ORIGINAL RESEARCH PAPER

Volume-6 | Issue-9 | September-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SERUM PARAOXONASE 1 IN TYPE 2 DIABETES MELLITUS COMPLICATED WITH NEPHROPATHY

Biochemistry

Simant Baliarsingh Department of Biochemistry, ESIC Medical College & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. - Corresponding Author

ABSTRACT

More than one third of Type 2 diabetes mellitus patients (T2DM) may progress to the stage of nephropathy, which might be attributed to oxidative stress. Paraoxonase 1(PON 1) levels are inversely related to oxidative stress. Fifty subjects each were selected from T2DM without complications (DM), with Nephropathy (DN) and Controls. Blood samples were analyzed for PON 1, Fasting blood sugar (FBS), HbA1c and Lipid profile. PON1 levels of the DN group were significantly decreased and of FBS, HbA1c, Total cholesterol and LDL-C levels significantly increased from the Control group (ANOVA $p<0.0001$). VLDL-C and Triglyceride levels were significantly lower in the Control group when compared to DN and DM groups (ANOVA $p< 0.001$). PON1 levels show a steep fall in diabetics going into nephropathy and therefore may act as an additional marker for the condition.

KEYWORDS

Type 2 diabetes mellitus, Paraoxonase 1, Diabetic nephropathy, Fasting blood sugar, Lipid profile

Introduction

Diabetes Mellitus type 2(T2DM) is one of the most common endocrine disorders affecting more than 135 million people in the world [1]. Diabetic nephropathy (DN) occurs approximately in one third of T2DM patients. In T2DM, microalbuminuria is less predictive of nephropathy and its progression to overt nephropathy, when compared to type 1 diabetes mellitus [2]. Therefore other modalities of assessing DN in T2DM need to be explored, a promising candidate for which is Paraoxonase (PON). PON is a serum esterase that hydrolyzes organophosphorus compounds, aromatic carboxylic acid esters and nerve gases [3] Human PON is in close relation with High density lipoprotein (HDL) [4]. PON is responsible for detoxification of lipid peroxides and it is thought that individuals with low paraoxonase activity may have a greater risk of developing atherosclerosis [5]. Studies in patients with T2DM have shown that they have significant decrease in serum paraoxonase activity without having a significantly lower HDL cholesterol concentration [6].The human body has three kinds of PONs (I, 2 and 3). We undertook the study of PON 1 in T2DM patients with and without nephropathy as detailed studies of PON in T2DM patients with complications is lacking, especially in the Indian subcontinent.

Materials and methods

A cross sectional study was conducted on patients attending the diabetic OPD of a tertiary care hospital in Bengaluru. The duration of the study was eighteen months from November 2016 to April 2017. The study was conducted on diagnosed cases of diabetic nephropathy, as well as uncomplicated diabetes mellitus. Age and sex matched voluntary blood donors attending the blood bank of the hospital were taken as controls. Each gave an informed consent and this study was approved by ethical and research committee of the associated Medical College.

In this study the subjects were divided into three groups with 50 adults of both sexes in each group:

1. Control group- Healthy, age and sex matched, non diabetic subjects.
2. Group I-Type 2 diabetes patients without nephropathy. (DM group)
3. Group II- Type 2 diabetes patients with diagnosed nephropathy without any other complications. (DN group)

Exclusion criteria were as follows:

1. Patients with Type 2 Diabetes mellitus with following complications acute coronary syndromes, cerebrovascular accide nts or stroke , severe peripheral vascular artery diseases
2. Diabetics with acute or chronic liver disease
3. Gestational diabetes.
4. Chronic inflammation & infection.

Relevant clinical history was taken and clinical examination was performed on all the subjects. Under aseptic precaution sampling of fasting blood was done by venipuncture- 5ml of blood was drawn out of which 1ml was transferred to vacutainer containing EDTA to be used for HbA1c analysis. Remaining portion of the blood collected was allowed to clot and then centrifuged and two ml of serum aliquoted and stored at -5° C until the time of assay of Paraoxonase. PON1 activity was estimated by the reaction of paraoxon hydrolysis into p-nitrophenol and diethylphosphate as formerly explained [6] Remaining serum was subjected to routine investigations that include fasting blood sugar (FBS), HbA1c, lipid profile which included Total cholesterol (TC), Low density lipoprotein- cholesterol (LDL-C), High density lipoprotein- cholesterol (HDL-C), Very low density lipoprotein- cholesterol (VLDL-C) and Triglycerides (TG). All these parameters were analysed by COBAS INTEGRA 400 auto analyzer (Roche, Germany).

The data was analyzed using Graph Pad Prism, version 6 for windows (GraphPad software, La Jolla California USA, www.graphpad.com). Results are expressed as mean±SD. One way analysis of Variance (ANOVA) was used for comparison between controls and study subjects which was followed by Post-hoc test (Tuckey's multiple comparison test) Relationship between variables was assessed by Karl Pearson's coefficient of correlation. A p-value of 0.05 or less was considered as statistically significant.

Results

As shown in Fig.1 Serum PON1 levels of the Control and DM groups were comparable, but the DN value was significantly decreased compared to both of them (ANOVA $p< 0.0001$).

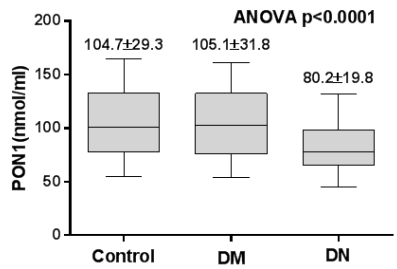

Fig.1 Comparision of PON 1 levels between the three groups. PON 1: Paraoxonase 1; DM: Diabetes Mellitus; DN: Diabetic Nephropathy. Values are expressed as Mean± SD, n=50.

The FBS of the Control, DM and DN groups were significantly different from each other at 95.3± 8.0, 212.6 ± 31.0 and 243.4 ± 35.8 mg/dl respectively (ANOVA $p< 0.0001$). The same was reflected in HbA1c levels where the three groups were significantly different from each other (5.0± 1.1, 9.8± 1.9 and 11.9± 2.1 % respectively; ANOVA $p< 0.0001$). TC (173.7 ± 7.1, 193.9± 23.4, 222.5± 26.7 mg/dl) and LDL-C (103.6± 19.8, 120.4 ± 25.9, 141.4± 35 mg/dl) levels were statistically different from each other in Control, DM and DN groups respectively (ANOVA $p< 0.001$for both). The VLDL-C level was significantly lower (ANOVA $p< 0.001$) in the Control group (25.2 ±

5.1mg/dl) when compared to DN and DM groups (32.6± 8.2 and 34.3± 4.4 mg/dl). TG also showed a similar pattern, the control, DM and DN, the levels being 123.1± 28.4, 163.7± 40.8, 171.9± 22.1 mg/dl respectively (ANOVA p <0.001). The HDL-C level in DN (46.4± 10.8 mg/dl) was comparable to that in DM (41.0± 5.5 mg/dl) but significantly more (ANOVA p=0.003) from the Control group (25.2± 5.1 mg/dl).

As shown in Table 1. PON 1 did not show a statistically significant correlation with any of the routine parameters in control as well as DM group, except Total Cholesterol (TC), which showed a slight negative correlation in the DM group. In the nephropathy group there was a moderate negative correlation of PON 1 with FBS, HbA1c, TC, TG, LDL-C, VLDL-C and a moderately positive correlation with HDL-C.

Table 1. Correlation of Paraoxonase 1 with regular parameters

	Control		DM		DN	
	r	p	r	p	r	p
FBS	-0.11	0.46	-0.19	0.18	-0.44	0.001***
HbA1C	-0.18	0.19	-0.03	0.82	-0.40	0.003**
TC	-0.13	0.35	-0.28	0.04*	-0.45	0.001***
TG	-0.10	0.58	-0.04	0.76	-0.42	0.002**
HDL-C	0.05	0.71	0.13	0.36	0.43	0.001***
LDL-C	-0.14	0.32	-0.22	0.10	-0.43	0.002**
VLDL-C	-0.15	0.30	-0.04	0.74	-0.41	0.003**

HDL: High density lipoprotein -cholesterol; FBS: Fasting blood sugar; LDL; Low density lipoprotein -cholesterol; TC: Total cholesterol; TG: Triglycerides; VLDL: Very low density lipoprotein –cholesterol; *>0.05, **>0.005, ***>0.001

Discussion

Hyperglycemia in T2DM has been implicated in the pathogenesis and progression of microvascular complications though the mechanism involved is not yet exactly clear. Oxidative stress is hypothesized to play an important role in the development of late diabetic complications [7]. To study the associated oxidative stress we estimated PON1- an antioxidant enzyme, in DM patients with and without nephropathy. PON1 was further correlated with routine parameters like FBS, HbA1c and fasting lipid profile.

In our study we found a significantly reduced PON1 levels in DN group when compared to both controls and DM group. Bharathi Mackness et al [8] and Jun-Wang et al [9] also obtained similar results in their study. PON 1 is an enzyme associated with HDL which prevents oxidation of LDL by free radicals produced due to oxidative stress. In our study there was no significant difference in PON1 levels between controls and DM group. This was in accordance with the study Vanitha Gowda et al [10]. The findings of our investigation suggest that loss in PON1 activity is an event occurring significantly only later in the stage of the disease.

Fasting blood sugar and HbA1c are both increased in DM and more so in complicated DM as observed in various studies- Alikor CA et al [11] and Randel PJ et al [12]. Our study supports these findings as the above mentioned parameters were found to be increased in diseased groups, with a greater elevation in DN when compared to DM group. Amongst the two, HbA1c levels is considered to provide the most accurate and reliable method to routinely assess the relative level of diabetes control. We found a graded elevation of HbA1c through the groups as they progressed towards disease. Our finding was in accordance with the study conducted by Attmann et al [13] who also found pronounced lipoproptein abnormalities in patients with increased HbA1c levels. The associations between blood glucose parameters and oxidative stress parameters were also observed. In the DN group both FBS and HbA1c levels showed an increase with decrease in PON1levels. This indicates that increasing oxidative stress is linked with deteriorating glycaemic control.

DN is associated with an altered lipid profile characterized by elevated TG levels even in the early stages of renal disease. In this study, the plasma total cholesterol, TG, LDL-C, VLDL-C were significantly higher in DN group than in controls and DM group. Similar results were obtained in studies by Arbhana.B et al [14] and Goldberg IJ[15]. Also correlation studies showed that these parameters clearly increased with a decrease in PON 1 in DN patients but not those having uncomplicated DM. We also observed that the level of HDL-C was significantly lower in DM with and without nephropathy, than in controls. Furthermore HDL-C showed a negative correlation with PON1 which was statistically significant only in the nephropathy group. Several factors are likely to be responsible for diabetic dyslipidaemia: insulin effects on liver apoprotein production, regulation of lipoprotein lipase, actions of cholesteryl ester transfer protein, and peripheral actions of insulin on adipose and muscle [15]. DM and its complications are associated with a greater risk of mortality from cardiovascular disease which is mainly due to dyslipidemia. A study by Bonnet et al [16] reported an independent deleterious influence of serum total cholesterol on the decline in renal function and progression of albuminuria. According to Martinez-Castelao et al [17], low levels of HDL-Cholesterol are a key feature of Type 2 diabetes.

The major limitations of our study are (i) the small sample size and (ii) non-inclusion of renal laboratory parameters and only depending on clinical diagnosis for grouping them into DN group.

Conclusion

PON1 levels, an indicator of oxidative stress, do not display a change in uncomplicated diabetes but show an acute fall with the advent of nephropathy and its progress in these patients. Thus PON1 might act as an additional marker for diabetic nephropathy in south Indian population.

References

1. Sharifi F, Sazandeh SH.(2004). Serum Ferritin in Type 2 Diabetes Mellitus and its relationship with HbA1c. Acta Medica Iranica, 42(2),142-145.
2. Powers AC. (2005). Diabetes mellitus. In: Kasper LS, Braunwald E, Fauci AS, Longo DL, Hauser SL, Jameson JL. Harrison's principle of Internal medicine. Newyork, McGraw-Hill.
3. Aldridge WN. (1953). Serum esterases 2: an enzyme hydrolysing diethyl p-nitrophenylphosphate (E600) and its identity with the A-esterase of mammalian sera. Biochem J,53,117-124.
4. Mackness MI, Hallam SD, Peard T, Warner S, Walker CH. (1985). The separation of sheep and human serum 'A'-esterase activity with the lipoprotein fraction by ultracentrifugation. Comp Biochem Physiol, 82B,675-677.
5. Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN. (1995). Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. Arteriosclerosis, thrombosis, and vascular biology,15(11),1812-8.
6. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. (1991).Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis,86,193-199.
7. Jakus V. (2000). The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl Lek Listy,101, 541–51.
8. Mackness B, Durrington N, Abuashia B. (2000) Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. Clinical science,98, 355- 63.
9. Jun W, Ming MY, Shi S, Tszkin NG, Yanbo L, Xiaomin L.(2013). Association of paraoxonase gene polymorphisms with diabetic nephropathy and retinopathy. Molecular medicine reports 8,1845-51
10. Vanitha G, Kusuma MN, Vasudha K S. (2013). Serum paraoxonase activity in type 2 diabetes mellitus and diabetic nephropathy. Int J of Applied research, 3(40), 351-53.
11. Alikor CA, Emem PC. (2015). Epidemiology of diabetes and impaired fasting glucose in a rural community of Nigerian Niger delta region. Niger J Med, 24(2),114-24.
12. Randle PJ. (1998) Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after thirty five years. Diabetes Metab Rev,14, 263-83.
13. Attman PO, Knight-Gibson C, Tavella M, Samuelsson O, Alaupovic P. (1998)The compositional abnormalities of lipoproteins in diabetic renal failure. Nephrol Dial Transplant,13,2833-41
14. Archana B, Sangeeta C , Shirish C , Harsh K. (2015).Study of renal and lipid profile in diabetic patients. Int J Pharm Bio Sci, 5(1),33-41
15. Goldberg IJ. (2001).Diabetic Dyslipidemia: Causes and Consequences.J Clin Endocrinol Metab, 86,965-71.
16. Bonnet F, Cooper ME.(2000). Potential influence of lipids in diabetic nephropathy:Insights from experimental data and clinical studies. Diabetes Metab,26, 254–64.
17. Martinez-Castelao A, Ramos R, Gonzalez MT, Castineira MJ. (2000). Dyslipidemia and cardiovascular risk factors in type 2 diabetes mellitus patients with associated diabetic nephropathy. Nefrologia, 22,51–8.