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Effect of Atorvastatin on Paraoxonase Activity in Patients with Hyperlipidemia

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Abstract: Paraoxonase is high density lipoprotein associated enzyme which prevents the oxidation of low density lipoprotein. In the present study we evaluated the effect of atorvastatin on serum paraoxonase levels in south Indian population with hyperlipidemia. The study was conducted on 59 newly diagnosed hyperlipidemic patients and 41 healthy controls. Hyperlipidemic patients were divided into two groups, group 1-before treatment and group 2-three months after receiving 10 mg atorvastatin daily. Serum paraoxonase and lipid profile were estimated in both cases and controls. Serum paraoxonase activity and high density lipoprotein levels were lower and total cholesterol, triglycerides and low density lipoproteins were high in hyperlipidemics compared to controls ($p<0.01$). Serum paraoxonase activity and high density lipoproteins levels were increased and total cholesterol, triglycerides and low density lipoprotein levels were decreased significantly in group 2 cases compared to group 1 cases ($p<0.01$). Serum paraoxonase correlated positively with high density lipoprotein ($R = 0.347$, $p<0.01$). Atorvastatin apart from favorably improving lipid profile, also improves paraoxonase activity significantly, this may suggest anti-atherogenic role of statins along with their antilipidemic function.

Key words: Paraoxonase, atorvastatin, hyperlipidemia, high density lipoprotein

INTRODUCTION

South Asians especially Indians show at increased risk for atherosclerosis and premature coronary heart disease (Sarkar and Madhsudhan, 2006). Hyperlipidemia is highly prevalent in Indian population and known to contribute towards increased mortality and morbidity related to cardiovascular and cerebrovascular disorders (Mackness and Durrington, 2003). Several antilipidemic drugs are in use which improves lipid profile favorably thus decreasing the rate of mortality and morbidity. Recently antilipidemic drugs like fenofibrates shown improvement in paraoxonase activity along with favorable improvement in lipid profile (Paragh *et al*., 2003). Serum paraoxonase is a calcium dependent high density lipoprotein associated esterase that is known to catalyze the hydrolysis of organophosphates. Serum paraoxonase is known to prevent protein oxidation by preventing homocysteinylation of protein (James *et al*., 2000) Paraoxonase decreases low density lipoprotein oxidation by its peroxidase activity and by preventing homocysteinylation of Apo B 100 (Aviram *et al*., 1998). Mackness *et al*. (1991) showed reduced paraoxonase activity in patients with hyperlipidemia. In the present study, we studied the activity of serum paraoxonase in south Indian population with hyperlipidemia and effect of atorvastatin on serum paraoxonase activity.

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MATERIALS AND METHODS

Subjects

The study was carried out on 59 newly diagnosed hyperlipidemics not associated with any systemic disease and 41 healthy controls. Mean age and sex of patients was 46±6 years and 48 males/11 females and that of controls was 42±7 years and 30 males/11 females, respectively. Informed consent was taken from all subjects involved and the study was approved by institutional review board. Hyperlipidemic patients were recruited from Kasturba Medical College hospital; who came for routine health check up. We have excluded all the cases with diabetes mellitus, chronic renal failure on conservative management or on hemodialysis and any other inflammatory conditions. We have included all those diagnosed with hyperlipidemia for the first time with or without associated conditions like hypertension or atherosclerosis related medical disorders. The paraoxonase activity was estimated at the time of diagnosis. All the hyperlipidemic subjects were treated with 10 mg of atorvastatin daily for the period of three months and advised to take low cholesterol diet. After three months, follow up was done and the lipid profile and paraoxonase activity was estimated. The hyperlipidemic patients were divided into two groups, group 1-cases at the time of diagnosis and group 2-cases three months after treatment with 10 mg atorvastatin daily.

Under aseptic conditions blood samples (5 mL) were drawn into plain vacutainers from ante-cubital veins of controls and cases. The collected blood was allowed to clot for 30 min and then centrifuged at 2000 g for 15 min for clear separation of serum. All assays were performed immediately after serum was separated.

Special chemical paraoxone was obtained from Sigma chemicals, St Louis, MO, USA. All other reagents were of analytical grade. Paraoxonase was estimated spectrophotometrically by the method described elsewhere with minimal modifications. Briefly, the assay mixture consists of 500 μL of 2.2 mM Paraoxone substrate in 0.1 M tris-HCl buffer, pH 8.0 containing 2 mM $CaCl_2$ and 50 μL of fresh serum specimen. The absorbance was monitored at 405 nm at 25°C. One unit (IU) of Paraoxonase activity is defined as 1 μmol of p-nitrophenol formed per min per liter at 25°C and activity was expressed as U L^{-1} of serum (Schiavon *et al*., 1996).

Fasting lipid profile was estimated by enzymatic kinetic assay method using automated analyzer, Hitachi model 912. Total cholesterol estimation was done by cholesterol oxidase method; HDL cholesterol was estimated by same method after precipitating the LDL, VLDL and Chylomicrons (Allain, 1974). Triglycerides were estimated by enzymatic mixture containing lipoprotein lipase, glycerol kinase and glycerol-3-phosphate oxidase and peroxidase (McGowan, 1983). Low density lipoprotein levels were calculated by using Fridewald's formula.

Statistical Analysis

The results were expressed as mean±Standard Deviation (SD). A p-value of <0.05 was considered statistically significant. Analysis of Variance (ANOVA) was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS AND DISCUSSION

As shown in the Table 1, serum total cholesterol, triglycerides, low density lipoprotein were elevated ($p<0.01$) and high density lipoprotein and paraoxonase levels were significantly low ($p<0.01$) in group 1 cases compared to healthy controls. There was significant decrease in total cholesterol, triglycerides, low density lipoprotein ($p<0.01$) and increase in high density lipoprotein ($p<0.01$) in group 2 cases compared to group 1 cases. Serum paraoxonase activity improved significantly in group 2 cases compared ($p<0.01$). There was positive correlation between high density cholesterol and paraoxonase activity in group 2 cases ($R = 0.347$, $p<0.01$) (Fig. 1).

Table 1: Serum paraoxonase and lipid profile parameters in healthy controls, cases before treatment (group 1) and three months after treatment (group 2)

Treatments	Total cholesterol	Triglycerides	High density lipoprotein	Low density lipoprotein	Paraoxonase ($U\ L^{-1}$)
	---------- mg dL^{-1} ----------				
Healthy controls					
(N = 41)	160±32	121±68	46±16	91±27	192±31
Group 1 cases					
(N = 59) (Hyperlipidaemics before treatment with atorvastatin)	213±49*	221±106*	40±11*	133±51*	45±29*
Group 2 cases					
(N = 59) (Hyperlipidaemiacs after treatment with atorvastatin)	185±31**	178±78**	53±11**	95±30**	180±42**

Values were expressed mean±SD, *: $p<0.01$ compared to healthy controls, **: $p<0.01$ compared to group 1 cases

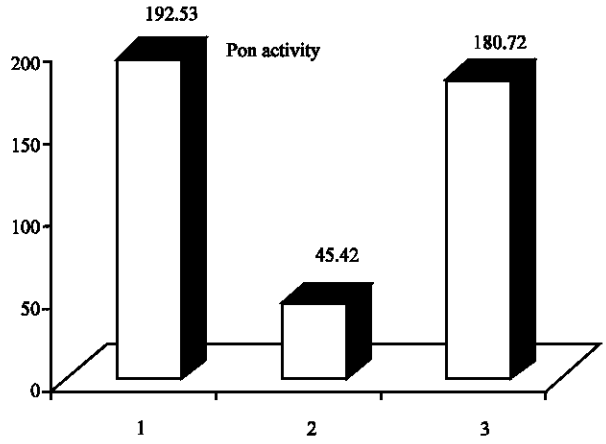

Fig. 1: Serum paraoxonase activity ($U\ L^{-1}$) in health control and hyperilidemic patients before and after treatment (1) healthy control, (2) hyperlipidemics before treatment and (3) hyperlipidemics three month after treatment

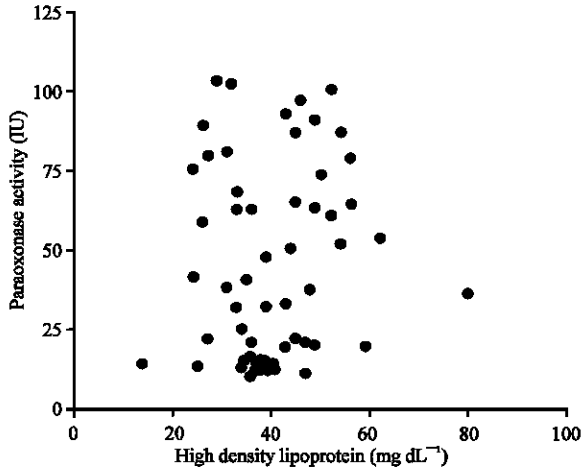

Fig. 2: Correlation between paraoxonase and high density lipoprotein three months after treatment with atorvastatin

In present study we found significant decrease in paraoxonase activity in hyperlipidemia patients. This decrease in paraoxonase activity may be associated with decrease in levels of high density lipoproteins, as this enzyme is associated with high density lipoproteins. Thus decrease in paraoxonase activity and increase in low density lipoproteins in hyperlipidemia may be favorable for atherogenesis process, thus predisposing them for premature coronary artery disease. The exact mechanism of anti-atherogenic function of high density lipoproteins and its associated components is not clear at present but the role of high density lipoprotein associated paraoxonase activity in this process is increasingly stressed in recent times (Watson *et al*., 1995).

Statins, a 3-hydroxy methyl glutaryl Coenzyme A reductase inhibitors are drugs of choice in different types of hyperlipidemia especially their role in favorably improving lipid profile. In our study, atorvastatin apart from favorably improving the lipid profile, it also improved the activity of paraoxonase (Fig. 1). This improvement in paraoxonase activity correlated positively with increased high density lipoproteins level (Fig. 2). Previous authors found improvement of paraoxonase activity with fenofibrates (Paragh *et al*., 2003). The improvement in paraoxonase activity by fenofibrates was due to induction of paraoxonase 1 gene promoter activation by fibrates (Gouedadr *et al*., 2003). We found improvement in paraoxonase activity on treatment with atorvastatin for three months in hyperlipidemic patients and it may implicates the role of statins in improving the paraoxonase activity and there by decreasing the rate of atrherogenesis in this population. However, long term follow-up studies are required to know the outcome of such treatment and it also requires specially designed study to understand the molecular mechanisms of statin in improving paraoxonase activity.

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