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Correlation Between Macrophage iNOS Activity and Serum Cholesterol Level – A CVD Risk Factor Among Indian Geriatric Women

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ABSTRACT

Ageing is a dynamic and progressive process; during aging, the functional and structural deterioration of the arterial wall advances so slowly that it often goes unnoticed. The accumulation of oxidative damage with age may occur via an increase in the generation of oxidized substances, reduction in antioxidant capacity, reduction of repairs to oxidative damage, decrease in the generation of oxidized macromolecules or a combination of these mechanisms","thus ageing is one important risk factors in cardiovascular diseases. Nitric oxide synthases (NOS) enzyme system is an important source of reactive substances in aging. Specially among women estrogen is particularly noteworthy for its intense and beneficial effect on endothelial function by increasing production and decreasing degradation of NO which is down regulated after menopause. Macrophages are the first inflammatory cells to invade atherosclerotic lesions, and they are the main component of atherosclerotic plaques. In present study the iNOS activity of cultured macrophages from geriatric women having hypercholesterolemia were evaluated critically with their different cardiovascular risk factors. The correlation coefficient value indicates that low iNOS activity has higher risks of having various cardiovascular disease risks (BMI(r=-0.466), WC (r=-0.468) Total Cholesterol(r=-0.392), Triglyceride(r=-0.304), LDL-C (r=-0.451),HDL-C (r=-0.519)etc).

INTRODUCTION:

Ageing is a dynamic and progressive process that is characterized by the occurrence of morphological, biochemical, functional and psychological changes in the organism [1].

During aging, the functional and structural deterioration of the arterial wall advances so slowly that it often goes unnoticed. Over time, aging reduces the functional reserve and gradually increases the susceptibility of the arterial wall to insults such as smoking, physical inactivity, diabetes, and other cardiovascular risk factors [2].In this scenario, genetic inheritance and its interaction with harmful or protective factors influence the rate of deterioration of the artery wall function through various mechanisms as such, for example, modulation of nitric oxide (NO) production [3].Among these modulating factors, estrogen is particularly noteworthy for its intense and beneficial effect on endothelial function by increasing production and decreasing degradation of NO.[4] In fact, significant impairment of endothelial-dependent arterial dilation becomes apparent at the age of menopause [5], after which a rapid loss of endothelial function occurs, contributing to a rise in the prevalence of hypertension to levels equal to or even higher than those found in men [6]. many different studies thus indicating the critical risk factors of CVD among geriatric women is higher than geriatric male[6].

On the other hand, the nitric oxide synthases (NOS) enzyme system is an important source of reactive substances in aging. Physiologically, the NOS converts L-arginine into nitric oxide, resulting in control of the tonus of the vascular musculature. There is evidence that the expression of NOS enzyme and bioavailability of L-arginine decrease during aging, which leads to the accumulation of superoxide radicals[7]. An excess of this radical stimulates decoupling of the NOS enzyme, resulting in contraction of the vessel. These situations limit the blood supply to the heart, alter the consumption of O2 in the myocardium and increase apoptosis of endothelial cells. The uncoupled NOS (eNOS) has been demonstrated in diseases such as arteriosclerosis, diabetes mellitus (DM) and high blood pressure (HBP) [8].

Macrophages are the first inflammatory cells to invade atherosclerotic lesions, and they are the main component of atherosclerotic plaques [9]. Inflammatory cytokines produced by macrophages stimulate the generation of endothelial adhesion molecules, proteases, and other mediators, which may enter systemic circulation in soluble forms [10]. Cytokines as inflammatory biomarkers, independent of cholesterol and regulators of blood pressure, could yield more information on different aspects of pathogenesis of atherosclerosis [11].Extensive ROS has been implicated in atherosclerosis by inducing the chronic activation of vascular endothelium and components of immune systems (Macrophage is one of them). It has been demonstrated that superoxide production from both macrophages and vascular cells plays a critical role in atherogenesis [12]. When ROS production exceeds the scavenging capacity of cellular antioxidant systems, the resulting oxidative stresses damage lipids, membranes, proteins, and DNAs [13].The accumulation of macrophages laden with cholesterol in the vascular intima is the hallmark of fatty plaque formation in atherosclerosis. Understanding the mechanisms involving macrophages is critical for the prognosis, diagnosis, and treatment of atherosclerosis. In contrast, some papers on atherosclerosis emphasise the fact that many genes involved in macrophages have “major and critical” functions for plaques, which complicates the process of determining useful biomarkers for atherosclerosis [13]. Here in this study we try to correlate the iNOS activity of cultured macrophage and their persisting CVD risks among geriatric women.

METHODOLOGY:

Study Subjects:

The experiment was conducted with the human subjects’ understanding and consent, as well as a statement that the responsible Ethical Committee has approved the experiments. 50 subjects (Post –menopausal geriatric women mean age 62.5 ±4.40 years) were studied; among them those who have hypercholesterolemia (Total Cholesterol >200mg/dl) were selected for further evaluation. Subjects with chronic renal, hepatic, cardiac, gastro-intestinal, skeletal, or endocrine diseases (except diabetes), acute critical illness were excluded from the study.

Anthropometric Assessments:

Blood pressure was measured after a 5-min rest period to the nearest 1 mmHg while participants were seated using a sphygmomanometer (*Infi Mercurial, India*). Height was measured barefoot to the nearest 1 mm using an anthropometric rod.

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Weight was measured to the nearest 0.1 kg using an electronic scale (*Atlas Weighing Equipments, India*) with subjects wearing light clothing. Waist circumference was measured mid-way between the iliac crest and lowest rib, to the nearest 1 mm using anthropometric tape, with the subject standing.

Biochemical Assessment:
Phlebotomy was performed prior to 11.00 h to obtain a fasting blood sample (5ml) from all subjects. Processed serum was stored and analyses for fasting blood glucose, hemoglobin, Total cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, Very low density lipoprotein (VLDL) cholesterol and triglycerides were performed using commercially available enzymatic methods (*Span Diagonostic ltd.India*).

Estimation of iNOS:
Peripheral blood mononuclear cells (PBMC) were isolated from fresh blood sample and cultured for seven days using standard protocol [14]. They were then utilized to estimate iNOS activity at 420μM using previously explained protocol [15].

Statistical analysis:
Data are expressed as mean ±SD. The Repeated measures ANOVA was applied for multiple comparisons. Univariate analysis of the effects of each risk factor on macrophage derived iNOS activity was performed. Test of significance of correlation coefficient were also performed.

RESULTS AND DISCUSSION:
Clinical Characteristics of subjects:
The subjects consisted of 50 geriatric women among them after considering their present clinical scenario 10 women were considered as targeted sample as they have hypercholesterolemia (283±65.75mg\dl)[according to American Heart Association criteria].The other baseline characteristics were shown in table-1and their biochemical profile in Figure-1.

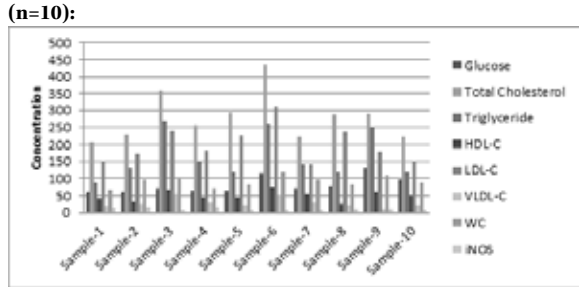
Table-1: Baseline Characteristics among 10 selected geriatric women:

VARIABLE	Mean (SD)
Age (years)	62.7 (4.40)
Waist Circumference (cm)	91.7 (16.075)
Mean Blood pressure(mm Hg)	156/91(9.16/8.30)
HDL-Cholesterol (High Density Lipoprotein-C) (mg\dl)	49.26 (14.00)
LDL- Cholesterol (Low Density Lipoprotein-C) (mg\dl)	199.15 (51.20)
VLDL-Cholesterol (Very Low Density Lipoprotein-C) (mg\dl)	28.95 (13.06)
Triglyceride (mg\dl)	162.0 (66.79)
Total Cholesterol (mg\dl)	283 (65.75)
Fasting Plasma Glucose (mg\dl)	80.85 (23.65)
Body Mass Index (kg\m^2)	25.987 (4.96)

Macrophage derived NOS activity and Coronary risk factors:
Mean iNOS activity among targeted population was 9.12±4.10. Macrophage –Derived NOS activity among geriatric women having hypercholesterolemia exhibited a inverse correlation with the different number of CVD risk factors. Univariate analysis showed strong inverse relationship with HDL-C level (r= -0.519 p>0.05), where as other factors are also showing weak inverse relationship with Total Cholesterol level (r= -0.392 p>0.05),Triglyceride level (r= -0.304 p>0.05), LDL-Cholesterol level (r= -0.451 p>0.05),VLDL- Cholesterol level (r= -0.291 p>0.05), BMI(r= -0.466 p>0.05),Waist Circumference (r= -0.468 p>0.05)and Blood pressure (r= -0.2757 p>0.05) .

In the present study the most important finding is the correlation between the CVD risk factors and iNOS activity of macrophages, which explain that increase in CVD risks also reduces the NOS activity on macrophages as well. Evidence suggests that cellular oxidation may occur in the aging process, participating in the genesis of many non-transmittable chronic diseases that affect elderly individuals [16]. Oxidative stress is considered as an independent risk factor for some diseases, such as osteoporosis. This increase in cellular oxidation leads to a greater incidence of cardiovascular diseases, cancer and neurodegenerative diseases[1]. The accumulation of oxidative damage with age may occur via an increase in the generation of oxidized substances, reduction in antioxidant capacity, reduction of repairs to oxidative damage, decrease in the generation of oxidized macromolecules or a combination of these mechanisms thus ageing is one important risk factors in atherosclerosis or cardiovascular diseases[17,18]. Here in this study though no conclusive relation were found with iNOS activity and ageing (r= +0.268 p>0.05), studying with more sample size may explore this results critically.

Figure 1: Biochemical profile of selected geriatric women (n=10):



There are a number of studies showing that insufficient NO production from the endothelium is associated with all major cardiovascular risk factors, such as hyperlipidemia, diabetes, hypertension, smoking and severity of atherosclerosis, and importantly also has a profound predictive value for the future atherosclerotic disease progression [1,2,3].Macrophages and their role in inflammatory vascular disease is only now coming in light. **O**ne of the key features of atherosclerotic lesions is the accumulation and oxidation of lipids in the vascular wall [19], Macrophages play crucial role in controlling lipid oxidation and plaque formation, which are the two main incidence in atherosclerosis [19].As macrophages are likely to be early mediators of the inflammatory cascade in atherosclerosis and other related diseases, the key initiating factors appears to be activation of the endothelium and subsequent endothelial dysfunction. [19] Different previous work have already been identified lipid metabolism and accumulation of lipid are crucial for atherosclerosis; thus presence of abnormal lipid profile have adverse effect on macrophage in regulating anti-inflammatory activity and good cardiovascular health [19,18].

On the other hand different research explore that macrophage induced NOS activity (as we consider iNOS) has two main role which are anti-viral [20] and anti-bacterial [21] but other than that iNOS activity among inflammatory cell like macrophages has beneficial actions include cytoprotection, decreased leukocyte adhesion, antiplatelet activity, reduced vascular permeability, anti-oxidant activity, and improved cardiac diastolic function[22]. Present study also indicates similar observation where correlation value indicates that low iNOS activity of macrophages has higher risk of having various cardiovascular risks factors and vise versa (BMI(r=-0.466 p>0.05), Total Cholesterol(r=-0.392 p>0.05), Triglyceride(r=-0.304 p>0.05), LDL-C (r=-0.451 p>0.05),HDL-C (r=-0.519 p>0.05)etc) though these all have shown insignificant correlation coefficient but still it has given one direction about relationship between iNOS activity and cardiovascular risks factors among hypercholesterolemic geriatric women.

CONCLUSION:

Thus in conclusion, the present study demonstrates that coronary risk factors are may be associated with an impairment of macrophage-induced NOS release in human macrophages. Further evaluation might also indicate their poor immunological responses which may also be correlated critically. Evaluation with more sample size may also provide one definite direction towards CVD risks and iNOS activity.

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