

Original Research Article

## **Study on dyslipidemia and oxidative stress in Elderly- A clinical approach to predict cardiovascular disease risk**

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**Abstract:** Aging is characterized by systemic deterioration of biomolecules and increased incidence of oxidative stress. In addition, occurrence of dyslipidemia in elderly significantly contributes the development of cardiovascular disease. It is conceivable that association of dyslipidemia with oxidative stress enhances the future the risk of cardiovascular disease (CVD) in older population. The aim is to estimate the plasma lipid profile and marker of oxidative stress in elderly and to determine their role in predicting CVD risk. The study population consists of 90 subjects. Out of 90 subjects, 60 healthy individuals were categorized into two groups: Group I (40-55 years) and Group II ( $\geq 56$  years) and statistically compared it with that of 30 younger controls (20-30 years) by using student's t-test. Plasma lipid profile levels were measured along with erythrocyte malondialdehyde (MDA) levels. Data were statistically analyzed using standard methods. Plasma total cholesterol, triglycerides, LDL-cholesterol HDL-cholesterol and MDA levels were significantly high ( $p < 0.05$ ) in group III subjects as compared to healthy controls. However, these levels were altered insignificantly ( $p < 0.1$ ) in group II subjects. Similarly, plasma CRP levels were found to be significantly high ( $P < 0.001$ ) in Group III as compared to younger controls. Thus, alteration in lipid profile along with enhanced oxidative stress authenticates the fact that these markers are more efficient than conventional lipid profile parameters in prediction of CVD risk in older population.

**Keywords:** Malondialdehyde, free radical, total cholesterol, HDL-cholesterol, inflammation

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### **INTRODUCTION:**

Progressive deterioration in physiological functions and metabolic processes are hallmarks of aging process which lead to morbidity and mortality [1]. Dyslipidemia or abnormal lipid profile with advancing of age is an alarming condition of future health complications predominantly cardiovascular diseases (CVD) such as myocardial infarction, atherosclerosis etc. [2, 3]. In agreement with the free radical theory of aging, reactive oxygen species (ROS) generated as byproducts of biological oxidations, lead to oxidative stress, which induce casual and cumulative oxidative damage to macromolecules inducing to cellular dysfunction with age and eventually cell death [4]. In addition, production of large amount of reactive oxygen species in the cells can evade or overwhelm the antioxidant protective mechanisms of cells and tissues, and produce major interrelated impaired cell

metabolism including lipid peroxidation, DNA strand breakage, rises in intracellular free  $Ca^{2+}$ , damage to membrane ion transporters and other specific proteins which have important implications for ageing, inflammation, CVD and a variety of other diseases [5].

Among various destructive events, lipid peroxidation has been found to be a major event in the development of various diseases with senescence [6, 7]. Malondialdehyde (MDA) is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that binding to these aldehydes to membrane protein may alter their function, tonicity, permeability, rigidity and integrity, and thereby may induce culprit effect [8].

Moreover, incidence of inflammation further enhances the frequency to develop CVD [9]. In this

context, the role of C-reactive protein as a marker of systemic inflammation, synthesized in liver and raised by many folds following acute inflammation, in various age related disorders including diabetes, osteoarthritis, hypertension, cancer, and myocardial infarction, cannot be neglected [10, 11].

It is plausible that there is a close link between systemic inflammation, oxidative stress and dyslipidemia in increasing the frequency of CVD risk in older population. In spite of improvement in our knowledge on psoriasis from pathologic point of view, the intimate mechanisms involving systemic inflammation, oxidative stress and dyslipidemia in older population making them more susceptible to develop future CVD are complex and not yet fully understood. In addition, as best of our knowledge, previous studies on elderly have not included dyslipidemia, oxidative stress and systemic inflammation in a single setting [2, 12, 13]. Therefore, the present work aims to evaluate the levels of C - reactive protein (CRP), MDA and lipid profile in different age group subjects and to determine their role in prediction of CVD risk in elderly.

#### MATERIAL AND METHOD:

90 healthy subjects of both the sex (15 males & 15 females in each group) and different age groups were selected randomly and categorized into three groups depending upon age i.e. Group I (younger people) includes 30 healthy subjects of age group 20 – 30 years, Group II includes 30 healthy subjects (middle aged people) of age group 40 – 55 years and Group III includes 30 healthy subjects (elderly) of age group 56 years onwards. These subjects were included in the study only after taking their informed consent and approval of protocol by ethics committee of college. Fasting blood samples (6 ml) were collected in EDTA vial from anticubital veins avoiding venostasis from each subject after taking the demographic information, history and limited physical examination such as age, sex, height, weight, blood pressure and confirmation of healthy state. Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) was calculated as  $B.M.I. = \text{weight (Kg)} / \text{Height (metre)}^2$ . Obese (B.M.I > 25), hypertensives (B.P. >120/80 mmHg) and smokers were excluded from the study.

For the estimation of study group parameters, plasma was separated from 4ml of the collected blood sample by centrifugation at 1000 g for 15 min at room temperature and stored at -80°C until use. Erythrocyte

malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate prepared from 2ml of whole blood. The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution formed a trimethine coloured substance, which was measured spectrophotometrically at 532 nm [14]. Plasma C-reactive protein (CRP) levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

Plasma lipid profile contents (Total Cholesterol, Triglycerides and HDL cholesterol) were analysed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald's formula [15].

$$LDL-C = TC - [(TG/5) + HDL-C]$$

#### 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  $\pm$  SD and 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.

#### RESULT:

In the present study, the demographic profile including mean age and blood pressure of the study group subjects, are depicted in Table 1. Elderly subjects have insignificant variation ( $p < 0.1$ ) with respect to blood pressure, as compared to healthy controls. Oxidative stress status and marker of systemic inflammation are presented in Table 2. Plasma CRP and erythrocyte MDA levels were found to be significantly high ( $P < 0.001$ ; 37.9% and  $p < 0.001$ ; 65.2% high) in Group III as compared to younger controls whereas these levels were increased insignificantly ( $p < 0.1$ ) in Group II subjects which reflect the role of inflammation and oxidative stress in aging process. Plasma lipid profile along with apolipoproteins levels, as depicted in Table 2 revealed that plasma total cholesterol, triglycerides and LDL-cholesterol levels were significantly high ( $p < 0.001$ , 48.5%, 45.1% and 63.38% high) only in Group III subjects as compared to younger controls. Similarly, HDL-cholesterol levels were altered significantly ( $p < 0.05$ , 26.6% low) in Group III subjects with respect to younger controls.

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

| S No | Particulars                     | Group I (n=30) | Group II (n=30) | Group III (n=30) |
|------|---------------------------------|----------------|-----------------|------------------|
| 1)   | Age (years)                     | 25.6 ± 2.3     | 46.5 ± 4.6      | 62.1 ± 4.8       |
| 2)   | M:F ratio                       | 1:1            | 1:1             | 1:1              |
| 3)   | Height (meter)                  | 1.57 ± 0.03    | 1.60 ± 0.02     | 1.58 ± 0.02      |
| 4)   | Weight (Kg)                     | 57.4 ± 2.3     | 60.8 ± 2.7      | 62.5 ± 2.6       |
| 5)   | B.M.I. (Kg/m <sup>2</sup> )     | 22.6 ± 1.2     | 24.4 ± 1.2 *    | 24.2 ± 0.7 *     |
| 6)   | Systolic blood pressure (mmHg)  | 107.0 ± 2.0    | 112.8 ± 2.15*   | 116.0 ± 2.4*     |
| 7)   | Diastolic blood pressure (mmHg) | 76.2 ± 1.3     | 77.5 ± 1.2*     | 78.5 ± 1.6*      |

Where,

\* P<0.1: Non significant

\*\* P< 0.05: Significant

**Table 2: Plasma lipid profile, markers of oxidative stress and CRP levels in study group subjects (Mean ± SD)**

| S.No | Particulars                  | Group I (n=30) | Group II (n=30) | Group III (n=30) |
|------|------------------------------|----------------|-----------------|------------------|
| 1.   | CRP (mg/L)                   | 2.74 ± 0.10    | 3.05 ± 0.13     | 3.78 ± 0.15****  |
| 2.   | Malondialdehyde (µmolMDA/ml) | 1.84 ± 0.12    | 2.25 ± 0.15     | 3.04 ± 0.16**    |
| 1.   | Total Cholesterol (mg/dl)    | 138.24 ± 11.6  | 176.45 ± 12.5   | 205.26 ± 15.0**  |
| 2.   | Triglycerides (mg/dl)        | 95.52 ± 11.30  | 118.80 ± 12.82  | 138.6 ± 16.2**   |
| 3.   | HDL cholesterol (mg/dl)      | 40.2 ± 3.80    | 36.6 ± 3.52     | 29.5 ± 3.05*     |
| 4.   | LDL cholesterol (mg/dl)      | 78.6 ± 8.24    | 102.5 ± 10.40   | 128.42 ± 11.8**  |

Where,

\* p<0.1 : Non-significant

\*\* p<0.05 : Significant

\*\* p<0.001 : Highly significant

**DISCUSSION:**

It has been well established that disturbance in systemic oxidative balance due to uncontrolled ROS production plays a crucial role in increasing the chances to develop future age related complications in older population [16]. ROS produced by endothelial cells and vascular smooth cells not only oxidize low density lipoprotein and initiate atherosclerotic event but also involve in cell membrane damage via lipid peroxidation which in turn play a crucial role in the development and progression of vascular complications in elderly [17]. In the present study, marked increase in erythrocyte MDA levels were observed in Group III subjects (p<0.001) as compared to healthy controls which clarify the etio-pathogenic role of ROS via lipid peroxidation, in shaping older population more susceptible to develop future incidence of CVD and its related complications.

Similar findings have been reported in previous studies carried out on elderly subjects with arthritis [6, 18].

According to them, lipid peroxides are toxic to the 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 and thereby leading to development of CVD complications in elderly with dyslipidemia [19-22]. It is conceivable that dyslipidemia and oxidative stress in elderly are associated with incidence of inflammation and the mechanism behind its complex interplay is responsible for the development of future CVD risk. In the present study plasma CRP levels were found to be significantly high in older population along abnormal lipid profile as compared to healthy controls which may contribute to CVD risk in elderly because it

stimulates macrophages to produce tissue factor, a pro-coagulant that is found in atherosclerotic plaques. The presence of CRP in atherosclerotic lesions also suggests a 'cause and effect' relationship between this acute phase reactant and coronary events [23, 24]. Furthermore, in response to an oxidative inflammatory effect, atherogenic complexes of autoantibodies to oxidized LDL are generated which enhances the accumulation of LDL in the endothelial wall and thereby enhances the CVD risk [25]. Interestingly, Liu *et al.* reported that CRP is a convenient and useful biomarker to predict early bacterial infection in older patients especially when other markers are atypical or not present [26].

In addition, the present study group subjects revealed a traditional CVD risk factor i.e. an abnormal lipid profile, characterized by an increase of plasma total cholesterol, triglycerides and LDL-C levels, and a reduction in HDL-C levels which enhances the future CVD risk in older population. It could be explained on the basis of less physical activity with advancing of age which may be considered as a secondary impact of aging [27, 28]. Reduction in HDL cholesterol levels, as observed in elderly, also exposed them to CVD risk because HDL particle is known not only for its ability to facilitate reverse cholesterol transport, but also due to its anti-thrombotic, anti-oxidant, anti-inflammatory, and endothelium-stabilizing properties that may benefit against atherosclerosis [29-32]. Our findings were in consistent with the findings of Shanmugasundaram *et al.*; and Saxena *et al.*; who also observed marked alteration in lipid profile content in elderly subjects [2, 9].

#### CONCLUSION:

Thus, considering the findings of present study, we concluded that high LDL cholesterol levels and decreased HDL cholesterol levels along with hypercholesterolemia as a traditional CVD risk factors should be included to predict CVD complication in elderly. Moreover, regular assessment of markers of systemic inflammation and oxidative stress are additional approach to provide clear clinical picture with advancing of age. Furthermore, counseling of older population to maintain healthy dietary pattern, life style modification, regular exercise and adoption of antioxidant rich diet are essential steps which may help the older population not only in the control of oxidative stress mediated biomolecular deterioration but also enable them to reduce the cardiovascular risks.

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