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Original Research Article

Age and sex related serum changes in nitric oxide and its correlation with plasma lipid profile and lipid peroxidation in healthy ageing individuals

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ABSTRACT

Background: The free radical theory of aging postulates that aging results from the accumulation of deleterious effects caused by free radicals, and the ability of an organism to cope with cellular damage induced by ROS plays an important role in determining organismal lifespan. GSH and SOD functions by donating the proton and in scavenging the superoxide radicals, thereby protecting the body against oxidative stress by scavenging the free radicals produced in the body. Glutathione peroxidase also have similar function, it reduce lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water.
Methods: Hundred healthy adults from staff and student community under the age group of 20 to 60 years were recruited. Approximately 5ml blood sample was collected and used for the analysis of lipid profile, MDA and antioxidant status using standard protocol.
Results: The lipid profile, MDA and antioxidant status were measured and compared with that of serum nitric oxide levels of 100 healthy individuals of the age 20-60 yrs. Men aged 20-29 years showed significantly higher NO levels compared to corresponding women. There is a significant reduction in total antioxidant capacity in elderly people. With the decrease in NO there is increase in MDA is observed.
Conclusions: There is a significant reduction in total antioxidant capacity in elderly people. This reduction in antioxidant capacity implies a defect in antioxidant system, may be due to a reduction in individual antioxidant or may be caused by a non-equilibrium or poor cooperation between them.

Keywords: Nitric oxide, Lipid peroxidation, Plasma lipid profile, Total antioxidant capacity

INTRODUCTION

The biological process of ageing affects the cells of the organism as well as the functions performed by the cells thereof. Aging of whole organisms is therefore a complex process that can be defined as a progressive deterioration of physiological function, an intrinsic age-related process of loss of viability and increase in vulnerability.[1,2] In almost every country, the proportion of people aged over 60 years is growing faster than any other age group, as a result of both longer life expectancy and declining fertility rates.

Nitric oxide is a signaling molecule, which is gaseous in form. It diffuses freely across membranes and thus is an ideal paracrine and autocrine signaling molecule.[3] Nitric oxide can both promote and inhibit lipid peroxidation. By itself, nitric oxide acts as a potent inhibitor of the lipid

peroxidation chain reaction by scavenging propagatory lipid peroxyl radicals. In addition, nitric oxide can also inhibit many potential initiators of lipid peroxidation, such as peroxidase enzymes. However, in the presence of superoxide, nitric oxide forms peroxynitrite, a powerful oxidant capable of initiating lipid peroxidation and oxidizing lipid soluble antioxidants.[4-7]

Plasma nitric oxide availability is impaired with advancing age in healthy adults.[8] As age progresses the Serum nitric oxide levels strongly correlate with serological markers such as those related with cardiovascular functions and lipid profile.[9]

The excess intake of fat and carbohydrate by the body, leads to their conversion into triacylglycerols in the liver. These triacylglycerols are packaged into VLDLs and released into the circulation for delivery to the various tissues (extra-hepatic) for storage or production of energy through oxidation. HDLs represent a heterogeneous population of lipoproteins which exert anti-inflammatory, antioxidant, and vasodilatory effects that together represent addition atheroprotective functions of HDL, along with reverse cholesterol transport and excretion in bile. Unfavorable changes in plasma lipids are strongly associated with the age-related increase in visceral abdominal adipose tissue.[10] In case of females upto the age of 45-50 (until they reach menopause) estrogen has an atheroprotective effect. But once they have attained menopause the protective effect of estrogen wears off. Thus, the serum lipid levels rise.[11]

The fundamental manifestation of the aging process is a progressive decline in the functional maintenance of tissue homeostasis and an increasing propensity to degenerative diseases and death.[12] There is an emerging consensus that aging is a multifactorial process, which is genetically determined and influenced epigenetically by environment.[13] Because of their potential to cause oxidative deterioration of DNA, protein, and lipid, Reactive Oxygen Species (ROS) have been implicated as one of the causative factors of aging.[14] Glutathione (GSH) and Superoxide Dismutase (SOD) functions by donating the proton and in scavenging the superoxide radicals, thereby protecting the body against oxidative stress by scavenging the free radicals produced in the body.

Glutathione peroxidase reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. Hence, the present study was undertaken to investigate age and sex related serum changes in nitric oxide and its correlation with the lipid profile, lipid peroxidation and antioxidant status in healthy ageing individuals.

METHODS

This study was conducted after the institutional ethical clearance and written, informed consent from all the participants. In the present study hundred healthy male and female subjects between 20 to 60 years were selected. About 5ml of blood was collected without anticoagulant in appropriate sterile vials and the serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at 4^{o}C.

Erythrocyte separation

500 µL of drawn heparinized blood was centrifuged at 3000 rpm for ten minutes and the upper plasma layer was separated out, and about 500 µL of normal saline was added to the erythrocyte layer, mixed well and centrifuged, again discarding the upper layer and adding fresh normal saline to the erythrocytes, this step was repeated two more times in order to wash the erythrocytes. Finally, 200 µL of the washed erythrocytes was taken and lysed with 600 µL of cold distilled water to lyse the erythrocytes and release the enzyme contained. Thus prepared 1:4 diluted erythrocytes were stored at 0-4 ^{o}C until use for biochemical analysis.

The estimation of Nitric Oxide was done by Griess method. Total cholesterol was estimated using CHOD-POD method, estimation of triglyceride was done by GPO-POD method. Standard commercial kits were used to estimate HDL and estimation of LDL is done using the formula LDL = TG-(HDL+VLDL). The VLDL was calculated using formula VLDL = TG/5.

Statistical analysis

The data obtained was expressed as Mean±SD. The data was statistically analyzed by one way ANOVA. The p value less than 0.05 was considered the level of significance.

RESULTS

The nitric oxide level was found to be significantly higher in men (p=0.0017) of the age between 20-40 years as compared to females of the same age. Similarly, in the age group of 40-60 years (Figure 1). The total cholesterol level was found to be significantly higher in women (p=0.0001) in both the age group as compared to that in men (Figure 2).

The triglyceride level was found to be significantly higher in men (p=0.012) of the age between 20-40 years as compared to females of the same age. Whereas, in the age group of 40-60 years, it was significantly higher in women (p=0.012, Figure 3).

There was no significant difference in HDL Cholesterol level between male and females belongs to both the age group (p=0.469, Figure 4). The LDL Cholesterol level was found to be significantly higher in men of both the age when compared to females of the same age (p=<0.0001, Figure 5).

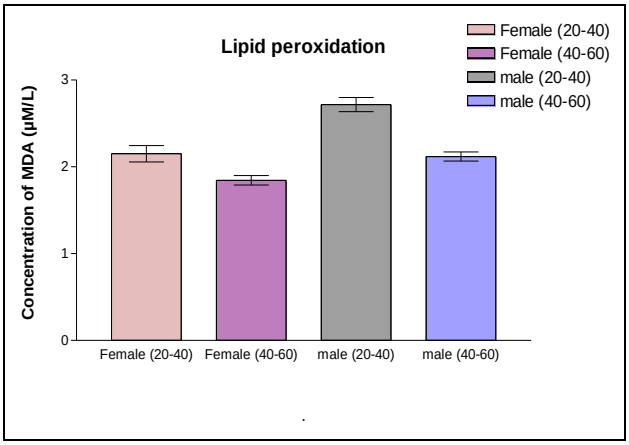

Figure 1: Comparison of lipid peroxidation between men and women belongs to different age group.

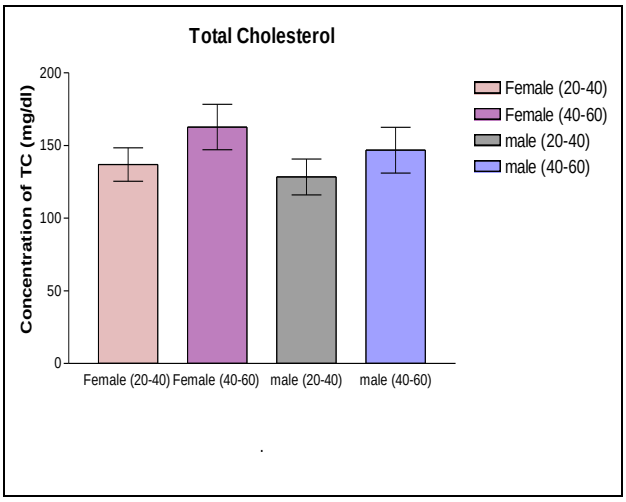

Figure 2: Comparison of total cholesterol between men and women belongs to different age group.

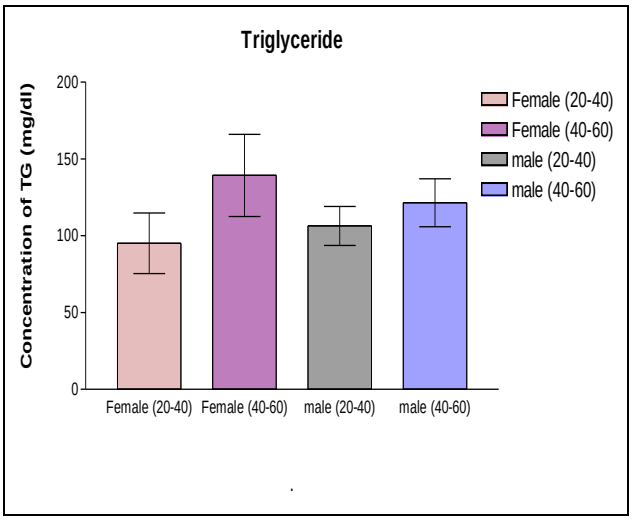

Figure 3: Comparison of triglyceride level between men and women belongs to different age group.

There was no significant difference in VLDL cholesterol level between male and females belongs to both the age group (p=0.282, figure 6). There was no significant difference in the sod level between male and females belongs to both the age group (p=0.465, figure 7).

The GSH level was found to be significantly higher in women (p=0.005) of the age between 20-40 years as compared to men of the same age. Whereas, in the age group of 40-60 years, it was significantly higher in men (p=0.0059, figure 8). The nitric oxide level was found to be significantly higher in men (p=0.001) of both the age group as compared to females of the same age (Figure 9).

The total antioxidant capacity was found to be significantly higher in men (p=0.0001) of the age between 20-40 years as compared to females of the same age. Whereas, in the age group of 40-60 years, it was significantly higher in women (p=<0.0001, figure 10).

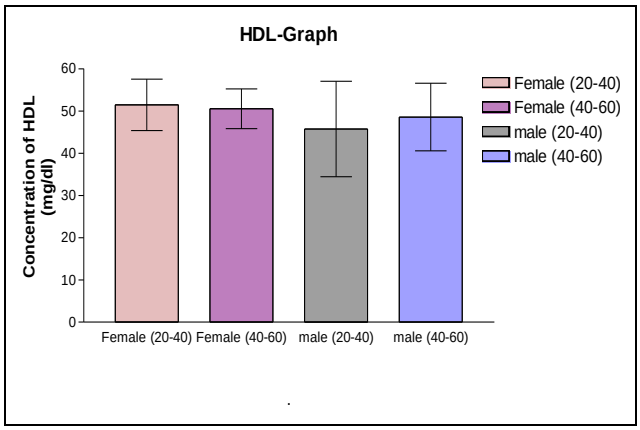

Figure 4: Comparison of HDL cholesterol level between men and women belongs to different age group.

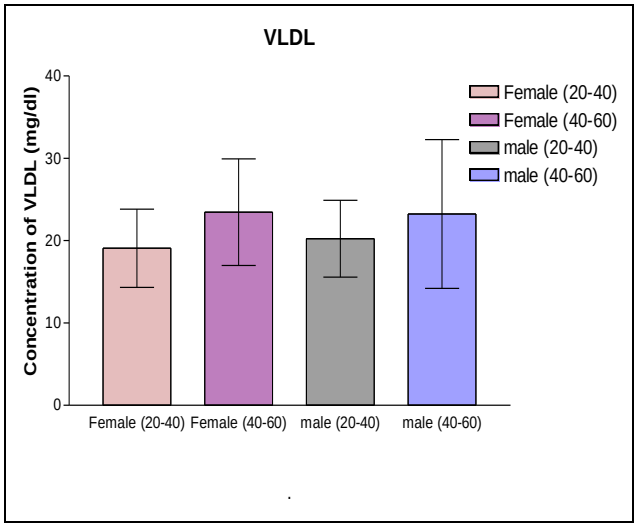

Figure 5: Comparison of LDL Cholesterol level between men and women belongs to different age group.

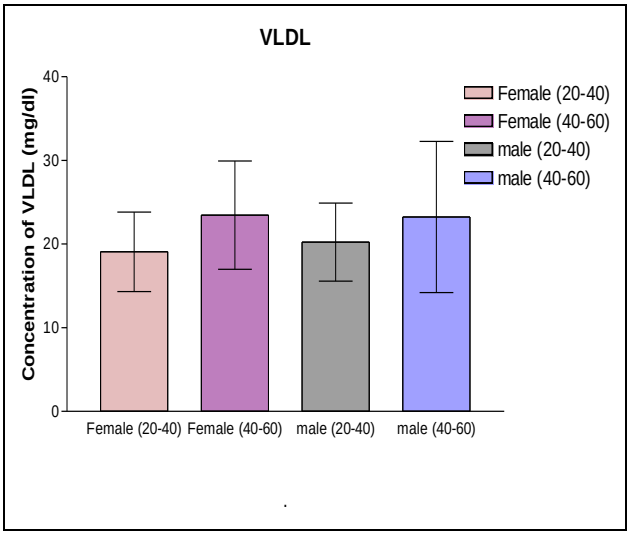

Figure 6: Comparison of VLDL Cholesterol level between men and women belongs to different age group.

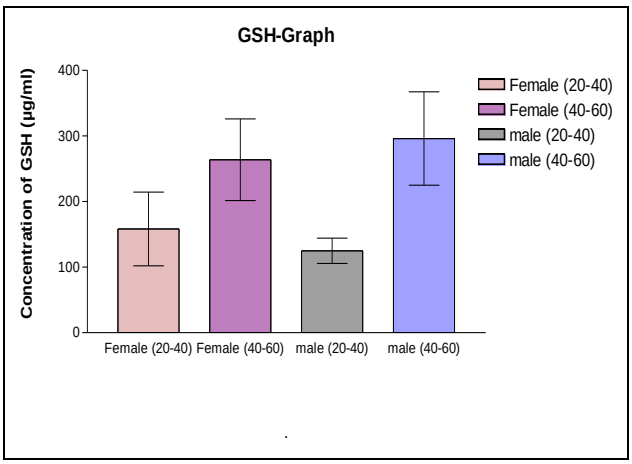

Figure 7: Comparison of SOD level between men and women belongs to different age group.

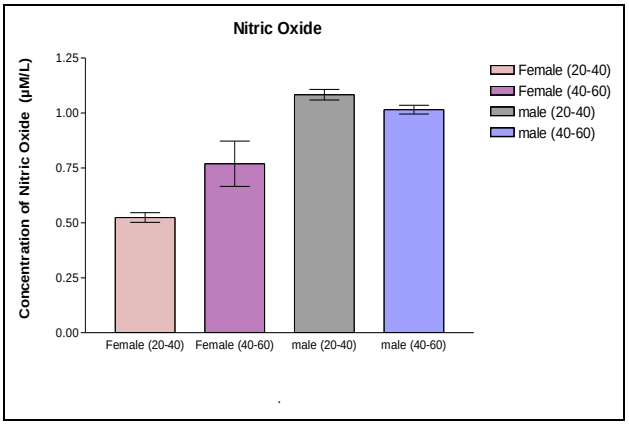

Figure 8: Comparison of GSH level between men and women belongs to different age group.

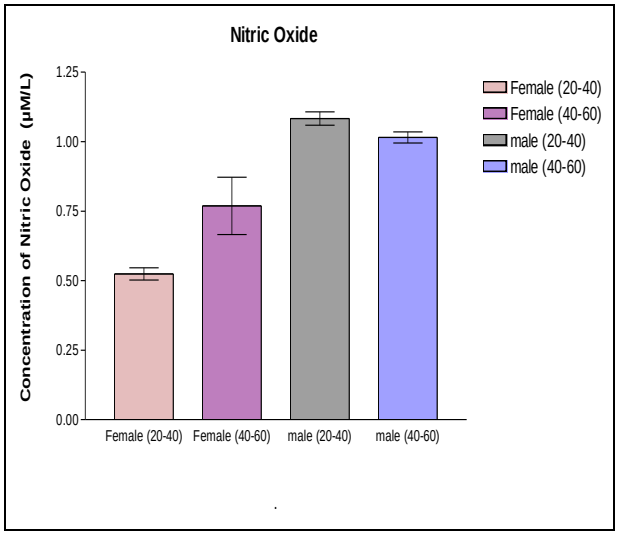

Figure 9: Comparison of nitric oxide level between men and women belongs to different age group.

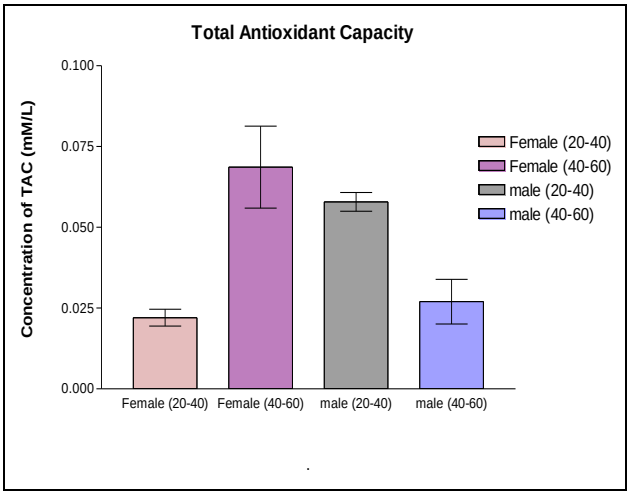

Figure 10: Comparison of total antioxidant capacity level between men and women belongs to different age group.

DISCUSSION

In the present study, we found that the plasma nitric oxide availability is impaired with advancing age in healthy individuals. Men aged 20–40 years have significantly higher NOx levels compared to older men in the age group 40-60. The men in the age group of 20-40 will also have a significantly high level of NO in their serum when compared to the corresponding women of the same age. It was similar to the studies by Ghasemi A et al, and others reported that, men aged 20-29 years had significantly higher NOx levels compared to corresponding women.[15-17]

The LDL level in the females of age group of 20-40 years had the lower levels of LDL when compared to older women of 40-60 years, this may be due to the atheroprotective effect of estrogen. We also found that

the levels of LDL were significantly higher in older males thereby predisposing them to various cardiovascular diseases. This is similar to the results of Nabatchian F, et al reported that, oxidative stress enhances the likelihood of LDL oxidation and atherosclerotic plaque development.[18]

The VLDL levels were almost equal in the older age group of 40-60 years, but there is a contrast when observed in younger age group - the levels of VLDL are marginally higher in case of males than females probably because of the atheroprotective effect of oestrogen, thus our finding with regard to VLDL is consistent with the findings of Buege JA and others who reported that, estrogen is associated with reduced subclinical atherosclerosis progression in healthy postmenopausal women. This association is partially mediated by its beneficial effect on lipids. The total cholesterol levels in both the older age group as well as the younger age group; females have the higher levels of cholesterol.[19,20]

There is a significant reduction in total antioxidant capacity in elderly people. This reduction in antioxidant capacity implies a defect in antioxidant system, may be due to a reduction in individual antioxidant or may be caused by a non-equilibrium or poor cooperation between them. By itself, nitric oxide acts as a potent inhibitor of the lipid peroxidation chain reaction by scavenging propagatory lipid peroxyl radicals. It also inhibits many potential initiators of lipid peroxidation, such as peroxidase enzymes. With the decrease in NO there is increase in MDA

CONCLUSION

In the present study lipid profile, MDA and antioxidant status were measured and compared with that of serum nitric oxide level. From the results, it was concluded that, men aged 20–40 years will have significantly higher NOx levels compared to corresponding women. There will be significant reduction in total antioxidant capacity in elderly people. With the decrease in NO there is increase in MDA.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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