CORRELATION BETWEEN SERUM hs-CRP AND LDL CHOLESTEROL AS A PREDICTOR OF CARDIOVASCULAR DISEASES

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ABSTRACT

BACKGROUND

Of novel risk factors for cardiovascular disease currently under investigation, High-Sensitivity C-Reactive Protein (hs-CRP) is the most promising. Circulating levels of C-Reactive Protein (CRP) may constitute an independent risk factor for cardiovascular disease. How CRP as a risk factor is involved in cardiovascular disease is still to be discovered. In order to determine the better diagnostic marker and their probable role in the pathogenesis of IHD in comparison to serum LDL-C, we evaluated hsCRP.

METHODS

For this study, 90 patients of myocardial infarction and 90 controls irrespective of age and sex were studied for these parameters over a period of 1 year. Lipid profile and hs-CRP was estimated by using commercial kit on autoanalyzer and Elisa reader.

RESULT

The statistical analysis showed that the serum hsCRP was significantly raised in myocardial infarction cases than controls ($p<0.005$), but LDL-C was not ($p>0.019$).

CONCLUSION

On the basis of present study, it may be concluded that the serum hsCRP contribute as an independent risk factor for atherosclerosis and other CVD.

KEYWORDS

Myocardial Infarction, Atherosclerosis, hsCRP, LDL-C, CVD.

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INTRODUCTION

Cardiovascular Diseases (CVDs) are the biggest cause of death worldwide. Over the past two decades, deaths from CVDs have been declining in high-income countries, but have increased at an astonishingly fast rate in Low- and Middle-Income Countries (LMIC). CVDs are largely preventable. Both, population wide measures and improved access to individual health care interventions can result in a major reduction in the health and socioeconomic burden caused by these diseases and their risk factors.(1) For more than 30 years blood pressure, smoking status, hyperlipidaemia and the presence or absence of diabetes was considered as cardiovascular risk factor. These core traditional risk factors for heart disease and stroke derive largely from the ground breaking Framingham Heart Study that first provided the conceptual basis for cardiovascular risk factors in the early 1960s.(2) Due to extensive research and identification of the aetiopathogenic basis of Cardiovascular Disease (CVD) as well as the diverse mechanisms implicated in the onset and progression of atherosclerosis, current studies in this area focus in the characterization of biomarkers for the early detection of the inflammatory activation underlying this process.

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The atherosclerosis is major cause of IHD and other CVDs. It is a chronic inflammatory response of the arterial wall initiated by injury to the endothelium. Moreover, lesion progression is sustained by interaction between modified lipoproteins (eg. oxidized LDL), monocyte-derived macrophage (Foam cells), T-lymphocytes and the normal cellular constituents of the arterial wall. Atherosclerosis is characterized by thickening of the arterial wall, which protrudes into and obstructs the vascular lumen.(3) The biomolecule with the greater body of research both from a molecular and epidemiological perspective is C-Reactive Protein (CRP), a plasma protein of the pentraxin family and an acute phase reactant, which displays high sensitivity as a general inflammation marker.(4) Numerous studies have demonstrated the active participation of this molecule in the atherogenic process.(5) and due to the discovery of high-sensitivity techniques for its determination, its stable plasmatic concentrations and its relatively low costs, it may be of great use in the identification of patients at high risk as a prognostic indicator and even as a therapeutic target in large populations.

More than 20 prospective epidemiologic studies have demonstrated that hs-CRP independently predicts vascular risk, 6 cohort studies have confirmed that hs-CRP evaluation adds prognostic information beyond that available from the Framingham Risk Score and 8 cohort studies have demonstrated additive prognostic value at all levels of metabolic syndrome or in the prediction of type 2 diabetes. Low Density Lipoprotein (LDL), which is called bad cholesterol, rich in cholesterol ester, participates in the atherosclerotic process. Dyslipidaemia with particular reference to LDL cholesterol has also been considered as risk factor for development of atherosclerosis.(6)

Although, there are plenty of studies which confirmed the hs-CRP as predictor of CVD, but its comparative importance with LDL-C is still a far cry. The present study is an attempt to establish a correlation between serum LDL-C and hs-CRP, which has greater importance.

MATERIAL AND METHODS

This study was conducted in the Biochemistry Department of Katihar Medical College and Hospital. Ninety patients irrespective of age and sex diagnosed as myocardial infarction clinically as well as by ECG and cardiac markers were chosen as cases. The controls were selected from the persons, neither suffering from any type of cardiovascular disease nor taking any drug from same region. Both the cases and controls were selected by a simple random method. After noting the name, age and sex, venous samples were drawn after 12 hours of overnight fasting. Serum was separated and assays were performed within 24 hours. Serum hs-CRP was measured by sandwich Enzyme Linked Immunosorbent Assay (ELISA).[7] Serum total cholesterol, Triglyceride (TG) and High Density Lipoprotein (HDL) were assayed by Cholesterol Oxidase-Peroxidase (CHOD-PAP), Glycerol-3-Phosphate Oxidase (GPO) and polyanion precipitation methods respectively using semi-autoanalyzer. Serum Very Low Density Lipoprotein (VLDL) was calculated by dividing the value of TG by 5 and serum LDL was obtained by Friedewald equation.[8] The value of hsCRP >3mg/L.[9] was considered as high risk for cardiovascular diseases. The data for biochemical analysis was subjected to standard statistical analysis such as student 't' test using the Statistical Package for Social Science (SPSS) 11.5 software.

RESULTS AND DISCUSSION

Results are given in the tables. In cases, the mean value of hsCRP was found to be 3.7222±0.62401 and in control 0.5778±0.16517 ($p<0.005$) Table 1. The mean value of LDL-C among cases and control were estimated as 97.34±18.60057 and 89.33±9.00312 respectively ($p>0.019$). The increase level of hs-CRP was highly significant as compared to their normal counterpart. Although, the level of LDL-C among the cases was significantly increased as compared to their control, but the level was not highly elevated.

	Concentration of hsCRP (mg/L) (Mean±SD)
IHD Cases Control	3.7222±0.62401 0.5778±0.16517
P value	0.005

Table 1: Comparison of Mean of hs-CRP between the cases Suffering from Myocardial Infarction and Controls

	Concentration of LDL-C (mg/dL) (Mean±SD)
IHD Cases Control	97.34±18.60057 89.33±9.00312
p value	0.019

Table 2: Comparison of Mean of LDL-C between the cases Suffering from Myocardial Infarction and Controls

These results suggest that serum hsCRP level was significantly higher in cases of myocardial infarction. Inflammation has a key role in the pathophysiology of atherosclerosis.[10,11] Macrophages present in the atherogenous plaque lead to release of additional mediators like cytokines and chemokines, which in turn increase the plasma concentration of CRP which amplify inflammatory and procoagulant responses.[12] Therefore, markers of inflammation such as CRP have been investigated for risk estimation of cardiovascular event. However, in atherosclerosis, low-grade inflammation has been recognized, so CRP concentration are often lower than measuring range of traditional CRP assay. Due to this for suspected cardiac cases hs-CRP (High Sensitivity CRP) was measured. Many assay techniques for hs-CRP measurement are now commercially available, which give accurate and reproducible results.

JUPITER study.[13] demonstrate the usefulness of CRP for the identification of subjects in risk and they also hint towards its potential role as a therapeutic target in the atherosclerotic process.[14] In this aspect, hs-CRP is more than a simple biomarker and current findings tightly link this protein with the CVD. Its implementation is based on the guidelines suggested by the NACB, which delimit its application to a certain population at risk and sets a cut-off point for its serum levels. However, many aspects still remain to be elucidated, requiring the assessment of CRP behaviour across ethnic groups (Asians, Africans and Hispanics) since most studies have been limited to European and North American cohorts. Likewise, further research would clarify the true role of CRP in the development of CVD.[15] Furthermore, research is being expanded to further age groups, analyse the impact of hs-CRP in coronary event prognosis and decipher the phenomena linking it to the atherogenic process in order to exploit its potential efficacy as a therapeutic target.[16,17] The answers to these matters would allow the confirmation of the feasibility of hs-CRP quantification and the formulation of management guidelines for our patients, based on the measurement and the clinical picture of each individual.

Because of its clinical importance in atherogenesis, LDL is the focus of current guidelines for the determination of the risk of cardiovascular disease. It is noteworthy that only half of all patients with coronary heart disease have any one of the established risk factors like–hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, marked obesity and physical inactivity. Braunwald in his scholarly lecture has listed some emerging cardiovascular risk factors, C-reactive proteins being one of them.[18] It has been shown that maximum myocardial infarction cases have serum hsCRP level more than 3 mg/L, though they have normal serum LDL level. After extensive data analysis, Willerson inferred that high CRP/Low LDL-C persons are at higher absolute risk than low CRP/high LDL-C persons.[19]

European Society of Cardiology guidelines for the prevention of heart disease strongly endorse cholesterol screening. Those same guidelines are silent on C-reactive protein.[20] Inflammation is a fundamental component of atherosclerosis.[21] For more than a decade, data from large-scale prospective cohorts in the USA and Europe have consistently indicated that the predictive value of the inflammatory biomarker C-reactive protein is at least as large as that of cholesterol.[22,23] This observation is important since half of all heart attacks and strokes occur among those with

average if not low cholesterol levels. That C-reactive protein and lipids are equal contributors to vascular risk has recently been confirmed in an elegant 2012 meta-analysis published in the New England Journal of Medicine by the Emerging Risk Factors Collaboration that analysed data from 38 prospective studies and included 166 596 men and women without prior disease.[24] In 2008, in a fully parallel manner the JUPITER trial answered this crucial question in primary prevention for those who had elevated levels of C-reactive protein, but who otherwise would not qualify for statin therapy as they already had levels of LDL-C below treatment thresholds.[25]

In brief, among 17802 individuals with LDL-C 3.36 mmol/L (Median ¼ 2.7 mmol/L), but who were identified at increased vascular risk due to C-reactive protein levels >2 mg/L (Median 4.1 mg/L), rosuvastatin reduced major vascular events by 44% (P 0.0001) and all-cause mortality by 20% (P¼ 0.02). JUPITER also extended the statin literature in primary prevention to include women and non-Caucasian participants, all of whom experienced similar risk reductions. While there was no relationship in JUPITER between baseline LDL-C and subsequent benefit (An observation consistent with many studies in secondary prevention), those with sequentially higher baseline C-reactive protein values in JUPITER had higher absolute risk.[26-28]

Therefore, it can be suggested that the serum hs-CRP level is a strong predictor of cardiovascular events than the serum LDL-C. Therefore, future research should continue to more thoroughly study the effects of the reduction of serum hs-CRP levels. The designation of new risk factors stemming from advances in the comprehension of the inflammatory physiopathology of CVD has led research to try and elucidate which of these novel and emergent elements display all required criteria to be considered true risk factors and which have solely exhibited a casual statistical association. High sensitivity C-reactive protein is one of the numerous molecules that fit this description, but its properties and features have led it to become one of the main targets for researchers worldwide.

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STUDY OF ADENOSINE DEAMINASE AND LYMPHOCYTE/NEUTROPHIL RATIO IN COMBINATION AS DIAGNOSTIC TOOL FOR TUBERCULAR PLEURAL EFFUSION

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ABSTRACT: Adenosine deaminase, considered one of the key enzyme of purine metabolism, has been used in work up of lymphocytic pleural effusion. Low level of ADA <40IU/L essentially excludes tuberculosis from consideration as differential diagnosis of pleural effusion cases. ADA >50IU/L specially when combined with Lymphocytic/neutrophil ratio >0.75 in pleural fluid is useful test in the diagnosis of tubercular pleurisy.

AIM AND OBJECTIVE: To suggest a better diagnostic tool in the diagnosis of pleural effusion of tubercular origin by estimating the activity of ADA along with L/N ratio in pleural effusion.

METHOD: Biochemical, cytological and microbiology studies were done by obtaining pleural fluid by thoracocentesis in 100 patients after excluding pleural effusion cases of malignancy, transudative effusion.

RESULT: 84 cases were tubercular and had high level of ADA in comparison to rest of 16 non-tubercular cases. At level of 50 IU/L of ADA activity test had sensitivity of 97.6%, specificity 87.5%, positive predictive value 97.6%, negative predictive value 87.5%which increased to 100% and 92.8%, 98.6%, and 100% respectively in combination with test of Lymphocytic/Neutrophilic ratio >0.75.

CONCLUSION: ADA level with L/N ratio can be important investigation in diagnosis of tubercular pleural effusion cases.

KEYWORDS: Tuberculosis, Pleural Effusion, Adenosine Deaminase.

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INTRODUCTION: Tuberculosis is one of the oldest and commonest infectious disease known to mankind. In India and third world countries, it has always been a major health problems. In spite of presence of effective drugs, epidemiological data show worldwide rise in incidence since emergence of acquired immunodeficiency syndrome (AIDS). Being commonest pulmonary tuberculosis is often associated with effusion, which is second most common extra pulmonary clinical manifestation it. In India most common cause of pleural effusion is tuberculosis. Delayed in diagnosis or untreated cases may develop in to active tuberculosis and poor prognosis thus it is of extreme importance to diagnose and give treatment to the cases as early as possible.

Tuberculous pleurisy is thought to be the result of a delayed hypersensitivity reaction in response to the presence of mycobacterial antigen in response of mycobacterial antigen in pleural space. This immunologic reaction causes the stimulation and differentiation of lymphocyte which release lymphokines which in turn activate macrophages for enhanced bactericidal effect.[1]

In addition to routine investigations, chest X-ray thoracocentesis is required to ascertain the nature of effusion and to differentiate it from manifestation of other etiologies.

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Malignancies, infectious diseases, pulmonary embolism collegen vascular diseases, sarcoidosis, uremia are few of other causes of pleural effusion.Definitive diagnosis of tubercular pleural effusion is done by tubercular bacilli or pleural granulomas demonstration in effusion fluid or pleural biopsy specimen or sputum.

Adenosine deaminase is a useful chemical biomarker as screening test specially in endemic areas, origin of which is unknown and tuberculosis is virtually excluded if levels of ADA are very low. The determination of ADA activity was first proposed as serological marker for lung cancer in 1970.[2] Later, Piras et al in 1978[3] reported the usefulness of ADA in diagnosing tubercular pleurisy. The presence of ADA in pleural fluid reflects the cellular immune response in pleural cavity and in particularly the activation of T lymphocyte. Several studies have suggested that an elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of90-100% and a specifity of 89- 100% when Giusti method is used.[4,5,6,7] The reported cut off value for ADA (Total) varies from 47 to 60 IU/L.[8,9,10]

Several studies have shown that ADA activity, especially when combined with differential leucocyte count and lymphocyte/ neutrophil ratio in pleural fluid remain a useful test in the diagnosis of tuberculous pleuritis. When the lymphocyte to neutrophils ratio (L/N ratio) >0.75 in pleural fluid was considered together with ADA activity >50IU/L, the results improved considerably for the diagnosis of tubercular pleuritis.[11]

This study made an attempt to investigate the role of Adenosine deaminase activity and lymphocytic/neutrophil ratio in combination to diagnosis of tubercular pleural effusion.

MATERIALS AND METHODS: The present hospital based case control study was undertaken in Department of Biochemistry Katihar Medical College and Hospital Katihar Bihar during the period from December 2007 to April 2009.

During this period of time 100 subjects were taken from admitted patients suffering from pleural effusion and selected by simple random method. Patients were informed about study and written consent was taken before any investigation. All exudative pleural effusion cases were included and patients with transudative pleural effusion e.g. congestive heart failure, rheumatoid arthritis were excluded from this study.Along with thorough clinical history and examination cases were investigated for following:

Haematological Examination: Haemoglobin, Total Leucocyte count, Differential leucocyte count, Erythrocyte sediment rate

- Chest X ray, P. A. View.
- Mantoux test.
- Sputum for AFB.

Pleural Fluid Examination: Differential count and Lymphocyte/Neutrophil ratio by microscope, LDH, Activity of Adenosine deaminase by using commercial kit, Z N stain for AFB, protein concentration.

Diagnostic thoracocentesis was performed to obtain pleural fluid. Chest radiograph was obtained and localization of effusion was done. Physical examination such as diminished breath sound at the base of affected lung and decreased percussion note were performed to define the place. Patient were asked to take upright and sitting position with arms up and forward. Puncture site was marked with pen in mid-scapular line two rib interspaces down from upper end of effusion. Skin was cleaned with antiseptic solution over an area of 4 inches in all direction. Skin was anaesthetized by injecting 2% lidocaine site was punctured with 18 guaze needle attached to 50–60ml syringe containing heparin 1ml, advanced until feeling a slight give was obtained.

Estimation of Adenosine Deaminase activity in pleural fluid was done by method described by Guisti and Galanti. Only fresh collection were used. Berthelot reaction is the basis of calorimetric method in which ammonia and inosine produced due to ADA reaction on adenosine.Blue indophenol complex is produced due to reaction between ammonia, phenol and hypochlorite in alkaline medium.

Light's criteria.[12] (Pleural fluid protein/serum protein >0.5; pleural effusion fluid LDH/serum LDH >0.6) was used to exclude transudative pleural effusion. Cases of pleural effusion associated with malignancy/presence of cytological or histological evidence of malignancy were reviewed and included.

Diagnosis of tubercular pleural effusion was made on the basis of first or more than one of following criteria in addition to already proved diagnosed cases of TB:

1. Identification of Mycobacterium tuberculosis in pleural fluid or in sputum.
2. Clinical and radiological evidence of TB.
3. Clinically presenting with signs and symptoms consistent with TB exclusion of other clinical entity.
4. Definite clinical and radiological improvement in 6 to 8 weeks after administration of antitubercular treatment.

Method Analysis: Data were presented as frequency, percentage and mean±2SD. Student t test was applied to determine the significance of biochemical parameters between two groups. Pearson coefficient correlation were calculated for relationship between measured parameters, p value of <0.05 considered as significant. Data was analysed using statistical package program SPSS 15.0.

RESULTS AND DISCUSSION: In one and half years of the study period 100 cases of pleural effusion were studied and included by using simple random method. Out of 100 cases 84 were confirmed cases of tubercular pleural effusion and rest 16 cases were non-tubercular pleural effusion cases.

Among 84 tubercular pleural effusion patients 49(58.3%) were male and 35(41.6%) females. Subjects were classified in age groups <5, 5-15, >15 and mean age was 26.07 with SD±18.93277 (Range 4 to 67 years) (Table 1).

35.71%cases among tubercular pleural effusion (n=84) were positive for sputum smear (Table 2). All cases of non-tubercular pleural effusion were negative for sputum smear. Table 2 shows sensitivity and specificity of sputum smear for tubercular pleural effusion.

With Cut off level of ADA >50 IU/L, out of 84 cases of tubercular effusion 82 and 2 cases of non-tubercular cases had level of ADA >50 IU/L (Table 3). This cut off level of 50 IU/L had sensitivity of 97.6%, specificity of 87.5%, positive predictive value 97.6% and negative predictive value 87.5%. Observation made in this study were similar to previous studies.[12,13,14]

Table 4 shows that 81 cases of tubercular pleural effusion and 2 cases of non-tubercular had Lymphocytic/Neutrophil ratio >0.75 out of total 84 cases and 16 cases respectively. Sensitivity was 96.4% and Specificity 87.7%. Positive Predictive value and Negative Predictive Value was 97.5% and 82.3% respectively.

Various studies.[11] had shown that combining ADA activity and Lymphocytic/Neutrophil ratio for diagnosis of tubercular pleural effusion increases sensitivity and specificity of these tests. In our study combination of these two had increased sensitivity (100%), specificity (92.8%), positive predictive value (98.6%) and negative predictive value (100%).

Table 6 shows comparison of mean between L/N ratio and ADA activity in pleural fluid in patients suffering from tuberculosis. Statistical analysis done by paired sample t test which concludes that two parameter positively correlate significantly (p=0.000).

The mean value of ADA activity in tubercular patients remains within the cut-off value and mean value of L/N ration in these patients are >0.75.

CONCLUSION: Tubercular pleural effusion traditionally diagnosed by identification of Mycobacterium tuberculosis in pleural fluid or culture of biopsy specimen. Clinical diagnosis are needed to be made before availability of lab results and this presents difficulty in practice. ADA level activity estimation is a simple, inexpensive, and in conjugation with L/N ration highly specific and sensitive test. Estimation of ADA along with lymphocytic/neutrophil ratio will provide less time consuming diagnosis of tubercular pleuritis and should be routinely included in pleural fluid examination.

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Ethical Clearance: The study was approved by ethical committee of Katihar Medical College, Katihar, Bihar.

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Age Group (years)	Male	Female	Total (% of cases n=84)
<5	0	3	3(3.5)
5-15	20	14	34(40.4)
>15	29	18	47(55.9)
Total	49	35	84

Table 1: Distribution of Cases of Tuberculosis according to Age and Gender

Sputum smear	Tubercular	Non-Tubercular	Total
Positive	30	0	30
Negative	54	16	70
Total	84	16	100

Table 2: Sensitivity and Specificity of Sputum smear for Pleural effusion

Sensitivity = 35.71%, Specificity = 100%, Positive predictive value = 100%,
Negative predictive value = 22.86%

ADA level	Tubercular	Non-Tubercular	Total
ADA >50 IU/L	82	2	84
ADA <50 IU/L	2	14	16
Total	**84**	**16**	**100**

Table 3: Sensitivity and Specificity of ADA Pleural fluid >50 IU/L

Sensitivity =97.6%, Specificity =87.5%, Positive predictive value =97.6%,
Negative predictive value = 87.5%

L/N ratio	Tubercular	Non-Tubercular	Total
>0.75	81	2	83
<0.75	3	14	17
Total	**84**	**16**	**100**

Table 4: Sensitivity and Specificity of L/N ratio for Pleural Fluid >0.75

Sensitivity = 96.42%, Specificity = 87.70 %, Positive Predictive Value = 97.59 %,
Negative Predictive Value = 82.35%

ADA and L/N ratio	Tubercular	Non-Tubercular	Total
ADA >50 IU/L and L/N >0.75	75	1	76
ADA <50 IU/L and L/N <0.75	0	13	13
Total	**75**	**14**	**89**

Table 5: Sensitivity and Specificity of ADA activity as an etiological marker for Pleural effusion in conjugation with L/N ratio (Lymphocyte/Neutrophil)

Sensitivity = 100 %, Specificity = 92.85 %, Positive Predictive Value 98.68%
Negative Predictive Value = 100%

	Mean ± 2SD
L/N ratio	1.2900 ± 2.73746
ADA level	53.6229± 9.22786
P Value	0.000

Table 6: Comparison of Mean between L/N ratio and ADA activity in Tubercular Pleural effusion

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To,
Dr Md Faizur Rahman,
Assistant Professor,
Department of Biochemistry,
Katihar Medical College, Katihar (Bihar)

Dear author/s

I have pleasure to inform you that your following paper has been accepted for publication in Indian Journal of Public Health Research & Development

STUDY OF ADENOSINE DEAMINASE ACTIVITY IN TUBERCULOUR PLEURAL EFFUSION AND OTHER RESPIRATORY DISEASES

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With regards

Yours sincerely

Prof R K Sharma
Editor

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Study of Adenosine Deaminase Activity in Tuberculour Pleural Effusion and other Respiratory Diseases

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ABSTRACT

Introduction: Pleural effusion frequently presents a diagnostic problem to establish the aetiology in spite of good history taking , thorough clinical, radilological, full examination of aspirated fluid and pleural biopsy. Increased level of Adenosine deaminase activity has been found to be associated with tubercular pleural effusion. **Aim and Objective**: To suggest estimating the activity of ADA as a diagnostic tool in cases of pleural effusion and compare its activity between tubercular and non tubercular origin. **Method**: Biochemical , cytological and microbiology studies was done by obtaining pleural fluid by thoracocentesis in 100 patients after excluding pleural effusion cases of malignancy, transudative effusion. 84 cases were tubercular and had high level of ADA in comparison to rest of 16 nontubercular cases . At level of 50 IU/L of ADA activity test had sensitivity of 97.6% , specificity 87.5%, positive predictive value 97.6%, negative predictive value 87.5 %. **Conclusion**: ADA level > 50IU/L in pleural effusion fluid can be important investigation in diagnosis of tubercular pleural effusion cases.

Keywords: Tuberculosis, Pleural effusion, Adenosine deaminase

INTRODUCTION

Tuberculosis , one of the oldest disease known to mankind remains a worldwide public health problem despite the fact that causative organism *Mycobacterium tuberculosis* was discovered more than hundred years ago and highly effective drugs and vaccines are available making tuberculosis a preventable and curable . If properly treated tuberculosis caused by drug susceptible strains is curable and if left untreated, can be fatal within five years in 50-60% of cases.

Tubercular Pleural effusion is a common in primary tuberculosis fluid accumulate in pleural space as a result of the delayed hypersensitivity reaction to tubercular protein . Pleural effusion frequently represents a diagnostic problem and definite diagnosis of tubercular effusion can be difficult because of low sensitivity and/or specificity of non invasive traditional diagnostic tools. The diagnosis of pulmonary tuberculosis is confirmed mainly by sputum examination for acid fast bacilli . For etiological confirmation of pleural diseases various investigation like pleural aspiration , fluid biochemistry , cytology and culture , mantoux test , pleural biopsy and histopathology , adenosine deaminase and various techniques such as polymerase chain reaction (PCR) ,lysozymes have been studied previously. There is need of simple, rapid and reliable techniques to diagnose tuberculosis quickly as well as accurately .

Adenine deaminase (ADA) is an enzyme involved in purine metabolism and is essential for proliferation and differentiation of lymphoid especially T cells. The rise of ADA in pleural fluid of tubercular pleurisy is of unknown origin . The determination of ADA activity was first proposed as serological marker for lung cancer in 1970[1]. Later Piras et al in 1978 reported the usefulness of ADA in Diagnosing tubercular pleurisy[2]. ADA estimation in pleural fluid has emerged as a reliable biomarker in recent years specially when

there is suspicion of tuberculosis in endemic areas . This study was conducted to assess the diagnostic value of adenine deaminase level in pleural fluid in pulmonary tuberculosis and compare activity of ADA pleural effusion between of tubercular and non tubercular origin.

MATERIALS & METHODS

The present hospital based case control study was undertaken in the Department of Biochemistry Katihar Medical College and Hospital, Katihar , Bihar and conducted during the period from December 2007 to April 2009 after approval by institutional ethical committee. The subjects were admitted patients suffering from pleural effusion and selected by simple random method. Patients were informed about risks and benefits of study and written consent was taken before study .

Out of two hundred and fifty cases of pleural effusion hundred patients were included in study by simple random method and along with thorough clinical history, examination were investigated for following –

- Haematological examination Haemoglobin , Total Leucocyte count , Differential leucocyte count , Erythrocyte sediment rate
- Chest X ray , P.A. view
- Mantoux test
- Sputum for AFB
- Pleural fluid examination for activity of Adenosine deaminase by using commercial kit , Z N stain for AFB , protein concentration

To obtain pleural fluid diagnostic thoracocentesis was performed . Chest radiographs were done to localise the pleural effusion. Physical examination such as diminished breath sound at the base of affected lung and decreased percussion note were performed to define the place . Patient were asked to take upright and sitting position with arms up and forward . Puncture site was marked with pen in mid-scapular line two rib interspaces down from upper end of effusion . Skin was cleaned with antiseptic solution over an area of 4 inches in all direction . Skin was anaesthetized by injecting 2% lidocaine site was punctured with 18 guaze needle attached to 50 – 60 ml syringe containing heparin 1 ml , advanced until feeling a slight give was obtained.

Estimation of Adenosine Deaminase activity in pleural fluid was done by method described by Guisti and Galanti[3] . Only fresh collection were used . Berthelot reaction is the basis of calorimetric method in which ammonia and inosine produced due to ADA reaction on adenosine . Blue indophenol complex is produced due to reaction between ammonia, phenol and hypochlorite in alkaline medium.

Diagnosis of tubercular pleural effusion was made on the basis of first or more than one of following criteria in addition to already proved diagnosed cases of TB

1. Identification of Mycobacterium tuberculosis in pleural fluid or in sputum
2. Clinical and radiological evidence of TB
3. Clinically presenting with signs and symptoms consistent with TB exclusion of other clinical entity
4. Definite clinical and radiological improvement in 6 to 8 weeks after administration of antitubercular treatment

Transudative pleural effusion cases were excluded by using Light's criteria[4] (pleural fluid protein / serum protein > 0.5 ; pleural effusion fluid LDH / serum LDH > 0.6). Cases of pleural effusion associated with malignancy / presence of cytological or histological evidence of malignancy were reviewed and included as non tubercular cases .

Method analysis : Data were presented as frequency, percentage and mean ± 2SD. Student t test was applied to determine the significance of biochemical parameters between two groups . Pearson coefficient correlation were calculated for relationship between measured parameters , p value of < 0.05 considered as significant . Data was analysed using statistical package program SPSS 15.0 .

RESULTS

In present study total 100 cases were included by simple random method of pleural effusion during one and half year of study period. 84 cases of tubercular origin diagnosed by history , sputum results, pleural fluid results and rest were cases of non tubercular causes comprising of malignancy , collagen vascular disorders etc.

Out of 100 cases 62 were males and 38 were females (table 1) . Number of cases of both tubercular and non tubercular in age groups < 5, 5-15 , > 15 years were 3 , 41 , and 56 respectively (table 2).

Table 3 shows that Sputum smear was positive for 30 cases out of 84 cases of tuberculosis and all the non tubercular were negative for it. Sensitivity and specificity for Sputum smear for pleural effusion was 35.71% and 100 % respectively (table 3) Positive predictive value and negative predictive value were 100% and 22.86% for same .

Adenosine deaminase level >50 IU/L were taken as a cut off point and all the 84 cases of tuberculosis had level >50 IU/L . 2 cases out of 16 cases of non tubercular pleural effusion had level of ADA > 50IU/L . Sensitivity and specificity were 100% and 87.5 % respectively for this test with positive predictive value of 97.7% and negative predictive value 100% % (table 5).

DISCUSSION

Tuberculosis is common cause of pleural effusion especially in developing countries like [4]with trend of increasing incidence world wide [5]. It is difficult to establish the etiological diagnosis because of low sensitivity of the various diagnostic tools [6]. Culture for acid – fast bacilli are positive in 20 to 30% of pleural fluid samples and in 50 to 80 % of pleural biopsy specimens [7,8] . The sensitivity of polymerase chain reaction for active disease is 78%[9]. The cutaneous response to putrefied protein derivative may also be negative in one third of the patients[10]. Thus in spite of good history thorough clinical and radiological examination of patients and full examination of aspirated fluid and pleural biopsy , there is need of simple rapid and reliable diagnostic test to establish the aetiology of pleural effusion . Considering this , a prospective study was designed to find out how much pleural fluid ADA level could be helpful in establishing the diagnosis of pleural effusion.

Age wise distribution showed that number of cases of pleural effusion was more in > 15 years of age group(table 1) and prevalence was more in male (table 2).

The common diagnostic tools that are used for diagnosis of tuberculosis are sputum for AFB and Mantoux test but the sensitivity of these tests was found to be low with high level of specificity (table 3 and 4). Several previous studies scrutinized that about one third of patients with tuberculous pleural effusion can have negative tuberculin skin test[11] . Jay S.J. [8] after their study concluded that a negative tuberculin test does not excludes the diagnosis and it may be negative in one third of tuberculosis patients. There may be several false positive and negative result of sputum smear microscopy due to various problems in collecting , processing or interpreting sputum smears or because of administrative errors .

Adeniosine deaminase level in tubercular pleural effusion ranged from 47.5- 59.4 IU/L with a mean level of 52.9IU/L while in non tubercular group it ranged from 14.6 – 25.9IU/L with a mean level of 18.6 IU/L(p = 0.000) which was highly significant (table 6) . This result was in accordance with previous studies [4,12] .

In comparison to other diagnostic tools like sputum smear or AFB staining , sensitivity and specificity of ADA activity for diagnosing tubercular pleural effusion was 100 % and 87.5% with positive and negative predictive value 97.7 % and 100 % respectively . Previous studies have also shown sensitivity and specificity of 90% and 100% for value of ADA in pleural fluid using different cut off levels [13,14,15].

Table 1 : Distribution of Cases of Pleural effusion according to Gender

	Tubercular	Non tubercular	Total
Male	49	13	62
Female	35	3	38
Total	84	16	100

Table 2 : Distribution of Cases suffering from Pleural effusion according to Age

Age Group (years)	Tubercular (% of cases n=84)	Non tubercular (% of cases n=16)	Total
<5	3(3.5)	0(0)	3
5- 15	34(40.4)	7(43.8)	41
>15	47(55.9)	9(56.2)	56
Total	84	16	100

Table 3 : Sensitivity and Specificity of Sputum smear for Pleural effusion

Sputum smear	Tubercular	Non Tubercular	Total
Positive	30	0	30
Negative	54	16	70
Total	84	16	100

Sensitivity = 35.71 % , Specificity = 100 % , Positive predictive value = 100 % ,

Negative predictive value = 22.86 %

Table 4 : Sensitivity and Specificity of Mantaux test for tubercular pleural effusion

Mantaux test	Tubercular	Non Tubercular	Total
Positive	45	1	84
Negative	39	15	16
Total	84	16	100

Sensitivity =53.57 % , Specificity = 93.75 % , Positive predictive value =97.83 % ,

Negative predictive value = 27.78 %

Table 5 : Sensitivity and Specificity of Adenosine deaminase test for tubercular pleural effusion

ADA test	Tubercular	Non Tubercular	Total
Positive	84	2	84
Negative	0	14	16
Total	84	16	100

Sensitivity =100 % , Specificity = 87.5 % , Positive predictive value =97.7% ,

Negative predictive value = 100%

Table 6 : Comparison of mean of ADA activity in Pleural fluid in different groups

	ADA activity in pleural fluids	p value
Sputum positive patients	54.48±7.81236	0.066
Sputum negative patients	52.51±7.37326	
Mantaux positive	53.4077±7.30686	0.894
Mantaux negative	53.5487±10.9141	
Cases of pleural effusion of tubercular origin	52.9000±8.0169	<0.0001
Cases of pleural effusion of non-tubercular origin	18.6015±8.0169	

CONCLUSION

In present study it has been clearly shown that ADA level in most tubercular patients is between 47-60 IU/L. This test has 100% sensitivity and 87.5 specificty for diagnosing tubercular etiology . The method of ADA estimation is simple, cheap, does not require expensive equipment or elaborate laboratory arrangement except a simple colorimeter and takes only 2 hours. Present study

shows that highly sensitive and specific test like ADA estimation should be employed routinely to differentiate between tubercular and non tubercular etiology on the patients of pleural effusion.

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Conflict of Interest Statement: We certiify that there is no conflict of interest.

Source of Funding: No funds were required in this study.

Ethical Clearance: The study was approved by ethical committee of Katihar Medical College, Katihar, Bihar.

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ORIGINAL RESEARCH ARTICLE

Hyper Homocysteinemia and Dislipidemia as Risk Factors of Patients with Ischaemic Stroke: A Hospital Based Cross Sectional Study

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Abstract: -

***Aims:** Aim of our study was to evaluate the hyperhomocysteinemia and dyslipidemia as a risk factors of patients with ischemic stroke.*

***Methods:** A detail history, clinical examination and relevant investigations were performed to all cases. The lipid profile of the study sample was analyzed according to the ATP III classification for identification of dyslipidemia. Dyslipidemia (LDL >130 mg/dl, TC>200 mg/dl, HDL<40 mg/dl) as per ATP III guidelines was presented in all patients. Homocystein level were measured as EDTA samples were collected and transported in ice packs. Samples centrifuged immediately, refrigerated and properly thawed before estimation. Homocysteine enzyme immunoassay was done by ELISA (Transasia). Measuring range of calibration was from 2-50 µmol/L.*

***Results:** Data was analyzed with the help of MS- office software.*

***Conclusions:** This study was concluded middle age group peoples (41-60 years) were greatly affected with ischemic stroke. Hypertension and diabetes mellitus were the major baseline risk factors. Hyper homocysteinemia was the major risk factor than dyslipidemia of patients with ischemic stroke. Dyslipidemic patients of ischaemic stroke had more increased total cholesterol level than TG, LDL and HDL.*

***Key words:** Hyper homocysteinemia, dyslipidemia, ischaemic stroke.*

Introduction: -

Stroke is a clinically defined syndrome of rapidly developing symptoms or signs of focal loss of cerebral function with no apparent cause other than that of vascular origin. The syndrome varies in severity from recovery in a day, through incomplete recovery, to severe disability, to death [1]. Estimates from Indian Council of Medical Research (ICMR) indicate that there were 9,30,985 cases of stroke in India with 6,39,455 deaths and 6.4 million Disability Adjusted Life Years (DALY) lost in 2004 [2]. By the year 2020, stroke and coronary artery disease together are expected to be the leading causes of lost healthy life years [3]. Hyperhomocysteinemia, has been identified as being associated with vascular disease, including cerebrovascular disease [4,5]. Many casecontrol and cohort studies have identified a strong, independent and dose-related association between moderately elevated homocysteine and atherosclerotic vascular disease, including stroke [6-8]. The pathologic process may be considered in terms of the more basic or primary disorder, i.e. atherosclerosis, hypertensive arteriosclerotic change, arteritis, aneurysmal dilation, and developmental malformation [9]. Older age, family history of thrombotic stroke, diabetes melli-tus, hypertension, tobacco smoking, abnormal blood cholesterol [particularly, low high-density lipoprotein (HDL) and/or high low-density lipopro-tein (LDL)], and other factors are either proven or probable risk factors for ischemic stroke [10].

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Aims of our study was to evaluate the hyper homocysteinemia and dyslipidemia as a risk factors associated with patients of ischemic stroke.

Materials & Methods: -

A total of 100 subjects with ischemic stroke (60: males, 40: females) were enrolled in this study. Data was collected in the department of Medicine, Katihar Medical College, Katihar during a period from January 2017 to December 2017. Attendants of entire subjects signed an inform consent approved by institutional ethical committee was sought. Laboratory investigations were performed in the department of Biochemistry, Katihar Medical College, Katihar. Bihar, India.

Methods: -

Data was selected by using the random sampling methods**.** A detail history, clinical examinations and relevant investigations were performed to all cases.

Inclusion Criteria: -

A hundred patients between age 20 to >60 years of age and verified by CT scan/ MRI brain were included in the study. Diagnosis of ischemic stroke was strictly verified within 48 hours.

Exclusion Criteria: -

Stroke patients with aetiology other than thrombotic episodes confirmed either by clinical examination or by investigations, history of syncopal attacks, infective or metastatic disorders, other systemic disorders were excluded from this study.

Procedures:-

All patients were examined by a neurologist and they had performed Cranial Tomography (CT) or Magnetic Resonance Imaging (MRI). Clinical information including age, sex, history or current evidence of Hypertension (HTN) [systolic blood pressure (SBP): 150mmHg and diastolic BP: 90mmHg], Diabetes Mellitus (DM) [fasting blood glucose 3.5-5.5 mmol/L], cardiac disease, were recorded for all subjects.

The lipid profile of the study sample was analyzed according to the ATP III classification for identification of dyslipidemia. Dyslipidemia (LDL >130 mg/dl, TC>200 mg/dl, HDL<40 mg/dl) as per ATP III guidelines was presented in all patients. Homocystein level: EDTA samples were collected and transported in ice packs. Samples centrifuged immediately, refrigerated and properly thawed before estimation. Homocysteine enzyme immunoassay was done by ELISA (Transasia). Measuring range of calibration is from 2-50 µmol/L.

Statistical Analysis: -

Data was analyzed by the use of simple statistical methods with the help of MS-Office software.

Observations: -

In this study, we were selected a total of 100 patients (60: males, 40: females) with age group of 20 to greater than 60 years. Male and female ratio was 3:2.

Table 1:- Age group of patients with ischaemic stroke

Age group (yrs)	*No. of patients*	*Percentage*
20-40	*15*	*15%*
41-60	*55*	*55%*
>60	*30*	*30%*

In this study, majority of cases 55(55%) were in age group of 41-60 years. 30(30%) cases were in age group of >60 years. And 15(15%) cases with ischemic stroke were in age group of 20-40 years.

Table 2: -Baseline risk factors of patients with ischemic stroke (N=100)

Risk factors	*No. of cases*	*Percentage*
Hypertension	*45*	*45%*
Ischemic heart disease	*15*	*15%*
Valvular heart disease	*5*	*5%*
Diabetes mellitus	*35*	*35%*
Total	*100*	*100%*

In this study, majority of patients 45(45%) of ischemic stroke had baseline risk factors was hypertension. 35(35%) cases had diabetes mellitus, 15(15%) patients had ischemic heart disease. And 5(5%) patients had valvular heart disease.

Table 3: - Distribution of risk factors of patients with ischemic stroke

Risk factors	*No. of cases*	*Percentage*
Hyper homocysteinemia	*55*	*55%*
Dyslipidemia	*45*	*45%*
Total	*100*	*100%*

In this study, majority of patients 55(55%) with ischemic stroke hyperhomocysteinemia. And 45(45%) had dyslipidemia.

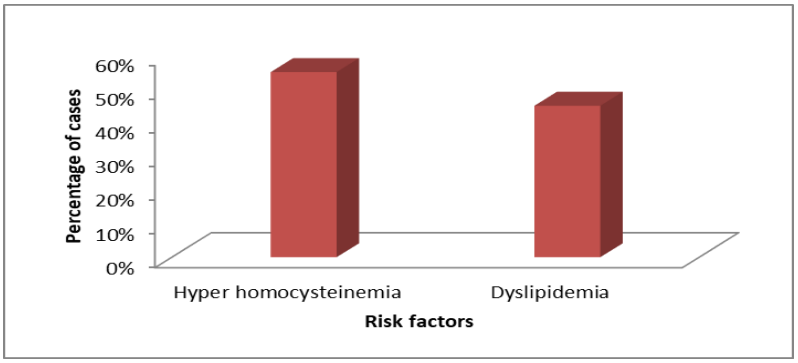

Figure.1. Risk factors associate with patients with ischemic stroke

Table 4:- Homocysteine level of patients with ischemic stroke (N=55)

Homocystein level(µmol/L)	*No. of patients*	*Percentage*
>20	*38*	*69.09%*
15-20	*12*	*21.81%*
10-15	*5*	*9.09%*
Total	*55*	*100%*

In this study, Out of 55 ischemic stroke patients, majority of patients with ischemic stroke 38(69.09%) had hyperhomocysteinemia (>20µmol/L). 12(21.81%) cases had moderate hyperhomocysteinemia and 5(9.09%) cases had mild hyperhomocysteinemia.

Table 5:- Dyslipidemia of patients with ischemic stroke (N=45)

Dyslipidemia	*No. of patients*	*Percentage*
Elevated total cholesterol	*25*	*55.55%*
Elevated triglyceride	*3*	*6.66%*
Elevated LDL	*2*	*4.44%*
Low HDL	*11*	*24.44%*
High total cholesterol & low LDL	*4*	*8.88%*
Total	*45*	*100%*

In this study, out of 45 dyslipidemic patients, majority of cases 25(55.55%) had elevated total cholesterol. 11(24.44%) had low HDL. 4(8.88%) cases had high total cholesterol and low LDL. 3(6.66%) had elevated triglyceride. And 2(4.44%) cases had elevated LDL.

Discussion: -

Stroke is also a leading cause of morbidity, with 20% of survivors requiring institutional care after 3 months and 15-30% remaining permanently disabled [11]. Of all strokes, 88% are classified as ischemic, and the remainder 12% comprise of hemorrhagic, either subarachnoid (9%) or intracerebral (3%) [12].

In this present study, we were evaluated the risk factors of ischemic stroke. This study was conducted in department of Biochemistry, Katihar Medical College, Katihar, Bihar, India. In this present study we were seen that majority of cases 55(55%) with ischemic stroke were age 41-60 years. Mid age group cases were commonly affected with ischemic stroke. Male and female ration was 3:2. Male were more affected with ischemic stroke than female. Our study supported the finding of study of Cynthia A, et al (2014). Cynthia A, et al (2014) were studied on cases with ischemic stroke and they found that 66% male and 34% female were suffered with ischemic stroke [13].

In this present study, major baseline risk factors of cases with ischemic stroke were hypertension 45(45%), diabetes mellitus 35(35%). Cynthia A, et al (2014) supported the findings of our study. In their study, major risk factors was hypertension (55%) and diabetes mellitus (30%) [13].

In our present, hyperhomocysteinemia was the major risk factors of ischemic stroke. 55(55%) patients of ischemic stroke had hyperhomocystenemia.

Classic homocysteinaemia is due to deficiency of the enzyme cystathionine β-synthase. Other causes of elevated levels of homocysteine are N5, N10-methylene THFA reductase (MTFR) deficiency and deficiency of cobalamide (vitamin B12) coenzyme synthesis. Severe hyperhomocysteinaemia (levels >100 µmol/L) has been related to early onset of arteriosclerosis and thromboembolic events including stroke and moderate hyperhomocysteinaemia (20-100 µmol/L) has been suggested to be a vascular risk factor. Many case control and cohort studies have identified a strong, independent and dose-related association between

moderately elevated homocysteine and atherosclerotic vascular disease including stroke [14-16]. The possible mechanism maybe the increasing homocysteine level caused neurotoxicity, endothelial dysfunction and other associated prothrombotic factors, which is reported to be an independent predictor of poor outcome in patients with ischaemic stroke [17,18].

In our study, out of 55 cases of ischemic stroke with hyperhomocysteinemia, majority of cases 38(69.09%) cases had homocysrtein level > 20 µmol/L, 12(21.81%) cases had homocystein level between 15-20 µmol/L.

In 1999, a prospective study done by Bots et al [19] in a community with 7983 cases of atherosclerotic diseases, including strokes have found a positive association with high homocysteine values. These patients were observed under four years of follow up.

Although, severe hyperhomocysteinaemia is clearly related to atherosclerosis, it is less clear whether mild-to-moderate elevation in plasma homocysteine level is a risk factor for cardiovascular disease and death. Kark and colleagues [20] followed a cohort of 1,788 men and women aged 50 years or older for a decade. Compared with the lowest levels of plasma total homocysteine (less than 8.5 micromole/l), there was a significant increasing risk for death from any cause for patients with high homocysteine levels. An extensive and statistically rigorous meta-analysis by Barbara Voetsch, et al [21] in 2002 found that hyperhomocysteinaemia is associated with atherothrombotic disease and venous thrombosis. This study also showed that even mild elevations of homocysteine increase ischaemic stroke risk.

Even though many studies are in favour of hyperhomocysteinaemia as an independent risk factor in ischaemic stroke, few studies suggest that elevation of homocysteine after ischaemic stroke is due to the disease process itself. Study done by D. J. Meikeljohn et al in 2001 showed that homocysteine concentrations are not elevated after recent atherothrombotic stroke, but rise in the convalescent period [22] and suggested that an increase in methylation reactions after tissue injury results in conversion of methionine to S-Adenosyl homocysteine, which leads to generation of homocysteine [23].

Dyslipidemia is a primary major risk factor for CAD and ischemic stroke [24]. It causes insulin resistance which results in increased levels of plasma triglyc-erides and LDL-c and a decreased concentration of HDL-c, as an important risk factor for peripheral vascular disease [25], stroke, and CAD [26,27]. Serum HDL-c has anti-atherogenic properties with ability to trigger the flux of cholesterol from peripheral cells to the liver and thus having a protective effect [28]. Lipid-modifying therapy with statins has de-finitively established that reduction of LDL-c re-duces cardiovascular risk. Statins benefit stroke survivors as well. Lipid lowering agents may slow progression of atherosclerotic plaque growth and may possibly cause a regression in plaque formation [29].

In our present study, dyslipidemia was the second major risk factor of ischemic stroke 45(45%) cases with ischemic stroke had dyslipidemia. Out of 45 patients of ischemic stroke with dyslipidemia, elevated total cholesterol 25(55.55%) was the major causes of dyslipidemia in ischemic stroke patients. And others were 11(24.44%) low HDL, 4(8.88%) high total cholesterol and low LDL, 3(6.66%) had elevated triglyceride, 2(4.44%) elevated LDL.

Ischemic stroke, which is the most commonly occuring cerebrovascular accident, is mostly due to thromboembolism secondary to atherosclerosis in the major arteries. Nikolai first proposed a link between cholesterol and atherosclerosis in 1912. In a research, oft cited as a breakthrough research in atherosclerosis in the 20th century, Nikolai and his team proved the obstructive pathophysiology in atherosclerosis occurs as a result of increased cholesterol levels.[30] However, though there exist several risk factors for cerebrovascular disease, atherosclerosis is recognized as one of the leading causes of brain ischemia [13].

Tian et al. 2014 found that cholesterol total, LDL, HDL related with the incidence of acute ischaemic stroke [31]. Kurth et al. 2007 and Shahar et al. 2003 reported that cholestreol total, LDL, HDL related with risk factor of ischaemic stroke [32.33]. Shahar et al. 2003 and Bowman et al. 2003, reported a decreased relation between lipid and stroke [33,34].

Conclusion: -

Our study concluded that middle age group peoples (41-60 years) were greatly affected with ischemic stroke. Males were more affected than females. Hypertension and diabetes mellitus were the major baseline risk factors. Hyper homocysteinemia was the major risk factor of patients with ischemic stroke. And second major risk factor was the dyslipidemia. Dyslipidemic patients of ischaemic stroke had more increased total cholesterol level than TG, LDL and HDL.

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Study on Lipid Profile of Obese Female Hypothyroid Patients: A Hospital based prospective study

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Abstract

Objectives: This present study was to evaluate the lipid profile of hypothyroid obese female patients receiving levothyroxine.

Methods: A detail history, clinical examination and relevant investigation were performed to all cases receiving thyroxine. Fasting venous blood samples were collected, centrifuged promptly, and separated sera was stored at -20˚C. TSH, FT3, FT4 measured by ELISA Method using BeneSphera manufactured by Avantor Performance Material India Limited and TC, TG and LDL were measured using SEIMENS kits by fully automated biochemistry anayser CPC 240.

Results: Data was analyzed by using latest version SPSS software. Mean ± S.D. was observed. Unpaired t – test was applied. T value and P value were observed. P value was considered less than or equal to 0.05 ($p \leq 0.05$) for significant differences.

Conclusions: Hypothyroid obese women had mild increased BMI, But, greater increase of TSH level with respect to euthyriod women. FT3 and FT4 were markedly reduced in hypothyroid women. Total cholesterol, triglyceride and LDL were greatly increased in hypothyroid women with respect to euthyroid women. Thus, it proves that levothyroxine therapy is beneficial for the normalisation thyroid as well as lipid parameter.

Keywords: Obese female, euthyroid, hypothyroid, levothyroxine, lipid profile.

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INTRODUCTION

Hypothyroidism occurs at a much higher frequency than widely recognised. The National Health and Nutrition Examination Survey (NHANES III) survey concluded from their data base of 17,353 patients that 9.2% had "clinically significant thyroid disease" based on biochemical criteria [1]. That number included both hypothyroid and hyperthyroid patients; however hyperthyroid patients frequently end up hypothyroid after ablative therapy. In addition, in the large scale Colorado Thyroid Disease Study 9.5% of results exceeded the upper range limit for TSH [2]. Within those percentages of biochemically hypothyroid patients there will be some that are not clinically hypothyroid. By the same token, some of the people having TSH within range will be clinically hypothyroid.

Further evidence of the magnitude of thyroid problems comes from an ATA estimate that "20 million Americans have some form of thyroid disease" and that "up to 60% are unaware of their condition" [3]. It was also noted that "women are 5 to 8 times more likely than men to have thyroid problems" [3].

In a survey from 110 countries (mostly in developing countries), hyperthyroidism and hypothyroidism were considered responsible for the morbidity at large [4]. TSH regulates the synthesis and secretion of the thyroid hormone through the hypothalamic-pituitary-thyroid axis [5] and is considered the primary indicator to assess thyroid function [6]. Currently, thyrotropin (TSH), free thyroxine (FT4), or FT4 combined with total triiodothyronine (TT3) is recommended for use as indicators in laboratory testing to assess thyroid function clinically (e.g., in the guidelines of the American Thyroid Association (ATA)) [7].

Several scientific studies have shown that reference ranges based on the specific thyroid test results of individuals were approximately half those of population ranges. This applies to all thyroid tests [8,9]. So, trying to identify abnormality by comparing an individual's TSH, FT4, or FT3 test result to the much wider reference range for the entire population can be very misleading [10] Furthermore, the current upper range limit for TSH, calculated from group data, has been purposely set even higher than would be expected from the normal distribution, in order to avoid excessive false positive diagnoses, and instead may result in excessive false negative diagnoses[10, 11].

Disorders of the metabolism of lipoproteins, including lipoprotein overproduction and deficiency are classified as dyslipidemia. These may manifest in one or more

of the following ways, a raised total cholesterol (CH) levels, a raised low density lipoprotein (LDL)cholesterol levels, a raised triglyceride (TG) levels and a decreased high density lipoprotein (HDL)cholesterol levels. Lipoproteins are a family of lipid carrying, water soluble proteins includingchylomicrons (CM), high, intermediate, low and very low density lipoproteins (HDL, IDL, LDL,VLDL) which are responsible for the transport of cholesterol (CH), cholesterol esters (CE), phospholipids and triglycerides throughout the Circulation.[12]

Aim of our study was to evaluate the biochemical profile of obese women with

hypothyroidisms who were received levothyroxine.

MATERIAL AND METHODS

This present study was conducted in department of Biochemistry, Katihar Medical College and Hospital, Katihar, Bihar, India during a period from January 2018 to July 2018.

Entire subjects signed an informed consent approved by institutional ethical committee of Katihar Medical College, Katihar, Bihar was sought.

A total of 60 obese women patients (30: euthyroidism, 30 : hypothyroidism) with age group 40 to 50 years were enrolled in this study. All the hypothyroidism patients were receiving levothyroxine treatment. Patients who were BMI < 30 kg/m^2 or not receiving thyroxine preparation or history of alcohol abuse, smokers, patients receiving drugs such as oestrogens, diuretics and beta-blockers, patients with familial or secondary dyslipidaemia, diabetic mellitus and renal, hepatic or other systemic diseases were excluded.

METHODS

A detail history, clinical examinations and relevant investigations were performed to all cases. Impaired thyroid function was recorded.

Procedures

Fasting venous blood samples were collected, centrifuged promptly, and separated sera was stored at -20˚C. TSH, FT3, FT4 measured by ELISA method using BeneSphera manufactured by Avantor Performance meterial India limited and TC, TG and LDL were measured using SEIMENS kits by fully automated biochemistry analyser CPC 240. Euthyroid subjects were defined as those having normal serum free T4 and TSH levels.

STATISTICAL ANALYSIS

Data was analyzed by using latest version SPSS software. Mean ± S.D. was observed. Unpaired t – test was applied. T value and P value were observed. P value was considered less than or equal to 0.05 ($p \leq 0.05$)for significant differences.

OBSERVATIONS

Mean age of euthyroid women was 44.9 and hypothyroid women was 46.06. When mean ± standard deviation (S.D) of euthyroid subjects were compared with mean ± S.D of hypothyroid subjects. P value was found to be 0.0885. Which is greater than 0.05. That was non significant.

Mean ± S.D of BMI of euthyroid subjects and hypothyroid subjects was compared. P value was found to be less than 0.05. It was significantly differences.

Table.1. Comparison of all parameters of euthyroid and hypothyroid obese females.

Parameter	**Euthyroid (N=30)**	**Hypothyroid (N=30)**	**T - value**	**P – value**
Age (years)	44.9±2.468	46.06±2.741	1.732	0.0885(Non significant)
BMI (Kg/m^2)	34.44±0.666	34.93±0.85	2.484	0.015 (Significant)
TSH (Mu/L)	2.92±0.466	6.01±0.947	16.009	< 0.0001 (extremely significant)
FT4 (ng/dl)	3.19±0.133	1.55±0.083	56.852	<0.0001 (extremely significant)
FT3 (ng/dl)	3.49±0.131	2.47±0.277	18.143	<0.0001 (extremely significant)
Total cholesterol (mg/dL)	224.72±32.668	249.4±24.831	3.294	0.0017 (very significant)
Triglyceride (mg/dL)	122.66±37.882	201.02±34.000	8.432	<0.0001 (extremely significant)
LDL (mg/dL)	94.63±10.760	121.46±17.694	7.097	<0.0001 (extremely significant)

Similarly, when mean ± S.D of TSH was compared between euthyroid and hypothyroid subjects. Data was extremely significant ($p< 0.0001$). It was showed that TSH level of hypothyroid women were greatly increased.

There was extremely significant differences ($p < 0.0001$) seen in between FT4 level of euthyroid and hypothyroid subject. It was showed that FT4 level was greatly reduced in hyptothyroid subject who were continuously received levothyroxine.

Similarly, FT3 level was extremely significant decreased in hypothyroid subjects with respect to euthyroid subjects ($p < 0.0001$).

There was a very significant difference seen between total cholesterol of euthyroid and hypothyroid subjects ($p< 0.0017$). Total cholesterol level was greatly increased in hypothyroid subjects.

Mean of triglyceride level was greatly increased in hypothyroid women. It was extremely significant differences ($p< 0.0001$) between euthyroid and hypothyroid women.

Similarly, mean LDL level was greatly increased in hypothyroid women. P value was found to be less than 0.0001. It was extremely significant differences.

DISCUSSIONS

TSH is considered the most important indicator for the evaluation of thyroid function [6]. FT3 and FT4 are the active biological state in plasma, and therefore, FT3 and FT4 are considered to be sensitive and meaningful indicators for the diagnosis of thyroid disease. [18]

Hypothyroidism accounts for about 2% of all casesof hyperlipidemia, and is second only to diabetesmellitus as a cause of secondary hyperlipidemia[13].Various other studies**[14]**have also reported thatdyslipidemia is commonly associated withhypothyroidism. Jung et al [**15]**and Duntas **[16]**have observed higher levels of total cholesterol andLDL-cholesterol in both subclinical and overt hypothyroidism. The effect of hypothyroidism inlipid metabolism is more marked in patients withhigher serum TSH levels i.e. patient with overthypothyroidism and observed significant correlation between raised TSH levels and serum total cholesterol and LDL cholesterol.**[17]**

In this present study, mean age of euthyroid women and hypothyroid women were 44.9 and 46.06 years respectively. All the cases were belonged in age 40 to 50 years.

AmitSaxena, et al. conducted a study on dyslipidemia of patients with hypothyroid and observed that maximum cases belonged to the age group of 21-30 years and minimum number of patients was in the 61-70 years.[12]

In our present study, all cases were continuously taking levothyroxine, TSH level was significantly increased ($p<0.0001$) in cases with hypothyroid with respect to euthyroid cases. BMI was mildly significant ($p=0.015$) increase in hypothyroid cases.FT4 and FT3 level was extremely significant decreased ($p<0.0001$) in hypothyroid cases than euthyroid cases.

Hongi Li, et al. (2014) were studied on among the 500 patients diagnosed with hyperthyroidism, 500 patients diagnosed with hypothyroidism, and 1,673 healthy persons, we analysed the Pearson correlations for serums TT3, TT4, FT3, and FT4 with TSH. FT4 is found to be associated with TSH at the maximum level in healthy people. The correlation between TT4 and TSH in patients diagnosed with hyperthyroidism or hypothyroidism is the maximum, which suggests that FT4 and TSH are the most valuable indicators to consider in a healthy population, and TT4 and TSH are the most valuable indicators to consider in patients with hyperthyroidism or hypothyroidism.[18]

The role of serum T3 is limited, either TT3 or FT3, because they are generally normal in patients diagnosed with hypothyroidism. This is mainly due to the increased TSH and the functional role generated by the increased conversion of type 2 iodinated thyroninedeiodinase to residual thyroid tissue. Because 80% of T3 comes from deiodination of T4, the T4 levels increase and in theory T3 levels should also increase concomitantly. However, in patients diagnosed with hypothyroidism who are treated long-term with levothyroxine (L-T4), serum T3 is usually maintained at a stable level, which suggests that energy metabolism is changed by a T4 dependent pathway [19]. Therefore, T4 is more reliable than T3 in assessing thyroid function in patients with

hypothyroidism. From Castellano's study, the analyses of five serum indicators, including TT4, TT3, FT4, FT3, and TSH, in patients with hypothyroidism showed that the correlation between TT4, FT4, and TSH was closer than the correlation of TT3, FT3, and TSH with

TSHwhich was the most important indicator in the diagnosis of hypothyroidism [6]. Thus, it can be interpreted that T4 is more suitable than T3 in assessing thyroid function in hypothyroidism patients. In a case report, a 59-year-old woman diagnosed with hypothyroidism was treated with LT4,and TSH concentrations expectedly fell three months later. However, after stopping L-T4 treatment, TSH level increased quickly but with a still higher FT4 and FT3 status[20].

In this present study, total cholesterol level was statistically very significant increased in hypothyroid cases with respect to euthyroid ($p<0.0017$). Triglyceride level and LDL were statistically extremely significant increased in hypothyroid women with respect to euthyroidwomen($p<0.0001$).

Hypothyroidism leads to atherogenic lipid abnormalities as well as a number of other cardiovascular risk factors. Levothyroxine treatment may reduce serum cholesterol and thereby decrease the incidence of coronary artery disease, stroke, and peripheral vascular disease. Various other authors (Monzani et al, Akbar et al)[21,22] also reported significant reduction in the levels of lipid parameters following levothyroxine replacement therapy, thus supporting our results.

Our findings was also supported the findings of Asranna, et al, [23] who were observed a mild increase in HDL from mean pretreatment levels of 41.14 to 43.43 mg/dl after replacement therapy with levothyroxine.

CONCLUSIONS

This present study concluded that the obese women cases who were receiving levothyroxine, had mild increased BMI, But, greater increase of TSH level with respect to euthyriod women. FT3 and FT4 were markedly reduced in hypothyroid women. Total cholesterol, triglyceride and LDL were greatly increased in hypothyroid women with respect to euthyroid women. Thus, it proves that levothyroxine therapy is beneficial for the normalisation thyroid as well as lipid parameter.

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Diabetes Accelerates Age- Related Lipid Profile Disturbances in Cardiovascular Complications

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Abstract

Cardio Vascular Complications (CVC) are predominant problems both in ageing and diabetes. The present study focuses on the effect of human ageing and diabetes, with special reference to serum lipid profile levels during cardiovascular complications in two age groups namely, 45±5 years and 65±5 years independent of obesity hypertension and nephropathy. The serum samples of study and control group includes estimation of serum total cholesterol, serum triglycerides, HDL-C, LDL-C and VLDL-C. Results shows, that the changes in the levels of lipid parameters follow a near similar trend in the two non-diabetic age groups with cardiovascular complications. Except the HDL-C which decline, total cholesterol, LDL-C, VLDL and triglycerides increases.

In diabetes, however, the said lipid parameters are not so similarly affected in the stated age subjects. One interesting observation is the near similar levels of lipid parameters found in diabetic 45±5 y and non-diabetic 65±5 y age groups with Cardiovascular complications. It is, therefore, suggested that diabetes accelerates age related disturbances in the lipid profile.

Key Words: Diabetes, Ageing, Lipid profile and CVC

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Introduction

Cardiovascular complications are predominant problems both in ageing and diabetes. The risk factors continue to predict Cardiovascular disease in older individuals[1-4] including lipoprotein abnormalities & diabetes along with other complications.

There is ample evidence that total Cholesterol, LDL, cholesterol and HDL – Cholesterol levels are all univariant predictors of risk of coronary disease in both men and women over 65 years of age.[1-6] HDL in particular is an important predictor of risk[7,8], because HDL & triglycerides levels are strongly and inversely, related it would not be surprising that triglycerides continue to be predictors of coronary risk in older individuals.

Patients over 65 years of age with established coronary diseases represent a group of particular interest. Elevated Cholesterol levels substantially increase the risk of recurrent myocardial infarction (MI) or death in such men and women.[6] In the Framingham study, Cholesterol levels over 275 mg/dl (7.1.mmol/L) were associated with a fourfold increase in risk of recurrent infarction or in coronary death, and almost a threefold increase in risk from all-cause mortality compared with Cholesterol levels less than 200 mg/dl.

Lipoproteins are altered in diabetes. The quantitative changes most commonly seen are an increase in the TG-rich lipoproteins and a decrease in HDL.[9-12] These changes can be seen at and even before the diagnosis of diabetes.

Despite the relative lack of attention to diabetes mellitus in the literature on geriatrics, this is the third most prevalent life threatening disease among older people after atherosclerosis and cancer. Approximately 20% of those over age 80 are diabetic[18] and there is 17% prevalence of "mild and easy to treat" diabetes among Finns over age 85.[20] According to the International Diabetes Federation bulletin (2000), India had nearly 33 million diabetic constituting around 1-2% of the total population. According to WHO estimates, India would have 75 million diabetics by 2020.

Due to the ageing of the global population, the prevalence of diabetes and the combination of diabetes and advanced age is expected to increase considerably. Diabetes appears to be an important risk factor for significant cognitive decline and dementia in the elderly. The challenge for the next decades will be to unravel the complex interaction between the mechanisms of ageing and diabetes.[19]

The present study focuses on the effects of human ageing and diabetes, with special reference to serum level lipid profile during CVC in two age groups namely, 45±5 years and 65±5 years, independent of

obesity hypertension and nephropathy. The parameters include estimation of serum total cholesterol; HDL – C, LDL – C, VLDL and triglycerides.

Research Design and Methods

The studies were carried out in human subjects at Katihar Medical College & Hospital, Katihar. Individuals consent and management permission were duly obtained.

Types of Subjects

The subjects selected for this study were non-obese, non-hypertensive & free from nephropathy. The entire subjects were divided into seven groups according to their age and patho-physiological state as shown in the Table below.

No. of Subjects: Minimum 15 in each categories.

S. No	Age group & Patho-physiological state.	Fasting Plasma glucose level
1	25±5 y – Non-diabetic, without CVC	60 - 100 mg/dl
2	45±5 y – Non-diabetic without CVC	70 - 100 mg/dl
3	45±5 y – Non – diabetic with CVC	70 - 100 mg / dl
4	45±5 y – Diabetic with CVC	150 - 220 mg / dl
5	65±5 – Non-diabetic without CVC	70 - 110 mg/dl
6	65±5 – Non – diabetic with CVC	70 - 110 mg / dl
7	65±5 – Diabetic with CVC	140 - 217 mg / dl

Methods

Serum total Cholesterol, HDL, Cholesterol & Triglycerides were estimated by enzymatic method. The VLDL was determined simply from the Triglycerides divided by five & LDL was determined by using Frieldewald equation. *LDL – C = S-total Cholesterol – HDL + VLDL*

Estimation of Total Cholesterol: The free Cholesterol produced by hydrolysis of Cholesteryl ester & preexisting free Cholesterol are oxidized by Cholesterol oxidase to liberate H_2O_2, which reacts with 4 – amino antipyrine (4AAP) to form a quinoneimine, a red color compound which is read at 510 nm.

HDL – Cholesterol – Estimation: In the presence of Phosphotungstate and divalent Cation i.e. magnesium LDL, VLDL, and Chylomicrons are precipitated. After centrifugation the HDL Cholesterol remains present in the supernatant and it is estimated by using Cholesterol reagent.

Triglycerides Estimation: Triglycerides is hydrolyzed by lipase into glycerol & free fatty acid. Glycerol reacts with ATP in presence of Glycerol kinase to form Glycerol 3 phosphate which further reacts with O2 catalyzed by Glycerol Oxidase to form dihydroxy acetone phosphate & H_2O_2. This H_2O_2 again reacts with 4 – aminoantipyrine in the presence of 3, 5 – dichloro – 2 – hydroxybenzene Sulfonate Catalyzed by peroxidase to form a pink Coloured quinoneimine dye. The absorbance is recorded in green filter.

Results

Results show that the change in the levels of lipid parameters follow a near similar trend in the two non-diabetic age groups with Cardiovascular complications as shown in the Table 1. The data shown in the table are the mean value of all 15 subjects with ±S.E.M. Except the HDL-C decline, total Cholesterol, LDL – C, VLDL and triglycerides increase with age.

Among non-diabetic middle aged subjects the level of cholesterol was found 203.5±3.37 which was further increased to 237±5.85in same age group with CVC. In old age non-diabetic people it was 224±3.72, which further increased to 260.73±4.79. Although the value of cholesterol among non-diabetic, middle aged and old aged people was within normal limit, but when it were compared with their younger group the increased was significant. Similarly the LDL-C, VLDL-C and triglycerides also increased significantly with age when compared to there younger counterpart. The value were further increased among the subjects with CVC in comparison to the subjects without CVC of same age group. Level of HDL-C was found in decreasing order in similar fashion.

Among the diabetic subjects with CVC of both age group the level of cholesterol, LDL-C, VLDL-C and triglycerides was very much higher than non-diabetic subjects of same age group without CVC. Although these levels was higher among diabetic subjects of 65±5 y age groups to the diabetic subjects of 45±5 y group, but difference was not very high. But the level of HDL-C was in decreasing order.

However, among diabetic the said lipid parameters are not so similarly affected in the stated age subjects. One interesting observation is the near similar levels of lipid parameters found in diabetic 45±5 y and non-diabetic 65±5 y age groups with Cardiovascular complications.

Table 1: Lipid profile in different age group with and without cardiovascular complication in diabetic and non-diabetic subjects

Age (in years)	25±5	45±5			65±5		
Physiological State	**Non-diabetic without CVC**	**Non-diabetic without CVC**	**Non-diabetic with CVC**	**Diabetic with CVC**	**Non-diabetic without CVC**	**Non-diabetic without CVC**	**Diabetic with CVC**
Serum total cholesterol (mg/dl)	174.46±6.90	203.5±3.37	237.8±5.85	271.6±3.74	224.0±3.72	260.73±4.79	281.53±8.63
S. HDL-C (mg/dl)	60.53±1.34	56.7±0.79	48.26±1.02	47.26±0.93	56.53±1.08	49.8±0.71	46.53±0.79
S. LDL-C(mg/dl)	89.86±5.43	113.4±1.85	143.13±5.98	170.2±3.08	122.73±2.79	157.4±5.05	179.53±7.89
S. VLDL-C (mg/dl)	24.73±1.09	33.4±1.22	45.73±0.95	50.6±1.39	43.8±1.0	52.46±2.12	54.13±1.40
S. Triglycerides (mg/dl)	122.33±5.36	162.0±6.13	228.73±4.79	253.0±6.98	219.0±5.02	266.06±10.38	270.66±6.67

All the values in the table are mean±S.E.M.

CVC – Cardiovascular complication.

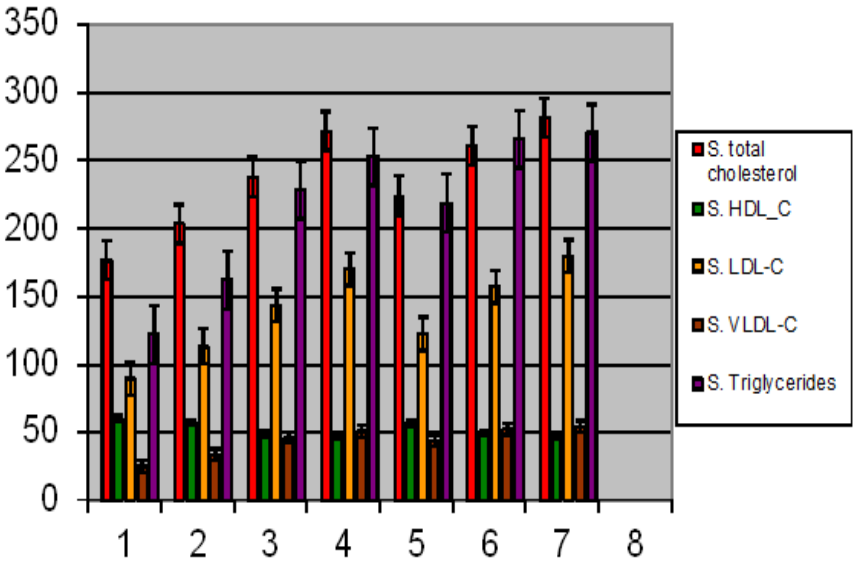

Fig. Shows alteration of lipid profile in ageing & diabtes with & without CVC

Discussion

It is well known that the disorders of lipoprotein metabolism result from abnormal synthesis, processing or Catabolism of Plasma lipoprotein particles. More than half of patients with angiographically confirmed coronary heart disease before age 60 years have a familial lipoprotein disorders. This association is most striking – among younger patients and declines with increasing age at first myocardial infarction. This suggests the presence of genetic factors that accelerate age – associated Cardiovascular changes seen in the general population. Four types of lipoprotein abnormalities are observed: elevated LDL – Cholesterol; reduced HDL Cholesterol, increased triglycerides and VLDL – Cholesterol.

The metabolic syndrome i.e. the clustering of high serum triglyceride, small dense LDL-particles, low serum HDL-cholesterol levels, hypertension, insulin resistance and a prothrombotic state, is an important risk factor for the development of CVD in older persons including very old people.

Cholesterol levels over 240 mg / dl are associated with a three fold increased risk of death from ischaemic heart disease in men relative to cholesterol levels below 200 mg/dl, and there is a continuous risk gradient as the

cholesterol rises. Elevated total Cholesterol primarily reflects elevated LDL Cholesterol which constitutes 70 percent of serum Cholesterol. Low HDL levels, usually accompanied by elevated plasma triglyceride levels represent the most common dyslipidemia associated with CHD.

There is an overall inverse relationship between serum triglycerides & HDL Cholesterol levels in the general population. This relationship primarily arises from the fact that high serum triglycerides stimulate Cholesterol ester transfer from HDL to triglyceride rich lipo-proteins an effect mediated by plasma C E transfer protein.

Effect of Diabetes on lipoproteins: The commonest abnormality in diabetes is hypertriglyceridemia due to an excess of very low density lipoprotein.(VLDL)[9]. Lipoprotein lipase depends for its full activity on insulin, and VLDL clearance is reduced in poorly controlled patients with IDDM. In NIDDM patients, there is also overproduction of VLDL & apoprotein (apoB). Insulin deficiency or resistance increases production of non-esterified fatty acids from adipose tissue by the action of hormone sensitive lipase and these provide a substrate for hepatic triglyceride synthesis Hypertriglyceridemia in diabetes, therefore, usually responds to intensified insulin treatment.

LDL levels are also raised in association with poor glycemic control, but a substantial improvement in blood glucose is required to lower LDL.[14] Insulin stimulates LDL receptor activity[15], increasing LDL clearance, while non enzymatic glycosylation of apo B reduces its affinity for the receptor, thereby, slowing down LDL removal.[16] HDL levels vary inversely with VLDL. Since reduced lipoprotein lipase activity impairs Catabolism of VLDL and hence transfer of lipids and apoproteins to HDL. In NIDDM, HDL levels are low especially in association with hyper triglyceridemia, whereas in IDDM the levels are normal. Glycosylation of HDL occurs in vivo and in animal studies glycosylated HDL is removed faster from the circulation.[17]

Shared mechanism of Pathogenesis of CVC during diabetes and ageing: Both ageing and diabetes are associated with a loss in function of cardiovascular system and increased CVC risk factor. Epidemiological data from the Multiple Risk Factor indicate that the risk for the cardiovascular death is increased two to three fold in type-2 diabetic individuals. Moreover, after a first myocardial infarction, cardiovascular morbidity and mortality are increased in patients with diabetes compared with non-diabetic patients.[21] The reason why the diabetic patients have such a high risk for CVC are probably multifactorial and include dyslipidemia, hypertension, inflammation, oxidative stress and accumulation of AGEs. Typically, patients with type-2 diabetes are characterized by hyper triglyceridemia and low HDL-cholesterol levels. Diabetic patients also exhibit alteration in post prandial lipid transport.[22]

One commonly reported problems of CVC in both ageing and diabetes is a reduction in the ability of the peripheral vasculature to vasodilate.[23] A similar mechanism might be involved in reducing vascular reactivity with age and diabetes.

The mechanism for reduced resting and post ischaemic blood flow with both ageing and diabetes has been linked to a reduction of the vascular endothelial cells ability to produce nitric oxide, a potent vasodilator substance.[24] This may be due to a defect in nitric oxide synthesis, decreased nitric oxide sensitivity or reduced availability of L-arginine, the precursor of nitric oxide. Ageing and diabetes effect endothelial function by a similar mechanism, so that it might be inferred that diabetes potentiated the loss in endothelial function with age.[25]

Non-enzymatic protein glycation is also a common feature in both ageing and diabetes. Glucose irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs) as a function of glucose concentration and time.[26,28] The formation of AGEs is also associated with the increased production of ROS[29], thus, linking the pathophysiological model of non-enzymatic glycation to oxidative stress. In addition, increased circulating levels of AGEs and glycation of basement membranes of vessel walls may affect vascular function, as endothelial oxidative damage and endothelial dysfunction have been observed in the presence of AGEs.[30] Furthermore, AGEs may be responsible for quenching of the vasodilating compound and nitric oxide.[27] In tissues affected by diabetic complications the amounts of AGEs are generally increased, leading to structural changes in the extracellular matrix, as well as to modifications to cell membranes and intracellular components.[26,28]

The important correlation observed in this study, which lies in the serum lipid and lipoprotein components among elderly and diabetics is, the changes which observed with non-diabetic elderly at the age of 60-65 years with CVC, match to those found among diabetics at the age of 40-45 years with CVC. Serum total cholesterol increases with age, CVC and diabetes. Interestingly, S-total cholesterol among diabetic middle age patients with CVC appears to be similar to those found in non-diabetic and diabetic elders with CVC, suggesting that

diabetes coupled with cardiovascular disorders accelerate the cholesterol elevation much earlier. Similarly LDL-C and VLDL-C are also found to go up with age, CVC and diabetes. However, HDL-C decreases with age and diabetes in CVC pointing to the fact that the good cholesterol starts depleting as a function of age. Diabetes and cardiovascular disorders only add fuel to the fire in this depletion. Likewise, the triglycerides also increase with age, which is further enhanced by diabetes.

On the basis of the present study and evidences available from the various other investigation regarding the changes in lipoprotein during ageing and diabetes, it may be suggested that diabetes accelerates age related disturbances in the lipid profile.

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ORIGINAL RESEARCH ARTICLE

Study on Dyslipideamia among the Patient of North Bihar suffering from Hypothyroidism.

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Abstract:

Hypothyroidism is most common endocrine disorders, associated with an unfavorable effect on lipids. Dyslipidemia is common metabolic abnormalities in hypothyroidism with marked increase in circulating Total cholesterol, Triglycerides, VLDL and low density lipoprotein (LDL-C). This study was carried out on patient attending in Katihar Medical College & Hospital. The entire subjects were categorised into three groups. The lipid profile of all the subjects were estimated and compared with normal control groups without having any type of thyroid and other endocrine complications. All the lipid profile parameters were significantly increased except HDL among the hypothyroidism subjects. Increase was more in cholesterol and LDL values among subjects suffering from hypothyroidism. HDL levels were low among hypothyroids. Finally it appeared that the hypothyroidism is strongly responsible for the alteration in lipid profile.

Key words: Hypothyroidism , TC, TG, LDL-C, VLDL-C, HDL-C, Dyslipidemia.

Introduction:

Hypothyroidism is by far the most common thyroid disorder in the adult population and is more common in older women [1]. Thyroid disease is associated with various metabolic abnormalities due to effect of thyroid hormones on all major metabolic pathways by directly or indirectly modifying the other regulatory hormones such as insulin or catecholamine [2]. Hypothyroidism is associated with hypercholesterolemia, hyper triglyceridemia with marked increased in circulating cholesterol concentration and low density lipoprotein (LDL-C) and apolipoprotein B (ApoB) due to decreased LDL receptor in the liver [3,4,5]. Indeed, even within the normal range of thyroid-stimulating hormone (TSH) values, a linear increase in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) and a linear decrease in high-density lipoprotein cholesterol (HDL-C) levels has been observed with increasing TSH [6]. In hypothyroidism dyslipidemia, co-existing metabolic abnormalities in combination of hormone induced hemodynamic alterations lead to cardiovascular diseases.

Material and Methods:

This cross-sectional study was conducted on 100 subjects having same socioeconomic status, cultural and food habits in the Department of Biochemistry in Katihar Medical College and Hospital in collaboration with the Department of Medicine. Permission was taken from the concerned authority and consent were also taken from the subject for this study.

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The patients were divided into two groups. Group A consisting of 50 subjects presented with only Hypothyroidism (HY). Group B consisted of Age and sex matched fifty healthy people without any history or symptoms of diabetes & hypothyroidism and other metabolic disorders were chosen as the control group.

Serum free T3 (fT3), T4 (fT4), TSH by competitive enzyme linked immunosorbent assay [7,8]. Total cholesterols were estimated quantitatively by CHOD-PAP technique as described by Allian C.C (1974) [9]. Triacylglycerol were estimated quantitatively by GPO-ESPAS technique as described by Buccolo G and David M (1973) [10]. High density lipoproteins were estimated quantitatively by PEG-PAP method [11]. The LDL level was estimated by using the Friedewald formula by subtracting the amount of cholesterol associated with other molecules, like HDL and VLDL [12,13].

Interpretation of the data was done by statistical Software like SPSS-19.0 and Microsoft Office Excel.

Result:

The present study is an attempt to establish a correlation in the alteration of the lipoproteins within the study groups. The table shown here focussed the mean with standard error of mean of total cholesterol (TC), triglycerides (TG), very low density Lipoprotein cholesterol (VLDL-C), low density Lipoprotein cholesterol (LDL-C) and high density Lipoprotein cholesterol (HDL-C) were compared among the study group and the control group. The HDL cholesterol was high in the HY group when compared with the control and other groups (41.20±0.3647 mg/dl). All other parameters were significantly increased among the hypothyroid subjects (HY) when compared to control subject.

Table-1: Comparison of Mean for the lipoprotein parameters among study groups and control.

Groups	TC (mg/dl) Mean ± S.E.M	TG (mg/dl) Mean ± S.E.M	LDL (mg/dl) Mean ± S.E.M	VLDL (mg/dl) Mean ± S.E.M	HDL (mg/dl) Mean ± S.E.M
Hypothyroidism (HY)	313.56 ± 1.83	321.86 ± 2.49	209.08 ± 1.69	64.41 ± 0.50	39.82 ± 0.24
Control (C)	178.74 ± 1.70	190.50 ± 1.44	103.02 ± 1.67	38.10 ± 0.36	43.50 ± 0.71

Discussion:

Thyroid hormone enhances the absorption, production and utilization of glucose. Diabetes mellitus appears to influence thyroid function at several sites, from hypothalamic control of TSH, release to T3, production from T4 in the target tissue [14,15]. There is a lowered T3:T4 ratio in the diabetic group. Uncontrolled hyperglycemia with ketosis lowers T4 and T3 levels and rT3 is elevated. The mechanisms of carbohydrate derangements in hypothyroidism are unclear [16].

The present studies also showed that all the serum lipoprotein parameters were significantly increased among the hypothyroid subjects when compared to control subject. The Rotterdam population-based cohort study showed that in hypothyroidism, with its accompanying hypercholesterolemia and hypertension shows a strong association with cardiovascular diseases in elderly population specially in women [17]. Parle et al made a cross-sectional study in southern UK found that dyslipidemia was a singular risk factor for development of atherosclerosis even though HDL level was not reduced [18]. Whickham survey studied the thyroid function in a large cohort of randomly selected adult subjects [19]. This mainstay study identified that, after 20 year of follow-up, the progression of subclinical to overt hypothyroidism occurred with major changes in the lipoprotein fractions which lead to complications [20].

A study was conducted in Regional Hospital Hamirpur, Himachal Pradesh, India where the level of high density lipoprotein (HDL) was significantly decreased and level of low density (LDL), triglycerides and very low density lipoprotein (VLDL) increased in subclinical and clinical hypothyroid diabetic patients. We concluded that insulin sensitivity act as a mediator of thyroid induced lipid changes in diabetic patients [21]. Jeong Rang Park et al in his study showed Primary hypothyroidism and type 2 diabetes are both typically associated with the increased level of triglycerides[19].

When the subjects are having co morbid diseases like hypothyroidism which per se causes severe lipid metabolic alterations our study clearly pointed towards significant alterations of lipoprotein parameters in those subjects who are having more than 5 years duration of diabetes, Saunders et al [1978), Weissel et al (1980) Gavin et al (1981) support the findings of our study [22,23,24,25,26].

Conclusion:

This study finally concluded a marked alteration in lipoprotein parameters in the subjects suffering from HY. Hypothyroidism is found to occur commonly in T2DM subjects' mostly subclinical variant (SH). Although the triglyceride level were significantly increased in all three cohorts but it was more in only Diabetic subjects. The level of Total cholesterol, LDL and VLDL were increased among diabetics and Hypothyroidism and it was further enhanced among the subjects suffering from Diabetes and hypothyroidism both.
So that the whole work may be summarized that the diabetes and hypothyroidism both have a significant role in alteration of lipoprotein levels and their collective presence have more alteration.

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Research Article

Pattern of Serum Vitamin D in Hospitalised Patients: A Retrospective Study

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Abstract

Background: To find out the prevalence of vitamin D deficiency in patients hospitalised in two tertiary care centres for various ailments in Eastern Bihar and North Bengal. **Methods**: Hospitalised patients in MGM Medical College, Kishanganj, Bihar and Medical North Bengal Clinic, Siliguri, West Bengal (Jan 2014 to Dec 2014) who underwent blood sampling for vitamin D estimation in their work up for various ailments were included in the study. **Result**: Out of 108 patients, 65 were female and 43 were male in the age group ranging from 1 month to 85 years. Maximum number of patients was in the age group of 41 to 60 years. Seventy two percent patients had low vitamin D levels with 54.63% having frank deficiency and 17.59% had insufficient levels. Diabetes mellitus and/or hypertension were the most common diseases associated with hypovitaminosis D followed by diseases of respiratory system. **Conclusion**: Vitamin D deficiency was seen in 72 % of the subjects with female preponderance. No age was spared as the age of the subjects ranged from 1 month to 85 years with majority in the 41 to 60 years age group. Among subjects with hypovitaminosis D, diabetes mellitus and /or hypertension were the most commonly encountered diseases.

Key words: Vitamin D, Hypovitaminosis D, Diabetes, Hypertension

Introduction

Vitamin D deficiency is one of the most widespread nutritional deficiencies in the world and in the Indian subcontinent despite of plenty of sunshine it prevails in epidemic proportions. As per the report of International Osteoporosis Foundation in North India, 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff and 84% of pregnant women were found to have hypovitaminosis D [1]. Various research papers have attributed many reasons for the epidemic. Some of the important factors which contribute to the above scenario in India include socioreligious and cultural restrictions towards adequate sun exposure [2], vegetarianism [2], increased office hours in urban India [2], unplanned unspaced pregnancies [3], burqa system in Muslims [3] etc. Vitamin D whose active form is 1, 25 dihydroxy cholecalciferol is a steroid hormone. It works through specialised receptors called VDRs (vitamin D receptors) [4]. VDRs are present in almost every tissue of the body including bones, intestines, kidneys, liver, heart brain, skin, osteoblasts, activated T and B lymphocytes, gonads, prostate, breast and mononuclear cells. Hence its deficiency can involve almost any tissue in the disease process [5].

Widespread prevalence of vitamin D deficiency in India is a well known fact. This study was carried out to know the level of vitamin D in the population of this region, which has very limited data so far in this regard.

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Material and Methods

Present study is a multicentric retrospective study done in MGM Medical College, Kishanganj, Bihar and Medica North Bengal Clinic, Siliguri, West Bengal. One hundred and eight subjects were included in the study, out of which 43 patients were male and 65 were female. All indoor patients who were admitted either in MGM Medical College Kishanganj, Bihar or in Medica North Bengal Clinic, Siliguri, West Bengal and who underwent blood sampling for vitamin D estimation in their work up for various ailments from January 2014 to December 2014, were included in the study. The purpose of the study was to find out the status of vitamin D3 in these patients who were admitted in the hospital for various diseases. Those patients who were taking vitamin D3 or steroids in any form were excluded. Socio economic status was not a bar and patients from all socio economic status were included. The data of vitamin D assay of the above patients in the 1 year period were extracted from the hospital information system and medical record department (MRD). Only those patients whose vitamin D3 levels were estimated from the laboratories of the respective hospitals or from reputed laboratories were included in the study. The cut off levels used in our study for defining sufficiency / deficiency was based on recommendation by Michael F Holick et al [6-10], which was as follows (a) Vitamin D deficiency: Level <20 ng/ml (b) Insufficiency: Level between 21 – 29 ng/ml and (c) sufficient: level of 30ng/ml and more.

Results

Hundred and eight patients were included in our study out of which 43 patients were male and 65 were female. The age group of our patients ranged from one month to 85 years.

Table 1: Age and Sex Distribution of the study population.

Age group	Male (number)	Female (number)	Total (number)
<1 year	4	9	13
1 – 20 years	8	10	18
21 – 40 years	6	10	16
41 – 60 years	9	23	32
61 – 80 years	12	14	26
>80 years	2	1	3

Maximum number of patients was in the age group of 41 – 60 years followed by 61 – 80 years (Table 1)

Table 2: Serum Vitamin D level in the study population (n=108).

Vitamin D level	Male (percent)	Female (percent)	Total (percent)
<20 ng/ml	21(19.44%)	38 (35.19%)	59 (54.63%)
21 – 29 ng/ml	8 (7.41%)	11(10%)	19 (17.59%)
>=30 ng/ml	15(13.89%)	15(13.89%)	30 (27.78%)

As depicted in table number 2 above, out of the 108 patients vitamin D deficiency (<20 ng/ml) was seen in 59 patients whereas 19 had insufficient vitamin D levels (21 – 29 ng/ml) and 30 patients had normal vitamin D levels (30 ng/ml and more). In our study 72 % patients had low vitamin D levels with 54.63 % having frank deficiency and 17.59 % had insufficient levels. Sufficient level of vitamin D was found in 27.78 %.

Mean value of vitamin D in our subjects was 23.17ng/ml. Out of the 59 patients with vitamin D below 20 ng /ml, 21 patients were male and 38 were female. Eight male patients and 11 female patients were found to have vitamin D levels between 21-29 ng/ml. And 15 male and 15 female patients had normal vitamin D levels.

Histogram showing pattern of Vitamin D level in the study population (n=108).

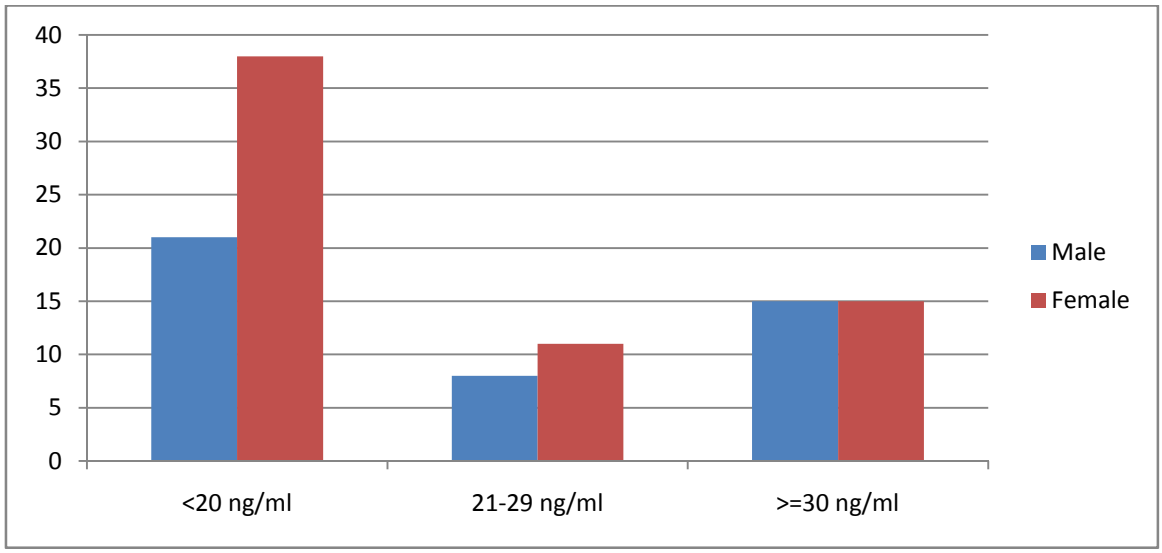

Table3: Disease pattern in Vitamin D deficient subjects, insufficiency & sufficient Vitamin D levels patients.

Diseases	Vitamin D deficient	Vitamin D insufficiency	Sufficient Vitamin D levels
Diabetes	8	1	1
Diabetes with Hypertension	8	4	5
Hypertension	7	4	7
Acute Respiratory Tract Infection	9	3	6
Acute gastrointestinal tract infection	3	0	0
Acute muscle pain	0	1	0
Anaemia	0	2	6
Spondylosis	8	2	1
Irritable Bowel Syndrome	0	0	1
Acid Peptic Disorder	2	0	2
Allergic Rhinitis	2	1	0
Carcinoma lung	0	1	0
Fibroid uterus	1	0	0
Cholelithiasis	2	0	0
Fracture neck of femur	0	0	1
Acute Coronary Syndrome	1	0	0
COPD	1	0	0
Bronchial Asthma	3	0	0
Bronchiolitis	4	0	0

- Twenty three patients (21.29%) out of 108 who were vitamin D deficient (level below 20 ng/ml) had diabetes mellitus and/or hypertension. Nineteen patients (17.59%) had diseases related to respiratory system (Acute Respiratory Tract Infection, Allergic Rhinitis, Bronchiolitis, COPD and Bronchial Asthma) and eight patients (7.41%) had spondylosis (Cervical / Lumbar).
- In the group having insufficient levels of vitamin D (level between 21 to 29 ng/ml) 9 patients (8.33%) had diabetes mellitus and /or hypertension, three (2.77%) had respiratory tract infection. Carcinoma lung was seen in one patient.
- In patients having sufficient levels of vitamin D (30 ng/ml and more), eight patients (7.41%) had diabetes mellitus and /or hypertension whereas anemia was seen in 6 (5.55%) patients.

In the three different categories of serum vitamin D levels measured in the study population (deficient, insufficient and adequate), the patients with diabetes mellitus and diabetes mellitus with hypertension were distributed as per the table depicted below. (Table 4)

Table 4: Vitamin D levels in diabetic subjects.

Disease	Vitamin D deficiency	Vitamin D insufficiency	Adequate Vitamin D
Diabetes	8	1	1
Diabetes and Hypertension	8	4	5
Total	**16**	**5**	**6**

Discussion

Vitamin D deficiency is wide spread in individuals irrespective of their age, gender, race and geography as is evident from the innumerable number of publications worldwide in this regard. Vitamin D functions in the body through both an endocrine mechanism (regulation of calcium absorption) and an autocrine mechanism (facilitation of gene expression).

The former acts through circulating calcitriol, whereas the latter, which accounts for more than 80% of the metabolic utilization of the vitamin each day, produces, uses, and degrades calcitriol exclusively intracellularly. In addition to diseases like rickets and osteoporosis the consequences of low 25(OH) D status include increased risk of various chronic diseases ranging from hypertension to diabetes to cancer [11].

There is a large body of epidemiologic data showing an inverse association between incident cancer risk and antecedently measured serum 25(OH) D [12-15]. This evidence has been accumulated for such cancers as prostate, colon, breast, lung and marrow/lymphoma, among others. Although cancer is not an uncommon entity in this part of the country, in our study only one subject had cancer. The reason could be the small sample size.

In the days when rickets was rampant, children with this disorder frequently died of respiratory infections. Calcitriol in its autocrine role has been recognized for roughly 20 years as playing a role in various aspects of the immune response [16,17]. In our study too, 16 patients (14.81%) had Acute Respiratory Tract Infection along with low vitamin D levels (<30 ng/ml).

Both type 1 and type 2 diabetes have been associated with low vitamin D status, both current and antecedent [18-20]. The association of vitamin D status and hypertension is particularly strong. Both control trials and meta-analyses have shown a protective effect of high calcium intake for both pregnancy-related and essential hypertension [21-25]. In our study too, out of total 108 subjects, 32 patients (30%) had diabetes mellitus and /or hypertension in association with hypovitaminosis D (<30 ng/ml). The other major group having hypovitaminosis was that with spondylosis (9%).

Conclusions

To conclude, vitamin D deficiency was seen in 72% of our patients with a mean value of 23.17ng/ml. This problem does not spare any age group and is found in a wide spectrum of illnesses. This further reiterates the fact that hypovitaminosis D is a common problem in India and our region is no exception. The need of the hour is to spread awareness about the problem and evolve strategies to provide affordable vitamin D supplements and also fortify the food. The medical fraternity at large and the government can certainly bring this change, if the effort is sincere and in right earnest.

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Research Article

Vitamin D status in outpatient department patients: a retrospective study

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ABSTRACT

Background: Although there are innumerable studies on vitamin D deficiency in India, there is limited data in Eastern Bihar and North Bengal. Keeping this in view, the aim of our study is to find out the prevalence of vitamin D deficiency in our region.
Methods: Patients attending the outpatient departments (OPDs) in MGM Medical College, Kishanganj, Bihar, India and Medica North Bengal Clinic, Siliguri, West Bengal (January 2014 to December 2015) for various ailments and who were advised vitamin D estimation were included in our study.
Results: Out of 485 patients, 187 were male and 298 were female. Age of the study population ranged from 1 month to 83 years. Maximum number of patients was in the age group of 21 to 60 years. Vitamin D deficiency was seen in 74.44 % out of which 54.22% had frank deficiency and 20.22% had insufficient levels with 46.4% female and 28.04% male subject.
Conclusions: Prevalence of vitamin D deficiency is very high in our region that is in Eastern Bihar and North Bengal, as is reflected from our study. This is the pattern seen in other parts of our country too. Also, the deficiency is high in the age group 21 to 60 years and females outnumber the male.

Key words: Vitamin D, Hypovitaminosis D

INTRODUCTION

It has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency.[1] In India majority of its population lives in areas receiving ample sunlight throughout the year hence there was disbelief that vitamin D deficiency is uncommon.[2] However, from the data available in the published literature, vitamin D deficiency is very common in India in all the age groups and in both sexes, across the country with a prevalence of 50 to 90 percent. [3-5]

The major source of vitamin D for humans is exposure to sunlight.[1-6]Anything that diminishes the transmission of solar UVB radiation to the earth's surface or any factor that alters the penetration of UVB radiation into the skin will affect the cutaneous synthesis of vitamin D.[3,7]

Vitamin D is metabolised in the liver to 25(OH) D and then in the kidneys to its active form 1, $25(OH)_2$ D.[8-9] It is also recognised that many other tissues in the body, including macrophages, brain, colon, prostate, breast and other, have the enzymatic machinery to locally produce $1,25(OH)_2$ D.[10-14]

Hypovitaminosis D leads to increased risk of many diseases ranging from rickets, osteoporosis to many chronic diseases like diabetes, hypertension and cancer .[15]

Given the limited available data on the vitamin D status among the population of Eastern Bihar and North Bengal, our endeavour was to find out the prevalence of vitamin D deficiency in our region.

METHODS

This is a retrospective study conducted at Mata Gujri Memorial Medical College and Lions Seva Kendra, Kishanganj, Bihar and Medica North Bengal Clinic, Siliguri, West Bengal.

Both are tertiary care centres in Eastern Bihar and North Bengal respectively. All patients who underwent blood sampling for vitamin D estimation during their visit to outpatient department from January 2014 to December 2015 in the above centres were included in our study. The data of vitamin D assay of 485 patients in the 2 year period were extracted from the hospital information system and medical record department (MRD) and were reviewed extensively. In addition to our attempt to find out the prevalence of vitamin D deficiency in our region, the study population was further categorised on the basis of age and sex. The age group ranged from 1 month to 83 years which included 187 male and 298 female subjects.

The cut off levels used in our study for defining sufficiency / deficiency was based on recommendation by Holick MF et al 1,16-19, which is as follows(a) Vitamin D deficiency: Level <20 ng/ml (b) Insufficiency: Level between 21–29ng/ml and (c) sufficient: level of 30ng/ml and more.

RESULTS

A total of 485 patients who underwent vitamin D estimation were included in our study with 187 male subjects and 298 female subjects.

The age group of our subjects ranged from 1 month to 83 years. We had 14 subjects in age group less than 1 year with equal sex distribution. In the age group 1-20 years there were 46 male and 30 female subjects.

Maximum numbers of subjects were seen in the age group 21-40 and 41–60 years with 163 and 160 subjects respectively. There were 54 male and 109 female in the former and 49 male and 111 female in the latter group. In the age group 61–80 years there were 31 male and 36 female subjects. All five subjects in the age group >80 years were female.

Table 1: Age wise gender distribution of the study population (n = 485).

	Male	Female	Total (Percent)
<1 years	7	7	14 (2.89%)
1-20 years	46	30	76 (15.67%)
21-40 years	54	109	163 (33.61%)
41-60 years	49	111	160 (32.99%)
61-80 years	31	36	67 (13.81%)
>80 years	0	5	5 (1.03%)

Out of a total of 485, there were 187 male subjects (38.56%) and 298 female subjects (61.44%) as shown in Table 1. As it is clear from the above table that in age group <1 year there were 2.89% subjects, in 1-20 years there were 15.67% subjects, in 21-40 years there were 33.61% subjects, in 41-60 years there were 32.99% subjects, in the age group 61-80 years, there were 13.81% subjects whereas in the age group more than 80 years there were 1.03% subjects.

Out of 263 patients who had frank deficiency (vitamin D levels <20ng/dl), 92 were male and 171 were female, whereas out of 98 patients who had insufficient vitamin D levels (21 to 29ng/dl), 44 were male and 54 were female. One hundred and twenty four subjects had normal vitamin D levels out of which 51 were male and 73 were female subjects. Vitamin D deficiency was seen in 74.44% subjects out of which 54.22% had frank vitamin D deficiency (<20ng/dl) whereas 20.22% had insufficient vitamin D levels (21-29 ng/dl). About twenty five percent of the study population had normal vitamin D levels (>30 ng/dl). Out of 54.22% subjects who had frank vitamin D deficiency 18.96% were male and 35.26% were female, whereas out of 20.22% subjects who had insufficient vitamin D levels 9.08% were male and 11.14% were female. A total of 25.56% had normal vitamin D levels out of which 10.51% were male and 15.05% were female.

Out of the total study population of 485, 54.22% (n=263) had frank deficiency of vitamin D, 20.22% (n=98) had insufficient vitamin D levels and 25.56% (n=124) had normal vitamin D levels which has been shown in the pie diagram above. In the age group less than 1 year there were 14 subjects with 4 each in the deficient and insufficient categories with equal sex distribution. Six infants had normal vitamin D levels. In the group 1-20 years which numbered 76, more than half had levels

below 20 ng/dl, with 22 male and 19 female being vitamin D deficient. Sixteen subjects, 11 male and 5 female, had insufficient vitamin D levels in this group.

Table 2: Prevalence of vitamin D deficiency and its variation with gender (n=485).

	Male (Percent)	Female (Percent)	Total (Percent)
<20ng/dl	92 (18.96%)	171 (35.26%)	263 (54.22%)
21–29ng/dl	44 (9.08%)	54 (11.14%)	98 (20.22%)
> 30ng/dl	51 (10.51%)	73 (15.05%)	124 (25.56%)

Table 3: Pattern of vitamin D levels and its variation according to age and sex in the study population.

	<20ng/dl			21-29ng/dl			>= 30ng/dl		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
<1 year	2	2	4	2	2	4	3	3	6
1-20 years	22	19	41	11	5	16	13	6	19
21-40years	33	65	98	15	26	41	6	18	24
41-60years	23	62	85	11	15	26	15	34	49
61-80years	12	20	32	5	5	10	14	11	25
>80years	0	3	3	0	1	1	0	1	1

In the age group 21-40 years, this had maximum number of subjects (163) as many as 98 had frank vitamin D deficiency with two third female majority. In this age group, vitamin D insufficiency was found in 41subjects again with nearly two third female majority. A meagre 24 subjects had normal vitamin D levels.

In the next age category (41-60 years) which had 160 subjects, more than half were vitamin D deficient with female preponderance here too with 62 female subjects out of the total of 85. Twenty six were vitamin D insufficient in this group with 11 male and 15 female subjects. Forty nine subjects had normal vitamin D levels.

In the age group 61-80 years, there were 67 subjects. Thirty two had frank vitamin D deficiency with 20 female and 12 male subjects whereas 10 had insufficient levels with equal sex distribution. Eleven patients had normal vitamin D levels with male preponderance. Out of the five octogenarians, all were female. Three had frank vitamin D deficiency and one each had insufficient and normal levels. Pattern of vitamin D levels and its variation according to age and sex in the study population is shown in Table 3 and in the Histogram below.

DISCUSSION

Vitamin D is a unique nutrient whose deficiency causes one of the most widespread spectrum of human diseases ranging from those known from time immemorial like rickets and osteoporosis to the hundreds of diseases which are now linked to hypovitaminosis D, like diabetes, hypertension, various cancers, tuberculosis, preeclampsia, depression, etc.[20]

In present study, hypovitaminosis D (vitamin D deficiency and vitamin D insufficiency) was observed in 74.44% of the study population. This is similar to many published articles which relates to vitamin D deficiency in the Indian population.[21-25]

The mean value of vitamin D in our subject was 22.36ng/ml. Another recent Indian study involving a large number of subjects (n= 26,346) had similar finding.[21] In the study by Shah P et al the mean vitamin D_3 level was only 9.36ng/ml.[23]

Another important finding in our study is that hypovitaminosis was more common in females as compared to the males.

Extra attention to their diet as well as vitamin D supplementation is warranted to avoid long term complications in the female gender, keeping in mind the increased need due to pregnancy and lactation. In pesent study maximum `number of subjects was in the age group of 21-40 years followed closely by the age group 41-60 years. This is again similar to the observation made in previous studies.[21,23]

It is interesting to note that the decades spanning from 21 years to 60 years of age are one of the most productive years in the life of a human being. It is also the period which is most challenging and rewarding as well. Deficiency of a vital nutrient like vitamin D has the potential to have an adverse impact in this crucial phase of life. Hypovitaminosis D perhaps heralds a cascade which finally leads to the chronic diseases as described in the beginning.

CONCLUSION

To conclude, vitamin D deficiency was seen in 74.44% of our study population with a mean value of 22.36 ng/ml. This further reiterates the fact that hypovitaminosis D is a common problem in India and our region is no exception.

Although this problem does not spare any age group as seen in our study, the most affected age groups were those in the 21 -40 years and 41-60 years, with a female predominance. Hence, medically monitored supplementation of vitamin D on a regular basis in this age group along with lifestyle modifications may have a positive long term impact and perhaps act as 'vaccine' to prevent the diseases that are presently plaguing not only the Indian population, but the human civilization at large.

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Original Research Article

Study on correlation between high density lipoprotein and cardiac markers in patients of type II diabetes mellitus complicated with hypertension

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Abstract

Problem statement: Diabetes mellitus is characterized by loss of the insulin-producing beta cells of the isolates of Langerhans is the pancreas, leading to insulin deficiency. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Plasma homocysteine is considered to be a maker of endothelial dysfunction and suggested to be a causative factor for atherosclerosis and arterial stiffness. It is clear that hyperhomocysteinemia can promote atherosclerosis. **Methods:** A prospective short study was undertaken to find out the correlation between high density lipoprotein and cardiac markers in various age groups, this study was carried out randomly in 100 patients having Type-II Diabetic Mellitus complicated with hypertension who was attending the OPD in the department of Medicine in MGM Medical College & L.S.K. Hospital, Kishanganj. The duration of the study was, from June2015 to October 2016. The data collection was carried out after selection of sampling unit according to the inclusion and exclusion criteria. The biochemical test parameters were measured using appropriate and standard with standard operating protocol in the clinical biochemistry laboratory of the institute. The SPSS statistical package was used for analysis. ± standard deviation was recorded. P value <0.05 was considered significant. **Results:** The age of 100 cases enrolled in this study ranged between 17 to 76 years. The 2% of cases was of age group of <20 yrs., 18% were between 21 to 40 yrs, 55% were between 41 to 60 yrs. and 25% were >60 yrs. of age group. The majority of cases 62% were male and 38% were female. Suggesting that the type-II complicated with pretension is more common in males than the females. Out of 100 cases studied 22 patients were having abnormal homocysteine and HDL. Most of the patients with abnormal homocysteine and HDL is having positive CRP (19 out of 22 that means approximately 79%) however only 21% (3 cases out of 22) were having negative CRP. The 19 cases out of 22 having positive CRP, abnormal homocysteine and HDL, 15 were male and 4 were female. Out of these 19 cases having positive CRP abnormal homocysteine and HDL. One patient was of age group between 0-20 yrs., 4 were age group between 20-40 yrs., 8 were of age between 41 to 60 yrs. and remaining 6 were of >60 yrs. of age. Out of 3 cases having negative CRP with abnormal homocysteine and HDL, 2 were and 1 was female. The 2 cases of it was of age group between 41 to 60 yrs., 1 was of >60 yrs. of age. **Conclusion:** No significant correlation has been found between homocysteine level and CRP. There was significant correlation with HDL levels and CRP.

Key Word: Diabetes mellitus, Atherosclerosis.

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INTRODUCTION

The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediate autoimmune attack. [1] There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight wen onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children of adults, but was traditionally termed

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"juvenile diabetes" because a majority of these diabetes cases were in children. "Brittle" diabetes, also known as unstable diabetes or labile diabetes is a term that was traditionally used to describe to dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. [2] There are many reasons for type 1 diabetes to be accompanied by irregular and unpredictable hyperglycemias, frequently with ketosis, and sometimes serious hypoglycemia's, including an impaired counter-regulatory response to hypoglycemia, occult infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease).[2] These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes.[3]

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion.[4] The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%-5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20% - 50% of affected women develop type 2 diabetes later in life. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. A 2008 study completed in the U.S. found the number of American women entering pregnancy with pre-existing diabetes is increasing. In fact, the rate of diabetes in expectant mothers has more than doubled in the past six years. [5] This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential for the children of diabetic mothers to become diabetic in the future. Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend may years in a state of pre-diabetes which has been termed "America's largest healthcare epidemic." [6,7] DM, Cooke DW *et al*. 2008.[8] Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, bases on age rather than etiology. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associate with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The ICD-10 (1992) diagnostic entity, malnutrition-related diabetes mellitus (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999. [7]

METHODOLOGY

A prospective short study was undertaken to find out the correlation between high density lipoprotein and cardiac markers in various age groups among patients attending Medicine OPD an diabetic clinic in M.G.M. Medical College and L.S.K. Hospital, Kishanganj, Bihar.

Study design: A prospective short study.

Study area/setting: Medicine OPD and Diabetic Clinic in M.G.M. Medical College and L.S.K. Hospital, Kishanganj (Bihar). Patients were heterogeneous and immigrated from different districts and states.

Study duration: The duration of the study was, from June2015 to October 2016.

Data Collection: The data collection was carried out after selection of sampling unit according to the inclusion and exclusion criteria. The biochemical test parameters were measured using appropriate and standard with standard operating protocol in the clinical biochemistry laboratory of the institute. The SPSS statistical package was used for analysis. $\pm$ standard deviation was recorded. P value <0.05 was considered significant.

Study Population: A prospective short study was undertaken to find out the correlation between high

density lipoprotein and cardiac markers in various age groups, this study was carried out randomly in 100 patients having Type-II Diabetic Mellitus complicated with hypertension who was attending the OPD in the department of Medicine in MGM Medical College and L.S.K. Hospital, Kishanganj.

RESULTS

Table 1: Distribution of patients with negative CRP with abnormal Homocysteine and HDL

Sex	CRP (-ve), abnormal Homo. and HDL
Male	2
Female	1

P Value = 0.56(non-significant)

Table 2: Distribution of study patients with positive CRP abnormal Homocysteine and HDL according to sex

Sex	CRP (+ve) abnormal Homo. and HDL
Male	15
Female	4

P Value = 0.011 (significant)

Table 3: Distribution of study patients having positive CRP with normal Homocysteine and abnormal HDL according to age group

Age Group	CRP (+ve) abnormal HDL and normal Homo.
0 - 20	0
21 - 40	3
41 – 60	12
>60	5

P Value = 0.001 (significant)

Table 4: Distribution of Total Cholesterol in the study population According to age group and family history

Age Group	Positive Family H/O	Negative Family H/O
0 - 20	2	0
21 – 40	11	7
41 – 60	24	31
>60	10	15

P Value = 0.21 (non-significant)

Table 5: Distribution of total cholesterol in the study population According to age group

Age Group	Normal	Boarder Line High	High
0 – 20	2	0	0
21 – 40	15	2	1
41 – 60	40	8	7
>60	20	3	2

P Value = 0.92 (non-significant)

Table 6: Distribution of LDL in the study population according to age groups

Age Group	Normal	Boarder Line High	High	Very High
0 - 20	2	0	0	0
21 – 40	15	3	0	0
41 – 60	43	11	1	0
>60	20	3	2	0

P Value = 0.66 (non-significant)

Table 7: Distribution of TG in the study population according to age group

Age Group	Normal	Boarder Line High	High	Very High
0 - 20	2	0	0	0
21 – 40	16	2	0	0
41 – 60	47	7	1	0
>60	22	2	0	1

P Value = 0.87 (non-significant)

DISCUSSION

The study was conducted to establish any correlation between high density lipoprotein and other cardiac markers in subject of Type-II DM complicated with HT. this study was carried out randomly in 100 patients having Type-II Diabetic Mellitus complicated with hypertension who was attending the OPD in the department of Medicine in MGM Medical College and L.S.K. Hospital, Kishanganj.

The age of 100 cases enrolled in this study ranged between 17 to 76 years. The 2% of cases was of age group of <20 yrs., 18% were between 21 to 40 yrs, 55% were between 41 to 60 yrs. and 25% were >60 yrs. of age group. The majority of cases 62% were male and 38% were female. Suggesting that the type-II complicated with pretension is more common in males than the females (Table and Fig.-1).

Out of 100 cases studied 22 patients were having abnormal homocysteine and HDL. Most of the patients with abnormal homocysteine and HDL is having positive CRP (19 out of 22 that means approximately 79%) however only 21% (3 cases out of 22) were having negative CRP. The 19 cases out of 22 having positive CRP, abnormal homocysteine and HDL, 15 were male and 4 were female. Out of these 19 cases having positive CRP abnormal homocysteine and HDL. One patient was of age group between 0-20 yrs., 4 were age group between 20-40 yrs., 8 were of age between 41 to 60 yrs. and remaining 6 were of >60 yrs. of age. Out of 3 cases having negative CRP with abnormal homocysteine and HDL, 2 were and 1 was female. The 2 cases of it was of age group between 41 to 60 yrs., 1 was of >60 yrs. of age. Majority of patients (79%) abnormal homocysteine and HSL had some inflammation causing positive CRP however only few cases were not having inflammation but were having abnormal homocysteine and HDL.

Out of 100 cases studied 20% of the cases were having positive CRP and abnormal HDL but normal homocysteine. 13 out of them were male and 7 were female. Out of these 20 cases, 3 were of age group 21 to 40 yrs., 12 were of 40 to 60 yrs. and 5 were of >60 yrs. of age group.

Out of 100 cases studied only 3% of the cases were having positive CRP, abnormal homocysteine and normal

HDL, 1 out of 3 as male and 2 were female. Out of 3 cases, 1 of age group 41 to 60yrs. and 2 were of age group >60yrs.

77% of the cases were having normal Total Cholesterol, 12% were having borderline high T. Cholesterol and only 10% were having high Total Cholesterol levels. Among the normal Total Cholesterol levels, 2 cases were of age group 0-20yrs., 15 were of between 21-40yrs., 40 were of between 41 to 60yrs., and 20 cases were of >60 yrs. of age group. Among the borderline high Total Cholesterol none of them were of age <20rs., 2 were of age group 21 to 40 yrs., 8 were of 41 to 60 yrs., and 3 were of 60yrs., among between 21 t 41yrs., 7 were of 41 to 60 yrs. and 2 were of >60 yrs. of age.

CONCLUSION

The present work entitled 'study the correlation between HDL and cardiac markers in patients of type-2 diabetic mellitus complicated with Hypertension had been carried out on 100 subjects chosen randomly from the OPD in the department of Medicine of MGM Medical College and L.S.K. Hospital, Kishanganj (Bihar) in the year 2011-2012.

No significant correlation has been found between homocysteine level and CRP. There is significant correlation with HDL levels and CRP.

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Original Research Article

Correlation of Dyslipideamia among Hypothyroidism and Type II Diabetes Mellitus

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Abstract: Hypothyroidism and diabetes mellitus (DM) are the two most common endocrine disorders, which are on simultaneous rise. Dyslipidemia is common metabolic abnormalities in hypothyroidism and diabetes mellitus with marked increase in circulating low density lipoprotein (LDL-C). Several studies have demonstrated significant variations in dyslipidemia in Type II Diabetes Mellitus with Hypothyroidism**.** This study was carried out on patient attending in Katihar Medical College & Hospital. The entire subjects were categorised into three groups. The lipid profile of all the groups were estimated and compared with normal control groups without having any type of diabetic or thyroid complication. All the lipid profile parameters were significantly increased except HDL among the diabetics, hypothyroidism, and diabetics with hypothyroidism subjects. Increase was more in cholesterol and LDL values among subjects suffering from both diabetes and hypothyroidism. HDL levels were lowest among the diabetics and further decreased among the diabetic hypothyroids. Finally it appeared that both the endocrine disorders are equally responsible for the alteration in lipid profile & theirs cumulative effects further added fuel to the fire.
Keywords: T2DM, Hypothyroidism, TC, TG, LDL-C, VLDL-C, HDL-C.

INTRODUCTION:

The occurrence of diabetes mellitus (DM) has increased dramatically over past two decades from an estimated 30 million cases in 1985 to 177 million in 2000. Based on current trends, more than 360 million individuals will have diabetes by the year 2030 [1].

'Diabetic dyslipidaemia' is characterised by high level of plasma triglyceride (TG), and low density lipoprotein (LDL) concentrations with low level of High density cholesterol (HDL-C) due to reduced action of insulin at the tissue level or due to insulin resistance [2]. Diabetic dyslipidemia increases the risk of atherosclerosis particularly, if glycaemic control is poor, which in-turn is an important risk factor for coronary heart disease (CHD) [3].

Hypothyroidism is by far the most common thyroid disorder in the adult population and is more common in older women [4]. Thyroid disease is associated with various metabolic abnormalities due to effect of thyroid hormones on all major metabolic pathways by directly or indirectly modifying the other regulatory hormones such as insulin or catecholamine [5]. Hypothyroidism is associated with hypercholesterolemia, hypertriglyceridemia with marked increased in circulating cholesterol concentration and low density lipoprotein (LDL-C) and apolipoprotein B(ApoB) due to decreased LDL receptor in the liver [6,7,8]. In hypothyroidism dyslipidemia, co-existing metabolic abnormalities in combination of hormone induced hemodynamic alterations lead to cardiovascular diseases.

Thyroid dysfunction and diabetes mellitus (DM) are the two most common endocrine disorders. DM and thyroid disease appear to be closely linked [9]. Thyroid hormone enhances the absorption, production and utilization of glucose. Often latent diabetes may be unmasked by hyperthyroidism, while hypoglycaemia is sometimes a manifestation of hypothyroidism. Diabetes mellitus appears to influence thyroid function at several sites, from hypothalamic control of TSH, release to T3, production from T4 in the target tissue. The best studied effect is the lowering of circulating T3 in diabetics **[**10, 11**].** Apart from genetic link between thyroid disorders

and Diabetes mellitus, thyroid hormones (TH) also have well described effects on glucose and lipid metabolism. Thyroid hormones have short- and long-term interaction with the regulatory network for energy homeostasis and via direct interaction with insulin regulation causes glucose disposal in peripheral tissues [12].

RESEARCH DESIGN AND METHODS:

Subjects:

This cross-sectional study was conducted on 150 subjects & 50 control having same socioeconomic status, cultural and food habits in the Department of Biochemistry in Katihar Medical College and Hospital in collaboration with the Department of Medicine.

The patients were divided into four groups. Group A consisting of 50 subjects presented with only Type 2 Diabetes Mellitus (T2DM) Group B having 50 subjects who were suffering from only Hypothyroidism (HY). Group C consisted of patients suffering from both type 2 Diabetes mellitus and Hypothyroidism. Age and sex matched fifty healthy people without any history or symptoms of diabetes & hypothyroidism and other metabolic disorders were chosen as the controls were kept in D group.

The diagnosis of diabetes mellitus was based on World Health Organization (WHO) criteria i.e. a fasting plasma glucose of 126 mg/dl (7.0 m mole/L) after a minimum 12-hour fast, with symptoms of diabetes and a 2 hours of post prandial glucose level of more than or equal to 200 mg/dl (11.1mmole/L) [14].

Study Design:

All the biochemical estimations (Plasma Glucose & S. Lipid Profile) were done by using fully automated biochemistry analyzer Turbo-Chem by Awareness Technology, Inc. Reagents were used commercially available ready to use Kits supplied by CPC Diagnostics[17-22]. Instructions from manufacturers were followed for the estimations. Thyroid hormones were estimated by Chemiluminescence Immunoassay methods, machine and kits supplied by Monobind Inc USA [15-16].

Interpretation of the data was done by statistical Software like SPSS-19.0 and Microsoft Office Excel.

RESULT ANALYSIS AND DISCUSSION:

The present study is an attempt to establish a correlation in the alteration of the lipoproteins within the study groups. The table shown here focussed the mean with standard error of mean of Fasting glucose, Serum TSH, total cholesterol (TC), triglycerides (TG), very low density Lipoprotein cholesterol(VLDL-C), low density Lipoprotein cholesterol (LDL-C) and high density Lipoprotein cholesterol (HDL-C) were compared among the three study groups (T2DM, HY, and T2DM with HY) and the control group. In T2DM with hypothyroidism group there was marked increase in TG level (350.02±5.12mg/dl) with lowest HDL level (31.74±0.28 mg/dl). The lipoprotein parameters were significantly increased among the Diabetic subjects except serum HDL level which was significantly decreased when compared to their non-diabetic control group. The HDL cholesterol was high in the HY group when compared with the control and other groups (40.22±0.24 mg/dl). All other parameters were significantly increased among the hypothyroid subjects (HY) when compared to control subject. In T2DM + HY group all the serum lipoprotein parameters were significantly increased among the Diabetic hypothyroid subjects except serum HDL level which was significantly decreased when compared to their non-diabetic control group.

Table-I Mean and standard error of mean for the Fasting P. Glucose, serum TSH & Serum Lipid Profile among study groups and control

Groups	P. Glucose (F) mg/dl	TC (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TSH (µIU/ml)
Type-2Diabetes Mellitus(T2D)	192.72±3.82	310.12±4.26	329.46±4.63	65.89±0.92	216.92±4.12	30.28±0.27	3.93±0.11
Hypothyroidism(HY)	92.50±1.00	312.58±1.77	321.86±2.49	64.37±0.49	207.76±1.68	40.22±024	21.06±1.57
T2DM With Hypothyroidism (T2DM+HY)	191.86±1.00	337.92±4.79	350.02±5.12	70.00±1.02	23617±4.09	31.74±0.28	24.32±0.99
Control (C)	86.7±1.007	175.66±1.38	169.62±1.76	33.82±0.36	99.58±1.52	40.80±0.53	3.94±0.109

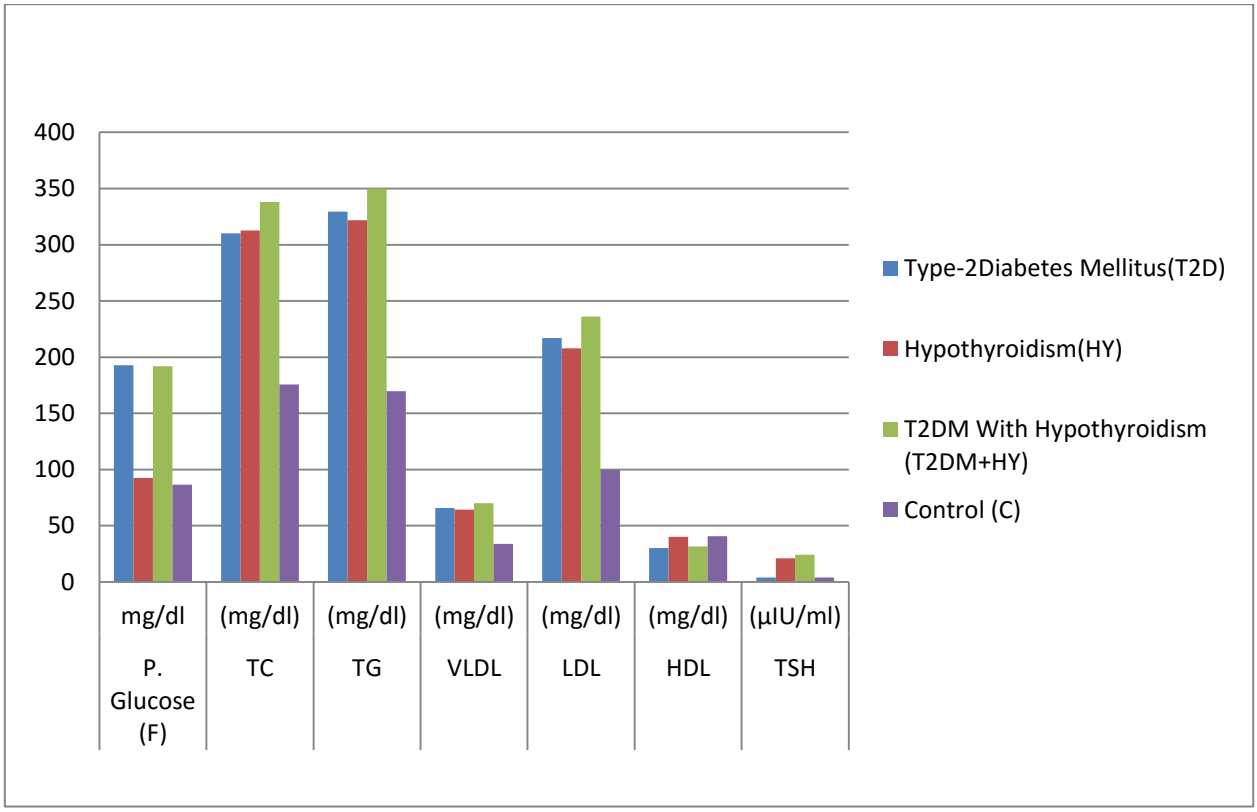

Fig-1: Graph showing Mean for the Fasting P. Glucose, serum TSH & Serum Lipid Profile among study groups and control

Thyroid hormone enhances the absorption, production and utilization of glucose. Diabetes mellitus appears to influence thyroid function at several sites, from hypothalamic control of TSH, release to T3, production from T4 in the target tissue. **[**10, 11**]** There is a lowered T3:T4 ratio in the diabetic group. Uncontrolled hyperglycemia with ketosis lowers T4 and T3 levels and rT3 is elevated. The mechanisms of carbohydrate derangements in hypothyroidism are unclear [3**].**

The diabetic (T2DM), hypothyroid (HY) and diabetic with hypothyroidism (T2DM with HY) groups have been compared with the control group (C) to find the changes in the lipoprotein parameters.

The table reflects that all the serum lipoprotein parameters were significantly increased among the Diabetic subjects except serum HDL level which was significantly decreased when compared to their non-diabetic control group. Our findings were consistent with findings of studies by Miller *et al.;* [23] and The Framingham Study [24]. They established that serum total cholesterol increases in diabetics with age secondary to increasing LDL-C. Arshag D Mooradian in 2009 [3] supported our findings that Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance.

The present studies also showed that all the serum lipoprotein parameters were significantly increased among the hypothyroid subjects when compared to control subject. The Rotterdam population-based cohort study showed that in hypothyroidism, with its accompanying hypercholesterolemia and hypertension shows a strong association with cardiovascular diseases in elderly population especially in women [25]**.** Parle *et al.;* made a cross-sectional study in southern UK found that dyslipidemia was a singular risk factor for development of atherosclerosis even though HDL level was not reduced [26] **.**Whickham survey studied the thyroid function in a large cohort of randomly selected adult subjects [27]. This mainstay study identified that, after 20 year of follow-up, the progression of subclinical to overt hypothyroidism occurred with major changes in the lipoprotein fractions which lead to complications [28].

In this study it was observed that all the serum lipoprotein parameters were significantly increased among the Diabetic hypothyroid subjects except serum

HDL level which was significantly decreased when compared to their non-diabetic control group. The serum HDL level in diabetic hypothyroid group was (31.74±0.286) which is slightly higher than the diabetic group. B M Singh and Goswami in 2010 in their study found that Patients with hypothyroidism demonstrated insulin resistance and dyslipidemia as observed by higher cholesterol and triglyceride levels respectively as compared to the controls. Thyroid dysfunction leads to alterations in glucose and lipid metabolism which is an important risk factor for cardiovascular diseases [29].

A study was conducted in Regional Hospital Hamirpur, Himachal Pradesh, India where the level of high density lipoprotein (HDL) was significantly decreased and level of low density (LDL), triglycerides and very low density lipoprotein (VLDL) increased in subclinical and clinical hypothyroid diabetic patients. We concluded that insulin sensitivity act as a mediator of thyroid induced lipid changes in diabetic patients [30]. Jeong Rang Park *et al.;* in his study showed Primary hypothyroidism and type 2 diabetes are both typically associated with the increased level of triglycerides [27].

In this study it is found that all the serum lipoprotein parameters were significantly increased among both the Diabetes mellitus subjects and diabetic hypothyroid subjects except HDL which was significantly lower in Diabetics than diabetic hypothyroids. The triglyceride levels were more in only diabetic with hypothyroidism patients. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles [3]. The prevalence of low HDL cholesterol level in those with diabetes mellitus was almost twice as high as the prevalence in non-diabetic. Thus, both men and women with diabetes had an increased prevalence of hypertriglyceridemia and low HDL cholesterol levels, but their total cholesterol and LDL cholesterol levels did not differ from those in patients with both endocrinal disorders [31]. The hypercholesterolemia of hypothyroidism is a well-known risk factor for cardiovascular atherosclerotic disease that will aggravate the macroangiopathic and perhaps also the microangiopathic complications of long-standing diabetes mellitus. Studies done by Mason *et al.;* as early as 1930 had first revealed the fact which was further justified by studies done by Rosenman in 1952,Kurland *et al.;* in 1955, Dorey *et al.;* in 1981 and Abrams JJ in 1981 [32-36].

All the serum lipoprotein parameters were significantly increased among the diabetic mellitus subjects when compared with only hypothyroid subjects except serum TC level which was raised in both the groups but did not show a significant variability. Our findings of this study among T2DM and HY groups were corroborating with the Miller GJ *et al.;* in 1977, Arshag D Mooradian in 2009, Jeong Rang Park *et al.;* in 2005 and others [23, 3, 27]. Studies under taken by, Hecht and Gershberg in 1968, Lendrum *et al.;* in 1975, Saunders *et al.;* in 1978, Weissel *et al.;* in 1980 Gavin *et al.;* in 1981 support the findings of our study [37-41].

The benefits of identifying thyroid dysfunction at an early stage in Type 2 DM, and even in a symptomatic patient are considerable because progression to overt thyroid dysfunction is associated with consequent morbidity including the adverse effects on lipid and bone metabolism which proves that overt hypothyroidism in T2DM has much more deleterious effects [42, 43]. Consecutively many studies done by Tunbridge *et al.;* in 1977, Feely in 1979, Gray *et al.;* in 1980 in the same decade supported our findings [28, 44-46].

CONCLUSION

This study finally concluded a marked alteration in lipoprotein parameters in the subjects suffering from both T2DM with HY. Hypothyroidism is found to occur commonly in T2DM subject's mostly subclinical variant (SH). Although, triglyceride levels were significantly increased in all three cohorts, but it was highest in diabetic subjects with hypothyroidsm. The level of Total cholesterol, LDL and VLDL were increased among diabetics and Hypothyroidism and it was further enhanced among the subjects suffering from Diabetes and hypothyroidism both. So that the whole work may be summarized that the diabetes and hypothyroidism both have a significant role in alteration of lipoprotein levels and their collective presence have greater effect.

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ROLE OF HbA1c IN DETERMINING GLYCAEMIC CONTROL IN DIABETES MELLITUS

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ABSTRACT

BACKGROUND

Diabetes mellitus is disorder of metabolism characterised by chronic hyperglycaemia resulting from an absolute/relative insufficiency of insulin secretion, insulin action or most commonly both.[1] Type 2 diabetes is more common and account for 90-95% of diabetic patient.[2] Severity of diabetes triggers a vast group of complications, both microvascular or macrovascular.[3]

MATERIALS AND METHODS

Glycosylated Haemoglobin (HbA1c) is a form of haemoglobin, which used primarily to identify the average plasma glucose concentration over prolonged periods of time (8 to 12 weeks).[4] The early diagnosis and glycaemic control in diabetes mellitus is important because control of blood sugar level can reduce the risk of long-term complications (Babcock Irvin C, et al; 2000)[5] and may improve treatment (Larsen ML, et al; 1990).[4]

RESULTS AND CONCLUSION

The primary aim of this work is to establish the role of HbA1c in monitoring the progression of disease and glycaemic control in diabetic patients.

KEYWORDS

Glycosylated Haemoglobin, Diabetes Mellitus, Hyperglycaemia, Blood Glucose, Insulin.

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BACKGROUND: Diabetes mellitus is one of the main threats to human health in twenty first century (Zimmet 2000).[6] Over the past several decades, diabetes mellitus has become a major health problem worldwide reaching epidemic proportions in many developing countries especially in India. Diabetes mellitus is a disorder of metabolism characterised by chronic hyperglycaemia resulting from absolute/relative insufficiency in insulin secretion, insulin action or most commonly both. The chronic hyperglycaemia of diabetes and attendant metabolic deregulation maybe associated with a vast group of complications with secondary damage in multiple organ systems especially the kidneys, eyes, nerves, heart and blood vessels.[1] The worldwide prevalence of diabetes has risen dramatically over past two decades. Based on current trends, more than 360 million individuals will have diabetes by the year 2030 (Harrison, 17th edition).[7] The complications of diabetes are influenced not only by the duration of diabetes, but also by the average level of chronic glycaemia.

Acute life-threatening consequences of uncontrolled diabetes are hyperglycaemia with ketoacidosis or nonketotic hyperosmolar syndrome.

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AIM AND OBJECTIVES: Long-term complications of diabetes include retinopathy, nephropathy, peripheral neuropathy and autonomic neuropathy. There is also an increased incidence of atherosclerosis, cardiovascular disease, peripheral arterial disease, cerebrovascular disease, hypertension and abnormalities of lipoprotein metabolism. (Diabetic Care, 1997).[1,8,9]

Glycosylated Haemoglobin (HbA1c): Is a form of haemoglobin, which used primarily to identify the average plasma glucose concentration over prolonged periods of time (8 to 12 weeks).[10] It is formed by a non-enzymatic glycation of haemoglobin. Diagnosing diabetes mellitus by fasting and postprandial plasma glucose are not suitable for acutely ill patients. A single fasting blood glucose measurement only gives an indication of the patient's immediate past (Last 1-2 hours) condition and may not represent the true glycaemic control. HbA1c level provides a representation of blood glucose levels over the preceding several months and does not require the patients to fast or undergo glucose challenges. The early diagnosis and glycaemic control in diabetes mellitus is important because control of blood sugar level can reduce the risk of long-term complications (Babcock Irvin C, et al; 2000) and may improve treatment (Larsen ML, et al; 1990). But, within day biological variability of plasma glucose might unveil disturbance of glucose metabolism, but HbA1c cannot.

Diabetes Control and Complication Trial (DCCT), a great extent study has demonstrated that the 10% stable reduction in HbA1c determines 35% risk reduction for retinopathy, a 25-44% risk reduction for nephropathy and

30% risk reduction for neuropathy (Lorenza Calisti et al, 2005).[11]

MATERIAL AND METHODS: The present work "Role of HbA1c in determining glycaemic control in diabetes mellitus" has been carried out in the Department of Biochemistry and Central Laboratory, Katihar Medical College, Katihar, on the clinically diagnosed cases of diabetes mellitus and control group. The patients were categorised in three groups on the basis of taking drugs regularly or not, on diet control or not, and on regular exercise or not. These cases were selected from Dutdoor and Indoor Department of Medicine, Katihar Medical College and Hospital.

In the present study, 50 cases of known diabetics were selected from different age groups and of both sexes, ranging from 30 to 60 years. 20 cases of non-diabetics healthy individuals has been selected as control group. Their consent was taken. In the selection of control group (Healthy Individuals), care had been taken to match the range of age with that of the test group (Diabetic patients). The diagnosis of diabetes mellitus was made on the basis of history and laboratory investigations of urine and blood. The criteria for diagnosis of diabetes mellitus were of patients having the fasting blood glucose equal or more than to 126 mg/dL and postprandial blood glucose value equal or more than 200 mg/dL and HbA1c level more than or equal to 6.5% (ADA-2010).[12,13] The patients were studied as follows:

a. Detailed history taking and clinical examination,
b. Plasma glucose and HbA1c levels were estimated in blood.

Estimation of Glucose Level in Blood is done by Glucose Oxidase Peroxidase (GOD-POD) method.

Estimation of Glycosylated Haemoglobin is done by Ion Exchange Resin Method.

RESULTS AND DISCUSSION: This work "Role of HbA1c in determining glycaemic control in diabetes mellitus" was done on 50 patients and 20 controls having age between 30-60 years and of both sexes in the Department of Biochemistry and Central Laboratory, Katihar Medical College, Katihar, and following observations were made. The age wise mean blood glucose and HbA1c among control group shows that the maximum mean of all the variables were found among age group 51-60 years and minimum were among 30-40 years. It shows that there is general tendency of increasing blood plasma glucose and HbA1c with increasing age.

The fasting blood glucose levels varied from 68.7-88.25 mg/dL (mean±S.E.M., 79.96±1.11), postprandial blood glucose level varied from 77-101 mg/dL (mean±S.E.M., 90.57±1.51) and glycosylated haemoglobin ranged between 4.50 to 6.11% (mean±S.E.M., 5.30±0.05). The fasting blood glucose level is well within normal limits (<100 mg/dL) among control group that is comparable to W.H.O. expert committee report on diabetes mellitus.

A positive correlation of blood plasma glucose with age and HbA1c was found in this study among normal healthy individuals. In diabetic patients, there were 50 patients of both sexes in study group of age ranging from 30-60 years. The maximum incidence of diabetes mellitus was in 41-50 years of age groups in this study that was 49.33%. Incidence of diabetes is found more in male in comparison to female. The age and sex incidence in this work is nearly similar to that mentioned in W.H.O. expert committee report on diabetes mellitus. To assess glycaemic control and its relation to serum HbA1c, the diabetic group was divided into 3-subgroups depending upon regular medication, diet control and regular exercise.

The Entire Diabetic Patients were divided into Three Groups:

Group I: Diabetic patients on regular medication, diet control and on regular exercise (Good Glycaemic Control).

Group II: Diabetic patients on regular medication, without diet control and on irregular exercise (Poor Glycaemic Control).

Group III: Diabetic patients on irregular medication, without diet control and on irregular exercise (Bad Glycaemic Control).

Group I: Diabetic Patients on Regular Medication, Diet Control and on Regular Exercise: The fasting blood glucose levels varied from 86.5 to 111.25 mg/dL with (mean±S.E.M., 99.30±1.50), postprandial blood glucose levels varied from 102.25 to 127.5 mg/dL (mean±S.E.M., 113.10±1.6) and glycosylated haemoglobin (HbA1c) levels in diabetic, which are on regular medication, diet control and regular exercise varied from 5.90 to 7.15% (mean±S.E.M., 6.38±0.82). It was observed that HbA1c was within the range of control. The figures in this study are slightly lower than the Chandalia et al (1980)[14] and Raheja et al (1981).[15] This may be due to differences in the criteria adopted for classifying the degree of control. Chandalia et al (1980) had considered good control if fasting and postprandial blood plasma glucose were <120 mg/dL and 145 mg/dL, respectively. Raheja et al (1981) considered good control if 2-hrs. postprandial blood plasma glucose level was <140 mg/dL.

Group II: Diabetic Patients on Regular Medication, Without Diet Control and on Irregular Exercise: Fasting blood glucose levels varied from 99.7 to 117.5 mg/dL with (mean±S.E.M., 110.5±1.72), postprandial blood glucose levels varied from 121.5 to 157.75 mg/dL (mean±S.E.M., 136.36±2.75). The Glycosylated Haemoglobin (HbA1c) in this group ranged between 7.65 to 9.75% (mean±S.E.M., 8.70±0.17). The result shows that HbA1c increases more in this group in comparison to the level among good glycaemic control group (Group I).

Group III: Diabetic Patients on Irregular Medication, Without Diet Control and on Irregular Exercise: Fasting blood glucose levels varied from 111.00 to 173.75 mg/dL with (mean±S.E.M., 139.16±4.56), postprandial blood glucose levels varied from 144.75 to 247.50 mg/dL with (mean±S.E.M., 181.30±7.55) and glycosylated

haemoglobin level among this group ranged between 9.00 o 10.76% with (mean±S.E.M., 9.83±0.14).

The level of blood glucose and HbA1c among this group was increased significantly when compared to other groups. Scobie et al (1981) observed that a rise in blood plasma glucose concentration of 2.5 mmol/L (45 mg/dL) could produce a significant increase in HbA1c concentration of almost (1%) of total haemoglobin and this increase appeared 10 days after the hyperglycaemia and remained high until 30 days.[16]

Similarly, Svendsen et al (1979) reported that HbA1c increased in diabetic patients within hours after blood glucose concentration was raised by means of an intravenous infusion of glucose. They showed that HbA1c value returned to pre-infusion levels after incubation of blood samples for 17 hrs. in glucose free solution.[17]

CONCLUSION: The entire work may be summarised as follows:

1. The glycosylated haemoglobin was in normal range among the control group, but there was a positive correlation of blood glucose and HbA1c with age.
2. Among the diabetic patients with good glycaemic control, the HbA1c levels was also within normal range with positive correlation with blood sugar control.
3. It was observed that among the poor and bad glycaemic control groups, the HbA1c levels was increased with the increase in their blood glucose level indicating the glycation increases with the persistent increase in blood glucose level.
4. It was observed that lifestyle changes can dramatically reduce the incidence of diabetes and slow the HbA1c increase in both non-diabetic and diabetic individuals. Broadly adopted lifestyle changes should therefore reduce diabetes-related complications.

As we know that lifestyle changes can dramatically reduce the incidence of diabetes and slow the HbA1c increase in both non-diabetic and diabetic individuals. Broadly adopted lifestyle changes should therefore reduce diabetes-related complications. So, on the basis of entire work, finally, it maybe concluded that the HbA1c test provides crucial information about glycaemic control and progression of disease (Complications) in patients with diabetes. It seems to be considered the most significant parameters for monitoring diabetic control and institution of appropriate drugs for the management of the diabetes and prevention of its complications.

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Original Research Article

Role of FNAC in Diagnosis of Palpable Subcutaneous Nodules

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Abstract: Role of FNAC in subcutaneous nodules is for the rapid, non-invasive diagnosis of primary tumors, tumor recurrence, metastatic tumors & the distinction between a reactive process likely to resolve spontaneously or respond to conservative treatment. It has limited role in diagnosis of primary tumor of skin & subcutis to ease of surgical excision. Only a few series of FNAC of subcutaneous nodules have been reported. In our study FNAC was done in 200 cases of palpable subcutaneous nodules that had come to the dept. of pathology, KMCH, Katihar in between January 2015 - December 2016. FNAC was done by using 22-23 gauge needle, smears were stained by MGG, PAP & H&E stains and evaluated with clinical & radiological correlation. Histopathological correlations were done wherever possible. There were 130 males (65%) & 70 females(35%) from age ranging between 1-72 yrs evaluated. Aspirations done from different sites of the body, most common was neck, shoulder & abdomen. Out of 200 cases, benign neoplasm were 124(62%), cystic lesions were 36(18%), infective were 32(16%) & malignant were 08(4%). Lipoma was the commonest subcutaneous nodule followed by benign cystic lesions.
Keywords: FNAC, subcutaneous nodules, tumours

INTRODUCTION

In the era of modern diagnostic cytopathology, the practice of FNAC is clear advantages to patients, doctors & taxpayers. The technique is relatively painless, produces a speedy result, and is inexpensive [1]. The interest followed on preoperative diagnosis of neoplasm, benign or malignant, in any organ or tissues of the body and also valuable in the diagnosis of infections, cystic, inflammatory & degenerative conditions [2]. Intraopertative cytology as an alternative to frozen section examination using these days with a comparable level of accuracy [3].

The main indication of FNAC in tumor and tumor like lesions of subcutis is Investigation of primary tumours, suspected metastatic malignancy, recurrent tumours and the distinction between reactive process and neoplasia. It is a rapid, simple and convenient method for investigation of nodules, indurations and thickenings related to surgical scars or elsewhere in the skin or subcutis in patients with known malignancy [4]. Multiple sampling from different parts of large heterogeneous lesions is also possible without complications & hospitalization is not necessary. By using rapid staining procedures, a preliminary diagnosis will be made within short time and surgery can be avoided if lesion proves to be non neoplastic, or delayed for convenience if it is benign. In the cases of metastatic malignancy it allows pre-operative staging and planning of the extent of surgery. By doing FNAC instead of surgical biopsy seeding of tumor cells to uninvolved tissue may be minimized [4, 5]. However the differentiation between few skin adnexal tumor and metastatic malignancy can sometimes be difficult. However, from a clinical point of view, the distinction between primary and metastatic tumor is the essential information sought [4].

MATERIAL AND METHODS

FNAC was done in 200 cases of palpable subcutaneous nodules that have come to Department of Pathology, Katihar Medical College, Katihar in between January 2015 to December 2016. FNAC was performed using 22- 23 Gauge needle after proper aseptic precaution by both with aspiration & without aspiration method. In some cases of skin tumor insertion of needle was done parallel/tangential for more precise specimen collection. Multiple aspirations were done in few cases.

Air dried smears were stained by MGG stain & wet fixed smears were stained by PAP & H&E stain. MGG stained smears highlight cytoplasmic & stromal details whereas PAP & H&E stained smears give excellent nuclear details. Cytohistological correlation was done, wherever possible.

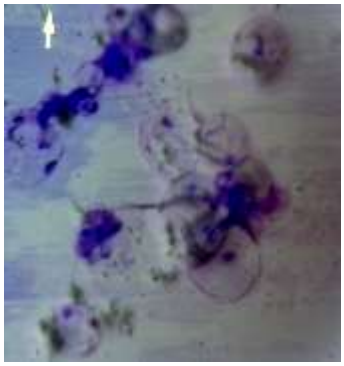
OBSERVATIONS

Table 1: 200 FNAC cases of subcutaneous nodules were studied with following observations

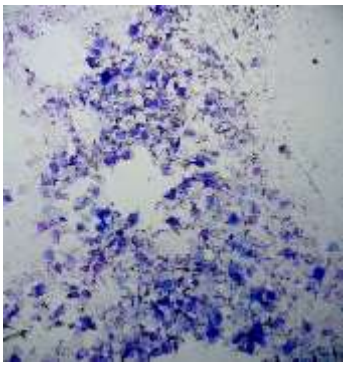
S.No.	Cytological diagnosis	Cytological typing	No. of cases	Age (years)	Sex	Salient cytological features
1.	Lipoma & its variants	Benign	105	10-72	78 M 27 F	Mature adipocytes in the background of fat droplets
2.	Epidermal inclusion cyst	Benign	28	5-64	18 M 10 F	Benign squamous cells, anucleate squames, inflammatory cells & debris
3.	Ganglion	Benign	06	10-55	04 M 02 F	A few pale histiocytes like cells in the background of myxoid material
4.	Calcinosis cutis	Benign	02	24-60	02 F	Areas of calcified masses, crystals & histiocytes
5.	Cold abscess	infective	26	10-45	12 M 14 F	Epitheloid granulomas with granular necrosis & langhan's giant cells
6.	Filarial nodules	infective	02	20-40	01 M 01 F	Filarial worm & microfilaria with eosinophils
7.	Abscess	infective	04	08-36	03 M 01 F	Intact & disintegrated neutrophils with areas of necrosis
8.	Foreign body granuloma	Benign	01	40	01 M	Granulomas with foreign body type of giant cells
9.	Neurofibroma	Benign	06	20- 40	05 M 01 F	Wavy pattern of spindle cells with fibrillar stroma & nuclear palisading
10.	Endometriosis	Benign	01	32	01 F	Biphasic tissue fragment, sheet of glandular epithelial cells & spindle cell stromal tissue
11.	Cutaneous cylindroma	Benign	01	56	01 F	Pseudopapillary fragments of cohesive basaloid epithelial cells
12.	Benign vascular tumor	Benign	04	1-45	02 M 02 F	Strands of endothelial cells in hemorrhagic background
13.	Benign Fibrous histiocytoma	Benign	06	18-40	04 M 02 F	Benign fibroblasts with histiocytes
14.	Malignant vascular tumour	Malignant	01	66	01 F	Atypical spindle & epitheloid cells with fragmented vessels
15.	Basal cell carcinoma	Malignant	03	35-60	01 M 02 F	Cohesive basal cell fragments with nuclear palisading
16.	Metastatic adenocarcinoma	Malignant	01	70	01 F	pleomorphic malignant cells in clusters & glandular pattern
17.	Squamous cell carcinoma	Malignant	02	50-72	01 M 01 F	pleomorphic malignant squamous cells in sheets & scattered singly as well
18.	Dysgerminoma, metastatic	Malignant	01	32	01 F	Round to oval cells, mostly dispersed with prominent nucleoli & lymphocytes in tigroid background

Distribution of patients, according to age, sex, cytological typing & cytomorphological diagnosis

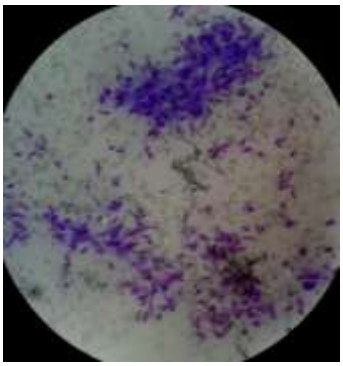
Out of 200 cases, benign neoplasm were 124 (62%), cystic lesions 36 (18%), infective 32 (16%) & malignant 08(4%). Among the neoplasms Lipoma & its variants were commonest followed by cystic lesions.



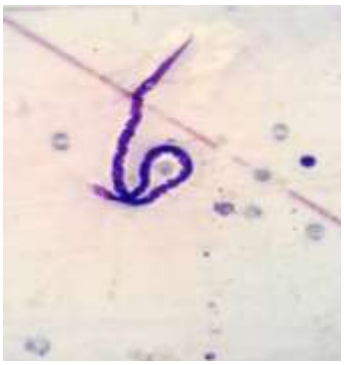
Lipoma 40 x



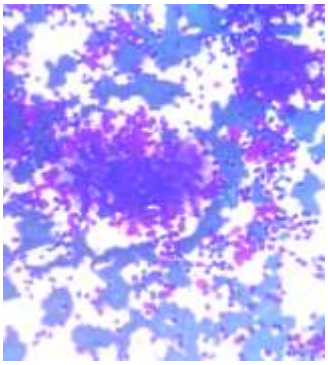
Epidermal inclusion cyst 10 x



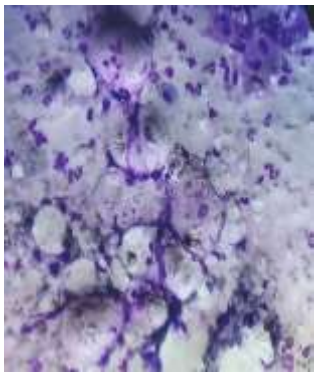
Neurofibroma 40 x



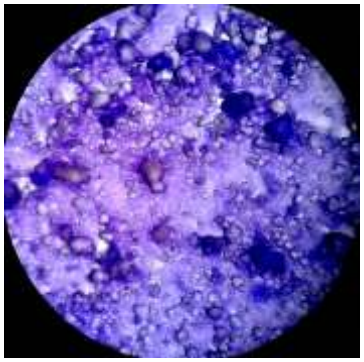
Microfilaria 40x



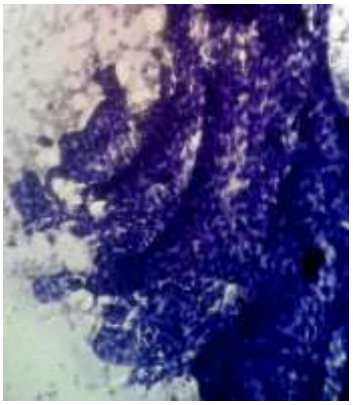
Cold abscess 40 x

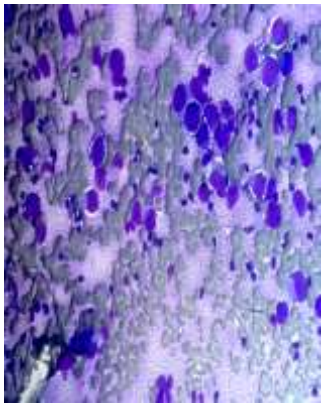


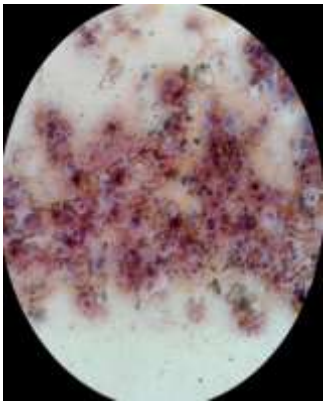
Infected Epidermal inclusion cyst 40 x

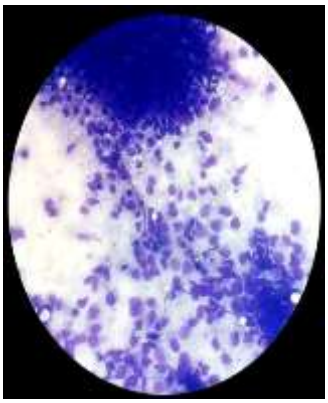


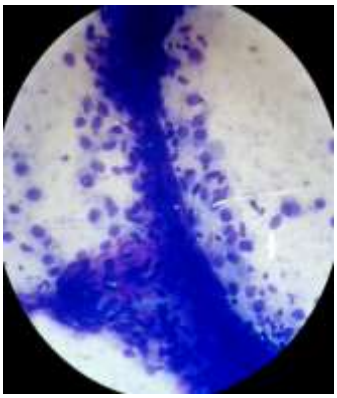
Calcinosis cutis 10 x


Basal cell carcinoma 40 x


Dysgerminoma,met 10 x


Squamous cell carcinoma 10 x


Malignant vascular tumour 10 x


Malignant vascular tumour 40 x

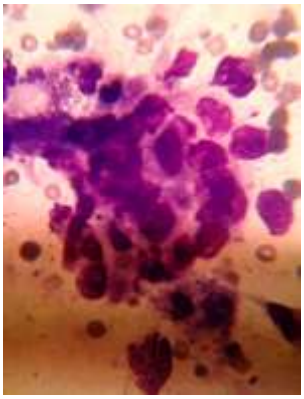

Adeno carcinoma,met 40 x

Figure-1

DISCUSSION

Role of FNAC and cytodiagnosis of skin & subcutaneous nodules has found limited application by some workers due to the ease of surgical excision and argued that it should be restricted for assessment of suspected metastatic malignancy and recurrent lesions [4, 6]. However, studies by Rekhi B *et al* [7], Liu K L *et al* [8], Layfield L J *et al* [6] & Domanski H *et al* [9] clearly established the role of cytology in this field with highly sensitive & specific tumor detection in their study group.

FNAC is routinely used as a screening test. It gives fairly accurate results regarding the nature of lesions, especially when supported by appropriate clinical findings and radiology [10]. Adequate FNA sampling and sufficient cellularity with preserved cytomorphological details are pre requisites for avoiding false negative results. It was observed that the patients who had benign and non-neoplastic lesions were relatively younger than malignant cases.

The overall incidence of cutaneous and subcutaneous metastasis has been reported to range from 0.7% to 10%. Although any region of skin can be involved, metastasis generally tends to occur close to the site of the primary malignancy [11]. The present study included 200 patients, 130 males & 70 females of age groups 1-72 years. It does not have any false positve /false negative results in broadly categorizing the lesion as inflammatory, neoplastic, benign, malignant, reactive and cystic with radiological and clinical correlation. Typing particularly of benign cystic lesion has always been difficult on cytology. The vast volume of tissue called soft tissue compartment is represented by fat, fibrous tissue, blood vessels, skeletal muscles & the peripheral nervous system.[12] In present study, we have taken only superficial lesions. Diseases presenting as tumor like masses in the compartment are challenging, as these tissues can harbour their own mesenchymal tumors & they also provide a hospitable environment for secondary deposits of epithelial, melanocytic & even lymphoid parentage & secondly, non-neoplastic inflammatory masses, cysts or reactive condition at this site add to the complexity of condition which must enter in the differential diagnosis. Open surgical biopsy procedures, unless meticulously planned with care by skilled personnel, are not without adverse

or hazardous effects [4, 12]. A retrospective analysis of FNA material by Akerman *et al* from the orthopeadic Oncology Group, Lund University Hospital over the last 20 years, revealed that diagnostic aspirates were obtained from 475 out of 517 soft tissue tumors (92%). A correct diagnosis with regard to benign versus malignant lesion was made in 447 (94%) of the 475 diagnostic aspirates. The main reasons for obtaining insufficient material were the presence of large cystic or necrotic areas, highly vascular lesions or a collagenous background matrix [9]. Borasji *et al* [13] also reported similar accuracy figures in a retrospective study of 342 cases from the musculo-skeletal tumor group at the Karolinska Hospital, Stockholm. As with FNA material from other sites, it is critical to have a multidisciplinary approach when evaluating aspired material from soft tissue. The patient age, location, size, mobility and anatomic location of the mass, the clinical presentation (rapid vs. slow growth) along with the radiographic findings were correlated with the cytologic features and high sensitivity & specificity is reported [5, 8]. Benign lesions are generally small circumscribed & cutaneous or superficial masses whereas malignant lesions are more often large, infilterative & deep seated [6, 8].

CONCLUSION

FNAC of cutaneous & subcutaneous nodules plays an important role in rapid confirmation of the diagnosis and avoiding unnecessary surgical intervention in the majority of cases. Diagnosis of metastasis can be made easily and promptly. It is a simple and inexpensive technique with high sensitivity and specificity and has proved to be very useful in quick confirmation of the nature of the nodular skin lesion, so can provide purposeful accurate information to clinicians.

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ORIGINAL RESEARCH PAPER
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VITAMIN D DEFICIENCY IN TYPE 2 DIABETES MELLITUS PATIENTS: A HOSPITAL BASED OBSERVATIONAL STUDY.

Biochemistry

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ABSTRACT

Objectives: This present study was to evaluate the fasting blood sugar levels, BMI, HbA1C and vitamin D deficiency in type 2 diabetes mellitus patients.

Methods: 10 ml of venous blood was drawn after 8-10 hours of fasting of patients. It was then transferred to plain vial (5ml) and EDTA vial (5ml). After clotting, the samples were centrifuged at 3000 rpm for 10 minutes. Estimation of Fasting plasma sugar was done in TurboChem 240 auto analyzer using standard kits of Randox. And it was estimated by Glucose Oxidase-Peroxidase method. 25-hydroxyvitamin D serum level was measured by using the Instant vitamin D (CLIA) method using neo lumax semiautoanalyser machine. Estimation of HbA1c was performed by using identi HbA1c (Glycosylated Haemoglobin) Standard Set. BMI was calculated by the formula: BMI(kg / m2) = Weight(Kgms) / Height(m2).

Results: Data was analysed by using Instat 3 software. Mean and standard deviations were observed. Unpaired t test was applied. P value was taken less than or equal to 0.05 for significant differences ($p \leq 0.05$).

Conclusions: A significant relationship of vitamin D deficiency was found in type 2 diabetes mellitus patients. And also found a significant relation in FBS, HbA1C, BMI with type 2 diabetes mellitus. Hence, prompt investigation should be performed for the diagnosis of vitamin D deficiency in type 2 diabetes mellitus. So that, supplementation of vitamin D would be advise on proper time for the management of vitamin D deficiency in type 2 diabetes mellitus patients.

KEYWORDS

type 2 diabetes mellitus, vitamin D deficiency, FBS, HbA1C, BMI, age

INTRODUCTION

Vitamin D deficiency is estimated to affect over 1 billion people worldwide, and its prevalence is increasing in conjunction with Type 2 diabetes (T2D), obesity, and derangements in metabolic traits [1]. Recent studies have examined the physiological functions of Vitamin D beyond its well-established role in musculoskeletal health [3]. In addition to findings of oncologic and immunologic associations, Vitamin D deficiency is associated with metabolic derangements and T2D [2,3,4]. A serum level of <20 ng/ml (50 nmol/L) 25(OH)D is considered as Vitamin D deficiency, between 20 and 30 ng/ml as its insufficient level and higher than 30 ng/ml as its desirable or sufficient level [5].

Extensive studies focus on T2DM as the pathological defect after the deficiency of vitamin D takes place were the dominant. However, substantive studies need to be explored more to validate the strong relationship of cause and effect between vitamin D and its predisposing risk factors. The literature review is focusing on T2DM pathophysiology regulates the defect of bioavailability of vitamin D and lead to the pathological effects. Many observational studies are linking between vitamin D and glucose homeostasis impairment that is predisposed to T2DM that has been received great attention and plays a significant role in alteration of insulin mechanism [6,7].

Forouhi et al. [8] in the current issue of *Diabetes*. The sun is the primary source of vitamin D, which is synthesized endogenously in skin to produce cholecalficerol (vitamin D3), although a small proportion (_20%) of vitamin D comes through diet from a limited range of foods (in the form of ergocalciferol [vitamin D2] and vitamin D3) [9]. The main marker of vitamin D status is the metabolite 25-hydroxyvitamin D [25(OH)D], which is synthesized in the liver. The epidemiology of vitamin D status is inverse to that of diabetes, since blood levels of 25(OH)D decline with age and are lower in populations with increased skin pigmentation, such as African Americans and South Asians, and in people with obesity, while diabetes increases with age and obesity and is higher in these ethnic groups [10].

In a number of studies, it has been observed that 25-hydroxyvitamin D serum level is significantly lower in diabetic patients than healthy individuals. [11,12] Vitamin D affects the production and secretion of insulin as well as insulin sensitivity [13]. Objective of our study was to correlate the various parameter like BMI, HbA1C with vitamin D deficiency in type 2 diabetes mellitus patients.

MATERIALS & METHODS

This present study was conducted in Department of Biochemistry with the collaboration of Department of Medicine, Katihar Medical College and Hospital, Katihar, Bihar, India during a period from September 2018 to February 2019.

Entire subjects signed an informed consent approved by institutional ethical committee of KMCH, Katihar was sought.

A total of 100 subjects of type 2 diabetes mellitus with age group 40 years to 70 years with irrespective of sex were enrolled in this study. Patients with bone disorders, hepatic and renal diseases were excluded from this study.

A detail history, clinical examinations and relevant investigations were performed to all patients with type 2 diabetes mellitus.

METHODS:

Estimation of fasting plasma glucose:

10 ml of venous blood was drawn after 8-10 hours of fasting. It was then transferred to plain vial (5ml) and EDTA vial (5ml). After clotting, the samples were centrifuged at 3000 rpm for 10 minutes. Estimation of Fasting plasma sugar was done in using auto analyser Turbochem 240. And it was estimated by Glucose Oxidase-Peroxidase method.

Estimation of vitamin D: 25-hydroxyvitamin D serum level was measured by using the Instant vitamin D (CLIA) method using neo lumax semiautoanalyser machine and 30–100 ng/mL was determined as the natural amount. Moreover, samples having vit D (OH) <25 ng/mL was determined as vitamin D deficiency, samples having vit D <30 ng/mL was determined as inadequacy of vitamin D, and vit D >30 ng/mL was defined as adequacy of vitamin D [9].

Instant Vitamin-D (CLIA):

Assay Protocol:

Add 25 ul Calibrators/ Controls/Sample into appropriately labelled wells

↓

Add 100 ul Releasing Reagent into all the wells (Yellow colour)

↓ Mix

Incubate for 30 mins at R.T (20 – 26 degree C)

↓

Wash the wells 5 times with 350 ul of working Wash Buffer

↓

Add 100 ul Enzyme conjugate (Pink Colour)
↓ Do Not Mix.
Incubate for 30 mins at R.T (20 – 26 degree C)
↓
Wash the wells 5 times with 350 ul of working Wash Buffer
↓
Add 100 ul Working Signal Reagent
↓
Incubate for 5 mins at R.T (20 – 26 degree C in dark)
↓
Take the Reading on Radiance/ Neo-Lumax/ Lumax

Estimation of HbA1C:
Procedure for estimation of HbA1c by using identi HbA1c (Glycosylated Haemoglobin) Standard Set.

Preparation:
a. Remove 1 Kit of the Standard Set from the cold storage (2-8ºC) and allow to come to room temperature.
b. The Set consists of One vial of 2mL of "0" Value Standard (Level 1) which is a liquid ready to use and 4 vials each of 0.5mL of different levels (Level 2, Level 3, Level 4 & Level 5) of the Standard which are lyophilized material.

Reconstituting the Lyophilised Standard vials (Level 2, Level 3, Level 4 & Level 5):
c. Gently tap the caps of the vial containing the lyophilized Standards so that any portion adhering to the cap is dislodged.
d. Reconstitute one vial at a time.
e. Gently remove the cap and keep it upright on a clean surface.
f. Add 0.5 mL of de-ionised water to the vial.
g. Recap the vial and gently swirl it to dissolve the contents.
h. Gently invert the vial back and forth several times to ensure uniformity of the dissolved material.
i. Similarly reconstitute the remaining 3 vials also.
j. Keep the vials at Room Temperature for 30 minutes to ensure complete dissolution of the lyophilized material.

Handling "0" Standard (Level 1):
k. The "0" Standard (Level 1) is a liquid which is ready to use.
l. Gently remove the cap and keep it upright on a clean surface.
m. Aliquot 200 uL of the solution into micro centrifuge cups. Store it at 2-8ºC. Do not store it at -20ºC.
n. Mark each vial with a suitable marking to denote the content (eg GHL1 14/01 – to indicate HbA1c Standard Level 1, date of reconstitution & Lot No)

Aliquoting "Reconstituted Lyophilised" Standard (Level 2, Level 3, Level 4 & Level 5)
o. Aliquot One Level of Standard at a time.
p. Aliquot 50uL of the solution into Micro Centrifuge cups.
q. Mark each vial with a suitable marking to denote the content (eg GHL2 14/01 – to indicate HbA1c Standard Level 2, date of reconstitution & Lot No)
r. Similarly aliquot the remaining 3 vials of the reconstituted Standards also.
s. Store the aliquoted vials in the freezer compartment of the refrigerator.

Suggestion: Use different coloured micro centrifuge cups for each level of the Standard set for easy identification and to reduce the chances of error when using the aliquoted vials for Calibration.

Usage:
1) Take one aliquot vial of 200uL of the "0" Standard (Level 1) from 2-8ºC storage.
2) Take one aliquot vial each of the aliquoted ((Level 2, Level 3, Level 4 & Level 5) Standard set from the freezer compartment
3) Allow the vials to come to Room Temperature
4) Check the vials visually to ensure that there is no frozen material and the contents have completely thawed.
5) Gently mix the content using pipette by aspirating/dispensing few times
6) Standard Level 1 should be loaded directly (Point 1).
7) Take 5uL of from each thawed Standard aliquots (i.e Level 2, Level 3, Level 4 & Level 5) and add to 500uL of De-ionized Water. Allow to stand for 5 minutes for complete lysing of standards. Mix gently & ensure the solution is homogenous. Use the contents to perform Calibration.
8) Discard the vials on completion of successful Calibration.

Precautions: a. When mixing by swirling or inverting, ensure that no foaming takes place. b. Do not mix vigorously. d. Do not shake vigorously. e. Store the reconstituted material/aliquoted material/thawed material away from light. f. The frozen aliquots can be stored for 90 days in frozen condition. g. Thawed aliquots should be discarded at the end of the day. h. They should not be frozen again or stored at 2-8ºC. i. They should not be reused.

Figure.1. HbA1c Standard set (Aliquoted vials being stored in -20ºC).

BMI was calculated by the formula: BMI(kg / m2) = Weight(Kgms) / Height(m2)

STATISTICAL ANALYSIS
Data was analysed by using Instat 3 software. Mean and standard deviations were observed. Unpaired t test was applied. P value was taken less than or equal to 0.05 for significant differences ($p \leq 0.05$).

OBSERVATIONS
In this present study, a total of 100 type 2 diabetes mellitus patients with age group 45 to 70 years were enrolled. Out of 100 patients, group A had 50 patients with vitamin D level less than 20ng/ml. And group B had also 50 patients with vitamin D level more than 20 ng/ml. Out of 100 patients, 60% was males and 40% was females. And male and female ratio was 3:2.

Table.1. Various parameter of type 2 diabetes mellitus patients.

Parameters	Vit D< 20ng/ml (Group : A)	Vit D ≥20ng/ml (Group : B)	t-value	P-value
	Mean ± S.D (N=50)	Mean ± S.D (N=50)		
Age	63.38±6.378	57.96±7.188	3.990	0.0001
FBS	248.12±25.719	167.94±18.443	17.915	<0.0001
HBA1C	8.06±1.300	6.94±1.434	4.091	<0.0001
BMI	27.0±1.565	28.46±1.832	4.285	<0.0001

Mean age of group A patients was 62.6±5.206 years and group B was 56.8±6.145 years. And it was statistically extremely significant ($p<0.0001$).

Similarly, when mean value of FBS of group A 248.12±25.719 and group B 167.94±18.443 was compared. P value was found to be less than 0.0001. it shows extremely significant differences. When mean value of HBA1C of group A 8.06±1.300 and group B 6.94±1.434 was compared. P value was found to be less than 0.0001. it shows extremely significant differences. Similarly, when mean value of BMI of group A 27.000±1.565 and group B 28.46±1.832 was compared. P value was found to be less than 0.0001. it shows extremely significant differences.

DISCUSSIONS
Vitamin D, as a critical and essential micronutrient for human health, has received widespread attention for numerous non skeletal effects, including its potential in pancreatic insulin secretion and insulin action [14]. Epidemiological studies indicate that vitamin D deficiency is widespread in those with diabetes [15]. There is also ample evidence to suggest that a low level of serum 25-hydroxyvitamin D [25(OH)D], a generally accepted indicator of vitamin D status, is inversely associated with impaired glucose tolerance (IGT) and diabetes [16]. Moreover, higher vitamin D intakes are significantly associated with a lower risk of type 2 diabetes (T2DM) [17] and vitamin D and calcium supplementation can even improve glucose homeostasis in adults with impaired fasting glucose (IFG) [18].

Type 2 diabetes is characterized by impaired pancreatic b-cell function, insulin resistance, and systemic inflammation, and there is evidence that vitamin D modulates these mechanisms. Several lines of evidence support a role for vitamin in pancreatic b-cell function and regulation of insulin secretion. In in vitro and in vivo studies, vitamin D deficiency impairs glucose-mediated insulin secretion in rat pancreatic b-cells, whereas vitamin D supplementation restores insulin secretion [19,20,21,22,23]. Vitamin D may have a direct effect on b-cell function mediated by binding of the circulating active form, 1,25- dihydroxy vitamin D [1,25(OH)2D], to the vitamin D receptor, which is expressed in pancreatic b cells [24,25]. Furthermore, mice lacking a functional vitamin D receptor show impaired glucose stimulated insulin secretion, attributed to a reduction in insulin biosynthesis [26]. Importantly, activation of vitamin D may occur within the b-cell by the 25-hydroxyvitamin D–1a-hydroxylase enzyme (CYP27B1), which is expressed in b-cells, thereby allowing for a paracrine effect of circulating 25- hydroxyvitamin D [25(OH)D] [27].

In this present study, a total of 100 type 2 diabetes mellitus patients with age group 45 to 70 years were enrolled. And it was categorized into two groups (group A and group B). Group A had 50 patients with vitamin D level less than 20ng/ml. And group B had also 50 patients with vitamin D level more than 20 ng/ml. Patients with vitamin D level < 20 ng/ml was mean age 62.6±5.206 years and patients with vitamin D level > 20ng/ml was 56.8±6.145 years. And it was statistically extremely significant ($p<0.0001$). Out of 100 patients, 60% were males and 40% were females.

Bajaj AH et al. studied on correlation of vitamin D deficiency with type 2 diabetes mellitus patients and found that 25(OH)D was quantified in a total of 144 patients with a mean age of 55.95±10.95 in the diabetic patients and 44.42±16.79 in the non-diabetic patients. Among the participants, 52.7% were males in the diabetic group and 54.2% were males in the non-diabetic group [28].

In our present study, mean value of FBS of group A (248.12±25.719) and group B (167.94±18.443) was extremely statistical differences ($p<0.000$). Mean value of HBA1C of group A (8.06±1.300) and group B (6.94±1.434) was also statically significant differences ($p<0.0001$). Similarly, mean value of BMI of group A (27.000±1.565) and group B (28.46±1.832) was also statistically significant ($p<0.0001$).

Ghavam, et al. [29] studied on HbA1c and vitamin D deficiency in type 2 diabetic patients and stated that an inverse linear relationship between vitamin D with HbA1C ($P < 0.37$), FBS, ($P < 0.64$), BMI ($P < 0.59$), and disease duration ($P < 0.1$). There was also a direct linear relationship between HbA1C with FBS and disease duration ($P < 0.000$ and $P < 0.000$) and an inverse linear relationship between HbA1C and BMI ($P < 0.41$). Taheri et al. conducted a study on type 2 diabetes mellitus patients and concluded that vitamin D deficiency plays an important role in the pathogenesis of type 2 diabetes.

Suboptimal vitamin D status has emerged as a potential contributor to the pathophysiology of type 2 diabetes, with several lines of evidence supporting a role for vitamin D in pancreatic b-cell function and insulin sensitivity [30]. Several trials have examined the effect of vitamin supplementation (with or without calcium) on glycemia and insulin sensitivity in patients with type 2 diabetes; results are summarized in recent meta-analyses [31]. George et al. [31] performed a meta-analysis of trials among patients with type 2 diabetes or impaired glucose tolerance and noted a significant reduction of fasting glucose (~6 mg/dL) and improvement in insulin sensitivity in patients given vitamin D supplementation vs placebo. Ameta-analysis by Seida et al. [32] reported non statistically significant improvements in hemoglobin A1c (HbA1c), fasting glucose, and insulin sensitivity. In 2017, three meta-analyses of trials using vitamin in patients with type 2 diabetes reported concordant results [33]. In the first study, Wu et al. [33] found that vitamin D supplementation reduced HbA1c significantly (24 trials) and reduced fasting glucose non significantly (18 trials). Among patients with baseline 25-hydroxyvitamin D [25(OH)D],20 ng/mL, reductions in HbA1c and fasting glucose were significant. In the second study, Mirhosseini et al. [34] reported statistically significant reductions in HbA1c, fasting glucose, and insulin resistance (assessed by Homeostatic Model Assessment of Insulin Resistance) after vitamin D supplementation compared with placebo in an analysis of 24 trials. Finally, Krul-Poel et al. [35] reported no statistically significant improvements in HbA1c and fasting glucose favouring vitamin D. Although the summary results from these study support a beneficial effect of vitamin D supplementation on glycemia and insulin sensitivity in patients with type 2 diabetes.

CONCLUSIONS

This present study concluded that a significant relationship of vitamin D deficiency in type 2 diabetes mellitus patients. And also found a significant relation in HbA1C, BMI with type 2 diabetes mellitus. Hence, prompt investigation should be performed for the diagnosis of vitamin D deficiency in type 2 diabetes mellitus. So that, supplementation of vitamin D would be advise on proper time for the management of vitamin D deficiency in type 2 diabetes mellitus patients.

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Clinical and Biochemical Profile of Neonates with Hyperbilirubinemia in a Tertiary Care Center

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ABSTRACT
Background: *Neonatal hyperbilirubinemia, though of common occurrence, is a significant medical condition not only because of its impact on hospital discharge but more importantly because of its potential to cause serious long term neurological complications.*
Method: *In this retrospective study, data of 258 neonates treated for neonatal hyperbilirubinemia in the neonatal unit of MGM Medical College, Kishanganj, Bihar during the period January 2016 to December 2016 were analyzed and taken up for the study.*
Results: *In our study ABO incompatibility was the most common cause of neonatal hyperbilirubinemia followed by idiopathic, prematurity, Rh incompatibility and glucose 6 phosphate dehydrogenase deficiency, in addition to other minor causes. Male preponderance was seen. Unfortunately bilirubin encephalopathy (Kernicterus) was seen in a couple of cases.*
Conclusion: *ABO incompatibility is a very common cause of neonatal hyperbilirubinemia. Although historically Rh incompatibility has been accorded much importance, ABO incompatibility should alert the attending doctors about the impending risk of significant neonatal jaundice. If discharged early a written protocol should be followed where a revisit should be planed within 1 to 3 days for babies with any risk factor so that hyperbilirubinemia , if any, is detected and treated accordingly to prevent long term neurological morbidity.*
Key Words: *Neonates, Hyperbilirubinemia, ABO incompatibility, Prematurity, Jaundice, G6PD.*

INTRODUCTION

Neonatal jaundice is observed during the first week of life in 60% of full term infants and 80% of preterm infants(1). It is one of the most common causes of readmission in neonates and also a case of 'LAMA – Left Against Medical Advice' because of delay in discharge.

Neonatal Jaundice is broadly classified as physiologic and non physiologic hyperbilirubinemia; in the former the level rises to 6 to 8 mg/dl by 3-5 days of age and may reach up to 12 mg/dl and then falls. Non physiologic hyperbilirubinemia is one in which onset of jaundice is before 24hours of age or persists beyond 8 days. In fact any elevation of serum bilirubin that requires phototherapy is non physiologic (2).

This study was conducted to know the profile of neonatal hyperbilirubinemia in babies admitted in a

tertiary care hospital in Bihar, India. Although there are innumerable studies on neonatal jaundice worldwide, there are hardly any studies from this region.

MATERIALS AND METHODS

This retrospective study was conducted in MGM Medical College and LSK Hospital, Kishanganj, Bihar. The data was collected from the medical record section of the college.

A total of two hundred and fifty eight neonates were taken up for the study. Those babies who were admitted in the neonatal unit (NICU) during the period January 2016 to December 2016for the treatment of hyperbilirubinemia with bilirubin level of ≥14 mg/dl in case of term babies and ≥12 mg/dl in case of preterm babies were considered for the study.

All other details like history, physical examination, laboratory tests and those requiring phototherapy and /or exchange transfusion, were collected from the medical records and were thoroughly analyzed.

Details of laboratory investigation done which includeed – total bilirubin (conjugated and unconjugated bilirubin), blood group of mother and neonate, Hb, TC, DC, and CRP (sepsis screening), G6PD status, TSH levels were collected and analyzed in our study.

RESULTS

After thoroughly analyzing the data, a total of 258 neonates with hyperbilirubinemia – with bilirubin level ≥ 14 mg/dl in case of term babies and ≥12 mg/dl in case of preterm babies were included in the study. It was seen that out of 258 neonates 144 (55.8%) were male and 114 (44.2%) were female and 195 (75.58%) were full term babies whereas 63 (24.42%) were preterm babies. (Table 1)

Out of 195 full term babies 109 (55.90%) had bilirubin level between 14 to 17 mg/dl, 67 (34.36%) had bilirubin level between >17 to 20 mg/dl whereas 19 (9.74%) neonates had bilirubin level more than 20 mg/dl.

Amongst the 63 preterm babies 31 (49.21%) had bilirubin level between 12 to 15 mg/dl whereas 25 (39.68 %) babies had bilirubin level between >15 to 19 mg/dl and 7 (11.11%) had bilirubin levels more than 19 mg/dl. (Table 2)

ABO incompatibility was observed in 68 (26.36%) babies, out of which 37 (54.41%) were male and 31 (45.59%) were female. RH incompatibility was observed in 20 (7.75%) babies with 12 (60%) male and 8 (40%) female.

In 50 (19.38%) babies the cause of hyperbilirubinemia was not known. Out of these 50 babies 26 (52%) were male and 24 (48%) were female.

Out of 63 premature babies, in 46 (17.83%) other causes of hyperbilirubinemia were ruled out and prematurity itself was assigned as a cause of hyperbilirubinemia. Out of these 46 babies 27 (58.75) were male and 19 (41.3%) were female.

Sepsis was the cause of hyperbilirubinemia in 26 (10.08%) babies among them 15 (57.7%) were male and 11(42.3%) with female.

G6PD deficiency was observed in 18 (6.98%) babies among them 10 (55.6%) were male and 8 (44.4%) were female

There were 11 (4.26%) infant of diabetic mother who had hyperbilirubinemia out of which 6 (54.5%) were male and 5 (45.5%) were female.

Breast milk jaundice was seen in 8 (3.10%) babies out of which 5 (62.5%) were male and 3 (37.5%) were female.

Hyperbilirubinemia with cephalhematoma was observed in 4 (1.55%) babies with equal male 2 (50%) and female 2 (50%) distribution.

Hypothyroidism was the cause of neonatal hyperbilirubinemia in 3 cases with identical number seen in polycythemia as well. Gender distribution in case of hypothyroidism was 1(33.3%) male baby and 2(66.7%) female babies whereas it was just the opposite in case of polycythemia where 2 (66.7%) babies were male and 1 (33.3%) was female.

One (0.39%) baby with Down's syndrome also had hyperbilirubinemia. (Table 3)

Features of bilirubin encephalopathy (kernicterus) was seen in 2 neonates and both the babies had G6PD deficiency too.

Amongst the 258 neonates all the babies had received phototherapy as a part of the treatment for hyperbilirubinemia whereas only 21 babies had to undergo exchange transfusion.

Table 1. Gender distribution in neonates with hyperbilirubinemia

Gender	No of Neonates N = 258	(%)
Male	144	55.8
Female	114	44.2

Figure 1. Pie chart showing gender distribution in neonates with hyperbilirubinemia

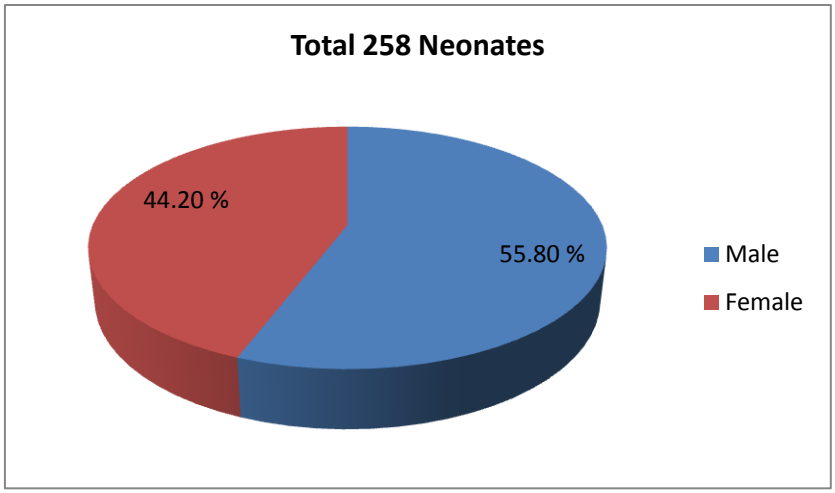


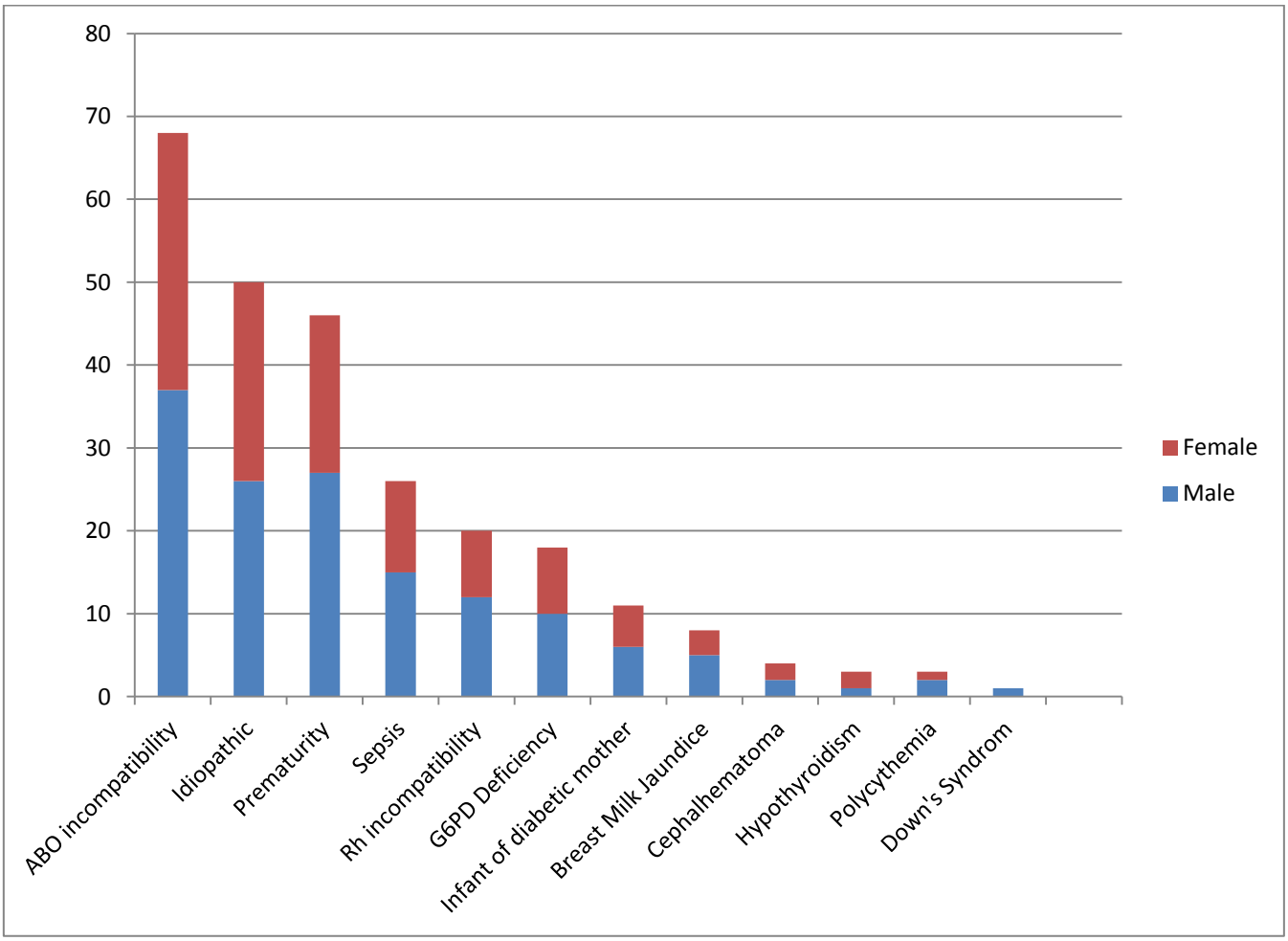
Table 2. Serum bilirubin levels in Term and Preterm Neonates

Full Term Neonates		Pre Term Neonates	
195 (75.58%)		63 (24.42%)	
Serum Bilirubin	No of neonates	Serum Bilirubin	No of neonates
14 – 17 mg/dl	109	12 – 15 mg/dl	31
> 17 – 20 mg/dl	67	>15 – 19 mg/dl	25
> 20 mg/dl	19	> 19 mg/dl	7

Table 3. Causes of Neonatal Hyperbilirubinemia

Various Causes	No of Neonates with Percentage		Male babies No with Percentage		Female babies No with Percentage	
ABO incompatibility	68	26.36 %	37	54.41 %	31	45.59 %
Idiopathic	50	19.38 %	26	52 %	24	48 %
Prematurity	46	17.83 %	27	58.7 %	19	41.3 %
Sepsis	26	10.08 %	15	57.7 %	11	42.3 %
Rh incompatibility	20	7.75 %	12	60 %	8	40 %
G6PD Deficiency	18	6.98 %	10	55.6 %	8	44.4 %
Infant of DM mother	11	4.26 %	6	54.5 %	5	45.5 %
Breast Milk Jaundice	8	3.10 %	5	62.5 %	3	37.5 %
Cephalhematoma	4	1.55 %	2	50 %	2	50 %
Hypothyroidism	3	1.16 %	1	33.3 %	2	66.6 %
Polycythemia	3	1.16 %	2	66.6 %	1	33.3 %
Down's syndrom	1	0.39 %	1	100 %	0	0 %

Figure 2. Bar diagram showing different causes of hyperbilirubinemia in neonates and their gender distribution



DISCUSSION

Hyperbilirubinemia is quite common in newborn and multiple factors are responsible for occurrence of neonatal hyperbilirubinemia. A review article published in North America suggested that the etiology of neonatal hyperbilirubinemia is multifactorial [(3)], we also got similar results. In our study out of 258 babies 144 were male and 114 were female. Male preponderance was observed in previous Publications too [(4,5)].

ABO incompatibility was the most common cause of pathological hyperbilirubinemia in our study with 26.36 % of babies having ABO incompatibility similar to the results of other studies. Study done by Anil Shetty et al: Neonatal hyperbilirubinemia in a tertiary care hospital showed ABO incompatibility as the most common cause of hyperbilirubinemia [(6, 7)]. Similar study done by Mishra at al: Hematological profile in neonatal jaundice showed 20% of neonates develop hyperbilirubinemia due to ABO incompatibility [(4,8)].

Idiopathic as an etiology was observed in 15.5% cases in a study by Singh S K et al [(9)]. In our study, in 19.38% cases, a definite cause could not be ascertained. Similar findings were observed in an Iranian study where 118 neonates were investigated for the cause of neonatal hyperbilirubinemia and the researchers revealed that in 25.4% of neonates the etiology could not be ascertained [(10)]. A Canadian study also revealed that in the majority of neonatal hyperbilirubinemia cases the underlying cause was not identified[(11)]. Many authors have unable to establish the etiology of hyperbilirubinemia in more than half of the cases in their series[(12)].

Prematurity is an important cause of neonatal hyperbilirubinemia and has been well documented in the literature[(13,14,15)]. In our study too as many as 18% of the cases were due to prematurity.

Sepsis is a significant cause of neonatal hyperbilirubinemia and the fact is supported by a very large number of literature published worldwide [16, 17].

Another important cause of hyperbilirubinemia is Rh incompatibility. Rh incompatibility has been shown as a risk factor for hyperbilirubinemia in newborns in many studies [4,9,18,19].

G6PD deficiency is a fairly common cause of neonatal hyperbilirubinemia but not investigated routinely in developing countries particularly because of the cost involved, as also observed in a study by Anil Shetty et al [5]. G6PD deficiency can lead to severe neonatal hyperbilirubinemia, as reported in many studies. In a study conducted by Basoi S et al in a tertiary care hospital in West Bengal showed that 14.68% of the newborn were G6PD deficient and 23.8% of them developed severe neonatal hyperbilirubinemia compared to 12.5% of non G6PD deficient who developed severe neonatal hyperbilirubinemia [20]. A cohort study was carried out to access the association between G6PD deficiency and neonatal hyperbilirubinemia. Data suggest that the G6PD deficient neonates are at increased risk of hyperbilirubinemia even in the nursery free from agent that can potentially cause hemolysis to G6PD deficient red cells.[21] Although hemolysis may be observed in neonates who have G6PD deficient and are jaundiced. [22] Other mechanisms appear to play a more important role in the development of hyperbilirubinemia. [23, 24, 25]

Infant born to diabetic mothers are also prone to hyperbilirubinemia. In our study too we had 11 neonates born to diabetic mother who had hyperbilirubinemia. Studies shows that large for gestational age infant of diabetic mother are at increased risk of hyperbilirubinemia then average for gestational age infant of diabetic mother and infant of non diabetic mothers and that increased heme turnover is a significant factor in the pathogenesis [26].

Breast milk jaundice occurs later in the newborn period with the bilirubin level usually peaking in the 6th to 14th day of life [27]. This late onset jaundice may develop in up to one third of healthy breast fed infants[28]. The underlying cause of breast milk jaundice is not clearly understood. Substance in maternal milk suggests beta glucuronidases and non esterified fatty acids, may inhibit normal bilirubin metabolism [29-32].

Cephalhematoma is a rare but not uncommon cause of hyperbilirubinemia. In our study we had 4 babies with Cephalhematoma leading to hyperbilirubinemia. Hypothyroidism polycythemia and Down's syndrome can also lead to hyperbilirubinemia in many neonates and this is well documented in many studies. In our study too few cases were seem to develop hyperbilirubinemia due to the above three causes.

CONCLUSION

ABO incompatibility is very common cause of neonatal hyperbilirubinemia. Although historically Rh incompatibility has been accorded much importance, ABO incompatibility should alert the attending doctor about the impending risk of significant neonatal jaundice. If discharged early written protocol should be followed where a revisit should be planned within 1 to 3 days for babies with any risk factors so that hyperbilirubinemia if any is detected and treated accordingly to prevent long term neurological morbidity. In this era where India has achieved significant advancement in the field of Medical Science, even a few cases of bilirubin encephalopathy and its associated sequelae is a grim reminder of the state of affairs of our health delivery system and ignorance among the population at large. Much needs to be done to spread awareness particularly in the rural areas where people still approach traditional healers to treat jaundice leading to delay in timely medical interventions.

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Pattern of glucose 6 phosphate dehydrogenase deficiency in neonates with hyperbilirubinemia in a tertiary care center

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Abstract
Background: G6PD deficiency leading to neonatal hyperbilirubinemia is not an uncommon disorder in India. However it is highly under diagnosed and poorly reported.
Method: In this retrospective study, data of 108 neonates treated for neonatal hyperbilirubinemia and also screened for G6PD deficiency in the neonatal unit of MGM Medical College, Kishanganj, Bihar and Medica North Bengal Clinic, Siliguri, West Bengal during the period January 2016 to December 2016 were analysed and taken up for the study.
Results: Out of 108 neonates who were screened for G6PD deficiency, 11.1 1% were found to be deficient with a male predominance. Bilirubin encephalopathy was seen in 6 babies. ABO incompatibility was the most common cause of neonatal hyperbilirubinemia.
Conclusion: Screening for G6PD deficiency routinely in all cases of neonatal hyperbilirubinemia or at least in cases where more common causes are ruled out could go a long way in preventing lifelong consequences of bilirubin toxicity and reducing the burden of handicapped children to the parents and the society at large.

Keywords: hyperbilirubinemia, MGM, LSK

1. Introduction
Jaundice is observed in more than 80% of all healthy new newborns during the first week after birth [1, 2]. Although this condition must have been noticed by caregivers throughout the centuries but the first scientific description of neonatal jaundice was made by Baumes in later part of the 18th century in Paris. Although most jaundiced infants are otherwise perfectly healthy, they make us anxious because bilirubin is potentially toxic to the central nervous system [3]. Hyperbilirubinemia may cause bilirubin encephalopathy with disastrous consequences in some infants.
In day to day clinical practice in India in addition to history taking and clinical examination, a neonate with neonatal jaundice is commonly subjected to the following lab investigations: hemoglobin, sepsis screening, blood group including mothers and serum bilirubin with fractions. However hyperbilirubinemia in newborn period can be associated with severe illness such as hemolytic disease, metabolic and endocrine disorders, anatomic abnormalities of the liver and infections.
Glucose 6 phosphate dehydrogenase deficiency is the most frequent human enzyme defect estimated in approximately 400 million individuals worldwide [4, 5]. The incidence of neonatal jaundice is several folds higher in G6PD deficient infants compared with those who are sufficient [6].
This study was conducted primarily to highlight the importance of G6PD in all jaundiced neonates to facilitate early diagnosis and prevent long term morbidity.

2. Method
This is a retrospective study conducted in MGM Medical College and LSK Hospital Kishanganj, Bihar and Medica North Bengal clinic, Siliguri, West Bengal. The data were collected from record sections of the two centers. In our study we have included only those cases who were hospitalized for the treatment of hyperbilirubinemia with bilirubin level ≥14 mg/dl in case of term babies and ≥12 mg/dl in case of preterm babies and each one of them screened for G6PD deficiency during the period January 2016 to December 2016.
All the vital information like history, physical examination laboratory data and those requiring phototherapy and/or exchange transfusion were collected from the records and were analysed thoroughly.
From the medical record each file was studied for birth weight, age at the time of presentation, gestational age, G6PD status, total bilirubin including direct and indirect, blood group and Rh typing of baby and mother, CRP, Hb, TC, DC (sepsis screening), TSH, presence of cephalhematoma and reticulocyte count.

3. Results
In this retrospective study involving 108 neonates who were hospitalised with hyperbilirubinemia and were screened for G6PD deficiency during the period January 2016 to December 2016, 62 (57.41%) were male and 46 (42.55%) were female.
Eight (7.4%) babies were large for gestational age, 66 (61.1%) were appropriate for gestational age whereas 34 (31.5%) neonates were small for their gestational age.
Among the 108 neonates 87 (80.6%) neonates were term

babies whereas 21 (19.4%) were preterm. Out of the 87 full term babies 49 had bilirubin level between 14 to 17 mg/dl, 35 had bilirubin level between >17 to 20 mg/dl, one neonates had bilirubin level between >20-25 mg/dl and 2 babies had bilirubin levels more than 25 mg/dl. There were 21 preterm babies out of which 12 babies had bilirubin level between 12 to 17 mg/dl, 6 babies had bilirubin level between >17 to 20 mg/dl whereas 2 babies had bilirubin level >20 – 25 mg/dl and one baby had bilirubin level >25 mg/dl.

The age at the time of presentation was less than 3 days in case of 8 (7.4%) whereas 69 (63.9%) neonates presented between day 3 to day 7 and 31 (28.7%) neonates presented after 7 days of age.

Among the various causes of hyperbilirubinemia ABO incompatibility was found to be the most common cause. In our study 26 (24.07%) neonates had hyperbilirubinemia due to ABO incompatibility. Sixteen (61.54%) were male and 10 (38.46%) were female.

In 22 babies, the cause of hyperbilirubinemia was not known - 12 (54.55%) among them were male and 10 (45.45%) were female. Out of 17 premature babies who had hyperbilirubinemia, 9 (52.94%) were male and 8 (47.06 %) were female. In our study 10 (9.26%) neonates with hyperbilirubinemia had sepsis with equal male and female distribution. Rh incompatibility was seen in 9 (8.33%) babies among whom 4 (44.4%) were male and 5 (55.6%) were female.G6PD deficiency was seen in 12 (11.11%) babies with male predominance: where 9 (75%) were male and 3 (25%) were female. Out of these 12 babies all had serum bilirubin level approaching or beyond 20 mg/dl.

Other causes of hyperbilirubinemia in our study were as follows: Breast milk jaundice 6 (5.56%), cephalhematoma 3 (2.78 %), hypothyroidism 2 (1.8 5%) and polycythemia 1 (0.9 3%).

In case of breast milk jaundice gender distribution was equal that is 3 male and 3 female babies. In case of cephalhematoma 2 neonates were male and one was female whereas in case of hypothyroidism both the babies were male. One female baby had polycythemia.

Kernicterus was found in 6 babies, out of which 5 were male and 1 was female and among them 4 were G6PD deficient and one each had Rh incompatibility and ABO incompatibility. The latter two were home delivered.

All the babies were given double surface phototherapy covering the eyes and genitalia. Exchange transfusion was necessary in 10 cases.

4. Discussion

Our study involved 108 neonates who were treated for neonatal hyperbilirubinemia with varied etiology. All of them were subjected to screening for glucose 6 phosphate dehydrogenase deficiency. The most common cause of hyperbilirubinemia was ABO incompatibility followed by idiopathic, prematurity, G6PD deficiency, sepsis, Rh incompatibility, breast milk jaundice, cephalhematoma, hypothyroidism and polycythemia. Similar observations with regard to etiology was seen in several studies [7, 8].

Although glucose 6 phosphate dehydrogenase deficiency was not amongst the most common causes of hyperbilirubinemia, this study was undertaken primarily because of the fact that there is no study on G6PD deficiency and hyperbilirubinemia in a region of eastern Bihar and adjoining North Bengal.

Hyperbilirubinemia in newborn with glucose 6 phosphate dehydrogenase deficiency is a serious clinical problem because of the severity and unpredictability of its course (9). It has been observed that G6PD deficiency neonates are at increased risk for hyperbilirubinemia even in the nursery, free from agent that can potentially cause haemolysis to G6PD deficiency red cells [10].

Jaundice in G6PD deficient neonates is considered to be due to an imbalance between the production and conjugation of bilirubin, with a tendency for inefficient bilirubin conjugation. Borderline premature infants are at special risk of the bilirubin production conjugation imbalance [11].

G6PD deficiency is an X-linked disease that primarily affects men. Women may be affected if they are homozygous, which occurs in populations in which the frequency of G6PD deficiency is quite high. Heterozygous women (carriers) can experience clinical disease as a result of X- chromosome inactivation, gene mosaicism or hemizygosity [12]. In our study too, there was male preponderance with 9 male and 3 female who has G6PD deficiency.

G6PD deficiency can lead to an increased risk and early onset of hyperbilirubinemia [13, 14] which may require phototherapy or exchange transfusion [4, 14]. In certain population hyperbilirubinemia secondary to G6PD deficiency results in an increased risk of kernicterus and death [15, 16]. Whereas in other populations this has not been observed [17]. This may reflect genetic mutations specific to different ethnic groups [17, 18].

The highest prevalence of G6PD deficiency is reported in Africa, Southern Europe, The Middle East, Southeast Asia and the central and Southern Pacific Islands; however G6PD deficiency has now migrated to become a worldwide disease [19].

Deficiency of this enzyme was reported from India more than 50 years back. The prevalence varies from 2.3 to 27% with an overall prevalence of 7.7% in different tribal group [20]. A study by Bisoi *et al* reported G6PD deficiency in 14.68% of live newborns in Kolkata, West Bengal [21]. In our study, out of 108 neonates 12 babies 11.1 1% were deficient in G6PD.

Since the subject evaluated in our study belonged to a mixed population including Bengali, Bihari, Surjapuris, Adivasi, Gorkhas, Koch Rajbanshi etc of Eastern Bihar and adjoining North Bengal practicing Hinduism, Islam, Buddhism and Christianity, commenting on their ethnicity and tribal status is beyond the scope of this study.

In a study by Vandana Rai and Pradeep Kumar [22], G6PD deficiency in Muslim community of Junapur district of Uttar Pradesh was 13%. Similar data were reported from Muslim populations of other neighboring Asian countries: Bangladesh 3.3 to 20% [23], Pakistan 1.07 to 3.17% [24], Malaysia 3.3 to 17% [25] and Indonesia 2.7 to 17.5% [26].

In the US Pilot Kernicterus Registry, 20.8% of newborns cared for as healthy infants readmitted within a week of discharge with acute bilirubin encephalopathy were subsequently diagnosed to have G6PD deficiency [27]. Similar findings from a survey from the United Kingdom and Ireland reported hundred and eight newborns with extreme neonatal hyperbilirubinemia (30 mg/dl) in whom G6PD deficiency

independently increased the risk of encephalopathy many fold [28].

In our study 6 babies had presented with or developed features of bilirubin encephalopathy with 4 of them being G6PD deficient. Exchange transfusion was necessary in 10 babies.

5. Conclusion

G6PD deficiency leading to neonatal hyperbilirubinemia is not an uncommon disorder in India as seen from several publications. Delay in recognition can lead to rapid progression of severe hyperbilirubinemia and consequent bilirubin induced neurological damage. However due to lack of parental awareness about G6PD deficiency and an ongoing practice among clinicians of 'not including' G6PD screening routinely in jaundiced neonates, the condition is highly under diagnosed and poorly reported with negligible research in this field in our country. Screening of all neonates with neonatal jaundice for G6PD deficiency would be ideal. However lack of facilities in all centers coupled with financial constraints are likely to play spoilsport. So screening for G6PD deficiency could perhaps be done routinely in all those cases in which other more common causes are ruled out. This step alone could go a long way in preventing lifelong consequences of bilirubin toxicity and reducing the burden of handicapped children to the parents and society at large.

Table 1: Gender distribution in neonates with hyperbilirubinemia

Gender	No of Neonates	
	N = 108	(%)
Male	62	57.41%
Female	46	42.59%

Fig 1: Pie diagram showing gender distribution in neonates with hyperbilirubinemia.

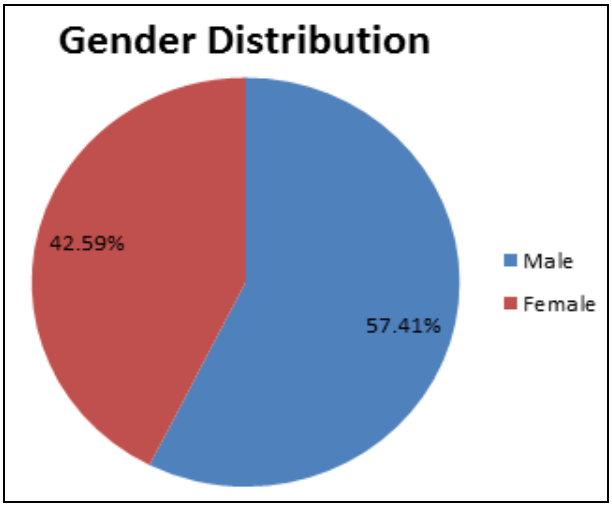

Table 2: Serum bilirubin levels in Term and Preterm Neonates

Full Term Neonates		Pre Term Neonates	
87 (80.6%)		21 (19.4%)	
Serum Bilirubin	No of neonates	Serum Bilirubin	No of neonates
14 – 17 mg/dl	49	12 – 17 mg/dl	12
> 17 – 20 mg/dl	35	>17 – 20 mg/dl	6
> 20 – 25 mg/dl	1	> 20 - 25 mg/dl	2
>25 mg/dl	2	>25 mg/dl	1

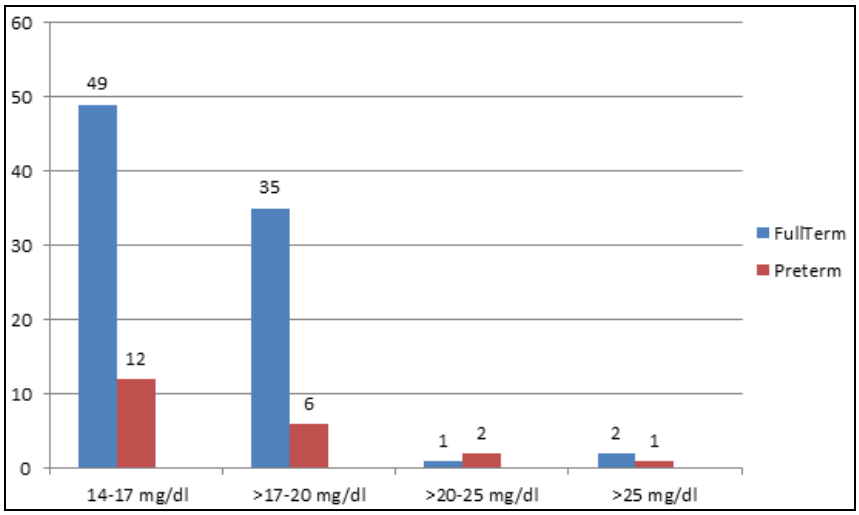

Fig 2: Bar diagram showing serum bilirubin levels in term and preterm neonates.

Table 3: Birth weight and age at the time of presationation.

Birth Weight		Age at the time of presentation	
Weight	No of neonates with percentage	Age	No of neonates with percentage
LGA	8 (7.4%)	<3 days	8 (7.4%)
AGA	66 (66.1%)	3 – 7 days	69 (63.9%)
SGA	34 (31.5%)	>7 days	31 (28.7%)

Table 4: Causes of Neonatal Hyperbilirubinemia

Various Causes	No of Neonates with Percentage		Male babies No with Percentage		Female babies No with Percentage	
ABO incompatibility	26	24.07%	16	61.54%	10	38.46%
Idiopathic	22	20.37%	12	54.55%	10	45.45%
Prematurity	17	15.74%	9	52.94%	8	47.06%
G6PD Deficiency	12	11.11%	9	75%	3	25%
Sepsis	10	9.26%	5	50%	5	50%
Rh incompatibility	9	8.33%	4	44.4%	5	55.6%
Breast Milk Jaundice	6	5.56%	3	50%	3	50%
Cephalhematoma	3	2.78%	2	66.67%	1	33.33%
Hypothyroidism	2	1.85%	2	100%	0	0%
Polycythemia	1	0.93%	0	0%	1	100%

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