ORIGINAL ARTICLE

Thiamine deficiency and its correlation with dyslipidaemia in diabetics with microalbuminuria

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Abstract

Objective: To measure and correlate the levels of thiamine and dyslipidaemia in microalbuminuric diabetics.

Methods: Cross-sectional comparative study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, from January 2009 to December 2010, and comprised 60 known diabetic patients, who were inducted from diabetic clinics of Rawalpindi. These patients were divided into three equal groups, with group I (n=20) being normal healthhy individuals, group II comprised of microalbuminurics type 2 diabetics (n=20) and group III (n=20) were macroalbuminuric type 2 diabetics, based on their albumin excretion rate. The healthy volunteers (n=20) had blood glucose less than 6 mmol/L and were inducted as the comparison group. Fasting blood samples of diabetic and control groups were analysed for glucose, glycosylated haemoglobin, lipid profile, thiamine chloride and thiamine monophosphate. Besides, 24-hour urine samples were analysed for microalbuminuria, thiamine chloride and thiamine monophosphate.

Results: Plasma thiamine chloride and thiamine monophosphate levels were found to be significantly ($p<0.001$) reduced in the diabetics (n=60) compared to the controls (n=20). Furthermore, there was a progressive decline in these levels with increasing albuminuria; the lowest being in the macroalbuminuric group (group IV). Urinary thiamine levels were significantly ($p<0.001$) higher in the diabetics compared to the controls. These changes were more pronounced as albuminuria level increased; the highest being in group IV. The parameters of lipid profile, including triglycerides, total cholesterol and low-density lipoprotein cholesterol, were significantly ($p<0.001$) higher in diabetics and showed progressive increase with worsening albuminuria. Whereas, the high-density lipoprotein cholesterol levels were significantly ($p<0.001$) reduced in diabetics and showed progressive decline as the microalbuminuria status worsened. Furthermore, a significant negative correlation was found between plasma thiamine and all the parameters of lipid profile except high-density lipoprotein cholesterol which had a significant positive correlation. A significant linear regression of microalbuminuria on plasma thiamine was also found.

Conclusion: Thiamine levels were reduced in the diabetic population and this reduction in thiamine level was negatively correlated with lipid profile in microalbuminuric diabetics.

Keywords: Diabetes mellitus, Dyslipidaemia, Microalbuminuria, Thiamine chloride, Thiamine monophosphate.
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Introduction

Thiamine is an indispensable coenzyme of several steps of the intermediary metabolism. Diabetes is believed to be a thiamine-deficient state due to amplified glucose metabolism.[1] The main mechanisms causing diabetic complications include the activation of polyol pathway, formation of advanced glycation end products (AGES), activation of protein kinase C (PKC) and increased flux through the hexosamine biosynthetic pathway (HBP).[1] These processes are triggered by increased concentrations of triosephosphate intermediates of glycolysis[2,3] and can be suppressed by decreasing the accumulation of triosephosphates. This may be done by the activation of reductive pentose phosphate pathway (PPP) by high-dose thiamine therapy that would increase transketolase (TK) activity and stimulate the conversion of glyceraldehyde-3-phosphate (GA3P) and fructose-6-phosphate (F6P) to ribose-5-phosphate (R5P), thus reducing the risk of the development of diabetic complications.

Although the main abnormality seen in type 2 diabetes mellitus (DM) is abnormal glucose metabolism and is responsible for most of the symptoms and complications of diabetes, but it is believed that the pathogenesis of type 2 DM is mainly linked to disordered lipid metabolism.[4] Many factors are responsible for the genesis of post-prandial lipid abnormalities in type 2 diabetics. Lipoprotein lipase (LPL) is the key enzyme in the catabolism of both exogenous and endogenous triglyceride-rich lipoproteins.[5] Thiamine can cause reversal of

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dyslipidaemia by reducing the levels of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) which is a hexosamine pathway intermediate. UDP-GlcNAc causes glycosylation of the transcription factor Sp1. In its unglycosylated state, the Specificity protein (Sp1) transcription factor activates the expression of LPL.[6] Furthermore, glycosylation of Sp1 also increases the transcription of the rate limiting enzyme of the fatty acid synthesis, namely, acetyl CoA carboxylase (ACC). Thus, by reducing the levels of UDP-GlcNAc, hepatic fatty acid synthesis can also be reduced.[7] Another effect of thiamine on decreasing dyslipidaemia is by diverting the flow of glucose and its metabolites away from the hexosamine pathway. In the liver of transgenic mice, over-expression of glutamine:fructose-6-phosphate amidotransferase (GFAT) is seen.[8] This is the rate limiting enzyme of the hexosamine pathway. The activation of the hexosamine pathway caused by hyperglycaemia leads to the induction of lipogenic enzymes like glycerol-3-phosphate dehydrogenase (GPDH), fatty acid synthase (FAS) and ACC.[9] High-dose thiamine can induce the expression of TK and saturate it with thiamine pyrophosphate (TPP), thus enhancing its activity. Once the depletion of TK is countered, it can activate the PPP and divert the flow of glucose and its metabolites away from the hexosamine pathway.[10]

Diabetic nephropathy is characterised by albuminuria as a consequence of glomerular endothelial damage and progression due to tubulointerstitial inflammation and fibrosis.[11] It is believed that if the accumulation of triosephosphates as seen in hyperglycaemia is countered, it would prevent the multiple pathogenic pathways leading to the development of diabetic nephropathy. Thiamine can prevent the accumulation of triosephosphates through the induction of TK synthesis which, in turn, reverse the multiple pathways leading to diabetic nephrophathy.

The current study set out to explore the levels of thiamine in our diabetic population and to see the effects of its deficiency, if found, on the lipid profile of diabetics. Dyslipidaemia is one of the major known compounding factors associated with diabetes causing diabetic complications leading to increased morbidity and mortality. If the thiamine deficiency is associated with dyslipidaemia, then improvement in its level may reduce diabetic complications associated with deranged lipid profile.

Patients and Methods

The cross-sectional comparative study was conducted at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, from January 2009 to December 2010. Non-probability convenience sampling was used, and patients were enrolled from the diabetic clinics of Military Hospital and Holy Family Hospital, Rawalpindi. The subjects were divided into four groups. Group I (n=20) consisted of normal healthy individuals; group II (n=20) comprised normoalbuminuric type 2 diabetics; group III (n=20) had microalbuminuric type 2 diabetics; and group IV (n=20) consisted of overt albuminuric type 2 diabetic subjects. The albuminuria status was divided as follows: (i) normoalbuminuric: albumin excretion rate (AER)<30mg/24h; (ii) microalbuminuric: AER between 30-300 mg/24h; (iii) overt albuminuric: AER>300 mg/24h.

Known type 2 diabetics of both genders between the ages of 18 and 65 years, with a body mass index (BMI) between 19-40 kg/m^2, glycosylated haemoglobin (HbA1c) less than 10% and a history of 5 or more years were included in the study. Age and gender matched healthy volunteers having fasting blood glucose level below 108 mg/dl were inducted in the normal control group.

Patients with significant co-morbidities like chronic liver disease, ischaemic heart disease, those having undergone major transplant surgery etc., subjects who had participated in an interventional study within the preceding 30 days, patients with end-stage renal disease (creatinine clearance less than 10 ml/min), patients taking B-complex supplements and pregnant or lactating women were excluded from the study.

Samples of 10ml venous blood and 24-hour urine were collected after getting informed written consent from the subjects. Blood was transferred to plain tubes and tubes containing ethylenediaminetetraacetic acid (EDTA). One to two ml of whole blood was stored separately, while the rest of it was centrifuged at 5000 revolutions per minute (RPM). Plasma, serum and urine were separated and stored at -80°C for estimation. All the tubes were pre-labelled with the particulars of the subjects and the date of sampling. Plasma glucose was estimated by enzymatic colorimetric method using glucose oxidase enzyme (levels < 6.0mmol/L were considered normal).[12,13] Glycosylated haemoglobin was determined by column chromatography with cation exchange resins (values <6.5% were considered normal).[14] Serum triglycerides were estimated by enzymatic colorimetric method using glycerol kinase (levels <1.7 mmol/L taken as normal).[15] Total cholesterol was measured by enzymatic colorimetric method using

cholesterol oxidase (values <5.16 mmol/L considered normal).[16] High-density lipoprotein (HDL) cholesterol was measured by direct method employing cholesterol oxidase in the first step and a surfactant acting on the HDL in the second step (level >1.42 mmol/L in males and >1.68 mmol/L in females were taken as normal).[17] Low-density lipoprotein cholesterol was determined by using polyvinyl sulphates (PVS) method (values <2.5 mmol/L considered normal).[18] Thiamine chloride and thiamine monophosphate (TMP) in the plasma and urine were determined by high-performance liquid chromatography (HPLC) with fluorimetric detection and precolumn derivatisation to thiochromes.[19] Urinary albumin excretion (UAE) was measured by immunoturbidimetry.

For statistical analysis, SPSS 17 and Microsoft Excel worksheet 2010 with Add on Statistical Package were used. Mean and standard error of mean (SEM) were used to describe numeric variables. Analysis of variance (ANOVA) was applied to find out significant differences among the groups. ANOVA was followed by Post Hoc Tuckey's test for multiple comparisons among the groups. A p-value less than 0.05 was considered significant, and a p-value less than 0.01 was considered highly significant.

Results

The mean ± SEM values for glycaemic status, thiamine status and lipid profile were measured for all the groups (Table-1). There was a significant difference in the mean levels of parameters of the glycaemic status in all the groups except groups III and IV where though means plasma glucose and glycosylated haemoglobin (HbA1c) were higher, in group IV, but the difference was insignificant (Table-2). The mean difference between all the parameters of lipid profile was significant in all groups except groups II and III where the difference between total cholesterol was not significant (Table-3). The mean difference in plasma thiamine chloride level was significant in all groups except between groups I and II and groups III and IV. The mean difference in urinary thiamine chloride was significant between groups I and III and groups I and IV, while no significant

Table-1: Mean ± SEM levels of various parameters of glycaemic status, lipid profile thiamine status and albuminuria of different subjects in each group.

Variable	Group I	Group II	Group III	Group IV
Plasma Glucose (mg/dl)	78.40 ± 1.75	162.80 ± 6.68	242.30 ± 2.21	262.95 ± 5.23
HbA1c (%)	3.14 ± 0.11	5.02 ± 0.18	7.54 ± 0.27	8.29 ± 0.30
Triglycerides (mg/dl)	104.71 ± 1.87	219.90 ± 3.92	263.88 ± 4.70	316.66 ± 5.65
Total cholesterol (mg/dl)	150.80 ± 3.76	226.19 ± 5.64	248.81 ± 6.20	373.22 ± 9.30
LDL cholesterol (mg/dl)	52.98 ± 0.94	132.45 ± 2.35	158.94 ± 2.82	222.52 ± 3.94
HDL cholesterol (mg/dl)	75.69 ± 0.95	37.84 ± 0.47	25.23 ± 0.32	19.40 ± 0.24
Plasma thiamine chloride (nmol/L)	7.33 ± 0.36	6.83 ± 0.31	5.52 ± 0.28	4.68 ± 0.21
Urinary thiamine chloride (ug/day)	42.85 ± 3.50	60.0 ± 4.90	71.99 ± 5.88	79.19 ± 6.46
Thiamine monophosphate(nmol/L)	8.37 ± 0.36	6.12 ± 0.28	4.38 ± 0.28	2.71 ± 0.25
Albuminuria (mg/day)	27.91 ± 2.26	27.37 ± 2.21	220.52±17.84	330.78 ± 26.76

SEM: Standard error of mean.
HbA1c: Glycosylated haemoglobin.
LDL: Low-density lipoprotein.
HDL: High-density lipoprotein.

Table-2: Mean difference in plasma levels of various parameters of glycaemic and thiamine status in different groups.

Group comparison	Glycosylated haemoglobin		Plasma glucose		Plasma thiamine chloride		Urinary thiamine chloride		Plasma thiamine monophosphate	
	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value
Groups I and II	-1.88**	≤0.001	-84.40**	0.000	0.50NS	0.633	-17.14NS	0.110	2.24***	0.000
Groups I and III	-4.40**	≤0.001	-163.90**	0.000	1.80***	0.000	-29.14***	0.001	3.99***	0.000
Groups I and IV	-5.15**	≤0.001	-184.55**	0.000	2.64***	0.000	-36.34***	0.000	5.66***	0.000
Groups II and III	-2.51**	≤0.001	-79.50**	0.000	1.30**	0.013	-12.00NS	0.385	1.744***	0.000

NS $p > 0.05$ = not significant.
** $p \leq 0.001$ = highly significant.
*** $p \leq 0.001$ = very highly significant.

Table-3 Mean difference in plasma levels of various parameters of lipid profile in different groups.

Group Comparison	Triglycerides		Total cholesterol		LDL		HDL	
	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value
Groups I and II	-115.18**	0.000	-75.40**	0.000	-79.47**	0.000	37.84**	0.000
Groups I and III	-159.16**	0.000	-98.01**	0.000	-105.96**	0.000	50.46**	0.000
Groups I and IV	-211.94**	0.000	-222.42**	0.000	-169.53**	0.000	56.28**	0.000
Groups II and III	-43.98**	0.000	-22.61NS	0.77	-26.49**	0.000	12.61**	0.000
Groups II and IV	-96.76**	0.000	-147.02**	0.000	-90.06**	0.000	18.44**	0.000
Groups III and IV	-52.78**	0.000	-124.04**	0.000	-63.58**	0.000	5.82**	0.000

NS $p > 0.05$ = not significant.
** $p \leq 0.001$ = very highly significant.
LDL: Low-density lipoprotein.
HDL: High-density lipoprotein.

Table-4: Correlation coefficients between thiamine and the parameters of lipid profile in different groups.

Groups	Variable	Coefficient/significance	Lipid profile			
			TG	TC	HDL	LDL
I	Thiamine	r[1]	-0.90***	-0.86***	0.87***	-0.85***
		p[2]	0.000	0.000	0.000	0.000
II	Thiamine	r[1]	-0.86***	-0.89***	0.83***	-0.84***
		p[2]	0.000	0.000	0.000	0.000
III	Thiamine	r[1]	-0.76	-0.78***	0.68***	-0.76***
		p[2]	0.000***	0.000	0.001	0.000
IV	Thiamine	r[1]	-0.75***	-0.70***	0.70***	-0.65**
		p[2]	0.000	0.001	0.001	0.002

[1] r — Pearson's correlation coefficient.
[2] p — probability value.
** $p \leq 0.05$ = significant.
*** $p \leq 0.001$ = very highly significant.
TG: Triglycerides. TC: Total cholesterol. HDL: High-density lipoprotein. LDL: Low-density lipoprotein.

difference was found between other groups. A significant mean difference was found in plasma TMP levels in all the groups.

Thiamine was found to have a highly significant negative correlation with triglycerides, total cholesterol and LDL cholesterol, while it had a highly significant positive correlation with HDL cholesterol in all groups (Table-4).

In all the groups, a significant linear regression ($p<0.001$) of microalbuminuria on plasma thiamine was found.

Group I: Microalbuminuria (mg/24 hours) = -5.60 x plasma thiamine (nmol/L) + 68.96

Group II: Microalbuminuria (mg/24 hours) = -5.76 x plasma thiamine (nmol/L) + 66.74

Group III: Microalbuminuria (mg/24 hours) = -44.51 x plasma thiamine (nmol/L) + 466.29

Group IV: Microalbuminuria (mg/24 hours) = -95.85 x plasma thiamine (nmol/L) + 779.54.

Discussion

The study showed reduced levels of plasma thiamine chloride, TMP and packed red blood cell (RBC) TK levels in diabetic patients compared to the controls. The decline in plasma thiamine and TK levels worsened with progressively increasing UAE as the level of plasma thiamine and RBC TK was lower in microalbuminuric diabetics compared to normoalbuminuric diabetic, and the lowest in macroalbuminuric diabetics. Moreover, there was an increased urinary excretion of thiamine in diabetic patients which correlated with progressive increase in the UAE. This is in close association with a study conducted on streptozotocin (STZ)-induced diabetic rats where decreased levels of thiamine and TK were seen in diabetic animals compared to the controls.[10] Another study showed decreased levels of plasma thiamine and TK in Dutch diabetic population.[20]

In a Britain-based study, the level of thiamine in blood was found to be reduced while urinary thiamine was raised in the diabetic population compared to the controls.[21] Since there is great variation in the diet, baseline prevalence of thiamine and genetic susceptibility in different populations, therefore, there was need to find out the thiamine status in our population. In this regard, a pilot study was conducted on 40 patients in 2009, which also showed a markedly reduced level of plasma thiamine in Pakistani diabetic population.[22] The study was done on type 2 diabetics with microalbuminuria only. In our study, we included type 2 diabetics having normoalbuminuria, microalbuminuria and macroalbuminuria in order to correlate the degree of thiamine deficiency with the extent of UAE. Our results further supported the finding that thiamine levels were reduced in Pakistani diabetic population, and also brought to notice the association between thiamine deficiency and the status of albuminuria in our diabetic population.

We studied the levels of triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol in all the groups of our study and found them to be significantly elevated in diabetics compared to the controls except for HDL cholesterol, which was significantly lower in the diabetics. Studies conducted to see the lipid profile of diabetic patients consistently found dyslipidaemic profile characterised by elevated triglycerides, total cholesterol and various fractions of cholesterol, like LDL, while HDL was reduced.[23,24] Our findings of lipid profile were in concordance with these studies. Dyslipidaemia was seen in diabetics with normoalbuminuria and worsened progressively in the microalbuminuric and macroalbuminuric groups; the last one being the worst. This was in line with previous studies conducted to evaluate the association between dyslipidaemia and microalbuminuria. In one such study, it was found that in patients with normoalbuminuria the progression of renal disease was related to LDL cholesterol, while in patients with microalbuminuria the progression of nephropathy was related to triglyceride content of very low-density lipoprotein (VLDL) particles. In macroalbuminuric subjects, the progression of diabetic nephropathy was related to the size of LDL particles.[25] Another study conducted in Taiwan showed raised triglyceride levels in all stages of albuminuria, while the apolipoprotein content varied from stage to stage.[26] However, our study found a consistent rise in total cholesterol, LDL cholesterol and triglycerides, and a progressive decline in HDL cholesterol levels with increasing albuminuria.

We then studied the effect of thiamine deficiency on dyslipidaemia in diabetics and found a significant linear regression of all parameters of lipid profile on thiamine. It was seen that thiamine had a significant negative correlation with triglycerides, total cholesterol and LDL cholesterol and a significant positive correlation with HDL cholesterol. This is in concordance with studies where a correlation between dyslipidaemia and thiamine deficiency in diabetic subjects was found. In an animal study, HDL levels were reduced and triglycerides and total cholesterol levels were increased in thiamine-deficient diabetic rats.[7] In the same study it was further found that high-dose thiamine administration normalised the triglyceride and total cholesterol levels, but no effect was found on HDL.[7] Another study showed normalisation of both plasma triglyceride and total cholesterol levels after thiamine administration.[21] However, a more recent study showed normalisation of plasma triglyceride levels upon thiamine administration, but no effect was seen on total cholesterol level.[27]

Conclusion

There was a deficiency of plasma thiamine in diabetic patients as the excretion of thiamine was increased in them. The deficiency was greater in those who had increased albuminuria. Furthermore, a positive correlation was found between thiamine deficiency and dyslipidaemia in diabetics. Thus, with increased deficiency of thiamine, there is greater risk of deranged lipid profile in these patients.

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