IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 8 Ser. 8 (August. 2019), PP 55-59
www.iosrjournals.org

Study of Serum Paraoxonase Levels in Alcoholic and Non-Alcoholic Type-2 Diabetes Mellitus

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Abstract: ***Introduction****:Diabetes mellitus is one of the most common disease encountered in the present health scenario. Hyperglycemia and free radicals cause oxidative damage leading to microvascular complications . Type 1 diabetes mellitus is associated with an elevated level of oxidative stress, increased susceptibility to coronary heart disease, and reductions in PON1 (Paraoxonase) concentration and activity. In humans, light drinking upregulates, whereas heavy drinking downregulates PON activity and its expression, irrespective of its genetic polymorphism.*
AIM*: To study antioxidant status by measuring serum paraoxonase levels in alcoholic and non-alcoholic type-2diabetesmellitus.*
Materials &Methods*: Study was conducted on 60 subjects among them 20 were normal healthy individuals,20 were Non alcoholic type-2 diabetes mellitus patients and another 20 were Alcoholic type-2 diabetes mellitus patients.*
Results: *There is a decrease in serum paraoxonase levels in patients suffering from diabetes mellitus when compared to controls,but is not statistically significant . There is a significant decrease in serum paraoxonase levels in patients suffering from diabetes mellitus with alcoholism when compared to non alcoholic diabetes mellitus patients.*
Conclusion*: Serum paraoxonase levels are decreased in patients suffering from diabetes mellitus when compared to controls ,but not statistically significant . Serum paraoxonase levels are decreased in patients suffering from diabetes mellitus with alcoholism when compared to non-alcoholic diabetes mellitus patients suggesting the effect of alcohol in decreasing serum paraoxonase levels in Diabetics increasing the risk of vascular complications.*
Keywords*: Diabetes mellitus, Alcoholism, serum paraoxonase activity(PON 1).*

Date of Submission: 03-08-2019 Date of acceptance: 19-08-2019

I. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels **(American Diabetes Association.,2007)**. It is a complex disorder which has an aberrant metabolism of carbohydrates, lipid and proteins due to either relative or absolute deficiency of insulin in secretion or action. These result in hyperglycemia, glycosuria, ketonuria and ketoacidosis **(Joslins 2005)[5]**. There are various experimental studies suggesting the over-production of reactive oxygen and nitrogen species to be involved in the initiation and development of vascular complications in diabetes **.** Free radicals are reactive chemical substances, which can cause oxidative injury by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. Under normal circumstances there is a critical balance in generation of oxygen free radicals and antioxidant defence mechanism to protect them from free radical toxicity **(Halliwell B et al 2004)[4].** Breach in this balance leads to oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue mechanism in a wide spectrum of human disease. The oxidative modification of low-density lipoprotein (LDL) in the artery wall is believed to be central to the pathogenesis of atherosclerosis**.** There are reports in animal experiments to show that alcohol intake further aggravates the generation of free radicals.
Paraoxonase enzyme (EC3.1.8.1) belongs to the class of hydrolases and the family of esterases. **(Mackness et al., 1996).** The paraoxonase gene family has three known members PONI, PON2 and PON3, located on long arm of chromosome 7 between q 21.3 and q 22.1 in human **(Primo-Parma et al., 1996).** The genes share considerable structural similarity. PON1, PON2 and PON3 share approximately 60% identity at the amino acid level and about 70% identity at nucleotide level. Codon 106 (lysine) present in PON1 is missing in all PON2 and PON3 **(Mackness et al., 2002).**

I) Paraoxanase-1 (PON1):

Paraoxonase-1 is a liver and plasma enzyme most studied because of its ability to hydrolyze the active metabolites of several organophosphorus insecticides. The discovery that PON1 can also metabolize oxidized phospholipids has spurred research on its possible role in coronary heart disease and atherosclerosis. Additionally, its potential roles in metabolizing pharmaceutical drugs are also being explored. PON1 displays several polymorphisms that influence both its level of expression and its catalytic activity, thus determining the rates at which a given individual will detoxify a specific insecticide, metabolize harmful oxidized lipids, and activate or inactivate specific drugs .

II) Paraoxonase-2 (PON2):

Paraoxonase-2 (PON2) is not associated with HDL **.** It is a cellular antioxidant, expressed in a wide variety of cell type and which is capable of retarding the oxidation of mildly oxidized-low density lipoprotein (ox-LDL)**.** PON2 is ubiquitously expressed in mammalian tissues PON2 is more widely expressed and is found in a number of tissues including brain, liver, kidney and testis **(Busch et al., 1999).**

III) Paraoxonase-3 (PON3):

Paraoxonase-3 is primarily synthesized in the liver and is associated with HDL fractions of human and rabbit serum and is absent from LDL **(Reddy et al., 2001).** PON3 is not regulated by oxidized lipids and is not affected by atherogenic diet like PON1 **.**

Paraoxonase-1 enzymatic activity

Paraoxonase-1 has a number of enzymatic activities: Paraoxonase (Paraoxon Substrate),: Arylesterase (Phenylacetate Substrate), Lactonase (Lactone Substrate) activities and antioxidant activity.

Type 1 diabetes mellitus is associated with an elevated level of oxidative stress, increased susceptibility to coronary heart disease, and reductions in PON1 concentration and paraoxonase activity. It has been reported that 67% of type-I diabetics have a low level of paraoxonase activity, irrespective of HDL cholesterol concentration, compared with 50% of the healthy population **(Mackness et al., 2002)[7].**

Drinking 40 g/day of alcohol increased both PON1 activity and mass. There was no difference between red wine and beer suggesting that it is not the red wine polyphenols alone that cause the effect**.** Similar results were obtained in a study that examined the effect of drinking alcoholic compared with non-alcoholic beer. Only alcoholic beer had a positive effect on PON1 activity .Moderate drinking significantly increases serum HDL-cholesterol and apoAI concentrations, which may account for the observed increase in PON1 concentration. Increased serumPON1 may be one of the factors underlying the reduced CHD risk in moderate drinkers **(Sierksma et al.,2002)[12].** In humans, light drinking upregulates, whereas heavy drinking down regulates PON activity and its expression, irrespective of its genetic polymorphism **(Rao MN et al 2003)[10].**

Hence the study is undertaken to find out the effect of alcoholism and free radical generation in patients of Diabetes Mellitus by measuring and comparing the levels of Serum Paraoxonase levels in diabetes mellitus patients with and without alcoholism.

II. Materials And Methods

The study was conducted on 60 subjects in the age group of 35- 60yrs of both genders Among them 20 were normal healthy individuals who formed the control group. Twenty (20) patients suffering from Type-2Diabetes Mellitus without history of alcoholic intake formed the study group -1.Twenty(20)patients suffering from Type-2 Diabetes Mellitus with Alcoholism formed the study group-2.

Inclusion criteria:

1. Normal healthy individuals as controls.
2. Cases are divided into 2 groups: GROUP 1 comprises of patients suffering from TYPE 2 DIABETES MELLITUS ONLY. GROUP 2 comprises of patients suffering from TYPE 2 DIABETES MELLITUS WITH ALCOHOLISM (>70ml/day)
3. Diabetes mellitus patients who are on treatment with and without complications like coronary artery disease, neuropathy and nephropathy are included. History of alcoholism is taken and subjects are divided accordingly.

Exclusion criteria:

Diabetes mellitus patients with severe acute complications and women with pregnancy are excluded.

Collection of blood sample for analysis:

A fasting (12 hours) venous blood sample (5ml) was drawn from the patients and controls into a sterile disposable syringe which was transferred into centrifuge tubes and was allowed to clot for 30 minutes. A post prandial venous blood samples are also collected from same patients .The samples were centrifuged at 3000 rotations per minute for 10 minutes and the serum was separated and collected from the centrifuge tubes and analyzed within one hour as follows.

Estimation of serum paraoxonase activity: principle:

Serum Paraoxonase activity was measured by using 5.5 mM/L p-nitrophenyl acetate (Sigma chemicals, USA) as a substrate, the increase in the absorbance of p-nitrophenol formed at 412 nm was measured by using ELICO spectrophotometer.

The activity of Paraoxonase was measured in 20 mM/L Tris buffer at pH 8.0 and which contains 1 mmol calcium chloride. The generated product of p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole per cm at pH 8.0. Results are expressed as U/ml. (1U,1 nmol p-95 nitrophenol formed per minute) or U/L(1U, 1umol of p-nitrophenol formed per minute).

Analysis of PON1 Activity:

Reagents:

The reagents mixture included (Tris – HCL buffer (20mmol/L, pH 8.0, containing 1mmol/L CaCl2and 5.5 mmol/L p-nitrophenyl acetate)

Procedure:

To 350 µL of reagent mixture, 10µL of serum was added and incubated for 17 seconds.The hydrolysis rates of the substrate were determined by measuring the released *p*-nitrophenol at 412nm at 37°C for 3 minutes on continuously recording spectrophotometer. Blank was included to correct for the spontaneous hydrolysis of the substrate.

Enzyme activities were expressed in international units per litre(U/L) of sera. An international unit is the amount of hydrolysed substrate in µmol per minute. Extinction coefficient of *p*-nitrophenol at 412 nmis 17,000/mol/cm.

Normal reference value: Serum paraoxonase:- 150-466 U/L

III. Results

Serum paraoxonase levels was measured in control group and study group -1 and study group -2. All the values are expressed as Mean $\pm$ SD . Mean values are compared between control group and study group-1 (Table-1). Mean values are compared between the two study groups 1 and 2 (Table-2). Statistical analysis is done by student t test and the statistical significance is expressed as p value $\leq$0.05. The statistical analysis is done by SSPS statistical system.

Mean Serum Paraoxonase levels are less in study group I (cases withType II diabetes mellitus without alcohol intake) when compared to control group but it is not statistically significant (p= 0.355). (Table-1)

Table-1

Comparison of controls with group 1 of cases (diabetes mellitus only)

S.No	INVESTIGATION		CONTROL (n=20)	CASES (GROUP I) (n=20)
01	SERUM PARAOXONASE	MEAN	262.8	240
		SD	43.96	40.354
		t-Test	0.9363	
		p-Value	0.3550	

Table-2

Comparison of 2 groups of cases (Type II diabetes and Type II diabetes mellitus with alcoholism)

S.No	INVESTIGATION		CASES (GROUP 1)	CASES (GROUP 2)
01	SERUM PARAOXONASE	MEAN	240	163.75
		SD	40.354	34.871
		t-Test	4.3928	
		p-Value	0.0001	

Table 1: shows the comparative data of antioxidant status (PARAOXONASE) in control and cases of type 2 Diabetes 262.80 ± 43.96 and 240 ± 40.354 respectively. Mean Serum Paraoxonase levels are less in study group I (cases withType II diabetes mellitus without alcohol intake) when compared to control group but it is not statistically significant (p= 0.355). P value is insignificant in this parameter as the value is >0.005.

Table 2: shows the comparative data of antioxidant status (PARAOXONASE) in 2 groups of cases. Group1 and group 2 values are 240 ± 40.354 and 163.75 ± 34.871 respectively. Mean Serum Paraoxonase levels are less in study group II when compared to Study Group I which is statistically significant (p= 0.0001) P value is highly significant in this parameter as the value is <0.05

IV. Discussion

In our study we have found a decrease in serum paraoxonase in diabetes when compared to controls but is not statistically significant . Type 1 diabetes mellitus is associated with an elevated level of oxidative stress, increased susceptibility to coronary heart disease, and reductions in PON1 concentration and paraoxonase activity. It has been reported that 67% of type-I diabetics have a low level of paraoxonase activity, irrespective of HDL cholesterol concentration, compared with50% of the healthy population **(Mackness et al., 2002)**[7].

Type 2 diabetics present with a lower paraoxonase activity and a lower ratio of paraoxonase activity to HDL cholesterol level than those found in healthy control subjects. The mechanism by which PON1 is reduced in diabetes is poorly understood, but may be associated with an increase in blood glucose concentration and altered glycation of HDL and/or PON1, lower rate of synthesis or higher rate of catabolism, or increased oxidative stress **(Mackness et al., 2002)**[7]**.**

Glycation can both inactivate PON1 and increase lipid peroxidation in HDL. Glycated HDL also has a reduced ability to protect against oxidation. In support of the above in vitro data, PON1 activity and concentration were decreased in studies of healthy subjects with elevated fasting glucose levels.

Boemi et al., (2001)[3] showed that activity is reduced to an extent that can alter antioxidant capacity in vitro, suggesting an association with the tendency towards increased oxidative stress in diabetics.

In addition, diabetic patients who present with complications such as coronary heart disease, retinopathy or neuropathy have lower paraoxonase activity than diabetics without these complications . It has also been observed that paraoxonase activity is lower in diabetic patients undergoing hemodialysis than in non-diabetic patients undergoing hemodialysis**.**

It is believed that there are many pathogenic mechanisms that contribute to the development of diabetic complications especially diabetic nephropathy and retinopathy including excessive oxidant damage.

Several mechanisms may lead to increased oxidative stress in diabetes. Firstly, hyperglycemia may increase the generation of free radicals through the ability of glucose to yield oxidizing intermediates such as superoxide anion, hydroxyl radicals and hydrogen peroxide. Secondly, antioxidant defenses are reduced in diabetes. Moreover, type 2 diabetes is often associated with various metabolic derangement including obesity, hypertension and dyslipidemia. The feature of the dyslipidemia in type 2 diabetes is the presence of small dense LDL which is prone to oxidative damage **(Robertson., 2004)**[12]**.**

Increased susceptibility of LDL oxidation is another contributing factor for the development of diabetic complications. Mildly oxidized LDL induces the activation of nuclear transcription factor via a cAMP dependent mechanism and consequently induces endothelial cells to express monocyte specific chemoattractants, adhesion molecules and colony stimulating factors. It is well known that HDL protects LDL oxidation, this protection is impaired in diabetic patients. Also serum PON was found to decrease the susceptibility of LDL to lipid peroxidation thus, it could prevent the induction of inflammatory response in arterial cell wall **(Mackness et al.,1991a)**[7]**.**

In our study we have found statistically significant decrease in serum paraoxonase levels in chronically drinking diabetics when compared to Type 2 DM only. These individuals were consuming >70ml/day of alcohol which categorises them as heavy drinkers.

In humans, light drinking up regulates, whereas heavy drinking down regulates PON activity and its expression, irrespective of its genetic polymorphism **(Rao MN et al 2003)**[10]**.**

Light ethanol feeding caused a 20–25% increase in PON activity in both serum and liver and a 59% increase in the level of liver PON mRNA compared with pair-fed control rats . In contrast, heavy ethanol feeding caused a 25% (P <.05) decrease in serum and liver PON activities with a 51% (P <.01) decrease in liver PON mRNA level. Light drinkers had a 39.5% (P <.001) higher, whereas heavy drinkers had a 45% (P <.001) lower serum PON activity compared with nondrinkers. Light to moderate alcohol consumption in humans increases the activity of paraoxonase in serum, and the enzymatic activity was strongly correlated with concomitant increases in concentrations of HDL-C and Apo A-I **(Tol AA et al 2001)**[14].

The reduced risk of coronary artery disease associated with moderate alcohol consumption may be explained by its ability to increase plasma HDL. Apart from this, moderate ethanol increases PON1, an HDL-associated antiatherogenic enzyme, that has its cardioprotective action because it (1) hydrolyzes oxidized lipids in OxLDL in serum and macrophages, a prerequisite for the onset of atherosclerosis, (2) inhibits cholesterol uptake by macrophages from OxLDL **(Rao MN et al 2003)[10]**, (3) attenuates macrophage cholesterol biosynthesis and (4) stimulates macrophage cholesterol efflux **(Rao MN et al 2000)[11].**

However, heavy alcohol consumption (70–90 g/d) resulted in a significant decrease in PON1 and protein thiols. **(Prakash M et al 2007)[9]**. It is well known that PON1 loses its activity in the oxidative environment. Therefore, any factors that affect the status of oxidative stress will also affect PON1 activity status. For example, antiphospholipid antibodies increase oxidative stress in experimental mouse model with decreased PON activity . On the other hand, statins commonly used for hypercholesterolemia increase serum PON1 activity by reducing oxidative stress **(Deakin S et al 2003)[4]**. Paraoxonase 1 (PON) may contribute to the cardioprotective action of high-density lipoprotein (HDL) because it inhibits low-density lipoprotein (LDL) oxidation, a prerequisite for the onset of atherosclerosis. Because light drinking and heavy drinking have diametrically opposite effects on cardioprotection, scientists have determined the effects of ethanol dosage on rat serum PON activity and its hepatic expression. Furthermore, they have investigated PON activity and polymorphism in human light and heavy drinkers.

V. Conclusion

Serum Paraoxonase levels are decreased in patients suffering from type 2 diabetes mellitus when compared with controls which is not statistically significant . Serum Paraoxonase levels are decreased with statistical significance in chronic alcoholics with diabetes mellitus when compared to non-alcoholic diabetes mellitus patients suggesting the effect of alcohol in decreasing Paraoxonase levels in Diabetics increasing the risk of vascular complications.

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Dr.M. Anil Babu MD. " Study of Serum Paraoxonase Levels in Alcoholic and Non-Alcoholic Type-2 Diabetes Mellitus." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 8, 2019, pp 55-59.