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Abbreviations and symbols must be standard and SI units should be used throughout. Whenever possible drugs should be given their approved generic name. Acronyms should be used sparingly. Statistical analysis must explain the methods used. Reference should follow the Vancouver format. In the text they should appear as numbers starting at 1. At the end of the paper they should be listed (double spaced) in numerical order corresponding to the orders of citation in the text. All authors should be quoted for papers with upto six authors, for papers with more than six authors the first six only should be quoted followed by et al.

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2. Parkin DM, Clayton D, Blook RJ, Massyer E, Fried HP, Iranov E, et al. Childhood Leukaemia in Europe after Chernobyl : 5 years follow-up. Br J Cancer 1996; 73 : 1006-12.

Chapter in a Book :

1. Phyllyps SJ, Whisnant JP. Hypertension and Stroke. In : Lurgh JH, Brennes BM, editors. Hypertension : Pathophysiology, diagnosis and management. 2nd ed. New York : Raven Press; 1995. p. 465-78.

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PERITONEOVENOUS SHUNT FOR THE REFRACTORY ASCITES: A CASE REPORT

MOHAMMAD MOHIBUL AZIZ[1], ROBERT AHMED KHAN[2]

Introduction

Refractory ascites is an incapacitating condition in patients with chronic liver disease or advanced malignancy. It may produce symptoms like abdominal distension, early satiety, respiratory embarrassment, impaired mobility and lethargy[1]. Successful relief of these symptoms is often difficult to obtain. Conventional medical therapy has focused on use of diuretics and therapeutic paracentesis. Despite the use of these methods some patients progressively become resistant to therapy and develop refractory ascites. New modalities of treatment such as Peritoneovenous shunt (PVS) or Transjugular intrahepatic portosystemic shunt (TIPS) are applied to these group of patients for their palliation. We report on the use of PVS in a patient with troublesome malignant ascites that resulted in palliation of his symptoms. To the best of our knowledge there was no report of such case previously in our country.

Case Report

Mr. M a 50 year old gentleman presented with 2 years history of upper abdominal pain, post-prandial discomfort, anorexia and weight loss. For the last 2 months he had developed abdominal distension. Gradually the distension became so severe that he experienced great difficulty in breathing and he could not lie flat. There was no history of alcohol intake, jaundice, haematemesis or melaena.

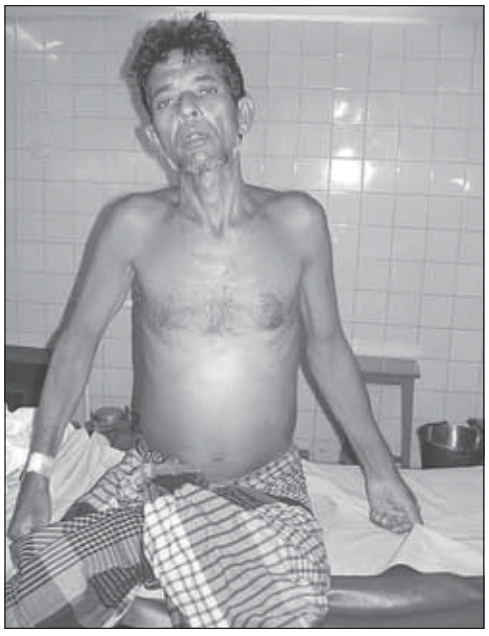

Fig.-1: *Picture of the patient.*

On examination his pulse was 84/min, blood pressure 125/85 mm of Hg and respiratory rate 28/min. No Virchow's lymph node. Abdomen was hugely distended and tense, fluid thrill was present. Liver was palpable and no abdominal mass was detected. Few tiny nodules were felt on the left side of the pelvis on digital rectal examination.

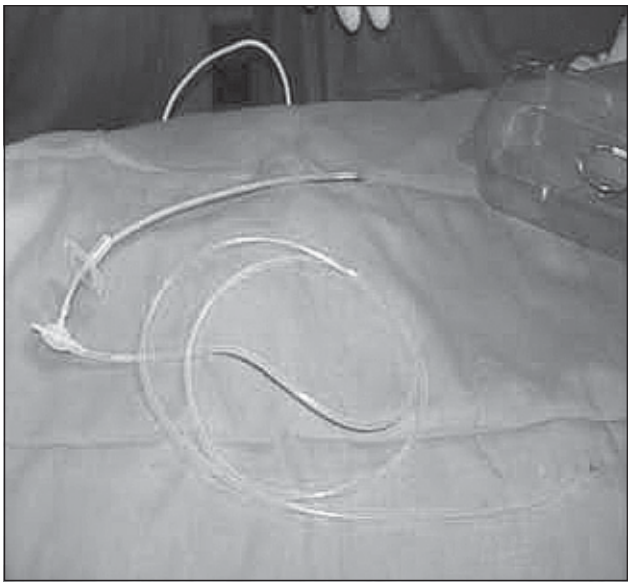

Fig.-2: *Shunt assembled.*

His haemoglobin was 8 gm/dl, liver function tests, prothrombin time and electrolytes were normal. Chest x-ray was normal. Abdominal ultrasound revealed gross ascites, liver parenchyma was coarse but no focal lesion was detected. No other pathology was detected in the abdomen. Upper GI endoscopy showed an ulcero-cauliflower like lesion at the posterior wall of the stomach. Biopsy from the lesion revealed adenocarcinoma. Ascitic fluid tap showed straw coloured fluid with high protein content (exudate in

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nature). Cytology showed cell count- 30/cmm (lymphocyte & mesothelial cells), no malignant cell was detected. Repeated aspiration of 1.0-1.5 litre of fluid was done to reduce difficulty of breathing but relief was only short lived.

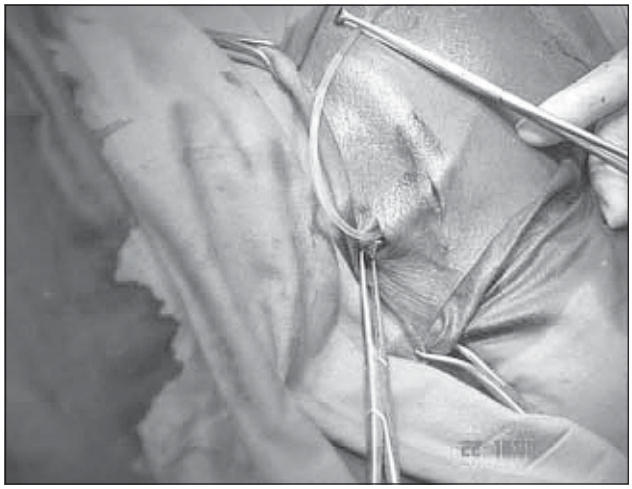

Fig.-3: *Long limb before introducing in to the vein.*

Laparotomy revealed a large tumour at the posterior wall of the body of the stomach. Whole peritoneal cavity (both visceral and parietal peritoneum) was studded with innumerable deposits. Peritoneovenous shunt was done. Pudenz diaphragm valve shunt was used. The short limb was placed in the subhepatic space, the flash chamber over the right costal margin and the long arm was introduced into the superior venacava via the right external jugular and right internal jugular vein. The tube was tunneled through the subcutaneous tissue of the right chest wall and connected with the flash chamber.

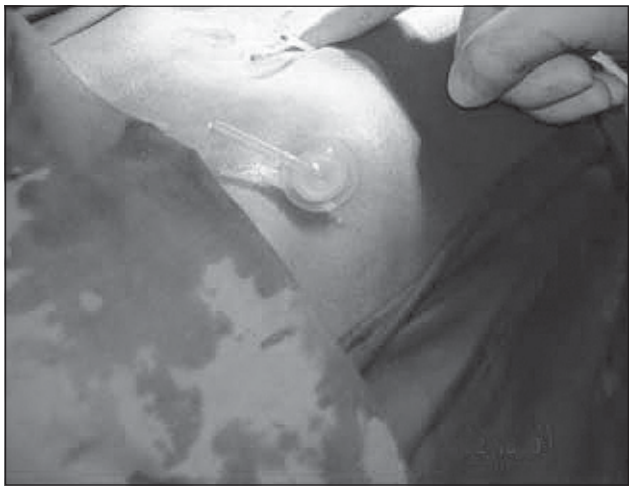

Fig.-4: *Long limb before connection with the flush chamber.*

Postoperatively oral feeding was started on 3rd postoperative day and the patient was released on the 9th postoperative day. One month follow up showed markedly improved symptoms, with moderate abdominal distension.

Discussion

We have reported a case of refractory ascites due to malignancy treated by peritoneovenous shunt. Approximately 10-20% patients with ascites respond to dietary sodium restriction alone and the majority of the remaining patients respond to diuretic therapy. However about 10% patients do not respond to above measures develop refractory ascites which imparts significant morbidity to these patients [2] . Many interventions for refractory ascites have been applied for the palliation of symptoms of this group of patients. Interventions like large volume paracentesis, surgical portosystemic shunting, ascitic fluid filtration and re-infusion, peritoneovenous shunt, transjugular intrahepatic portosystemic shunt and novel treatment like use of ANP(atrial natriuretic peptide), V2(vasopressin) receptor antagonist, OPC-3126, niravoline, adenosine 1 receptor antagonist etc. have been tried [2]. Each intervention has advocates and alleged differences in efficacy and differing profiles in complications. In short, after failing medical management there is no consensus which interventional therapy provides the best palliation with least morbidity.

The use of PVS was first described by Smith in 1962. Although initially successful they tended to block. The success of the Leveen shunt presented in 1974 renewed interest in PVS. Later in 1979 Lund and Newkirk published their experience with Denver shunt [3] . These systems, which are activated by pressure gradient between the peritoneal cavity and the venous circulation, serve as a one-way valve and autogenously re-infuse ascitic fluid. Initially the PVS was devised for the treatment of refractory ascites due to alcoholic cirrhosis but later the indications were extended to other conditions like malignant ascites, chylous ascites, nephrogenic ascites and ascites secondary to myelofibrosis [4]. Complications reported after PVS include variceal bleeding, sepsis, adult respiratory distress syndrome, cardiac failure and DIC [5]. However the rate of complication is relatively low in patients with malignant ascites in comparison with cirrhotic ascites and there is no adverse effect on survival time [6].

Our patient presented with symptomatic ascites, which was secondary to advanced carcinoma stomach. Medical treatment and repeated paracentesis failed to improve his symptoms. We performed a laparotomy

and could not resect the tumour and placeed a PVS for the ascites. One month post operative review showed moderate ascites and marked reduction of symptoms . In Sooriakumaran P et al [3] series 10 out of 12 patients had total symptomatic improvement. In another series [7] of cirrhotic patients , 126 out of 140 showed symptomatic improvement .However, in that series 38 patients had recurrence of symptoms with in two years. Acturial one year survival was 81.4 % and the survival was proportional to the state of liver function. Ideal PVS like Leveen or Denver shunt are not locally available so we had to look for other available options. There are reports of modification of PVS by using patients own resources where long saphenous vein is used as a drainage system and natural valve in the saphenous orifice ensures one-way ascites flow[8]. Commercially available hydrocephalus shunts were not found to be satisfactory for long-term control of ascites as they leak at low pressure or present a high impedance to flow[9]. For this reason we used a low pressure diaphragm valve hydrocephalus shunt as it's valve opens at low pressure and provides low impedance to flow. With our limited resources in this way we were able to relief the symptoms of this terminally ill patient.

Conclusion

Refractory ascites can result in very troublesome symptoms for patients who may otherwise have some time to live. We think PVS is an effective palliative technique for these patients.

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ORIGINAL ARTICLES

SONOLOGIC EVALUATION OF IN-PATIENTS BY GASTROENTEROLOGY TRAINEE WITH A PORTABLE MACHINE IN WARD AND RADIOLOGIST IN RADIOLOGY DEPARTMENT: A PROSPECTIVE COMPARATIVE STUDY

BIMAL CHANDRA SHIL[1], MADHUSUDHAN SAHA[2], MAHMUD HASAN[3]

Abstract:

Ultrasound is accepted as a first line imaging investigation for many gastroenterological disorders. This study was designed to compare the findings of a gastroenterology trainee having short training on ultrasound with a small portable machine in ward, those of radiologists in radiology department on the same patients with the final diagnosis.

Initially the gastroenterology trainee was trained on abdominal ultrasound by an experienced sonologist. Then the trainee carried out ultrasound examination of the admitted patients independently with a small portable machine in gastroenterology department. Then radiologists in radiology department using a sophisticated machine examined the patients. The patients were thoroughly investigated to reach the final diagnosis. All findings of patients were recorded.

One hundred and six patients were enrolled in this study. Among 21 cases of cirrhosis of liver, the trainee gastroenterologist detected 18 (85.7%) and radiologist detected 17 (80.9%) cases in this series. In this study, in 25 patients with SOL in liver the trainee and radiologist detected 22 (84.6%) and 24 (92.3%) cases of SOL respectively . Both the trainee and radiologist detected all (38) cases of ascites. Among 25 cases of biliary dilatation both the trainee and radiologist detected all, radiologist could find out causes of biliary dilatation in 19(76%) and trainee in 11(44%) cases. Both the trainee and radiologists detected all cases of cholelithiasis. Among 18 cases of abdominal mass lesion of possible gut, lymph node or other origin the trainee detected 17(94.4%) and the radiologist detected eight (44.4%) cases.

Overall diagnosis of patients by the trainee was high and comparable to that of the radiologist. So patients of gastroenterology should undergo prompt ultrasound examination in ward by the gastroenterologists. Ultrasound training should be part of training program for gastroenterology trainee.

Introduction:

Diagnostic Sonography, considered by some to be a "mature" technology in the late 1980s, is experiencing a breath-taking period of technological advancement. Diagnostic sonography's ability to image flow and soft tissue in real time is unique among imaging techniques.[1]

Ultrasound (US) is the name given to high frequency sound waves, over 20,000 cycles per second (20 kHz). These waves, inaudible to humans, can be transmitted in beams and are used to scan the tissues of the body. The reflected ultrasound pulses detected by the transducer need to be amplified in the scanner. The information is stored in a computer and displayed on a video (television) monitor.[2]

Transabdominal ultrasonography (US) is widely used to assess morphologic integrity of parenchymal abdominal organs such as the liver, bile ducts, pancreas, kidneys, spleen, and abdominal blood vessels etc.[3] It is accepted as a first line imaging investigation for many disorders suspected to be gastroenterological in origin, being a low cost, painless, repeatable procedure requiring little preparation and limited patient cooperation, and with no known harmful effects at diagnostic frequencies.[4] When needed, ultrasound can be performed quickly and at the bed side. [1]

Use of ultrasound in the investigation of gastrointestinal masses have shown it to be useful for the localization of the lesion within the

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gastrointestinal tract and for any associated abnormalities such as metastases.[5] Hepatic sonography is useful to detect the mass lesions in liver (>1cm diameter). Real time ultrasound is the imaging modality of choice for evaluation of the gall bladder and biliary ducts. It has a 95% sensitivity for the detection of cholelithiasis.It is 95% accurate in the detection of biliary ductal dilatation although it may not be able to determine whether the obstruction is due to a stone, tumor, or stricture. [6]

Although computerized tomography (CT) remains the more sensitive for evaluating pancreatic disease, modern ultrasound technology and new scanning techniques are reestablishing sonography as a useful and clinically relevant pancreatic imaging technique. Sonography may detect acute or chronic pancreatitis or reveal pancreatic masses.[1, 7]

Use of ultrasound improves the rate of correct diagnoses from 70% to 83%, in patients with acute abdominal pain.[8] Ultrasonography can detect as little as 100ml of ascites.[6] The efficacy and effectiveness of ultrasound in evaluating patients suspected of having blunt abdominal trauma are near of computed tomography (CT) and diagnostic peritoneal lavage[9]. Ultrasonography gives a good diagnostic yield compared with intravenous urography and its high sensitivity coupled with the rapidity and relative ease with which it is performed make it an attractive modality for urological investigations.[10] Female pelvic ultrasound permits the rapid, confident and cost effective diagnosis of a full spectrum of pathology in patients of all ages.[11] US-guided fine needle aspiration biopsy of focal hepatic lesions is used extensively with high accuracy.[4,12]

Abdominal ultrasound training is considered to be a necessary part of the European Diploma in Gastroenterology.[13,14] European Union of Medical Specialists (EUMS) has the support of its Radiology Section in this. [14] In United States of America (USA), basic training of ultrasound is recommended for gastroenterologists.[15] In United Kingdom (UK) the Joint Committee on Higher Medical Training (JCHMAT) includes ultrasound imaging in gastroenterology curriculum.[13] For surgical and medical gastroenterologists, the prospect of their being able to utilize sonography as a direct extension of clinical examination is exciting.[16]

A prospective study of ultrasound scans done by attending surgeons for patients with abdominal symptoms showed 85% diagnostic accuracy.[8] Another prospective double blind study comparing the accuracy of Radiologists and Radiographers in routine abdominal ultrasound showed that the radiographers are as competent as the radiologists at routine abdominal ultrasound.[17]

With the above background it was thought that an assessment of the ability of a trainee gastroenterologist with a short training in abdominal ultrasound in detecting gastroenterological abnormalities in the ward with a small portable machine and to compare the findings of trainee, those of trained radiologist using a more sophisticated machine in radiology department with the final diagnosis may be undertaken.

Aims of the study:

1. To assess the value of routine use of abdominal ultrasonography for preliminary screening in patients admitted in gastroenterology unit.
2. To compare the sonologic findings in patients with gastrointestinal, hepatobiliary and pancreatic diseases performed by a trainee gastroenterologist in ward with a portable machine, those of radiologists in the radiology department with a more sophisticated machine with the final diagnosis.
3. To assess how much training in ultrasound is required for a gastroenterologist for efficient interpretation of US scan.

Materials and Methods:

The study was conducted in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka. It was performed during the period of July 2003 to November 2004.

Patients admitted in department of gastroenterology having indication for abdominal ultrasound were included in this study. Indications of ultrasound examination were-Liver disease, abdominal pain,unexplained ascites, abdominal mass, suspected space occupying lesion in liver and spleen, chronic diarrhea, and obstructive jaundice. Patients who had ultrasound scan performed recently were excluded from this study.

The study was a prospective study to compare the ultrasound findings of a gastroenterology trainee having short training on US and those of radiologists in Radiology department. The gastroenterology trainee initially was trained about diagnostic ultrasound by an experienced sonologist in gastroenterology ward. According to recommendation of WHO scientific committee it included 200 abdominal ultrasound examinations, which were carried out by me under direct supervision of the sonologist. Following training, ultrasound examination was performed by

the trainee independently with a small portable machine (Toshiba Sonolayer SAL 32B Machine with linear probe of 3.5 MHz frequency). In the department of Radiology, ultrasound was carried out by radiologist with a more sophisticated machine (Siemens Sonoline Prima Machine with curvilinear probe of 3.5 MHz frequency and linear probe of 7.5 MHz frequency) with better image and resolution facilities.

Total 106 patients were enrolled in this study. All patients underwent detailed clinical history and examination after admission in the ward by the trainee. Following clinical examination, all the patients were subjected to sonologic evaluation in the ward by the trainee with the portable machine. Findings were recorded and then these patients were sent to Radiology department with clinical record files with full history and available investigation reports. The radiologist performed US examination but he was unaware of US findings of the trainee. Sonographic findings by radiologist were also recorded. Thorough investigations were carried out to reach the final diagnosis of the patients. Then the final diagnoses were compared and evaluated with the sonographic findings of trainee gastroenterologist and those of radiologist.

The data obtained from the study were entered into excel database and significance of differences were estimated by using appropriate statistical methods. Computer based SPSS (Statistical Package for Social Science) Version 11.0 software package was used for all the analysis. Comparison between two groups was done by chi-square test, p value of less than 0.05 was considered as significant

Results:

A total of one hundred and six cases were included in this study. Out of them 60 cases (56.6%) were male and 46 cases (43.4%) were female with age range of 16-75 years. The mean age of these patients was 45.5 years. The final diagnoses and findings of trainee gastroenterologist and radiologist are arranged in following tables:

Table-I

showing distribution of space occupying lesio (SOL) in liver

Final diagnosis	Ultrasound (US) by Trainee	Ultrasound (US) By Radiologist
Liver abscess (n = 7)	7	7
HCC (n = 8)	6	8
Metastatic lesions in liver (n= 5)	4	5
Hepatic cyst (n = 3) (a) Simple	1	1
(b) Hydatid cyst	2	2
Hemangioma (n=2)	2	1

Table-II

Showing patterns of diffuse liver disease:

Final diagnosis	US by Trainee	By Radiologist
Cirrhosis of liver(n=21)	18	17
HCC andMetastasis (n= 3)	3	3
Fatty liver (Bright liver) (n= 6)	6	6

Table-III

Showing Gall bladder disease patterns:

Final diagnosis	US by Trainee	By radiologist
Cholelithiasis (n = 6)	6	6
Gall bladder mass (n = 3)	2	3

Table-IV

Showing patterns of portal venous disorder:

Disorder	US by Trainee	By Radiologist
Dilated portal vein suggestive of portal hypertension (n=14)	14	10
Portal venous Thrombosis (n= 2)	0	2

Table-V

Showing finding of accumulation of fluid in peritoneal cavity and pleural space:

Disorder	US by Trainee	By Radiologist
Ascites (n=38)	38	38
Pleural effusion		
Right side (n=5)		
Left side (n=2)	5	5
	0	2

Table-VI

Showing causes of biliary dilatation (n=25)

Final diagnosis	US by Trainee	By Radiologist
Biliary Ascariasis(n =5)	3	4
Choledocholithiasis (n= 8)	4	7
Ca head of pancreas (n =5)	2	5
Post Surgical biliary Stricture (n=1)	0	0
Cholangiocarcinoma(n= 2)	0	1
Duodenal adenocarcinoma (n=1)	1*	0
Gall bladder malignancy infiltrating to CBD (n= 2)	1	2
Cause undetermined (n= 1)	-	-

*ultrasound reveals gut mass.

Table-VII

Showing patterns of pancreatic diseases:

Final Diagnosis	US by Trainee	By Radiologist
Acute Pancreatitis (n= 2)	2	2
Chronic Pancreatitis without calcification(n= 4)	2	3
Chronic CalcificPancreatitis (n = 3)	2	3
Ca head of Pancreas(n=5)	2	5

Table-VIII

Shows distribution of abdominal mass lesions

Final diagnosis	US by Trainee	By Radiologist
Ca Stomach (n= 1)	1*	1*
Carcinoma Gall bladder (n= 3)	2	3
Carcinoma duodenum (n=1)	1*	0
Carcinoma colon (n= 2)	2*	0
Renal cell Carcinoma infiltrating liver (n= 1)	1	0
Abdominal Tuberculosis (n= 5)	5*	1*
Para aortic lymphadenopathy (n=3)	3	2
Mass due to IBD (n=1)	1*	1*
Undetermined origin(Adenocarcinoma) (n=1)	1*	0

*ultrasound reveals gut mass.

Table-IX

Showing distribution of clinical organomegaly, sonologic findings by trainee gastroenterologist and radiologist:

Clinical Organomegaly	US by Trainee	By Radiologist
Hepatomegaly(n = 63)	59	44
Splenomegaly(n = 21)	20	15

Table-X

Showing distribution of correct diagnosis by the trainee gastroenterologist and radiologist:

Total cases	106
Correct diagnosis by trainee	83(78.3%)
Correct diagnosis by radiologist	69(65.1%)

Discussion:

Abdominal ultrasound is an excellent imaging modality for many gastroenterological disorders being a low cost, painless, repeatable procedure requiring little preparation and limited patient cooperation, and with no known harmful effects at diagnostic frequencies[4]. Newer handheld instruments will revolutionize medical practice by becoming an integral part of initial evaluation by virtually all physicians[1].

In this study, total number of patients with space occupying lesions in liver was 25. Most SOL size was more than three cm (>cm) in diameter in our study. The difference between the rate of detection of SOL in liver by trainee and radiologist was not significant (p=0.297). US scan is a powerful tool to allow sonologists to reach an early diagnosis of HCC. Screening of cirrhotic patients twice annually by ultrasound and alpha-fetoprotein (AFP) estimation is well established now[18,19]. Here radiologist could detect all cases of hepatic neoplasm, but the trainee detected 76% cases. The size of most SOL detected by the trainee was >3 cm, but possibly radiologist could detect smaller lesions by the more sophisticated machine of Radiology department. Saha[20] detected all cases of SOL in liver but the radiologist could detect 73% cases only in his study. A retrospective study on various imaging procedures used for diagnosis of small HCC (<3cm) was evaluated in Japan. The overall sensitivity of sonography was 84%[21].

Among 30 cases of diffuse liver disease, final diagnoses of 21 cases were cirrhosis of liver. Among them gastroenterology trainee detected 18 (85.7%) cases and radiologist 17 (80.9%) cases. Both the trainee and radiologist detected all three HCC and metastatic liver (heterogenous liver) and six fatty liver. Sonography was 88% accurate in assigning the correct pattern to the corresponding pathology (sensitivity 89%, specificity 86%, P<0.001) in a study conducted on 110 patients with wide variety of diffuse liver disease processes [22]. Sonography is the least expensive modality to diagnose fatty liver disease and can detect with a sensitivity of 83% and specificity of 100% when fat is present in more than 30% each lobule[23]. Saha[20] detected all the cases of diffuse liver disease in his study. This is similar with our finding.

Six patients were found to have cholelithiasis, these were not confirmed by other investigations. Both the trainee and radiologist could detect all the cases. Three cases were finally diagnosed as carcinoma gall bladder. The trainee could diagnose two of three cases of gall bladder mass, radiologist detected all of them. Sonography is the imaging method of choice for the initial evaluation of all suspected diseases of the gall bladder. It is highly reliable in detecting tiny gall stones (95% sensitivity)[1.6].

Among 25 patients of biliary dilatation, both the trainee and radiologist detected all the cases. Over all, the trainee could detect cause of dilation in 44% cases and radiologist in 76% cases. The image and resolution quality of US machine used by the trainee was lesser than the non-portable machine used by the radiologist, so smaller lesions may be missed by the trainee. Saha[20] detected 95% cases of biliary dilatation and etiology in 50% case in his study. In this study the trainee could find out choledocholithiasis in 50% cases. Ultrasonography usually demonstrates stones in the bile duct only in 30-50% cases[24].

In this study, 14 cases were found to have dilated portal vein (>13mm) suggestive of portal hypertension. All cases were associated with cirrhosis of liver diagnosed on clinical basis and laboratory investigations. The gastroenterology trainee found all of them, but radiologist missed four cases, (p=0.031). As the trainee gastroenterologist was fully aware of clinical details of patients, this may have impact on the diagnosis. The prevalence of varices in cirrhosis is proportional to the severity of portal hypertension and liver disease. Therefore cirrhosis patients should be screened when there is clinical evidence of portal hypertension, e.g. platelet count <1,00,000 or an

enlarged portal vein diameter >13mm on ultrasound[25]. Two cases were diagnosed to have portal vein thrombosis, confirmed with Doppler ultrasound study. Radiologist detected all of them but trainee detected none.

In the present series, 38 patients had ascites, these were not confirmed by other investigations. Both the gastroenterology trainee and radiologist detected ascites in all cases. Ultrasonography is highly sensitive to detect ascites. It can detect as little as 100ml of ascites[25]. Saha[20] could detect 97% of ascites cases in his study.

In this series, total 14 patients were found to have pancreatic disease. The gastroenterology trainee could detect eight (57%) of 14 cases and radiologist could diagnose 13(92.8%) cases. Both the trainee and radiologist detected both cases of acute pancreatitis one associated with pseudocyst. Among seven cases of chronic pancreatitis, trainee found four (57%) cases. But the radiologist found six cases (85.7%). More than 90% patients with acute pancreatitis have sonographic abnormalities[1]. Abdominal ultrasound may demonstrate calcifications, pancreatic atrophy or a markedly dilated pancreatic duct in chronic pancreatitis. The procedure has a limited ability to visualize the pancreas because bowel gas may obstruct the view[6]. Out of five cases of pancreatic carcinoma, the trainee could detect two cases (40%) and radiologist detected all five cases (100%). Saha[20] found 75% pancreatic mass lesions in his series. In this series, detection rate of pancreatic diseases by the trainee is quite low; it may be due to small sample size, lesser image quality of old portable machine and less efficiency of trainee.

In 18 cases of abdominal mass lesions, gastroenterology trainee detected 17 (94.4%) cases and radiologist could detect only eight (44.4%) cases. Ultrasound revealed gut mass lesions only, not the etiologies in respect of carcinoma stomach, duodenum, colon, abdominal tuberculosis and mass due to IBD. The difference of detection rate was significant (p=0.002). A retrospective study was made by Barker et al[5] in John Radcliff Hospital, Oxford on 104 adult patients with a palpable abdominal mass. Sixty-nine patients had an abnormality responsible for the clinically palpable mass and 35 patients did not. A prospective study on 227 patients was carried out by Hollerbach et al[3] in Germany. The overall sensitivity of US was 76%, whereas the positive predictive value was 98%. Overall specificity was 95%. The negative predictive value for bowel disorders was only 58%. Conclusion of the study was that positive US findings are useful for the diagnosis of bowel processes. US are highly predictive albeit not disease specific. Negative US examinations, however, do not exclude pathologic bowel processes. In the present series, the trainee gastroenterlogist could detect abdominal mass lesions more efficiently than radiologist. Possibly, clinical evaluation before US has impact on it. Besides this, radiologists might not have enough time to concentrate on every patient due to high workload. Saha[20] found 86% abdominal mass and radiologist detected 36% only in his study, this finding coincided well with the present series.

In this study 63 patients had clinical hepatomegaly. With defined cut off value the gastroenterology trainee found 59(93.6%) and radiologist found 44(69.8%) cases of hepatomegaly. The difference between the rates of detection was significant (p=0.001). This finding is similar with that of Saha[20]. Twenty-one patients were found to have clinical splenomegaly. With cut of value of 12.5 cm the trainee found 20(95.2%) and radiologist found 15(71.4%) on ultrasound. Detection rate is better than radiologist (p=0.038). This finding also well coincides with finding of Saha[20].Clinical evaluation before US by the trainee and high work burden on radiologist possibly explain the better detection rate by the trainee.

Ultrasound diagnoses of study population by trainee, those of radiologist and final diagnosis were compared to evaluate the diagnostic value of routine ultrasound after clinical evaluation by the clinician. Here the gastroenterology trainee could reach the correct diagnosis in 83 cases (78.3%) after sonologic examination and radiologist could diagnose 69 cases (65.1%). Using the chi square test on SPSS program assessed the significance of difference between gastroenterology trainee and that of radiologist. The difference was significant (p= 0.03).

The trainee gastroenterologist showed equal accuracy with the radiologist in detecting hepatic abscess, liver cysts, fatty liver, ascites, SOL in spleen, cholelithiasis and biliary dilatation. The trainee could detect the lesions better than radiologist in respect of hemangioma liver, cirrhosis of liver, portal hypertension and abdominal mass lesions. The radiologist was superior in diagnosing lesions like HCC, metastatic lesions in liver, carcinoma gall bladder, portal vein thrombosis, pancreatic diseases and in detecting etiologies of biliary dilatation. In this study gastroenterology trainee performed US with a small old portable machine (Toshiba Sonolayer SAL 32B) with a larger linear probe with 3.5 MHZ frequencies. Small portable machine has limited

ability of resolution, image quality and memory, which imposes some limits on the quality of examination[16]. Radiologist performed US in his department with a more sophisticated machine (Siemens Sonoline Prima) with two probes (curvilinear and linear) of different frequency (3.5 MHz and 7.5 MHz respectively). It's image quality, resolution is superior to the portable machine. Inspite of this, overall performance of trainee gastroenterologist is comparable with that of radiologist as the trainee was aware of full clinical details of patients but the radiologist knew brief summary of the same. Besides this, the trainee could spend much time for each patient during US keeping clinical information in mind but it might not be possible for the radiologist due to work burden and long awaiting list of patients seeking US in Radiology department.

In this study it is seen that the trainee in a very short time at bedside could detect most of gross pathology. So, patients of gastrointestinal, hepatobiliary and pancreatic disorders should undergo prompt ultrasound examination as a preliminary screening in ward by the clinician, which will increase greatly the diagnostic yield. Many developed and developing countries like Japan, Singapore, Germany, Austria, and India etc had already taken up this approach. Clinicians, surgeons, obstetricians and radiographers showed equal efficiency in US scan in relation to radiologist in various centers. The result in the present series validates the recommendation of inclusion of training in ultrasound in training program for trainee gastroenterologist made by the American Gastroenterological Association[15], European Board of Gastroenterology[14], and the council of the British Society of Gastroenterology[13]. In this perspective, it can be recommended that the training program should be part of the training of gastroenterologists seeking postgraduation in our country also.

As there is increasing interest in the patient focused and one stop approaches to the patients' management, so gastroenterologist should be trained on US. It is possible, gastroenterologist can be able to perform US examination in the ward as a routine and in emergency situations. This will lead to great impact on patients' early and proper management. Those more complicated cases may be referred to radiology department for evaluation. This will reduce the workload of radiologist and undue sufferings of patients in fact.

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KIMURA'S DISEASE: A CASE REPORT WITH BRIEF REVIEW

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Abstract:

A 12 year old boy, hailing from Uttara, Dhaka, presents with a painless, slowly growing mass over left parotid region along with marked eosinophilia. He was diagnosed as a case of Kimura's disease. This is probably the first reported case from Bangladesh of this rare entity which is commonly found in young Asian men of Chinese or Japanese origin. The typical picture is a triad of painless swelling with adenopathy in the head and neck region, marked eosinophilia and increased serum IgE level. Confirmed diagnosis is achieved by biopsy. Treatment modalities include steroids, cytotoxic drugs, radiotherapy and surgery. Although recurrence of the lesion is common, the disorder is benign in nature with no evidence of malignant transformation and the prognosis is excellent.

Introduction:

Kimura's disease (KD) is a rare, chronic inflammatory disorder of unknown etiology. Usually, there are unilateral painless subcutaneous masses in the head and neck region with associated adenopathy, eosinophilia and high levels of serum IgE. It is endemic in Asia, primarily affecting Chinese or Japanese men during the second and third decades of life.[1, 2] Many patients of KD diagnosed in other parts of the world have this ethnic origin. Few cases have been identified in other races as well including whites[3], Arabs[4] and Indians[5]. However, no case of KD has been reported from Bangladesh yet. In this paper, KD in a young Bangladeshi boy is reported and a brief review of the disease is discussed.

Case report:

A 12 year old boy presents with a painless swelling over the left parotid region for 2 months which was gradually increasing in size. There was no history of fever, cough, rash, sore throat or any constitutional symptoms. He had no problem in chewing and deglutition.

On examination, the boy was apparently healthy with average body built and nutritional status. There was a swelling over the left parotid region which was irregular, 7X5 cm in size, lobulated, non-tender, firm and not fixed with underlying structures or overlying skin. The skin over the swelling was normal. Lymph nodes were not enlarged. No abnormalities were found on examination of the oral cavity, teeth, ears and eyes. There was no evidence of facial palsy. Rest of the examination revealed normal findings.

Investigation: CBC: Hemoglobin 12.1%, TC 8400/cmm (Neutrophils 37%, Lymphocytes 38%, Eosinophils 21% and Monocyte 4%), Platelet 2,50,000/cmm and ESR 11 mm in 1st hour. RBS was 5.5 mmol/L and serum creatinine was 1.1 mg/dl. Urinalysis was normal, no proteinuria. X-ray of the chest and ultrasonogram of whole abdomen were also normal. FNAC from the lesion was done twice and both reported reactive hyperplasia.

Biopsy from the lesion was advised, but the patient refused. Instead, his father, who worked in Sweden, took him there for further evaluation. Extensive investigations were undertaken including a search for HIV, TB, filariasis and sarcoidosis, but the results were negative. By this time there was involvement of cervical lymph nodes. Biopsy was taken from the lymph node and bone marrow. The lymph node showed dense infiltration of lymphocyte with germinal centre formation, foci of eosinophilic infiltrate, vascular proliferation and presence of few Warthin-Finkeldey-type giant cells. There was eosinophilic infiltration in bone marrow. Depending on these histological features, typical clinical picture and peripheral eosinophilia, a diagnosis of Kimura's disease was made. The patient is now getting steroid.

Discussion:

Synonyms: Eosinophilic granuloma of soft tissue, eosinophilic hyperplastic lymphogranuloma, eosinophilic lymphofolliculosis, eosinophilic lymphofollicular granuloma, eosinophilic lymphoid granuloma.

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Background: KD was first described by Kim and Szeto in China in 1937 and was termed "eosinophilic hyperplastic lymphogranuloma".[6] The disorder received its current name after Kimura and his associates had provided a systematic description of it in 1948.[1]

Epidemiology: KD is endemic in the Far East. Most patients are of Chinese or Japanese origin, but it has been reported in other areas and ethnic groups as well.[3] Young and middle aged men of second and third decades are commonly affected.[7] Median age is 26 years with a male to female ratio of greater than 3:1.[8,9,10]

Pathogenesis: The pathogenesis of KD is still unknown. Probable mechanisms include an allergic or hypersensitivity response to a persistent antigenic stimulus and alterations of immune regulation.[3,11] Specifically there is marked proliferation of CD4 cells. Activated Helper T cells (Type 2) produce eosinotrophic cytokines such as granulocyte macrophage colony-stimulating factor, tumor necrosis factor-á, IL-4 and IL-5 resulting in high serum level of IgE and marked eosinophilia.[7,11-13] High levels of circulating eosinophilic cationic protein and major basic protein along with high tissue concentrations of IgE have been found in patients with active stage of KD.[14] Although no specific antigen has been detected to initiate this process, it is speculated that a viral, fungal (specially *Candida albicans*) or parasitic trigger may be responsible.[11,15]

Pathology: In 1989, the histological features of KD were classified in 3 groups - constant, frequent and rare. The constant features are preserved architecture of the lymph node, florid germinal centers, dense eosinophilic infiltration in a background of abundant lymphocytes and plasma cells, and an increased amount of postcapillary venules in the paracortex. The frequent features include sclerosis, karyocytosis in both the germinal centers and the paracortex, vascularization of the germinal centers, proteinaceous deposits in germinal centers, necrosis of germinal centers, eosinophilic microabscesses, and atrophic venules in sclerotic areas. Warthin-Finkeldey-type polykaryocytes are a common feature. Progressive transformation of germinal centers is a rare feature. Immunostaining findings included deposition of IgE in a reticular fashion in germinal centers and IgE coated nondegranulated mast cells.[3,9,10,14,16]

Clinical features: Typically, there are slowly enlarging painless masses in the head and neck region commonly involving subcutaneous soft tissues, lymph nodes (periauricular, axillary, epitrochlear and inguinal) and parotid and submandibular salivary glands.[7-10] Rarely, oral mucosa, auricle, scalp, orbit and ocular adnexa, limbs and trunk, kidneys and peripheral nerves may be involved.[10,11,17-19] There may be associated pruritus of the overlying skin and dermatitis. Skin lesions may present as reddish brown papules.[10] The lesion of KD gradually enlarges over a period of months to years.

It is generally a benign and self-limiting disease but may be complicated by renal involvement where nephrotic syndrome is the commonest presentation.[18,19] Various histological patterns of renal involvement is seen, e.g. minimal change disease, focal segmental glomerulosclerosis, mesangioproliferative glomerulonephritis, membranous nephropathy, IgM nephropathy, IgA nephropathy etc.[18] Occasionally, renal complication may be evident prior to subcutaneous lesions which may delay the diagnosis.[18] KD is also reported to be associated with systemic connective tissue disease.[3]

Differential diagnoses:[7,11]

1. Angiolymphoid hyperplasia with eosinophilia (ALHE)
2. Eosinophilic granuloma
3. Mikulicz's disease
4. Hodgkin's disease
5. Follicular lymphoma
6. Angioimmunoblastic lymphadenopathy
7. Salivary gland tumor
8. Acute non-lymphocytic leukaemia
9. Nodal metastasis
10. TB and other infectious lymph node enlargement (e.g. Toxoplasmosis)

All these differential diagnoses can be excluded by careful clinical evaluation and biopsy reports. Only ALHE may cause diagnostic difficulties.

Laboratory investigations: Peripheral eosinophilia and elevated serum IgE levels are consistently found.[1,3,11] Proteinuria may occur in 12 to 16% patients indicating associated renal complication.[18,19] Imaging studies including CT and MRI scans are useful to determine the extent and progression of the disease.[20] FNAC is useful to diagnose recurrent lesions,[3] but may be misleading in some cases[5]. Biopsy usually confirms the diagnosis.[5]

Differentiating from ALHE:

Angiolymphoid hyperplasia with eosinophilia (ALHE) is now considered a separate disorder from KD.[3,8,21] It is a rare, chronic, benign vascular tumor typically presenting in white women during 3rd and 4th decades as small intracutaneous nodules with heavy eosinophil infiltrate.[7,21] Lymphadenopathy, salivary gland involvement and elevated serum IgE levels are rare in ALHE. Blood eosinophilia is noted in less than 10% of cases.[16] Histologically ALHE shows increased vascular proliferation lined with plump, epithelioid appearing endothelial cells with nuclei of varied size and shape and hemosiderin deposits. Presence of

inflammation around medium sized vessels is also characteristic. Fibrosis is less marked. By contrast, reactive germinal centers and eosinophilic microabscess are found in KD, but not in ALHE.[10,11]

Treatment: KD is a benign lesion. Unless the lesion is symptomatic or disfiguring, treatment is not required. Surgical excision is the treatment of choice if localized and may be curative.[5] In many cases a diagnosis of KD is made after the biopsy of the lesion, thus the patient is treated before diagnosis.[3] However, recurrence is common (about 25%) after surgery.[3]

Other treatment modalities include oral or intralesional steroid, cyclosporine, pentoxifylline, radiotherapy, cryotherapy, laser fulguration etc. but their outcome varies. In addition, their long term complications and recurrence of the lesion after discontinuation pose limitation to their use.[3,11,22,23] Systemic steroids may be indicated in frequent relapses or in cases complicated by nephrotic syndrome.[5,24] Steroid should be started at high doses and then tapered to effect.[3,24] Radiation may be considered in cases refractory to medical and surgical treatments and where surgery is not possible.[3,5,25]

Prognosis: The prognosis is excellent with no potential for malignant transformation but spontaneous involution is rare.[3] The lesion may persist or recur frequently despite treatment. Sometimes without treatment it may give rise to a large and disfiguring lesion.

Conclusion:

The diagnosis of KD is difficult as many clinicians and pathologists are unfamiliar with it. As a result, patients have to undergo extensive tests before KD is suspected. Patients are usually worried about malignancies while the disorder is benign. In many cases, biopsy becomes essential to establish the diagnosis.

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FOCAL CORTICAL DYSPLASIA WITH STROKE

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This is a case of a man with acute onset right hemiparesis and recurrent convulsion due to stroke and cortical dysplasia, which has not previously been reported in Bangladesh.

Case report

A 38 years old, diabetic, normotensive, smoker, right handed male working as Ansar at Ansar VDP Head Quarter, Dhaka, was admitted to Neurology unit of BIRDEM on 16.01.2004 with the complaints of right sided weakness and slurring of speech for three weeks. Initially he got admitted into a Medical College Hospital on the day of his illness and was diagnosed as a case of stroke with right hemiparesis with DM-T2. CT scan of brain was not done. For his uncontrolled blood glucose level he was referred to BIRDEM hospital.

On admission his pulse was 80 pm; BP 160/100 mmHg, slurred speech, GCS-11, Right, VII nerve palsy (Upper motor type), muscle power reduced (MRC 3/5) on right side both proximal and distal group with extensor planter response: Other clinical finding were unremarkable.

His random blood glucose was - 28 mmol/l, CT scan of brain done on day of admission was inconclusive as to the nature brain lesion (Fig 1), other relevant investigations were normal.

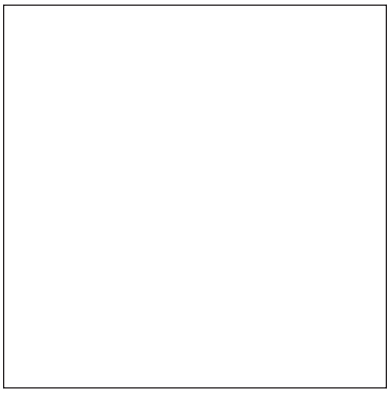

Fig.-1: *CT scan of Head*

An MRI of brain was planned but patient suddenly developed simple partial seizure, which was treated with carbamazepine, Eventually MRI of brain was done and focal cortical dysplasia with small cerebral infarct in left parietal lobe was reported (Fig 2). He

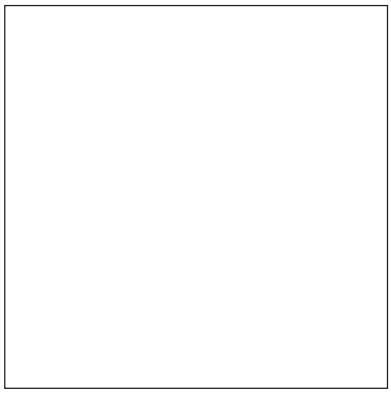

Fig.-2: *MRI of Brain (FLAIR).*

was discharged on 24.1.2006 with carbamazepine, aspirin, insulin, amlodipine and advised for physiotherapy and to quit smoking.

Discussion

Cortical dysplasia[1] is a group of developmental defect due to abnormal morphogenesis or defect in the regional specification of neuroepithelium or defect in apoptosis. There is great deal of interest in the interaction, of environmental and genetic defect in the development of cortex. Cortical dysplasia may be localized or generalized. A classification of cortical dysplasia is given in table-l.

Table-I

a.	Diffuse
	Lissencephaly
	Polymicrogyria
	Pachygyria
	Hemimegalencephaly
	Tuberous sclerosis
	Band heterotropia
	Periventricular nodular dysplasia
b.	Focal
	Isolated focal cortical dysplasia
	Schizencephaly
	Microdysplasia
	Heterotropia

Clinical features

Depends on type of lesion. Small focal lesion usually remain. asymptomatic, may present with focal seizure. Diffuse cortical dysplasia may present with

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developmental delay, static encephalopathy[3] or severe seizure. Heterotropia may present with pyramidal sign.

Treatment

Anticonvulsants

Surgery in refractory seizure.

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IMPROVED METHOD OF DIRECT MICROSCOPY FOR DETECTION OF ACID-FAST BACILLI IN SPUTUM

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Abstract

Microscopy of direct smears for acid-fast bacilli (AFB) is the most commonly used method for diagnosis of TB. However, direct smear microscopy of sputum, though rapid, has low sensitivity and there is a need for improved method. We have compared microscopy of smear made directly from sputum, with microscopy of sputum made after liquefaction and concentration of sputum with Bleach (commercially available as Chlortec, containing 5.25% NaOCl), microscopy of smear after processing of sputum with N-acetyl L-cystein-NaOH (NALC) and after petroffs digestion and concentration methods.

Three consecutive sputum from 300 clinically and radiologically diagnosed pulmonary tuberculosis patients were collected from indoor and outdoor patient department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, and institute of Tuberculosis control and research centre, Chankharpul, Dhaka, and the study performed in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.Among the 300 clinically and radiologically diagnosed pulmonary tuberculosis, cases, 162 (54%) cases had AFB positive smears using direct Ziehl-Neelsen's (ZN) staining.

After processing of sputum with different concentrating technique and then stained with ZN stain the number of positive patients was increased to 184 (61.33%) with Bleach and NALC methods and 182 (60.66%) with petroffs method. Though the sensitivity of Bleach and other concentrating methods are similar, Bleach method took less time to completely homogenize the mucoid sputum then did the NALC and petroffs methods. Bleach is also easily available, cheaper than other concentrating methods with improved laboratory safety. Therefore, the Bleach concentrating method may provide better result in TB laboratories of developing countries like Bangladesh.

Introduction:

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. It is the world's leading cause of death from a single infectious agent[4]. It is still a problem in developing countries, where the prevalence is extremely high; even developed countries show an increase in prevalence, as a consequence of the AIDS epidemic and other social factors. Each year nearly one percent of the world's population is newly infected with TB, 5 to10 percent of them become sick or infectious at some time during their life[11]. Currently there are about 10 million new cases of tuberculosis every year with 3 million deaths occurring worldwide. More than 90% of global TB cases and death occur in developing world. Forty percent of the world's TB cases live in South East Asian Region where 3 million cases and 7,00,000 deaths occur every year.

The diagnosis of tuberculosis is hampered by the sloe growth of M. tuberculosis. Culture of mycobacteria is the reference method for detection of tubercle bacilli but it is slow and need special safety procedures in the laboratory. Serologic techniques may be useful in some clinical situations but both there sensitivity and specificity are unsatisfactory[3]. Nucleic acid amplification methods are most promising, but the technology is very much expensive. So microscopy of direct smears for acid fast bacilli (AFB) remains "the gold standard" for most laboratories. The specificities of positive acid fast smear is high (99.3% to 99.9%) but the sensitivity is low ranging from 22% to 78%[7].

Therefore, there is a need to detect methods for improvement of diagnosis of pulmonary tuberculosis by techniques that are appropriate in developing countries.

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Digestion of sputum with sodium hypochlorite (NaOCl, 5.25%) gave the best recovery of AFB (2) and concentration of bacilli by centrifugation of sputum increased the recovery rate of mycobacteria (Ratnam and March, 1986). Improved recovery of mycobacteria after treatment with NaOCl might be attributable to changes in surface properties of the mycobacteria (*ie* charge and hydrophobicity), and for denaturing of sputum constituents leading to flocculation and subsequent increased sedimentation rate of mycobacteria[5]. Gebre et al., (1995) reported NaOCl not only increased the sensitivity of sputum microscopy but also acts as a potent disinfectant, thus eliminates the risk of laboratory infection, especially in laboratories with inadequate safety standards. It is also easily available, inexpensive and approximately requires 20-30minutes to perform the procedure. NaOCl solution can be preserved for a longer period in room temperature. Disadvantage of the method is the specimen cannot be cultured after processing in this method as it kills almost all mycobacteria.

Material and Method:

Patients and control subjects were selected from indoor and outpatient department of Bangabandhu sheikh Mujib Medical University (BSMMU) and Institute of tuberculosis Control and Training, Chankharpool, Dhaka.Sputum samples on 3 consecutive days were collected from 300 clinically and radiologically diagnosed pulmonary tuberculosis patients. We have compared microscopy of smear made directly from sputum, with microscopy of sputum made after liquefaction and concentration of sputum with Bleach (commercially available as Chlortec, containing 5.25% NaOCl), microscopy of smear after processing of sputum with N-acetyl L-cystein-NaOH (NALC) and after Petroffs digestion and concentration methods. Only samples that were processed with NALC method were cultured in L-J media.

Laboratory Procedure

A direct smear from each specimen was prepared and stained with Ziehl-Neelsen's (Z-N) stain (Appendix-II). and the remainder was divided equally into three 15-ml screw-capped tubes. The tubes were labeled A, B and C respectively, tube A was used for the NaOCl method, tube B was used for the N-acetyl L-cysteine method and tube C for petroffs method of sputum processing. In all three cases smear was made from the processed sputum and ZN stain done.

Processing of sputum with Bleach concentration: (Gebre et al, 1995)

1. A portion of the sputum (2 ml) was taken to 15 ml screw capped tube and mixed with equal volume of commercially available household bleach (5.25%). The mixture was kept stand at room temperature for 10 minutes and shaken at regular intervals for proper digestion of sputum.

   The samples were centrifuged at 3000rpm for 15 minutes. The supernatant was discarded.

2. Smear were prepared from the pellets, air-dried, heat fixed and stained by Ziehl-Neelsen's method and examined under oil immersion objective in the light microscope.

Digestion procedure by Petroff's method: (Kent & Kubica,1985)

1. Two ml of sputum was taken in a 15 ml plastic centrifuge tube to which 4ml of 4% NaOH and was added.
2. The tube was kept stand for 15 minutes at room temperature and shaken at regular interval for proper digestion of sputum.
3. The tube was then centrifuged at 3000 rpm for 15 minutes, and the supernatant was discarded.
4. The sediment was resuspended with 10ml sterile saline.
5. Then the tube was centrifuged at 3000 rpm for 15 minutes, supernatant was discarded and smear was made from the deposit and stained with ZN stain.

Digestion procedure by N-acetyl L-cysteine method: (Kent & Kubica, 1985)

1. Three ml of sputum was taken in a 50 ml plastic centrifuge tube to which equal volume of NALC-NaOH solution (Appendix-III) was added.
2. The content of the tube was mixed properly with shaking for liquefaction of sputum.
3. The tube was kept stand for 15 minutes at room temperature for decontamination.
4. Digested-decontaminated specimen was then diluted up to the 50 ml mark with sterile phosphate buffer (Appendix-IV), pH 7.0, to minimize the continuing action of NaOH.
5. The tube was then centrifuged at 3000 rpm for 15 minutes
6. After centrifugation, supernatant was discarded and pellets were used for ZN staining and culture.

7. Two to three loopfulls of sediment was taken in to Lowenstein-Jensen media (LJ) media for mycobacterial culture.
8. A loopfull of sediment was also taken to prepare a smear for ZN stain.

Results:

sputum from 300 pulmonary TB patients were studied microscopically on three consecutive days for AFB prepared directly from sputum and after sputum processing with Bleach, NALC and Petroffs concentrating technique respectively. By direct smear preparation method 162(54%) patients were found AFB positive, of them 6 patients were missed for AFB on the 1st day, which were found positive on 2nd and 3rd day, and 3 patients were missed for AFB on the 2nd day, which were found positive on the 3rd day. By using concentrating technique in sputum processing with Bleach, NALC and Petroffs methods, no samples were missed on any consecutive three days, 184 (61.33%) patients were found AFB positive with Bleach, and NALC methods and 182(60.66%) patients were found AFB positive with Petroffs method (Table:I. Thus 22(7.33%) AFB positive cases were detected by Bleach and NALC concentrating technique and 20(6.66%) AFB positive cases were detected by Petroffs concentrating technique, which were false negative by direct smear preparation procedure for AFB staining ($p<0.05$).

Microscopic finding revealed that maximum AFB positive patients, 129 (79.62%) were found as (+++) followed (++++) (9.25%), (+) (6.79%) and (++) (4.32%) by direct smear preparation (Table:I). When using Bleach and NALC concentrating technique 159(86.41%) sputum positive patients were detected as (++++), 3 cases detected as (+++), 4 cases detected as(++) and 18 sputum positive cases detected as (+). By using petroffs method 157(86.26%) positive patients were detected as (++++). The smear positive patients, those were not identified by direct method preparation due to the presence of lower number of mycobacterium in sputum that were detected as (+) and (++) by concentrating technique.

Table-I

Microscopic findings of AFB in sputum using different methods (N=300).

Methods	No. of positive cases in			Total positive cases
	1st day	2nd day	3rd day	
Direct	156	159	162	162(54%)
Bleach	184	184	184	184 (61.33%)
Petroffs	182	182	182	182 (60.66%)
NALC	184	184	184	184 (61.33%)

NALC = N-acetyl L-cysteine.

Table-II

Number of patients according to AFB positivity in sputum using different methods of processing (N=300)

Methods	Number of patients according to AFB positivity				Total positive
	(+)	(++)	(+++)	(++++)	
Direct	11	7	129	15	162(54%)
Bleach	18	4	3	159	184(61.33%)
Petroffs	16	5	4	157	182(60.66%)
NALC	18	4	3	161	184(61.33%)

NALC = N-acetyl L-cysteine.

Discussion:

Despite all the advances made in the treatment and management, tuberculosis still remains as one of the major public health problem, particularly in the developing countries. Tuberculosis has been a grave health problem in Bangladesh with adverse social & economic consequences.

Current recommendations for the control of tuberculosis emphasize case detection so as to allow treatment of patients and thereby limit the transmission of the bacilli[10]. The mainstay for its control is the rapid and accurate identification of the infected individuals. The simplest rapid method is the detection of acid-fast bacilli by microscopy. In developing countries, microscopy of sputum is by far the fastest, cheapest and more reliable method for diagnosis of pulmonary tuberculosis. Although simple, rapid and economical, the estimated detection limit of microscopy is 10^4 bacilli/ml of sputum. Smear sensitivity may be influenced by a variety of factors including type of specimen, efficiency of decontamination and concentration procedures, types of staining procedures and experience of microscopists. So the range of acid fast smear sensitivity is quite wide from 22% to 78%[7]. The specificity of positive acid fast smear is high (99.3% to 99.9%).As a result large number of patient with pulmonary TB, and around 75% of extrapulmonary TB are smear negative, and culture methods take several weeks to become positive[12].

Digestion of sputum with Bleach (NaOCl) gave the best recovery of AFB[2] and concentration of bacilli by centrifugation of sputum increased the recovery of mycobacteria[8]. Improved recovery of mycobacteria after treatment with Bleach might be attributable to change in surface properties of the mycobacteria (*ie* charge and hydrophobicity), and for denaturing of sputum constituents leading to flocculation and subsequent increased sedimentation rate of mycobacteria[5]. The major advantage of the Bleach method is the higher density of bacilli per microscope field obtained after concentration of the sample and the reduction of debris present in sputum, leaving a free field for bacterial detection.

Among the 300 cases of pulmonary tuberculosis, 162 (54%) had AFB positive smears using direct Ziehl-Neelsen's (ZN) staining. After processing of sputum with different concentrating technique and then stained with ZN stain the number of positive patients was increased to 184 (61.33%) with Bleach and NALC methods and 182 (60.66%) with Petroffs method. Though the sensitivity of Bleach and other concentrating methods are similar, Bleach method took less time to completely homogenize the mucoid sputum then did the NALC and Petroffs methods. Bleach is also easily available, cheaper than other concentrating methods with improved laboratory safety. Therefore, the Bleach method may provide better result in TB laboratories of developing countries

Table VI: Results of sputum positive for AFB patient on three consecutive days by different methods. (N=300).

Sputum from 300 pulmonary TB patients were studied microscopically on three consecutive days for AFB prepared directly from sputum and after sputum processing with Bleach, NALC and Petroffs concentrating technique respectively. By direct smear preparation method 162(54%) patients were found AFB positive, of them 6 patients were missed for AFB on the 1st day, which were found positive on 2nd and 3rd day, and 3 patients were missed for AFB on the 2nd day, which were found positive on the 3rd day. By using concentrating technique in sputum processing with Bleach, NALC and Petroffs methods, no samples were missed on any consecutive three days, 184 (61.33%) patients were found AFB positive with NaOCl, and NALC methods and 182(60.66%) patients were found AFB positive with Petroffs method. All AFB positive smears by direct method were also positive by household bleach concentration method and other concentrating methods. The number of positive cases increased by 22(7.33%) using the Bleach and NALC method and 20(6.66%) positive patients were increased by Petroffs method ($p<0.05$). The results of sputum microscopy using Bleach treatment correlate with other studies. Ängeby et al. (2000) reported the use of Bleach method increased the number of positive cases for acid fast bacilli by 5% than compared with direct method. Romulo et al. (1997) showed Bleach treatment increased the number of positive cases by10%. Improved recovery of mycobacteria after treatment with Bleach due to change in surface properties of the mycobacteria (*ie* charge and hydrophobicity), and for denaturing of sputum constituents with subsequent increased sedimentation rate of mycobacteria (5).

There was also a marked increase in the average number of AFB seen in sputum per microscope field in the smears prepared after digestion with NaOCl and other concentration methods. Smears which were graded microscopically as (+) by direct method increased to (+++) or (++++) after concentrating methods. All AFB positive smears by direct method were also positive by Bleach and other concentrating methods. Twenty two AFB negative cases by direct method were positive by Bleach and other

concentration methods, and were found mainly as (+) and few as (++). Two AFB positive cases which were not detected by Petroffs method were also recovered by Bleach method and NALC method. Other studies also showed that there was a significant increase in the average number of AFB seen per field in the smears prepared after the concentration methods. Nyein et al, 2001 reported that smears that were graded (+) by direct method increased to (+++)or (++++)after concentration with Bleach treatmentThe higher density of bacilli per microscope field obtained after concentration of sample and the reduction of debris present in the sputum, leaving a free field for bacterial detection.

Conclusion and Recommendations:

This study suggests that digestion of sputum with sodium hypochlorite and concentration by centrifugation increase the sensitivity of direct microscopy with improved laboratory safety. The method is simple and requires only NaOCl, which is easily available. Thus the application of this method is feasible and useful especially in resource poor settings.

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A COMPARATIVE STUDY OF DONOR SCREENING TESTS BY RAPID CHROMATOGRAPHY AND ELISA METHOD FOR HBV, HCV & HIV- A TERTIARY HOSPITAL TRANSFUSION MEDICINE CENTER BASED STUDY

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Abstract:

3,600 (HbsAg, anti-HCV, anti HIV) negative and 100 (67 HbsAg, 27 anti HCV, 06 anti HIV) reactive blood donor samples on Rapid chromatography screening tests was retested by ELISA screening method in the transfusion medicine department of BSMMU within the period from 13.07.2005 to 27.02.2006. Only 03 HbsAg positive, 02 anti- HCV positive and no anti HIV positive was found by ELISA within negative donor samples. It means that if Rapid chromatography was used instead of ELISA for screening of HBV, HCV and HIV, the chance of transmission was 0.08% for HBV, 0.05% for HCV more than that of ELISA. The study also shows that Rapid test by immuno chromatography give more false positive than false negative results, which is not a major problem in donor screening. False positive result found were 44.7% for HbsAg 74% for anti HCV and 0% for anti HIV compared to ELISA test. But positive results in ELISA were not confirmed by any further test procedure. Based on above results, cost and time requirement of ELISA advocacy may be made in favour of Rapid test for blood donor screening at present situation.

Key words: Screening, Rapid immuno chromatography, ELISA, Comparision, Advocacy.

Introduction:

Screening of blood donors for, HBV, HCV, Syphillies and malaria is made compulsory by law in Bangladesh.[1] The aim of screening is to make transfused blood free from above infectious agents as far as possible because they produce post transfusion infection. For HBV screening the marker used universally are HbsAg alone or with totalanti HBc. In our country alone HBsAg is accepted marker. For HCV and HIV—anti HCV and anti –HIV I & II are accepted markers like other countries. As a method ELISA for the detection of above markers is accepted best for donor screening. Donors found reactive for HbsAg, anti-HCV and anti HIV I & II by ELISA are deferred from donation. ELISA method like other biologic tests may give false positive or negative results. The false positive results are not a major problem in donor screening except unnecessary reduction of blood donor from donor pool. So, require confirmation. But false negative results are dangerous in donor screening as it open the door to transmit infection. ELISA capable of detecting 0.25ng/ml HbsAg even by using monoclonal antibody cannot detect all cases of HBV infection. Anti-HBC included in some countries in adition to HbsAg. HBV is transmitted from donors who are HbsAg negative but anti HBc positive[2]. Transmission by HbsAg and anti-HBc negative donors still occur[3]. Even some reports indicate that HBV-DNA is detected by PCR in individuals who have no HBV marker[4], incubation or window period may be responsible for it. Like false positive, false negative results are found in anti-HCV testing by ELISA method which may be confirmed by RIBAs or PCR but not suitable for donor screening. Use of two sequential different screening assay is an alternative approach and found to be reliable for donor screening[5], but not affordable for all country including Bangladesh. Serrogate test ALT may detect some carriers of hepatitis who do not have speceific markers[6]. HIV — ELISA may give false negative like false positive result- confirmed by Western blot or PCR but not suitable for screening. It is true that ELISA screening can not give 100% safety from HBV, HCV or HIV .It is a time consuming method. It requires costly reagents, equipments and set up in a transfution medicine center. Other methods like RIBAS, PCR ete highly costly, complex and not recommended for screening.

Now a days, a new precise method of identifying markers of HBV, HCV and HIV have been introduced known as 'Rapid Chromatography, Which is a form of

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membrane immunoassay. It is a modified enzyme method but precise. It is now widely used as a rapid test for screening in centers with small number of donors[7].

In the present study, we have tried to make comparision between effectiveness in screening between Rapid and ELISA method. Donor samples found negative/positive in Rapid test was retested by ELISA. Results in two methods was compared, time required for screening and cost of test was also compared, finally advocacy was made.

Methods and materials:

The study was conducted within the period from 13.7.2005 to 27.2.2006 in the department of transfusion medicine, BSMMU. A total of 3,700 donor samples was first tested by Rapid chromatography. Rapid negative samples (3,600) and positive samples (100) were preserved separately at 4^0C within one hour and tested by ELISA method within 72 hours of collection. In ELISA, the reactive samples in duplicate test was taken as positive and no further confirmatory procedure was adopted. The reagent used for rapid chromatography was from Accu-Biotec and Excell (China) and for ELISA reagents used are of 3rd generation from India, and Korea. The specificity and sensitivity cited in literature was more or less same in two methods.

Aim of the study:

The aim of the study is to make a comparision of the positive or negative results obtained in Rapid Chromatography with the results found in ELISA method and to make a comment about the method of testing suitable for the screening of HBV, HCV and HIV in Blood Transfusion Centers at the present socio-economic condition.

Result Of The Study: are summarized in tables

Table-I

Reference HBsAg

Method		ELISA		Total Results
Rapid chromatography	Result	Positive	Negative	
	Positive	27	40	67
	Negative	03	3,630	3,633
Total Results	30	3,670	3,700	

Table-II

Reference anti-HCV

Method		ELISA		Total Results
Rapid chromatography	Result	Positive	Negative	
	Positive	07	20	27
	Negative	02	3,671	3,673
Total Results	09	3,691	3,700	

Table-III

Reference anti-HIV

Method		ELISA		Total Results
Rapid chromatography	Result	Positive	Negative	
	Positive	0	06	06
	Negative	0	3,694	3,694
Total Results	0	3,700	3,700	

Table-IV

Percentage of false negative in Rapid (Chance of transmission).

Test for	Total samples	Rapid Negative	ELISA Positive	Percent
HBV	3,700	3,633	03	0.08%
HCV	3,700	3,673	02	0.05%
HIV	3,700	3,694	0	0%
	3,700		05	0.13%

• False negative in Rapid is 0.13% (5 within 3,700 donor)

Table-V

Percentage of False Positive in Rapid (Change of missing donor):

Test for	Total samples	Rapid Positive	ELISA Negative	Percent
HBV	3,700	67	30	44.7%
HCV	3,700	27	20	74.0%
HIV	3,700	06	06	0%
	3,700		56	1.5%

• False positive in Rapid is 1.5% (56 within 3,700 donor)

Discussion:

A total of 3,700 samples was tested in both Rapid chromatography & ELISA for HbaAg, anti HCV and anti HIV. Rapid test was done first and there after the positive/negative samples was retested by ELISA with a view to compare the result of Rapid test with ELISA. Undoubtedly, ELISA is a superior and established method of screening blood donor for HBV, HCV & HIV. The present study (table 1, 2 & 3) also proves the superiority of ELISA.

In case of HBaAg, within 3,633 Rapid-Negative samples only 3 were positive in ELISA and within 67 Rapid- Positive samples 37 were positive in ELISA. In case of anti HCV, within 3,673 Rapid-Negative samples only 02 were positive in ELISA and within 27 Rapid-Positive samples 07 was positive in ELISA. For anti HIV, within 3,694 Rapid-Negative donors no one was found positive in ELISA & within 06 Rapid positive donors no one was positive in ELISA.

If only Rapid method was used the chance of transmission of HBV, HCV & HIV due to false negative were only 05 (0.13%) within 3,700 donors (table-4). As a screening method even ELISA cannot ensure 100% prevention.

The study also shows that Rapid method gives more false positive result compared to ELISA (table-5) & is 1.5% (56 within 3,700 donors), which is significant. But it is not a major problem in donor screening as it bears no risk of transmitting infection. But it unnecessarily reduces the donor from donor pool. The problem can be overcome by retesting the positive samples by ELISA & re-recruiting the negative donors in donor pool-if only Rapid test used for mass screening.

If cost of per test is compared Rapid test is half/ one third of ELISA .If time requirement is compared, Rapid test require time only One-Sixth or One-Seventh that of ELISA testing.

In Bangladesh, the blood transfusion centers are still at primitive level of development. Most of the centers are suffering from the lack of accommodation, shortage of manpower and also lack of fund. Cost sharing has been restricted. The conciousness of people and also of Surgons and clinicians about transfusion is still not up to date. Most of the donations are relative[8]. Surgeons send requisition from operation theatre and most of the requisitions demand unnecessarily fresh blood as soon as possible after giving donor sample for screening and matching. We suppose such is the situation in most developing and underdeveloped countries. Because of this problem, National module have proposed Rapid screening for centers receiving few donations at district level. If ELISA screening is to be performed donation or donor samples must reach blood bank at least 12 hours before supply and screening of post donation samples only. Most donations must be voluntary.

Considering all these, we advocate in favour of Rapid screening by Rapid Chromatography at present situation which may be shifted to ELISA method with improvement of consciousness in future if felt necessary.

Conclusion:

In spite of superiority of ELISA there is no major problem to use Rapid Chromatography for screening HBV, HCV and HIV markers of blood donors.

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EVALUATION OF SEROLOGICAL TEST FOR THE DIAGNOSIS OF TUBERCULOSIS USING THE (38KDA + 16KDA) ANTIGEN

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Abstract:

We evaluate an enzyme linked immunosorbent assay (ELISA) for the measurement of IgG antibody in human serum to the (38kDa + 16kDa) antigen of Mycobacterium tuberculosis *from Omega Diagnostics to diagnose tuberculosis. The study was carried out in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, July 2006. . A total of 160 serum samples of tuberculosis patients and control subjects were studied. 72 sera from both microscopy and culture positive pulmonary tuberculosis patients and sera from 46 both microscopy and culture negative pulmonary TB patients; those were diagnosed by combination of clinically, radiologically and with high ESR findings. Sera of 21 non TB respiratory disease control patients and sera of 21 healthy control subjects were studied. Among 72 microscopy and culture positive pulmonary TB patients, 55(76.381%) cases showed positive response, of which 31(43.05%) cases showed high positive and 24(33.33%) showed low positive response. Among 46 microscopy and culture negative pulmonary TB patients, 24(52.17%) cases showed positive response, of which 7(15.21%) showed high positive and 17(36.95%) showed low positive response. Out of 21 diseases control 4 (19.04%) cases and out of 21 healthy control subjects 3(14.2%) cases showed only low positive response. The specificity was 85.71%. The main diagnostic benefit of serology rests in the rapid diagnosis of approximately 52.17% cases of microscopy and culture negative pulmonary tuberculosis However, in view of seronegativity of microscopy positive pulmonary tuberculosis cases, antibody detection by serology can not replace microscopic examination for diagnosis of tuberculosis. A positive result could potentially aid in clinical decision making in selected symptomatic patients.*

Introduction:

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. It is the world's leading cause of death from a single infectious agent (5). Tuberculosis is still the top killer in our country among infectious diseases. Nearly 2.21% of the population become infected every year, 3, 00,000 progress to disease and at least 70,000 people die of TB each year (14). Currently, the only sure criterion for definite diagnosis of TB is the demonstration of the presence of tubercle bacilli in clinical specimens. This is based on traditional methods, microscopy with Ziehl-Neelsen's (Z-N) acid fast stain and laboratory culture of *M.tuberculosis* on Lowenstein-Jensen (L-J) medium (9). However, Z-N staining lacks sensitivity, only 40-60% of truly active pulmonary tuberculosis cases being confirmed even with optimal staining and satisfactory microscopic examination.Other methods for diagnosis of Tuberculosis include Culture, Tuberculin test, Chest X-ray, Histopathology, PCR and Serology. Culture techniques are complex and time consuming, require several (4-8) weeks to yield growth of *M. tuberculosis* and also lack sensitivity, particularly in smear negative cases.

Though PCR is highly sensitive, 88 to 100 % (11) the technique gives false positive results (specificity of PCR is 80%) due to contamination with DNA fragments from previous PCRs debris from nonviable bacilli (10). The Tuberculin test is a well-established and widely used test for determining infection with tubercle bacilli. But it can not differentiate between a recent and past infection and also exposure to *M. tuberculosis* and active disease (1). Chest X-ray can be useful in the diagnosis of tuberculosis. But chest lesions identified by radiograph is not specific and cannot identify the causal agent (6). Serological

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techniques are easy to perform and require simple reagents and could be helpful in smear or culture negative Patients.

The humoral response to different proteinaceous antigens of *M tuberculosis* is heterogeneous among patients with active disease, and this has originated in the proposal to use a combination of several specific antigens to find an efficient serodiagnostic test for tuberculosis (4).

The 38 kDa antigen has been reported as the single most antigen for the serodiagnosis of tuberculosis (7). This antigen is poorly expressed in M bovis than in *M tuberculosis* and is present in one tenth of the concentration of that in *M. tuberculosis*. (2). As *M. africanum* is only limited in some countries of East and West Africa, it is specific for *M tuberculosis* in other parts of the world.

The 16kDa antigen is also present in TB-complex bacteria. Both of the 16kDa and the 38 kDa antigens are highly specific recombinant antigen of *M tuberculosis* complex that has been expressed and purified from *Escherichia coli* by recombinant DNA technology. Wilkinson et al. (1997) while evaluating 38 kDa have reported sensitivity of 73% and specificity of 95%. The 16 kDa antigen has been shown to increase the sensitivity when used in combination with 38 kDa antigen as compared to using the 38 kDa alone and usually does not affect the high specificity of the test. Mahmood et al. (2000) have reported a sensitivity of 73% and a specificity of 90% during evaluation of Pathozyme-TB Complex Plus which incorporates these two (38kDa+16kDa) antigens. The study was done to see serum IgG response against (38kDa + 16kDa) antigen among tuberculosis patients.

Patient's Selection Criteria:

A total of 160 serum samples of tuberculosis patients and control subjects were studied. 72 sera from both microscopy and culture positive pulmonary tuberculosis patients and sera from 46 both microscopy and culture negative pulmonary TB patients; those were diagnosed by combination of clinically, radiologically and with high ESR findings.

Exclution Criteria:

Patients on anti-tuberculosis treatment, immunocompromised conditions with malignancy or any chronic illnesses were excluded. Patients under steroid or immunosuppressive therapy were excluded from the study.

Selection of Control Subjects:

Disease control subjects: Twenty-one patients with non-TB respiratory diseases, including pneumonia (n=8), acute bronchitis (n=7), pleurisy (n=4), asthma (n=2), were screened by radiographic techniques and diagnosed according to the standard clinical practice for each disease were included in the study.

Healthy control subjects: These groups consisted of healthy blood donor population and medical students with no past history of tuberculosis or any other chronic illness.

Collection of Samples:

Sputum samples were collected for bacteriological examination. A direct smear from each sputum was prepared and stained with Ziehl-Neelsen's (Z-N) stain and culture done in L-J medium. Blood samples collected in the test tube were allowed to stand for one hour and then the serum was separated by centrifugation and collected in sterile eppendorf tubes, and preserved at -20ºc until used for antibody detection by ELISA.

Detection of anti TB IgG antibody response against (38kDa+16kDa) antigen in serum by ELISA using Pathozyme TB complex Plus:

Reagent preparation:

1. All the kit components and test sera were brought to room temperature prior to the start of test
2. Wash buffer reagent was diluted 1:20.
3. The kit control sera were run with each batch of specimens to check the performance and determine the cut-off value.

Test Procedure:

1. Test serum was diluted 1:50 by adding 20µl of serum to 1000µl of dilution buffer.
2. 100µl of diluted samples and the control were dispensed in appropriate well. The plate was then incubated for 60 minutes at 37°C.
3. Well contents were then discarded and washed three times.
4. 100il of the conjugate was dispensed into each well, and incubated at 37°C for 30 minutes.
5. At the end of incubation the plate was again washed three times with wash buffer in previous manner.
6. 100il of the TMB (Tetramethyl Benzidine) Substrate was dispensed into each well and incubated in the dark for 15 minutes at 37°C.
7. The reaction was stopped by adding 100il of stop Solution (Sulphuric Acid) into each well. This produced color change from blue to yellow in well containing the enzyme, which indicated the presence of anti Mycobacterium tuberculosis complex antibodies.

8. Absorbance of each well was measured immediately after stopping the reaction with an automated plate reader set at 450nm wavelength and blanked on air.

Calculation:

For each test and control sera Optical Density (OD) was determined. The serum IgG values (units/ml) were calculated by using Biography software.

Interpretation of result:

Serounits	IgG
Negative result:	Less than 200U/ml
Low positive result:	200U/ml to 450U/ml
High positive result:	Greater than 450U/ml

Result:

Among 72 microscopy and culture positive pulmonary TB patients, 55(76.381%) cases showed positive response, of which 31(43.05%) cases showed high positive and 24(33.33%) showed low positive response. Among 46 microscopy and culture negative pulmonary TB patients, 24(52.17%) cases showed positive response, of which 7(15.21%) showed high positive and 17(36.95%) showed low positive response. The sensitivity and specificity of (38kDa+16 kDa) antibody test for diagnosis of tuberculosis among different groups of study population. The sensitivity was 76.38% among microscopy and culture positive and 52.17% among microscopy and culture negative pulmonary TB cases. The specificity was 85.71%.

Table-I

Antibody(IgG) level to (38kDa+16 kDa) antigen of M.tuberculosis among study population.

Study population	Positive response		
	High positive	Low positive	Total +veresponse
Pulmonary TB patients (n=118)	38(32.20)	41(34.75)	79(66.94)
Extra Pulmonary TB patients(n=21)	3(14.28)	5(23.80)	8(38.09)
Non TB disease control(n=21)	00	04(19.04)	04(19.1)
Healthy control(n=21)	00	03(14.28)	03(14.2)

Negative result = IgG level less than 200U/ml
Low positive result = IgG level 200U/ml to 450U/ml
High positive result = IgG level greater than 450U/ml
Figures within parenthesis indicate row percentage.

Table-II

Performance of (38kDa+16 kDa) antigen for diagnosis of tuberculosis among different groups of study population.

Study population(N=139)	Microscopy and culture result	No. of patient with +ve Ab response	Sensitivity(%)	Specificity(%)
Pulmonarytuberculosis	Micr & cul+ve (n=72)	55	76.38	85.71
	Micr & culture -ve(n=46)	24	52.17	

Micr & cul +ve = Microscopy and culture positive
Micr & cul -ve = Microscopy and culture negative.
Patient with +ve Ab response = Patient with positive antibody response

Discussion:

Despite all the advances made in the treatment and management, tuberculosis still remains as one of the major public health problem, particularly in the developing countries. Tuberculosis has been a grave health problem in Bangladesh with adverse social & economic consequences.

Immunological methods use the specific humoral and cellular responses of the host to infer the presence of infection or disease. Since the introduction of ELISA in 1972 and the availability of monoclonal antibodies as well as purified antigens, the serological diagnosis of tuberculosis has become more promising (3). To develop a standard serological test for tuberculosis, different antigens were tried, but the observed immune responses were heterogeneous.

It is accepted that TB patients produce antibodies to more than one pertinacious antigen. Wide spectrums of humoral responses exist in TB patients, depending upon the disease stage, the patient's immunological background, and/or the differential gene expression of different strains of *Mycobacterium tuberculosis*. Thus, some investigators suggest the use of a combination of specific purified antigens for TB serodiagnosis.

A combination of two or more antigens further improves the utility of serological testing, especially in sputum negative cases of the disease. Antigens used in different combinations also yield an improvement in the sensitivity and specificity of the assay. The use of 16kDa antigen has been shown to increase the sensitivity when combined with 38 kDa antigen compared to using the 38kDa alone but does not affect the high specificity of the test, (13). Mahmood et al 2000. have reported a sensitivity of 73% and a specificity of 90% during evaluation of Pathozyme-TB Complex Plus which incorporates these two, 16kDa and the 38 kDa antigens antigens. Both of the 16kDa and the 38 kDa antigens are highly specific recombinant antigen of *M tuberculosis* complex that has been expressed and purified from *Escherichia coli* by recombinant DNA technology.

In this study, the antibody response (76.38%) among microscopy & culture positive pulmonary TB was higher than microscopy and culture negative group (52.17%) (Table-II). The higher rate of seropositivity for the microscopy & culture positive TB patients compared to that for the microscopy & culture negative TB patients may be due to more bacillary load exposed to immune system leading to more antibody production. The cause of negative response among microscopy & culture positive patients of pulmonary TB might be due to intracellular nature of the bacilli resulting in less or no exposure to the immune system, or it may be due to immunosuppressive condition of the patients. The results of antibody response of this study correlate with other studies.

The sensitivity of (38kDa + 16kDa) antigen was 76.38% in smear and culture positive cases and was 52.17% in smear and culture negative cases. The specificity of the test was 85.71% (Table-II). Wilkinson et al. (1997) carried out ELISA with (38 kDa + 16 kDa) antigen and found a sensitivity of 63%, which increased to 73.5% when culture confirmed tuberculosis cases were considered. The findings correlates well with Lopez et al. (1996), they evaluated with Pathozyme TB complex plus with smear negative tuberculosis and found 40% sensitivity and 96% specificity. Mahmood et al. (2000) have reported a sensitivity of 73% and a specificity of 90% in sputum positive tuberculosis patient during evaluation of Pathozyme-TB Complex Plus which incorporates these two to (38 kDa + 16 kDa) antigens. Where as, Siddique et al. (1994) found 85.71% sensitivity in culture confirmed tuberculosis cases with a specificity of 100%.

Conclusion and Recommendations

This study has been carried out to see the usefulness of serum IgG for diagnosis of TB patients. By using Pathozyme TB complex Plus, serum IgG against (38kDa+16kDa) antigen of *M. tuberculosis* were detected by microscopy and culture positive and microscopy and culture negative patients of both pulmonary and extra pulmonary tuberculosis. The sensitivity was 76.38% in smear and culture positive cases and was 52.17% in smear and culture negative cases. The specificity of the test was 85.71% .

The main diagnostic benefit of serology rests in the rapid diagnosis of approximately 52.17% cases of microscopy and culture negative pulmonary tuberculosis. However, in view of seronegativity of microscopy positive pulmonary tuberculosis cases, antibody detection by serology can not replace microscopic examination for diagnosis of tuberculosis. A positive result could potentially aid in clinical decision making in selected symptomatic patients.

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REVIEW ARTICLE

DENGUE: PAST, PRESENT AND FUTURE – A REVIEW

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Introduction

Dengue is a mosquito borne acute viral illness, caused by one of four closely related, but antigenically distinct, virus serotypes 1 through 4 (DEN-1, DEN-2, DEN-3, and DEN-4), of the genus flavivirus and is a frequent cause of febrile illnesses in many countries in tropical and subtropical regions [1, 2]. Infection with one of these serotypes does not provide cross-protective immunity, so persons living in a dengue-endemic area can have four dengue infections during their lifetimes. Infection with dengue viruses produces a spectrum of clinical illness ranging from a nonspecific viral syndrome to severe and fatal hemorrhagic disease. Since its first recognition during the last quarter of eighteenth century, periodic outbreak has been reported from both developed and developing countries with Asia always remaining of highest endemicity [3, 4]. Included in the spectrum of clinical illness are dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Important risk factors for DHF include the strain and serotype of the infecting virus, as well as the age, immune status, and genetic predisposition of the patient. Recovery from infection by one provides lifelong immunity against that serotypes but confers only partial and transient protection against subsequent infection by the other three. There is good evidence that sequential infection increases the risk of more serious disease resulting in dengue haemorrhagic fever (DHF) [5].

Epidemiology

The viral etiology of dengue was established by the 1940s, and records of dengue-like illness date back more than 200 years since Benjamin Rush from Philadelphia first described it as 'breakbone fever' in 1780 [6]. DF has been known for more than a century in the tropical areas of the Southeast Asia and the Western Pacific regions [7]. Major changes in the epidemiology of dengue virus infections began after World War II and have continued to date. Given current estimates of over 100 million infections worldwide each year and over 2.5 billion individuals at risk for infection [8], the dengue viruses are now arguably the most important arthropod-borne viruses from a medical and public health perspective.

Both epidemic and endemic transmission of dengue viruses are maintained through a human-mosquito-human cycle involving mosquitoes of the genus Aedes [9]. Susceptible humans become infected after being bitten by an infected female Aedes mosquito. Viremia in humans begins towards the end of a four to six-day incubation period and persists until fever abates, which is typically three to seven days [8, 10]. An uninfected Aedes mosquito may acquire the virus after feeding on the subject during this viremic period. The mosquito has an incubation period of eight to 12 days before it is capable of transmitting the virus to susceptible people. Once infected, mosquitoes carry the virus for their lifespan and remain infective for humans. Aedes aegypti mosquitoes are the principal vector for the transmission of dengue virus and have many characteristics that make them ideal for dissemination of the virus [9, 11]. Aedes aegypti typically breed in or close to houses, laying eggs in both man-made and natural water containers. The typical flight distance is relatively short. Aedes aegypti are daytime feeders that prefer to bite humans and are frequently unnoticed. They are easily interrupted in their feeding and move on to another host, frequently taking multiple blood meals in a single breeding cycle. Thus, an infected Aedes aegypti mosquito may transmit dengue virus to several individuals in a small area. For these reasons, family members who are at home during the day, typically women and young children, are thought to be at particularly high risk for infection.

Aedes albopictus mosquitoes are a competent vector for the transmission of dengue virus under both experimental and natural conditions [9] but are less likely to do so since they do not bite humans as frequently as Aedes aegypti and more often breed in outdoor water containers. Aedes albopictus also are more tolerant of the cold and have a wider geographic distribution than Aedes aegypti [12]. Infected female mosquitoes may also transmit the virus to their offspring by transovarial (via the eggs) transmission [13] and Aedes aegypti eggs are highly resistant to desiccation and can survive for extended periods[14].

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Patterns of Transmission: Epidemic and Hyper Endemic transmission:

Epidemic dengue transmission occurs when the introduction of dengue virus into a region is an isolated event involving a single virus strain. If sufficiently large populations of susceptible hosts and mosquitoes are present, transmission of dengue is explosive, leading to a recognizable epidemic. The incidence of infection among susceptible individuals often reaches 25 to 50 percent [15], and can be considerably higher. Epidemic activity is currently the predominant pattern of dengue virus transmission in smaller island nations, certain areas of South America and Africa, and in the areas of Asia where dengue virus transmission has recently reemerged. The incidence of dengue virus infections in these locations varies considerably from year to year. Intervals of several years or more usually pass between epidemics, allowing the number of susceptible individuals to accumulate so that the next epidemic can be perpetuated. Herd immunity, changes in weather, and mosquito control efforts can all contribute to the termination of the epidemic [9].

Hyper endemic transmission refers to the continuous circulation of multiple dengue virus serotypes in the same area. This requires the year-round presence of competent vector mosquitoes and either a large population base or steady movement of individuals into the area to maintain a pool of susceptible individuals. Seasonal variation in virus transmission is common. The incidence of infection also varies from year to year, with increased dengue transmission at intervals of three to four years, but this variation is not as dramatic as in areas where transmission predominantly follows the epidemic pattern. Between five and ten percent of the susceptible population experiences dengue virus infection annually in some regions [16]. Urban areas are particularly affected [17].

Factors Influencing Transmission

Factors of transmission are increased vector density, increased movement of vectors and viruses, shorter incubation period in the mosquito, population overgrowth and overcrowding, poorly planned urbanization, poor sanitation and water distribution, improper disposal of plastic bags, containers, tires etc., modern transportation, lack of vector (mosquito) control and increased duration and magnitude of viremia in humans [9].

Factors Contributing to the Reemergence

The emergence of DF and DHF as a public health problem has largely been a result of human behaviors including population growth, poorly planned urbanization associated with overcrowding, poor water distribution and poor sanitation, changing lifestyles, such as increased reliance on plastic containers and tires, modern transportation with increased movement of viruses, mosquitoes, and susceptible humans and lack of effective mosquito control[8].

Distribution

Currently Aedes aegypti and dengue viruses are endemic in every continent except Europe and Antarctica [18], although epidemic DHF occurs predominantly in Asia and the Americas.

Southeast Asia: Aedes aegypti are present throughout the region, extending to southern China and the south of the island of Taiwan. Hyperendemic transmission of all four dengue serotypes, with cases of DHF, has been present in Thailand, Vietnam, and Indonesia for over 40 years. Epidemic dengue reemerged in China during the 1980s and the 1990s after an absence of several decades, and was associated with the first occurrence of DHF in that country[19].

Dengue virus transmission occurs year-round, but typically reaches a peak between June through November. More than 200,000 cases of DHF were reported from the region in 1998, with Vietnam accounting for the majority of cases. The geographic distribution of disease within the region expanded in the year 1997 to 1998[20].

South Asia: A. aegypti are widely distributed in India, Pakistan, and Sri Lanka. Dengue virus transmission, particularly in India and Sri Lanka, increased substantially during the 1980s and 1990s. Hyperendemic circulation of all four dengue serotypes appears to be established, and outbreaks of DHF have become more frequent.

Western Pacific islands: A. aegypti are present in most of the region. Hyperendemic transmission of all four dengue serotypes is present in Indonesia, Malaysia, and the Philippines.

Australia: A. aegypti are present in the northeastern corner of Australia. Dengue viruses are not endemic to the continent, but periodic introduction of dengue viruses from neighboring islands led to four epidemics in urban areas of north Queensland during the 1990[20].

African and Eastern Mediterranean Regions: A. aegypti are present in much of sub-Saharan Africa and the Middle East. Several outbreaks were reported from east Africa and the Middle East during the 1990 [18].

North America: A. aegypti are present in most areas of Mexico and in the southeastern United States. In

1998, over 23,000 cases of dengue infection were reported from Mexico, including 372 cases of DHF and 14 deaths [21].

Central America: A. aegypti and hyperendemic transmission of all four dengue virus serotypes are present throughout the region. Nicaragua reported the second highest number of cases of DF (13,000) and DHF (432) among the countries in this region in 1998[21]. Outbreaks of dengue virus infections occurred in Costa Rica, El Salvador, Guatemala, and Nicaragua during 2000 [22].

Caribbean: A. aegypti is present throughout the region. Hyperendemic circulation of dengue virus serotypes 1, 2, and 4 has been present on the larger islands for several decades. Dengue virus serotype 3 was detected in the region in 1998 after an absence of several decades. In Puerto Rico, peak dengue virus transmission usually occurs between October and December; over 17,000 cases of dengue virus infection were reported there in 1998 [21].

South America: A. aegypti is present in every South American country except Chile [18]. Hyperendemic circulation of dengue virus serotypes 1, 2, and 4 is present in the north of the continent, and reintroduction of dengue virus serotype 3 was detected in Brazil and Venezuela during 2000. In 1998, more than 500,000 cases of dengue virus infection were reported in Brazil. However, DHF has been very infrequent in Brazil, and has not been observed in Peru despite the occurrence of documented secondary infections with dengue virus serotype 2 [23].

Current Trends

In the 1980s, DHF began a second expansion into Asia when Sri Lanka, India, and the Maldive Islands had their first major DHF epidemics; Pakistan first reported an epidemic of dengue fever in 1994. The recent epidemics in Sri Lanka and India were associated with multiple dengue virus serotypes, but DEN-3 was predominant and was genetically distinct from DEN-3 viruses previously isolated from infected persons in those countries. After an absence of 35 years, epidemic dengue fever occurred in both Taiwan and the People's Republic of China in the 1980s. The People's Republic of China had a series of epidemics caused by all four serotypes, and its first major epidemic of DHF, caused by DEN-2, was reported on Hainan Island in 1985. Singapore also had a resurgence of DF/DHF from 1990 to 1994 after a successful control program had prevented significant transmission for over 20 years. In other countries of Asia where DHF is endemic, the epidemics have become progressively larger in the last 15 years. Dengue remains a public health problem in Thailand and Singapore as well [24].

In the Pacific, dengue viruses were reintroduced in the early 1970s after an absence of more than 25 years. Epidemic activity caused by all four serotypes has intensified in recent years with major epidemics of DHF on several islands. Epidemics of DHF and DSS were first recognized in the Philippines in 1956 where Dengue virus types 2, 3 and 4 were isolated [25].

Despite poor surveillance for dengue in Africa, epidemic dengue fever caused by all four serotypes has increased dramatically since 1980. Most activity has occurred in East Africa, and major epidemics were reported for the first time in the Seychelles (1977), Kenya (1982, DEN-2), Mozambique (1985, DEN-3), Djibouti (1991-92, DEN-2), Somalia (1982, 1993, DEN-2), and Saudi Arabia (1994, DEN-2).

In India, first epidemic of dengue hemorrhagic fever (DHF) was reported in Kolkata (DEN-2) in 1963 [26]. Delhi witnessed dengue epidemics in 1967 (DEN-2), 1970 (DEN-1&3), 1982 (DEN-1&2), 1988 (DEN-2) and in 1991[27-29].

The first documented outbreak of dengue fever (DF) in Bangladesh was in 1965 when it was called 'Dhaka fever'[30]. Another epidemic fever with features closely mimicking that of dengue hemorrhagic fever (DHF) occurred again in 1968 in areas of Bangladesh bordering Myanmar [31]. Subsequent entomological and serological studies have indicated the continued presence of dengue virus in the country [32-35]. Subsequently, in 1977 and in 1982, some cases were documented in clandestine surveys [35]. The first formal sero-epidemiological study for the presence of dengue in Bangladesh was done in 1996 through 1997 at Chittagong medical college hospital (CMCH), Chittagong, where 13.75% of fever cases were found to be sero-positive for dengue infection [36]. Later on in 1998 and 1999 sporadic cases of dengue were coming up in the media, especially from Dhaka city [37] In Bangladesh, an outbreak of an acute febrile illness clinically suspected as dengue and dengue haemorrhagic fever (DHF) occurred in and around Dhaka city during the summer of 1999 [38]. Serological evidence of dengue virus infection was found in the majority of these cases. Since then dengue cases are being reported every year from different hospitals and institute in different media and series [38-41].

Susceptibility and Resistance

Susceptibility is universal, but children generally have milder illness than adults. All four dengue serotypes produce clinically identical disease, and all can produce DHF and DSS in decreasing order of

frequency: serotypes 2, 3, 4, and 1. Individuals infected with one strain maintain lifelong homotypic immunity while remaining susceptible to infections with other heterotypic strains. Interestingly, DHF/DSS is more likely to develop if an individual previously infected with one serotype is later inoculated with a different viral strain. DHF and DSS usually occur as a second dengue infection in children and in infants born to dengue-immune mothers. Repeated episodes of DHF/DSS have not been described in the same individual. The patient may recover rapidly after volume replacement but shock may recur during the period of excessive capillary permeability. The case fatality rate in DHF can be as low as 0.2% if detected early and treated. Once shock has set in, the fatality rate may be as high as 12% to 44% [42].

Pathogenesis

Virus enters into the human body during bites of infective female Aedes or Albopictus mosquitoes and replicates in the mononuclear phagocytes cell of the infected person. Megakaryocytes in the bone marrow are also affected and so thrombocytopenia occurs. In DHF, it spreads throughout the body and is found in liver, lungs, kidney, spleen, lymph node, heart and CNS. Pathogenesis of DHF is not clear but difference of viral virulence in different serotypes, difference in genetic susceptibility and host response and immunological response are held responsible. DHF is usually found in individuals who had a previous experience with at least one of the four serotypes of dengue virus. This leads to the hypothesis of heterotypic antibodies from a previous dengue infection promoting the viral replication within the mononuclear leucocytes, the phenomenon of antibody-dependent enhancement [43]. Furthermore, the immunologic processes aimed at eliminating dengue virus infected cells can result in release of histamine and substances with vasoactive and procoagulant properties, the release of interferon-gamma, and the activation of complement [44].

The distribution of virus in humans with natural dengue virus infection has been studied in blood, biopsy, and autopsy specimens obtained late in the viremic period or after viremia. Peripheral blood mononuclear cells have the highest yield for virus. In one study, virus was detected in peripheral blood mononuclear cells in 23 percent of 332 samples compared to only 7 percent of corresponding plasma samples[45]. Megakaryocytes in the bone marrow are also affected and so thrombocytopenia occurs. In DHF, it spreads throughout the body and is found in liver, lungs, kidney, spleen, lymph node, heart and CNS. The yield of dengue virus from tissues obtained at autopsy has generally been low. However, in one study using the most sensitive techniques for virus isolation, virus was isolated most often (4 of 16 cases) from liver tissue[46]. Antigen staining has suggested that the predominant cell types infected are macrophages in the skin and Kupffer cells in the liver[47].

Immune responses and viral clearance: Both innate and adaptive immune responses induced by dengue virus infection are likely to play a role in the clearance of infection. Infection of monocytes and fibroblasts in vitro induces production of interferon alpha and beta, respectively [48]. Consistent with these observations, elevated serum levels of interferon alpha have been demonstrated in children with dengue virus infection in Thailand [49]. Neutralization clearly requires a threshold level of antibodies; when the concentration of antibodies is below this threshold, the uptake of antibody-bound virus by cells that express immunoglobulin receptors is paradoxically increased, a process termed antibody-dependent enhancement (ADE) of infection [50].

Primary versus secondary dengue infection: Infection with one of the four serotypes of dengue virus (primary infection) provides life-long immunity to infection with a virus of the same (homologous) serotype. However, immunity to the other (heterologous) dengue serotypes is transient, and individuals can subsequently be infected with another dengue serotype (secondary infection). Several studies have reported that higher peak plasma virus titers in secondary dengue infections were associated with more severe illness [51].

Factors Influencing Disease Severity

Most dengue virus infections produce mild, nonspecific symptoms or classic dengue fever. The more severe manifestations, DHF and DSS occur in less than 1% of dengue virus infections.

Viral factors: Although DHF can occur during infection with any of the four dengue serotypes, several prospective studies have suggested that the risk is highest with dengue 2 viruses [51]. Genetic analyses of dengue virus isolates from the Western hemisphere strongly suggest that DHF only occurs during infection with viruses that fall into specific genotypes within each dengue serotype [23].

Prior dengue exposure: Epidemiologic studies have shown that the risk of severe disease (DHF/DSS) is significantly higher during a secondary dengue virus infection than during a primary infection. A prospective study in Myanmar from 1984 to 1988 found a relative risk of DSS in secondary infections of 82 to

103 [52]. The increased risk of DHF in secondary dengue virus infections is felt to reflect the differences in immune responses between primary and secondary dengue virus infections described above: antibody-dependent enhancement of infection; enhanced immune complex formation; and/or accelerated T lymphocyte responses.

Age: The risk for DHF appears to decline with age, especially after age 11 years. During the 1981 epidemic of DHF in Cuba, the modal age of DHF cases and deaths was four years, although the frequency of secondary dengue infections was similar in those 4 to 40 years of age [53]. A specific population at higher risk for DHF in endemic areas is infants, particularly those between 6 and 12 months of age. These children acquire dengue virus-specific antibodies transplacentally, and become susceptible to primary dengue virus infection when antibody levels decline below the neutralization threshold [54].

Nutritional status: Unlike other infectious diseases, DHF/DSS is less common in malnourished children than in well-nourished children. As an example, malnutrition, as determined by weight for age, was noted in 13% of 100 Thai children with DHF compared to 33% of 184 healthy Thai children and 71% of 125 Thai children with other infectious diseases admitted to the same hospital [55]. This negative association may be related to suppression of cellular immunity in malnutrition.

Genetic factors: Epidemiologic studies in Cuba showed that DHF occurred more often in whites than in blacks [53]. DHF has also been associated with specific HLA genes in studies from Thailand [56].

Pathophysiologic Basis of Specific Disease Manifestations

Capillary leak syndrome: Infection by one serotype produces non-neutralizing antibody and when subsequently, there is infection by another serotype a vigorous antigen antibody reaction occurs, which causes increased activation of complements and kinins and release of chemical mediators, which leads to increased capillary permeability and plasma leakage and so on. Plasma leakage, due to an increase in capillary permeability, is a cardinal feature of DHF but is absent in dengue fever. The enhanced capillary permeability appears to be due to endothelial cell dysfunction rather than injury, as electron microscopy demonstrated a widening of the endothelial tight junctions [57].

Blood and bone marrow: Leukopenia, thrombocytopenia, and a hemorrhagic diathesis are the typical hematologic findings in dengue virus infections. Leukopenia is apparent early in illness, and is of similar degree in DHF and dengue fever [1]. It is thought to represent a direct effect of dengue virus on the bone marrow. Bone marrow biopsies of children in Thailand with DHF revealed suppression of hematopoiesis early in the illness, with marrow recovery and hypercellularity in the late stage and during early clinical recovery [58]. Haemorrhage in DHF or DSS occurs due to involvement of megakaryocyte that reduces platelet, antigen antibody reaction leading to destruction of platelet, endothelial injury causing platelet adhesion, platelet function abnormality and disseminated intravascular coagulation. Some degree of thrombocytopenia is common in both dengue fever and DHF, but marked thrombocytopenia (<100,000 platelets/mm^3) is used to define DHF. Multiple factors are thought to contribute to the fall in platelet count which is most severe late in the illness [1]. In one study, 10 of 11 Thai children with DHF had a shortened platelet survival time, ranging from 6.5 to 53 hours [59].

Manifestations of the hemorrhagic diathesis in dengue virus infections range from a positive tourniquet test to life-threatening hemorrhage. Fatal DHF may be associated with diffuse petechial hemorrhages involving the stomach, skin, heart, intestine, and lungs [60]. Despite the nomenclature, however, the occurrence of hemorrhage does not define DHF as compared to dengue fever since a positive tourniquet test may occur with equal frequency in the two disorders [1]. Several different mechanisms, possibly acting synergistically, contribute to bleeding tendency of dengue virus infections. Both the vasculopathy and thrombocytopenia create a predisposition to bleeding. A coagulopathy also may play a role in severe infections. In one series, for example, the fractional catabolic rate of fibrinogen was abnormally high in 8 of 12 children with grade 2 DHF and 14 of 17 with grade 3 or 4 DHF [61].

Liver: Elevations of serum aminotransferases that are usually mild are common in dengue virus infections [1]. Typical pathologic findings in the livers of fatal cases of dengue include hepatocellular necrosis and Councilman bodies with relatively little inflammatory cell infiltration, similar to the findings in early yellow fever virus infection [60].

Clinical Features

Dengue virus infection may be asymptomatic or symptomatic. Symptomatic manifestation may occur as DF which is also known as classic dengue, DHF or DSS. DF is a severe, flu-like illness that affects infants, young children and adults, but seldom causes

death [3]. Symptoms typically develop between four and seven days after the bite of an infected mosquito, although the incubation period may be as short as three days or as long as 14 days. Dengue can essentially be excluded as the cause of symptoms in a traveler developing an illness more than 14 days after returning from a dengue-endemic country [62]. Classic dengue fever is an acute febrile illness accompanied by headache, usually frontal, retro orbital pain, and marked muscle and joint pains, which evoked the term "break-bone fever". Some patients report severe backache (back-break fever), sore throat, or abdominal pain, which can be severe enough to be confused with appendicitis. Fever typically lasts for five to seven days. Some patients display a biphasic ("saddleback") fever curve, with the second febrile phase lasting one to two days, although this was reported in only 5 to 6 %of patients in several studies [63]. These patients are lethargic with accompanying anorexia and nausea and vomiting. The febrile period may also be followed by a period of marked fatigue that can last for days to weeks. About day 5 or 6, fever subsides and a generalized maculopapular rash may appear which rash may become diffusely erythematous with clear areas scattered in between, the so-called "islands of white in sea of red". In this situation the clinical differentiation from other viral ill nesses may not be possible, recovery is rapid and the need for supportive treatment is minimal. [64]. The rash may be pruritic and heals with desquamation.

The clinical features of dengue fever vary according to the age of the patient. Infants and young children may have a non-specific febrile illness with rash. Older children and adults may have either a mild febrile syndrome or the classical incapacitating disease with abrupt onset and high fever, severe headache, pain behind the eyes, muscle and joint pains, and rash [3]. Objective physical and lab findings during this acute febrile phase include a relative bradycardia, lymphadenopathy, conjunctival injection with ocular globe tenderness and leukopenia with relative lymphocytosis. Minor bleeding phenomena such as epistaxis, petechiae, and gingival bleeding may occur at any time during and following the febrile phase. Major bleeding phenomena such as menorrhagia and GI hemorrhage can occur; this has been associated with pathologic changes of peptic ulcer disease.

Dengue hemorrhagic fever (DHF): A case can be diagnosed as DHF if along with other clinical features, there are one or more of the following: bleeding from mucosa or injection site, purpura, petechae or echymosis, haematemesis or melaena, thrombocytopenia (platelet <100000/mm^3) and evidence of plasma leakage due to increased capillary permeability. Evidences of plasma leakage are hypoproteinaemia, pleural effusion and ascites and >20% rise in haematocrit for age and sex. Illness is often biphasic, beginning abruptly with fever, malaise, headache, anorexia, nausea and vomiting, cough, and facial flushing. Severe bone and limb pain are often absent. The liver may be enlarged in 10% of cases. In moderate DHF cases, all signs and symptoms abate after the fever subsides. In severe cases, the patient's condition may suddenly deteriorate after a few days of fever; the temperature drops, followed by signs of circulatory failure, and the patient may rapidly go into a critical state of shock and die within 12-24 hours, or quickly recover following appropriate volume replacement therapy. Gastrointestinal bleeding or menorrhagia in patients with DHF, and occasionally in patients with dengue fever as well, can be severe enough to require blood transfusion. Factors that contribute to bleeding include thrombocytopenia due to decreased platelet survival [59] and, in severe cases, frank disseminated intravascular coagulation. Plasma leakage in DHF is important to manage with aggressive intravascular volume repletion to prevent or reverse hypovolemic shock [65].

Elevated transaminase levels and occasionally hepatic failure and encephalopathy, hypoalbuminemia, hyponatremia, especially in adults and DIC are common features. In severe cases, pleural effusions and ascites correlate with hypoproteinemia and marked liver dysfunction.

Dengue shock syndrome (DSS): A case can be diagnosed as DSS if along with clinical features of DHF, there are features of shock e.g. pinched face, sunken eyes, cold and clammy skin, rapid and shallow respiration, rapid and weak pulse, narrow pulse pressure (<20mm Hg), hypotension, restlessness, lethargy and oliguria. Patient may become unconscious and death may occur in 12-24 hours or recovery may occur in 2-3 days.

The clinical features of dengue virus infection vary from an asymptomatic infection to a febrile flu like infection, DF to more severe form like DHF which can lead to DSS [66]. The clinical variability is poorly understood and seems to be related to the age, sex and the immunologic and nutritional status of the patient. DHF is most likely to develop in immune-competent, well-nourished girls between the ages of 7 and 12 years [67].

A hemorrhagic tendency can be elicited frequently by the tourniquet test. This test is performed by inflating a blood pressure cuff on the arm to midway between systolic and diastolic blood pressures for five

minutes. The skin below the cuff is examined for petechiae, and a finding of greater than 20 petechiae in a one square inch area is considered positive.

DHF is the most serious manifestation of dengue virus infection. The cardinal features of DHF which distinguish this form of dengue from classic DF are [68]: (1) Increased vascular permeability (plasma leakage syndrome) evidenced by hemoconcentration (20 percent or greater rise in hematocrit above baseline value), pleural effusion, or ascites. (2) Marked thrombocytopenia (less than 100,000 cells/mm^3), associated with a bleeding tendency (a positive tourniquet test representing the minimum to meet this criterion) and (3) Hepatomegaly and/or abnormal liver function tests.

Plasma leakage is the most specific and life-threatening feature of DHF. The increase in vascular permeability develops rapidly, over a period of hours. In patients with marked plasma leakage, shock may develop, especially if supportive treatment is delayed. This clinical presentation is referred to as DSS and is associated with a case-fatality rate of 12 percent even with aggressive therapy [69]. Plasma leakage usually occurs between three and seven days after the onset of illness. This coincides with the extreme depression of the platelet count and elevation of the liver function tests, although abnormalities in these parameters are usually detectable before the onset of plasma leakage [1]. Abdominal pain is also reported to precede the onset of plasma leakage in approximately 60 percent of adults and children with DHF [70].

The severity of hemorrhagic manifestations is quite variable among patients with DHF. Spontaneous petechiae or ecchymoses were noted in approximately half of adults and children with DHF in Cuba [70]. Other less-frequent hemorrhagic manifestations reported in these studies included: hematemesis (15 to 30%), metrorrhagia (40% of adult women), melena (5 to 10%), and epistaxis (10%).

Liver failure or CNS dysfunction or both may rarely dominate the clinical picture in a patient with acute dengue virus infection [71]. Signs of CNS dysfunction vary from lethargy to coma and may be accompanied by seizures. Rare cases of encephalopathy have been attributed to dengue virus infections. True encephalitis has been reported, with detection of dengue virus in brain tissue [72].

The physical examination in patients with DF is generally nonspecific. Fever and rash arecommon features. Conjunctival injection, pharyngeal erythema, lymphadenopathy, and hepatomegaly are observed in 20 to 50 percent of patients but splenomegaly is uncommon [73].

Clinical Diagnosis and Differential Diagnosis

The diagnosis of acute dengue virus infection requires a clinical suspicion for the disease. In the developing countries, laboratory confirmation is typically not available for most cases. In the developed countries, laboratory confirmation is usually available only in specialized reference laboratories and is often not sufficiently timely to assist in the management of the illness.

Dengue virus infection should be considered in the differential diagnosis of a febrile illness in any patient who has resided in or traveled to an appropriate region in the two weeks before the onset of illness. In patients with the features of DF, the differential diagnosis includes: influenza, enteroviral infection, measles, and rubella. In the appropriate epidemiologic settings, malaria, leptospirosis, and typhoid fever must also be considered.

The clinical criteria for diagnosis are as follows: (1) fever; (2) haemorrhagic manifestations, including a positive tourniquet test result and a major or minor bleeding phenomenon; (3) hepatic enlargement; (4) shock (high pulse rate and narrowing of the pulse pressure to 20 mmHg or less, or hypotension). The laboratory criteria include (5) thrombocytopenia ($<100,000/mm^3$), and (6) haemoconcentration (haematocrit increase >20%). Thrombocytopenia with concurrent high haematocrit levels differentiates DHF from classic DF. In developing countries, DHF is frequently diagnosed based upon the clinical case definition established by the World Health Organization (WHO) [68]. In regions and seasons with a high incidence of DHF, the positive predictive value of the clinical definition is high. Laboratory tests confirm dengue virus infection in as many as 90 percent of such cases [74].

Table-I

WHO Classification of dengue fever

Grades	Clinical features
Grade I	Fever, constitutional symptoms, positive tourniquet test
Grade II	Grade I + spontaneous bleeding (skin, gums, GI tract)
Grade III*	Grade II + circulatory failure and agitation
Grade IV*	Grade III + profound shock (unrecordable blood pressure)

* Dengue shock syndrome

Laboratory confirmation of the diagnosis

Along with clinical features, some of the laboratory features are essential for diagnosis. Complete blood count including platelet count and haematocrit may reveal thrombocytopenia, leukopenia and rise in haematocrit. Isolation of virus can be done within 5 days of fever. Anti-dengue IgM and IgG antibody can be detected. Positive anti-dengue-IgM antibody indicates recent or primary infection. About 80% becomes positive in 5th day and about 99% becomes positive in 10th day and rises a peak in about 2 weeks but becomes undetectable over 2-3 months. On the other hand, positive anti-dengue-IgG antibody indicates secondary infection, is usually high in DHF or DSS, and rises to a peak in about 2 weeks but declines over 3-6 months.

Other investigations like x-ray chest, ultrasonography of whole abdomen, liver and kidney function test may be required.

Confirmation of acute dengue virus infection can be accomplished using either serologic or virologic methods.

Serologic tests: The most frequently used serologic tests for the diagnosis of acute dengue virus infection are the hemagglutination inhibition (HI) assay and IgG or IgM enzyme immunoassays. Complement fixation and neutralizing antibody assays are more technically demanding and are used in specialized laboratories only.

The HI assay historically has been and remains the gold standard for serologic testing for dengue virus-specific antibodies [68]. Analysis of paired acute and convalescent serum samples is essential; a fourfold or greater rise in HI antibody titer between acute and convalescent samples defines acute infection. However, cross-reactivity with other flaviviruses has been reported. In primary dengue virus infection, HI antibodies develop late (after the fifth day of illness) and reach titers of less than 1:1250 in the convalescent phase. By contrast, HI antibodies rise early in secondary dengue virus infection and reach titers above 1:1250 in the convalescent phase.

In a primary infection dengue haemagglutination inhibition antibody titer is generally less than 1:20 in a sample collected within the first 4 days after the onset of symptoms. In the convalescent phase sample (collected 1 to 4 weeks after the onset of symptoms) a fourfold or greater rise in antibody titer is detected, with antibody titer of 1:1280 [75]. A secondary dengue infection is characterized by the rapid appearance of broadly cross-reactive antibodies. Haemagglutination inhibition titers of 1:20 in the acute-phase sample rise to 1:2560 in the convalescent phase sample. An antibody titer of 1:1280 in the acute-phase sample without a fourfold or greater increase in the second sample also is considered presumptive of recent infection. An improved and less time-consuming method is a capture enzyme-linked immunosorbent assay that can detect specific anti-dengue IgM in a single acute-phase sample [76].

mmunoassays for the detection of dengue virus-specific IgG antibodies have demonstrated sensitivity and specificity of approximately 99 percent and 96 percent, respectively, compared to the HI assay [16].

Virus detection: Isolation of dengue virus or detection of dengue viral RNA or protein in an acute phase serum or tissue specimen provides the most definitive confirmation of infection. However, the importance of specimen timing and quality and the technical demands of these assays limit their clinical applications. Serum or plasma is the preferred specimens for virus isolation, although virus can occasionally be isolated from liver tissues after clearance of virus from the serum [77]. Regardless of the specific method used, optimal detection is achieved when specimens are obtained early after the onset of symptoms, during the febrile period. In one study of children in Thailand, dengue viruses could be isolated from all plasma samples obtained at least two days before defervescence but from no samples obtained two or more days after fevers resolved [78]. A number of investigators have described techniques for the detection of dengue viral RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) [79]. Although technically demanding and not widely available, RT-PCR is the only method that can detect virus within a clinically meaningful period (one to two days). RT-PCR appears to be approximately equal to or slightly better in sensitivity than mosquito inoculation for the detection of dengue virus in serum or EDTA plasma [80].

Recommended approach to the diagnosis of acute dengue virus infection: When dengue virus infection is suspected on clinical grounds, the patient should be treated empirically as appropriate for the symptoms and signs present. The recommended approach to confirm the diagnosis includes the following [68]:

1) An acute phase serum or EDTA plasma sample should be obtained. If a reference laboratory is readily available, the IgM immunoassay (MAC-ELISA or equivalent) is the procedure of choice for rapid confirmation of the diagnosis. However, failure to detect dengue virus-specific IgM antibodies should be considered an indeterminate result if the specimen

was obtained within the first six days of illness, especially if the patient was febrile at the time of blood collection.

(2) A convalescent phase serum sample should be obtained at least 10 to 14 days after the acute phase serum. The acute and convalescent specimens should be analyzed together by HI assay or IgG immunoassay to provide definitive serologic testing for acute dengue virus infection.

(3) Virus isolation and RT-PCR should generally be performed only when needed for epidemiologic purposes or as part of clinical research studies.

Treatment and Control

There is no specific treatment for dengue fever. However, careful clinical management by experienced physicians and nurses frequently saves the lives of DHF patients. With appropriate intensive supportive therapy, mortality may be reduced to less than 1%. Maintenance of the circulating fluid volume is the central feature of DHF case management. For patients with DHF/DSS, measures to correct hypovolemia, hypoxia, shock and DIC[3] can reduce complications and death. Modalities include nonsalicylate antipyretics, oxygen, and electrolyte and crystalloid and/or colloid fluid replacement (plasma or plasma expanders for severe shock or for continued rise in haematocrit despite vigorous IV fluid administration). The rate of fluid (initially 10-20 ml/kg/hr for hypovolemic shock) should be determined by serial microhematocrits. Red blood cell and platelet transfusions and fresh plasma are indicated for severe bleeding and decreasing haematocrit values.

A protocol for intravenous fluid therapy has been developed by the World Health Organization based upon clinical experience mainly in children from Southeast Asia [81]. For patients with shock, an initial bolus of five percent dextrose in normal saline (10 to 20 ml/kg of body weight) infused rapidly is recommended, followed by continuous infusion (10 to 20 ml/kg per hour) until vital signs and urine output normalize. The infusion rate can then be gradually reduced until it matches plasma fluid losses.

Patients with DF require rest, oral fluids to compensate for losses via diarrhoea or vomiting, analgesics, and antipyretics for high fever (acetaminophen but not aspirin, so that platelet function is not impaired). Steroids in DSS are not helpful [69]. With the earliest suspicion of threatened severe illness, an intravenous line should be placed so that fluids can be provided. Monitoring of blood pressure, haematocrit, platelet count, haemorrhagic manifestations, urinary output, and level of consciousness is important. Plasma leakage in DHF is very rapid and the haematocrit may continue to rise even while intravenous fluids are being administered; however, the "leaky capillary" period is short and intravenous fluids are usually required for only 1-2 days [82]. There is great variability from patient to patient, and the physician must adjust treatment using serial haematocrit, blood pressure, and urinary output data [83]. Insufficient volume replacement will allow worsening shock, acidosis, and disseminated intravascular coagulation, while fluid overload will produce massive effusions, respiratory compromise, and congestive heart failure. Because patients have loss of plasma (through increased vascular permeability into the serous spaces) they must be given isotonic solutions and plasma expanders, such as Ringer's acetate or Ringer's lactate, plasma protein fraction, and Dextran [84]. The recommended amount of total fluid replacement in 24 h is approximately the volume required for maintenance, plus replacement of 5% of bodyweight deficit, but this volume is not administered uniformly throughout the 24 h. A bolus of 10-20 ml of an isotonic solution per kg bodyweight is given in case of shock, and repeated every 30 min until circulation improves and urinary output is adequate. Vital signs should be measured every 30-60 min and haematocrit every 2-4 h, then less frequently as the patient's condition stabilizes [82].

Placement of a central-venous-pressure line is hazardous in patients with haemorrhagic tendencies but may be necessary, especially when more than 60 ml/kg of fluids has been given without improvement. An expert in a special care area should insert the line. It is used to estimate filling pressures and to guide further intravenous fluid administration. An arterial line will help in the assessment of arterial blood gases, acid base status, coagulation profiles, and electrolytes in the haemodynamically unstable patient, helping to identify early respiratory compromise.

Instructions for the treatment of DHF: Cases of DHF should be observed hourly. Serial platelet count and haematocrit values should be estimated daily or on alternate days. Timely ORS or isotonic crystalloid IV fluid can prevent shock or lessen its severity. If the patient's condition becomes worse despite giving 20ml/kg/hour for one hour, crystalloid solution should be replaced with colloid solution such as dextran or plasma but as soon as improvement occurs, it should be replaced with crystalloid. If improvement occurs speed should be reduced from 20 ml to 10ml, then to 6ml and finally to 3 ml/kg/

hour. If haematocrit falls, blood transfusion can be given 10 ml/kg/hour and then crystalloid IV fluid at the same rate. In case of severe bleeding fresh blood transfusion may require to give at a rate of 20 ml/kg/hour for about 2 hours and then crystalloid at 10 ml/kg/hour for about 30-60 minutes and then speed can be reduced. Of course, oxygen should be given in case of shock and acidosis should be corrected. Role of steroid is controversial, consensus is not to use steroid. Monitoring should be continued for at least a day after defervescence. Once the patient begins to recover, extravasated fluid is rapidly reabsorbed, causing a drop in haematocrit.

Signs of recovery: Pulse, blood pressure and respiratory rate become stable, fever subsides, appetite returns, vomiting or internal or external bleeding does not occur and urinary output becomes nomal and haematocrit become stable.Indications for hospitalization: Patients should be hospitalized if there is signs of significant dehydration, which include tachycardia, reduced peripheral pulse volume, cool or pale skin, increased capillary refill time (>2 second), oliguria, narrow pulse pressure (< 20 mm Hg), changes in mental status, hypotension, sudden rise or continuously elevated haematocrit despite administration of fluids. Patients with suspected dengue can be safely managed as outpatients as long as close clinical observation is assured. Daily outpatient visits may be needed to permit serial assessment of blood pressure, hematocrit, and platelet count. One study in Malaysia over a two month period tested a standard treatment protocol for patients with suspected dengue [85].

Criteria for discharging patients from hospital: Absence of fever for 24 h and a return of appetite, improvement in the clinical picture, hospital care for at least 3 days after recovery from shock, no respiratory distress from pleural effusion or ascites, stable haematocrit, and platelet count greater than 50,000/mm^3.

Complications of DHF and DSS: Complications are renal failure, hepatitis, HUS, DIC, bone marrow failure, secondary infection and CNS manifestations like altered consciousness, convulsion, encephalopathy, paresis, spasticity and intracranial haemorrhage. Encephalopathy and liver failure are uncommon manifestations of DHF which are associated with a high mortality rate [71].

Prognosis

Prognosis of DF is excellent but in DHF mortality rate is 5 -10% but with proper management with physiologic fluid replacement and supportive care can reduce mortality from 40-50% to 1-2%. Platelet counts less than 50,000 mm^3 and prolonged prothrombin times are poor prognostic indicators.

Mosquito Control

Prevention and control of dengue virus infection and its arthropod vector relies on insecticides, barrier measures protective clothing, bed netting, and insect repellents are advised. Mosquito control is the most effective approach to the prevention of dengue transmission. Insecticide spraying, in response to dengue outbreaks, is not highly effective against A. aegypti mosquitoes, which frequently breed inside houses. Community-based approaches involving education of the population in efforts to reduce breeding sites, such as discarded tires and other containers that accumulate standing water, have shown some promise [86].

Future Outlook

No dengue vaccine is available. Live-attenuated vaccines for dengue types 1, 2, and 4 have been developed but are not yet commercially available. Recently, however, attenuated candidate vaccine viruses have been developed in Thailand. These vaccines are safe and immunogenic when given in various formulations, including a quadrivalent vaccine for all four dengue virus serotypes. Efficacy trials in human volunteers have yet to be initiated. Research is also being conducted to develop second-generation recombinant vaccine viruses; the Thailand attenuated viruses are used as a template. Therefore, an effective dengue vaccine for public use will not be available for 5 to 10 years. Preliminary clinical studies of candidate attenuated vaccines developed in Thailand were encouraging [87], but follow-up studies indicate that additional effort is required before large scale clinical studies can be pursued[88].

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CASE REPORTS

CASE REPORT: TUBERCULOUS BREAST ABSCESS MIMICKING PYOGENIC BREAST ABSCESS

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A 27-year-old non-diabetic woman presented with 2-week history of pain and swelling in her upper and outer part of the left breast on 13th September 2003. It was red, tender and hot. Hb%, TC, DC, ESR and fasting blood sugar was within normal range. Based on the clinical diagnosis of pyogenic breast abscess, Flucloxacillin was instituted. Due to lack of clinical improvement, incision and drainage of the abscess was done. Gram stain and culture of the pus showed profuse growth of *Staphylococcus aureus* and culture for AFB was also sent. Histopathologic examination of the affected skin revealed features of non -specific chronic inflammation and no evidence of malignancy or granuloma was noted. Although her symptoms abated, a non- healing ulcer developed which took one month of regular dressing to heal apparently. Six weeks later, no growth of AFB was found in culture media.

Four months later, she again developed the same complaints but with a greater magnitude. Routine blood tests were unremarkable. Ultrasonogram of breast showed hypoechoic masses in the left axillary region possibly abscess (? matted lymph node).

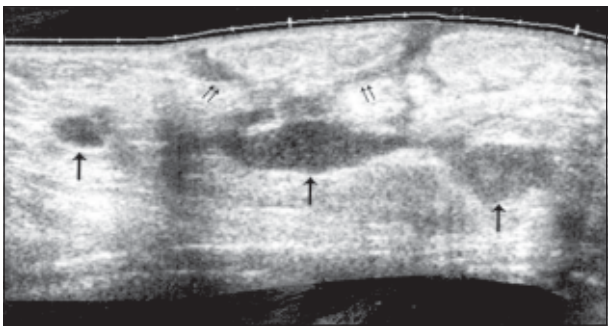

Fig.-1: *Shows hypoechoic masses in the breast.*

Incision and drainage was done to relieve her symptoms and the specimen was sent for Gram staining and c/s. Tuberculin skin test result was 06 mm. Gram Stain and culture revealed the profuse growth of *Staphylococcus aureus* and *E. coli.* Appropriate antibiotic was started according to sensitivity report. After continuing antibiotics for several weeks along with dressing, the ulcer healed incompletely.

Histopathology of the necrotic material showed features of suppurative lymphadenitis without any evidence of granulomatous inflammation. She complained occasional flare-up and partial remission of the disease with different courses of antibiotics.

On September 2005, she developed a discharging sinus at the same site following incision & drainage of the abscess. She was again treated with different courses of antibiotics, but no improvement occurred at that occasion. Repeated histopathology was done on 20th September and showed the epidermis is thick and the dermis contains a fistulous tract presented by granulomatous tissue. The granulomatous tissue contains epithelioid cell debris. Hence the diagnosis is tuberculous sinus tract.

After confirmation of the diagnosis, the patient received standard antituberculous chemotherapy for six months.She is currently being followed-up and the symptoms and signs are improved with no sign of disease activity.

Discussion

The significance of breast tuberculosis is due to rare occurrence and mistaken identity with breast cancer and pyogenic breast abscess. The first case of mammary tuberculosis was recorded by Sir Astley Cooper in 1829, who called it 'scrofulous swelling of the bosom' [1].It is more common in developing countries. Breast tuberculosis is rare in the western countries, incidence being <0.1 per cent of breast lesions examined histologically. But, with the global spread of AIDS, mammary tuberculosis may no longer be uncommon in the developed world (as an AIDS defining condition). Tuberculosis constitutes approximately 0.025–0.1% of all surgically treated diseases of the breast; however, this ratio is higher in underdeveloped countries. Tuberculosis of the breast in males is even rarer and is not a recognized condition[2].

Breast tissue is remarkably resistant to tuberculosis. This is due to the fact that, like skeletal muscles and

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spleen, it provides infertile environment for the survival and multiplication of tubercle bacilli. The breast may become infected in a variety of ways e.g., (i) haematogenous, (ii) lymphatic, (iii) spread from contiguous structures, (iv) direct inoculation, and (v) ductal infection. Of these, the most accepted view for spread of infection is centripetal lymphatic spread [1].

Mammary tuberculosis may be primary or secondary to a lesion elsewhere in the body, however, it is generally believed that infection of the breast is usually secondary to a tuberculous focus elsewhere, which may not be clinically or radiologically apparent. Primary infection of the breast may occur through skin abrasions or through the duct openings on the nipple. Direct extension from contiguous structures like the underlying ribs is another possible mode of infection. Whether the axillary lymph node was the site of primary infection or secondary to the mammary tuberculosis is a contentious point [1,2].

Clinically the lesion can be classified into four types [3].

1) Nodular type: The lump is the commonest presentation in breast tuberculosis. These breast lumps are mostly misdiagnosed as fibroadenoma, fibroadenosis or malignancy. Lump in breast tuberculosis is also common in upper outer quadrant of breast as in carcinoma .Multiple lumps are less frequent. Tubercular lumps are irregular, ill defined, hard similar to that seen in carcinoma. Pain is usually dull, constant ache and is more frequent than in carcinoma patients. Tubercular ulcer over the breast skin and tubercular breast abscess with or without discharging sinuses are other common forms of clinical presentation of breast tuberculosis. Peau d' orange is often seen in patients with extensive axillary nodal tuberculosis. Purulent nipple discharge or persistent discharging sinus may be the rare presenting feature[3,4,5].

2) Sclerosing type: usually occurs in the older patient. The lesion is hard and fibrous. The course is very chronic & the breast may be small & hard & there may be nipple retraction mimicking carcinoma of breast. Sinus is not usually present (3,5,6)

3) Cystic type: rarely manifest as a large cystic swelling[3].

4) Atypical form: a superficial ulcer may form on the nipple & which may spread on the breast (3).

X-ray chest may reveal evidence of active or healed lesion in the lungs, but it is present only in few cases. Mantoux skin test is usually positive in adults in endemic areas; therefore it is of no diagnostic value. Mammogram is of limited use as findings in breast tuberculosis are nonspecific. Furthermore they are often indistinguishable from those seen in malignancy. Ultrasonography is useful in characterizing the ill-defined densities shown on mammography and differentiating the cystic from the solid mass. It reveals heterogeneous, hypoechoic, fluid containing masses with internally floating and echogenic material in the breast parenchyma or retromammary region. Ultrasound guided fine needle aspiration can be done for cytological and microbiological studies.

Fine needle aspiration cytology (FNAC) from the breast lesion continues to remain an important diagnostic tool of breast tuberculosis. Approximately 73 per cent cases of breast tuberculosis can be diagnosed on FNAC when both epithelioid cell granulomas and necrosis are present. In tubercular breast abscess, FNAC may be inconclusive and the FNA picture may be dominated by acute inflammatory exudates. Histological findings include epitheloid cell granulomas with caseous necrosis in the specimen. Core needle biopsy yields a good sample often yielding a positive diagnosis. However, open biopsy (incision or excision) of breast lump, ulcer, sinus or from the wall of a suspected tubercular breast abscess cavity almost always confirms breast tuberculosis(1,2).

The principal differential diagnosis to be considered is carcinoma, although other diseases of the breast, such as fatty necrosis, plasma cell mastitis, periareolar abscess, actinomycosis,and blastomycosis should also be considered. After microbiologic or histologic confirmation of the diagnosis of tuberculosis of the breast, treatment should include nodule excision or drainage in the case of abscess, and therapy with antituberculosis drugs.

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ROLE OF HIGH-DOSE DEXAMETHASONE IN THE TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Summary

The role of high-dose dexamethasone in the treatment of immune thrombocytopenic purpura in adults is under trial and yet to be established. We have given a four consecutive days trial of 40 mg oral dexamethasone per day in six patients in the Department of Medicine, Bangabandhu Sheikh Mujib Medical University & other private hospitals. We selected the patient according to American Society of Hematology Guideline and were enrolled between 1st January to 30th June 2006. A response was defined as an increase in the platelet count of at least 30,000/cu.mm & a platelet count of more than 50,000/cu.mm by day 10 after the initiation of the treatment. A susta ined response was defined as platelet count of more than 50,000/mm^3 6 months after the initial treatment. The treatment protocol was well tolerated and satisfactory with tolerable side effects. A four-day course of 40 mg oral dexamethasone is an effective initial therapy for adults with idiopathic thrombocytopenic purpura (ITP).

Introduction

Idiopathic thrombocytopenic purpura, also known as primary immune thrombocytopenic purpura, is an acquired disease of adults and children that is defined by a low platelet count in the absence of other causes of thrombocytopenia. The estimated incidence is 100 cases per 1 million persons per year, and about half of these cases occur in children.[1] The low platelet count is due to its destruction by several autoantibodies in the reticuloendothelial system of spleen, liver and bone marrow. It now appears that a significant number of ITP patients (up to 40%) experience decreased production in addition to peripheral destruction.[2] Prednisolone, in a dose equivalent to 1-2 mg/kg body weight, is the conventional initial treatment for ITP. Although this regimen raises the platelet count in about 75 percent of adults, [3] relapses are frequent whenever an attempt is made to reduce the dose. Sustained remission is not satisfactory.[4-5] Moreover; prolonged corticosteroid use is associated with many serious side effects.

A short course high-dose dexamethasone has been tried in refractory ITP patients with variable success.[6-8] We tested the therapeutic effectiveness of high-dose oral dexamethasone (40 mg per day for four consecutive days) as an initial treatment modality in ITP.

Materials and Methods

Six consecutive cases of ITP were selected at the Bangabandhu Sheikh Mujib Medical University & private hospitals between January 2006 to June 2006 according to the practice guidelines of the American Society of Hematology.[9]

Patient selection criteria:

a) Platelet count<20,000/mm^3

b) Platelet count<50,000/mm^3 with significant mucosal bleeding.

Exclusion criteria:

a) Relapsed ITP b) Treatment with corticosteroids during the previous 6 months c) H/O serious side effects during previous steroid use i.e., psychosis or avascular necrosis of bone d) Uncontrolled hypertension e) Diabetes mellitus f) Active infection of bone g) Pregnancy.

A photograph of purpuric rash on the forearm of one patient is shown in figure-1.

Patients received 40 mg of oral dexamethasone daily for four consecutive days. Criteria selected for an initial response were an increase in the platelet counts of at least 30,000mm/^{3}, a platelet count of more than 50,000 mm/^{3} by day 10 after the initiation

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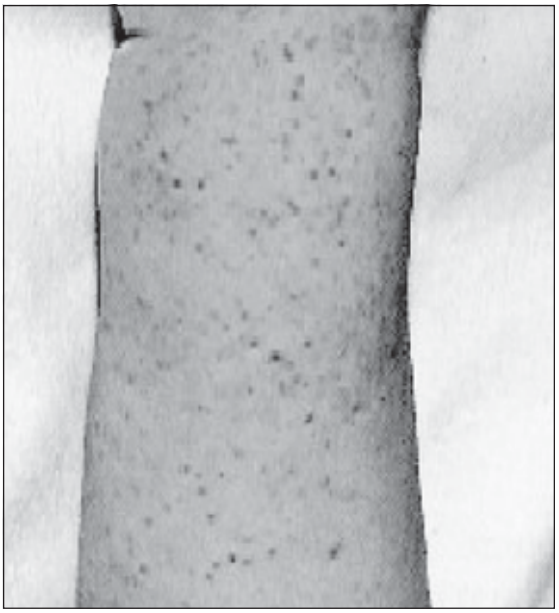

of treatment, and cessation of bleeding. Unresponsiveness was defined as an increase in the platelet count of less than 30,000/mm^3 or a platelet count of 50,000/mm^3 or less by day 10.Patients were considered to switch on other treatments if there was no response to dexamethasone. If the patient had a platelet count of more than 50,000/mm^3 after four days of dexamethasone treatment, no further treatment was given. The patients had outpatient follow up visits monthly for two months and then every two to three months.

Complete blood counts were obtained at recruitment; during high-dose dexamethasone treatment; on day 3, 4, 10 and during follow-up visits. Fasting and postprandial blood glucose levels were measured at recruitment and after treatment with high-dose dexamethasone. Urinalysis, ANA, RA and dengue antibody were done at the time of recruitment. Bone marrow aspiration was performed in one patient in order to rule out differential diagnosis.

Results

The median age of these six patients was 37 years; 4 were female, and 2 were male. They were non-diabetic and non-hypertensive. Particulars of one patient (presented with gum bleeding, purpura & epistaxis) shown in Table -I.

Five other patients were diagnosed as a case of ITP based on the clinical features & lab findings & supported by bone marrow study. Response of platelet count after initiation of high-dose oral dexamethasone is shown in the table-II.

Table-I

Particulars of one patient (presented with gum bleeding, purpura & epistaxis).

Lab data	On recruitment	3rdday of treatment	4thday of treatment	10thday of treatment	3 months after treatment
Hb	12.75gm/dl	12gm/dl	12.75gm/dl		
TLC	8000/cu.mm	15000/cu.mm	12,000/cu.mm		
DC (%)					
Polymorph	60	82	80	3,00,000/mm^3	67
Lymphocyte	30	12	12		27
Eosinophil	06	04	02		03
Monocyte	04	06	05		03
Basophil	00	80,000/mm^3	2,00,000/mm^3		2,15,000/mm^3
Platelet count	30,000/mm^3				
PBF	WBC and RBC: normal, Platelets are reduced				WBC and RBC: normal, Platelets are adequate.
ESR	15mm/1sthour	12mm/1sthour			13mm/1sthr
FBS	5 mmol/l	5.1 mmol/l		5.7 mmo/l	5.5 mmol/l
Urinalysis	Protein: nil, RBC: Occasional				Normal
Bone marrow study	Megakaryocytes are normal, lymphocytes and plasma cells are increased				
ANA, RA, Dengue antibody	Negative				

Table-II

Response of platelet count after initiation of high-dose oral dexamethasone.

Serial no	On recruitment	3rd day of treatment	4th day of treatment	10th day of treatment	3 months after treatment
One	16,000/mm^3	50,000/mm^3	1,50,000/mm^3	3,00,000/mm^3	2,25,000/mm^3
Two	15,000/mm^3	45,000/mm^3	2,00,000/mm^3	3,45,000/mm^3	1,80,000/mm^3
Three	10,000/mm^3	40,000/mm^3	1,00,000/mm^3	1,15,000/mm^3	2,75,000/mm^3
Four	30,000/mm^3	55,000/mm^3	1,15,000/mm^3	2,00,000/mm^3	4,00,000/mm^3
Five	20,000/mm^3	44,000/mm^3	1,00,000/mm^3	3,25,000/mm^3	1,90,000/mm^3

Discussion:

Since idiopathic (immune) thrombocytopenia in adults is usually a chronic condition, with few spontaneous remission, the goal of treatment is not cure, but to maintain a hemostatically safe platelet level. The indication for treatment should be based not merely on platelet count, but also clinical indices of bleeding.[11] Plenty of studies have been done on corticosteroid treatment in adults with idiopathic thrombocytopenic purpura.[12] Spontaneous remissions are rare, and the response rate ranges from 65 to 85 percent. The lack of a substantial increase in the platelet count by three weeks is generally considered to indicate treatment failure, although responses

have been observed after six months in a few patients.[13] Sustained responses after the discontinuation of conventional corticosteroid therapy occur in 5 to 30 percent of patients.[14]

In adult patients with relapses of chronic immune thrombocytopenic purpura, the role of high dose dexamethasone is controversial. Some studies showed encouraging results, e.g, Khouri et al.[15] reported complete remission that lasted for four years in two of the three adults who had had relapses of chronic ITP while others have failed to confirm these.[16-19]

In summary, we assessed the effectiveness and side effects of a four-day course of high-dose oral dexamethasone as initial treatment in 6 adults with ITP. Virtually every patient responded well to this treatment and within three months of follow-up, none showed any clinical or lab signs of relapse.

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ASSOCIATION OF DIABETIC RETINOPATHY WITH MICROALBUMINURIA IN TYPE - 2 DIABETES MELLITUS

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Abstract:

Important clinical concomitant of diabetic retinopathy is diabetic nephropathy. Nearly all Patients with diabetic nephropathy also develop retinopathy. But lower percentage of patients with retinopathy usually has evidence of renal involvement. The study was conducted in the department of Ophthalmology and Endocrine clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU) and BIRDEM from July 2002 to June 2004 to see the relation between Diabetic Retinopathy with Microalbuminuria in Type - 2 Diabetes Mellitus by excluding other risk factors effecting microalbuminuria (hypertension, urinary tract infection, febrile illness, kidney diseases and pregnancy).

This prospective case control study was performed upon 70 patients of type-2 diabetes mellitus, among whom 35(27 male and 8 female) had diabetic retinopathy and remaining 35 (29 male and 6 female) had no diabetic retinopathy as control. Mean age (±SD) of DR (Diabetic Retinopathy) patients was 47±7.39 years and mean age (±SD) of control was 47.76±4.97years. All the newly detected patients of type-2 DM were examined thoroughly and investigated by fundus fluorescein angiogram. Microalbumin level in urine was determined by immunological method .The study showed higher rate (n=19, 54.29%) of microalbuminuria in diabetic retinopathy patients than the patients without diabetic retinopathy (n=10, 28.57%) which was statistically significant (P< 0.05). So diabetic retinopathy is associated with diabetic nephropathy (microalbuminuria). Fundal examination and determination of microalbuminuria in all newly detected patients with type 2 diabetes mellitus should be done.

Introduction:

Diabetic retinopathy is a common cause of blindness under age 65 years. Chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and non vascular complications. The vascular complications are further subdivided into microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications. Individuals with diabetic nephropathy almost always have diabetic retinopathy also[1,2].

Clinically the most important screening tool for identifying early nephropathy is detection of microalbuminuria. Clinical studies and histopathologic correlations[1,2] have shown that diabetic retinopathy is almost invariably present in the patient with nephropathy, the invariability being such that the absence of retinopathy should lead to serious question as to accuracy of kidney diagnosis. Discussions of origin of the glomerular lesions lead inevitably to a consideration of the causes of small blood vessel changes in diabetics. These changes are similar histochemically and in staining properties to the lesions observed in the retina and the kidney. They are considered part of a generalized systemic involvement of the small blood vessels, now well known as diabetic microangiopathy. It is only because changes in the retina and the glomerulus produce obvious clinical disturbances in critical areas that they have aroused an earlier and greater interest among clinicians and investigators.Thomsen[3] showed a progressive decrease of the frequency of normal glomeruli obtained at renal biopsy corresponding to the increase in the frequency of clinical retinopathy. Sixty-nine percent of glomeruli of patients without retinopathy appeared normal,whereas no glomeruli were normal in those with proliferative retinopathy. Klein[4] study excluded the factors that could effect UAE (Urinary Albumin Excretion) and they found significant difference between patients who had no

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retinopathy and patients with PDR (Proliferative Diabetic Retinopathy).

Type-2 DM is very common in our population. So far we know there was no such study in our population. This study was conducted to see the relation between diabetic retinopathy and microalbuminuria in type-2 diabetes mellitus.

Materials And Methods:

The study was carried out in BIRDEM, department of Ophthalmology and Endocrine clinic of BSMMU Dhaka from July, 2002 to June 2004. All patients were evaluated by detailed history, thorough examination and relevant investigations. Ocular examinations include slit- lamp biomicroscopy with+90 D Volk lens, direct and indirect ophthalmoscopy. Investigations include color fundus photography, fundus fluorescein angiography and urine for microalbumin by immunological method. ACR (Albumin creatinine Ratio) in Urine and UC (Urinary Creatinine) levels were also measured.

The study was undertaken upon 70 patients of type-2 diabetes mellitus who were newly detected, untreated,normotensive without any anti-hypertensive drug and antidiabetic therapy, and who had risk factors influencing microalbuminuria (hypertension, urinary tract infection,febrile illness, kidney diseases and pregnancy) were excluded. Among them 35 had diabetes mellitus with retinopathy and remaining 35 were age,sex and socioeconomically matched diabetes mellitus individuals without diabetic retinopathy as control.

Observations & Results:

Age of the 35 case studied patients ranged from 30 to 65 years.12 (34.30%) patients belonged to 30 to 45 years age group.21 (60%)patients were within 46 to 55 years age group and 2(5.7%) patients belonged to over 55 years age group(table-I). Mean age (±SD) of DR (Diabetic Retinopathy) patients was 47±7.39 years and mean age (±SD) of control was 47.76±4.97years. Among 35 studied cases 27(77.14%) patients were male and 8(22.86%) were female. Among 35 control patients 29(82.86%) were male and 6(17.14%) female (table-II).

Fundoscopic findings were variable among 35 DR patients. Microaneurysms were found among 15(42.86%) patients,retinal haemorrhage among 26(74.29%),retinal edema among 2(5.7%),hard exudates among 8(22.86%)and macular edema among 4(11.43%).

Among 35 diabetic retinopathy patients, 19(54.29%) had microalbuminuria, whereas 16(45.71%) had no microalbuminuria. Among 35 without diabetic retinopathy control individuals, 10(28.57%) had microalbuminuria whereas 25(71.43%) had no microalbuminuria. Distribution of microalbuminuria is shown in table III and table IV. There was statistically significant difference between two groups (case and control) (P< 0.05).

Distribution of UC level and ACR In urine among study population are shown in table V and table VI respectively

Table I

Age distribution among study population

Group	Case (diabetic retinopathy present)		Control (diabetic retinopathy absent)	
	No.	Percentage	No.	Percentage
30-45 years	12	34.30	10	28.5
46-55 years	21	60	22	63
56-65 years	02	5.7	03	8.5

Table II

Sex distribution among study population among study population

Group	Case(diabetic retinopathy present)		Control (diabetic retinopathy absent)	
	No.	Percentage	No.	Percentage
Male	27	77.14	29	82.86
Female	08	22.86	06	17.14

Table III

Distribution of Microalbuminuria among study population

Group	Present	Absent
Case(diabetic retinopathy present)	19(54.29%)	16(45.71%)
Control (diabetic retinopathy absent)	10(28.57%)	25(71.43%)

Table IV

Level of Microalbuminuria among study population

Group	30-100 mg/day	101-200 mg/day	201-300 mg/day
Case(diabetic13 retinopathy present)	05	01	
Control (diabetic retinopathy absent)	05	03	02

Table-V

Distribution of Urinary Creatinine level among study population

Group	≥ 1.5 g/24 hr.	<1.5 g/24 hr.	P<0.05
Case			
(DR present)	22 (62.86%)	13(37.14%)	Control (DR
absent)	9 (25.7%)	26(74.30%)	

Table-VI

Distribution of ACR in urine among study population

Group	>30mg per gm	<30mg per gm	
Case (DR present)	20(57.14%)	15(42.86%)	P >0.05
Control (DR absent)	15 (42.86%)	20(57.14%)	

Discussion:

Diabetic retinopathy and nephropathy both are important diabetic microangiopatthy. All DR patients were non proliferative. In fact in newly detected DM patients prolierarative DR was not supposed to be present. Microalbumin level in urine should not be expected to be high in newly detected diabetic and non proliferative DR patients what we found in our study (table-IV). Maximum patients are within the level 30-100 mg/day. Similar results are found in other studies also [4,5,6,7,8].

Savage *et al*[5]. demonstrated significant relation between UAE and retinopathy (P< 0.001). Wirta *et al.*[6] found significant relation between background DR and microalbuminuria. Nonproliferative DR and microalbuminuria have beem found to be related in type -2 diabetic subjects in cross sectional studies (Esmatjes *et al.*[7]). Chen *et al*[8].found relation between progression of DR and increasing proteinuria in a longitudinal study in NIDDM patients. It is suggested that diabetic nephropathy increases the risk of DR development not only by elevating the blood pressure but also by the serum level of fibrinogen. Eggertsen *et al.*[9] found 42% of NIDDM patients with diabetic retinopathy and mainly of background type, and 34% with microalbuminuric. In our study, 54.29% patients with DR had microalbuminuria and 28.57% patients without DR had microalbuminuria.

Several methods for detection of urinary microalbumin are available for screening which give comparable results. The gold standard is the 24 – hour urine collection which if accompanied by serum and urine creatinine, also allows calculation of creatinine clearance rate and serves as a reference for future comparison. Somewhat more convenient for the patient is the albumin/creatinine ratio on a spot urine sample (normal<30mg albumin per gram creatinine) or albumin excretion rate in a timed specimen (4 hours or overnight, normal <20mg albumin per minute). Measuring albumin without relating it to duration of collection or creatinine concentration is less sensitive and specific because of dilution variability.

Several studies indicated that ACR may be regarded as the risk marker through which all of the risk factors express themselves but in type-2 DM proteinuria is not generally found to be an independent predictor of subsequent incidence of DR.In my study 20(57.14%) out of 35 DR patients had urinary ACR higher than 30 mg per gram which was not statistically significant but statistically significant was the incidence of >30mg per gm urinary ACR in 17(89.5%) out of 19 diabetic nephropathy patients. Again UC level higher than 1.5gm per 24 hour was found in 22(62.9%) out of 35 DR patients and 17(89.5%) out of 19 diabetic nephropathy patients. There was statistically significant difference between case and control groups (P < 0.05).

It is facile to explain the chicken and egg problem by assuming renal involvement and diabetic retinopathy to be parallel disease processes. Renal involvement and diabetic retinopathy have same risk factors in common and these two complications may mutually or unilaterally modify the relationships between these risk factors and renal involvement or diabetic retinopathy.

This study showed diabetic retinopathy is associated with microalbuminuria (early diabetic nephropathy) which was statistically significant. Fundal examination and determination of microalbuminuria in all newly detected patients with type 2 diabetes mellitus should be done.

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