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Role of cardiac biomarkers in assessing risk in chronic kidney disease

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Abstract

Aim: To find out whether assessment of blood levels of troponin I and cardiac enzymes can be utilised to detect high-risk cases of chronic kidney disease (CKD).

Materials and methods: 109 patients diagnosed to have CKD who were undergoing dialysis or attending the OP of the department of nephrology were studied. Blood levels of different cardiac biomarkers were estimated for all the patients. Patients were staged by calculating eGFR. Correlation between the cardiac biomarker levels and stages of CKD was done statistically.

Results: Statistically significant correlation existed between stage of kidney disease and troponin I levels (R value -0.1.5).

Conclusion: Estimation of cardiac markers to assess the cardiac status of CKD patients will be beneficial in detecting patients having cardiac problems complicating CKD.

Introduction

There has been an increase end-stage renal disease (ESRD) patients all over the world[1]. Among the CKD population, CAD is highly prevalent. Prevalence has been estimated to vary from 15% to 73%. The wide range in prevalence is mostly because many cardiac disease cases present asymptomatically. It is estimated that > 50% of patients – particularly diabetics – are asymptomatic[2].

Cardiac disease is also the major cause of death in patients with ESRD, accounting for about 45% of all deaths[3]. There has been a dramatic 40-fold increase in death rates among dialysis patients, as compared to the general population as was first reported by Sarnak and Levey[5]. This appears to be associated with the heavy burden of cardiovascular disease (CVD) among patients with ESRD[6]. The prevalence of CVD at initiation of dialysis also has increased dramatically from 25% in 1984 to 40% by 1999, in United States[7]. But, despite advances in chronic heart failure treatment, the prognosis of these patients remains poor[8].

Even though many studies show the importance of renal function tests and other biochemical parameters in assessing prognosis in CKD patients, less emphasis has been placed on biomarkers of cardiac function. Hence a study to assess the relationship between cardiac biomarker levels in blood and renal function will help in diagnosing the high-risk group among CKD patients. This will also help to divert resources to the more needed group of patients in the society as CKD is becoming a very common public health problem especially among the elderly.

Material and methods

109 patients, including 32 (38.5%) females and 87 (61.5%) males were studied. Mean value of the different parameters studied are given in Table I.

Table I: Mean values of the parameters studied.

	Mean	Studied deviation
Age	45.69 years + SD	13.76
Urea	96.04 mg%	35.1, g%
Creatinine	7 mg%	3.6 mg%
AST	38.67 U/L	23.74 U/L
CK-MB	11.831 U/L	14.28 IU/L
Troponin 1	5.24 ng/ml	13.02 ng/ml

After calculating eGFR from serum creatinine level using MDRD formula, patients were staged. Patients belonged to stages II, III, IV and V. There were no patients of stage I. Distribution of patients is as follows: stage V – n - 80, stage IV – n - 11, stage III – n - 14, stage II – n - 4 and stage I – n - 0.

Among cardiac biomarkers tested, means of AST and CK MB levels were within normal limits (mean AST – 38.67U/ml, CK MB - 11.831 U/ml). Mean troponin-I level was found to be high – 5.24 ng/ml.

Stagewise distribution of blood levels of the different parameters is given in Table II.

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Table II: Stagewise distribution of blood levels of the different parameters.

		N	Studied deviation	P value
Trioponin I	Stage II	4	.042	
	III	14	.492	
	IV	11	9.82	
	V	80	14.57	
	Total	109	13.02	0.329
AST	Stage II	4	1.59	
	III	14	32.07	
	IV	11	22.32	
	V	80	22.54	
	Total	109	23.74	0.175
CK-MB	Stage II	4	10.5	
	III	14	15.28	
	IV	11	10.29	
	V	80	14.16	
	Total	109	14.28	.024

Among the different parameters tested, the mean troponin I level increased with higher stages. Stage II - 0.043 ng/ml, stage III - 0.198 ng/ml, stage IV - 4.64 ng/ml, and stage V - 6.46 ng/ml. Mean AST level was not found to increase with stage of disease. Highest mean AST level was seen in stage IV. Similarly in the case of CK-MB also, highest mean value was found in stage III.

Levels of these cardiac biomarkers were compared between the 4 stages of kidney disease by ANOVA using SPSS version 16 (Table III).

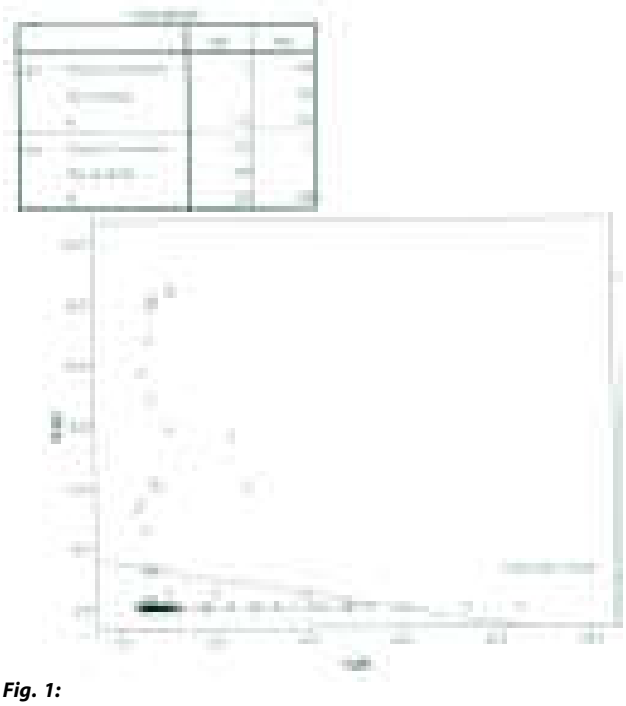

Fig. 1:

Table III: Comparison of markers between different stages using ANOVA.

	Significance
Troponin I	.329
AST	.175
CK-MB	.024

CK MB values showed significant variation between the 4 stages (p - .024). The mean of each of these parameters in each stage was compared with the means in other stages using Bonferroni method also. This also showed statistically significant results in the case of CK-MB. At the same time, variation in troponin I, and AST values between the 4 stages was not significant statistically.

Correlation of troponin I, CK MB, and AST with eGFR which is indicative of stage of kidney disease was done by Pearson correlation. Correlation was found to exist between eGFR and troponin I level but it was not found to be statistically significant.

Discussion

Recent publications show an increase in the extent of CKD in the general population[9]. There are reports on the increasing burden of CVD associated with the CKD population also[10]. In fact, 5 - 10 times higher rate of death has been documented among CKD patients which is assumed to be because of CVD[5]. Among cardiac diseases, ischaemic heart disease (39%), congestive heart failure (41%), arrhythmia (31%), and other heart diseases (63%) have also been documented to be on the rise among CKD patients in various reports[5,11]. Chronic kidney disease patients showed a 5-times higher rate of hospitalisation for congestive heart failure, greater than that of non-CKD patients[5]. These point to the fact that monitoring for markers of ischaemic heart disease is not sufficient to identify the high-risk group among CKD patients.

Estimation of B-type natriuretic peptide measurement (BNP and NT-proBNP) can be done for the diagnosis of acute decompensated HF[12]. To reduce cardiovascular complications in diabetic patients, the American Diabetes Association (ADA) and the American Heart Association (AHA) have recommended the performance of lipid studies and glycosylated haemoglobin testing during routine monitoring of CKD patients[13,14,15].

Detailed studies have shown high levels of biomarkers for myocardial ischaemia among CKD patients[11,12]. Much of this increase has been attributed to reduced renal clearance, structural alterations of the cardiac muscle during uraemia, myocardial cell injury due to overproduction and release of pro-inflammatory cytokines, particularly tumour necrosis

factor-alpha, interleukin (IL)-1 and IL-6 and not myocardial ischaemia as such[16].

The main disadvantage in this study was the gross disparity in the number of patients belonging to each stage of CKD. As patients were selected not considering the stage of disease and they were staged afterwards, equal number could not be included in all the stages. There were no patients of stage I probably because our hospital is a tertiary care centre and only higher stages reach here.

Even though increase in mean level of troponin I with increasing stage of CKD and correlation with regard to CK-MB was documented in this study also, the high difference in the number of patients belonging to each group must be the reason for the difference not being statistically significant. Statistically significant difference can be documented only by doing a more detailed study including more patients belonging to each stage of the disease. A study that includes analysis of parameters for assessing other cardiac disorders along with myocardial ischaemia will be ideal for risk stratification.

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RESEARCH ARTICLE

'Lipid Profile in Female Breast Cancer: a study from Kerala, South India'

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Abstract

The incidence of breast cancer is on the rise in India.Exposure levels and prevalence of established risk factors may be different in developing and developed nations. The role of dyslipidemia in breast cancer initiation is not completely understood. This study aims to compare the serum lipid levels in female breast cancer patients with normal healthy controls in a rural district in Kerala.

Materials and Methods

Fasting lipid profile of 113 histologically proven female breast cancer patients and 88 healthy females were studied. Statistical analysis were performed using SPSS version 16.

Results

The median age at diagnosis of breast cancer in the study population was 48 years (Standard deviation 10.04). The youngest patient was aged 27 years, and the oldest patient, 82 years. 56.63% (64 out of 113) of the patients were postmenopausal. High levels of Low density lipoprotein (LDL), Triglycerides (TG) and total cholesterol (TC) were observed in breast cancer patients compared to healthy control.

Conclusion

This study supports the hypothesis that total cholesterol, LDL-C and triglycerides are important risk factors in the development of breast cancer. Higher consumption of animal fat and red meat, make the population of Kerala prone to a host of diseases, among them the development of breast cancer.

Background

INTRODUCTION

The incidence of breast cancer is on the rise in India [1]. Aetiology of breast cancer is multifactorial, and includes genotype and lifestyle-related factors. Important lifestyle factors believed to contribute towards the development of breast cancer include obesity, decreased physical activity and excessive consumption of animal fat and alcohol [2]. The role of lipoprotein fractions in the development and proliferation of breast cancer has been reported in many in vitro studies [3, 4,5]. The role of lipid fractions in cancer has extensively studied in developed countries, but it is still a matter of controversy [6, 7, 8]. Exposure levels and prevalence of established risk factors may be different in developing and developed nations.

This study aims to compare the serum lipid levels in female breast cancer patients with normal healthy controls in a rural district in Kerala.

Materials and Methods

This study was conducted in the Department of Biochemistry, Government Medical College,Thrissur. The study group consisted of 113 histologically proven female breast cancer patients and controls were 88 healthy females. 113 consecutive breast cancer patients who underwent surgery in the New Medical College Hospital between October 2012 and December 2013 were selected as cases. Those who were on lipid lowering therapy were excluded from the study population. Patient data including age, menopausal status, grade of the tumor, side which was affected, height and weight were collected. The study was approved by the Institutional Research Committee.

Blood samples were collected after overnight fasting (> 8 hours) by venipuncture. Estimation of serum lipid profile (Total cholesterol-CHOD-POD method; Triglycerides-GPO-POD method, HDL-Cholesterol (HDL-C) - indirect method by selective precipitation of low density lipoprotein cholesterol by phosphotungstate and MgCl2) was carried out using EM 360 Autoanalyser (Transasia) utilizing kits provided by Agappe diagnostics. LDL cholesterol (LDL-C) was calculated using the Friedewald formula.

Statistical analysis was performed using SPSS version 16. Data was analyzed using Student's t test.

p-value less than 0.05 is considered statistically significant.

Results

The median age at diagnosis of breast cancer in the study population was 48 years (Standard deviation 10.04). The youngest patient was aged 27 years, and the oldest patient, 82 years. 56.63% (64 out of 113) of the patients were postmenopausal. 59.29% (67 out of 113) had right-sided tumors and 55.75% (63 out of 113) had grade 2 disease. Age distribution is shown in Table 2 and comparison of lipid profiles of cases and controls in Table 3.

Table: 1 General characteristics of study population

	Cases(N-113)	**Controls(n-88)**
Age		
Menopausal Status		
Premenopausal	49	30
Post-menopausal	64	58
BMI	22.90±1.51	22.82±1.65
Side of breast affected		
Right	67	
Left	46	
Grade of the tumor		
I	14	
II	63	
III	36	
Diet History		
Non Vegetarian	106	84
Vegetarian	7	4

Table 2. Age distribution of patients and controls

Age	**Cases N (%)**	**Controls N (%)**
≤ 30	02(01.7)	0
31-40	20(17.6)	08(09.0)
41-50	47(41.6)	24(27.3)
51-60	28(24.8)	42(47.7)
61-70	12(10.6)	14(06.2)
≥ 70	04(03.5)	0
Total	**113**	**88**

Table 3. Comparison of lipid parameters among cases and controls

	Cases (Mean ± SD)	Controls (Mean ± SD)	p-value
Total Cholesterol	203.43±46.19	192.93±39.6	0.004
HDL-C	62.25±19.74	62.23±21.85	0.946
LDL-C	111.48±44.46	98.80±39.11	0.044
TG	146.56±57.0	169.48±67.4	0.034

Discussion

Carcinoma breast is now the commonest oncological disease among women in Kerala. Changes in lifestyle and diet are thought to be associated with an increase in breast cancer in developing countries [9]. Local eating habits with increased consumption of animal fat, alcohol and sedentary life style have been attributed to many cancers including breast cancer. An association with serum lipids and lipid fractions has been reported in many cancers [10, 11].

Median age of breast cancer patients in the study group was 48 years with around 41.6% (47 out of 113) in the perimenousal age group. It has been reported that this is also the age when prevalence of hyperlipidemia rises among women [12].

In the current study we found that total cholesterol, LDL-C and TG were significantly associated with breast cancer compared to the age-matched control group. This result concurs with the findings of previous studies [11, 13, 14, 15, 16, 17]. No association was observed between HDL-C and breast cancer. Our results are not in agreement with some studies that have failed to show a statistically significant association between total cholesterol and breast cancer [18, 19].

Since endogenous sex steroids are significantly related to the development of breast cancer, it has been hypothesized that cholesterol is an important risk factor for the development of breast cancer [20].It has been reported that low HDL-C is a marker of relative androgen excess [21]. If there is excess androgens in the body, aromatization of these will promote breast cancer development. Elevated levels of LDL-C may result in increased lipid peroxidation, and low HDL-C may also cause accumulation of reactive oxygen species and free radicals, thus favouring tissue injury, and in turn carcinogenesis [22].

Conclusion

Our study supports the hypothesis that total cholesterol, LDL-C and triglycerides are important risk factors in the development of breast cancer. It also reinforces the importance of control of these factors and thereby reducing the incidence and mortality associated with breast cancer.Higher consumption of animal fat and red meat make the population of Kerala prone to a host of diseases, among them the development of breast cancer.

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STUDY OF THE RISK FACTORS FOR THE DEVELOPMENT OF RETINOPATHY IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT FAMILY HISTORY

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ABSTRACT

Diabetic retinopathy (DR) is one of the most common and specific complications of type 2 diabetes mellitus (T2DM).[1-3] It is estimated that almost half of the patients with T2DM have some degree of retinopathy at any given time and 75 % of T2DM develop DR after 15 year duration of diabetes.[4,5] Even though visual morbidity from diabetes is a significant public health problem, this is largely preventable and treatable if managed with timely intervention, and thus the quality of life can be preserved. Hence it is important to precisely identify the risk factors for the development and progression of DR. Though risk factors of DR such as duration of diabetes, glycemic control, hypertension and dyslipidemia are extensively studied, other incompletely defined factors also may be involved in the development of complications because many people with long standing diabetes never develop any complications like nephropathy or retinopathy.[5] A few previous studies in the Western population investigated the role of heredity in the development and progression of DR.[6] Very few data are available from the Indian subcontinent.[7] This study examined the role of family history in the prevalence and severity of DR and, analysed whether there is any difference in the risk factor pattern like hyperglycemia, Hb A_1C, microalbuminuria, dyslipidemia and obesity in patients with DR in relation to family history.

KEY WORDS: Diabetic retinopathy, family history, microalbuminuria, hyperglycemia, HbA_1C, dyslipidemia, obesity.

INTRODUCTION

The prevalence of chronic, non-communicable diseases is increasing at a frightening rate. Diabetes with its devastating complications is rapidly emerging as a global health problem that threatens to assume a pandemic level by 2030 by involving 366 million population.[1] In India an epidemic increase in T2DM has been reported by the World Health Organization.[2] T2DM is a leading cause of morbidity and mortality due to its macro vascular and micro vascular complications. Diabetic retinopathy (DR) is one of the most common and specific complications of T2DM [3]. It is estimated that almost half of the patients with T2DM have some degree of retinopathy at any given time and 75 % of T2DM develop DR after 15 year duration of diabetes.[4, 5] The UK Prospective Diabetes Study reported 35% prevalence of DR in diabetes population.[6] Visual morbidity from diabetes is a significant public health problem; however this is largely preventable and treatable if managed with timely intervention, the quality of life can be preserved hence it is important to precisely identify the risk factors for the development and progression of DR.

Many of the risk factors of DR are extensively studied and which emphasis the role of duration of diabetes, glycemic control, hypertension and dyslipidemia.[7] But other incompletely defined factors also may be involved in the development of complications because many people with long standing diabetes never develop any complications like nephropathy or retinopathy. A Few previous studies mention about the role of heredity in the development and progression of DR.[8, 9] Moreover, the data about the risk factors are mostly derived from the studies on Western population. The data available from the Indian subcontinent are almost scanty.[10] This study examined the role of family history in the prevalence and severity of DR and whether there is any difference in the risk factor pattern like hyperglycemia, Hb A_1C, microalbuminuria, dyslipidemia and obesity in patients with DR in relation to family history.

MATERIALS AND METHODS

Subjects

A cross-sectional study was undertaken on 200 known diabetic patients attending the Medicine OP Clinic of the Department of General Medicine, Government Medical College, Thrissur. Each patient was examined by the attending physician and detailed family history

about the diabetes and other basic demographic data were collected using a questionnaire. Informed consent was obtained from all study subjects. Anthropometric measurements like height, weight and body mass index (BMI) were carried out using standard techniques.[8] Blood pressure (BP) was recorded twice (5 min apart) in the sitting position in the right arm to the nearest 2 mm Hg with a mercury sphygmomanometer and the mean was taken as the final reading. Hypertension was diagnosed in subjects who were on anti-hypertensive medication or had systolic BP ≥140 mmHg or diastolic BP ≥ 90 mmHg.[9]

Methods

A fasting blood sample of 8 ml was obtained after an overnight fast of 8 hrs. by venipuncture for fasting blood glucose, lipid profile, glycated hemoglobin (HbA_1C) and, a 90-minutes post prandial sample was obtained after a standard South Indian breakfast, for investigations like hyperglycemia, HbA_1C, microalbuminuria, dyslipidemia and obesity. Fasting urine samples were also collected for microalbumin determination. Estimation of plasma glucose and serum lipids were carried out in EM 360 Fully Automated Biochemistry Analyser of M/s. Erba-Manneihm utilizing the kits, calibrators and controls supplied by M/s. Transasia Diagnostics, Mumbai. Hb A_1C was estimated by column chromatography. Low-density lipoprotein (LDL-C) cholesterol was calculated using the Friedewald formula. Microalbumin concentration was measured using an immuno turbidimetric assay. The diagnostic criterion for macroalbuminuria was albumin excretion ≥300 mg/g creatinine.[10]

A comprehensive ocular examination was carried out in all study subjects by an Ophthalmologist. The patients were categorized with a score of 0 to 5 according to the degree of their retinopathy as follows: 0-No retinopathy, 1-Mild non-proliferative diabetic retinopathy (NPDR), 2-Moderate NPDR, 3-Severe NPDR, 4-Very severe NPDR and 5-Proliferative diabetic retinopathy (PDR). Statistical analyses were done using Chi square test and two-tailed, independent ‘t’ test using SPSS version 17.0 software program.[11]

RESULTS

The study population in the present study constituted almost equal number of males and females. There was no statistically significant difference between the family histories of diabetes in both sexes. In this study, it was noticed that the incidence of development of DR is less in patients without family history.

Table 1: Incidence of DR in T2DM Patients with and without Family History.

Family History	No DR	Mild DR	Moderate DR	Severe DR	PDR
With Family History(N=140)	27 (13.5%)	50 (25%)	60 (30%)		3 (1.5%)
Without Family (N= 60)	51 (25.5%)	7 (3.5%)	2 (1%)	0 (0%)	0 (0%)

Table 2: Age and Gender-wise Distribution of T2DM Patients with and without Family istory

Family History	Gender		Age Groups	
		41-50 yrs.	51-60yrs.	61-70yrs.
With (N=140)	Men	18	27	25
	Women	22	34	14
Without (N= 60)	Men	10	10	9
	Women	11	12	8

Out of 200 patients, 60 patients were without family history and 140 patients with family history. Out of 140 (80.7%) 113 developed DR against 9 (15%) out of 60 patients in the group without family history of T2DM ($p<0.0001$).

Table 3: Gender-wise Distribution of T2DM Patients with and without Family History.

Sex	Without family history of Diabetes	With family history of Diabetes	Total
Male	29 (14.5%)	70(35%)	99
Female	31(15%)	70(35%)	101
Total	60	140	200

Table 4: Age-wise Distribution of T2DM patients with and without Family History

Age	With family history of Diabetes	Without family history of Diabetes	Total
40-50	40 (28.5.5%)	21 (34.6%)	61
51-60	61(43.57%)	22 (36%)	83
61-70	39 (27.8%)	17 (26.6%)	56
Total	140	60	200

Table 5- Comparison of the Clinical and Biochemical Characteristics of T2DM patients (Mean ± SD) using the Student's "t" Test

Variables	No DR	DR	p value
Age	53.10 ± 9.34	56 ± 9.4	0.018
BMI	24.05±3.15	24.45±2.95	NS
FBG	145.14 ± 38.86	171.10 ±56.77	0.0001
PPBG	179.63 ± 56.27	206.47± 69.17	0.005
HbA1C	7.72±1.78	8.58 ± 1.92	0.002
Microalbumin	26±49.5	137.05 ±81.47	0.001
TC	222.63±44.38	230.62±49.82	NS
HDL-C	58.5 ±16.95	52.44±14.51	0.008
LDL-C	131.05±42.17	143.43±46.63	<0.05
TG	165.40 ±58.69	173.77±62.12	NS

ApoA1	136.72 ± 35.33	147.28 ±66.07	NS
ApoB	112.72 ± 45.87	109.95±43.04	NS

In patients without family history 78% had mild DR and none had severe DR or PDR where as 54.4% of patients showed moderate to severe PDR and PDR.A total of 122 patients had DR which constitutes 61%. There is a significant difference between the groups with DR and without DR in the risk factor pattern.

DISCUSSION

In the present study, 200patients with T2DM were studied out of which 61% had DR. Out of these, 140 patients were having a family history of diabetes for the first degree relatives. Previous studies from India reported prevalence of DR ranging from 17.6-26.8%.[12, 13] The finding from our study is comparable with the reports from Liverpool Diabetes Eye Study (33.6%) and Diabetic Eye Disease in Tayside-Sctotland study (55.5%). The higher percentage of DR in the present study may be explained by strong genetic component in the etiology of T2DM and its complications. About 92%of patients with DR had a family history of diabetes for the first degree relative. The severity of DR was also found to be associated with family history. Majority (77.8%) of patients without family history was in the mild DR group and none had severe DR or PDR. But in patients with family history, moderate to severe DR accounted for 53.1% and PDR for 2.7%. Previous studies on parental influence on T2DM in the off springs observed a younger age of onset of T2DM and a greater chance of developing later complications.[14-17] The other findings on the major risk factors for the development of DR are consistent with previous studies. The role of glycemic control indicated by FBG, PPBG and HbA_1C is well recognized. Our study also shows a significant association between DR and glycemic control. But contrary to the previous studies, the duration, gender and blood pressure do not show significant association with DR. Dyslipidemia as an additional risk factor had been attempted in several studies but results were inconsistent. The preset study observed a significant association between HDL-C and LDL-C but not with TC, TG, apo-A and apo-B. Role of micro-albuminuria as a risk factor is also a matter of dispute.[18-20] The present study is in favour of micro-albuminuria substantiating the fact that urinary albumin is a marker of generalized vascular involvement in T2DM.

CONCLUSIONS

In the present study, a significant association between the prevalence and severity of DR with family history was found. Hence a more prudent control of other risk factors like glycemic

indices, dyslipidemia and micro-albuminuria may be advised for diabetic patients with family history as they are at greater risk for the development and progression of DR.

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ANTI-THYROID PEROXIDASE ANTIBODY PREVALENCE IN REPRODUCTIVE AGE GROUP FEMALES- A STUDY FROM CENTRAL KERALA, INDIA

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ABSTRACT

BACKGROUND
Assessment of serum TSH and anti-TPO antibody titre will play an important role in the early detection of hypothyroidism and autoimmune thyroid disorders, thus helping in the initiation of appropriate treatment. Anti-TPO antibody-positive subjects can also be evaluated for other autoimmune disorders. The present study was proposed to assess the prevalence of anti-TPO antibodies in asymptomatic women of reproductive age.

MATERIALS AND METHODS
The study consisted of 200 asymptomatic females in reproductive age group from a tertiary care center in Central Kerala. TSH and anti-TPO were measured using chemiluminescence immunoassay system for all the participants.

RESULTS
A total of 23.5% participants in the study were anti-TPO positive. TSH had a statistically significant correlation with anti-TPO in anti-TPO positive group (r=0.306; p=0.0362). About 10 participants (21.28%) in this group had high TSH values suggestive of hypothyroidism. Maximum number of anti-TPO positive participants were found in 45-49 years age group, but the mean value was highest in 35-44 years age group. These two groups also had high TSH levels. Levels of anti-TPO are associated with TSH values indicating a negative impact on thyroid function. It can lead to hypothyroidism or subclinical hypothyroidism, which in turn affect fertility, pregnancy and other reproductive outcomes.

CONCLUSION
The study shows the need for screening for anti-TPO antibodies in all women of reproductive age group to estimate the risk of infertility among them.

KEYWORDS
Anti-TPO antibodies, Thyroid Autoimmunity, Anti-thyroid Antibodies.

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BACKGROUND

Autoimmunity is one of the most frequent cause of thyroid dysfunction in women of reproductive age. About 5-15% of euthyroid women and up to 2% of euthyroid men have thyroid antibodies and these individuals are at increased risk of developing thyroid dysfunction.[1] Prevalence of thyroid antibodies like antithyroid peroxidase antibodies (anti-TPO) was related to low rate of fertilisation, implantation and pregnancy.[2] Autoimmune thyroiditis is considered to be one of the aetiological factors for hypothyroidism in a population[3] and this condition was found to be significantly linked with infertility.[4] Thyroid autoimmunity was found to be highly prevalent in women with polycystic ovary syndrome cementing the importance of screening for anti-TPO antibodies in women.[5]

To our knowledge, studies on anti-TPO antibody titre in young asymptomatic females in central region of Kerala are negligent. Assessment of serum TSH and anti-TPO antibody titre will play an important role in the early detection of hypothyroidism and autoimmune thyroid disorders and also in the initiation of appropriate treatment for prevention of complications. Anti-TPO antibody-positive subjects can also be evaluated for other autoimmune disorders. The present study was proposed to assess the prevalence of anti-TPO antibodies in asymptomatic women of reproductive age.

MATERIALS AND METHODS

The cross-sectional study consisted of 200 asymptomatic females in reproductive age group, all of whom were either students or staff of a tertiary healthcare centre. The study was conducted from September 2015 to August 2016. Potential participants were excluded if they were-

- Pregnant.
- Had already diagnosed thyroid disease.
- Currently receiving thyroid medications.
- Not willing to participate in the study.

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The participants were grouped into four based on age. Medical and family histories of the patients were collected using a semi-structured questionnaire. TSH and anti-TPO were measured using chemiluminescence immunoassay system- Roche Cobas E411. Appropriate calibrators were used for validating the test.

For analyses, the reference value taken was <35 IU/mL for anti-TPO antibodies and 0.34-4.25 μIU/mL for serum TSH. Exploratory analysis was done using EPI Info version 1. TSH and anti-TPO levels were analysed using multivariate linear regression models after log transformation due to skewness of their distribution in their original scale. Mean ± SD was used for other parameters. Age-wise prevalence of TSH and anti-TPO was compared using Chi-square test. Hypothyroidism state of the participants was analysed on the basis of TSH level alone due to economic constraints.

RESULTS

In this cross-sectional study, 200 participants from the department were evaluated for the presence of anti-thyroid peroxidase antibody. The mean age (mean ± SD) of the participating women were 32.92 ± 11.82 years. More than half of the participants (65.5%) were staff of the department, while the rest were students. About 17% of the participants had a family history of thyroid disorders. Most of them had a regular menstrual cycle and only 7% reported irregularities in periods. Infertility rate was also low in the group with only 3.5% diagnosed as having the condition. About 3% of the women in the study had a history of preterm delivery. Mean values of the different biochemical parameters of the participants are given in Table 1.

Parameter	Mean
Fasting blood sugar (mg/dL)	90.57
Total cholesterol (mg/dL)	198.17
Triglycerides (mg/dL)	105.15
HDL (mg/dL)	58.38
LDL (mg/dL)	118.88
TSH (μ IU/mL)	2.51
Anti-TPO (IU/mL)	68.12

Table 1. Mean Values of the Different Biochemical Parameters of the Participants

TSH and anti-TPO antibodies had a statistically significant correlation (r=0.192, p=0.006)*. But, the antibodies were not significantly correlated with BMI.

Anti-TPO Positive Prevalence

A total of 47 women (23.5%) in the study group were anti-TPO positive with a mean age (mean ± SD) of 34.55 ± 12.09 years. More than 25% of these women in the anti-TPO positive group had a family history of thyroid dysfunction. The mean values of parameters in this group are given in Table 2.

Parameters	Mean
BMI (kg/m^2)	22.7
Fasting blood sugar (mg/dL)	91.43
Total cholesterol (mg/dL)	200.64
Triglycerides (mg/dL)	125.04
HDL (mg/dL)	55.23
LDL (mg/dL)	115.57
TSH (μ IU/mL)	3.57
Anti-TPO (IU/mL)	249.8

Table 2. Mean Values of Different Parameters in Anti-TPO +ve Participants

Correlation between the different parameters in the group is given in Table 3. TSH had a statistically significant correlation with anti-TPO in anti-TPO positive group (r=0.306; p=0.0362). BMI and FBS were not significantly associated with these antibodies. About 10 participants (21.28%) in this group had high TSH values suggestive of hypothyroidism.

	Anti-TPO	
	R	p
TSH	0.306	0.0362*
BMI	0.262	0.076
FBS	0.064	0.183

Table 3. Correlation between Different Parameters and Anti-TPO in Anti-TPO Positive Group

The anti-TPO positive group was further categorised on the basis of family history of thyroid dysfunction. The mean TSH value was 2.30 μ IU/mL, while that of anti-TPO was high (196.33 IU/mL) in participants with a family history of thyroid disorders. The correlation shown between parameters like BMI and FBS with anti-TPO in this group (anti-TPO positive with family history) was not statistically significant (FBS- r=0.036, p=0.909; BMI- r=0.172, p=0.593). There was a statistically significant correlation between TSH and anti-TPO when the participant had a family history of thyroid disorders (r=0.630, p=0.028*).

Age-Specific Prevalence

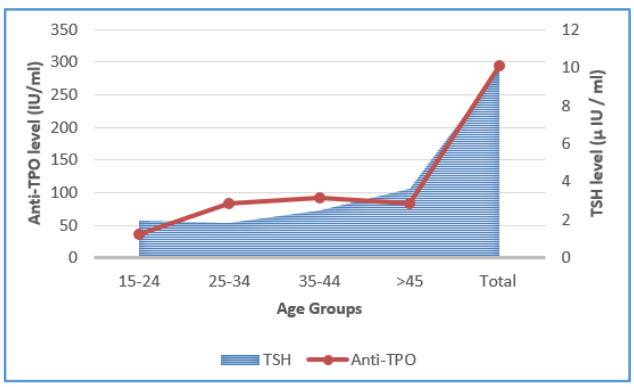
The study subjects were divided into different age groups- 15-24, 25-34, 35-44 and 45-49 years. The mean ± SD values for different parameters are given in Table 4.

	15-24 yrs.	25-34 yrs.	35-44 yrs.	Above 45 yrs.
Mean age (years)	19.32 ± 1.41	29.52 ± 2.21	39.27 ± 2.77	47.6 ± 1.46
Mean body weight (kg)	46.56 ± 6.62	57.45 ± 5.99	61.87 ± 9.33	58.93 ± 6.90
Mean BMI (kg/m^2)	19.61 ± 2.44	23.61 ± 3.04	25.16 ± 3.50	24.67 ± 2.49
Family history (no:)	11	7	6	10
FBS (mg/dL)	77.72 ± 10.88	88.17 ± 8.40	95.22 ± 18.17	105.11 ± 20.64
TSH (μ IU/mL)	1.95 ± 2.15	1.82 ± 1.10	2.52 ± 2.02	3.63 ± 7.20

Anti-TPO (IU/mL)	35.41 ± 62.29	84.03 ± 173.05	91.22 ± 181.38	83.93 ± 144.36
Anti-TPO +ve (no:)	15	5	11	16

Table 4. Mean Values (Mean ± SD) of Different Parameters in Different Age Groups. (Family History and Anti-TPO +ve are Represented as Numbers)

Maximum number of anti-TPO positive participants were found in above 45 years group, but the mean value was highest in 35-44 years group. These two groups also had high TSH levels. Chi-square test between TSH and anti-TPO in the different age groups did not reveal a statistically significant association between the groups ($\chi 2=0.989$, $p<0.005$). The levels of anti-TPO antibodies and TSH in relation to age distribution is given in below figure.



Rate of Anti-TPO Antibodies and TSH in Relation to Age Distribution

Prevalence of Hypothyroidism

A total of 18 participants (9%) had TSH value higher than the normal range suggesting presence of hypothyroidism. Mean age (mean ± SD) of this group was 38.89 ± 10.59 years. Mean TSH value in the group was 9.69 ± 11.37 µ IU/mL. Ten participants (55.55%) in this group had high anti-TPO levels (mean ± SD=192.68 ± 244.28 IU/mL). But, a statistically significant association was lacking between TSH and anti-TPO in this group ($r=0.014$, $p=0.954$). There was no significant association between TSH and BMI in this group with clinical hypothyroidism.

DISCUSSION

Thyroid function abnormalities like hypothyroidism and hyperthyroidism are common in Kerala and the values are higher when compared to reports from other parts of the world.[3,6,7,8] Autoimmune-related thyroid dysfunctions caused by antibodies are one of the most common cause of thyroid diseases.[9] In general population, 8-27% are reported to have anti-TPO antibodies and a high titre of these antibodies is present in 89.9% of patients with autoimmune thyroid disorders.[10,11] Present study show 23.5% of the participants to be anti-TPO positive with a mean ± SD value of 68.12 ± 137.62 IU/mL. It is known that most of the autoimmune diseases and their pathologies remain hidden for several years before their clinical manifestation. Anti-TPO antibodies in the serum are considered as a valuable indicator of autoimmune thyroid disease as they precede the development of disease phenotype.[9,12] Thus, the presence of these antibodies in an asymptomatic individual, especially in females in reproductive age group should not be neglected. The prevalence of antibodies though lesser than that reported in a pilot study points towards a real increase in autoimmune thyroid disease in the population and should be further evaluated with well-designed prospective studies.[13]

TSH values were not significantly different in anti-TPO positive and anti-TPO negative groups, although studies have reported occurrence of thyroid insufficiency in women with autoimmune antibodies.[14] Some values of anti-TPO antibodies in our study were very high in the range of >600 IU/mL, multiple times higher than the acceptable upper limit. This maybe a reflection of an active autoimmune process going on in the thyroid gland with the implication of future thyroid dysfunction. It is possible that thyroid autoimmunity is present in the form of asymptomatic, subclinical hypothyroidism in many patients as reported by Lata et al.[15] Nested case-control study conducted by Hutfless et al showed that these antibodies had increasing prevalence before the clinical diagnosis of the disease.[12]

Earlier studies had reported increasing levels of anti-TPO antibodies and serum TSH levels with advancing age.[16,17,18] This increase in TSH with age might be due to increased presence of anti-TPO antibodies in the participants. The present study did show an increased prevalence in the age group above 45 years. But, this cannot be directly related to the prevalence of diseases as the study focused on indices and not on prevalence of autoimmune thyroid diseases. When anti-TPO positive group was excluded, no age-dependent change was noticed in our study. Data from this study can be used as a reference for future investigations and also for comparisons with other cohorts who have a high risk of thyroid dysfunction and autoimmunity.

Positive family history of thyroid dysfunction is associated with elevated anti-TPO antibodies.[19] In the present study, 34% of the participants with a family history of thyroid disorders had elevated anti-TPO antibodies. Further, majority of the patients with hypothyroidism (55.56%) had elevated amount of anti-TPO antibodies, although a statistically significant relation could not be established between TSH and anti-TPO antibodies. Many studies do show a significant positive correlation between anti-TPO antibodies and TSH.[3,19] But, the increased risk of developing thyroid dysfunctions with increased levels of anti-TPO antibodies is more or less well established. This is a single-centre study and has not included other antibodies involved in thyroid autoimmunity. A higher sample size and a long-term follow up study are warranted to give a better picture of the effect of autoimmunity in the overall health and quality of life.

Matalon et al[20] found that anti-TPO antibodies, even when not associated with overt thyroid dysfunction, cause poor reproductive outcomes. Autoimmunity due to these antibodies is one of the important factors in infertility and

anti-TPO antibody positive women are found to have spontaneous miscarriages, recurrent abortion, lower fertilisation, implantation and pregnancy rate.[2,15,21] The changes in fertility and maintenance of pregnancy maybe channelled by triggering the immune system or by increasing the risk of subclinical hypothyroidism, which later progresses to clinically overt hypothyroidism.[22,23] This may explain why the mean value of TSH is skewed to the higher side of normal range in anti-TPO positive women in our study. As 64% of the participants are not married or are yet to plan for a child, this evaluation and study are significant. With autoimmunity and thyroid dysfunction related to negative reproductive outcomes, early diagnosis is warranted to prevent infertility and other pregnancy-related issues. Preclinical identification of this disease is now possible using antibody testing. Anti-TPO as an indicator of increased risk of thyroid diseases, thus gains importance, particularly in females of reproductive age group. More studies are required to explore the predictive value and clinical management options for women with elevated anti-TPO antibodies to improve the pregnancy outcomes and overall health.

CONCLUSION

The present study shows that about one in four women in the reproductive age have higher levels of anti-TPO antibodies. Most of these women are euthyroid and asymptomatic. Levels of anti-TPO are associated with TSH values indicating a negative impact on thyroid function. It can lead to thyroid dysfunction, which in turn affect fertility, pregnancy and other reproductive outcomes. Early diagnosis and treatment of the autoimmunity will help to improve the reproductive outcomes in women in reproductive age group. More than half of the participants in our study (64%) are not married or are yet to plan for a child making this study and evaluation very relevant. This study can be a reference for future prospective studies and comparisons particularly in people with an elevated risk of thyroid autoimmunity and other autoimmune disorders.

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ASSOCIATION OF SERUM URIC ACID LEVELS AND THE COMPONENTS OF METABOLIC SYNDROME- A COMPARATIVE STUDY

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ABSTRACT

BACKGROUND

Metabolic syndrome is an emerging threat in our population. Metabolic syndrome is characterised by central obesity, hypertriglyceridaemia, low HDL cholesterol, hyperglycaemia and hypertension. Serum uric acid level has been reported to be associated with various cardiovascular complications of metabolic syndrome. However, its direct relationship with metabolic syndrome remains controversial. Thus, we planned this study to find the association of serum uric acid with all the components of metabolic syndrome.

MATERIALS AND METHODS

The study was a comparative study conducted among patients attending obesity clinic in Thrissur from March 2014 to March 2015. 56 subjects with metabolic syndrome and 54 subjects without metabolic syndrome between the age group 25-65yrs. with BMI ≥25kg/m^2 were included in the study. Fasting blood glucose, lipid profile and uric acid estimation were done by analysers in the Central Laboratory, Government Medical College, Thrissur. Data analysis was done using SPSS software. The tests were statistically significant, if 'p' value <0.05.

RESULTS

Metabolic syndrome was observed in 50.9% of obese patients in our study. The mean serum uric acid levels were 6.07±1.61 and 4.62±1.33 in metabolic and non-metabolic syndrome respectively and were found to be statistically significant. The association of mean uric acid levels with BMI, FBS and triglycerides were statistically significant.

CONCLUSION

A significant positive association of uric acid with body mass index, waist circumference, triglycerides and fasting blood glucose. The association of uric acid with various components of metabolic syndrome support that it might be taken as a marker for metabolic syndrome.

KEYWORDS

High-Density Cholesterol (HDL), Low-Density Cholesterol (LDL), Triglycerides (TG), Fasting Blood Sugar (FBS), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP).

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BACKGROUND

The metabolic syndrome refers to a clustering of metabolic factors including central obesity, glucose intolerance, hyperinsulinaemia, low HDL, high triglycerides and hypertension.[1] For the past two decades, the prevalence of metabolic syndrome has increased worldwide including developing countries like India due to the changing lifestyle.[2] Recent studies show the prevalence of metabolic syndrome is 25.8% among Asian and 53.2% among obese persons.[3,4,5,6]The prevalence rate of metabolic syndrome was 33.5% in a community study conducted among urban eastern civilians in India.[3] Old age, female gender and general obesity significantly contributed the risk of metabolic syndrome in their study.

Uric acid is the final breakdown product of catabolism of the purine nucleotides. Uric acid is a non-protein nitrogenous compound, which is cleared from the body by the kidney.[7] Purines formed from the catabolism of dietary nucleic acid are directly converted to uric acid. The bulk of uric acid arises from the degradation of nucleic acids. Uric acid is primarily produced in the liver and small intestine. Serum urate levels vary with rates of purine biosynthesis and their excretion. The normal uric acid level is 3-7mg/dL.

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The daily excretion of uric acid is 500-600mg. Serum urate varies with age, sex,height, body weight, blood pressure, renal function and alcohol intake. Exercise, alcohol, obesity and purine diet are the common causes of increased urate production in humans.

The increased mortality and morbidity in metabolic syndrome is due to the atherosclerotic cardiovascular diseases, hypertension and diabetes mellitus.[7,8,9,10,11] Elevated serum uric acid levels are significantly associated with insulin resistance of metabolic syndrome[12] and its components.[13]Decreased renal uric acid clearance due to hyperinsulinaemia is the reason for elevated uric acid levels in metabolic syndrome.[14] Hyperuricaemia along with other components of metabolic syndrome may act synergistically increasing the cardiometabolic risk.[13]Recently, serum uric acid has been proposed as a marker of oxidative stress, which is considered to be one of the major mechanism for the development of metabolic syndrome and cardiovascular diseases.[15]The positive association of serum uric acid with the components of metabolic syndrome remains controversial. So, we investigated the association of serum uric acid with all the components of metabolic syndrome in obese patients in our population.

Aim- To study the association between serum uric acid levels and the components of metabolic syndrome.

MATERIALS AND METHODS

This study was conducted among 110 patients from March 2014 to March 2015 at the obesity clinic, Thrissur, Kerala, India. 56 subjects with metabolic syndrome and 54 subjects without metabolic syndrome were taken. Sample size was calculated using the following formula taking 80% power and error fixed at 5%.

$$\text{Sample size} = \frac{(Z\alpha + Z\beta)^2\ 2SD^2}{d^2}$$

The institutional ethical committee approved the study and informed consent was taken. All patients in the age group 25-65 years with BMI ≥25 kg/m^2 were included in the study. Diagnosis of the metabolic syndrome was made by the IDF (International Diabetes Federation) criteria.

Central Obesity- (defined as waist circumference ≥90cm for Indian men and ≥80cm for Indian women).

Plus any of the following features

Raised Triglycerides Level- ≥150mg/dL (1.7mmol/L) or specific treatment for this abnormality.

a. **Reduced HDL Cholesterol**-<40mg/dL(1.03mmol/L) in males and <50mg/dL(1.29mmol/L) in females or specific treatment for this lipid abnormality.
b. **Raised Blood Pressure**- Systolic BP ≥130 or diastolic BP ≥85mm of Hg or treatment of previously-diagnosed hypertension.
c. **Raised Fasting Plasma Glucose (FPG)**- ≥100mg/dL (5.6mmol/L)or previously-diagnosed type 2 diabetes mellitus.

The following patients were excluded from the study-

1. Patients with a history of hypothyroidism, hyperparathyroidism, diabetes insipidus and glucose-6-phosphate dehydrogenase deficiency, liver diseases, renal diseases and alcoholism.
2. Patients taking medications like salicylates, diuretics or uricosuric drugs.
3. Pregnant and lactating mothers.

Those patients taking antihypertensive, antidyslipidemic or antidiabetic drugs were considered to have hypertension, hypercholesterolaemia, hypertriglyceridaemia and diabetes mellitus.

Anthropometric and Blood Pressure Measurement- Weight was measured in kilograms using a weighing machine. Height was taken in centimetre. Body Mass Index (BMI) was calculated from the formula BMI = body weight in kg/height in m^2.Waist circumference was measured at the midpoint between the lowest part of costal margin and superior border of the iliac crest. Blood pressure was recorded on the right arm of patients seated at rest using a sphygmomanometer.

Laboratory Measurements- The venous blood sample was collected in the morning after 8-12 hours fasting. Fasting blood glucose levels was estimated by glucose oxidase-peroxidase method. Total serum cholesterol was measured by cholesterol oxidase endpoint enzymatic method. Glycerol phosphate oxidase method (Trinder method) was used to determine serum triglycerides. HDL cholesterol was measured by modified polyvinyl sulfonic acid and polyethylene glycol methyl ether coupled classic precipitation method. LDL cholesterol was calculated from the values of total cholesterol, HDL cholesterol and triglycerides using Friedewald's formulae. Serum uric acid was determined by uricase peroxidase method. All biochemical parameters were determined in Transasia fully automated analyser, Central Laboratory, Government Medical College, Thrissur.

Data Analysis

The Statistical Package for the Social Sciences 18.0 (SPSS) for windows software was used for statistical calculations. Continuous variables were expressed as mean ± SD. Qualitative variables were expressed as a percentage. Comparison of group means of the different variables was done using Student's t-test. Chi-square test was done to find out the association between different components in subjects with and without metabolic syndrome and also to detect the association between hyperuricaemia and metabolic syndrome. The tests were statistically significant, if 'p' value <0.05.

RESULTS

The mean age of the study populations was 40.52 ± 10.42 years and 55% of the subjects were females. The mean age of metabolic syndrome patients was 42.22 ± 9.85 and the mean age of non-metabolic syndrome was 36.86 ± 10.76 and this difference was found to be statistically significant (p=0.01).

78.2% and 21.8% of study populations were having BMI ≥30 and BMI <30, respectively. Metabolic syndrome was diagnosed in 50.9% (n=56) of obese patients in our study. A nonsignificant increase of metabolic syndrome was observed among females. 74.4% of patients with metabolic syndrome have a BMI >30.

The anthropometric parameters of all the patients were shown in the Table1. Table 2 shows that the BMI of patients with metabolic syndrome and non-metabolic syndrome were 38.20 ±7.92 and 34.71 ± 7.01 respectively and was statistically significant (p<0.02). Height, weight and waist circumference of patients with metabolic syndrome and non-metabolic syndrome were not statistically significant.

Table 3 shows the biochemical parameters and blood pressure of all the study subjects. Serum total cholesterol, HDL cholesterol and LDL cholesterol levels in patients with metabolic and non-metabolic syndrome were statistically not significant (p >0.05). Systolic and diastolic blood pressure, serum fasting blood sugar and triglyceride levels in patients with metabolic and non-metabolic syndrome were statistically significant(p<0.05).

Table 5 shows the mean serum uric acid levels of male and female in both groups. The mean uric acid level in subjects with metabolic syndrome was 6.07 ± 1.61 and that in patients without metabolic syndrome subjects was 4.62 ± 1.33. The difference was found to be statistically significant (p<0.001). The difference was statistically significant in both groups.

Table 6 shows the association of mean uric acid levels with different components of metabolic syndrome.The association of mean uric acid levels with BMI, FBS and triglycerides was statistically significant. The association of mean uric acid levels with HDL cholesterol and hypertension was not statistically significant.

Parameter	Total (n=110) (Mean ±S.D.)
Weight (kg)	96 ± 24.80
Height (meter)	1.60 ± 0.08
BMI	37.08 ± 7.70
Waist circumference (cm)	106.48 ± 11.98

Table 1. Anthropometric Parameters of Study Subjects

n = number of subjects.

Parameter	Metabolic Syndrome (Mean± SD)	Non-Metabolic Syndrome (Mean± SD)	p value
Weight (kg)	98.7±26.62	90.20±19.70	0.09
Height (meter)	1.59±0.08	1.61±0.07	0.41
BMI	38.2±7.91	34.70±70	0.02*
Waist circumference(cm)	107.73±11.9	103.92±11.70	0.12

Table 2. Anthropometric Measurements among Subjects with and without Metabolic Syndrome

(*p= <0.05 significant).

Parameter(Normal Range)	Total (n=110) (Mean ±S.D.)
Fasting blood sugar (70-110 mg/dL)	122.85±48.32
Systolic blood pressure (130 mmHg)	133.95±12.08
Diastolic blood pressure (90 mmHg)	85.43±8.03
Total cholesterol (150-200 mg/dL)	198.91±32.62
Triglycerides (50-150 mg/dL)	135.93±44.61
High-density lipoprotein (30-75 mg/dL)	38.24±7.31
Low-density lipoprotein (60-150 mg/dL)	135.95±30.54

Table 3. Biochemical Parameters of Study Subjects

N= number of subjects.

Parameter	Metabolic Syndrome(Mean ± SD)	Non-Metabolic Syndrome(Mean ± SD)	p value
Fasting blood sugar (mg/dL)	131 ± 48.06	106 ± 44.92	0.01*
Systolic blood pressure (mm of Hg)	138.7 ± 10.21	124.17 ± 9.52	0.001*
Diastolic blood pressure (mm of Hg)	87.95 ± 7.82	80.25 ± 5.72	0.001*
Triglycerides (mg/dL)	146.01 ± 48.91	115 ± 23.51	0.001*
High-density lipoprotein (mg/dL)	37.5 ± 6.12	39.6 ± 9.091	0.15
Total cholesterol (mg/dL)	198.9 ± 36.51	198.41 ± 23.21	0.925
Low-density lipoprotein (mg/dL)	133 ± 33.81	141 ± 21.72	0.19

Table 4. Biochemical Parameters of Study Subjects

P value <0.05 significant.

Gender	Metabolic Syndrome Mean± SD	Non-Metabolic Syndrome Mean± SD	p Value
Male	6.38 ± 1.59	5.09 ± 1.37	0.007
Female	5.82 ± 1.62	4.29 ± 1.18	0.001
Total	**6.07± 1.61**	**4.62± 1.33**	**0.001**

Table 5. Uric Acid Levels in Males and Females with Metabolic Syndrome and Non-Metabolic Syndrome

(*p= <0.05, significant).

Variables	Groups	n	Mean ± SD	p value
BMI	<30	86	5.87 ± 1.62	
	>30	24	4.71 ± 1.52	0.002*
FBS	≥100	78	5.95 ± 1.72	
	<100	32	4.79 ± 1.44	0.001*
BP	≥130/85	61	5.87 ± 1.72	0.06
	<130/85	49	5.29 ± 1.66	
HDL	Low	87	5.62 ± 1.71	
	High	23	5.52 ± 1.71	0.823
TG	>150	37	6.15 ± 1.91	
	<150	73	5.31 ± 1.51	0.013*

Table 6. Association of Mean Uric Acid Levels with BMI, Blood Pressure, Fasting Blood Sugar, High-Density Lipoprotein and Triglycerides

(*p= <0.05, significant).

	Metabolic Syndrome	Non-Metabolic Syndrome		
Uric Acid	**(%)**	**(%)**	**p**	**Odds Ratio**
>6.8 mg/dL	35	11		
<6.8 mg/dL	21	43	0.001	6.51
	56	54		

Table 7. Association of Metabolic and Non-Metabolic Syndrome with Hyperuricaemia

(*p= <0.05 significant).

DISCUSSION

Older age, females over the age of 45, general obesity, hypertension, glucose intolerance and hypertriglyceridaemia were significantly associated with metabolic syndrome in our study. Older age, female gender, general obesity, hypercholesterolaemia, glucose intolerance and hypertriglyceridaemia significantly contributed to increased risk of metabolic syndrome.[3] Metabolic syndrome was diagnosed in 50.9% of the study population in our study.

The prevalence rate of metabolic syndrome was 53.2% in the CURES study.[16] The significant difference in the mean age group of subjects with and without metabolic syndrome (p<0.01) showed that the prevalence of metabolic syndrome was higher among older age groups. CURES study also observed that the prevalence of metabolic syndrome was higher among older age groups.

Obese individuals were five times more likely to have metabolic syndrome compared with those having normal weight.[17]There was significant difference between the mean BMI of subjects with and without metabolic syndrome in our study (p <0.02). 78.2% of our studied patients have a BMI of more than 30. As the degree of obesity increases, obesity related diseases like cardiovascular diseases, type2 diabetes mellitus and metabolic syndrome becomes greater.[17]

The mean values of FBS in subjects with and without metabolic syndrome were 131 ± 48.06 and 106 ± 44.9 and this difference was statistically significant (p<0.01). Glucose intolerance was found to be associated significantly with metabolic syndrome and has a very high risk of developing the syndrome. There was a significant difference in the mean blood pressure in subjects with and without metabolic syndrome. The mean HDL value was not statistically significant among subjects with metabolic syndrome and non-metabolic syndrome. There was no significant association seen between total cholesterol and LDL among our study groups. Similar results were observed by Shi-Dou-Lin et al in their study in Taiwan.[13]

We observed that the serum uric acid was significantly higher in patients with metabolic syndrome than with non-metabolic syndrome in our study. We studied the association between uric acid and different components of the metabolic syndrome. Elevated serum uric acid level was significantly associated with BMI, hypertriglyceridaemia and diabetes in our study. Previously, some authors also studied the association of uric acid and components of metabolic syndrome. Elevated serum uric acid level was higher in subjects with abnormal triglycerides, waist circumference, low HDL cholesterol, blood sugar and blood pressure than in patients with normal levels.[13]The most significant association was seen between uric acid and hypertriglyceridaemia in their study.

Individuals with metabolic syndrome had significantly higher fasting plasma glucose, blood pressure, triglycerides, waist circumference, low HDL cholesterol than those without metabolic syndrome(p <0.001).[18] Multiple logistic regression analysis revealed that hyperuricaemia

was an important risk factor of metabolic syndrome in their study. The association of serum uric acid with low HDL cholesterol was not statistically significant in our study.

Higher waist circumference and BMI were associated with higher insulin resistance and reduced renal uric acid clearance leading to hyperuricaemia.[14] HDL cholesterol was negatively associated with insulin resistance in their study.

Uric acid was significantly and positively associated with fasting blood glucose levels in both sexes and the associations remained after adjusting the confounding factors like BMI, waist circumference, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol.[18] The uric acid level has shown a significant positive correlation with all components of metabolic syndrome except low HDL cholesterol and diastolic BP in our study.

The serum uric acid concentration can be considered as an important predictive factor for metabolic syndrome.[14] The risk of metabolic syndrome has been increased with elevated serum uric acid concentrations in their study.

Significant higher uric acid value was observed in subjects with metabolic syndrome in comparison with subject to non-metabolic syndrome.[19]The same study proved a significant positive correlation of uric acid with blood glucose level. LDL cholesterol and total cholesterol did not show any significant correlation with serum uric acid levels in their study. The findings of our study also consistent with the above studies.

CONCLUSION

- The study showed that patients with metabolic syndrome have higher uric acid levels.
- A significant positive association of uric acid with body mass index, waist circumference, triglycerides and fasting blood glucose.
- The association of uric acid with various components of metabolic syndrome support that it might be considered as a marker for metabolic syndrome.

Limitations of the Study- We have not included control groups in our study. We used standard reference values for comparison.

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Comparison of Metabolic Bone Markers in Diabetic and Non-Diabetic Chronic Kidney Diseases in Government Medical College, Thrissur, Kerala, India

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ABSTRACT

BACKGROUND

The term 'Chronic Kidney Disease-Mineral and Bone Disorder' (CKD-MBD) has been used to describe clinically, the abnormalities in the bone and mineral metabolism associated with CKD. In CKD, serum levels of metabolic bone disease markers generally reflect a high bone turnover state (hyperphosphatemia, hypocalcaemia, hypersecretion of PTH, increased ALP). However, it has been noted that in diabetic CKD patients on regular haemodialysis, there is an impaired secretion of PTH when compared to the non-diabetics on haemodialysis. In this study we intend to evaluate the serum bone markers in both diabetic and non-diabetic CHD patients. If a significant association can be demonstrated between diabetes mellitus and a low bone turnover state, then treatment guidelines can be tailored accordingly in the diabetic CHD patients.

METHODS

A hospital based cross-sectional study was done on 150 patients attending the Dialysis Unit of Govt. Medical College, Thrissur district, Kerala, India, from March 2014 to March 2015. Estimation of serum FBS, creatinine, calcium, phosphorus, ALP and PTH was done.

RESULTS

The mean levels of serum phosphorus and PTH are significantly lower in the diabetic CHD population than in the non-diabetics, but mean serum ALP is significantly higher in the diabetic CHD patients. Statistical significance is seen in the serum metabolic bone disease markers except calcium among diabetic and non-diabetic chronic kidney disease.

CONCLUSIONS

The serum levels of PTH and phosphorus were found to be significantly lower in diabetic CHD patients than in their non-diabetic counterparts. Serum ALP levels were significantly higher in the diabetics. This demonstrates that a relative hypoparathyroidism is prevalent among the diabetic CHD patients and hence, prevention of deterioration of the already existing low turnover bone disease in such patients should be the treatment motto. Avoidance of oral calcium supplements, vitamin D supplements and increased calcium in the dialysate would be ideal, since these can lead to hypercalcemia and further suppress the PTH secretion.

KEYWORDS

Diabetes Mellitus, Chronic Kidney Disease, Bone Markers

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BACKGROUND

Chronic kidney disease (CKD) comprises of a broad spectrum of different pathophysiological processes, which are associated with abnormalities in the various functions of the kidney and a resultant progressive decline in the glomerular filtration rate (GFR). CKD stage 5 or end-stage renal disease (ESRD) represents a stage of CKD where due to the decreasing kidney function there is an accumulation of fluid, electrolytes and various toxins that are normally removed by the renal system. Those CKD patients who have been undergoing maintenance haemodialysis (MHD) for a period of not less than three months are termed chronic haemodialysis (CHD) patients.

A wide range of complications can be seen associated with CKD, of which the most common include anemia, malnutrition, and hypertension and bone disease. The term 'Chronic Kidney Disease-Mineral and Bone Disorder' (CKD-MBD) has been used to describe clinically a broad syndrome associated with CKD which manifests as abnormalities in the bone and mineral metabolism and / or extra-skeletal calcification.[1] The kidney plays an important role in the normal metabolism of the minerals calcium and phosphorus. Any disturbances which might affect the functioning of this organ can result in an imbalance in the calcium and phosphorus metabolism which can in turn cause disorders of the bone.

The effects of the CKD complications that arise from disturbances in the metabolism of calcium and phosphorus are most evident in the skeleton and the vascular bed. Secondary hyperparathyroidism (SHPTH) is a commonly observed complication among the CKD patients. It is characterized by an excessive level of parathyroid hormone (PTH) in the serum, parathyroid hyperplasia, hypocalcaemia and hyperphosphatemia.[2] This disorder can eventually lead to clinically significant long term consequences[3] and increase the risk for cardiovascular morbidity and mortality in the CKD population.[4,5]

Diabetes mellitus (DM), one of the most common chronic metabolic diseases, has been on the increase worldwide and in India. This can be attributed to the increasing obesity and sedentary lifestyle which have now become the most important risk factors associated with DM.[6] It has been noted in studies that DM is one of the two leading causes of CKD in India.[7] In CKD, hyperphosphatemia is encountered with due to the decreased excretion of phosphorus by the kidneys. Also, inadequate production of the active form of vitamin D by the kidney results in decreased levels of serum calcium. Both these factors together cause hyper secretion of PTH in an attempt to normalize the levels of serum calcium and serum phosphorus. Alkaline phosphatase (ALP) levels are also found to be increased in CKD due to the high bone turnover state induced by PTH over activity.[8] However, it has been demonstrated in various studies that diabetic CKD patients on regular haemodialysis have an impaired secretion of PTH when compared to the non-diabetics on haemodialysis.[9]

Chronic kidney disease is a common disease seen in our country. Since Kerala is the unofficial 'diabetic capital of India', the subject of whether DM has any effect on the levels of metabolic bone disease markers in serum is one of medical importance. This can help in altering treatment plans accordingly and thereby decreasing morbidity and mortality associated with mineral and bone disorders seen in CKD. There have been very few studies conducted concerning this subject in India. Hence, this study is being done to prove a relation between DM with serum PTH, calcium, phosphorus and ALP in CHD patients attending the Dialysis Unit of Govt. Medical College, Thrissur.

Objectives

1. To study the prevalence of DM in CHD patients.
2. To evaluate the effect of DM on serum levels of metabolic bone disease markers (PTH, calcium, phosphorus, ALP) in CHD patients.

METHODS

The study was a hospital based cross-sectional study done among patients attending the Dialysis Unit of Govt. Medical College, Thrissur district, Kerala, India, from March 2014 to March 2015. Sample size was calculated using the formula,

$$Sample\ size = (z\alpha)^2 pq/d^2$$

zα = 1.96
p = 41 % [7]
q = 59 % (1 - p)
d = 8.2 (20 % p)

Substituting the values in the above formula we get 138.2039 which is the minimum sample size required. A total of 150 patients were included in the study to increase the sensitivity of the study.

Inclusion Criteria

CKD patients irrespective of cause, aged between 18-80 yrs, undergoing haemodialysis > 3 months

Exclusion Criteria

Subjects diagnosed with primary hyperparathyroidism and those with liver disease.

Written informed consent from the patients satisfying the inclusion criteria were obtained. On admission, data regarding the baseline characteristics of each patient - Age, Sex, history of DM, duration of haemodialysis was collected. The patients were grouped according to diabetic status (diabetic or non-diabetic). Venous blood samples were drawn from the patient before the start of haemodialysis for the estimation of the various biochemical parameters in metabolic bone disease. Estimation of serum FBS, creatinine, calcium, phosphorus and ALP was done using Fully Automated Clinical Chemistry Analyzer Erba-Mannheim 360 and estimation of PTH in the serum sample was done by Enzyme linked immunosorbent assay (ELISA) using Rayto RT-2100C Micro plate reader and BioRad PW40 Washer.

All the data collected was coded and analysed using SPSS 18.0 statistical software. The frequency distribution and descriptive statistics of the study population was done. The quantitative variables were presented as mean ±standard deviation (SD). The qualitative variables were expressed in percentage. Comparison of the means of serum PTH, calcium, phosphorus, ALP and magnesium among diabetic and non-diabetic CHD patients was done using Student's t-test. Comparison of the means of serum PTH, calcium, phosphorus, and ALP among the different age categories was done using one-way analysis of variance (ANOVA). All the tests were 2-tailed and taken to be statistically significant if p value < 0.05.

Statistical Analysis

All the data collected was coded and analyzed using SPSS 18.0 statistical software. Comparison of the means of serum PTH, calcium, phosphorus and ALP among diabetic and non-diabetic CHD patients was done using Student's t-test.

RESULTS

The mean age of the study population was found to be 50.27 ± 12.85 years. The mean age of the male study subjects was 52.45 ± 11.73 years, and that of the female study subjects was 45.65 ± 13.97 years. The number of male and females were found to be 102 (68 %) and 48 (32 %) respectively. The characteristics of the study population are described in Table 1.

Parameter	Mean ± SD	Minimum	Maximum
Age (years)	50.27 ±12.85	22	78
Duration of haemodialysis (years)	3.57 ± 1.77	1	8
Creatinine (mg / dL)	8.74 ± 2.72	3	16.8
Fasting blood sugar (mg / dL)	98.74 ± 28.76	64	173
Parathyroid hormone (pg / mL)	357.27 ± 222.58	47	831
Calcium (mg / dL)	7.92 ± 0.81	5	10
Phosphorus (mg / dL)	5.04 ± 1.48	1.8	8.3
Alkaline phosphatase (U / L)	281.33 ± 202.45	46	1048

Table 1. Characteristics of the Study Population

Among the 150 CHD study subjects, there were 67 diabetics and 83 non-diabetic patients. The prevalence of DM among CHD patients was found to be 44.70 %.

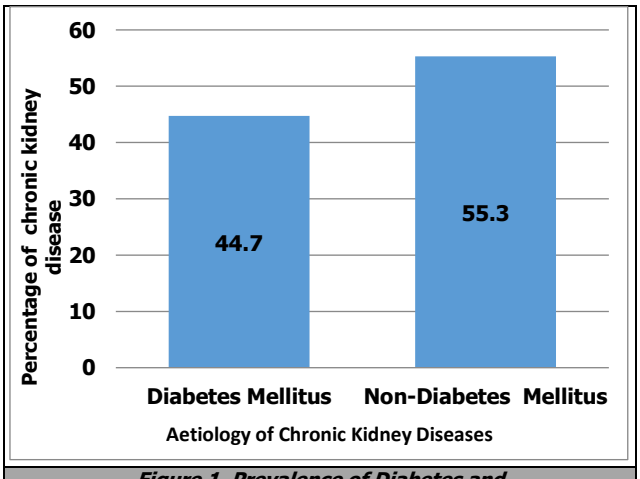

Figure 1. Prevalence of Diabetes and Non-Diabetes in Chronic Kidney Disease

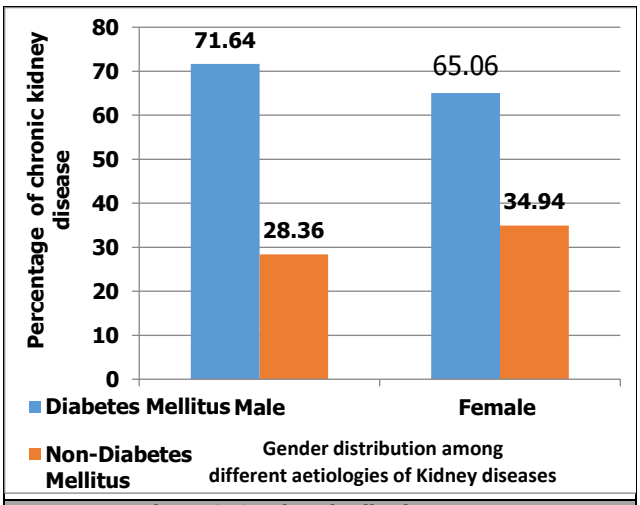

Figure 2. Gender Distribution among Diabetics and Non-Diabetics

Parameter	Diabetics (n = 67) Mean ± SD	Non-Diabetics (n = 83) Mean ± SD	t	Mean Difference	p
Parathyroid hormone (pg / mL)	146.58 ± 51.04	527.35 ± 149.59	19.09	380.77	0.001
Calcium (mg / dL)	7.84 ± 0.84	7.99 ± 0.78	1.14	0.15	0.256
Phosphorus (mg / dL)	4.53 ±1.56	5.45 ± 1.28	3.95	0.91	0.001
Alkaline phosphatase (U / L)	344.93 ± 224.14	230 ± 167.54	-3.59	-114.92	0.001

Table 2. Serum Metabolic Bone Disease Markers in Diabetic and Non-Diabetic CHD Patients (Student's t-Test)

The mean levels of serum phosphorus and PTH are significantly lower in the diabetic CHD population than in the non-diabetics, but mean serum ALP is significantly higher in the diabetic CHD patients. Statistical significance is seen in the serum metabolic bone disease markers except calcium among diabetic and non-diabetic chronic kidney disease.

DISCUSSION

Chronic kidney disease is a chronic non-communicable condition which is commonly encountered in the present day scenario. An increasing prevalence of CKD is being observed in the developed Western countries through the last couple of decades. This could be attributed to the increasing life expectancy and the rise in the prevalence of lifestyle diseases like DM and hypertension (HTN).[10] The developing nations are not far behind in this aspect with the easy availability of better medical facilities, lack of exercise and the increasingly sedentary lifestyle.

It has been estimated that there are around 55,000 patients who are on dialysis in India. This population is growing at an alarming trend of 10 - 20 % annually.[11] DM, HTN, autoimmune disease, old age, previous episode of acute kidney injury and family history of renal disease are some of the risk factors predisposing to CKD. Data collected from around the world, between 1990 and 2002, showed that DM and HTN alone accounted for the majority of kidney failure cases (44.6 % due to DM and 26.9 % due to HTN).[12] It has been seen in studies that the aetiological spectrum of primary disease among CKD patients in India comprise of

DM (41 %), HTN (22 %), chronic glomerulonephritis (16 %), chronic interstitial disease (5.4 %), ischaemic nephropathy (5.4 %), obstructive uropathy (2.7%), miscellaneous (2.7 %) and other unknown causes (5.4 %).[13] Variations have been observed from region-to-region in this distribution pattern.[14] Nevertheless, it has now been reported that DM and HTN account for about 40-60% of CKD cases in India, with diabetic nephropathy emerging as the major cause.[15]

Improving Global Outcomes (KDIGO) Controversies Conference on 'Definition, Evaluation and Classification of Renal Osteodystrophy' held in 2005 recommended that the term renal Osteodystrophy (ROD) be used solely for defining the bone pathology associated with CKD. It was also proposed that the many clinical, biochemical and imaging abnormalities that have been identified as correlates of ROD, be defined more broadly as a clinical entity or syndrome called 'Chronic Kidney Disease-Mineral and Bone Disorder.[16]

The PTH levels in circulation have been used as an indicator of the bone turnover. It is used together with measurements of serum calcium, serum phosphorus and serum ALP, to help evaluate, diagnose and guide the treatment in CKD-MBD. According to the KDIGO workgroup which evaluated the clinical utility of biomarkers in the assessment of CKD-MBD, serum PTH levels used along with serum total ALP levels were established to be helpful in predicting the bone turnover. bALP may have a distinct advantage over the total ALP,[17] but to overcome the high cost of measurement of bALP it was suggested that the total ALP may be used. In CKD-MBD, the laboratory parameters most strongly associated with morbidity and mortality is the serum PTH, serum calcium and serum phosphorus levels.[18] Even though bone biopsy is considered the gold standard for the diagnosis of ROD, biochemical markers like PTH, calcium, phosphorus and ALP may be used as alternative diagnostic tools. Studies have shown that when liver disease has been excluded, intact PTH (iPTH) and ALP can be used for the diagnosis of CKD-MBD.[19]

Many hypotheses have been put forward to explain the relative hypoparathyroidism seen in diabetic CHD patients when compared to their non-diabetic counterparts. It has been noted that DM is accompanied by an increased risk of bone disease.[20] Studies have shown that the poor metabolic control associated with DM can result in alterations of calcium homeostasis.[21] Diabetic patients present a lower level of serum PTH and calcium values than the non-diabetic population. Paula FJ t al, have documented the inhibitory effect that poor metabolic control has on low calcium-mediated PTH secretion.[22] Low PTH concentrations can result in decreased bone formation and weak bones which lead to a higher risk of vertebral fractures in diabetic patients.[23] It has been observed in studies that the low calcium levels are associated with a decreased bone mass.[24] Diabetes mellitus being one of the leading causes of CKD has been constantly evaluated for its role in the development of CKD-MBD. The increasing prevalence of DM in Kerala imparts a great deal of medical significance to this disease and its effects.

In the present cross-sectional study among CHD patients, the prevalence and effects of DM on serum levels of PTH, calcium, phosphorus and ALP was evaluated. It was seen that among the total 150 subjects who took part in this study, 68% was male and 32% was female. The average age of the study subjects was 50.27 ± 12.85 years. Rajapurkar MM et al, conducted a study on 'what do we know about chronic kidney disease in India: first report of the Indian CKD registry' and it was seen that the mean age of CKD was 50.1 ± 14.6 years, and the male female ratio was 70:30.[15] The mean age in studies conducted outside India was found to be 46 ± 18 years.[25]

The prevalence of DM among the study subjects was found to be 44.7 %. Dash SC et al, conducted a study on 'Incidence of chronic kidney disease in India' and found that DM had a prevalence of 41 % among the CKD population. [13] However, in a study conducted by Joy MS et al, in the United States of America, it was seen that DM accounted for about 44.6 % cases of CKD.[12] The increased prevalence in our study population which approximates that seen in the developed countries could be explained by the fact that diabetes is gaining a stronger foothold in our population with the increasing consumption of junk food and sedentary lifestyle. These factors can lead to obesity which is a major risk factor for DM.

Among the diabetic CHD patients, 71.64 % were males and 28.36% were females. The mean age of the diabetic CHD population was found to be 52.81 ± 11.92 years. It was also noted that the majority of diabetics (32.84 %) in the study population were in the age category of 41 - 50 years.

The mean levels of PTH and phosphorus were seen to be significantly lower among the diabetics than the non-diabetics in the study population. However, the mean level of ALP was found to be significantly higher in the diabetics. In this study, the mean PTH value among the diabetic CHD patients was found to be below 300 pg / mL, which is the target cut-off for CKD stage 5 patients on MHD as per the NKF K/DOQI guidelines.[26] On the other hand, the mean serum PTH values among the non-diabetics were found to be higher than the target value. A statistical significance was demonstrated in the serum levels of PTH among diabetic and non-diabetic CHD patients ($p = 0.001$). This is similar to the findings by Dan S et al, among the South Indian population.[14] It has been postulated that there are two mechanisms behind the relative hypoparathyroidism caused by DM. The suppression of PTH secretion can be considered to be due to hyperinsulinemia or hyperglycaemia seen in DM, or the presence of advanced glycation end products (AGEs).[14] Sugimoto T et al, in their study demonstrated that increasing the concentration of glucose in the medium of cultured bovine parathyroid cells caused an inhibition of the secretion of PTH by the cells.[27] Likewise, in another study conducted by Clowes JA et al, it was seen that induction of hyperinsulinemia resulted in a decrease in the PTH values.[28] Makita Z et al, in their studies noted that the level of AGEs was higher in diabetics ESRD patients than both the non-diabetic ESRD patients and the diabetic non-ESRD patients.[29] The excessive accumulation of AGEs in the tissues is seen to suppress the secretion of PTH in response to hypocalcaemia and also the osteoblastic activity resulting in adynamic bone disease.[30]

The mean serum calcium level of the whole study population was found to be below the target range of 8.4-9.5 mg/dL recommended in the NKF K/DOQI guidelines.[26] Both the diabetics and non-diabetics had a mean serum calcium value in the hypocalcaemic range and no statistical significance could be demonstrated in the serum calcium levels among diabetic and non-diabetic CHD patients. This is similar to findings in other studies done both in India and abroad in countries like Egypt.[14,25]

A statistically significant difference was seen in the serum phosphorus levels among diabetic and non-diabetic CHD patients in this study. The mean phosphorus level of the study population was found to be within the target range recommended by NKF K/DOQI (3.5 - 5.5 mg / dL).[26] It was noted that in the diabetics the mean phosphorus level was within the target range but in the non-diabetics it was near the upper limit of the target range. A study by Aubia J et al, has shown that there can be a relative hyperphosphaturia in DM which may affect the hyperphosphatemia normally seen with CKD.[31] The resultant lower levels of serum phosphorus could in turn have a protective effect against secondary hyperparathyroidism. But even small increases in the high normal ranges of serum levels of phosphorus in CKD patients with DM can increase their risk for cardiovascular mortality and morbidity.[32]

The mean serum total ALP level among the study subjects was found to be above the cut-off of the target range (> 128 U / L). Both the diabetic and non-diabetics had mean ALP values above the target range. It was noted that there was a statistical significance in the serum ALP levels among diabetic and non-diabetic CHD patients. This could be explained by the increased bone turnover seen in CKD-MBD. This is similar to the findings seen in a study done by Nasri H et al.[25]

From the above observations, it can be inferred that statistical significance was noted in the serum levels of PTH, Phosphorus and ALP among diabetic and non-diabetic CHD patients. Thus it can be said that a relative hypoparathyroidism is more prevalent among diabetic CHD patients that could manifest as adynamic bone disease. Care should be taken while treating diabetic CHD patients with bone disease. Oral calcium supplements, vitamin D supplements and increased calcium in the dialysate should be avoided since they can lead to hypercalcemia. This can further suppress the PTH secretion and result in aggravation of the already existing low turnover bone disease in such patients.

Limitations

The present study was a hospital based study. A larger community-based study could give a better idea of the CKD population in the Thrissur district of Kerala. Even though KDIGO have suggested that total ALP along with the other biochemical parameters is sufficient to make a diagnosis of CKD-MBD, the use of high sensitivity bone specific ALP would make the study more specific.

CONCLUSIONS

The prevalence of DM among CHD patients was found to be 44.70%. 71.64 % of the male CHD subjects were found to be diabetic whereas, among the female CHD subjects, the prevalence was about 65.06 %.

A statistically significant association was demonstrated in the serum PTH ($p = 0.001$), phosphorus ($p = 0.001$) and ALP ($p = 0.001$) levels among diabetic and non-diabetic CHD patient. No statistical significance could be demonstrated between DM and serum calcium ($p=0.256$).

The serum levels of PTH and phosphorus were found to be significantly lower in diabetic CHD patients than in their non-diabetic counterparts. Serum ALP levels were significantly higher in the diabetics. This demonstrates that a relative hypoparathyroidism is prevalent among the diabetic CHD patients and so care should be taken while treating such patients. Prevention of deterioration of the already existing low turnover bone disease in such patients should be the treatment motto. Avoidance of oral calcium supplements, vitamin D supplements and increased calcium in the dialysate would be ideal, since these can lead to hypercalcemia and further suppress the PTH secretion.

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The prevalence of hypothyroidism in diagnosed cases of cholelithiasis

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Abstract

Introduction: The aim of this study was to evaluate the prevalence of hypothyroidism in diagnosed cases of cholelithiasis using the patient's ultrasonography data and to find the prevalence of obesity in gallstone disease.

Materials and Methods: This study was conducted in Govt. medical college, Thrissur. Two hundred and sixty five study subjects were selected from the patients who were attending wards and outpatient department (OPD) of General Surgery with diagnosed cases of cholelithiasis by ultrasound of abdomen. All the participants were subjected to clinical examination and lab investigations. Serum thyroid-stimulating hormone (TSH) was used to assess thyroid function and thereby hypothyroidism. Body Mass Index (BMI) was used to assess obesity.

Results: High prevalence of hypothyroidism (23%) was observed in gallstone disease. In this study only 6% gallstone disease subjects were obese. We also found that 73% of the study subjects with hypothyroidism were obese.

Conclusion: Hypothyroidism may be suggested as a risk factor for developing gallstone disease. Gallstone patients should be checked for serum TSH because of high incidence of hypothyroidism.

Keywords: Cholelithiasis, Dyslipidaemia, Gallstone Disease, Hypothyroidism, Thyroxine.

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Introduction

Gall stone is the most common biliary pathology both in India and western countries. Autopsy report has shown a prevalence of gallstones from 11to36%. In India high prevalence is reported in northern population.[1] The prevalence of gallstones is related to many factors, including age, gender, and ethnic background. Patients with a common bile duct stone and gallbladder stone have, respectively; 7-foldand3-foldincreasesinthefrequencyof hypothyroidism.[2] This may be related to the triad: hypercholesterolemia, hypotonia of the gallbladder and reduced Bilirubin excretion. There are several explanations for a possible relation between hypothyroidism and gallstone disease. These explanations include the known link between thyroid failure and disturbances of lipid metabolism[3]that may consecutively lead to a change of the composition of the bile. Recent studies[4] also demonstrated low bile flow in hypothyroid subjects. Furthermore, the sphincter of Oddi expresses thyroid hormone receptors and thyroxine has a direct pro-relaxing effect on the sphincter. [5]Both low bile flow and sphincter of Oddi dysfunction are regarded as important functional mechanisms that may promote gallstone formation.[6]

In an animal model of rabbits in which a fatty diet induced gallstone formation, administering thyroxine was associated with a low gallstone weight, but did not dissolve the gallstones.[7] Experiments in rats confirmed a thyroxine effect on bile composition,[8] decreased hepatocytic bile salt excretion in hypothyroid state and relaxation of the sphincter of Oddi. Hence dyslipidaemia is suggested to have strong association with gallstones.

Many studies were conducted to investigate the association between thyroid function and gallstone disease in human beings.[2,3] No systematic studies were carried out to evaluate the prevalence of hypothyroidism in diagnosed cases of cholelithiasis. Therefore, the aim of this study was to evaluate the prevalence of hypothyroidism in diagnosed cases of cholelithiasis using patient's ultrasonography data attending surgery outpatient department (OPD) /causality of Govt. Medical College, Thrissur.

Materials and Methods

This study was conducted in Govt. medical college, Thrissur, Kerala over a period of one year. Two hundred and sixty five study subjects were selected from the patients who were attending wards and OPD of General Surgery with diagnosed cases of cholelithiasis by

ultrasound scan of abdomen [sample size is calculated using the formula $N= (Z\alpha)^2 p q/d^2$ where N= Sample size, p= prevalence according to study[9] q= 100- p

Zα= Z score of α error (i.e. 1.96 with an α error of 5%), D= clinically allowable error of 20% & power of 80% at a significance level of 0.05 D= (p × 20)/100]. Institutional scientific committee and ethical committee approval were obtained prior to the study. Written informed consent was obtained from all the participants and they were subjected to clinical examination and lab investigations. Serum TSH was used to assess thyroid function and thereby hypothyroidism. Serum TSH reference range of 0.350-4.940 uIU/ml was taken as normal. [10] BMI used to assess obesity. Obesity BMI (Quetelet index)>30 was taken as obese.

Results

Two hundred and sixty five subjects were selected for this study. Age of the study subjects ranged from 36–65 and their age distribution is given in Table 1. Middle aged population is the group mostly affected with gallstones.

Table 1: Age wise distribution of the study subjects

Age group	Frequency	Percent
<35	5	1.88
36-45	96	36.22
46-55	97	36.6
56-65	67	25.28
Total	265	100.0

Gender distribution is given in Table 2.

Table 2: Gender distribution

Sex	Frequency	Percent
Female	168	63.40
Male	97	36.60
Total	265	100.0

Female preponderance was noted in study population.

Prevalence of hypothyroidism in gallstone disease is given in Table 3.

Table 3: Prevalence of hypothyroidism in gallstone disease

	Gall Stone Disease Present
Hypothyroidism Present	61(23%)
Hypothyroidism Absent	204(77%)

Gender prevalence of hypothyroidism in gallstone disease is given in Table 4 and 5.

Table 4: Gender prevalence of hypothyroidism in gallstone disease – in male

	Gall Stone Disease Present
Male Hypothyroidism Present	32(33%)
Male Hypothyroidism Absent	65(67%)

Table 5: Gender prevalence of hypothyroidism in gallstone disease – in female

	Gall Stone Disease Present
Female Hypothyroidism Present	29(17%)
Female Hypothyroidism Absent	139(82%)

Prevalence of Obesity in gallstone disease is given in table 6.

Table 6: Prevalence of obesity in gallstone disease subjects with and without hypothyroidism

	Frequency		
Obese Subjects	30(6%)	Hypothyroidism Present	22(73%)
		Hypothyroidism Absent	8(27%)
Non obese Subjects	235(94%)	Hypothyroidism Present	39(17%)
		Hypothyroidism Absent	196(83%)

Discussion

In this study we observed a high prevalence of hypothyroidism in gallstone disease. Many studies have investigated possible associations between serum TSH levels and gallstone disease.

In a study conducted by Honore,[11] series of 668 female patients who had undergone cholecystectomy for gallstone disease, the proportion of treated hypothyroidismwas2.4% compared to 0.8% in the 782 controls.

Other studies found a proportion of

previously diagnosed hypothyroidism of 8% and 6% in patients having common bile duct and gallbladder stones respectively, compared to a proportion of only 1%in the controls.[10] The usage of thyroxine was even suspected to dissolve gallstones.[12] In an animal model of rabbits in whom a fatty diet induced gallstone formation, administering thyroxine was associated with a low gallstone weight, but did not dissolve the gallstones.[8] In a study conducted in North India by Watali et al[13] they observed that 14% of patients were hypothyroid in case group and 8% of the patients in control group. On comparing the two groups, there was no statistically significant difference in the prevalence of hypothyroidism (p value 0.175) between the two groups. However in the present study we observed that 23% of gallstone disease patients have hypothyroidism.

Previous studies that investigated the association between thyroid function and gallstone disease in human beings, were conducted in a series of patients with potential for selection bias that may have produced false positive results.[2,10] Furthermore, the statistical analyses were only controlled for age, but not for further confounders in both studies.[10,11] In a large case control study, no independent relation between thyroid disorders and gallstone formationwasfound.[2] Unfortunately, the exposure was only defined as previous history of thyroid disease, and assessments of the current thyroid function status were not included.

According to Singh et.al an advanced age, high BMI and serum lipids were identified as major independent risk factors for cholelithiasis[14]. However in our study only 6% gallstone disease subjects were obese. According to Sanyal and Raychaudhuri obesity and hypothyroidism are two common clinical conditions that have been linked together closely.[15] Our study is also in agreement with that as we found that 73% of the study subjects with hypothyroidism were obese.

Conclusion

High incidence of hypothyroidism was observed in patients with gallstone disease. Hypothyroidism may be suggested as a risk factor for developing gallstone disease. In this study male hypothyroids were of lager in number when compared with females in gallstone disease. Only 6% of gallstone subjects were noted obese. Gallstone patients should be checked for serum TSH because of high incidence of hypothyroidism.

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Serum Lipids and Apolipoproteins in Diabetic Retinopathy: A Case Control Study

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Abstract:
Aim*: To evaluate association of apolipoproteins A1 and B and fasting lipid profile (FLP) with diabetic retinopathy (DR) in adults.*
Materials and Methods: *This is a hospital based 9 months case control study of diabetic subjects (n= 169) by Ophthalmological examination and Biochemical parameters (Fasting plasma glucose (FPG), FPL , Apolipoproteins A1 and B) .Statistical analysis was done using SPSS version 17.*
Results*: Out of 169 diabetic subjects studied(mean age - 55.46±9.78 years; mean BMI - 24.25±3.71; Mean duration of diabetes - 10.17±7.45years) , 51.5% (87/169) had no signs of DR, 11.8% (20/169) had mild NPDR, 16.6% (28/169) had moderate NPDR, 6.5% (11/169) had severe NPDR and 13.6% (23/169) had proliferative DR. FPG (p-0.02), TC (p-0.009), LDL-C (p-0.053), HDL-C (p-0.001) and TG (p-0.0001) were found to have a statistically significant association with DR. ApoA1 (OR-0.996 and p-0.393) and apo B (OR-1.006 and p-0.145) showed no statistically significant association with DR apo B /Apo A1 ratio (OR-2.11 and p-0.048) showed a statistically significant association with DR. No significant correlation was observed between various lipid parameters and apolipoproteins with different stages of diabetic retinopathy.*
Conclusion*: The significant association of serum lipids and Apolipoprotein B/apo A-1 ratio with DR shown by this study indicates, along with glycemic control, correction of hyperlipidemia is important in preventing the development of DR. Besides, the Apo B / apo A -1 ratio can be used as an index of DR.*
Keywords*: Diabetic retinopathy, Serum Lipids, Apolipoprotein A1, Apolipoprotein B, Apolipoprotein B/ apo A-1 ratio (apo B/apo A-1 ratio*)

I. Introduction

Diabetic retinopathy (DR) is a common, specific microvascular complication of diabetes mellitus (DM) causing visual impairment which is preventable by screening and clinical management. A positive association has been shown between DR and duration of DM, poor glycemic control [1] and unhealthy lipid profile [2] . Recently apolipoprotein A1 (apo A-1) of antiatherogenic HDLand apolipoprotein B (apo B) of atherogenic LDL are found relevant to biophysiological changes of DR than traditional lipids. [3, 4, 5] There is an increasing prevalence of type 2 DM and DR in India. [6,7] Type 2 DM in Indians differ from the West in different aspects [2,4] indicating the importance of regional studies.

Aim

To evaluate and compare the association of apo A -1 , apo B and traditional lipid profile with diabetic retinopathy in adults with type 2 DM.

II. Materials and Methods

This was a hospital-based case-control study. The study group consisted of 169 type 2 DM patients who attended the Medicine OPD and/ or Retina Clinic of Government Medical College, Thrissur, from April 2012 to December 2012. Patients diagnosed with DR of any stage were taken as cases and type 2 DM patients without any evidence of retinopathy were taken as controls of this study.

Inclusion criteria: Type 2 DM patients with and without DR.

Exclusion Criteria: Type 2 DM patients with history of glaucoma, liver disease, previous vitreoretinal surgery and those with media opacity were excluded from the study.

Clinical Assessment: Clinical and treatment history, family history of having at least one first degree relative diagnosed of type 2 DM and demographic data were collected, and a complete physical examination was done. Diagnosis of type 2 DM was made according to WHO criteria. [8] A complete ophthalmological examination was performed including fundus examination with slit lamp biomicroscopy and indirect ophthalmoscopy, fundus colour photograph centered on the macula and fundus fluorescein angiography wherever indicated;

subsequently subjects were classified as cases (with DR) and controls (without DR). Cases were further divided into non-proliferative DR and proliferative DR, in which non proliferative DR was sub stratified into mild NPDR, moderate NPDR, severe NPDR and very severe NPDR according to the International Clinical Diabetic Retinopathy Disease Severity Scale. [9] The worse eye was used to determine the severity scale of a patient.

Anthropometric Measurements: Participants were weighed in light clothing without shoes and their heights measured. Body mass Index (BMI) was calculated as kilogram per meter square (kg/m^2).

Biochemical Analysis: Blood samples were collected after overnight fasting (> 8 hours) by venipuncture. Estimation of FPG (fasting plasma glucose) done by glucose oxidase - peroxidase based method and serum lipid profile (Total cholesterol – CHOD – PAP method; Triglycerides – GPO – PAP method, HDL – Cholesterol (HDL – C) – indirect method by selective precipitation of low density lipoprotein cholesterol by phosphotungstate and $MgCl_2$) was carried out using EM 360 Autoanalyser (Transasia) utilizing kits provided by Agappe diagnostics. LDL cholesterol (LDL-C) was calculated using the Friedewald formula. Serum apo A - 1 and apo B were measured by immune turbidimetry (Quantia).

Ethics: A written consent was obtained from each participant and the study was approved by Institutional Ethics Committee (IEC), Government Medical College, Thrissur, Kerala.

Statistics: Data analysis was performed with SPSS version 11.5. Baseline characteristics of participants with or without DR were compared using Chi-square test and t-test. Logistic regression was used to calculate adjusted odds ratio between serum lipids and apolipoproteins with DR.

III. Results:

General characteristics of study population: The diabetic patients (n=169) of this case control study showed mean age 55.46±9.78 years, BMI 24.25±3.71 and duration of type 2 DM 10.17±7.45 years. Out of 169 subjects 51.5% (87) had no signs of DR, 11.8% (20) had mild NPDR, 16.6% (28) had moderate NPDR, 6.5% (11) had severe NPDR and 13.6% (23) had proliferative DR. Analysis of various parameters of the study subjects are shown in Table 1.

Association of serum lipids and DR: Logistic regression analysis showed statistically significant association of FPG, Total Cholesterol, LDL- C, HDL- C and TG with DR. ApoA1 and B were found to differ considerably in patients with and without DR, but the differences were not statistically significant. However, apo B/apo A-1 ratio showed a statistically significant correlation with DR**.** (Table 1 and 2)

Association between serum lipids and stages of retinopathy: No statistically significant correlation was observed between various lipid parameters – both conventional lipid profiles and apolipoproteins with different stages of DR as shown in Table 3.

Association of various risk factors to development of DR: Family history of diabetes, hypertension, and smoking were found to be significantly related to the development of DR as shown in Table 4.

IV. Discussion

The aim of our study was to find out the association between serum lipids and apo A -1 and apo B with DR and this study showed statistically significant association of FPG (high levels) (see table 1), serum lipids (high total Cholesterol, high TG, and low HDL - C) and apo B/apo A-1 ratio (high) with DR. But there was no statistically significant independent association of apolipoprotein A - 1 and B with DR. These results are consistent with several other studies. [**1,2,7,10,11,12,13,14].** But in a recent study , Sasongko et al [3] reported statistically significant association between apo A1, apo B and apo B/apo A-1 ratio with DR.

The finding of significant association of apo B/apo -1 ratio and the lack of significant independent association of these apolipoproteins with DR supports the following concepts. Apo A-1 has anti-inflammatory and antioxidant actions [19.,20,21] and in addition it specifically inhibits oxidation of LDL to form oxidized LDL. The oxidized LDL will deteriorate antiplatelet and anti-inflammatory functions of endothelium. Hence we need a parameter that reflect the net effect produced from the interactions of apo A-1 and apo B which seems to be crucial in the pathogenesis of microangiopathy leading to DR. For that apo B/apo A-1 ratio will be useful and was proved by our study as well as several other studies [**1,3,14,15]**.

In Sasongko MB et al study **[15]** ,the apo B/apo A-1 ratio indicated association between measures of vascular functions like flicker light induced retinal arterial dilatation and retinal arterial tortuosity suggestive of its role in retinal microvasculature. Their study also showed significant differences between apo A1 and apo B with different stages of diabetic retinopathy. But in our study, there was no significant independent association of apo A1 and apo B with DR.

No significant association was found between either serum lipids or apolipoproteins with the severity of diabetic retinopathy in our study. But in DCCT/EDIC **[10]**, a study in a type 1 diabetic population, there was a positive association of TG and an inverse association of HDL-C with severity of DR, but no significant association was noted with total cholesterol (TC). In the CURES study, TC was found to be an independent risk factor for DR **[7]**. Sasongko et al [3] also established inverse association of HDL–C with any type of DR.

WESDR (Wisconsin Epidemiologic Study of Diabetic Retinopathy) [12] and a cross-sectional study from Bangalore [16] did not show any association between dyslipidemia with occurrence or severity of DR. No association was found between serum lipids and progression of DR in type 2 DM in the FIELD study [17]. According to Deguchi et al apoA1 ,apo B and apo B/ apo A-1 ratio are related to the development of Proliferative DR (p value 0.08, 0.02 and 0.004 respectively) [14]. Another study by Andina Hu et al also showed significant association between apolipoprotein ratio and proliferative DR[1].

Our study noted statistically significant association between family history of type 2 DM and DR. The DCCT study [10] reported familial clustering of DR (odds ratio of 5.4) in diabetic relatives of diabetic retinopathy subjects compared to those without retinopathy [18]. Both smoking and presence of hypertension were also significantly associated with DR. (see table 4)

Present study once again reaffirms high TC, high TG , low HDL -C, high LDL- C ,high apo B /apo A-1 ratio and family history of diabetes as potential risk factors of DR and hence the need of tight control of diabetes and lipid profile by diet, life style modifications and drugs to lessen or prevent the occurrence of DR. It also alert the need of optimum control of hypertension, quit the habit of smoking as these factors showed significant association with DR. In addition it lights up the potential of apo B /apo A-1 ratio as a marker of DR.

Our study has some limitations. 1)The study determined the scale of diabetic retinopathy based on clinical examination rather than from standard fundus photographs (2) Sample size may not be enough in various stages of retinopathy, to assess the relation of serum lipids and apolipoproteins with the severity of retinopathy (3)Direct methods of assay for LDL and HDL could have given a better estimate of these lipoproteins than the indirect methods used in this study (4) Since the study was hospital based it may not reflect the true status of all diabetic patients in the population. (5) This study showed a statistically significant association between apo B/apo A-1 ratio with diabetic retinopathy which indicates the need for doing a similar study with larger sample size to unravel the independent association of apo B and apo A - 1 with DR.

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Table 1 .General characteristics of study population

	No DR (Mean ±SD)	With DR (Mean ±SD)	Mean difference	P value
Age	55.01 ±9.79	55.93±9.8	-0.915	0.545
BMI	24.24±4.41	23.25±2.71	0.985	0.084
FPG	152.51±61.08	173.23±53.175	-20.726	0.020
TC	185.5±48.03	210.6±54.03	-2.509	0.002
LDL-C	101.24±44.46	126±48.25	-25.283	0.001
HDL-C	62.57±19.07	55.13±15.24	7.441	0.006
TG	129.33±53.997	164.77±63.36	-35.44	0.000
ApoA1	143.92±33.14	138.98±35.36	4.944	0.350
Apo B	115.82±37.88	125.38±43.69	-9.556	0.130
Apo B/Apo A1	0.853±0.36	0.974±0.44	-0.121	0.054

(DR-Diabetic retinopathy, SD-standard deviation, BMI-body mass index, FPG-fasting plasma glucose, TC : Total cholesterol, LDL-C --low density lipoprotein cholesterol, HDL-C -- high density lipoprotein cholesterol, TG-triglycerides, Apo A1-apolipoprotein A1, Apo B-apolipoprotein B)

Table 2. Association of serum lipids and retinopathy

	Adjusted Odds ratio (95% CI)	P value
Apo A1	0.996 (0.991 – 1.001)	0.393
Apo B	1.006 (1.002 - 1.010)	0.145
Apo B-Apo A1 ratio	2.110 (0.703 - 3.520)	0.048
TC	1.043 (1.026 - 1.059)	0.009
LDL	0.968 (0.951 - 0.984)	0.053
HDL	0.939 (0.921 - 0.957)	0.001

(LDL-C --low density lipoprotein cholesterol, HDL-C -- high density lipoprotein cholesterol, Apo A1-apolipoprotein A1, Apo B-apolipoprotein B)

Table 3. Association between serum lipids and stages of retinopathy

	Adjusted Odds ratio (95% Confidence Interval for OR)	
	No DR Vs NPDR	NPDR Vs PDR
Apo A1	1.010 (0.997-1.024)	1.009 (0.995-1.023)
Apo B	0.989 (0.978-0.999)	0.992 (0.981-1.002)
Apo B / A-1 ratio	0.206 (0.071-0.595)	0.291 (0.097-0.873)
TC	0.952 (0.914-0.981)	0.989 (0.954-1.025)
LDL	1.040 (0.996-1.087)	1.009 (0.970-1.050)
HDL	1.094 (1.039-1.152)	1.037 (0.988-1.089)

(DR-Diabetic Retinopathy, NPDR – Non-Proliferative Diabetic Retinopathy, PDR – Proliferative Diabetic Retinopathy, LDL-C --low density lipoprotein cholesterol, HDL-C -- high density lipoprotein cholesterol, Apo A1-apolipoprotein A1, Apo B-apolipoprotein B)

Table 4. Association of various risk factors to development of retinopathy

Risk factors	Odds Ratio	95% Confidence Interval
Family history of Diabetes(+/-)	1.846	0.999 - 3.409
History of hypertension(+/-)	2.717	1.444 - 5.11
Smoking(+/-)	2.105	1.016 - 4.361

Case Report

Crystals Hold the Clue

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Abstract

A 21-day-old male infant, born as the first child to a nonconsanguineous couple, presented with nonspecific symptoms, signs, and superimposed infection. Investigations conducted were not conclusive to arrive at a diagnosis. In 6 days, the infant succumbed to his condition. Postmortem samples were analyzed for metabolic substances, and liver biopsy was done. Urine metabolic screening showed the presence of amino acids and reducing substance. Further analysis proved the presence of galactose, generalized aminoaciduria, and liver biopsy with features of inborn error of metabolism. Further samples for higher investigations were not available, which draws attention to the need of being able to diagnose the condition early enough to save lives. We are suggesting a helpful, easy to perform, and cheap diagnostic test algorithm for diagnosing galactosemia in resource-poor settings.

Keywords: *Diagnostic test algorithm, galactosemia, osazone test for sugars, urine metabolic screening*

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Introduction

Inborn errors of metabolism are disorders which have features similar to each other and to that of infection, infestation, and intoxication. Good clinical acumen and competent laboratory are mandatory for early diagnosis and prompt intervention. The deranged metabolic pathway of common biological compounds cause deficiency of substances, accumulation of metabolic intermediates and undesired products formed from alternate metabolic pathways. These hinder and interrupt normal functioning of the human body.[1,2]

We came across an interesting and rare condition which was diagnosed at our laboratory. We would like to highlight a seldom used testing algorithm for diagnosing the same even in a resource-poor setting.

Case Report

A 21-day-old male infant was referred to our tertiary care center with the complaints of refractory hypoglycemia, jaundice, loose stools, and superimposed *Candida albicans* infection. The patient on presentation had tachypnea with normal oxygen saturation, hypotonia, and other systemic examination was normal. The child was born as the first child to a nonconsanguineous couple, after a previous first trimester abortion, with uneventful pregnancy, spontaneous onset of labor, and normal vaginal delivery at a primary health center. On day 1, the child developed fever and jaundice, and after ruling out maternal causes, the patient was referred to a higher center. At the higher center, the patient's jaundice subsided with phototherapy, but he developed seizures, loose stools, and later *C. albicans* infection. When they found refractory hypoglycemia and rapidly worsening condition, the patient was referred to a tertiary care center. The patient had normal liver function test, electrolytes, negative urine sugar, and ketone bodies. Regular expressed breast milk was given along with supplements. His condition worsened, requiring intensive care unit admission. Anemia was noticed and two subsequent matched blood transfusions were given. Electrolyte imbalance was corrected. The patient succumbed to the sepsis in spite of supportive management. No diagnosis could be reached at the time of demise. Postmortem urine sample was sent directly by bladder syringing, for metabolic screening.

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Liver tissue was sent for biopsy. Urine metabolic preliminary screening tests showed the presence of amino acids by ninhydrin test and reducing substances by Benedict's test. Common amino acidurias were ruled out. Urine amino acid chromatography result was generalized aminoaciduria when compared to an age-matched control. The most common reducing substance seen in urine was glucose, and for that, specific enzymatic test – glucose oxidase-peroxidase test was done, which reported negative. Sugar chromatography was done with the common reducing sugar standards. A spot corresponding to galactose standard was obtained. Osazone test yielded characteristic rhombic-shaped crystals arranged in the form of cartwheel which was similar to that with galactose standard [Figures 1 and 2]. There was no sample available for further confirmatory tests – mucic acid test and red blood cell enzyme assay. Liver biopsy result was nonconclusive but showed features of inborn error of metabolism. This brought us to conclude that the infant had an inborn error of metabolizing the reducing sugar galactose, which was inadvertently introduced into the body in the form of breast milk. From the rapid progression of the condition, it might have been complete deficiency of galactose-1-phosphate transferase termed "classic galactosemia."

Discussion

Galactosemia is an autosomal recessive metabolic disorder of carbohydrates, involving defective galactose-degrading enzymes – galactokinase, galactose-1-uridyl transferase, or Uridine diphosphate (UDP)-galactose epimerase. The sources of galactose in human beings are both endogenous (from breakdown of tissue glycoproteins and glycolipids) and exogenous (mostly from milk protein lactose which is broken down by intestinal flora into monomers, glucose and galactose). The pathophysiology lies in the body's lack of enzymes in Leloir pathway, to convert galactose into glucose and thus getting utilized. The accumulated galactose and its metabolites from alternative pathways cause tissue damage, especially in the liver, brain, and kidneys. Liver dysfunction causes refractory hypoglycemia and progresses soon to severe liver dysfunction and cirrhosis.[3,4] One of the metabolites, galactitol, gets irreversibly deposited in ocular lens, causing zonular cataracts. Accumulation in white blood cell decreases its bactericidal activity and predisposes to Gram-negative sepsis. It has been found that the damage starts *in utero* and gets worsened by milk intake after birth. By restricting lactose during pregnancy and following birth, the damages caused by the sepsis, clinically significant cataract, and severe liver dysfunction can be prevented.[5] The neurological and ovarian dysfunctions seem to be unaffected by diet control. In certain less symptomatic disease variants, like the Duarte type, the patient survives to adulthood.[6]

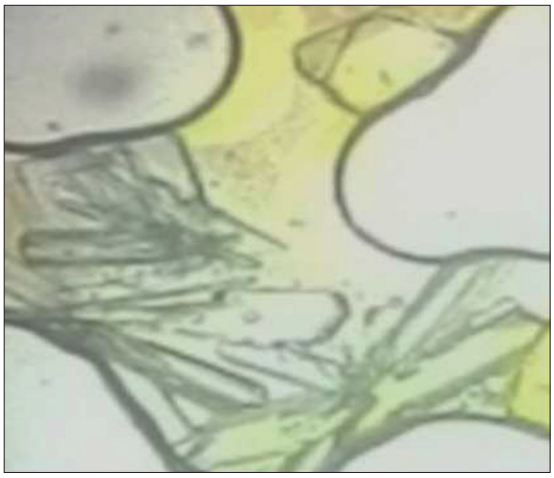

Figure 1: Osazone crystals from the patient sample when viewed under microscope showing rhombic-shaped crystals

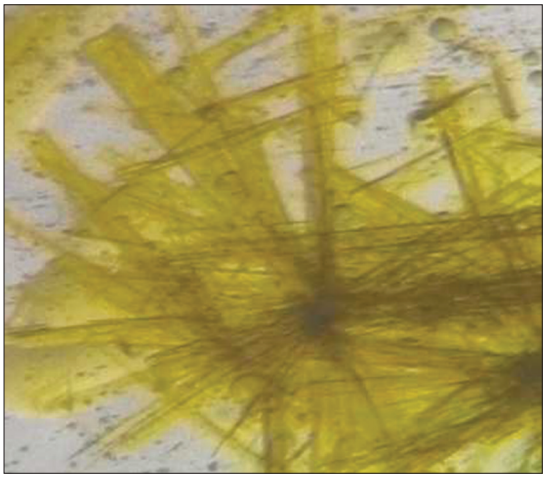

Figure 2: Osazone crystals of galactose standard when viewed under microscope showing similar rhombic-shaped crystals

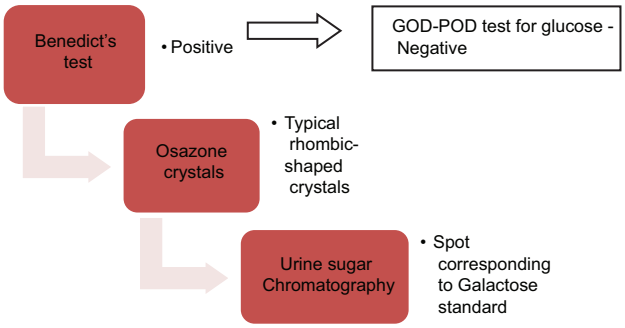

Figure 3: Diagnostic algorithm for Galactosemia

In this case, the presentation of the male infant with neonatal jaundice was treated successfully as physiological jaundice. Subsequent refractory

hypoglycemia and high serum insulin level and *C. albicans* sepsis with pneumonia made it difficult to understand the basic pathophysiology within the short span of time within which the patient succumbed. A report of absent urine-reducing sugars led the treating clinician to rule out carbohydrate-metabolizing defect. The fulminant *C. albicans* sepsis and pneumonia further made it difficult to delineate the symptoms of the underlying condition.

Looking back, refractory hypoglycemia presenting in the 1st week of life with worsening clinical condition and no obvious noxious factors point toward two differential diagnosis –glycogen storage disorders and galactosemia.[7] A history of one previous spontaneous abortion was a pointer toward a possible genetic defect. The nonspecific symptoms of vomiting, diarrhea, lethargy, failure to thrive, and seizures requiring multiple antiseizure medications indicated an ongoing pathology which was fueled by the milk intake. Both the conditions can present with similar clinical picture and clinicians depend on laboratory evidence to arrive at a definitive diagnosis. Enzyme assays are the answer to both, but it is not available at our setup. Microscopic examination of the liver tissue after differential staining can help to rule out one condition, but it is an invasive procedure and in this case was inconclusive. It however showed features of a metabolic disorder. Paper chromatography which finally helped to arrive at a diagnosis is only a surrogate test and had to be relied on, by which time the patient had expired. Galactosazone crystals seen in the urine sample were conclusive as the uniquely shaped crystals are diagnostic on their own. No further sample could be obtained for further confirmatory tests for galactose.

It called for the laboratory to try out the old and seldom used techniques in the absence of modern facilities to aid the clinician. We are putting forth a simple, economical diagnostic test algorithm for galactosemia, which can be performed by any medical practitioner as a bedside test [Figure 3].

Declaration of patient consent

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

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The laboratory supplies provided by Government of Kerala were used for the tests done as part of routine laboratory investigations.

Conflicts of interest

There are no conflicts of interest.

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