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Original Research Article

Relation of senescence with systemic inflammation and serum paraoxanase activity: A clinical approach

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Abstract: It has been documented that the incidence of inflammation along with oxidative stress in biological system leads to the development of age related complications. However, the mechanism exploring the conundrum of senescence with various sorts of health complications is still in obscure. Therefore, the present study was designed to evaluate the relation of serum paraoxonase (PON) with markers of systemic inflammation and lipid peroxidation in the blood samples of different age group subjects and to determine their relation in the prediction of cardiovascular disease risk. Marker of lipid peroxidation (malondialdehyde; MDA), serum paraoxonase, c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were estimated in selected 90 healthy subjects by using standard methods. Out of 90 subjects, 60 individuals were categorized into two groups: Group I (40-55 years) and Group II (≥ 56 years) and statistically compared it with that of 30 younger controls (20-30 years). Marked depletion in serum PON activity was observed in Group I and II as compared to healthy controls whereas erythrocyte MDA levels were increased significantly ($p<0.05$) in Group I and II subjects. However, serum CRP levels were increased significantly ($p<0.05$) only in Group II subjects whereas ESR levels were altered insignificantly ($p<0.01$) in Group I and II subjects as compare to controls. In addition, PON activity was inversely correlated with marker of systemic inflammation and lipid peroxidation. Thus, assessment of serum paraoxonase activity incorporation with marker of systemic inflammation is a crucial step in early diagnosis of cardiovascular complications in elderly. Therefore, regular monitoring of cardiac markers and antioxidant rich diet should be adopted with senescence.

Keywords: Paraoxonase, malondialdehyde, C - reactive protein, erythrocyte sedimenta- -tion rate, oxidative stress

INTRODUCTION:

Biological aging predisposes individuals to various morbidities through age related perturbation of systemic oxidative balance, i.e. uncontrolled free radicals production and increasing incidence inflammation. Among different types of chronic complications associated with age, cardiovascular disease (CVD) represents a major public health problem worldwide and, indeed, the main cause of morbidities in older people [1]. It has been well predicted that by the year 2020 there would be an almost 75% increase in the global cardiovascular disease burden, and thus, received much attention for early prediction of CVD in older population [2].

C-reactive protein (CRP), a marker of systemic inflammation and synthesized in liver, has been received considering attention in aging mediated inflammatory disorders such as CVD, osteoarthritis and cancer etc. [3-5]. Previous studies have demonstrated an association between age related disorders and inflammation as measured by plasma C-reactive protein

[6, 7]. In addition, emerging concepts reveal its relation with other inflammatory markers, vascular injury and endothelium dysfunction, and attract the researchers to clarify its role in advancing of age [8-11].

Apart from systemic inflammation, oxidative stress caused by imbalance of oxidant and antioxidant in elderly, forecasting a grim scenario for the evolving CVD complications with senescence [12]. The incidence of aging is a consequence of various sorts of destructive events caused by reactive oxygen species (ROS) which include biomolecular deterioration, DNA strand breakage, oxidative modification of lipoproteins, damage to endothelium, cartilage and membrane ion transporters [13].

In general, lipid peroxidation serves as a marker of oxidative damage caused by free radicals leading to cellular senescence [14]. In addition, lipid peroxidation has been implicated in the development of age related complications such as cardiovascular disease. The prime targets of peroxidation by ROS are the polyunsaturated fatty acids (PUFA) in the membrane lipids. Consequently, a variety of end products are produced, including reactive aldehydes such as malondialdehyde (MDA). The levels of MDA indicate the extent of lipid peroxidation [13, 15].

Paraoxonase, a glycoprotein, has not only antioxidant activity but also has anti-atherogenic function. It is synthesized mainly in the liver, as HDL-associated lipo-protective enzyme carried on apo A-I. It prevents oxidation of low-density lipoprotein (LDL). Its serum concentration is influenced by inflammatory changes. In addition, PON also hydrolyzes organophosphates like pesticides, neurotoxins, and arylesters [16]. Previous studies have shown that PON level alters in various age related complications such as cardiovascular diseases, musculoskeletal and neurological disorders [17, 18]. However, alteration in PON levels with aging process and in determining future risk of age related complications including CVD, is still in obscure, and has received much attention in order to explore hidden facts related to commencement of senescence.

Therefore, considering the role of aforesaid parameters in the development of various age related complications, the objectives of present study was to evaluate the relation of plasma paraoxonase activity with markers of systemic inflammation and lipid peroxidation in different age group North Indian subjects and to determine their role in focusing early prediction of age related disorders such as CVD risk.

METHODS:

In the present study, 90 healthy subjects were recruited and divided into 3 groups of 30 subjects each (on the basis of age) i.e. control group (younger people) includes 30 healthy subjects of age group 20 – 30 years, Group I includes 30 healthy subjects (middle aged people) of age group 40 – 55 years and Group II includes 30 healthy subjects (elderly) of age group 56 years onwards. In each group, 15 male and 15 female (1:1 ratio) were included. These subjects were recruited randomly after taking their informed consent and approval of protocol by ethics committee of college. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all the subjects. Height and weight were measured with subject barefoot and light dressed. The body mass index (BMI) was calculated as [BMI = weight (Kg) / Height ($metre^2$)].

Inclusion criteria: Subjects who gave informed consent for study, having no history of disease, neither under any medical treatment nor taking antioxidant supplement were included.

Exclusion criteria: Patients with diabetes mellitus, hypertension, renal insufficiency, hepatic disease or under any medicinal treatments were excluded. Pregnant and lactating women, smokers, obese (BMI > 25), hypertensives (B.P. >120/>80 mmHg) as per JNC 7^{th} guidelines and who did not follow the study instructions, were excluded from the study.

Fasting blood samples were collected in EDTA and plain vials from the anticubital vein of the subjects and processed immediately. Serum PON activity, CRP levels, markers of lipid peroxidation and erythrocyte sedimentation rate (ESR) were estimated in controls as well as in different age group subjects. The ESR levels were determined according to the Westergren method. Serum C-reactive protein (CRP) levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate.(19) The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, measured at 532 nm.

Seum paraoxonase activity was estimated by Gan *et al.;* method using p-nitrophenyl acetate (5.5 mM/L) as a substrate.(20) The increase in the absorbance of p-nitrophenol formed at 412 nm was measured spectrophtometrically. The activity of PON was measured in Tris buffer (20 mM/L; pH 8.0) containing 1mM $CaCl_2$. The generated product p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole/cm at pH 8.0. Results are expressed as Units/ml (1 nmol p-nitrophenol formed per minute).

Statistical Analysis:

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. The distribution of't'- probability was calculated depending on 'n' and significance of test was obtained. P value < 0.05 and < 0.001 were considered as significant and highly significant respectively. In addition, correlation analysis between aforesaid parameters was performed by using Pearson correlation test.

RESULTS:

In the present study, age, anthropometry and hematological profile of the study group subjects were depicted in Table 1. BMI measurement revealed insignificant increase ($p<0.1$) in elderly as compared to middle aged and younger controls. However, elderly subjects had significant variation in hemoglobin levels ($p<0.05$) with respect to younger controls indicating that elderly were more susceptible to have various sorts of complications other than CVD which include osteoarthritis, anemia etc. In addition, elderly subjects had significant variation in blood pressure ($p<0.05$) with respect to younger controls indicating that elderly were more susceptible to have future CVD risk.

Plasma CRP levels were found to increased significantly (41.81% high; $p<0.05$) only in Group II subjects whereas in Group I subjects plasma CRP level increases insignificantly (24.54% high; $p<0.1$). Marked occurrence of atherogenic profile and significant alteration in the levels of serum paraoxonase and erythrocyte MDA were observed in the study group subjects, as represented in Table 2. Serum paraoxonase activity was found to be significantly low ($p<0.05$ & $p<0.05$) in Group I and II subjects as compared to controls. Erythrocyte MDA levels were 20 % and 34.71% high in Group I and Group II respectively as compared to younger controls. These levels reveal continuous variation with increase in age but statistically these values were altering insignificantly, when these levels were compared with in Group I and II.

In addition, we also observed that serum PON activity was negatively correlated with serum CRP, MDA and ESR, DAS28, disease duration of RA (DDRA) whereas HDL cholesterol and TAA levels were positively correlated with PON activity, as shown in Table 3. These results clarify the role of PON reduction in enhancing the CVD risk with senescence most probably by its relation with oxidative stress and systemic inflammation.

Table 1: Demographic and hematological profile of study group subjects (n=90)

S No	Particulars	Control group (n=30)	Group I (n=30)	Group II (n=30)
1.	Age (years)	24.5 ± 2.4)	47.2 ± 5.0	64.6 ± 4.0
2.	M:F ratio	1:1	1:1	1:1
3.	Height (meter)	1.58 ± 0.02	1.60 ± 0.02	1.59 ± 0.03
4.	Weight (Kg)	58.5 ± 2.4	60.2 ± 2.8	62.7 ± 2.5
5.	B.M.I. (Kg/m^2)	22.8 ± 1.1	24.2 ± 1.1 *	24.0 ± 0.8 *
6.	Systolic blood pressure (mmHg)	108.0 ± 2.10	112.5 ± 2.35*	116.5 ± 2.5*
7.	Diastolic blood pressure (mmHg)	75.2 ± 1.4	75.7 ± 1.3*	78.2 ± 1.54*
8.	Hemoglobin (gm %)	13.15 ± 1.18	12.40 ± 0.85*	11.85 ± 0.72*
9.	ESR (mm/h)	13.5 ± 2.20	15.2 ± 2.4*	19.8 ± 2.7*
10.	RBC (millions/cumm)	3.8± 1.7	3.6 ± 1.4*	3.0 ± 1.7*
11.	WBC count (cells/cumm)	5.80 ± 1.24	5.52 ± 0.95*	5.36 ± 0.82*

Where, * P<0.1: Non significant, ** P< 0.05: Significant

Table 2: Plasma paraoxonase, markers of systemic inflammation and lipid peroxidation in different age group subjects (Mean± SD).

S.No	Particulars	Control group (n=30)	Group I (n=30)	Group II (n=30)
1.	CRP (mg/L)	3.30 ± 0.14	4.11 ± 0.17*	4.68 ± 0.20**
2.	Paraoxonase (U/ml)	225.4 ± 17.5	187.5 ± 17.0*	156.2 ± 15.8**
3.	Malondialdehyde (µmol MDA/ml)	2.65 ± 0.15	3.18 ± 0.20*	3.57 ± 0.26**

Where, *P<0.1: Non significant, ** P< 0.05: Significant

Table 3: Correlation coefficient between serum PON activity and other variables in Group I and II subjects

Particulars	ESR	MDA	CRP
PON in Group I	-0.258	- 0.425	-0.357
PON in Group II	-0.346	- 0.684	-0.562

DISCUSSION:

It has been reported that ROS are highly reactive and indiscriminate, and if not promptly neutralized, they can inflict major interrelated derangements of cell metabolism leading to aging process [4, 14]. Endothelial cells and vascular smooth cells produce ROS which oxidize low density lipoprotein and thereby initiate atherosclerosis. In addition, involvement of ROS in cell membrane damage via lipid peroxidation. Among reactive aldehydes, malondialdehyde (MDA) is an abundant product of lipid peroxidation and various studies have been reported regarding the etiopathological role of lipid peroxidation in aging and disease development [21]. MDA incorporation with other products of lipid peroxidation exerts its toxic effect on cellular components, and responsible for not only initiation of complex cascade that promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance [22]. In this context, marked increase in erythrocyte MDA were observed in group I and group II subjects ($p<0.005$) as compared to younger controls which clarify the etio-pathogenic role of ROS via lipid peroxidation, in shaping elderly more susceptible to develop future incidence of CVD complications. Similar findings have been reported in previous studies on older population as well as other age related complications [23-25].

In order to provide protection against harmful action of ROS, antioxidant defense system plays a crucial role and prevents the development of free radicals mediated biomolecular destruction. Apart from various other antioxidant enzymes, PON contributes to anti-atherogenic and antioxidant activity by regulating oxidation of LDL, by hydrolyzing specific oxidized phospholipids, cholesterol linoleate hydro peroxides, and by neutralizing hydrogen peroxide [16, 17, 26, 27]. Depletion in the PON activity may have significant effect in inducing rise in blood pressure with advancing of age. In the present study, serum PON activity was found to be decreased continuously in middle aged followed by elderly which reflects toward its utilization in preventing ROS mediated lipid peroxidation and its inactivation due to interaction of oxidized lipids with the PON free sulfhydryl group. Our findings are consistent with the findings of Gupta *et al.;* who also observed depleted activity of PON in elderly knee osteoarthritis patients and directed its role in prediction of CVD risk [28].

`In particular, free radicals mediated oxidative stress has been described as an important mechanism underlying inflammatory diseases. ROS, RNS and their intermediates serve as mediators of inflammation by enhancing various culprit events such as inhibition of glycolytic enzymes, reduction of antioxidant reserves in synovial fluid and activation of proteolytic enzymes to degrade cartilage [29]. C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein that participates in the systemic response to inflammation. It is an excellent biomarker for acute-phase response and has emerged as an important, powerful and characteristic predictor of future cardiovascular disease and metabolic abnormalities in ostensibly healthy men and women of advancing age [30-31]. Serum CRP was found to be increased continuously in middle aged followed by elderly which reflects toward the role of excessive ROS production mediated systemic inflammation and thereby increases the risk of CVD in elderly. In addition, CRP levels were negatively associated with PON activity which indicates that depletion in antioxidant reserve also facilitates the incidence of inflammation, a hallmark of CVD complication. Similarly, Bhattacharya et also observed elevated levels of serum CRP in elderly subjects and reported that elevated levels of CRP was associated with local inflammation in age related disorder [32].

CONCLUSION:

On the basis of findings of present study and consistent findings of previous studies, it is concluded that PON activity measurement is an important diagnostic test to predict CVD risk as PON activity is inversely associated with systemic inflammation and oxidative stress mediated lipid peroxidation in elderly subjects. Moreover, it is suggested that regular intake of antioxidant rich diet, normal exercise and life style modification can avert not only oxidative stress mediated aging process but also senescence related complications. Furthermore, inclusion of more investigations even at molecular basis also needed to get more accurate prediction and effective regulation of CVD burden as well.

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