SERUM PARAOXONASE ACTIVITY IN RENAL TRANSPLANT RECIPIENTS

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ABSTRACT

BACKGROUND

Serum paraoxonase is an enzyme synthesised in the liver. It is known to prevent atherosclerosis by inhibiting oxidation of low-density lipoprotein. Renal transplant recipients have increased tendency for developing atherosclerosis and cardiovascular disease. Reduced activity of serum paraoxonase contributes to accelerated atherosclerosis and increased cardiovascular complications in these patients.

The aim of this study was to estimate serum paraoxonase activity in renal transplant recipients and compare it with healthy controls.

MATERIALS AND METHODS

30 renal transplant recipients and 30 age and sex matched healthy controls were taken for the study. Serum paraoxonase activity, blood urea, serum creatinine and uric acid were estimated in these groups. The serum paraoxonase activity was correlated with urea, creatinine and uric acid levels.

RESULTS

Serum paraoxonase activity was reduced in renal transplant recipients compared to healthy controls. There was a negative correlation between paraoxonase activity and the levels of urea, creatinine and uric acid levels.

CONCLUSION

In this study, the paraoxonase activity was reduced in renal transplant recipients compared to controls. The increased cardiovascular disease in these patients could be due to reduced paraoxonase activity.

KEYWORDS

Paraoxonase, Renal Transplant Recipients, Atherosclerosis, Oxidised Low-Density Lipoprotein.

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BACKGROUND

Serum Paraoxonase (PON) (EC 3.1.8.1, aryldialkylphosphatase) is an enzyme associated with High-Density Lipoprotein (HDL). PON1 binds with HDL and contributes to antioxidant and antiatherosclerotic capabilities of HDL.[1] PON1 protects HDL from peroxidation and hence improves reverse cholesterol transport.[2] It has 354 amino acids. The molecular mass is 43 kDa. The PON family consists of Paraoxonase 1 (PON1), Paraoxonase 2 (PON2), Paraoxonase 3 (PON3) located on the long arm of chromosome 7.[3] The most studied member is PON1. It is synthesised in the liver and transported in the plasma by binding to high-density lipoprotein.[4] PON1 has been shown to protect from atherosclerosis by preventing oxidation of Low-Density Lipoprotein (LDL).[5]

Oxidation of Low-Density Lipoprotein (LDL) is an important step in atherosclerosis. Oxidised LDL (Ox-LDL) are taken up by the scavenger receptor mechanism resulting in cholesterol accumulation and subsequent foam cell formation.[6] Oxidised LDL is a potent chemo attractant for monocytes and a potent inhibitor of macrophage motility, thereby promoting retention of macrophages in the arterial wall.[7]

PON1 prevents LDL from lipid peroxidation. PON1 can accelerate both the breakdown of phospholipid hydroperoxides and platelet activating factor. PON1 also metabolises peroxides of cholesteryl ester.[8] PON1 was shown to inhibit cholesterol influx by reducing the formation of oxidised LDL. PON1 acts by decreasing macrophage uptake of oxidised LDL. It also inhibits cholesterol biosynthesis in macrophages.[9]

Renal transplantation is done to improve the quality and length of life of individuals with end-stage renal disease. Overall transplantation outcome has improved progressively over the last two decades and continues to improve.[10] Although, transplantation improves quality of life they have high rate of mortality when compared to general

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population.[11] Approximately, 50% of patients die with a functional transplant with approximately 50% of these deaths from cardiovascular disease.[12]

It has been reported that reduced PON1 activity could lead to enhanced atherosclerosis and cardiovascular disease in renal transplant recipients.[13] It has been found that PON1 activity was decreased in renal transplant recipients compared to controls.[14] It has been proposed that the enzyme activity is inhibited in the uraemic environment. The enzyme activity is inhibited through post-translational modification of the enzyme as a result of reactions with advanced glycation end products or urea-derived cyanate.[15] The increased risk of atherosclerosis and cardiovascular disease could be due to reduced PON1 activity. The aim of the study was to estimate the PON1 activity in renal transplant recipients and compare it with the healthy controls.

MATERIALS AND METHODS

The study was conducted on 30 renal transplant recipients attending the Outpatient and Inpatient Department of Nephrology at JSS Medical College and Hospital, Mysore. Institutional Ethical Committee approved the study and informed consent was taken. 30 age and sex matched healthy adults formed the control group. The age group was between 20-65 years. Patients with conditions that could affect the PON1 activity such as hepatic, respiratory and acute inflammatory conditions were excluded from the study.

5 mL of blood was collected under aseptic conditions. Blood was allowed to clot .Serum was separated using centrifuge at 3000 rpm for 10 minutes. Serum was used for analysis of various parameters.

Serum PON1 Activity- PON1 activity was determined by using p-nitrophenyl acetate as substrate (procured from Sigma Aldrich Chemicals). 50 µL of fresh serum sample was mixed with 5.5 mM 4-nitrophenyl acetate as the substrate in 20 mM Tris-HCl buffer containing 1 mM CaCl2 at pH of 8.0. The increase in the absorbance due to the formation of yellow 4-nitrophenol was monitored at 405 nm for 3 minutes. PON1 was taken as 1 U/mL when 1 nmol/mL of the product 4-nitrophenyl acetate was formed under the assay conditions.[16]

Blood urea was estimated by urease/glutamate dehydrogenase method.[17] Serum creatinine was estimated by modified Jaffe's method.[18] Uric acid was estimated by uricase method.[19] Blood urea, serum creatinine and uric acid were estimated using Randox Daytona autoanalyzer.

Statistical Analysis- The Statistical Package for the Social Sciences (SPSS) version 17.0 was used for statistical calculations. Independent sample t-test was used to compare mean values. Pearson's correlation coefficient analysis was used to find out the degree of correlation between parameters. The tests were considered statistically significant at the level of P <0.05.

RESULTS

The study was conducted on 30 renal transplant recipients and 30 age and sex matched healthy controls. Table 1 shows results of serum PON1 activity, blood urea, serum creatinine, uric acid expressed as mean ± SD. The age group was 20-65 years. The mean age group was 35.23 ± 7.38 in renal transplant recipients and 36.57 ± 9.41 is healthy controls.

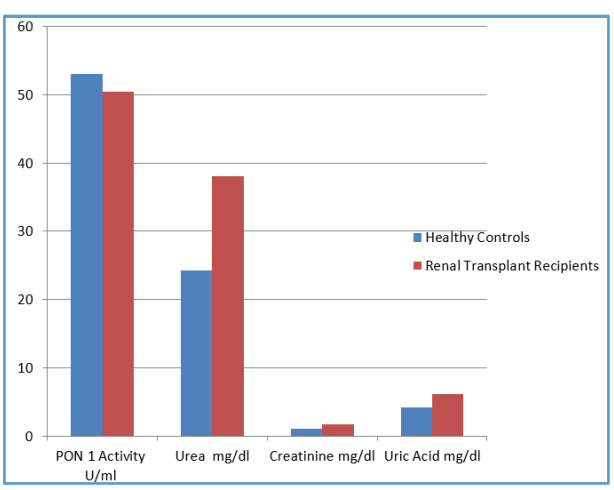
The serum PON1 activity was 50.42 ± 11.20 U/mL in renal transplant recipients and 53.09 ± 8.42 U/mL in healthy controls. The PON1 activity was decreased in renal transplant recipients when compared to controls, but was not statistically significant (p<0.301).

The blood urea was 38.13 ± 16.81 mg/dL in renal transplant recipients and 24.23 ± 3.41 mg/dL in controls. Blood urea was significantly increased in renal transplant recipients compared to control with p<0.001. Creatinine levels was 1.68 ± 0.69 mg/dL in renal transplant recipients and 1.05 ± 0.14 mg/dL in controls. There was a significant increase in the creatinine values in renal transplant recipients when compared to controls with p<0.001.

Uric acid was 6.23 ± 1.52 mg/dL in renal transplant recipients and 4.15 ± 0.67 in controls. The serum uric acid was significantly increased in renal transplant recipients when compared to controls with p<0.001. There was a negative correlation between serum PON1 activity and urea, creatinine and uric acid levels.

Parameters	Renal Transplant Recipients (N=30) (Mean ± SD)	Healthy Controls (N=30) (Mean ± SD)	p-value
PON1 activity (U/mL)	50.42 ± 11.20	53.09 ± 8.42	P <0.301
Urea (mg/dL)	38.13 ± 16.81	24.23 ± 3.41	P <0.001
Creatinine (mg/dL)	1.68 ± 0.69	1.05 ± 0.14	P <0.001
Uric acid (mg/dL)	6.23 ± 1.52	4.15 ± 0.67	P <0.001

Table 1. Comparison of Biochemical Parameters in Two Groups



Comparison of Biochemical Parameters in Two Groups

DISCUSSION

Renal transplant recipients are at increased risk for developing cardiovascular disease compared to the general population. Renal transplant recipients also have an increased incidence of atherosclerotic plaques and other vascular alterations. The high prevalence of cardiovascular disease leads to a substantial increase in cardiovascular mortality in renal transplant recipients.[20,21] Cardiovascular mortality remains 3-5 times higher in this patients compared to general population.[22] There is an accelerated tendency of atherosclerosis and cardiovascular disease in these subjects, which could be due to reduced serum PON1 activity. The enzyme activity is observed to be reduced in the uraemic environment due to post-translational modification of the enzyme.[15]

In the present study, serum PON1 activity was reduced in renal transplant recipients compared to healthy controls. Sztanek et al demonstrated that PON1 activity was reduced in renal transplant recipients compared to controls.[14]

Gungor et al suggested that reduced PON1 activity leads to arterial stiffness in renal transplant recipients. They found out that there is a role of reduced PON1 activity in the development of atherosclerosis and vascular stiffness in renal transplant recipients. It was suggested that PON1 activity could be used as a marker of arterial stiffness in renal transplant recipients.[13]

Oxidation of LDL is considered to play a major role in the development of atherosclerosis. OX-LDL inhibits the motility of tissue macrophages, thus leading to their "trapping" within the intima. OX-LDL is cytotoxic, which could promote endothelial dysfunction and the evolution of the fatty streak into a more advanced lesion.[23]

It has been found that there is increased prevalence of hyperlipidaemia after renal transplantation.[24] It has been suggested that there is increased plasma lipoprotein concentration and there is alteration in the composition of the lipoproteins.[25] There is increased susceptibility of LDL oxidation in renal transplant recipients.[26] Increased LDL oxidation leads to accelerated atherosclerosis occurring after transplantation. PON1 inhibits LDL oxidation and prevents the development of atherosclerosis in these patients. Reduced PON1 activity can lead to increased atherosclerosis and cardiovascular mortality in renal transplant recipients.

In another study, it was observed that there is association between serum PON1 activity and carotid atherosclerosis in renal transplant recipients. It was suggested that reduced PON1 activity is significantly associated with increased carotid artery intima media thickness in renal transplant recipients.[27] Accelerated atherosclerosis and cardiovascular complication in these patients could be due to reduced PON1 activity.

CONCLUSION

In this study, serum paraoxonase activity was reduced in renal transplant recipients compared to controls. Renal transplant recipients have increased tendency to develop atherosclerosis and cardiovascular disease. Cardiovascular disease is major cause of mortality and morbidity in these patients. The increased cardiovascular mortality could be due to reduced PON1 activity in these patients.

Further Scope of the Study- The present study demonstrates reduced PON1 activity in renal transplant recipients. We recommend further studies on cardiovascular disease in renal transplant recipients. The role of PON1 in the development of atherosclerosis and cardiovascular disease in renal transplant recipients can be studied in further studies. The increased cardiovascular mortality can be reduced by studying PON1 activity in these patients.

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