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Effect of Lifestyle Factors on Serum Antioxidant Levels in Apparently Healthy Individuals

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Lifestyle factors are among the multiple factors that initiate pathology of non-communicable diseases (NCD) which starts in young age and remains undetected till the clinical symptoms occur. Serum antioxidant levels in individuals with varied lifestyle habits were evaluated to determine the relationship between lifestyle factors, reactive oxygen species and development of NCD. The purpose of this study is to evaluate the role of serum antioxidants in the metabolic changes initiated by lifestyle choices of apparently healthy individuals. 104 healthy volunteers of age 20-30 years from South Indian Population were randomly selected after obtaining informed consent for the cross section observational study. The inclusion criteria were Fasting glucose <100mg/dl, Triglycerides <150mg/dl, Total cholesterol< 200mg/dl and Hemoglobin >12 g/dl (females) and >14g/dl (males). Lifestyle factors like age, gender, diet, socioeconomic condition, physical activity, psychological stress, smoking, alcohol were determined based on questionnaires. Fasting serum antioxidants- Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Coenzyme Q10 – single dilution method HPLC, serum uric acid and body mass index (BMI) were estimated. Pearson correlation and Spearman rho correlation was done using SPSS software version 17 with "p" value <0.05 were considered as significant. Mean of Age (25.30±3.214), BMI (22.5±4.65), Superoxide dismutase (U/mL) (181.78±17.9), Glutathione peroxidase (U/L) (8607.36±1237.2), Coenzyme Q10 (μg/L) (484.12±59.65) and Uric acid (mg/dL) (4.92±1.27) are as noted in the study group. A positive correlation was found between SOD, CoQ10 and increased dietary intake of fruits and vegetables, low BMI, higher Socio economic status. GPx and uric acid had a positive correlation with age, smokers and alcoholics, and negative correlation with physical activity. Gender wise differences significantly seen in GPx and Uric acid. Serum antioxidants are altered by the lifestyle choices of the study population.

Keywords: Antioxidants; Biomarker; Lifestyle Factors; Non communicable disease; Young Population.

Health is defined by WHO as "A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"[1]. Several factors like lifestyle choices, environment, genetic constitution, public health amenities play a major role in determining health status of individuals[2]. But individuals with underlying Non-communicable diseases (NCD) go unrecognized in initial stage, which occurs in young age, and are diagnosed only after symptoms occur as age and disease progresses. This is because there are no definite tests to evaluate the influence of the above mentioned various factors on an individual's health. Studies have shown that

lifestyle factors induce biochemical alterations[3] from a young age i.e. < 30 years[4,5]. Studies have also shown that reactive oxygen species by causing oxidative damage contributes to development of NCD[6].

Hence in this study the lifestyle practices, [non-modifiable-age, gender, ethnicity and modifiable-behavioral (smoking, alcohol, drug usage), material (socio-economic condition), psychosocial, diet and nutrition and physical activity][5] of the young subjects and their serum antioxidants[Enzyme System- Superoxide Dismutase, Glutathione system- Glutathione Peroxidase, Pro oxidants- Uric acid, and other antioxidant metabolites[7]-Coenzyme Q10] have been evaluated. This is to check for correlation between the two as both are among the major contributors of development of NCD. The aim is to see if the serum antioxidants are modified due to the metabolic changes initiated by the lifestyle practices that are known to contribute to development of NCD.

This current study was conducted on a group of apparently healthy individuals in a South Asian population of young adults without any proven detectable pre-existing cause for altered serum antioxidant level. Their lifestyle factors were analyzed and their serum antioxidant levels has been simultaneously estimated and correlation between them has been statistically determined with certain limitations.

MATERIALS AND METHODOLOGY

This cross-sectional observational study was conducted from July 2017 to June 2018 in a young south Indian population of Chennai, Tamil Nadu after getting prior approval from the Institutional ethics committee.

Apparently healthy volunteers i.e. with no history of any known disease and no complaints of any symptoms, under the age group of 20-30 years were randomly selected after providing complete information about this study and after getting informed consent in written format in their own preferred language in the presence of a witness. They were then tested for their fasting plasma glucose (FPG), fasting lipid profile and hemoglobin levels.

Inclusion criteria

Out of them 104 apparently healthy individuals (53 females and 51 males) with values within the range of FPG 70- 99 mg/dl (GOD POD Method), serum triglycerides <150mg/dl (Glycerol phosphate/peroxidase method), serum cholesterol< 200mg/dl (Cholesterol oxidase/peroxidase method) and hemoglobin 12-14 g/dl (Drabkin's method[8]) for females and >13.5-17g/dl for males were enrolled in the study. Subjects with values out of the specified range and with significant medical illness or history that can cause oxidative stress were excluded from the study.

The selected subjects were then evaluated for their modifiable lifestyle factors like dietary habits (dietary intake of fruits and vegetables)[9], socioeconomic condition(upper high, high, upper middle, lower middle, poor)[10], psychological stress(no stress, moderate, high chance for stress related changes)[11], physical activity and exercise (High, moderate, low-sedentary)[12],smoking(high, moderate, nonsmokers)[13] and alcohol consumption(high, moderate, non-consumers)[14] by questionnaire method. The non-modifiable factors: Age (20 – 30 years), gender (males and females), ethnicity (south Indian) and also body mass index (BMI)[15] were noted.

Questionnaires

The questionnaires which were internationally pre validated reliable and specific to the study factors were selected. The format was structured and close ended with multiple choice answers. They were easy to comprehend and were self administered in their preferred language.

Body mass index calculation

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2)[15] .They were classified in terms of: Normal 18.50- 22.99-1, Underweight <18.50 – 2, Overweight 23-24.99-3, Pre Obese 25-29.99-4, extreme obesity> 30-5 according to WHO table for Asians population.

Laboratory investigation

2ml of fasting (8-10 hours overnight) venous whole blood sample was collected in Light protected heparinized tubes aliquots prepared and processed for Superoxide dismutase-Arthur and Boyne method[16] (Reference range164-240 U/mL), Glutathione peroxidase -method based on Paglia and valentine[17] (Reference Range-4171-10881

U/L) in Spectro max e2, Coenzyme Q_{10} –single dilution method[18] in HPLC Shimadzu (Reference Range 433-1532 µg/L), Uric acid-Uricase / POD method(Male: 4.0-7.2mg/dL, Female: 2.7-6.5mg/dL) in fully automated analyzer.

Exclusion criteria

Subjects with diseases causing oxidative stress and low antioxidant levels like: neurodegenerative diseases,[19] cardiovascular diseases,[20] diabetes mellitus[21], hypertension,cancer[22], cerebrovascular diseases, dyslipidemia[23], liver diseases[24], tuberculosis, anemia and drug(nutritional supplements) intake history and family history of NCD.

Statistical analysis

Statistical analysis was done using SPSS Inc. version 17.0 IBM software. The mean and standard deviation was calculated for all numerical values. Pearson correlation was done with confidence interval (C.I) 95% and p value up to < 0.05 was taken as significant. For gender association Spearman Rho correlation study was performed with C.I 95% and p value < 0.05 was taken as significant.

RESULT AND DISCUSSION

In this study 104 individuals selected based on the criteria for apparently healthy status were studied and their lifestyle factors and antioxidant levels relationship were evaluated. The mean and standard deviation values for the age, BMI, and the four antioxidants studied are given in table 1.

Pearson correlation studies between the lifestyle factors and serum SOD, GPx, CoQ_{10} and Uric acid were done and the values are shown in Table 2. In this current study as age progresses GPx and Uric acid seems to increase and SOD seems to fall, but as this is a limited group of only 20-30 years, this needs more data in a larger age group for it to be conclusive. The findings for SOD and GPx are consistent with the studies conducted by Bolzan et al[25] and PP Singh et al[26] in terms of age and data among individuals with or without smoking. Kaikonnen et al[27] have shown positive influence of age on CoQ_{10} and uric acid relationship with age in our study is consistent with Chen et al[28].

Overall the dietary intake have positive influence on SOD and CoQ10 levels which is consistent with other studies conducted. Toaldo et al had similar results with SOD and uric acid and they also reported increase of GPx with fruit juice intake[29]. Gutierrez-Mariscal et al reported about the importance of dietary intake of fruits and vegetables and CoQ10 in preventing oxidative DNA damage[30].

Socio economic condition showed that with increasing socioeconomic betterment there was a statistically significant decrease of SOD, CoQ_{10} and uric acid but within reference range.

Table 1. Mean, median and standard deviation of numerical parameters in this study

Parameter	Values Mean± S.D
Age (in years)	25.30±3.214
BMI (kg/m^2)	22.5±4.65
Superoxide dismutase (U/mL)	181.78±17.9
Glutathione peroxidase (U/L)	8607.36±1237.2
Coenzyme Q_{10} (µg/L)	484.12±59.65
Uric acid (mg/dL)	4.92±1.27

Table 2. Pearson correlation study between serum antioxidants and the lifestyle factors

Antioxidant	Age	SE score	IPAQ score	FACET score	STRESS score	Smoking score	Alcohol score	BMI score
Superoxide	-.204*	-.198*	.191	.284**	-.331**	.216*	-.162	-.207*
dismutase	.038	.044	.052	.004	.001	.028	.101	.035
Glutathione	.266**	.059	-.857**	-.103	.155	-.594**	.765**	.039
peroxidase	.006	.549	.000	.300	.117	.000	.000	.691
Coenzyme Q_{10}	-.092	-.309**	.068	.358**	-.093	.258**	-.110	-.259**
	.354	.001	.492	.000	.348	.008	.267	.008
Uric acid	.340**	-.273**	-.344**	-.016	.043	-.381**	.436**	.733**
	.000	.005	.000	.876	.661	.000	.000	.000

* Significant at 0.05 level (2 tailed) ** Significant at 0.01 level (2 tailed)

When seen according to gender males showed the negative influence of social class only with CoQ_{10} and females showed negative influence of social class on uric acid alone. Mulholland et al study on the influence of social class on SOD and GPx showed the significant influence on GPx but not on SOD[31].

Decreased physical activity increased the GPx[25] and Uric acid with $p<0.05$ above the reference range. But SOD and CoQ_{10} did not show any significant influence in our study. Mena et al[32] have proven positive influence of physical activity on SOD and GPx. Sarmiento et al showed positive influence of physical activity on CoQ_{10} levels[33]. Psychological stress shows increase in SOD values in abnormal range whereas in low stress conditions values are within range consistent with similar studies[34].

Table 3. Spearman rho test for association between gender and antioxidant levels

Antioxidant	Gender
Superoxide dismutase	0.084
Correlation coefficient	0.396
Glutathione peroxidase	-.487**
Correlation coefficient	0
Coenzyme Q_{10}	0.006
Correlation coefficient	0.951
Uric acid	-.433**
Correlation coefficient	0

* Significant at 0.05 level (2 tailed)
** Significant at 0.01 level (2 tailed)

Smoking and alcoholism is more prevalent among male population than in female population in India but this level is slowly rising among female population. For association between gender and antioxidant levels Spearman's Rho test was performed, in that only GPx and uric acid showed significant difference within gender due to their influence on alcohol and smoking. Table 3 shows the results. And the statistical data was split to determine the significance of lifestyle factors influence on antioxidant levels in males and females separately as seen in table 4 and table 5.

Overall smoking had a positive influence on SOD, CoQ10 levels when values were within range ie nonsmokers. Smokers showed negative correlation with GPx and Uric acid. Conen et al[35] showed no correlation between uric acid and smoking. Niklowitz et al showed male smokers had increased levels of CoQ_{10} than female smokers[36].

The overall effect of alcohol consumption showed increased values of GPx and uric acid. In males alone when evaluated alcohol had positive correlation with increase in SOD, GPx and uric acid and showed negative association[27] with decrease CoQ_{10} values. Chien et al[37] and Lieber et al[38] showed similar finding regarding alcohol on uric acid. Mulholland et al showed no relationship between alcohol and SOD and GPx[31] which is different than the findings in our study.

Increased BMI being one of the risk factors for many NCD has been widely evaluated in patients with NCD. In this study lower the BMI with cut off at normal BMI range showed levels of SOD CoQ_{10} within range whereas uric acid[35,37]

Table 4. Pearson correlation study to determine the influence of lifestyle factors on serum antioxidants in males

Antioxidant	Age	SE score	IPAQ score	FACET score	STRESS score	Smoking score	Alcohol score	BMI score
Superoxide	-.380*	-0.239	0.112	.291*	.454**	.366**	.310*	-.275*
dismutase	0.006	0.091	0.434	0.038	0.001	0.008	0.027	0.051
Glutathione	0.051	0.129	-.850**	-0.173	0.036	-.599**	.884**	0.185
peroxidase	0.722	0.369	0	0.226	0.8	0	0	0.193
Coenzyme Q_{10}	-.296*	-.445**	0.132	.438**	-0.104	.431**	-.280*	-.361**
	0.035	0.001	0.356	0.001	0.467	0.002	0.047	0.009
Uric acid	0.129	-0.026	-.352*	-0.214	0.134	-.311*	.333**	.750**
	0.366	0.854	0.011	0.131	0.35	0.026	0.017	0

* Significant at 0.05 level (2 tailed)
** Significant at 0.01 level (2 tailed)

Table 5. Pearson correlation study to determine the influence of lifestyle factors on serum antioxidants in females

Antioxidant	Age	SE score	IPAQ score	FACET score	STRESS score	Smoking score	Alcohol score	BMI score
Superoxide	0.064	-0.172	.296*	.275*	-0.238	-0.081	0.075	-0.145
dismutase	0.65	0.217	0.031	0.047	0.086	0.564	0.596	0.299
Glutathione	0.15	0.045	-.805**	0.007	0.196	-0.206	0.224	-0.213
peroxidase	0.284	0.749	0	0.962	0.16	0.138	0.107	0.125
Coenzyme Q_{10}	0.062	0.168	0.031	.286*	-0.096	0.029	0.069	-0.141
	0.661	0.228	0.827	0.038	0.493	0.838	0.634	0.314
Uric acid	0.229	-.467**	-0.053	0.164	-0.107	-0.116	0.049	.866**
	0.1	0	0.704	0.24	0.446	0.408	0.729	0

* Significant at 0.05 level (2 tailed)
** Significant at 0.01 level (2 tailed)

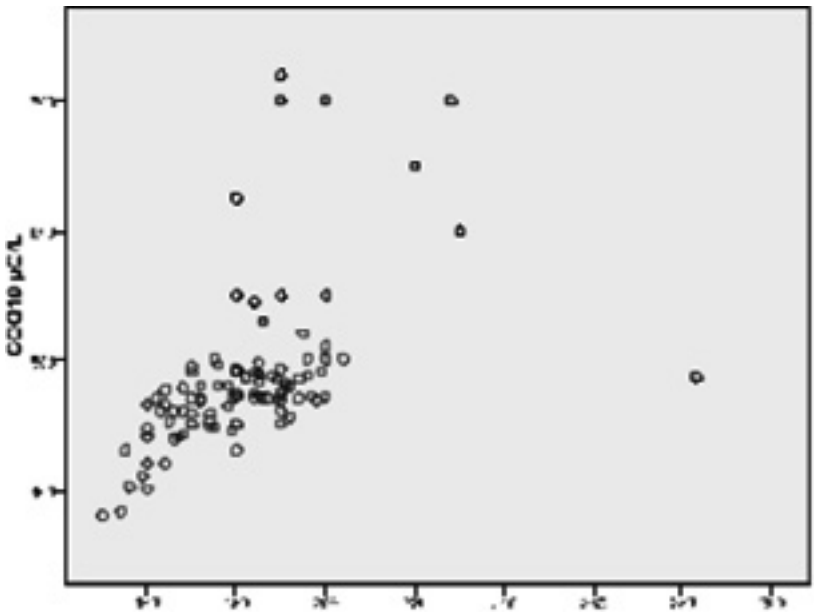

Fig. 1. Scatter plot diagram showing the significant relationship between SOD and CoQ10

seems to increase with increase in BMI. Same findings were seen when males and females data were separately analyzed. Marklund et al have also shown decreasing levels of SOD with increasing BMI[39].

Thus the findings in our study and the comparison with other studies show that most findings are consistent but certain discrepancies exist as explained above, which may need further detailed evaluation.

CONCLUSION

In this current study it is conclusive that serum antioxidant levels are significantly influenced by the different lifestyle choices. In considering modifiable lifestyle factors-betterment of socioeconomic status, low stress, low BMI, increased fruits and vegetables in diet and nonsmoking had appositive healthy influence on SOD and CoQ10, GPx and uric acid and showed the values within the reference range pointing as

good lifestyle factors. Typically sedentary lifestyle and alcohol consumption raised GPx and Uric acid values above the reference values(unhealthy trend maybe signifying compensatory mechanism) with statistical significance of $p<0.05$.Smoking had a negative relationship with GPx and Uric acid. From these findings we have come to the conclusion that individuals with good lifestyle and bad lifestyle choices have a line of demarcation determined by antioxidant levels. The main limitation of the study is that only few antioxidants were estimated and oxidant stress factor was not evaluated to equate to corresponding antioxidant levels and follow up of these patients were not possible. This study can be taken further by overcoming these limitations with a broader study group and adequate follow up.

Conflict of Interest

No conflict of interest.

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