (37.1%) male**.** Using of Facebook was most common among participants (90.7%) followed by using YouTube 86.3%, then WhatsApp 85.4%, Google 74.1%, Instagram 63.9%, Snapchat 37.1%, Imo 22.9%, Twitter 23.4% and viber17.1% respectively. Most of the students 123(60%) used social media for visiting different medical pages and groups, 118(57.56%) for following friends' posts, 54.1% of the respondents spent 3 to 6 hours (mean duration 4.5±2 hours) in social media. Majority51.7% of the respondents stated that social media had both positive and negative effects on academic performance; 21.5% mentioned about positive effects, 21% stated no effects and only 5.9% said that social media had only negative effects on academic performance. Duration of using social media was not associated with regularity (p= 0.3) and academic performance (p=0.403).

Conclusion: Social media has both positive and negative effect on the academic performance as well as social life of students. Respondents in their study felt that time management contributed towards negative academic performance in addition to excessive use of social media.

Keywords: Social media, Academic performance, Medical students

Introduction:

The modern era is embraced in cascade of information technology (IT). One of the recent most impactful IT phenomena is the emergence and spread of a sub-set of IT technologies referred to as social media.[1] Social denotes society-comprising of people and the groups and media is the medium of expressions. One of the biggest and most popular innovations of IT is social media.[2] Social media are, however, qualitatively different from traditional media and on-line communication systems. It has been defined in a variety of ways like Boyd & Ellison defined it as a "platform to create profiles, make explicit and traverse relationships".[3] According to Buffer Marketing Library, Face book, YouTube, What's Up, Messenger, We Chat, Instagram, Tumbler, Twitter, Viber, Pinterest, Snapchat these are most popular social media apps.[4] According to Statista 2019 the number of worldwide social media users reached 2.3 billion and is expected to grow to some 2.95 billion by 2020.[5] Another top level global data (Hootsuite) says, on average, people across the globe spend 2 hours and 16 minutes per day on social media and use an average of 8.9 platforms. At least 30 million people in Bangladesh are using social media.[6] Previous work has shown that between 67%-75% of college-aged individuals are users of social media and another study observed that 90% of college students are using facebook.[7]

Medical student's academic performance plays an important role in producing the best quality graduates who will become great practitioners and workforces responsible for the country's health development, thus the economic growth of a country enhances.[8] Academic performance alludes to how well a student is completing

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his or her assignments or studies and is effected by various internal and external classroom factors. Among these factors, facility-related factors such as classroom facilities and environment, internet access are the key external classroom factor.[9]

Internet is a very useful medium for quick access to information, especially for students. In particular, social networking sites facilitate students in communication, socialization, coordination, collaboration, and entertainment, but internet usage can cause addiction and lead to time-wasting, information overload, and physical isolation from society.[10,11,12] However it is an essential part of our society, which provides easy access to social, political, economic, and academic information's across the world without leaving their place of residence. However, there are drawbacks such as impaired academic performance, health problems, personal relationship problems, and social dysfunctions when it is used in a wrong and uncontrolled way.[13]

Social media also has different role in education.[14] There are several studies worldwide to explore the social media and its effects on life, few of them focused on academic performance of students, very little is known about effect on academic performance of medical students and their perception about SM on social life. So this study aimed to determine the effect of social media on academic performance of medical students as well as their perception about SM on social relationship.

Material and Methods:

A cross sectional descriptive study was conducted from October 2018 to March 2019 to see the effects of social media on academic performance of medical students. We selected 3rd, 4th and 5th year students of Bangladesh Medical College, Dhanmondi, Dhaka. Total 205 students were selected by purposive sampling, who have gone through at least one professional examination of MBBS curriculum, excluding those who were absent in the class during data collection and who did not appear in at least one professional examination yet. Academic performance was determined by regularity of the students with the current batch and by passing in the professional examination. Data were collected by a pretested, semi structured, self-administered written questionnaire after formal permission from the college authority and respective departments. An informed written consent was prepared consisting of proper explanation about study purpose and procedure and with assurance of maintaining proper confidentiality. Data collection had been carried out during class hour. Data were maintained and cleaned up and kept for analysis with proper confidentiality and had been revised, coded and tabulated. Then data had been analyzed by using statistical package for social science (SPSS), virsion 16. Frequency, percent distributions was shown in tables, graphs and figures; chi square test and other appropriate inferential statistics had been used to find association.

Results:

Table 1: Distribution of the respondents by age group, gender and academic year (n=205)

A) Age group	Frequency	Percent
<22 years	125	61.0
22-24 years	70	34.1
>24 years	10	4.9
Mean±SD 22.0±1.32, Age range: 19-27 years		
B) Gender		
Male	76	37.1
Female	129	62.9
C) Academic year		
3rd	101	49.3
4th	49	23.9
5th	55	26.8

Table 1A shows that among the 205 students, majority 61% were <22 years of age, 70(34.1%) were 22 to 24 years of age group and only 10(4.9%) were >24 years of age group. Mean age was 22±SD1.32 years. More than half 129(62.9%) of the students were female and rest 76(37.1%) male (Table 1B). Almost half of the students 101(49.3%) were 3rd year MBBS students, 49(23.9 %) were 4th year MBBS students and 55(26.8%) were 5th year MBBS students (Table 1C).

Table 2: Distribution of respondents by types of social media and device they use and average duration of using social media. (n=205)

A) Types of social media	Frequency	Percent
Face book	186	90.7
WhatsApp	175	85.4
Youtube	177	86.3
Google	152	74.1
Instagram	131	63.9
Snap chat	76	37.1
Imo	47	22.9
Twitter	48	23.4
Viber	35	17.1
B) Types of device used for social media		
Computer	29	14.1
Laptop	86	42.0
Mobile phone	199	97.1

**Multiple response

Table 2A shows that using of Facebook was most common among participants 90.7% followed by using YouTube 86.3%, then Whats App 85.4%, Google 74.1%, Instagram

63.9%, Snapchat 37.1%, Imo 22.9%, Twitter 23.4% and viber17.1% respectively.

Almost all the respondents 97.1% used smart mobile phone for social media followed by laptop or tablet 42.0% and Personal computer 14.1% as shown in Table 2B.

Table 3: Distribution of respondents by reasons behind using social media

Reason behind using social media	Frequency	Percent
Visit medical pages and group	123	60
Follow friends posts	118	57.56
Kill spare time	98	47.80
Study together in groups in fb messenger	82	40
News	78	38.04
Fashion and styles	41	20
Sports	30	14.6
Buy products	26	12.68
Others	10	4.87
*multiple response		

Table 3 shows that most of the students 123(60%) used social media for visiting different medical pages and groups, 118(57.56%) for following friend's posts, 98(47.80%) to kill spare time, 82(40%) students used social media for studying together in messenger groups, 78(38.04%) used to get news, 41(20%) and 30(14.6%) used for fashion and sports respectively.

Table 4: Distribution of respondents by average duration of using social media

Average duration of using social media	Frequency	Percent
< 3 hours	70	34.1
3-6 hours	111	54.1
> 6 hours	24	11.75
Mean±SD 4.50±2.5 hours, Min 0.5 hours, max 20 hours		

Table 4 shows that more than half 54.1% of the respondents spent 3 to 6 hours in social media, 34.1% less than 3 hours and only 11.75% used more than 6 hours per day in average.

Table 5: Distribution of the respondents by Academic performance in last professional examination and regularity (n=205)

A) Academic performance in last professional examination	Frequency	Percent
Passed	197	96.1
Referred	8	3.9
B) Regularity		
Regular	178	86.8%
Irregular	27	13.2%

Table 5A shows by academic performance in last professional examination, 197(96.1%) passed and only 8(3.9%) referred in one or more subjects.

Among the 205 respondents 178(86.8%) were regular in study and 13.2% students were irregular (Table 5B).

Table 6: Distribution of the respondents by effects of social media on academic performance according to student's opinion

Effects of social media on academic performance	Frequency	Percent
No effect	43	21
Effects positively	44	21.5
Effects negatively	12	5.9
Both	106	51.7
Total	205	100.0

Among the 205 respondents more than half 106(51.7%) stated that social media had both positive and negative effects on academic performance, 44(21.5%) mentioned about positive effects, 43(21%) stated no effects and only 12(5.9%) said that social media had only negative effects on academic performance (Table 6)

Table 7: Relation between total duration of using social media and academic performance in terms of regularity and outcome of professional examination

A) Average duration of using social media	Regular	Irregular	Total	p value
<3 hours	60 (85.7%)	10 (14.3%)	70 (100%)	P=0.381
3-6 hours	95 (85.6%)	16 (14.4%)	111 (100%)	
>6 hours	23 (95.8%)	1 (4.2%)	24 (100%)	
B) Average duration of using social media	**Passed**	**Referred**	**Total**	
<3 hours	69 (98.6%)	1 (1.4%)	70 (100 %)	P=0.403
3-6 hours	105 (94.6%)	6 (5.4%)	111 (100%)	
>6 hours	23 (95.8%)	1 (4.2%)	24 (100%)	

Table 7A shows, among the students using social media less than 3 hours 85.7% were regular and students using more than 6 hours 95.8% were regular. Duration of using social media was not significantly associated with regularity (p= 0.3). Among the 70 students who use social media less than 3 hours 98.6% passed and among 24 students who use social media more than 6 hours, 95.8%

passed in the last professional examination. No statistical association between duration of using social media with outcome of professional examination was found (p=0.403).

Table 8: Students' opinion about relation between average duration of using social media and effects on academic performance.

Effects of social media on academic performance	Average duration of use social media		
	< 3 hours	3 to 6 hours	>6 hours
No effect	20 (28.6%)	17(15.3%)	6(25.0%)
effects positively	16(22.9%)	23(20.7%)	5(20.8%)
effects negatively	9(12.9%)	3(2.7%)	0(.0%)
Both	25(35.7%)	68(61.3%)	13(54.2%)
Total	70(100.0%)	111(100.0%)	24(100.0%)

Here it is evident that average duration of using SM is associated with academic performance as p = 0.05 according to students' opinion.

Discussion:

Social media websites and applications as well as the number of students using them have witnessed a dramatic increase over the last decade and became an integral part of students' daily life. In the current study among the 205 students majority 61% were less than or equal to 22 years of age 34.1% were 23 to 24 years of age and only 4.9% were more than 24 years of age. Mean age was 22 years with SD±1.32 and more than half 62.9% of the students were female and rests 37.1% were male. According to academic year distribution of the students 49.3% were 3rd year MBBS students, 23.9% were 4th year and 26.8% were 5th year MBBS students (Table 1). The study[15] of Jazan university included 205 (45.1%) male students and 250 (54.9%) female students, the large majority of them were from the second medical year 139(30.5%) followed by those from the third year 110(24.2%), fourth year 102(22.4%) and finally fifth and sixth years 55(12.1%) and 49(10.8%) respectively.

Using of facebook was the most common in our study, that is 90.7% among the participants followed by using Whats App 175(85.4%) then YouTube 177(86.3%), Google 152(74.1%), Instagram 131(63.9%), Snapchat 76(37.1%), Imo 47(22.9%), Twitter 48(23.4%) and viber 35(17.1%) respectively (Table 2A). The study [15]done in Jazan University; showed that the most common used sites were facebook (53%) followed by using twitter (35.6%) then Instagram and WhatsApp (34.3%) and (28.8%) respectively. Whereas the study[16] conducted among the medical students of university of Sharjah, UAE found among 350 students (74% females and 26% males) majority of used Facebook (93.5 %) followed by YouTube (87.9%), Instagram (62%), Twitter (56.9%), Google+ (40.3%) and finally, LinkedIn (5.1%).

In our study all most all the respondents 199(97.1%) used smart mobile phone for social media followed by laptop or tablet 86(42.0%) and Personal computer 29(14.1%)as shown in Table 2B. The study of Jazan university [15] revealed the first ranked method to check SNSs (Social Networking Services) were smart phones (80.3%) followed by laptops (23.4%), Tablets (8.5%) and PCs (2.5%).

This study showed that the students 123(60%) used social media for visiting different medical pages and groups, 118(57.56%) for following friend's posts, 98(47.80%) used SM to kill spare time, 82(40%) students used social media for studying together in messenger groups, 78(38.04%) used to get news, 41(20%) and 30(14.6%) used for fashion and sports respectively (Table 3). The previous study[15] found that the most common cause for using social media was fun (65.9%) and the least common cause was avoiding stress and boring (13%). Similar picture was found in study[17] done by university of Babylon in Iraq, where they found that The two main reasons were visiting medical pages and groups (57.9%) and to communicate with friends and follow their posts and updates (54.4%). Other reasons were killing spare time (45.6%), studying in groups on facebook messenger (40.4%), news (38.6%), fashion and styles (22.8%) and sports (15.8%). Another study[18] in Bangladesh also found that the main purpose of using the Social media for non-academic purposes such as, communicate with others 26.0%, for chatting 51.3%.

In the current study more than half 111(54.1%) of the respondents spent 3 to 6 hours in social media, 70(34.1%) less than 3 hours and only 24(11.7%) used more than 6 hours per day (Table 4). In the study[19] conducted in Saudi Arabia, it was found that of internet users 23(38.3%) spent on average 4-6 hours per day in social media, 17 (28.3%) spent 1-3 hours, 16 (26.7%) spent more than 6 hours on internet for social media and four (6.7%) were not sure how much time they spent on internet for social media. Overall, the largest part of the respondents use social media 4-6 hours per day. Another study[20] found around 25.7% students were using Social networking sites for 1-2 hours per day whereas in the study[21] done by El-Badawy around 33% students were using social media for 1-3 hours a day. In the earlier mentioned study[15] the large majority 58.5% of students used social networks from 2 to 4 hours and the least percent 3.3% used them for more than 10 hours.

In our study out of the 205 respondents more than half 106(51.7%) stated that social media had both positive and negative effects on academic performance, 44(21.5%) mentioned about positive effects, 43(21%) stated no effects and only 12(5.9%) said that social media had only negative effects on academic performance (Table 6). Similarly, Aljabry AM and et al[15] in his study revealed, 88.1% of the respondents expressed that SNS have a positive impact on teaching and learning while 45.6% stated that they had a positive effect on academic

performance. While in another study[22] almost 40% of the students complains of poor academic performance as an effect of social media while 50% of the students find it to have no effect on academic performance. A study[23] from Khartoum University demonstrated, 91% respondents said that using the social media effected negatively on all students' academic performance which was dissimilar to our study. Another study[24] from Nigeria showed that 28% of users agreed that there was a negative effect of using the social sites on their personal academic performance.

Here it was evident in our study that average duration of using SM was associated with academic performance according to students' opinion ($p = 0.05$) as shown in Table-8. Another study[25] has shown a negative relation between usage of SNSs and GPA (Grade point average), which was similar to results from other studies[26] in this contexts that duration of time spent on SM was strongly and significantly negatively related to overall academic performance. Whereas previous study[21] concluded that excessive time spent on social networking sites significantly affects student's academic performance. The study[15] of Jazan University stated that both numbers of hours and using social media during lectures influenced academic performance of students negatively. Similarly the study [27] it was revealed that the use of social media had affected the academic performance of their respondents negatively, another study[28] in Kogi State University found out that the exposure of the students to social media have negative effect on their academic performance. Students who spend more time on social media are likely to perform poorly in their academics. But dissimilar picture was seen in the previous study[19] where they found a strong positive relationship between the use of social media and academic performance. Based on the study[29] it was found that social media usage displayed a positive skew where most students do not

In this study we couldn't find any statistical association between student's regularity and course achievement/ performance in professional examination with duration of using social media. Supporting this fact, several studies turned out that the internet occupies a specific place in the life of students. Majority of the students cannot leave their phone even during the class session. Majority of them spend more time in using internet every day, but it does not interfere with their educational process.

Conclusion

The current study concluded that facebook was the most common social sites used by medical students followed by whats app and youtube. Most of the students (60%) use social media for visiting different medical pages and groups. According to students' opinion social media has both positive and negative effect on the academic performance as well as social life. No association was found between duration of using social media and academic performance. Social networking sites facilitate students in communication, socialization, coordination, collaboration, and entertainment, but internet usage can cause addiction and lead to time-wasting, information overload, and physical isolation from society. So management of time is a crucial factor for the students to make a balance between use of social media and learning.

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Review Article

Hydranencephaly-the lost brain

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Abstract

Hydranencephaly is a clinical condition that presents with classic features of absent cerebral hemispheres that is replaced by a membrane bound sac containing the ependyma, cerebrospinal fluid and glial tissue, and is commonly misdiagnosed as an extreme case of hydrocephalus, alobar holoprosencephaly or porencephalic cysts. This article provides a comprehensive review of hydranencephaly including the presentation in an infant admitted in Bangladesh Medical College Hospital.

Keywords: Hydranencephaly, Severe hydrocephalus, Brain malformation.

Introduction:

Hydranencephaly (HE) is a rare condition where there is absence of the bilateral cerebral cortex within intact meninges and skull with the resulting cavity being filled with cerebrospinal fluid.[1] Postnatal cases are highly uncommon, with only 1 in 10000 live births are seen, and could be attributed to intrauterine demise of fetus.[2] Interestingly, cases have been reported where parts of the occipital and temporal lobe had developed.[3] Components such as the choroid plexus, cerebellum, thalamus, mid brain and basal ganglia are commonly unaffected.[4-5] Hemihydranencephaly is a rare occurrence where bilateral occlusion within carotid arteries causes only one lobe to be affected [6-7] and usually occurs around the second trimester and could be caused by a variety of reasons.[8-9]

Historical Background:

The first ever reported case was described by a French anatomist, Jean Cruveilhier[10] where he discussed his findings from two patients possibly suffering from HE from the years 1829 to 1835. HE was given its name by a German neuropathologist, Walther Spielmever, who based his research on a pair of twins afflicted with the condition, whose autopsy reports indicated some nerve tissue and hemorrhagic cerebral lesions which were accounted for by the presence of vascular anomalies such as blood vessels with very thin walls.[11] Other earlier case studies were able to successfully differentiate HE from anencephaly, based on involvement of the cerebral vault in the latter.[12] Teratogenic agents and a variety of infections have been associated with HE in both clinical and pathological contexts. The highest survival numbers ever reported in a study was 15 patients over a period of time, followed by another study which integrated findings from 37 publications and 76 patients, and reported survival rates of patients between the years 2000-2012.[13-14] HE accounts for 1% of all hydrocephalus cases, that have been incorrectly diagnosed.

The etiopathogenesis of hydranencephaly is still largely unknown but a few studies suggest early internal carotid artery involvement. Other research showed angiographic and autoptic observation of aplasia or hypoplasia in the distribution of internal carotid artery and therefore categorized HE to be a variant of circulatory developmental encephalopathy.[8,13,15]

There are two main hypotheses to explain this severe malformation, the first being the destructive theory, which explains destruction of cerebral hemispheres following partial or complete development of the ventricles which are then diminished in utero, possibly due to encephaloclastic activity. It most commonly occurs during the 2nd trimester of pregnancy, at a stage where fetal cerebral hemispheres have formed and just before development of the brain stem, mesencephalon and diencephalon.[5]

The other hypothesis indicates a dysontogenic pathway that causes disturbance in early organogenesis.[15] Molecular dysfunctions as reported with other cerebral anomalies may also be postulated i.e. a mutation in COL4A1 was found in a study and linked to porencephaly resembling hydranencephaly.[16] Cytogenetics revealed triploidy in two out of four patients in a study[13] but also concluded that cerebral artery occlusion on top of the supraglenoid level seemed to be the most likely hypothesis.

Most cases are diagnosed within 13-26 weeks into pregnancy, however some cases have been detected as early as before 12th gestational week,[17] which conjures theories of the occurrence of certain pathogenetic events in early neurogenic phase, that ultimately lead to HE.

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Etiopathogenesis:

There is formation of a pial-glial-ependymal membrane. There is compensatory accumulation of CSF that causes tremendous loss of neopallium and results in destruction of germinal layer of the neurons due to viral activity. It is the effect of viral agents on developing vasculature. The severe destruction depends on virus' components and the respective gestational period when infection happens. and the period during gestation when the infection occurs. The glutton virus is reported to produce this effect on experimental models.[18] Experimental studies of direct inoculation of the vaccine into fetal sheep produced lethal necrotizing encephalitis during 50-58 days of gestation. In later period it caused less severe multifocal encephalitis resulting in porencephalic cyst. It was shown that the germinal cells of Telencephalon are very susceptible to this infection and they are mostly growing at end of 1st trimester in lamb fetus. When there is necrosis of germinal layer, it leads to destruction of neopallia cortical plate resulting HE.[18]

Intrauterine infections, especially Toxoplasmosis[19] and some viruses i.e. Adenovirus, Enterovirus, Parvovirus, Herpes simplex, Epstein-Barr virus, Respiratory syncytial virus, Cytomegalovirus have been linked to some cases.[20-23] Toxin and drug exposures like smoking, cocaine abuse,[24-27] estrogen[28-29] and sodium valproate[30] among others, have also been reported. There is a study where HE is associated with young maternal age.[31] There is a case report where the demise of one twin caused vascular changes in the other twin, resulting in HE in the surviving fetus.[32-33] In another study they showed deficiency of fibrin stabilizing factor[34] and intracerebral hematoma[35] may be causal events. All the reported causes could disrupt the internal carotid artery circulation resulting in profound changes in brain. So some authors think that HE is not a brain malformation it is rather a disruption pathological events which causes ischemic circulation in the region of internal carotid artery.

Neuronal-Pathologic Features:

There is a severe lack of uniformity in post mortem analyses in the reported case studies where most display a variable amount of remnant of cortex. This is provided by posterior circulation and significantly the basal section of the temporal cortex, the medial frontal cortex, and the midline cortex tissue alongside the flax, with the inferomedial occipital areas being uninvolved. This lack of cortex displayed thin walled cysts that were filled with fluid. This slim membrane that replaces the cortical tissue, is largely made of nervous tissue at immature stages, without any viable neurons. These sacs tend to inflate because of pressure by fluid and microscopic analysis of such tissues revealed them to be gliotic or to be structurally abnormal, resulting in loss of appropriate function.[39] Some new studies have demonstrated that the stratum, brain stem, thalamus and cerebellum are unaffected and some basic histological structures are preserved.[36-38]

Clinical features:

The mother does not feel any significant symptoms during the pregnancy and the fetus usually has normal head circumferences and in some cases may be microcephalic. But sometimes, the head tends to enlarge due to malabsorption of continuously produced CSF (Cerebrospinal Fluid) by the choroid plexus. In some cases, the intracranial pressure rises so high resulting in and enlarged head which leads to flax cerebri rupture. The extremely low number of infants that survive this stage do not show and clinical signs in this initial period. Sucking swallowing reflux, leg and arm movement are usually present. However, there is feeble crying, feeding difficulty, hypotonia, with wide anterior frontanelle. After few more days these signs become more evident. There may be convulsions which may arise from remaining cortical rim or brain stem. The surviving kids usually have visual impairment, spastic diplegia and cognitive failure.

In 2014, a 3-month male baby was referred to Bangladesh Medical College and Hospital. His birth history was unknown as he was adopted by his foster parents from an orphanage. His OFC (Occipito-frontal Circumference) was 44cm and had episodes of seizure attack. All the Blood parameters were normal. CT scan was done and it showed typical features of HE. His both cerebral cortexes were absent, mid line and posterior fossa structures were preserved. His EEG (Electroencephalogram) showed flattened and dispersed activity with paroxysmal activity being sporadic and slow. He was operated for rapid enlargement of head, only aim was to nurse the child. He had repeated attacks of status epilepticus with increasing severity. Antiepileptics in highest and combined doses failed to control the attacks, it seemed like drug resistant epilepsy. There was no neurological improvement following Ventriculo peritoneal shunt surgery only head size was under control. The baby expired at the age of 9 months during seizure attack. No autopsy was done.

Diagnosis:

- HE (Fig-1) can be diagnosed at 21st and 23rd gestational week by USG, which shows the lack of the cerebral cortices, with mid brain and posterior fossa structures being present.[40] After delivery CT (Computed Tomography) and MRI (Magnetic Resonance Imaging) can diagnose HE and differentiate it from severe forms of congenital hydrocephalus and holoprosencephaly. An EEG is used to confirm seizures and their types and is relevant to treatment. Auditory and ocular testing is sometimes advised as well. Angio MRI can be done to detect abnormalities in cerebro vascular components.

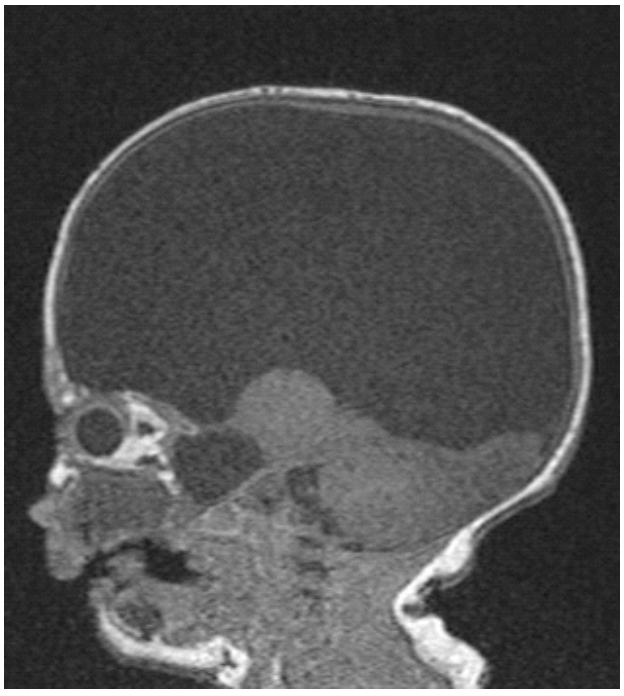

Fig 1: Hydranencepphaly

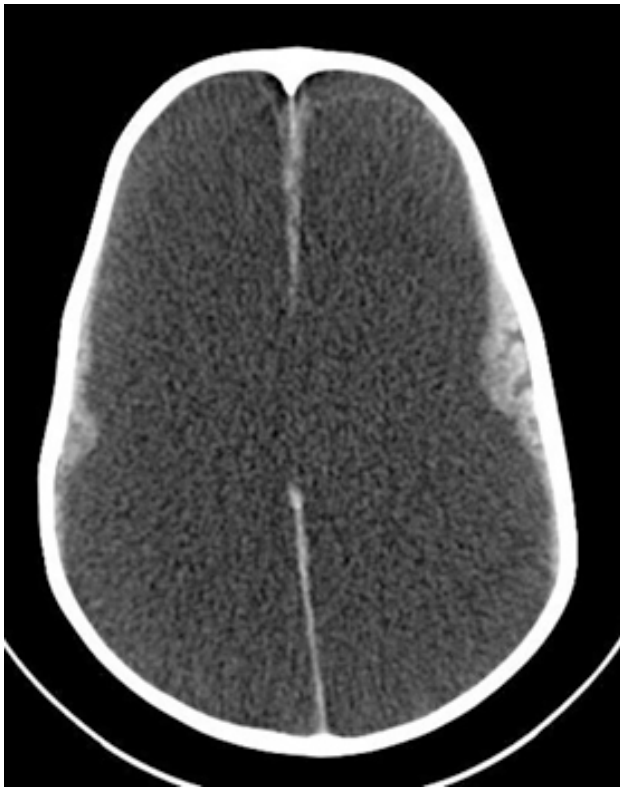

Fig 2: Extreme Hydrocephalus

Differential Diagnosis:

Severe Congenital Hydrocephalus (Fig 2) mimic HE, with a difference being the existence of a thin and intact cortical rim. The infants' head circumference is also taken into account as patients with HE have either normal or smaller measurements. An angiograph indicates whether there is bilateral occlusion of the internal carotid artery. There is difference of outcome of the babies affecting Hydrocephalus and HE. Where extreme Hydrocephalus babies shows neurological and radiological improvement after surgical treatment whereas HE babies shows none.[41]

In porencephaly (Fig-3), the sacs are usually situated in the mid cerebreal region and is linked to ischemic infarct resulting in cortical destruction may mimic HE. The difference is that the frontal and parieto-occipital cortex in case of Porencephalic cyst is retained. Head circumference of porencephalic infants are small and usually have facial deformities, unlike hydranencephaly patient.

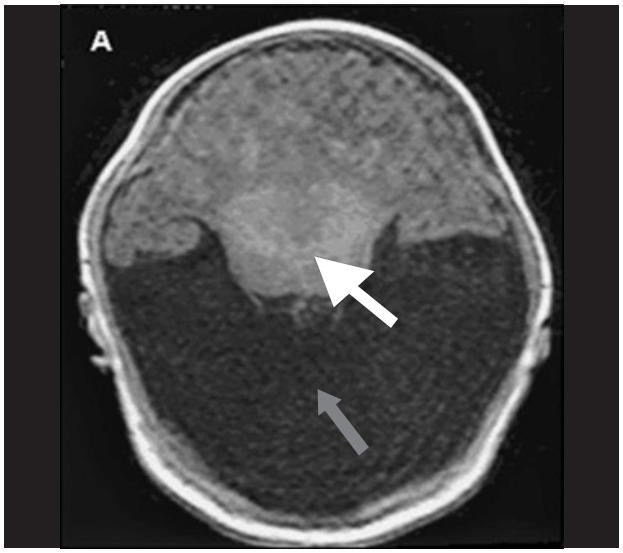

Fig 3: Porencephalic cyst

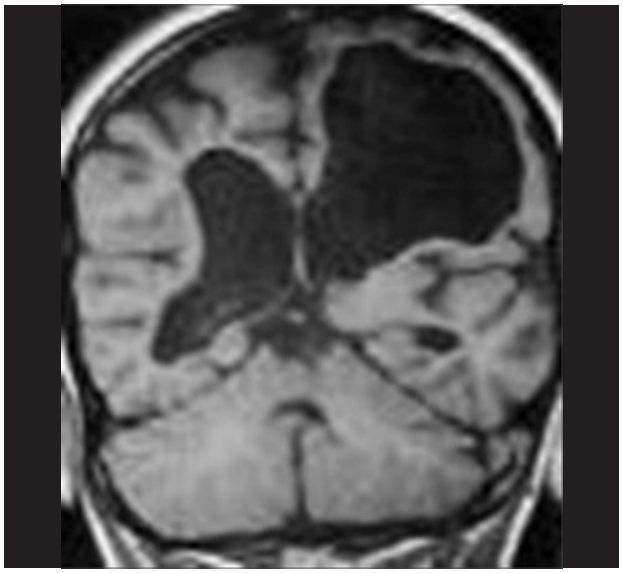

Fig 4: Holoprocencephaly

Holoprosencephaly (Fig 4), especially in alobar form is confused with alobar HE. In the former, slight fusion of the thalami is detected and the falx cerebri is absent.

HE sometimes associated with other disorders, Taori et al[42] reported a case study of HE, where the cerebellum, micro opthalmias and colobomas were involved. Watts et al[43] discussed a case study where there was presence of intracranial calcifications and chorio retinal dysplasia. Kelly et al[44] reviewed a case where there was cerebellar hypoplasia and presence of posterior masses that were calcified, and relevant to the posterior cerebral artery occlusion. HE has been linked to penoscrotal transposition, deletion at [13] (q22) [47] and renal dysplasia.[45-46] HE may be a part of autosomal recessive syndrome where there is Hydranencephaly and proliferating vasculopathy.[48-52]

Prognosis:

Prognosis of HE is extremely poor as most infants die in utero. In live births, death mostly occurs within the first year and multidrug resistant, seizure, development delay, repeated respiratory tract infections and spasticity are common. However, patients have been reported to have survival rates of 20[53] and 32 years.[13] Patient survival is contributed from brain stem.[2]

Treatment:

There is debate whether to perform any surgery as there is brain impairment in severe amounts. The most common surgical treatment is Ventriculo peritoneal shunt, and it reduces the tension and decreases cerebral volume. Sometimes Endoscopic third ventriculostomy and Choroid plexus coagulation is advocated. For the surviving kids Physiotherapy, nutritional care and management of seizures is required.

Conclusion:

HE is a rare disease and results in very poor life expectancy. Expecting women should be strictly monitored with USG. However proper diagnosis is very important as in fetal life it can offer the option for abortion. The next big issues are whether surgical treatment is viable, as well as the fact that no prevention methods of HE exists.

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Case Report

A rare presentation of hemoglobin D trait: Case report

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Abstract

Hemoglobin D heterozygotes *(*Hb D trait*)* are usually clinically silent condition with no anemia and normal red cell indices. But co-inheritance of Hb D with sickle cell or thalassemia produces clinically significant conditions like sickle cell anemia or thalassemia intermedia and chronic hemolytic anemia of moderate severity. Here we, present a case of symptomatic Hb D trait, who presented with hemolytic anemia and splenomegaly from Dhanmondi, Dhaka which is a rare phenomenon.

Keywords: Hemoglobin D trait, Hemolytic anemia.

Introduction:

There are several hemoglobin D variants, amongst them Hb D-Punjab (also known as Hb D- Los Angeles) is by far the 4th common hemoglobin variance.[1,2] Hemoglobin D disease is very rare and affects both sexes equally. The disease occurs most often in people whose ancestors come from Pakistan and Northwestern India and Iran. It also occurs in people from England, Ireland, Holland, Australia, China and the Middle East.[3, 4] Structural form of Hb D is β 121 Glu-Gln.[5] Subsequently, several hemoglobin's have been described that have the same electrophoresis pattern and solubility as Hb D, and each has a mutation within the β globin gene. These include (Hb D-Iran, β 22 Glu→Gln) Hb D-Bushman (β 16 Gly-Arg), Hb D-Ouled Rabah (β 19 Asn-Lys), Hb D-Granada (β 22 Glu-Val), Hb Iran (β 22 Glu-Gln), Hb D-Ibadan (β 87 Thr-Lys), Hb D-Los Angeles (β 121 Glu-Gln), and Hb D-Neath (β 121 Glu-Ala).[6-8] Hemoglobin D-Punjab occurs with greatest prevalence (2%) in Sikhs in Punjab, India, whereas Gujarat, the province in the west from where the case was reported, has a prevalence rate of 1%. Hb D occurs in four forms: heterozygous Hb D trait, Hb D-thalassemia, Hb S-D disease and the rare homozygous Hb D disease.[2, 9] Hb D has a S-like mobility on alkaline electrophoresis but co-migrates with HbA on acid pH. Osmotic fragility may be decreased. Blood films may show target cells.[10]

Hemoglobin D heterozygotes (Hb D trait) are usually asymptomatic with no anemia and normal red cell indices.[11] Hb D homozygotes (Hb D disease) manifests as mild hemolytic anemia and splenomegaly.[12] Here we present a case of symptomatic Hb D trait, who developed hemolytic anemia and splenomegaly which is a rare phenomenon. This case highlights the propensity for occurrence of rare phenotypes within our multi-ethnic population and emphasizes the importance of accurate genotyping to avoid erroneous counselling, and to plan an effective patient management strategy before complication evolves.

Case Presentation:

A 40-years-old lady hailing from Dhanmondi, Dhaka with no comorbid illness came with shortness of breath, which was insidious in onset and gradually progressive since 2 months and jaundice for 15 days' duration. She also had dizziness, easy fatigability and generalized weakness. It was also accompanied with left-sided abdominal pain since 2 months and passing dark colored stool intermittently for last 15 days. She had no history of chest pain or palpitations or weight loss or hematuria or hematochezia or hematemesis. She also had no history of leg ulcers or dark-colored urine. She gave a history of 1–2 episodes of jaundice in the past which subsided on its own without any medication. Patient also had a history of fever two months back prior to onset of above mentioned symptoms which lasted for one-week duration and subsided on its own. There was no past history of blood transfusion. She had 1 child. Patient was non-alcoholic and non-smoker. No history of consanguineous marriage was reported in the family but she had family history of similar complaints with grandfather and his younger brother (age 32 years) having recurrent episodes of jaundice. Although Hb electrophoresis couldn't be able to perform among family members as they lived in rural area and had financial issues. On general examination, no feature of hemolytic facies was observed. She had marked pallor and mild icterus. Her

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pulse was 74/min and blood pressure was 120/75 mm Hg. However, there was no edema, cyanosis, clubbing, or lymphadenopathy. Systemic examination revealed splenomegaly; spleen was palpable 6 cm below the left costal margin. Her rectal examination showed no hemorrhoid. Cardiovascular, respiratory, and nervous systems were normal.

Her initial investigations showed Hb (6.8 g/ dl) with a low packed cell volume (PCV) of 22.70%, Mean corpuscular volume (MCV) 72.20 fL, Mean corpuscular hemoglobin (MCH) 24.60 pg/cell, Mean corpuscular hemoglobin concentration (MCHC) 31.30 g/dL thus blood indices indicating microcytic hypochromic anemia. TLC 7970/mm^3, differential count (DLC) - polymorphs 65 %; lymphocytes 22%; monocytes 4%; eosinophils 1% & platelet 2,54,000/mm^3. Reticulocyte count was 1.5%. Serum LDH was high 314 U/L (reference: <247 U/L), serum total bilirubin was 5.18 mg/dL; direct was 0.74 mg/dL and indirect was 4.44 mg/dL indicating indirect hyperbilirubinemia in favor of hemolytic jaundice. Serum total protein level was 7.41 g/dL, albumin was 4.32 g/dL, globulin was 3.13 g/ dL, Albumin/Globulin (A/G) ratio was 1.38, serum glutamic-oxaloacetic transaminase (SGOT) was 24.0 U/L, and serum glutamic-pyruvic transaminase (SGPT) was 16.0 U/L ruling out any hepatocellular jaundice. The level of serum iron was 136 µg/dL (reference: 60-180 µg/dL in female), serum ferritin was 574.90 ng/ mL (reference: 11.0–306.8 ng/mL in female) and serum vitamin B12 level was 801.0 pg/mL (reference: 211-911 pg/mL). Other hematological investigations were as follows: direct and indirect Coombs tests and ANA were negative, which exclude the diagnosis of autoimmune hemolytic anemia. Osmotic fragility test was normal, serum G6PD and pyruvate enzyme levels were normal, and sickling test was negative. Serum immunoglobulin G level was 13.4 g/L (reference: 7.51-14.60 g/L) and immunoglobulin M level was 1.02 g/L (reference: 0.46-3.04 g/L), which were normal. Her urine routine analysis was normal, and stool routine examination did not show any occult blood or ova, parasite, or cyst. Other blood biochemistry levels were as follows: blood urea 14.0 mg/ dL (reference: 13-43 mg/dL), serum creatinine 0.81 mg/ dL (reference: 0.51-0.95 mg/dL), serum sodium 137.0 mmol/L (reference: 136-145 mmol/L) and serum potassium 4.0 mmol/L (reference: 3.5-5.1 mmol/L). Random blood sugar level was 6.8 mmol/L. Serum thyroid-stimulating hormone level was normal. Ultrasonography of abdomen showed cholelithiasis (8-mm-sized gall stone with posterior acoustic shadow) and splenomegaly (splenic span, 12.6 cm). Upper gastrointestinal endoscopy and Two-dimensional echocardiography were normal.

After clinical examination and subsequent investigations, possibility of hemoglobinopathies was considered as the cause of hemolytic anemia. Hemoglobin electrophoresis was ordered on blood sample collected before blood transfusion, and it showed abnormal band in Hb-D region suggestive of Hb D trait, with Hb-A, 59.5%, Hb-D 38%, and Hb-A2 2.5% [Figure 1].

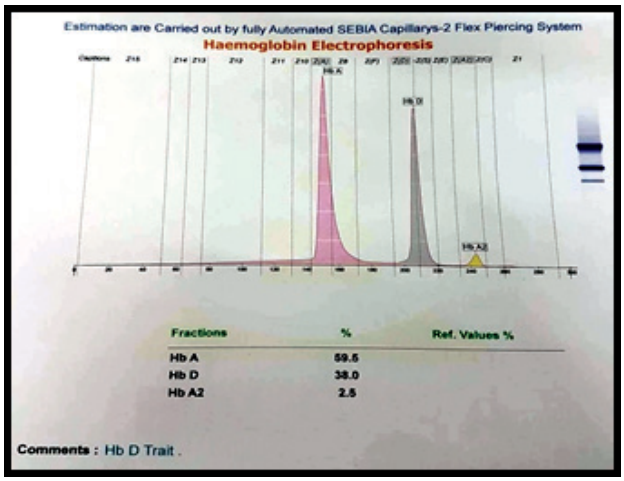

Figure 1: Hemoglobin electrophoresis showing Hb D trait

She was given two PCV transfusion and folate supplements. The patient was counseled regarding her diagnosis and was discharged on day 6 with Hb level 9.8 g/dl. Routine follow-up was scheduled for her subsequent visits to monitor her condition and assess her prognosis. She was also advised to consult with surgical specialist for management of gall stone.

Discussion:

Hb-D Punjab can be inherited as a homozygous component or as a heterozygous trait with normal Hb-A. The trait presents with no clinical or hematological alterations. However, when co-inherited with another variant of Hb such as sickle cell or thalassemia, it may present with clinically significant conditions often requiring hospital admissions and blood transfusions.[13] Although Hb-D is not uncommon in India, its homozygous form is very rare and very few cases have been reported.[14] Hb variants usually are the consequence of single amino acid substitutions caused by point mutations in genes encoding globin chains, resulting in a tetramer with different physicochemical characteristics.

According to the Globin Gene Server database, 1198 Hb variants were described until September 2014. Most of the Hb variants described do not cause symptomatic clinical manifestations; however, in some cases, they can be associated to relevant pathophysiology, for example, Hb-S. Hb-S is the most frequent Hb variant in the world; its clinical outcome is severe in homozygous or in association with other relatively common hemoglobinopathies, such as beta-thalassemia, Hb-C, or Hb-D.[15] The homozygous component Hb-DD, the rarest form of inheritance, is not commonly related to symptomatic cases, but occasionally individuals with this profile can develop mild-to-moderate hemolytic anemia and splenomegaly.[16,17] Hb D trait usually does not produce any clinical symptoms or hematological manifestations. Coinheritance with HbS mimics sickle cell disease.[18] She had HbD of only 16% with tests for sickling

and hemoglobin electrophoresis not suggestive of associated sickle cell disease. Our patient with Hb D trait manifested with hemolytic anemia and splenomegaly. The patient also had cholelithiasis, which is commonly seen with hemolytic anemia. Low-dose hydroxyurea (10 mg/kg/day) was found to be effective in reducing the clinical severity in patients with Hb-SD Punjab without any short-term toxicity,[19] but there has been no trial in patients with homozygous Hb-D Punjab. Some patients may eventually benefit from splenectomy.

Conclusion:

According to our extensive literature review and to the best of our knowledge Hemoglobin variants such as Hb-S and Hb-D are extremely rare in the multi-ethnic Bangladeshi population. However, demographic changes such as population migration, miscegenation causes new spectrum of inherited hemoglobin disorders to emerge. Therefore, it is important to make a precise genotype diagnosis to facilitate error free counselling and proper management Hb D disease. A combined data from electrophoresis at alkaline and acid pH, and the sickle solubility test enable definitive identification of HbA, HbF, HbS, HbC, and several others rare variants.

Declaration of Patient Consent:

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interests: There are no conflicts of interest.

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Case Report

Resection of bilateral large Tendo-Achilles Xanthomas with a Tendon Sparing Technique: A case report

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Abstract

Xanthomatosis is a pathological condition in which large benign tumors can occur in tendon, especially in the Tendo-Achilles. We report a case of twenty four years old housewife came from Norshingdi with large bilateral Tendo-Achilles, Left one is larger than right , presented to department of Orthopaedics on 18th September 2019. Xanthomas in which both tumors were resected. Approximately fifty percent of both Achilles tendons could be spared, so no reconstruction using autograft tendon was necessary. Postoperative treatment consisted of six weeks lower leg cast immobilization. After twelve weeks the patient was able to walk pain free with wear footwear without any difficulties. With fewer complications compared to total excision with augmentation. Twelve months after surgery our patient had no signs of recurrence of Achilles tendon swelling.

Keywords: Xanthomatosis; Tendon tumors; Tendo-Achilles; Benign tumor.

Introduction:

Large benign tumors in tendons are uncommon. Xanthomatosis is clinic-pathological condition in which such large tumors can occur. They are usually slow growing tumor. The cause is an accumulation of fatty lipids in tendons, especially the Tendo-Achilles. The benign bilateral Achilles tendon fat accumulation is an uncommon disease.[1, 2] It is mostly found in patients with heterozygous familial hypercholesterolemia (FH) or, less common, in patients with cerebro tendinosis xanthomasis.[3] In patients with heterozygous FH, Xanthomatosis becomes increasingly common from the third decade of life.[4] FH is characterized by increased LDL cholesterol, tendon Xanthomas, coronary disease and autosomal dominant vertical transmission.[1]

We report a case with large bilateral Tendo-Achilles Xanthomas, not known with FH, in which both tumors were surgically resected with a tendon sparing technique. This is contrasts to other reports that described the necessity of tendon reconstruction when surgically resecting large Xanthomas.[3,5]

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Case Presentation:

A twenty-four years old housewife presented at our hospital for a second opinion. She had complaints of pain and swelling in both Achilles tendons for a long time (Figure 1a). The swellings started several years back and were progressive. Finally she had difficulties in walking and increasing pain especially during long distance walking (> 2.5km) .

Medical history showed no history of episode of coronary heart disease. She was not known with FH. The primary treating orthopedic surgeon performed a non-invasive work up: the results of clinical presentations, (Details Lipid profile, Eye examination not done). Plain X-ray of ankle and an ultrasonography (Figure 1b & Ic) of both tumors were inconclusive. Subsequently a biopsy from the right Achilles tendon tumor was taken, which showed signs of an old hematoma. Although a malignancy was excluded, no clear diagnosis could be made. Conservative treatment with non-steroid anti-inflammatory drugs and cast immobilization did not cause any improvement and the patient was referred to us. Because the diagnosis was highly suggestive for Xanthomatosis, we decided to excise both tumors in two separate sessions, with the intention only to augment the Tendo-Achilles when it appeared necessary to remove more than fifty percent of the Tendo-Achilles. This was not the case in both procedures. Postoperative treatment consisted of two (02 wks) weeks non-weight bearing lower leg cast immobilization followed by four weeks with lower leg non-weight bearing cast. After this period the patient was treated by a physiotherapist to regain muscle strength and ankle mobility. After one week second procedure was done. After twelve weeks (12 wks) of procedure the patient had full range of motion of the ankle and was able to walk pain free

en wear footwear without any difficulties. She could perform a bilateral single foot heel raise. 12 months later the swellings did not recur and patient was able to walk long distances and works her daily works without any pain. Strength assessment of ankle plantar flexion with a hand-held dynamometer revealed strength of 200 Newton at the right ankle and 190 Newton at the left ankle. This corresponds to normal plantar flexion strength for an older woman.[6]

Pathology report after surgery confirmed that the characteristics of both excised tumors were consistent with those of Xanthomas. Patient's serum LDL cholesterol was obtained after the second surgical procedure. In our laboratory this was at the slight higher limits of normal (3.2 mmol/L).

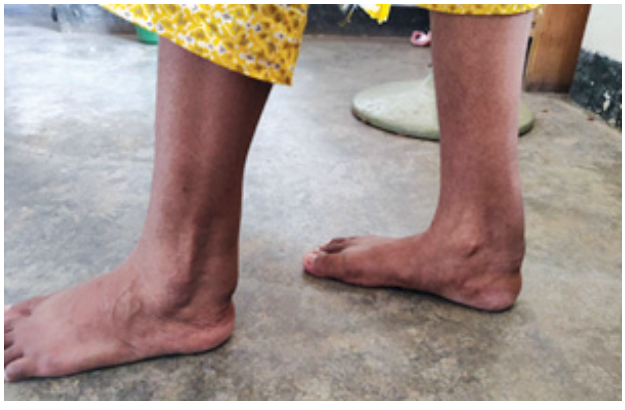

Figure 1a: Clinical presentation of bilateral large Tendo-Achilles xanthomas.

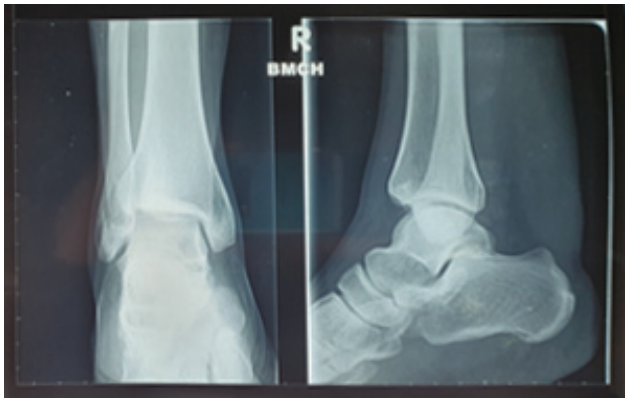

Figure 1b: X-ray of right ankle both AP & lateral view

USG of Ankle joint (Figure 1c) showed soft tissue swelling found in pre Achilles & retro-achilles area. Achilles tendon is thickened and heterogeneous. Minimal collection is seen around the tendon. No enhanced vascularity is seen. Same but little less finding are found in opposite Achilles tendon.

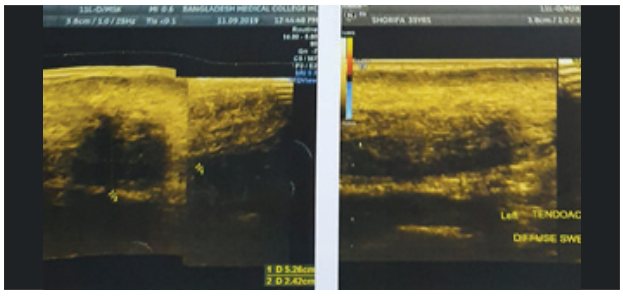

Figure 1c: Ultra sonogram view of the Achilles tendon xanthomas in both legs.

Operative Technique**:** Surgery was performed with the patient in prone position and under spinal anesthesia. A thigh tourniquet inflated to 240 mm Hg was used. A midline incision over the tumor was made. Subsequently, the tumor was mobilized and very carefully resected from the tendon until vital tendon tissue appeared (Figure 2 & 3). For both legs approximately fifty percent of the tendon could be spared, so no augmentation was necessary. In the left leg, slightly more tumor was resected. The wound was closed in layers.

Discussion:

Tendo-Achilles Xanthomatosis is not common disease. It is strongly linked to FH. Interestingly, our patient was not known with FH and possessed only two of the main characteristics of FH. She suffered HTN and developed Achilles tendon xanthomas. A raise of serum LDL cholesterol could not be found in multiple serum samples. However, this does not exclude a dyslipidemia.[7] Junyent et al. compared Achilles tendon sonography in patients with FH and in patients with non-FH dyslipidemias. It appeared that sonographic Xanthomas were only found in patients with FH. Therefore, they concluded Tendo-Achilles Xanthomatosis might be helpful in diagnosing FH.[8] Moreover, it may also be helpful in preventing cardiovascular disease as several authors suggested that Xanthomas are associated with a high risk for cardiovascular disease in patients with FH.[4,9] This is supported by a review of Fahey et al in which the authors showed that a majority of patients with Achilles tendon xanthomas had a family history of coronary disease.[3]

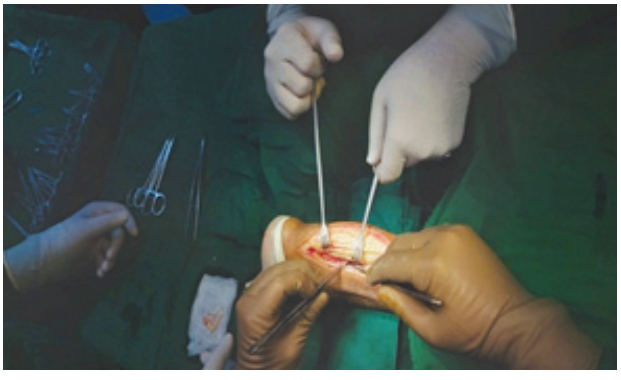

Figure 2a (I): The tumor of the left Achilles tendon exposed

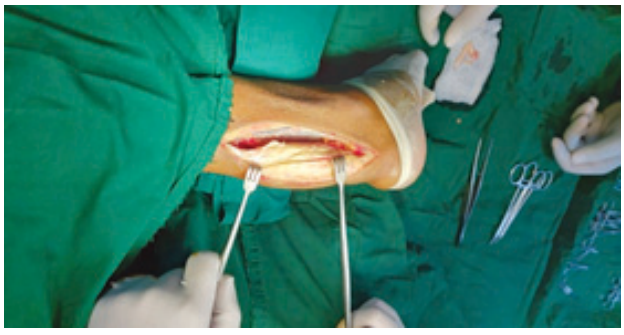

Figure 2a (II): The tumor of the left Achilles tendon exposed

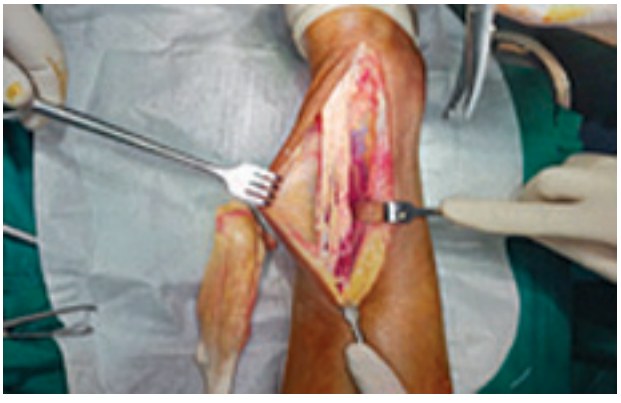

Figure 2b: Excision of tumor mass from Tendo-Achilles

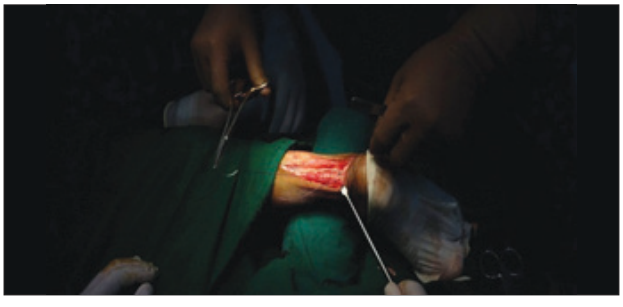

Figure 3: After resection approximately 50% of the tendon was intact and stitched.

We decided to perform a tendon sparing surgical resection of the tumor. However both tumors were excised macroscopically in total, its infiltration into the tendon made a subtotal resection inevitable. Carranza-Bencano et al. stated, in case of severe xanthomas infiltration, total resection is the best surgical technique [1] to avoid the risk of recurrence. As summarized by Huang et al. there are several procedures for Achilles tendon reconstruction. Autogeneous tendon grafts such as the peroneus brevis or flexor hallucis longus (FHL) are commonly used. The authors described a reconstruction with a tibialis posterior autograft, which was necessarily because of a large tendon defect after extensive resection [5]. We also performed an extensive resection resulting in a partial tendon defect, but decided to qualify the procedure as 'tendon sparing' (without reconstruction) when at least fifty percent of the cross-section tendon was saved. We believe this technique is a less invasive procedure, especially because other tendons (flexor hallusis longus, tibialis posterior and peroneus brevis tendon) and their functions remain intact, resulting in faster recovery. The patient started weight bearing after two weeks where others reported nonweight bearing periods of six weeks [3] or almost nine weeks [5]. Moreover, a subtotal tendon resection is associated with fewer complications compared to total excision with reconstruction [10]. For example, reconstruction of the Achilles tendon with the FHL tendon may lead to impaired flexion at the hallux interphalangeal joint and first metatarsophalangeal joint [3]. It has been suggested that a subtotal resection leads to a higher recurrence rate of the tumor [3]. In a follow-up period of 12 months, our patient had no signs of recurrence of Achilles tendon swelling. However, recurrence of the tumors in the next years cannot be excluded.

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Case Report

Outcome of exchange blood transfusion with reconstituted blood in hemolytic disease of newborn-A case report

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Abstract

Rhesus incompatibility, the most common cause of hemolytic disease of the newborn (HDN), is treatable with phototherapy but sometimes require neonatal exchange transfusion.Here, a case of Rhesus HDN clearly met criteria for exchange transfusion. An "AB" positive mother delivered a "B" negative neonate that quickly developed hyperbilirubinemia. The neonatal direct anti-globulin test was positive.Double volume exchange transfusion with reconstituted blood resulted in a favorable outcome.

Keywords: Reconstituted blood, HDN, Exchange transfusion, Hyperbilirubinemia, DCT, ICT.

Introduction:

An exchange transfusion (ET) is a medical procedure in which blood is removed and replaced with donor's plasma or whole blood.[1]ET removes circulating bilirubin, antibodies in plasma and antibody-coated sensitized red blood cells (RBCs), replacing them with RBCs compatible with maternal serum or neonates' serum and providing albumin with new bilirubin site.[2]The procedure is used to save the life of an adult or child with life-threatening blood abnormalities like sickle-cell disease and hemolytic disease of the newborn.[1]

In an Exchange transfusion, patient's blood is slowly withdrawn (usually about 5 to 20 mL at a time, depending on the patient's size and the severity of illness) and a slightly larger amount of fresh, pre-warmed blood or plasma flows into the patient's body. This cycle is repeated until the correct volume of blood has been replaced.[3]

Hemolytic disease of the newborn (HDN) is characterized by the presence of IgG antibodies in maternal circulation, which causes hemolysis in the fetus by crossing the placenta and sensitizing red cells for destruction by macrophages in the fetal spleen with consequent hyperbilirubinemia.[4]Hemolytic disease of the fetus and newborn (HDFN), also known as alloimmune HDFN or erythroblastosisfetalis.[5]Alloimmune HDFN primarily involves the major blood groups of Rhesus (Rh), A, B, AB, and O, although minor blood group incompatibilities (Kell, Duffy, MNS, P and Diego systems) can also result in significant disease.[6]Only maternal immunoglobulin G (IgG) causes HDFN, because transfer of maternal antibodies across the placenta. The etiology of ABO hemolytic disease of the newborn (ABO-HDN) is complex because anti-A and anti-B antibodies are composed mainly of IgM. Since only IgG antibodies cross the placenta, those pregnant women with high levels of IgG Anti-A, B, anti-A, or anti-B with an ABO incompatible fetus will be the ones to give birth to an infant with ABO-HDN.[7]

Before the practice of giving prophylactic anti-D to prevent the sensitization of Rh-negative pregnant women, Rh-associated HDN and hydrops fetalis were relatively common. The incidence of HDN ranges from one in 150 to 1:3000 births depending on the degree of anemia and level of bilirubin.[8]In the USA, 6.9% of all births are of infants with maternal-fetal ABO incompatibility and ABO-HDN is now the single most common cause of neonatal jaundice.[9]The hemolysis is widely accepted to follow a relatively benign course, rarely causing hydrops fetalis. Case reports of fetal hydrops secondary to ABO incompatibility are particularly rare, with only 9 cases reported to date.[10-14]Early detection and treatment of neonatal hyperbilirubinemia is important in prevention of bilirubin-induced encephalopathy.[15]

The goal of any therapy is to treat the etiology of the disease using the most effective but least invasive method. Several treatments are available for the management of HD of the newborn, including enteral or intravenous hydration, phototherapy, exchange transfusion, and IVIG.[16,17] According to the general guideline, severe anemia (hemoglobin <10 g/dL at birth) and/or severe hyperbilirubinemia in the first 48 hours of life have been suggested for exchange transfusion.[16] The procedure is effective, with approximately 25% of the bilirubin removed.[18]

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To identify infants who may be at risk for development of HD not known prenatally, blood typing and the direct antiglobulin test (DAT, or Coombs test) are completed. The DAT identifies antibodies that are attached to the infant's red blood cells, indicating an immune-dependent reaction.[16,19] The test will be positive regardless of the antibody type (anti-A, anti-B, or the rarer alloantibodies). It was shown that only 23% of infants with a positive DAT required phototherapy. However, it was also demonstrated that the stronger the positive result, the more likely the infant was to require treatment.[20]

A complete blood count is also necessary in the evaluation of HD. Depending on the timing of the study, anemia may be mild or severe. Reticulocytosis and an elevated nucleated red cell count as well as spherocytosis will often be present as the infant attempts to correct the anemia by generating new, immature red blood cells.[21]

As the red blood cells are destroyed, bilirubin is released and the infant's immature liver is unable to conjugate the large bilirubin load. Without removal in the gastrointestinal tract, the bilirubin levels rise, leading to clinical manifestation of jaundice within 12–24 hours of birth.[22]The initial anemia and subsequent hyperbilirubinemia are treated within the first week of life. However, chronic anemia often develops and should be followed-up at 6-8 weeks of life.[23]

Types of manual exchange transfusion are 1) Single volume exchange transfusion (SVET);2)Double volume exchange transfusion (DVET); 3) Exchange transfusion for sickle cell disease; 4) Partial exchange transfusion for polycythaemia.[24]

Anti-D prophylaxis (mostly administered postnatally) and advances in neonatal care have reduced the frequency of HDN by almost a factor of 10 to 1 in 21,000 births.[25]Deaths attributed to RhDalloimmunisation fell from 46/100,000 births before 1969, to 1.6/100,000 in 1990.[26]

Proper selection of donor's blood group is essential to prevent transfusion hazards. It is known that ABO antigen is fully developed at birth but the newborn baby does not produce ABO antibodies until 3 to 6 months of age. The ABO antibodies present in the serum of newborn babies are derived from mother's blood due to placental transfer. In case of transfusion of blood in newborn under 4 months of age, cross-matching of donor's blood is done with the mother's blood. We know, recipients same group of blood is always preferable in case of transfusion in adults or older children. But selection of blood for transfusion in the infants under 4 months of age depends on the mother's blood group as well. If the mothers blood group differs from the infant's blood group, the infants same group of blood may not be selected for transfusion. For example, if the mothers blood group is O and the newborn blood group is A or B, infants same group A or B group blood could not be transfused, because the anti-A & anti-B antibodies can be derived in the infant's serum from mother's blood which may react with the A or B antigen of the donor's blood. In this case O group packed RBCs should be selected for transfusion. O group whole blood may contain IgG Anti-A and anti-B antibodies in the plasma which can react with the A or B antigen of the infant's blood. So to avoid anti-A & anti-B antibodies in O group, plasma should be discarded and the packed RBCs should be transfused. In case of Rh-negative mother with Rh positive baby, Rh antibody may develop in mother's blood and Rh antibody may enter into baby's circulation, in this case the infant should be transfused with Rh-negative blood to avoid Rh antigen & antibody reaction. So for the selection of blood for transfusion in newborn baby up to the age of 4 months' mothers blood group is important to select the appropriate blood.[27] Whole blood used for exchange transfusion either compatible with neonates' serum or plasma or mother's serum is commonly used.[28]

ECT can be performed using many different combinations of blood components, including fresh whole blood and packed RBCs reconstituted with fresh frozen plasma (FFP). No single component is unequivocally the best Since fresh whole blood of the appropriate blood group is not always readily available, reconstituted blood is an alternative blood component for exchange transfusion. [29] In some conditions reconstituted blood that is A blood product used in transfusion therapy composed of components of blood (packed red blood cells plus plasma),which have been recombined after their separation and storage[30] may use for better outcome and safety of the patient.

Case Presentation:

A 04 days old baby boy was admitted with severe neonatal jaundice on 11 September 2019 at UAMCH at the department of pediatrics. Investigations revealed features of HDN. During admission, his total bilirubin was 19.7mg/dl, Direct and Indirect bilirubin was 1mg/dl and 18.7mg/dl respectively, Direct coombs test was positive. On examination baby was icteric face, heart rate was 124/min, respiratory rate 36/min. The reports are presented on Table 1.

Depends on baby's physical condition urgent blood demand was placed for exchange blood transfusion and specimen for cross-match was collected. His specimen was processed as per laid down protocol for 'urgent blood demand.

As a part of cross matching, blood grouping was done for both Baby and mother first. Initial rapid cell and serum grouping result of patient revealed group “B” Rh-D Positive and mother was “AB” Rh-D negative. Then planned for compatibility testing with patient's ABO matched rhesus negative blood.

Simultaneously compatibility with “B” Rhesus negative unit was undertaken and an abbreviated cross-match at

room temperature (RT) and 37°C major was compatible but minor was incompatible. Further compatibility test (Table 2) with another two "B" Rhesus negative unit was done which revealed same results due to present of allo-antibody coated fetal RBC. Then further compatibility test with Rhesus negative "O" and "AB" donor was proceeding which revealed same results that major was compatible and minor was incompatible. For better outcome and as a ideal protocol we decided to reconstituted blood unit with "O" Rhesus negative washed cell and "AB" Rhesus negative plasma for exchange blood transfusion (Table 3).

The reconstituted blood was prepared in the blood bank. First "O" grouped rhesus negative cell was washed with 0.9% normal saline and then suspended in "AB" grouped Plasma by standard method of preparation (SOP)-centrifugation and separation.

Post-exchange blood was collected for estimation of hemoglobin, hematocrit, bilirubin and direct antiglobulin test. Post-exchange indirect bilirubin fall and hemoglobin of neonate were also observed. (Figure 1)

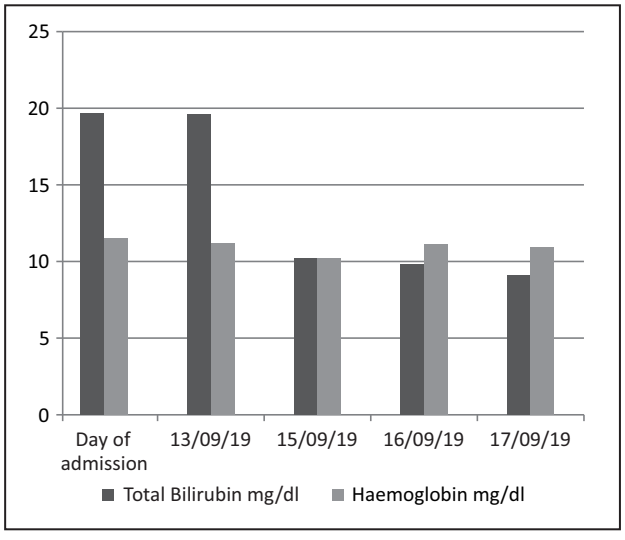

Figure 1: Bilirubin and Haemoglobin level before and after exchange transfusion

Table 1: Investigation reports

Name of the investigation		Results	Normal value	Unit
CBC	WBC	20.07	5000-15000	10^9/L
	N	17.6	2000-7500	10^9/L
	L	01.61	1500-5000	10^9/L
	M	01.00	200-800	10^9/L
	E	00.04	40-600	10^9/L
	B	00.00	0-100	10^9/L
	RBC	02.80	4.7	10^{12}/L
	Hb	12	10-13	gm/dl
	HCT	29.5	30-44	%
	MCV	105.4	76-96	Fl
	MCH	35	27-32	Pg
	MCHC	33.20	32-36	g/dl
	RDW	14.50	13.2+/-0.9	%
	Platelets	266	150000-400000	10^9/L
	MPV	09.20	7.5-12	Fl
Bilirubin	Total	19.6	0.2-1.3	mg/dl
	Direct	01	0.1-0.3	mg/dl
	Indirect	18.7	0.2-0.7	mg/dl
CRP		12	6	mg/L
Coombs	DCT	Positive		
	ICT	Positive		
Blood Group	**"B" Positive**			

Table 2: C ross Matching of A2 recipient

Donor		1	2	3	4	5
		"B"grouped Rhesus negative Donor 1	"B"grouped Rhesus negative Donor 2	"B"grouped Rhesus negative Donor 3	"AB"grouped Rhesus negative Donor 4	"O"grouped Rhesus negative Donor 5
Major Part (PS/DC)	With baby serum	Compatible	Compatible	Compatible	Compatible	Compatible
	With mother serum	Compatible	Compatible	Compatible	Compatible	Compatible
Minor part (PC/DS)		Incompatible	Incompatible	Incompatible	Incompatible	Incompatible

Table 3: Selection and Preparation of appropriate blood unit for exchange blood transfusion

ABO Blood group			Rhesus Blood group	Selection of blood
Blood group	**Cell contain**	**Plasma contain**	Cell doesn't contain Rh D antigen	
B	B Ag	A Antibody	Rhesus D negative	To follow ideal protocol and better outcome "B" group not selected
O	No Ag	A,B Antibody	Rhesus D negative	Select only "O"Cell as absence of ABO Antigen
AB	A,B Ag	No ABO Antibody	Rhesus D negative	Select only "AB" Plasma as absence of ABO Antibody

Discussion:

The incidence of severe neonatal jaundice within the first few hours of life (bilirubin above 10 mg/100 mL) is fairly common with a significant number requiring exchange transfusions. A 1 in 5 chance of ABO incompatibility between fetal red cells and maternal serum exists but the incidence of ABO HDN elsewhere is said to be uncommon occurring in 2% of all births.[13,10]Race has however been shown to have an effect on the incidence and severity of ABO HDN with a higher incidence and severity being observed among Blacks[31] and Latin Americans.[32]Thus we can expect the incidence and severity of ABO HDN to be higher in Nigeria.

The antibodies responsible for hemolysis can be naturally occurring (e.g, anti-A or anti-B antibodies) or can develop as a result of a sensitizing event such as pregnancy or transfusion. The most well recognized is rhesus alloimmunisation which begins with red blood cells from a rhesus-positive fetus crossing the placental barrier during pregnancy and delivery, and entering the maternal blood circulation. A rhesus-positive father and a rhesus-negative mother are required for this situation to develop. The incompatible antigens introduced result in a primary immune response and stimulate the production of maternal antibodies. Primary exposure can also be the result of amniocentesis, chorionic villus sampling and cordocentesis.

Several fetal rhesus antigens may cause alloimmunisation (c, C, d, D, e and E) and this can also occur with the Kell, Duffy, ABO and other blood group systems.[33] Consequently, ABO incompatibility is now the single largest cause of HDFN in the western world.[34]

There are rarely any problems during primary exposure but subsequent pregnancies result in large amounts of maternal anti-D antibodies being produced and the risk increases with each gestation. These are capable of crossing the placenta, where they affix to fetal red blood cells, which then become recognized as 'foreign' by the fetal immune system and haemolysed. If the rate of red cell destruction exceeds the rate of production it results in fetal anaemia which, if severe, can lead to fetal heart failure, fluid retention and swelling (hydrops). Red cell breakdown results in bilirubin release which is not a problem during fetal life as it is cleared by the placenta. After birth, however, the immature neonatal liver is not capable of handling a high bilirubin load and this can result in severe neonatal jaundice. High levels of jaundice if untreated can result in permanent brain damage (kernicterus) because of deposition of bilirubin in certain areas of the neonatal brain.[35]

By using reconstituted blood in this study, we further strengthened the concept. All cases studied before resulted in an approximate fall in indirect bilirubin by 52.01%, which is more than documented literature.[36,37]

The concept of reconstituted blood (O cells suspended in AB plasma) for exchange transfusion in HDN is safe and can be used, irrespective of the neonates' and mothers' blood group. We are supplying this reconstituted blood in routine for exchange transfusion. The concept is very easy to understand and simple to follow, and the results are also better than those of group-specific exchange transfusion in babies with HDN. The immunogenic complications and risks are very low. In Rh HDN group, we have chosen O negative cells suspended in AB plasma over group-specific Rh negative cells to avoid the controversy of ABO sub-grouping and because of the presence of weaker ABO antigens in newborns and full-term mothers and easy availability of O negative cells. In the remaining two groups, utility of reconstituted blood is well established. By use of reconstituted blood, hematocrit is also well adjusted according to the requirements.

Conclusion:

Reconstituted blood has superiority over whole blood in exchange transfusion in HDN as it fulfills all the therapeutic indications of exchange transfusion in a better way –viz., removal of bilirubin and antibody-coated RBCs from the neonates' circulation; better and safe survival of transfused RBCs; to establish normal hematocrit after exchange transfusion.

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College News

College Events:

- The National Mourning Day was observed on 45th death anniversary of Father of Nation Bangabandhu Sheikh Mujibur Rahman in Bangladesh Medical College and Hospital on 15th August 2020. Teachers, doctors, nurses, students of BMCH and officials & staffs of BMSRI participated in that event.
- 49th "Victory Day" of Bangladesh was celebrated in Bangladesh Medical College and Hospital premises on 16th December 2020. Hon'ble Minister, Ministry of Agriculture, Government of Peoples' Republic of Bangladesh Dr. Md. Abdur Razzak MP, Chairman, E.C, BMSRI inaugurated the mural of Bangabandhu Sheikh Mujibur Rahman, the Father of the Nation at BMC campus on 16th December 2020.

Obituary:

Sheikh A. Hafiz, Member of Executive Committee of BMSRI, FCA Partner Rahman Rahman Huq, Chartered Accountants died due to old age complications on 12th November 2020. Members of BMSRI, teachers, doctors, students and staff of BMC and BMCH expressed their deep condolence at the demise of this legendary person.

Seminar in BMC:

- World Diabetes Day was celebrated from 13th November to 15th November 2020 in the Bangladesh Medical College and Hospital. It was organized by Dr. Yasmin Aktar, Assistant Professor, Dept. of Endocrinology, BMC and Dr. Mustafa Aolad Hossain, RAP, Dept. of Medicine, BMCH. Theme for 2020 was "The nurse and diabetes".

Participation in the International Congress:

- Dr. Farha Rahman, Assistant Professor C. C, Dept. of Microbiology, Bangladesh Medical College attended the 9th International Congress of the Asia Pacific Society of Infection Control, (APSIC- 2019) held at Danang, Vietnam from 19th to 22nd March, 2019.

Following teachers of BMC have been promoted

Promoted to Professor

- Dr. Raihana Begum, Professor of the Department of Community Medicine
- Dr. Rashida Begum, Professor of the Department of Biochemistry
- Dr. Mosahef Uddin Ahmed, Professor of the Department of Forensic Medicine
- Dr. Rafat Nawaz, Professor of the Department of Gynae & Obst.
- Dr. Nasima Arjumand Banu, Professor of the Department of Gynae & Obst.
- Dr. Md. Tarek Alam, Professor of the Department of Medicine
- Dr. Md. Dabir Hossain, Professor of the Department of Medicine
- Dr. Rehnuma Tasnim Chowdhury, Professor of the Department of Pharmacology
- Dr. Farhana Alamgir, Professor of the Department of Microbiology
- Dr. Rezina Hamid, Professor of the Department of Neurosurgery
- Dr. Kamruzzaman, Professor of the Department of Orthopaedics

Promoted to Professor (Current Charge)

- Dr. Mahfuza Rahman, Professor (Current Charge) of the Department of Biochemistry
- Dr. Sohely Sharmin, Professor (Current Charge) of the Department of Microbiology
- Dr. Md. Saidur Rahman, Professor (Current Charge) of the Department of Pathology
- Dr. Fauzia Jahan, Professor (Current Charge) of the Department of Pathology
- Dr. Md. Amir Hossain, Professor (Current Charge) of the Department of Cardiology
- Dr. Saydur Rahman, Professor (Current Charge) of the Department of Orthopaedics

Promoted to Associate Professor

- Dr. Sadia Khanduker, Associate Professor of the Dept. of Biochemistry
- Dr. Farhana Shahid, Associate Professor of the Dept. of Forensic Medicine
- Dr. Shabnam Imam, Associate Professor of the Dept. of Community Medicine
- Dr. Mainul Alam Chaklader, Associate Professor of the Dept. of Community Medicine
- Dr. Sharmila Huda, Associate Professor of the Dept. of Pharmacology
- Dr. Rehana Khanam, Associate Professor of the Dept. of Pathology
- Dr. Azizun Nahar, Associate Professor of the Dept. of Microbiology
- Dr. Asma Habib, Associate Professor of the Dept. of Gynay & Obst.
- Dr. Halima Begum, Associate Professor of the Dept. of Radiology & Imaging

- Dr. Khondoker Ehsanul Arefin, Associate Professor of the Department of Paediatrics
- Dr. Rezwanur Rahman, Associate Professor of the Department of Nephrology

Promoted to Associate Professor (Current Charge)
- Dr. Farhana Hossain, Associate Professor (Current Charge) of the Department of Anatomy
- Dr. Saikat Barua, Associate Professor 9Current Charge) of the Department of Radiology & Imaging

Promoted to Assistant Professor:
- Dr. Farzana Maqsood, Assistant Professor of the Department of Anatomy
- Dr. Bushra Abrar, Assistant Professor of the Department of Biochemistry
- Dr. Raheena Akter, Assistant Professor of the Department of Physiology
- Dr. Tanzima Humayun, Assistant Professor of the Department of Pharmacology
- Dr. Zafrana Zahir, Assistant Professor of the Department of Community Medicine
- Dr. Sharmin Haque, Assistant Professor of the Department of Pathology
- Dr. Naheed Fatema, Assistant Professor of the Dept. of Gynae & Obst.
- Dr. Sadia Saber, Assistant Professor of the Department of Medicine
- Dr. Md. Elias Buiyan, Assistant Professor of the Department of Medicine
- Dr. Sanjida Huda, Assistant Professor of the Department of Forensic Medicine
- Dr. Shahed Haider Chowdhury, Assistant Professor of the Department of Ophthalmology
- Dr. Arman Ibne Haq, Assistant Professor of the Department of Psychiatry
- Dr. Yasmin Aktar, Assistant Professor of the Department of Endocrinology
- Dr. Ahsan Kabir Masoom, Assistant Professor of the Department of Paediatrics
- Dr. Shaikh Adnan Rakib, Assistant Professor of the Department of Surgery
- Dr. Saber Aminur Rahman, Assistant Professor (RS) of the Department of Surgery
- Dr. Nazmul Hossain Chowdhury, Assistant Professor of the Department of ENT
- Dr. A.T.M Zulfiqur Rahman, Assistant Professor of the Department of Orthopaedics
- Dr. Hasan Khalid Md. Munir, Assistant Professor (RS) of the Dept. of Orthopaedics

Promoted to Assistant Professor (Current Charge)
- Dr. Nargis Sultana, Assistant Professor (Current Charge) of the Department of Anatomy
- Dr. Farha Rahman, Assistant Professor (Current Charge) of the Department of Microbiology

Promoted to Registrar

Dr. Nabila Zohra Khan, Registrar of the Department of Paediatrics

Promoted to Curator

Dr. Mohammad Showkat Ali Shafait, Curator of the Department of Anatomy

Following teachers of BMC have been newly appointed
- Dr. Md. Khaled Noor, Professor of the Department of Paidiatrics (Neonatology)
- Dr. Md. Jamal Uddin, Professor of the Department of Dermatology (Ad-hoc)
- Dr. Mohammad Aftab Haleem, Assistant Professor of the Department of Neuromedicine
- Dr. Rifat Mahmud Khan, Lecturer of the Department of Community Medicine
- Dr. Md. Mahabub Alam Mondal, Lecturer of the Department of Forensic Medicine
- Dr. Abu Naser Siddiki, Lecturer of the Department of Pharmacology
- Dr. Tasmia Akter, Lecturer of the Department of Forensic Medicine
- Dr. Fuad Quader, Lecturer of the Department of Microbiology

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