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STUDY OF THE ACTIVITY OF SERUM PARAOXONASE AS A MARKER OF SEVERITY OF ATHEROSCLEROSIS IN POST MENOPAUSAL WOMEN AND ITS CORRELATION WITH LIPID PROFILE IN THESE WOMEN.

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ABSTRACT Coronary artery disease is one of the important causes of death among older women according to Global Burden of Disease 2010 Estimates. Altered lipid profile is a major risk factor of this disease. Paraoxonase is a serum esterase associated with HDL-C and contributes to protect against LDL-C oxidation. Post-menopausal women are known to have altered lipid profile so present study is designed to estimate serum Paraoxonase activity and correlate with lipid profile in post-menopausal women.

The study was conducted on 50 post-menopausal women with no history of conditions altering lipid profile and 50 healthy pre-menopausal controls. Serum Paraoxonase activity was estimated by spectrophotometric method and lipid profile by enzymatic assay methods.

Serum Paraoxonase activity and HDL-C levels were low and TC, and LDL-C levels were high in post-menopausal women compared to healthy pre-menopausal controls ($p<0.01$). Serum Paraoxonase levels were decreased in post-menopausal women compared to pre-menopausal women ($p<0.01$). Serum Paraoxonase activity correlated positively with HDL-C.

Decrease in HDL associated Paraoxonase activity with dyslipidaemias in post-menopausal women may accelerate LDL oxidation leading to atherogenesis.

Hence post-menopausal women with dyslipidaemia along with decreased serum Paraoxonase levels could be more prone and susceptible to accelerated atherogenesis leading to coronary artery disease.

KEYWORDS : Menopause, Paraoxonase, HDL-C

Introduction

Menopause

Menopause is a physiological process in women that occurs around 45-55 years old, which is defined as permanent cessation of menstruation by one year in row. The age of menopause depends on multiple factors such as number of ova in the female at birth, the frequency of loss of these ova throughout her life and the number of ovarian follicles required for maintaining the menstrual cycle. The diagnosis of menopause is retrospective and is established after a year without menstruation, and their symptoms may have different intensity for each woman.

During post menopause there is an abnormal atherogenic lipid profile characterized by increased lipoprotein cholesterol, low density (LDL-C), triglycerides (TG) and small dense LDL particles with reduced HDL-C [2,3] and elevated serum glucose and insulin, perhaps as a direct result of ovarian failure or indirectly as a result of central redistribution of body fat, and this favors the formation of atheromatous plaques and progression of coronary atherosclerosis and therefore cardiovascular disease incidence increases substantially in postmenopausal women. Other disorders such as obesity and metabolic syndrome also occurs at menopause, thus menopause is associated with a significant increase in the prevalence of cardiovascular disease in women.

HDL after Menopause

The high incidence of atherosclerosis and cardiovascular disease in women after menopause is mainly associated with a reduction in circulating oestrogen and an elevation in plasma LDL levels. Among postmenopausal women, an adverse lipoprotein pattern has been described, even though HDL cholesterol levels tend to be maintained or only slightly decreased.

However, compositional and structural alterations in HDL could be present and this might impact on HDL anti-atherogenic properties such as its capacity to inhibit LDL oxidation. The hypothesis that LDL oxidation plays a significant role in atherogenesis is supported by epidemiological data as well as studies in animal models receiving antioxidants.

Taking into consideration the antioxidant properties assigned to oestrogens, a reduction in their concentration could lead to an increase in lipid peroxidation. The increase in oxidative stress may influence endothelial injury and the susceptibility of lipoproteins to oxidation. If HDL becomes oxidized, its ability to protect LDL against oxidation in the sub endothelial space may be impaired. Several enzyme systems associated with HDL could be responsible for inactivating the reactive species within oxidized LDL and are mainly represented by Paraoxonase (PON). This HDL-associated enzyme might protect against the induction of inflammatory responses in cells of the arterial wall by destroying biologically active lipids in mildly oxidized LDL. It has been suggested that high – density lipoprotein has the capacity to reduce the oxidative modifications of LDL due to the presence of enzyme Paraoxonase. The enzyme is found to protect both HDL and low density lipoprotein (LDL) against peroxidation, which suggests a possible involvement of PON 1 in the anti atherogenic properties of HDL. It has been shown that invitro purified Paraoxonase decreased LDL lipid peroxidation [4] and that purified Paraoxonase significantly reduced the ability of mildly oxidized LDL to induce monocyte endothelial interactions [7]. Thus, a protective role of paraoxonase against atherosclerosis has been suggested as well as an explanation of protective role of HDL against LDL oxidation. Low serum PON 1 activity is associated with several risk factors for coronary heart diseases, including diabetes, hypercholesterolemia and smoking. In postmenopausal women, the increase in plasma triglycerides and, presumably, in VLDL particles would promote exchanges between lipoproteins, remodelling their composition.

The enzyme paraoxonase (PON1) bound to the HDL particle confers antioxidant properties to the lipoprotein, delaying the accumulation of lipid peroxides in LDL [10]. Several studies implicate PON1 in the development of atherosclerosis and coronary artery disease.

Paraoxonase

Paraoxonase (PON1) is a calcium-dependent serum esterase (354 amino acids) and in serum, is exclusively located on HDL[5]. It is a xenobiotic enzyme which hydrolyses organophosphorous compounds such as paraxone, unsaturated aliphatic esters, and aromatic carboxylic esters. Serum paraoxonase is a high density lipoprotein (HDL-C) associated enzyme synthesized mainly in liver, although the natural substrates for paraoxonase are unknown, paraoxonase decreases LDL-C oxidation by its paraoxonase activity[1] and by preventing homocysteinylation of Apo B-100. The biological role of HDL-C[16] is attributed to the presence of paraoxonase associated with it and contributes to the protective effect of this lipoprotein on LDL oxidation.

PON1 is synthesized and secreted by liver. PON1 tightly binds to HDL subfractions that also contain apoA-1 and apoJ or clusterin and it has the capacity to protect HDL and LDL against oxidation and preserves

their function.

Aims of the study

1. Study of activity of serum Paraoxanase
2. Correlation with lipid profile of post-menopausal women
3. Comparison of serum paroxonase activity between pre and post-menopausal women

Subjects

The study was conducted on 50 post-menopausal women with no history of hypertension and diabetes divided into 2 groups (Group 1 n=35 with low HDL-C ,Group 2 n=15 with normal HDL-C) and 50 healthy pre-menopausal women. Under aseptic conditions blood sample (5ml) was drawn into plain vacutainers. All assays were performed immediately after serum was separated.

Reagents and methods

Special chemicals like paraoxon were obtained from sigma chemicals. All other reagents were of analytical grade. Paraoxonase activity was estimated spectrophotometrically by the method described by Schiavon R et.al,[13] with minimal modifications. One unit (IU) of paraoxonase activity is defined as 1 µmol of p-nitrophenol formed per min per litre at 25 °C, and activity was expressed as U / L of serum.

Fasting lipid profile was estimated by enzymatic kinetic assay method[14,15] using automated analyzer. LDL-C levels were calculated by using Friedewald's formula.

Statistical analysis

The results were expressed as mean standard deviation (SD). A P value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS-17 (Chicago, USA). Independent samples t test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

Results

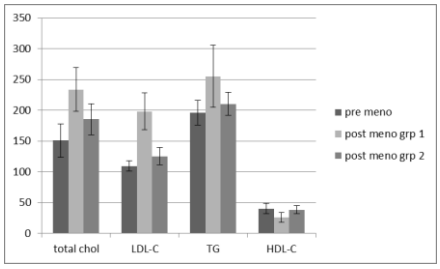
Serum Paraoxonase activity and HDL-C levels were low and TC, triglycerides and LDL-C levels were high in a group 1 when compared to healthy pre-menopausal controls ($p<0.01$). Serum Paraoxonase levels were decreased in all the 50 post-menopausal women compared to pre-menopausal women ($p<0.01$). #Group 2 had normal HDL-C but lower Serum Paraoxonase levels.

Table 1:

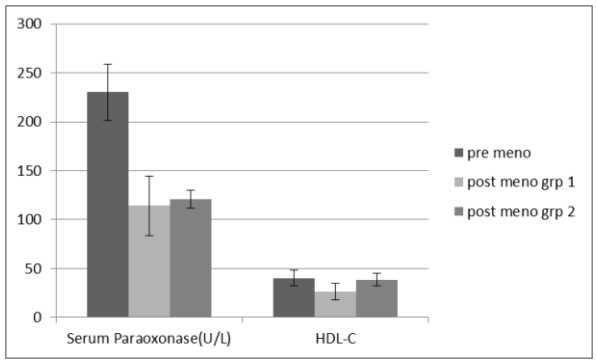
Parameters	Premenopausal women (n = 50)	post-menopausal women (group 1) (n =35)	Post-Menopausal women (Group 2) (n=15)	P value*
Serum Total cholesterol (mg/dl)	150.8 ± 27.2	233.5 ± 35.6	185.4±25.3	≤ 0.001
Serum LDL-C (mg/dl)	109.4 ± 8.6	198.2 ± 29.6	125.3±14.2	≤ 0.001
Serum Triglycerides(mg/dl)	196.1 ± 19.8	255.1 ± 50.3	210.4±18.6	≤ 0.001
Serum HDL-C (mg/dl)	40.2 ± 8.3	26.2 ± 8.1	38.4±6.3#	≤ 0.001
Serum Paraoxonase(U/L)	230.2 ± 28.7	114.3 ± 30.4	120.6±9.3	≤ 0.001

***P value < 0.001 compared to healthy Pre-menopausal women (Values expressed in mean ± SD).**

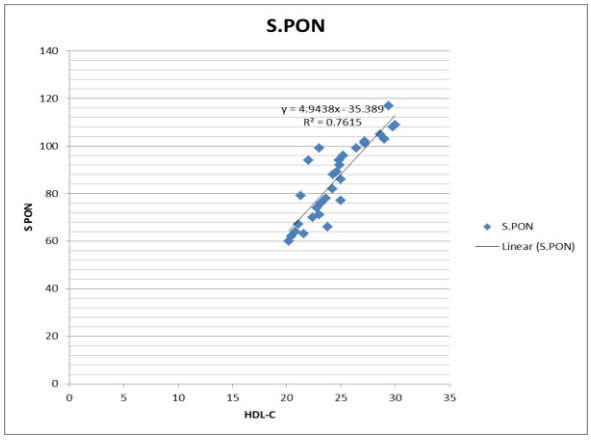
Comparison of lipid profile in post-menopausal and pre-menopausal women



Comparison of HDL-C and serum Paraoxonase in post-menopausal and pre-menopausal women



Correlation between serum paraoxonase activity and HDL-C level in post menopausal women



DISCUSSION

In our study we found significant decrease in Paraoxonase activity in post-menopausal women. This decrease in Paraoxonase activity may be associated with decrease in levels of HDL-C . Decrease in HDL associated Paraoxonase activity and increase in LDL-C in post-menopausal women may be favourable for atherogenesis process, thus predisposing post-menopausal women with dyslipidaemia for premature coronary artery disease.

The exact mechanism of antiatherogenic function of HDL and its associated components is not clear but the role of HDL associated paraoxonase activity in this process is increasingly stressed in recent times. Although several studies proposed the anti-oxidative and anti-atherogenic nature of Paraoxonase, the exact mechanism is still not clear. Recently the natural substrate and biological role of PON was reported by Jakubowski et al.[12]. Their study indicates role of Paraoxonase in hydrolysis of homocysteine thiolactone into homocysteine (homocysteine thiolactonase activity). Homocysteine thiolactone is unstable compound and can bind to proteins at lysine residues. This N-homocysteinylation of proteins alters protein's structure and increases its susceptibility to proteolysis. N-homocysteinylation of Paraoxonase (or other component of HDL regulating its activity like apolipoprotein a1) decreases PON activity. This decrease in Paraoxonase activity may initiate a positive feedback mechanism, since reduced Paraoxonase activity will cause further accumulation of homocysteine thiolactone and may augment protein homocysteinylation which increases the susceptibility of LDL-C to oxidation.

Some studies suggest that HDL from postmenopausal women may not exert its antioxidant action efficiently, independently from HDL cholesterol plasma levels[17].

This further increases atherogenesis and cardiovascular complications in post-menopausal women

Conclusion

In conclusion, decrease in HDL associated paraoxonase activity with dyslipidaemia in post-menopausal women may increase accelerated

LDL oxidation leading to atherogenesis. This study shows that the changes that occur in the lipid profile along with Paraoxonase activity after menopause is not friendly for the cardiovascular health of women. Hence post-menopausal women with dyslipidaemia along with decreased serum Paraoxonase activity could be more prone to coronary artery disease compared to pre-menopausal women.

So Paraoxonase can be used as marker for severity of atherosclerosis in post-menopausal women. Serum Paraoxonase can be more of a marker of early diagnosis of progressing atherosclerosis in post-menopausal women with normal HDL-C.

Research is going on oestrogen replacement in normally attained menopause to reduce atherogenic state. Further research is required to enhance the activity of enzyme and HDL-C and reduce LDL-C oxidation.

Conflict of interest

There is no conflict of interest.

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