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Original Article

Physiology Section

Change in Oxidative Stress of Normotensive Elderly Subjects Following Lifestyle Modifications

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ABSTRACT

Introduction: Oxidative stress is associated with aging, which ultimately causes deterioration of muscles. Antioxidant defense system deteriorates while enhancing accumulations of Reactive Oxygen Species (ROS) due to lipid peroxidation and altered enzyme activities in old age. Regular practice of yoga can maintain the antioxidants level of the body, even in stressful conditions.

Aim: The present study was designed to assess the effects of lifestyle technique on oxidative stress and lipid profile in normotensive elderly subjects.

Materials and Methods: Seventy four healthy elderly subjects (43 males and 31 females) 60 to 80 years of age were selected from the Santosh Medical College, Ghaziabad Uttar Pradesh, India, for three months lifestyle modification program which included morning walk, Nadi shodan pranayama, dietary restrictions and increased intake of water. Blood pressure and oxidative stress markers Glutathione (GSH), Super Oxide Dismutase (SOD) and Malondialdehyde (MDA) were recorded twice, one at baseline and another after three months of lifestyle modifications.

Results: Post lifestyle modifications technique values revealed a significant increase in GSH (88.03±9.58 ng/ml vs 93.12±9.17 ng/ml, $p < 0.0001$) and SOD (78.22±11.97 ng/ml vs 85.22±11.08 ng/ml, $p < 0.0001$), and a decline in MDA (5.28±0.52 m mol/ml vs 4.48± 0.69 m mol/ml, $p < 0.0001$) levels. Further, there was significant reduction in the systolic blood pressure ($p < 0.0001$) and diastolic blood pressure ($p < 0.0002$); besides all fasting lipids decreased significantly except High Density Lipids (HDL).

Conclusion: The findings of the present study show that lifestyle modification is helpful in reducing cardiovascular disease risk but also assuring for good health by decreasing oxidative stress level along with lipid profile. Further, all these modifications are easy to follow. However, more studies are required to make a generalized lifestyle modification program in normotensive elderly subjects.

Keywords: Aging, Antioxidants, Blood pressure, Yoga

INTRODUCTION

Oxidative stress is emerging as a causative factor for various diseases, including Cardiovascular Diseases (CVD). Moreover, the antioxidant defence decline, while oxidative stress enhanced by aging especially in geriatrics [1,2].

An oxygen molecule containing an unpaired electron in its outer orbit-produced by different processes of oxidation-is known as Reactive Oxygen Species (ROS). Free radicals are usually unstable and highly reactive due to unpaired electron. ROS contribute to different chemical reactions which eventually escort to divers pathological conditions [3,4]. Moreover, oxidative stress, defined as a disturbance in the balance between the production of ROS and antioxidant defenses [5]. Oxidative stress strongly influence lipids, protein and DNA in the central nervous system; besides ROS generated pathological damage increases with age especially in the last quarter of life span [6]. Furthermore, antioxidant defense system deteriorates while enhancing accumulations of ROS due to lipid peroxidation and altered enzyme activities in old age [7,8]. Essential hypertension is associated with impaired antioxidants status and the formation of free radicals [1]. Oxidative stress is associated with aging, which ultimately causes deterioration of muscles by two fundamental biological processes: increased production of ROS and decline in antioxidant enzymes. However, mild nonexhaustive exercise causes mild oxidative stress that stimulates the expression of certain antioxidant enzymes [9]. Oxidative stress plays an important role in adverse effects of obesity in CVD [10].

Severity of atherosclerosis is directly related to dyslipidaemia, hypertension and lipid peroxidation [11]. Oxidative stress might be considered as a novel therapeutic target for treating essential hypertension [12]. Blood pressure is affected by multiple dietary factors; therefore, modifications of diet can induce positive changes in blood pressure of both hypertensive patients and normotensive subjects [13]. Regular practise of yoga can maintain antioxidant level of the body even, in stressful conditions [14]. Exercise induces Super Oxide Dismutase (SOD) which serves to minimize superoxide anions (O_2^-) with nitrous oxide (NO) consequently benefits vascular functions [15]. Commencement of moderately sports activities, quitting smoking, maintaining blood pressure and body weight are separately associated with lower rates of death from all causes in middle and old age [16]. Physically unfit men are more likely to suffer from CVD than physically fit ones [17].

Therefore, the present study was designed to assess the effects of lifestyle modifications on oxidative stress, blood pressure and lipid profile in normotensive elderly subjects.

MATERIALS AND METHODS

Study Population

This interventional study included 74 healthy elderly subjects (43 males and 31 females) between 60 to 80 years of age. All elderly normotensive subjects were selected from Santosh Medical College and Hospital, Ghaziabad Uttar Pradesh, India. The study was conducted in the Santosh Medical College and Hospital, Ghaziabad, India from July 2014 to November 2015. Written consent of the participants in the local language was taken after clearly narrating the purpose and nature of research. Inclusion criteria for the study was normal blood pressure <140/90 mm Hg [18], body mass index 18.5-25 kg/m^2, non-smokers and non-alcoholic. Participant suffering with any kind of physical disability, chronic disease or taking any type of medicines was excluded from the study.

Lifestyle Modifications

Lifestyle interventions were implicated for three months time period [19] All participants were assigned to do the Nadi Shodhan Pranayama [20]. (forced one side nostril breathing) on an empty stomach for 20 minutes, early morning 6 days in a week under the supervision of yoga instructor. Along with it morning walk [16] of 2 miles daily for 6 days in a week and minimum sleep [21] of 5 to 6 hours was advised. In addition to it reduced intake of dietary salt [22] up to 100 m eq/day, lowering fat [22] intake up to 44 to 77 g and increased intake of water [23] 2 to 3 liters per day were suggested to all participants. An orientation program was organized before starting the research program for all the participants for better understanding of the lifestyle interventions. All the participants were instructed to come for 1 hour, early in the morning from 6am to 7am for 6 days in a week. The protocol of lifestyle modifications included 20 minutes yoga followed by 2 miles walk under the supervision of an authorized instructor. Investigators made interaction with every participant weekly; in addition, orientation programs were organized every month for the participants to keep their interest in research.

Measurements

All of the measurements and biochemical parameters were recorded early in the morning between 6 to 8 am with an empty stomach, twice, one at baseline and another after three months lifestyle modifications.

Blood Pressure

The participants were requested to sit in supine position comfortably and quietly for 10 minutes. After that blood pressure was recorded three times at the interval of 10 minutes by auscaltatory method with the help of the Sphygmomanometer (manufactured by Diamond Regular, India) [24].

Evaluation of Lipid Profile

Serum concentration of Total Cholesterol (TC), Triglycerides (TG) and HDL were estimated by CHOD-POD method, GPO-PAP method and CHOD-POD/ Phosphotungstate method respectively by using commercial kits manufactured by Erba Mannheim; Low Density Lipids (LDL) was calculated by using Friedewald's formula [25].

Estimation of Oxidative Stress

Oxidative stress markers Glutathione (GSH) and SOD were investigated by kits (Qayee-Biotechnology Co. Ltd) using Enzyme Linked Immunosorbent Assay (ELISA) [26,27]. Malondialdehyde (MDA) was estimated (TBARS method) by TBARS kit (Cayman chemical company) [28].

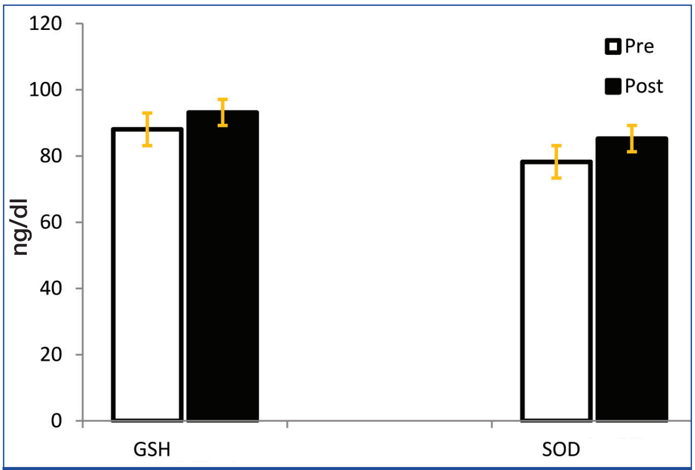
STATISTICAL ANALYSIS

The obtained data was expressed as Mean±SD (Standard deviation). Paired students t-test was used to analysis, statistical significance of data obtained before and after lifestyle modification. An association of oxidative stress markers (GSH, SOD and MDA) and Blood Pressure (SBP, DBP) was analysed using Pearson correlation coefficient test. A p-value < 0.05 was considered statistically significant. IBM SPSS Statistics 21.0 manufactured by IBM USA was used for entire calculations.

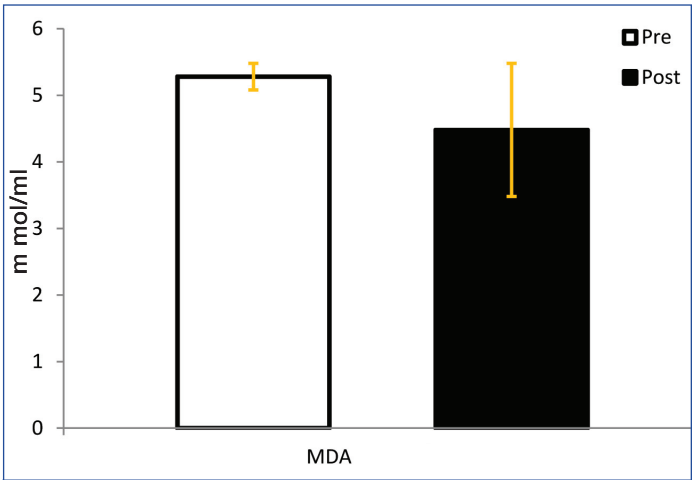
RESULTS

Total 74 elderly subjects aged 65.14±4.52 years, both males and females, completed the lifestyle modification program for three months. However, there was no significant change between the findings of males and females.

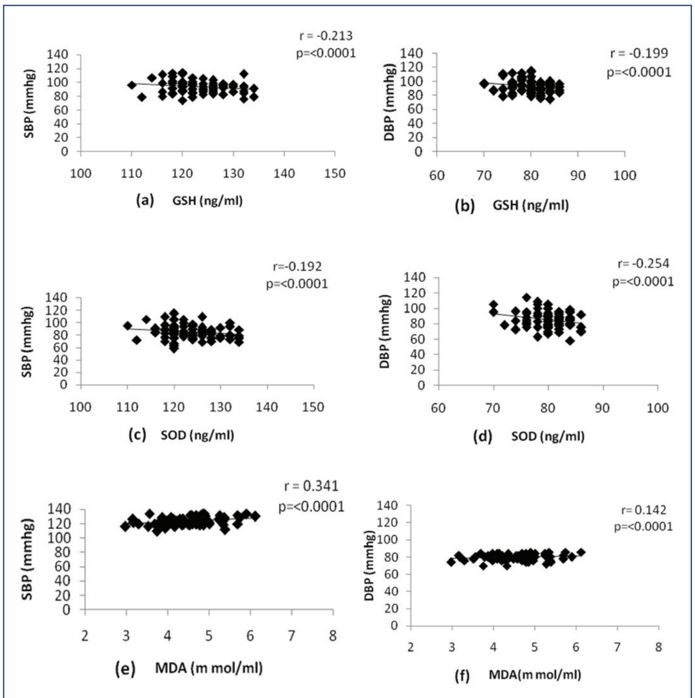
[Table/Fig-1] shows significant reduction in oxidative stress markers as baseline values of GSH increased from 88.03±9.58



[Table/Fig-1]: Changes in antioxidant status after lifestyle modifications. Pre- Before lifestyle modifications, Post – After lifestyle modifications, n= 74, GSH-Glutathione (p<0.0001), SOD- Superoxide dismutase (p<0.0001).



[Table/Fig-2]: Changes in MDA after lifestyle modifications. Pre- Before lifestyle modifications, Post – After lifestyle modifications, n= 74, MDA- Malondialdehyde (p<0.0001).



[Table/Fig-3]: Pearson correlation between SBP or DBP and GSH, SOD, and MDA in normotensive elderly subjects after following lifestyle modification. SBP-Systolic blood pressure, DBP-Diastolic blood pressure.

Parameters	Before Lifestyle modifications, n=74	After Lifestyle modifications, n=74	p-value
Weight (kg)	60.73 ± 7.98	57.53 ± 7.38	<0.0001*
BMI (kg/m²)	22.4 ± 2.01	21.25 ± 1.76	<0.0001*
Systolic blood pressure (mmHg)	127.92 ± 6.05	123.59 ± 5.48	<0.0001*
Diastolic blood pressure (mmHg)	81.65 ± 4.73	79.92 ± 3.68	<0.0002*
MAP (mmHg)	97.01 ± 4.48	94.51 ± 3.6	<0.0001*
HR (rpm)	78.3 ± 5.73	77.6 ± 6.14	<0.1259
TC (mg/dl)	204.31 ± 28.94	191.98 ± 23.23	<0.0001*
TG (mg/dl)	119.36 ± 23.89	111 ± 20.2	<0.0001*
HDL (mg/dl)	44.13 ± 6.53	47 ± 7.06	<0.0001*
LDL (mg/dl)	135.3 ± 24.38	122.72 ± 22.45	<0.0001*

[Table/Fig-4]: Comparison of pre and post lifestyle modifications values.
Data presented as Mean ± SD, MAP= mean arterial pressure, HR= heart rate, TC= total cholesterol, TG= triglycerides, HDL= high density lipids, LDL= low density lipids, SD= standard deviation of Mean. * = highly significant.

ng/ml to 93.12±9.17 ng/ml ($p < 0.0001$) and SOD enhanced from 78.22±11.97 ng/ml to 85.22±11.08 ng/ml ($p<0.0001$). On the other hand [Table/Fig-2] demonstrates the baseline values of MDA decreased significantly from 5.28±0.52 m mol/ml to 4.48± 0.69 m mol/ml ($p < 0.0001$). The results further reveal that GSH and SOD have negative while MDA has a positive correlation with both Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) [Table/Fig-3].

[Table/Fig-4] shows significant decrease of weight ($p <0.0001$) and body mass index ($p <0.0001$) after following lifestyle interventions. A significant decline of 4.31±0.57mmHg and 1.73± 1.05 mm Hg has been observed SBP and DBP, respectively. Besides, we observed significant reduction in mean arterial pressure (MAP); however, change in heart rate was not significant ($p <0.1259$). In addition, we observed a significant decrease of TC ($p <0.0001$), TG ($p <0.0001$) and LDL ($p <0.0001$), whereas a significant increase of HDL ($p <0.0001$) [Table/Fig-4].

DISCUSSION

It is well documented that oxidative stress is a harmful condition in which there is a loss of balance between oxidants and antioxidants as oxidants increased beyond the antioxidant status [4]. Moreover, uncontrolled ROS are the root cause of the diverse pathological conditions; various chronic diseases have been affected by ROS to different degrees [29,30]. The results of the current study showed that three months lifestyle modifications have significantly increased the antioxidant level of healthy elderly subjects [Table/Fig-1]. Previous studies of Gordon et al., Cheong et al., and Sinha et al., have shown similar enhancement in GSH and SOD [14,31,32]. SOD reacts with superoxide radical ($O_2^{\cdot-}$) and converts it into hydrogen peroxide (H_2O_2) and oxygen (O_2). However, GSH is an enzyme which detoxifies hydrogen peroxide into water (H_2O) and alcohol (ROH); this process is facilitated by reduced GSH which gets converted into oxidized GSH [33]. This enhancement of antioxidants seems to be due to the reason that exercise causes mild increase of ROS, which in turn enhances the expression of various antioxidants by activating redox-sensitive singling pathways [9]. Further, increased SOD causes decreasing $O_2^{\cdot-}$ activity which in turn enhances the endothelial derived NO and results enhanced expression of extracellular SOD [15]. Exercise induces various structural changes and remodelling of vascular wall results in improved functioning of endothelial cells [34]. Exercise stimulates endothelium to enhance the production of NO along with increased level of SOD that rectify the $O_2^{\cdot-}$ which further increases the bioavailability of NO [35]. Decreased level of antioxidant status has been found to be associated with hypertension since different free radicals while antioxidants play an important role in the progress of CVD [1]. Hence, improvements in SOD and GSH level in the present study after lifestyle modification program may be helpful in reducing the risk of CVD. Moreover, increased level of antioxidant through lifestyle modification might reduce the effects of aging as enhanced antioxidant level leads to decreasing the rate of deterioration of muscles, lipids peroxidation, CVD, target organ disease, DNA damage and aging process [6]. Age related oxidative damage cannot be controlled by antioxidant defense mechanisms; however, changes in lifestyle can reduce the oxidative stress [36]. Apart from this antioxidants level enhancement by lifestyle modifications is more beneficial as the reaction constant of superoxide with vitamin E and vitamin C is far less than the reaction constant of superoxide and SOD [37]. On the other hand, ROS target the lipids and induce lipid peroxidation process; which is a chain reaction and results in cell death.

Moreover, MDA is the end product of polyunsaturated fatty acid peroxidation and leads to further enhancement of lipid peroxidation. Nonetheless, MDA promotes various deteriorating cellular reactions which results in destruction of proteins and DNA. Therefore, increased level of MDA persuades aging process leading to damage to the structure of bio-molecules and various pathological disorders [38,39]. Age related changes cause elevation of ROS which further enhances the level of lipid peroxidation and reduces the level of antioxidants especially in elderly hypertensive. Moreover, there has been found a high level of MDA whereas decrease level of antioxidants Catalase and GSH in elderly people [8]. The reduction of MDA level, as observed in our study, is consistent with findings of previous studies of Patil et al. and Singh et al., [40,41]. This decrease of MDA may be due to improvement of antioxidant defense system as increased level of antioxidants decrease the ROS which reflects a reduction of lipid peroxidation [31]. Further, the decline of MDA may be helpful in decreasing risk for CVD as MDA, represents an independent indicator of risk for patients with stable coronary artery disease [42]. Furthermore, the present findings demonstrate a Negative correlation between SOD and GSH with both SBP and DBP. On the other hand, MDA has shown positive correlation with SBP as well as DBP, which is similar to previous studies where researchers showed the same correlation oxidative stress and blood pressure [12,31,34,40,41]. These changes seem to be due to regular physical exercise which decreases the age induced endothelial dysfunction – dependent vasorelaxation by an increment in nitric oxide release due to decreased oxidative stress [35,43]. In addition, correlation of blood pressure with oxidative stress as observed in our study may be due to increased level of ROS leads to destruction of endothelium, impaired endothelial functions, decrease vascular NO; though, various interventions which increase the NO production and reduce the genesis of superoxide have substantially decreased risk of CVD and vascular functioning in CVD patients in clinical studies [44].

Moreover, the results of the present study showed that lifestyle modifications produced a significant decline in SBP as well as DBP [Table/Fig-3]. The obtained results in the present study are very similar to earlier studies where LP Svetkey et al., Jiro et al., and Murthy et al., have shown a significant reduction in blood pressure after the implications of lifestyle interventions [45-47]. To the best of our knowledge, this is the first study of its kind, including yoga as well as walking modulation together with dietary restrictions. Yoga controls blood pressure by regulating hypothalamic pituitary adrenal axis and sympathetic nervous system; along with it yoga reduces stress, which stimulates parasympathetic activation and alteration in baroreceptors sensitivity that ultimately decreases BP in normotensive and hypertensive [48,49]. Physical exercise helps in the remodelling of arteries, improves bioavailability of NO as well as dilatation of smooth muscles. This may be due to improvement in antioxidants level of the body as ROS interrupt the synthesis and functioning of NO. Moreover, refined vascular function along with up regulated phosphorylation might be the cause of improved blood pressure [50]. Further, best control over blood pressure can be attenuated when multiple lifestyle modifications are integrated simultaneously [51].

These findings suggest that reduction in oxidative stress has a positive effect on blood pressure; and lifestyle modifications would be beneficial for elderly normotensive people. Lifestyle modification technique-even of short span has been found effective in reducing in TC, Triglycerides, LDL and increasing HDL [52]. Similarly, our study has shown a considerable decrease in lipid profile except increase in HDL levels. Previous studies have shown similar results as Paeolatti V et al., Yukihito H et al., recorded remarkable decreases in TC, TG and LDL while enhancement in HDL after life style modifications [35,53]. Similarly, Agarwal et al., Agte et al., and Lorengo AG et al., showed decrease in lipid profile after yoga practice [30,36,54]. Further, this decline of lipid profile may be due to a low caloric diet which induces weight loss reduces oxidative stress through improving glucose metabolism which may lead to decreased adiposity and cytokines secretion [55]. Moreover, the decrease of lipids profile might reduce the risk for CVD in elderly subjects as increased levels of lipids are independent risk factors of coronary heart disease; however, reducing lipids with exercise can decrease risk for coronary heart disease [37].

LIMITATION

In the present study, the small sample size was the most important limitation in assessment of lifestyle modifications in male and females separately. It warrants for more number of studies on a large number of populations to examine the effect on male and female individually to provide guidelines for lifestyle modifications.

CONCLUSION

The findings of the present study show that lifestyle modification is helpful in reducing cardiovascular disease risk but also assuring for good health by decreasing oxidative stress level along with lipid profile. Further, all these modifications are easy to follow. However, more studies are required to make a generalized lifestyle modification program for normotensive elderly subjects.

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