Dissertation on

EFFECT OF LIFESTYLE FACTORS ON SERUM ANTIOXIDANT LEVELS IN APPARENTLY HEALTHY INDIVIDUALS

Submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

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MADURANTHAGAM

TAMIL NADU

APRIL 2017

CERTIFICATION

This is to certify that "***Effect of lifestyle factors on serum antioxidant levels in apparently healthy individuals"*** is a bonafide work of ***Dr. R.Harini*** in partial fulfilment of the requirements for the ***M.D. Biochemistry*** (Branch XIII) examination of ***The Tamil Nadu Dr. M.G.R. Medical University*** to be held on April 2017.

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Head of department
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DECLARATION

I, ***Dr. R. Harini*** hereby declare that this dissertation "***Effect of lifestyle factors on serum antioxidant levels in apparently healthy individuals"*** is a presentation of my own work and that it has not been submitted anywhere for any award. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to literature and discussions.

This work was done under the guidance of ***Professor Dr.Aruna kumari.R MD,*** at Karpaga Vinayaga Institute of Medical Sciences, Maduranthagam.

Candidate's Name:Dr. R.Harini

Candidate's Signature:

Date:

In the capacity as guide for the candidate's dissertation work, I certify that the above statements are true to the best of my knowledge.

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"There are two kinds of teachers: the kind that fill you with so much quail shot that you can't move, and the kind that just gives you a little prod behind and you jump to the skies." — Robert Frost

By God's grace, I am blessed to be a student of a great teacher, who is always there to direct her students in the right direction, who does not put undue pressure and She is the one who taught the virtue of simplification and understanding in learning. She always greets us with a beautiful smile and shows that Knowledge is her power. She is none other than our esteemed and beloved, Head of department of Biochemistry and my guide, ***Professor Dr. Aruna Kumari. R. MD***.The word "***Gratitude"*** falls short for her. I express my deep, heartfelt and sincere thanks to her for her guidance in every process of this study.

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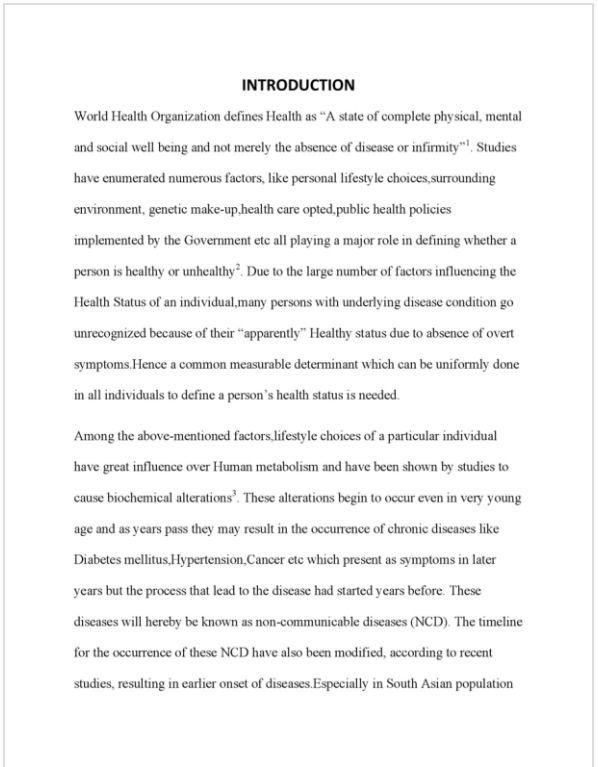
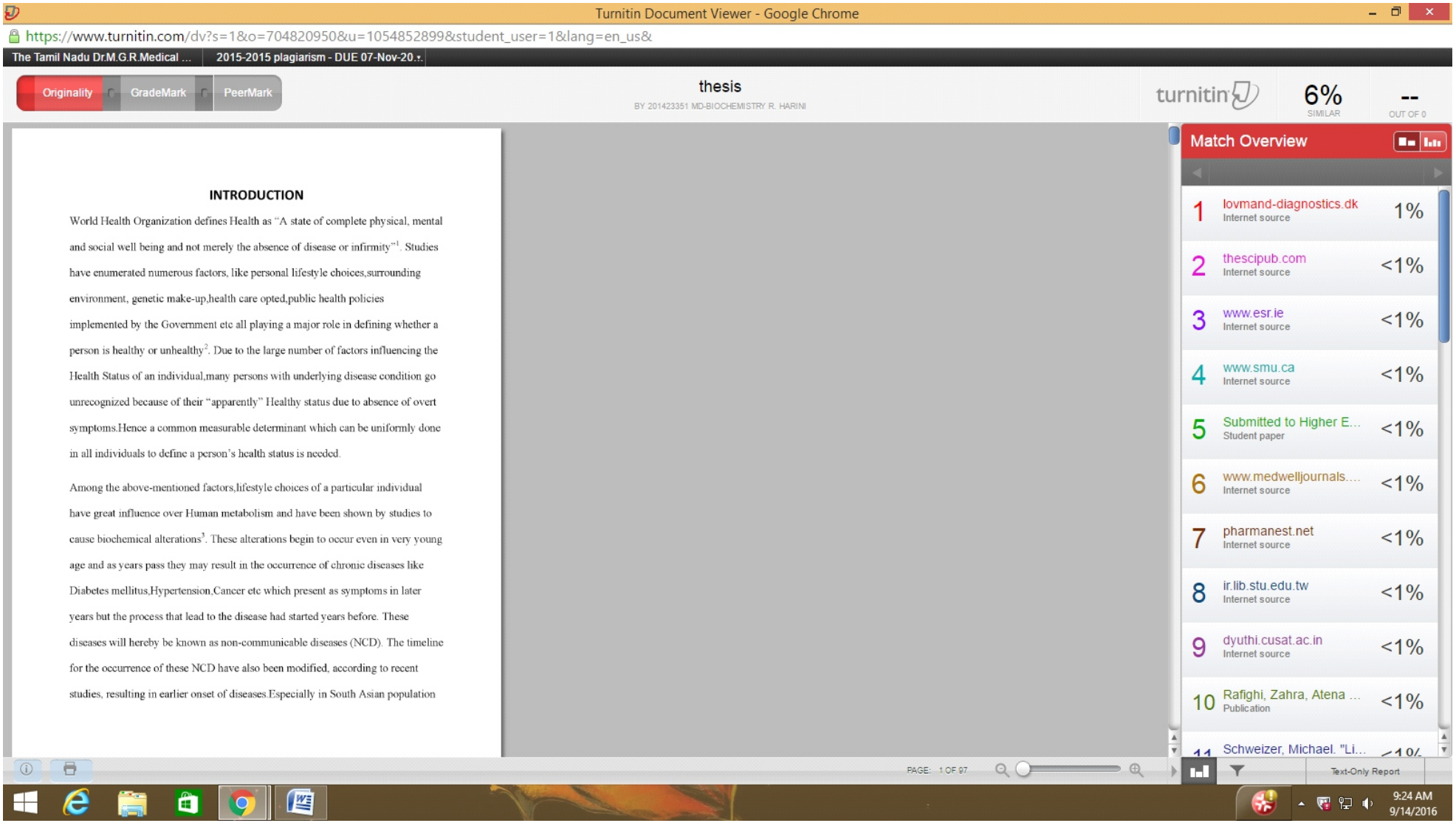


INTRODUCTION

World Health Organization defines Health as "A state of complete physical, mental and social well being and not merely the absence of disease or infirmity"[1]. Studies have enumerated numerous factors, like personal lifestyle choices,surrounding environment, genetic make-up,health care opted,public health policies implemented by the Government etc all playing a major role in defining whether a person is healthy or unhealthy[2]. Due to the large number of factors influencing the Health Status of an individual,many persons with underlying disease condition go unrecognized because of their "apparently" Healthy status due to absence of overt symptoms.Hence a common measurable determinant which can be uniformly done in all individuals to define a person's health status is needed.

Among the above-mentioned factors,lifestyle choices of a particular individual have great influence over Human metabolism and have been shown by studies to cause biochemical alterations[3]. These alterations begin to occur even in very young age and as years pass they may result in the occurrence of chronic diseases like Diabetes mellitus,Hypertension,Cancer etc which present as symptoms in later years but the process that lead to the disease had started years before. These diseases will hereby be known as non-communicable diseases (NCD). The timeline for the occurrence of these NCD have also been modified, according to recent studies, resulting in earlier onset of diseases.Especially in South Asian population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chronic disease's age at diagnosis has been decreasing and incidence has also been steadily increasing[4].

In this study the lifestyle factors of a particular individual is going to be studied in detail by using the appropriate tools. The lifestyle risk factors can be classified as non-modifiable like age gender ethnicity and modifiable which in turn can be further classified as behavioral, material, psychosocial factors, diet and nutrition and physical activity and exercise[5]. Behavioral factors include tobacco use, alcohol consumption, drug usage history etc. Material includes environment and living standards. These details will give an idea about the person's lifestyle choices but its influence on the individual's metabolism needs a marker which acts as a beacon that bridges internal metabolism and pathogenesis of chronic disease.

One of the common causal factor for the pathogenesis of chronic diseases are the damage by Reactive oxygen species(ROS).Reactive oxygen species are a collection of reactive molecules and free radicals derived from reactions involving oxygen. Apart from Apoptosis, ROS have both positive as well as negative influences over cellular metabolism.The positive effects include its actions on immunity and gene induction. The negative influences leads to derangement of cellular function and inflammatory reactions, which have, lead them to be implicated in the causation of many diseases.To defend our body from these

detrimental effects of oxidative damage we have Antioxidants circulating our body[6].

Antioxidant systems in this study can be classified briefly as Enzyme System, Glutathione system, Pro oxidants, and other antioxidant metabolites[7]. Among the antioxidant enzymes present in the Enzyme system, Superoxide Dismutase plays a wide role by acting on all cells exposed to oxygen in the human body. Its mechanism of action is to catalyze the dismutation of superoxide free radical and formation of oxygen and hydrogen peroxide therefore helping in the scavenging of harmful superoxide free radicals[6,7].

Glutathione peroxidase, which is a part of the Glutathione system of enzymes responsible for the scavenging of, released hydrogen peroxide by energy transfer method[6]. Carrying of electrons from reactive peroxides to Glutathione does this. Another antioxidant being estimated in this study is Uric acid which is both an antioxidant as well as a pro oxidant[7,8]. Uric acid mechanism of action is to scavenge the singlet oxygen,peroxyl radicals and hydroxyl radicals released and to protect RBC membrane from lipid peroxidation[9].

Another metabolite estimated as part of the antioxidant profile in this study is Coenzyme Q10 that acts as an energy carrier, going through a continuous cycle of oxidation and reduction. This helps in preventing lipid peroxidation, reducing

perferryl radical and singlet oxygen and in preventing the oxidation of bases present in DNA especially mitochondrial DNA. Coenzyme Q10 is superior to other antioxidants because of its process of inhibiting both the initiation and progress of lipid and protein oxidation thereby increasing its efficiency as an antioxidant. And also helps in the reformation of other antioxidants like Vitamin E and Vitamin C[10].

Therefore these serum antioxidants can very well be the potential markers of metabolic alteration in the body even in young age and if the internal milieu maintained by them gets disrupted due to the poor lifestyle choices, the body may go for compensation and if that compensation fails, the onset of overt disease may result.

Thus this study will focus on a group of apparently healthy individuals in a South Asian population of young adultswithout any proven antecedent cause for serum antioxidant level alterations. Their lifestyle factors will be studied in detail and their serum antioxidant levels of superoxide dismutase,glutathione peroxidase, uric acid and Coenzyme Q_{10} will be simultaneously estimated and correlation between them is going to be studied to establish them as biomarkers to predict the occurrence of Non communicable diseases.

AIMS AND OBJECTIVES

The aim of this study is to determine the influence of lifestyle factors on serum antioxidant levels in apparently healthy young adults of south Indian population.

The antioxidant levels to be measured are

Superoxide dismutase (SOD)

Glutathione peroxidase (GPx)

Coenzyme Q_{10} (CoQ_{10})

Uric acid

The main objectives of this study are:

To study the lifestyle factors in the individuals both Modifiable and Non-modifiable

To determine whether there is alteration in the levels of antioxidants based on their lifestyle choices

To determine the good and bad lifestyle choices based on the relationship between them

To determine whether the status of internal metabolism has been altered due to bad lifestyle choices

To determine whether serum antioxidants can be considered as biomarkers to predict the internal metabolism derangement in young adults.

REVIEW OF LITERATURE

Health is the cornerstone for all Human actions. To live happily with perfect harmony and synchronization with external and internal milieu is to be "Healthy".All the organizations being Governmental or Nongovernmental are formed to promote Healthy living. So what defines this perfect state is a balance between the good and harmful forces. There are many definitions for health world over but the more commonly known WHO definition states "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"[1]. But this is not widely accepted and it was criticized for its vagueness. It was stated that with newer understanding of the internal chemistry, external environment and the uniqueness of an individual this definition becomes unacceptable[11]. A revised WHO definition i.e. the "extent to which an individual or group is able to realize aspirations and satisfy needs, and to change or cope with the environment"[12]is now accepted more.

In Biochemistry point of view Health depends solely on maintaining the internal milieu by balancing the various interlocking biochemical reactions taking place in our body[13].But all definitions aside, the exact assessment of this state is the trickier part as Health has no physical measurable quality.

This has lead to Health being divided into many dimensions like Physical, Mental, social, vocal, etc, which are quantifiable and therefore can give an overall picture about the well being of an individual[14]. Physical includes healthy signs of external appearance of an individual like complexion, skin texture and hair quality. This can be analyzed by History taking, Clinical examination, Anthropometric measurements and Laboratory investigations. Mental includes an individual's behavior and attitude. Social defines the acceptance and position of an individual in his society.

The dimensions of Health though quantifiable have multiple interlinked factors influencing them. These factors can be enumerated as determinants of Health. They are Lifestyle, Genetic makeup, socioeconomic conditions, Environmental factors, Health services and Health promotion sectors, aging of population and other factors[5].

Genetic constitution determines the uniqueness of an individual among a population. As it influences all the metabolic reactions in our body, an individual's tendency for predisposition, occurrence and resistance to a particular disease, drug reactions and general adaptation to molecular insults can be studied. Lifestyle factors determine the health behavior. Davison et al define lifestyle as ″the aspects of health related behaviors and conditions which entail an element of personal action at the individual level ... strongly associated with the possibility of

individual choice and the triumph of self control over self indulgence"[15].Environmental influence deals with internal and external milieu synchronization leading to harmonious existence. This shows that Health is a state of dynamic equilibrium. Health services deals with the services rendered for the people by the Governing body. An Intersectoral co-ordination to ensure proper water supply, good roads, lighting, Food and agricultural, social welfare, education, rural and urban development etcare needed. Aging of a population determines the Health status, Burden of the aged population on developing economy etc. All these factorsnot only influence and determine the wellbeing status of a population but can also predict the risk for disease occurrence in a society.

Initially the "Germ theory" was considered the norm to explain disease occurrence. Then a new theory was put forth by Pettenkofer of Munich to explain the influence of Genetic, Social, Economic, Cultural, Lifestyle, Behavioral and Psychological factors on disease occurrence i.e. "Theory of multifactorial causation"[5]. This theory aims to explain the "web of causation of diseases" thereby substantiating the importance of these factors in determining whether an individual is healthy or not.

These other factors were then studied in more detail to determine their value as an attribute, depiction or exposure, which increases the chance for an undesirable

effect to be produced in an individual in the form of a disease causing morbidity or mortality. The factors were identified as “risk factors”.

These risk factors are further divided into modifiable and non-modifiable risk factors[5]. The non-modifiable risk factors are Age, Gender, Family history, Genetic makeup and Ethnicity. The modifiable risk factors are Diet, Physical activity, socioeconomic status, Environmental, Occupation, Psychological Stress and Substances dependence or abuse like cigarette, Alcohol and Drugs.

Among these factors the lifestyle component was studied in detail in this study. The lifestyle component includes Age, Gender, Ethnicity, Diet, Physical activity, socioeconomic status, psychological stress and substance usage[14].

Age group selected for this study was 20-30 years i.e. young adults. Major lifestyle choices are usually made as young adults only. Internal metabolism also peaks and slowly starts to decline after 25years[16]. The antioxidant levels in our body are usually in a healthy range at this age and slowly start to fall. Hence age plays an important role as far as risk factors for NCD are considered.

Gender has always been shown to play a major role in lifestyle choices. Antioxidants levels also vary among males and females. Internal metabolism and protein turnover and lean body mass varies among males and females[17]. This can have an influence in the antioxidant levels among males and females in our body.

Ethnicity defines a state where a category of people belongs to a common group based on their common language social and cultural practices and the society they live in. In this study South Indian population belonging to areas of Chennai and Maduranthagam in the State of Tamil Nadu were selected to be studied. Antioxidant levels in this population ethnicity to our knowledge is not widely studied and hence this study can act as stepping stone for further detailed studies, as South Asian population have high rates of incidence of non communicable diseases induced morbidity and mortality[18].

Diet and nutrition plays an important role in our antioxidant levels. Especially fruits and vegetable intake influences the antioxidant levels in our body[19]. Diet also forms an important criterion to maintain the internal metabolic equilibrium in our body.

Physical activity determines bodily movements that utilizes the musculoskeletal system and results in expenditure of energy. This plays a role in maintaining our body metabolism, weight, and removes stress and promotes healthy lifestyle. But exercise increases the release of free radicals, which may play a damaging role. But with regular exercise the generation of antioxidants increases and thereby reduces the oxidative stress[20]. Exercise increases free radical production by causing surge in catecholamine and lactic acid levels and secondary muscle damage. The catecholamines in an inactivated state produces oxygen radicals, whereas lactic

acid converts superoxide into damaging hydroxyl radicals and secondary muscle damage initiates inflammatory reaction cascade[20]. But the results are controversial in some studies and how the physical activity and inactivity in normal individuals without any training in exercise modifies the antioxidant levels is not seen in any study. Physical inactivity is identified as the fourth leading risk factor causing 3.2 million deaths all over the world.

Body mass index measurement is one of the important tools for predicting the risk for many non-communicable diseases. Obesity is a leading cause for morbidity and deaths due to NCD like cardiovascular diseases[21]. Obesity induces endothelial dysfunction and increased LDL oxidation, which may lead to oxidative stress. The endothelial Nitric oxide synthase deactivation because of lowered response of vasculature to shear stress in obese individuals may lead to further oxidative stress.

Socioeconomic status determines an individual's social habits and monetary status, which in turn determines other lifestyle choices like physical activity, diet and substance abuse[22]. When the lifestyle choices influence the adaptation against oxidative stress they increase or decrease the antioxidant levels. But studies showing the direct influence of socioeconomic classes on antioxidant level variation in a society especially in India have not been done according to our knowledge.

Psychosocial habits including psychological stress determines the influence of occupation, cultural habits, economy, religious beliefs etc on the psychological profile of an individual[23]. This may lead to stressful life events which can contribute to internal metabolism derangement and varying antioxidant levels.

Substance usage generally includes cigarette smoking, alcohol consumption, drug abuse, tobacco chewing, psychedelics consumption etc. And it's become highly prevalent in young adults[24]. Various studies have shown the influence of substance abuse over internal metabolism and oxidative stress and thereby altering antioxidant levels. In this study the effect of Cigarette smoking and alcohol consumption alone are studied, as the subjects included did not have any other substance usage history.

From the above description it can be seen that the lifestyle factors themselves have interlinked complex relationship[19,21]. Research has revealed this versatile relationship, but how this produces an impact in the maintaining of the internal milieu is not widely studied especially in South Indian population.

The knowledge about this complexity can go a long way in filling the lacunae in research thereby improving the chance for primary prevention of many diseases. Especially in case of Non communicable diseases (NCD) Primordial prevention i.e. action in the pre pathogenesis stage can be made possible[5]. This can be done by

determining the health lifestyle choices and eliminating or modifying the risk factors, which cause disease, disability, morbidity and premature mortality in individuals.

Non-communicable diseases are at present a global "Chronic emergency". Estimation shows 62% of total disease burden globally is due to NCDs out of them 52% i.e.36 million deaths and 43% disability adjusted life years (DALY) are seen[25]. Considerable loss of potentially productive years (35-64 years) is resulting due to the disease burden[5].

In India proportional mortality figures show 24% deaths occur due to cardiovascular diseases, 6% due to cancer, 2% due to diabetes, and 10% other NCD[26]. India at present tops with the highest number of diabetes cases in the world with an estimated 31.7 million suffering from Diabetes mellitus as of 2000[4].

This high incidence is attributed to a multifactorial causation including but not limited to all the factors discussed above i.e. lifestyle factors genetic environmental etc. The mean age of occurrence of these diseases have also decreased may be due to poor lifestyle choices early on in age.

As NCD have gaps in their natural history of occurrence like absence of known etiological agent, multifactorial causation, iceberg phenomenon and indefinite period of onset[14]. Though many theories as to the etiology of these diseases have

been put forth none of them are definite. But one of the most consistent causes appears to be the action of reactive oxygen species.

Reactive oxygen species (ROS) is a term used to refer to the reactive molecules and free radicals produced as by-products of the reactions involving Oxygen. In aerobic organisms production of ROS is part of normal physiology[7]. They are mainly generated during cellular respiration from mitochondrial electron transport chain (ETC) and also from the ETC present in endoplasmic reticulum and nuclear membrane. They are also generated from oxidoreductase enzymes, mono amino oxidases, lipo-oxygenases, oxidation catalyzed by metals, Cytochrome P_{450} etc[9].

Oxygen is more prone to produce free radicals because according to the atomic structure of oxygen, which is $1s^2\ 2s^2\ 2p^4$, the distribution of electrons in the outermost shell 2p will be 2 electrons in $2p_x$ 1 electron $2p_y$ and 1 electron in $2p_z$to attain the lowest energy requirements. Hence there are 2 unpaired electrons in two different orbital in the outer electron shell predisposing the reduction of oxygen by the addition of electrons to its outer orbitals. This leads to the formation of superoxide anion O_2^-, peroxide radical O_2^{2-}, hydrogen peroxide H_2O_2, hydroxyl radical OH, hydroxyl ion OH^- etc.

These reactive molecules and free radicals have both positive and negative properties making them indispensible in cellular metabolism. The positive actions

are activation of cell signaling cascade, gene expression, apoptosis, and also to act as inter and intra cellular messengers[9]. The negative actions occur when there is excess production of ROS causing the loss of natural capacity of cells to remove them from the body like in case of increased cellular metabolism or under extracellular stimuli like infection or environmental factors like tobacco smoke, radiation exposure etc. The negative actions include damage of lipids by causing lipid peroxidation and membrane lipoprotein oxidative damage, glycoxidation, specific damage to proteins and altered base production and fragmentation of DNA due to oxidation all consequentially leading to cell death and tissue injury and organ system dysfunction.

The ROS are produced from respiratory chain process especially in mitochondria. During the transfer of electrons from complex I to complex III, molecular oxygen undergoes univalent reduction thereby releasing ROS at the end of each electron addition step, thus Oxygen gets reduced to H_2O. it can be illustrated by the following equation:

$$\overset{e^-}{\rightarrow} \quad \overset{e^-}{\rightarrow} \quad \overset{e^-}{\rightarrow} \quad \overset{e}{\rightarrow}$$

$$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow \dot{O}H \rightarrow H_2O$$

The reactive intermediates superoxide anion, hydrogen peroxide and Hydroxyl radical are not usually formed because oxygen gets directly reduced to water by

Cytochrome oxidase using 4 electrons. In case of ETC being in a highly reduced state and the rate of reaction depends on ADP availability there occurs single electron leakage. This leakage especially increases during the transport of electrons by Ubiquinone (CoQ_{10}) to Complex III by the Q cycle[7]. This leads to increased production of Superoxide anion and other intermediates.

Superoxide anion is a relatively a molecule with low reactivity. But due to its conversion into peroxyl, alcoxyl, and hydroxyl radical it becomes highly reactive and potentially more injurious to cells. Hydrogen peroxide is another intermediate with low reactivity and it is usually neutral among all molecules in a cell as physiologically it is essential for activation of nuclear transcription factors, synthesis of thyroid hormones etc. But when it gets generated as a result of extracellular stimuli it gets the potential for injury. The extracellular stimulus which produces highly reactive H_2O_2 are cell signaling cascade factors, cytokines, growth factors, hormones, enzymes like fatty acyl oxidase, peroxismal oxidases, D amino acid oxidases, L hydroxyl acid oxidase and Cytochrome (Cyt) P_{450}, P_{450}reductase, and Cyt $b_5$reductase in Endoplasmic reticulum during certain settings. H_2O_2 is an important part of the process of Apoptosis[9]. It acts by modifying the permeability of mitochondrial membrane thereby causing the delivery of Cytochrome C from the Mitochondria leading to activation of caspases which in turn results in programmed cell death.

Transition metals like Iron (Fe) and Copper (Cu) are usually in the bound form but under pathological condition they get discharged from metalloproteins as redox reaction capable substances[8]. They act on Hydrogen peroxide leading to the formation of Hydroxyl radical. This hydroxyl radical causes damage by activation of Oncogenes and by damaging purines and pyrimidines leading to the generation of modified DNA nucleotides. Other than this Xanthine oxidase and phagocytes also lead to superoxide anion and hydrogen peroxide production. Dismutation of superoxide anion also produces H_2O_2,which can either be a spontaneous reaction under neutral pH conditions or by the action of superoxide dismutase enzyme.

The positive role of ROS being cell signaling takes place by sequential reduction oxidation reactions within and outside a cell facilitated by the reactive oxygen species production. They help in altering the redox potential of a cell and also act in modifying the protein structure by its action on cysteine and aromatic amino acids[9]. This oxidation of cysteine leads to disulfide formation, which helps in redox dependent signal transducing process. The role in Gene expression is also through the same process of protein modification as well as nuclear transcription factors NF-?Bactivation, which leads to, modified proteins binding to promoter region and gene expression[8].

Gene expression of enzymes like superoxide dismutase, hemeoxygenase, gamma glutamyl cysteine synthase, glutathione peroxidase etctakes place through this

process to promote cell protection and signal transduction. Hence they also act as second messengers for growth factors and cytokines.

The negative actions of radical mediated oxidative damage as seen earlier results in molecular damage to proteins, lipids, and DNA thereby are causing cellular damage in the form of membrane damage, mutation, metabolic dysfunction, electrolyte leakage etc finally resulting in cell death. This triggers the release of necrotic factors and proteases and reactive oxygen species from the damaged cell which further cause oxidative damage to adjacent cells. In the presence of inefficient repair mechanisms this condition gets worsened and contributes to the pathogenesis and worsening of many diseases like cancer, cardiovascular diseases, neurodegenerative diseases, metabolic diseases etc.

To maintain the balance so as not to cause injury and insult to cellular metabolism there are a system of enzymatic and non enzymatic substances called antioxidants. They form the natural defense system against ROS induced damage. The first defense against the ROS is the scavenging enzymes. They are Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase,heme peroxidase.

Then there are the non enzymatic compounds like alpha tocopherol, Beta carotene, glutathione,Ubiquinone,ceruloplasmin,transferrin, haptoglobin,lipoicacid,bilirubin,

thioredoxins, polyphenols metals like selenium etc. some compounds act as pro oxidant as well as antioxidant like Vitamin C and Uric acid[7].

Each antioxidant has a unique process of protecting of cells against ROS, for example antioxidants combat lipid peroxidation process of the ROS by two mechanisms namely Preventive and Chain breaking.

The Preventive antioxidants act by causing inhibition of the ROS production in the initiative stage itself thereby provides protection[7]. They act by

A. Stabilising or deactivation transition metals.

   Examples of antioxidants: transferrin, ferritin, Desferal,

B. Catalyzing reduction of hydrogen peroxide,

   Examples of antioxidants: catalase, glutathione peroxidases, pyruvate

C. Scavenging singlet oxygen species.

Examples of antioxidants: β-carotene, lycopene, bilirubin.

The chain breaking antioxidants act by inhibiting the propagative stage of lipid peroxidation by scavenging for peroxyl radicals.Examples: Tocopherol, ascorbate, uric acid,nitric oxide.

But all antioxidants together through interlinked reactions alone can completely eliminate the deleterious actions of ROS in the body. In this study the action of superoxide dismutase, Glutathione peroxidase, Coenzyme Q10 and uric acid which have interdependent mechanism of actions and how theycover the spectrum of defense against the reactive oxygen species shall be seen.

Superoxide dismutase (SOD)

This enzyme is the first level scavenger. It is a metalloprotein, which can be classified into three classes based on the enclosed metal. Copper and Zinc reactive cofactors containing SOD are seen in eukaryotes, Iron and Manganese are seen in prokaryotes and mitochondria, Nickel cofactor is seen in prokaryotes. In humans there are three forms of SOD present, they are SOD1 which contains copper and zinc and is present in cytoplasm, SOD2 which contains Manganese and is present in mitochondria and SOD3 which contains copper and zinc and is seen in extracellular tissues. The corresponding genes are located in chromosome 21q22.11, 6q25.3 and 4p15.3-p15.1.

Mechanism of action

Superoxide anion though has low reactivity it causes damage when it dismutes with itself or other radicals like nitric oxide and results in the formation of more dangerous toxic radicals[27]. It also reacts with transition metals releasing them as

reactive metals capable redox reactions example being its action on aconitase which is a iron-sulphur containing enzyme in TCA cycle, resulting in disruption of energy metabolism. This dismutation if it's spontaneous occurs at neutral pH and releases Hydrogen peroxide and oxygen. Superoxide dismutase enzyme action is necessary because of its capacity to protect the cells predisposed to be damaged. And the reaction rate is also greaterand highly efficient in the presence of SOD and is controlled only by one factor i.e. the chance for contact between the enzyme and superoxide.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Clinical importance of superoxide dismutase

The importance of SOD as not only an antioxidant but also as an important component in cellular metabolism is established by the occurrence of diseases in SOD gene deleted genetically engineered mice. Humans show evidence of neural disease amyotropic lateral schlerosis occurring in patients with SOD1 mutation. Other diseases linked with SOD are neural tube defects, COPD, hypertension etc.

Influence of lifestyle factors on SOD

Lifestyle factors have been shown to wield a great influence on Superoxide dismutase levels. According to Alejandro et al, who had conducted a study among

healthy individuals of a wide age group, superoxide dismutase decreases with age but the decrease is not linear and starts after 28 years of age. They also state that females have higher SOD values than males and that there is no change in SOD levels among smokers and non smokers[28].In another study conducted in healthy humans it was found that there was no association with age and SOD levels[29]. In a study conducted among older men with risk of Ischemic heart disease no association was found between age, smoking, alcohol, social class and body mass index (BMI)[30]. But in a study conducted among Indian population among farmers SOD showed significant association with age and the levels were found to decrease with increasing age[31].

Regarding diet and nutrition, the studies conducted among human population are very limited in number. Fruits and vegetable intake have been shown to be associated with SOD levels in our body[32].In one study conducted among healthy women it was found that SOD was significantly lower in the group where they consumed carotene deficient diet. Fruits and vegetables are rich in carotene and the group, which showed low SOD levels, had history of consumption of fruits and vegetable in a lower quantity when compared to the group having higher SOD[33].

Other studies were conducted in a controlled laboratory environment among rats. One such study fed the rats with casein semi-synthetic diet and it was found that the group with a calorie-restricted diet showed increase SOD levels and increased

corresponding mRNA levels[34]. Similarly another study in rats fed with casein diet but with different levels of salt level restrictions showed that high level of salt supplemented diet consuming rats had expressed decreased levels of CuSOD and MnSOD[35].Ketogenic diet fed rats showed no change in SOD levels in brain tissue[36]. This shows that the studies in laboratory rats are not conclusive and can be made use only as a model for further Human experimentation. One study has shown the increased activity of SOD with fruit juice i.e. grape juice intake[37]. There is severe dearth of studies conducted to evaluate the dietary role in SOD levels.

Regarding body mass index not many studies exist to associate it with SOD levels. One such study was conducted among randomly selected individuals in which their extra cellular SOD levels (EC SOD)and their genotypes showing common and high level variant types were evaluated. They proved high level variant of EC-SOD were seen in 4% of sample population and that they showed higher BMI and waist hip ratio and very high serum cholesterol and triglycerides levels and common EC-SOD levels were lower in Smokers and in individuals with high BMI and high waist-hip ratio[38]. In another study conducted among maternal population, SOD levels in placenta tissue homogenates were shown not to be significantly associated with BMI[39].

Physical activity as seen earlier has a complex relationship with antioxidant levels. On sudden exercise it had been found to increase oxidative stress, whereas regular

exercise is accompanied by the development of increased tolerance and adaptation therefore maintaining an increased healthy level of antioxidants. But in one study conducted in normal men and women a bout of physical activity showed increased immediate SOD levels and gradually decreasing levels. And in individuals with history regular physical activity increased SOD levels were seen[40]. This study also showed no difference in SOD levels among different age and gender groups and also in smokers. In a study conducted among regular cyclers SOD levels were higher in resting state as well as after an exercise session when compared to group of individuals showing sedentary lifestyle[41].

Studies in rats comparing the SOD levels and physical activity are numerous. They show that moderate exercise is associated with SOD gene upregualtion[42] and shows protective action on liver from oxidative injury[43] and also has cardio-protective actions[44,45]. A positive association between Endothelium derived Nitric oxide synthase and SOD levels have also been shown in a study conducted in mice[46].

Studies to evaluate the consequence of psychological stress and SOD levels are also very low despite theoretically proof of association between psychological stress oxidative stress and levels of antioxidants and also many disease pathology[47]. In human studies vitiligo patients were evaluated for their SOD levels and it was reported that emotional stress was elevated in these patients and they

had increased SOD levels[48]. In another study conducted among healthy population strong positive association was seen between mental stress and seminal plasma SOD levels[49]. There is also proven evidence for increased SOD levels associated with origination of many psychological diseases like Schizophrenia and bipolar disorders[50].

Studies have shown cigarette smoking being associated with increased SOD levels i.e. smokers showed higher SOD values when compared to non smokers[51]. But a similar study conducted India showed mean lowering of SOD levels with increasing age and duration of smoking[52].

Thus lifestyle factors have been shown to have major role in causing changes in the levels of SOD but it has been done mostly as animal studies or only individual factors have been studied separately and many lifestyle factors are yet to be studied especially in South Indian population. This lacuna, in the knowledge about the lifestyle choices influence on SOD, is proposed to be filled through this study.

Glutathione peroxidase:

Glutathione peroxidase (GPx) is a selenoprotein discovered by Mills in 1957. They belong to a group of enzymatic antioxidants with predominant preventive action on free radical release. There are seven isoenzymes identified so far[53]. Their main role is to remove hydrogen peroxide (H_2O_2) and other organic hydro-peroxides from

the body released from dismutation of superoxide radical. They act as the second line of defense after SOD in expelling the low reactive H_2O_2 before it gets converted into highly reactive hydroxyl radicals.

The seven isoforms are GPx1, GPx2, GPx3, GPx4, GPx5, GPx6 and GPx7[54]. GPx1 is coded by chromosome 3 p21.3 and is commonly present in all cells and its main action is to scavenge H_2O_2 and protect against oxidative stress. GPx2 is coded by chromosome 14 q24.1 and forms a part of gastro intestinal tissue oxidative stress defense system. GPx3 coded by chromosome 5 q23 is commonly seen extracellular. GPx4 coded by chromosome 19 p13.3 is otherwise known as phospholipid-hydroperoxide glutathione peroxidase (PHGPx) and plays an important role in removing lipid peroxides. GPx5 coded by chromosome 6 p21.32 is seen in epididymal cells and is helpful in protecting spermatozoa against oxidative stress. GPx6 coded by chromosome 6 p21 is seen in olfactory tissues. GPx7 coded by chromosome 1 p32 which is a non selecysteine containing GPx and is shown to have association with cancer etiopathogenesis of cancer especially eosophageal cancer[55].

Mechanism of action

GPx main action as preventive antioxidant is scavenging of hydroperoxides. It can be illustrated by the reaction:

Glutathione peroxidase

$$2GSH + H_2O_2 \xrightarrow{\text{Glutathione peroxidase}} GS\text{–}SG + 2H_2O$$

Glutathione peroxidase has an interrelationship with another preventive antioxidant enzyme called catalase in scavenging of hydroperoxides. Its first discovered role was to maintain red blood cell structural integrity and it was later found that hematological disorders occur in relation with GPx associated disorders[56]. Human GPx is said to have action on proliferation and activation of factors responsible for transcription process in cells, programmed cell death, and modulating inflammatory processes[57]. It also helps in preventing oxidative damage to DNA and therefore preventing oesophageal cancer[55].

Influence of lifestyle factors on glutathione peroxidase

Studies have shown association of age, sex, BMI, physical activity smoking, alcohol, social class and diet with GPx levels. But very few studies exist in that subject. In one study conducted among healthy individuals females have shown to have lower GPx when compared to males and also Gpx levels were found to increase with age. But smoking showed no significant association with GPx levels[28]. But in another study conducted among healthy population GPx levels were found to decrease with age in males but not in females[29]. In an ischemic heart disease risk present population GPx showed a trend of increased levels with age

and BMI but did not show statistical significance and showed statistically significant association with smoking and social class[30]. In a study conducted among Indian farmers GPx showed no change in levels upto middle age but started to fall after 60 years[31].

In maternal population a study shows increased levels of GPx in obese mothers but it showed no statistical significance[39]. In another study in maternal population GPx showed significant association with ethnicity i.e. higher levels were seen in African Americans when compared to Hispanics and Caucasians and also showed increased levels in increased dietary fat intake in third trimester[58]. Diet has been shown to wield a wider influence on GPx levels with Selenium levels in diet playing a major role by influencing GPx and mRNA levels but shows no influence on PHGPx levels[59]. Animal models have shown relationship between GPx levels in brain tissue and ketogenic diet i.e. rat fed on ketogenic diet showed four times increased levels of GPx in hippocampus but no change in GPx in cerebellum proving the protective action of GPx in children with convulsive disorders on ketogenic diet[60]. Fruit juice intake has also been shown to increase the activity of glutathione peroxidase[37].

Studies conducted in healthy individual with history of regular exercise in the form of cycling etc show that in their resting state in individuals who undergo regular physical exercise their GPx levels are higher than sedentary group[31]. In another

similar study in healthy individuals after a bout of exercise there was immediate surge of GPx and then the levels gradually attained basal levels and also on regular training the individuals maintained a higher level of GPx[40]. In a study conducted to evaluate the influence of physical activity in adolescents with Down syndrome showed that after 12 weeks of training they showed higher levels of GPx when compared to the sedentary group showing increased protection against redox actions[61].

The influence of alcohol consumption on GPx in human studies showed no change[30] but in a study conducted among rats fed with 20% ethanol and monitored liver homogenate GPx levels for 3, 6, and 9 weeks showed that the levels remained unchanged at the end of 3 weeks but after that it started to gradually rise thereby it can be assumed that increased GPx levels show adaptation against ethanol induced liver damage due to lipid peroxidation[62].

The action of emotional and psychological stress on GPx levels has not been done in any studies from our knowledge but in case of a study in vitiligo patients with emotional stress GPxlevels showed no change[48]. But this is not conclusive because of the chance for the existence of other pathological confounding factors.

Thus the studies showing the lifestyle influences and GPx association are very few in number and the results are also not conclusive and there are lots of potential for more research to be done.

Coenzyme Q_{10}

Coenzyme Q_{10} (CoQ_{10}) is also known as ubiquinone or ubidecarenone. They are a group of lipid soluble vitamin like substance present in the inner mitochondrial membrane of eukaryotes. Their main function is to perform redox coupling reaction to help in transfer of electrons for the generation of ATP in electron transport chain in mitochondria. It was discovered by Professor Frederick Crane and colleagues in 1957 and its chemical structure was defined by Dr Karl Folkers and colleagues in 1958[63].

Endogenous synthetic process of CoQ_{10}

It has 2 components namely benzoquinone and isoprene side chain. The precursors for the synthesis of the benzoquinone part are tyrosine and phenylalanine. And the isoprene side chain gets generated through the mevolonate pathway with acetyl CoA acting as its precursor[63]. The synthesis can be enumerated by the following steps of chemical reactions.

Benzoquinone synthetic pathway steps from Amino acid.

Phenylalanine hydroxylase

. Phenylalanine ⟹ Tyrosine

Tetra hydrobiopterin O_2 H_2O dihydrobiopterin

Aminotransferase

2. Tyrosine ⟹ 4 hydroxyphenylpyruvate

Prephenatedehydrogenase

3.4 hydroxyphenylpyruvate ⟺ Prephenate

Chorismatemutase

4.Prephenate ⟺ Chorismate

Chorismate pyruvate lyase

5. Chorismate ⟹ ***4 hydroxy benzoate***

Isoprene side chain synthetic pathway from acetyl CoA

Thiolase HMG CoA synthase

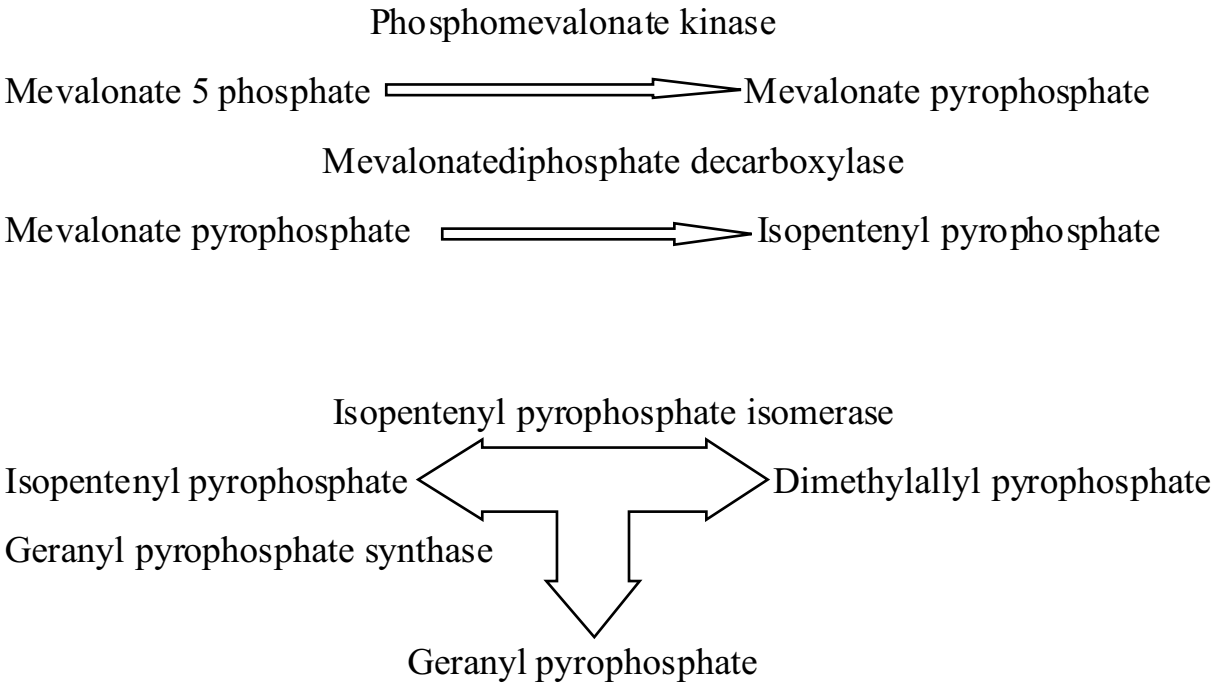
Acetyl CoA ⟹ Acetoacetyl CoA ⟹ Hydroxymethylglutaryl CoA (HMG CoA)

HMG CoA reductase

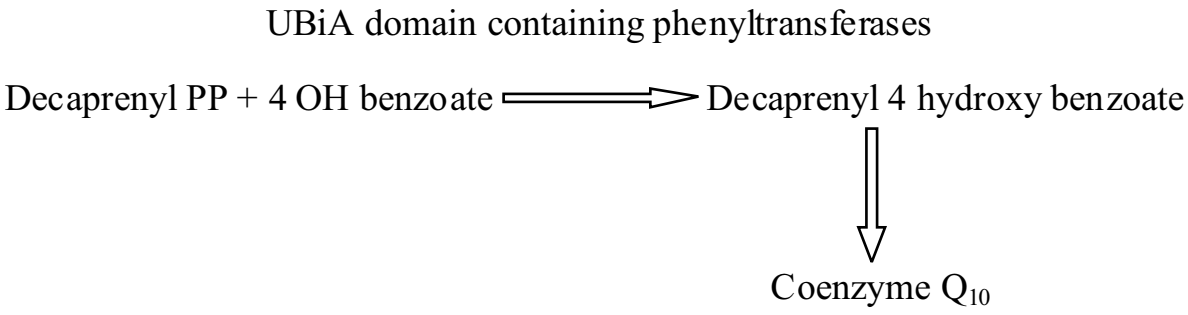
HMG CoA ⟹ Mevalonate

Mevalonate 5 kinase

Mevalonate ⟹ Mevolonate 5 phosphate



On sequential addition of Isopentenyl pyrophosphate to the products formed I each step, Polyprenyl pyrophosphate is synthesised. Depending on the number of units different Coenzyme Q are formed. When 10 units are added as a side chain they are known as Decaprenyl pyrophosphate (Decaprenyl PP) which is produced by the enzyme Decaprenyl diphosphate synthase. This Decaprenyl PP on reaction with 4 hydroxy benzoate forms Coenzyme Q_{10}.



Functions of Coenzyme Q_{10}

CoQ_{10} contains two functional components namely

1. Aerobic cellular respiration
2. Antioxidant[64]

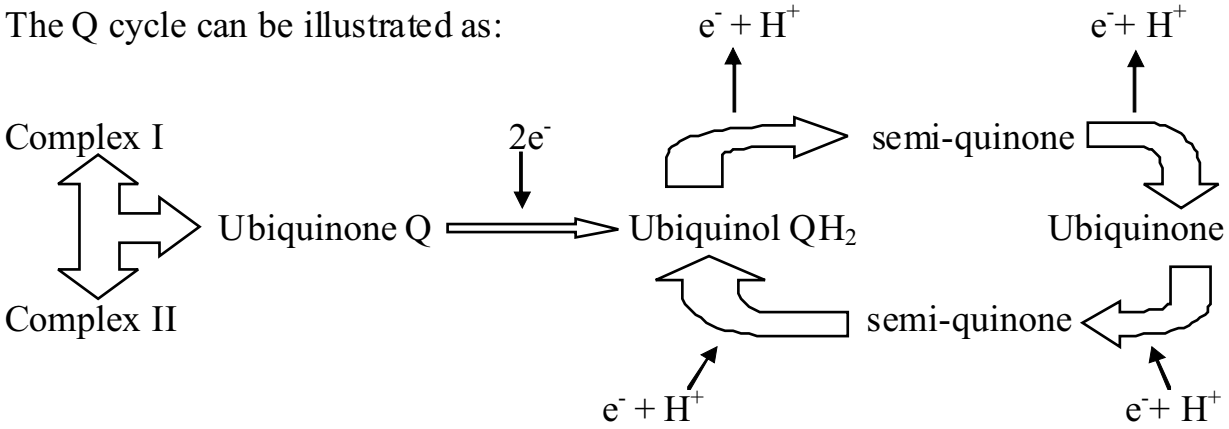
These functions are dependent on the reduction oxidation coupled reaction capacity of CoQ_{10}.

Due to this reaction CoQ_{10} can exist in three forms ie completely reduced form, semi reduced and oxidized form. They are otherwise known as Ubiquinol, Semi-quinone, and Ubiquinone respectively. The Ubiquinol is the active form and its production is the purpose of the redox reactions.

Role of CoQ_{10} in Electron transport chain[7]:

Ubiquinone accepts a pair of electrons from complex I (NADH-coenzyme Q oxidoreductase) or complex II (Succinate-Q oxidoreductase) to produce ubiquinol which enters into Q cycle to facilitate the switching from 2 electron carrier to single electron carrier Cytochrome C ie complex III (Coenzyme Q-cytochrome C oxidoreductase). This system plays an important role as a bridge between two electron donor system and one electron acceptor system.

The Q cycle can be illustrated as:



then finally the electron enters into Rieske iron sulphur protein complex and complex III takes over the chain.

Antioxidant role:

CoQ_{10} function as an antioxidant is based on its action in preventing lipid peroxidation by reacting with peroxyl radicals and removing them from the system

before they could have damaging actions[64]. This action is useful in preventing membrane lipids peroxidation as well as LDL oxidation thereby has protective action from from many diseases including cardiovascular disorders. It also helps in conversion of alpha tocopheroxyl, which is a prooxidant radical, to alpha tocopherol, the active form of Vitamin E[64]. There is also a theory stating that acorbic acid regeneration outside the cell is facilitated by $CoQ_{10.}$ As the process needs elctron transfer across the plasma membrane to regenerate from monodehydroascorbate, CoQ_{10} is hypothesized to play a major role in that process[10]. Other than these functions CoQ_{10} has a direct anti-atherogenic role by its action on endothelium and also by its action to reduce lipid hydroperoxide formation and atheroschlerotic lesion minimization as demonstrated by studies conducted in mice with Apolipoprotein E deficiency with high fat intake[64].

Clinical importance:

CoQ_{10} is proven to have influence over the clinical outcome of many diseases[65]. One of its main roles is ameliorating mitochondrial myopathies and encephalopathies. Studies have shown the importance of supplementation of CoQ_{10} in case of cardiovascular diseases including congestive heart failure cardiomyopathies, atheroschlerosis etc. Numerous studies show association between CoQ_{10} deficiency and onset of cancer, immunocompromised diseases, infertility and neurodegenerative diseases. Deficiency of ubiquinol is also reported

in diabetes mellitus. They have also been demonstrated to have action on gene expression and cell signaling by reduction oxidation coupling reactions generating H2O2 which stimulates transcription factors like NFkB which initiates gene expression[10,63].

Influence of lifestyle factors on CoQ_{10}

Theoretically and scientifically CoQ_{10} has been shown to improve the clinical outcome of many non-communicable diseases (NCD) by its various routes of defense against oxidative stress. Therefore the lifestyle risk factors for NCD can be hypothetically said to modify CoQ_{10} values too. Despite this possibility only very few studies exist to determine the scientific truth behind it. But these few studies conducted have shown good results but more research into this field is necessary.

In a study conducted among middle aged men and women CoQ_{10} is positively associated with age, male gender, diet i.e. intake of vegetables, roots and eggs, BMI, smoking, and alcohol consumption. And negative association is seen with conditioning exercise and diet i.e. intake of dairy products and fish[66]. Similar study conducted only among healthy females of three ethnic groups significant difference between the three groups but no association with age was noted[67].

In elderly women there was no relation with age, BMI, alcohol intake and socio economic status[68]. But in adolescents and premenopausal women adolescent girls

showed lower CoQ_{10} values and also this age wise difference was statistically significant when seen by ethnicity i.e. in Asians and Caucasians[69].

In a study conducted in health pregnant women CoQ_{10} was found to be higher in women who had no history of smoking and did more of exercise in third trimester[70]. CoQ_{10} levels seem to improve the sustainability and performance and decreases fatigue and also defend against exercise induced damage[71]. And CoQ_{10} supplementations have also been proven to decrease the oxidative damage induced by strenuous exercise and also improved endothelial functionality[72].

In a study conducted among health smokers in Europe male smokers showed higher values than female smokers[73] and in another study conducted among only female smokers the CoQ_{10} levels were decreased when compared to non smokers, therefore females have greater tendency for adverse effects due to smoking than males[74].

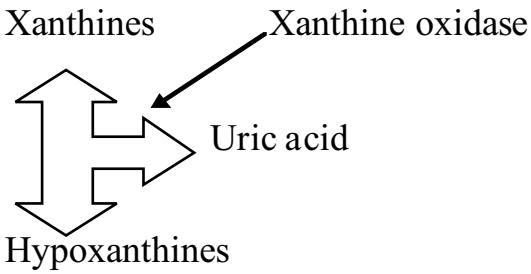
Mediterranean diet is proven to have influence over CoQ_{10} and also by increasing the levels they reduce DNA damage and with CoQ_{10} supplementation there is also stabilization of p53 in response to DNA damage[75]. Ethnic differences in CoQ_{10} levels were also noted by a study conducted among Kenyan population[76].

CoQ_{10} levels were also shown to be decreased in psychological disorders like depression and therefore may be acted upon by emotional stress component also[77].

Thus the above data has scratched only the surface in terms research showing association of lifestyle choices withCoQ_{10} and more detailed studies especially in Indian population is necessary to determine the scope of CoQ_{10} in the prevention of NCD.

Uric acid

Uric acid is the end product of purine metabolism[7]. It is formed as a result of xanthenes and hypoxanthines being degraded by xanthine oxidase enzyme. In higher mammals this happens to be the end step whereas in lower animals the reaction proceeds further with production of allantoin[13]. There is a theory stating that this suppression of allantoin step is an adaptive mechanism of higher mammals to utilize the antioxidant property of uric acid[13].



Uric acid is a strong reducing agent and has an interdependent relationship with vitamin C for its antioxidant actions especially in the presence of lipid peroxides in plasma[78].

Uric acid levels determine many clinical outcome of many diseases especially neuro-degeneration and insulin resistance[79,80].

High levels also known as hyperuricemis is seen due to increased purine and high fructose in diet, excessive and sudden weight loss, reduction in kidney function of excreting uric acid. The excess gets accumulated in joints to result in a disease called as Gouty arthritis. Other than these increased levels are associated with increased cardiovascular risk and increased insulin resistance in metabolic syndrome and type 2 diabetes mellitus[79,80].

Lower levels are associated with lower dietary zinc, iron and molybdenum due to their action as constituents for xanthine oxidase enzyme. In neurodegenerative diseases like multiple schlerosis and parkinson's disease variations in uric acid levels have been reported.

Uric acid action as pro-oxidant[78]

Uric acid has been proven to react with other radicals and form free radicals by its oxidant function especially with peroxynitrite. The radicals formed are the products of serial steps of uric acid degradation which includes urate anion, amino carbonyl ion etc. this action of uric acid as an oxidant seems to increase in the presence of lipid hydroperoxides and also excess Copper ions. They promote excess LDL oxidation and production of radicals causing oxidative damage. They

also cause induction of protein kinase p38 as part of intracellular signaling in adipocytes. This can be used to explain the direct action of uric acid on adipose tissue and its adverse effects. Pro-oxidant action of uric acid is also promoted by NOX dependent superoxides and reduction oxidation couple reaction signaling. But the relationship and actions are very complex and is not fully understood.

Uric acid as an antioxidant[7,78]

Uric acid has been shown to have action as an antioxidant by scavenging of singlet oxygen, erythrocyte membrane protection, hydroxyl radicals etc. the antioxidant function is fully sustainable only in a water loving environment like Plasma.

Uric acid have been proven to have major action on central nervous system by the studies conducted on association between uric acid levels and neurodegenerative disorders like multiple schlerosis and acute stroke. They have also been shown to predict the risk for occurrence of stroke. Its antioxidant action has been enumerated by studies in which peroxynitrite scavenging, decreasing the endothelial nitric oxide and also decreasing the neutrophil infiltration have all been attributed as modes of uric acid action.

Influence of lifestyle factors on uric acid levels

There is a great deficiency in the number of studies regarding uric acid and lifestyle factors in healthy individuals.Studies have been conducted to evaluate uric

acid levels along with other parameters to study its influence on age, BMI, gender, diet as a marker for prediction of diseases like metabolic syndrome, type 2 diabetes, cardiovascular disease etc[79,80,81]. The hyperuricemia in these cases were defined as >7 mg/dl in men and > 6 mg/dl in women[82]. The role of hyperuricemia in the prediction of these diseases is correlated with increased oxidative stress, endothelial dysfunction etc. From the cohort studies done to predict the association of hyperuricemia with the above mentioned diseases in previously healthy individuals, the association with lifestyle factors could be deciphered.

Such studies show that uric acid is positively correlated with age especially in women and is increased in males compared to women[83]. Many other studies confirm this finding in addition to showing the positive association of serum uric acid with body mass index, waist circumference and smoking and alcohol[81,84]. This finding also is consistent with different ethnic groups like Italians, Asians etc[84]. African population show increased serum uric acid levels[85].

Another study done among healthy individuals among African ethnicity in Seychelles show that serum uric acid are increased in men when compared to women and is significantly associated with age, BMI, and alcohol consumption. But there was no correlation with smoking[81].High association is seen between the metabolic syndrome risk factors and serum uric acid levels as evidenced by many studies[86].

In a separate study uric acid has been shown to decrease exercise induced oxidative stress in a healthy population sample. In this study administration of uric acid following a bout of strenuous exercise resulted in improving the overall free radical; scavenging property and decrease in oxidative stress[87].

Many studies have proven the increasing pattern of serum uric acid with increased alcohol consumption[85]. Diet with increased purine intake seems to increase the uric acid level but there are no studies to show the influence of diet and nutrition on uric acid as an antioxidant. One study where the influence of juice intake was associated with antioxidants it was seen that it had no significant relationship with uric acid levels[37].

. The concept of its antioxidant function is itself complex because of the paradoxical mechanism of urate redox shuttle[78]. Hence there are numerous studies where the serum uric acid levels were studied to determine its potential as marker for predicting many diseases but there are very few or no studies where the influence of only lifestyle factors were associated with uric acid with exclusion of other potential confounding factors.

In conclusion the literature available regarding lifestyle factors and their influence on the antioxidants pertaining to this study have been reviewed in detail, and some

obvious deficiencies and controversies have been noted. Some of the deficiencies, according to our knowledge, are:

There are no studies concerning normal south Indian population

Combination of all the lifestyle risk factors and antioxidant levels

There are very few or none showing the influence of socioeconomic condition on antioxidants

Daily physical activity effects on antioxidants. As studies exist to show the influence of exercise induced effect only.

Lack in studies concerning lifestyle risk factors and Coenzyme Q10

Influence of dietary intake of fruits and vegetables and antioxidants.

Only risk factor and disease prediction studies are seen in uric acid

And many studies also have given controversial results showing the presence of other unknown confounding factors.

Hence this field has more potential to be tapped and more studies are needed to fully understand the influence of lifestyle factors on serum antioxidant levels.

MATERIALS AND METHODOLOGY

This Cross-sectional Observational study conducted from February 2015 to June 2016 was started after getting approval from the Institutional ethical committee.

Healthy volunteers of the age group 20-30 years were randomly selected from KarpagaVinayaga Institute of Medical Sciences, Maduranthagamand they were tested for fasting serum glucose (FSG), fasting lipid profile and hemoglobin. Out of them 104 apparently healthy individuals with cut off values of FSG <100mg/dl, serum triglycerides <170mg/dl, serum cholesterol< 250mg/dl and hemoglobin >12 g/dl for females and >14g/dl for malesand with no significant medical illness that can cause oxidative stress were selected to participate in this study.

The study group consisted of 53 females and 51 males and every one of them were enrolled after providing complete information about this study and after getting consent in written format in their own preferred language in the presence of a witness.

The subjects were studied in terms of their modifiable and non-modifiable risk factors and their relationship with serum antioxidant levels. The study parameters used were:

Age (20 – 30 years)

Gender (males and females)

- Dietary(in terms of fruits and vegetable intake) using FACET questionnaire

  Socioeconomic condition by questionnaire

  Psychological stress by stress questionnaire

  Physical activity and exercise wise:low, Moderate, High.

  By International physical activity questionnaire[15]

  Smoking factor

  Alcohol intake

  Body mass index (BMI)

Exclusion criteria:

Subjects with diseases causing oxidative stress and low antioxidant levels like:

Neurodegenerative diseases[88]

Cardiovascular diseases[89]

Diabetes mellitus[90]

Hypertension

Cancer[91]

Cerebrovascular diseases

Dyslipidemia[92]

Liver diseases[93]

Tuberculosis

Anaemia

Drug intake history

Assessment parameters:

History

Physical Examination

Systems review

Questionnaires

Laboratory investigation:

Superoxide dismutase-Spectrophotometric method

Glutathione peroxidase -Spectrophotometric method

Coenzyme Q_{10} -HPLC

Uric acid-Uricase / POD method

Other investigations:

Fasting serum glucose

Estimated by Glucose oxidase/peroxidase method with accepted range of 70-100mg/dl

Fasting lipid profile

Total cholesterol

Estimated by Cholesterol oxidase/peroxidase method with accepted range of 130-250mg/dl

Serum triglycerides

Estimated by Glycerol phosphate/peroxidase method with accepted range of 60-170mg/dl

Hemoglobin

Estimated by Drabkin's method with accepted range of 12-14mg/dl for females and 13.5-17 mg/dl for males.

HISTORY:

History was elicited according to Pro forma enclosed in the annexure. Importance was given to their family history to look for history of non communicable diseases among their parents or siblings. Detailed drug history was taken to eliminate any drugs intake including multivitamins which can alter the antioxidant levels. History was the first level for the selection process of individuals to include them in the study. Only individuals who appeared healthy were asked to participate in the study.

GENERAL AND SYSTEMIC EXAMINATION:

General examination including Anthropometric measurements and systemic examinationwas done according to the proforma attached in the annexures.

QUESTIONNAIRES:

Internationally pre validated questionnaires with good reliability and specificity were selected. All the lifestyle factors were evaluated using independent questionnaires which were internationally recommended. . Care was taken to ensure that they cater to the needs of this study by making them specific to the age group and the life style factor under study and all the questions pertained to the issue in discussion. They were structured closed ended questionnaires with

multiple choice answers and in some cases dichotomous answers. They were easy to comprehend and were self administered in their preferred language.

FACET QUESTIONNAIRE[94] annexure 6

Five a day community evaluation tool is a well known and approved questionnaire to evaluate the dietary habits of adult of age group 15-69 years .This questionnaire can be self administered easy to comprehend and answer. It contains questions regarding the intake, eating behavior and attitude towards fruits and vegetables and knowledge of impact of fruits and vegetables intake on health i.e healthy eating habits are evaluated. The questionnaire is analyzed by using statistical methods and the study participants were scored into two groups. Table 1

Table 1:Scoring system for FACET questionnaire

Portion intake	Score
= 5 a day	2
< 5 a day	1

SOCIOECONOMIC STATUS QUESTIONNAIRE annexure 7

This questionnaire caters to both Urban and Rural population and also for all sections of the society[95]. This has a set of 22 questions and scoring system to classify the individuals according to their socioeconomic class. The scoring system used is as seen in table 2:

Table 2: Scoring system for socioeconomic class

SOCIAL STATUS	***SCORE***
UPPER HIGH	= 76
HIGH	61-75
UPPER MIDDLE	46-60
LOWER MIDDLE	31-45
POOR	16-30
VERY POOR OR BELOW POVERTY LINE	=15

The individuals are classified into these 6 classes and the comparison of the anti oxidant levels in each of these classes were done.

STRESS EVALUATION QUESTIONNAIRE Annexure 8

This questionnaire devised by the International stress Management Association UK contains a list of 25 questions for a simple psychometric analysis of the stress level of that particular individual. This is also self-administered. Table 3

Table 3: Scoring system for Stress questionnaire

Stress level	Score
Category 1	=4
Category 2	5-13
Category 3	=14

The category 1 denotes individuals who are having no or very little chance for stress related changes. The category 2 denotes that the likelihood of having stress related changes are high. The category 3 denotes that they have definite stress related changes and they would need intervention.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE annexure 9

This questionnaire devised for International use in all settings has two formats a LONG and a SHORT format[95]. In this study the easy to use Short format was use

to evaluate the physical activity of the individuals participating in the study. The scoring system has psychometric properties and uses a METS system of scoring. METS is defined as multiples of resting metabolic rate, i.e. the energy requirements calculation for each activity is done and then they are scored according to this system. Table 4

Table 4: Physical activity and METS scoring

MET	ACTIVITY
3.3	Walking
4.0	Moderate
8.0	Vigorous

The formula is applied according to the activity and then the Total Physical Activity Score is calculated.

Formula = resting metabolic rate x minutes spent in activity x number of days

Total physical activity MET-minutes/week = sum of the walking activity, moderate activity and vigorous activity/ week.

Then they are classified as seen in table 5

The sitting question at the end is used to denote sedentary lifestyle. They are expressed as median values or interquartile ranges

Table 5: Scoring system for IPAQ

Score	Requirement to fulfill
High	vigorous activity for minimum 3 days total physical; activity = 1500 MET-minutes/week ***OR*** 1 week of any combination of walking+moderate +Vigorous activities Total physical activity = 3000 MET-minutes/week.
Moderate	3 or more days of vigorous-intensity activity of at least 20 minutes per day ***OR*** 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day ***OR*** 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum Total physical activity of at least 600 MET-minutes/week.
Low	Not belonging to any of these above categories

SMOKING QUESTIONNAIRE

The classification was based on smoking manufactured or hand rolled tobacco or cigarettes. They were classified as in table

Where Pack-years is equal to smoking 20 cigarettes a day for 1 year[5]

Then comparison is made to determine the changes in antioxidant levels for each category as seen in table 6.

Table 6: Scoring for smoking categories

Category 1	1 or more pack years
Category 2	Not smoking now but used to smoke earlier.
Category 3	Not smoked at all, Occasional smoker, <1 pack year

ALCOHOL QUESTIONNAIRE

The individuals are classified based on their alcohol intake into categories as seen in table 7.

Table 7: Scoring for alcohol categories:

Never had a drink of alcohol	1
Low consumption (ethanol <20g/day)	2
High consumption (ethanol>20g/day)	3

In India, a standard drink corresponds to 10g of absolute alcohol. Based on the amount of absolute alcohol found in domestic liquor they were classified as detailed above. The content of absolute alcohol in common domestic liquoe are as follows

30ml straight spirits-10g

330ml of beer-10g

100ml of wine-10g

Then the category 2 and especially category 3 subjects are further evaluated with Alcohol uses disorders Identification test (AUDIT) that is the questionnaire recommended by WHO as a tool for picking up of early signs of hazardous drinking of alcohol. This screening questionnaire is used in this study to identify the level of hazardous drinking habits seen in individuals and compared to antioxidant levels. This screening test contains ten questions with separate score for each totaling to 40. A score of = 8 is considered as hazardous drinking. Annexure10

BODY MASS INDEX CALCULATION

Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).[5]

They were classified in terms of table 8

Table 8: WHO recommended BMI table for Asians

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

LABORATORY INVESTIGATION:

Sample collection and preparation:

2ml of whole blood venous sample were collected in Light protected heparinized tubes. And aliquoted into 5 ampoules and sample preparation was done according to each analyte estimation procedure.

SUPEROXIDE DISMUTASE

SAMPLE PREPARATION:

Whole blood samples in light protected Heparinized tubes were collected. 0.5mL of sample aliquot was taken in a clean dry test tube and centrifuged at 3000 rpm for 10minutes and then plasma aspirated.Then Erythrocytes were washed with 3 mL of 0.9% Sodium Chloride solution, centrifuging for 10 minutes at 3000 rpm and the same step was repeated four times.

Then the washed erythrocytes were made up to 2.0mL with cold redistilled water (4℃) to get the lysate, mixed and kept at 4℃ for 15 minutes. The lysate is then diluted with 0.01 mol/L Phosphate buffer at pH 7.0.This is done to ensure the inhibition percentage falls between 30% and 60%.

INHIBITION PERCENTAGE:

Function of the ratio between inhibitor and substrate, and not the absolute concentration of inhibitor.

% inhibition = [(normal activity – inhibited activity)/normal activity]

Dilution formula:

Final dilution factor = 100 i.e 1:100

Dilution factor = V_f/V_i

V_f– final volume

V_i – initial volume

100 = V_f/ 0.5mL

Final volume = 50mL

And then a 25 fold dilution of lysate is prepared.

25 × original volume after all process 2 mL = 50 mL

48 mL of 0.01mol/L of Phosphate buffer is added to 2.0 mL of lysate to get 25 fold dilution of lysate.

PRINCIPLE:

Superoxide dismutase increases the dismutation of the toxic superoxide radical

($O_2^{\bullet}$), which is produced during the oxidative process, to form hydrogen peroxide and molecular oxygen.

In this method xanthine and xanthine oxidase are added to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5- phenyltetrazolium chloride (I.N.T) to form a red formazan dye.

XANTHINE OXIDASE

XANTHINE ----------> URIC ACID + O_2?

O_2?

I.N.T ----------> FORMAZAN DYE

The activity of the Superoxide dismutase enzyme in whole blood is then measured by the degree of inhibition of the above reaction.

SUPEROXIDE DISMUTASE

O_2? + O_2? + $2H^+$ ----------> O_2 + H_2O_2

STABILITY AND PREPARATION OF REAGENTS

Mixed substratecontaining Xanthine and I.N.T was reconstituted and vial was stored at 2 to 8?C and its stability was 10 days.

Buffer containing N-cyclohexyl-3-aminopropanesulfonic acid and Ethylenediaminetetraacetic acid, was ready to use and was also stored at 2 to 8?C as it would remain stable at that temperature till its expiry.

Xanthine oxidase was reconstituted and the vial was stable for 2 weeks if stored at 2 to 8?C.

Standard is also reconstituted and serial dilutions were prepared as seen in table 9.

Table 9: standard preparation for SOD

STANDARD	VOLUME OF STANDARD SOLUTION	SAMPLE DILUENT
S6	Undiluted standard	-
S5	5 mL of S6	5 mL
S4	5 mL of S5	5 mL
S3	5 mL of S4	5 mL
S2	3 mL of S3	6 mL
S1	10 mL Sample diluent 0.01mol/L Phosphate buffer pH7.0	-

The diluted standards are stable if stored at 2 to 8?C for 2 weeks.Standards were used for calibration before each run.

All the samples to be processed and reagents were brought to room temperature before every run.

PROCEDURE

After setting the Wavelength at 505nm and temperature at 37?C the values were taken using a 1 cm path length glass cuvette and the measurement was made against air.

After setting these parameters the following procedure was followed as seen in table 10.

Table 10: Procedure for SOD estimation in UV-VIS Spectrophotometer

eagents	Sample diluent-S1	Standard S2	Standard S3	Standards S4	Standards S5	Standards S6	Diluted sample lysate - Test
) pipette ıto ıvette							
lank - ample iluent	0.05mL	-	-	-	-	-	-
tandard	-	0.05mL	0.05mL	0.05mL	0.05mL	0.05mL	-
est ˈiluted ımple ysate	-	-	-	-	-	-	0.05mL
fixed ıbstrate	1.7mL	1.7mL	1.7mL	1.7mL	1.7mL	1.7mL	1.7mL
fix well	Mix well	Mix well	Mix well	Mix well	Mix well	Mix well	Mix well
anthine xidase	0.25mL	0.25mL	0.25mL	0.25mL	0.25mL	0.25mL	0.25mL

As soon as the cuvette is placed inside the holder and the lid closed the timer is started. First reading taken at 30 seconds which is Abs 1 and then final absorbance reading taken at 3 minutes Abs 2

CALCULATION:

ΔA/min =(Abs2 –Abs 1)/3

Sample diluent rate (S1 rate)= rate at which no inhibition of reaction takes place ie 100% reaction rate

ΔA/min of Standards S2 to S6 and sample were calculated separately.Then they were converted into percentage of inhibition by the formula:

% inhibition of standards S2 to S6 =100 – (ΔA of standards S2 to S6 ×100)/ (ΔA of S1)

% inhibition of Sample =100 – (ΔA of sample ×100)/ (ΔA of S1)

Then a Graph of standard curve was plotted with X axis percentage inbition of standard and Y axis Log_{10} (standard concentration in SOD units/mL)

Then using the graph the according to percentage of inhibition value of the sample the SOD units/mL were calculated.

SOD units/mL of Whole blood = SOD units/mL from standard curve× dilution factor

REFERENCE RANGE

164-240 U/mL

GLUTATHIONE PEROXIDASE

METHOD

Manual method based on Paglia and Valentine.

REAGENTS PREPARATION AND THEIR STABILITY

Reagent was prepared by reconstituting one vial of concentrate reagent, containing Glutathione, Glutathione reductase and NADPH, with 6.5 mL of Buffer which contains Phosphate buffer and Ethylenediaminetetraacetic acid. This prepared reagent solution is stable for 48 hours at 2?C to 8?C.

Buffer solution is stable at 2?C to 8?C till its expiry date.

Cumenehydroperoxide is prepared by diluting 10 µL of given concentrate solution with 10mL of saline and was shaken vigorously to dissolve it completely. This dilute solution was prepared fresh daily. The Concentrate solution was stable when stored at 2?C to 8?C till its expiry date.

The diluting agent for sample preparation was prepared by diluting the concentrate solution given with 200 mL of redistilled water

SAMPLE PREPARATION

Venous blood sampling was done and was collected in light protected Heparinised tubes. 0.05 mL aliquot of whole blood was diluted with 1 mL diluting agent,which was provided with the estimation kit, and was incubated for 5 minutes at 25?C and then 1 ml of Hemoglobin reagent was added, mixed well and assayed within 20 minutes of adding the Hemoglobin reagent.

PRINCIPLE

Glutathione gets oxidized by CumeneHydroperoxide by the enzyme glutathione peroxidase.Then on addition of Glutathione reductase and NADPH the oxidized Glutathione gets converted into reduced form and NADPH gets converted into $NADP^+$ due to oxidation. The descending absorbance value at 340nm is measured and from that the level of Glutathione peroxidase is calculated.

Glutathione peroxidase

$$2GSH + ROOH \text{ ---------- } > ROH + GSSG + H_2O$$

Glutathione reductase

$$GSSG + NADPH + H^+ \text{ ---------- } > NADP^+ + 2GSH$$

PROCEDURE

After setting the Wavelength at 340nm and temperature at 37?C the values were taken using a 1 cm path length glass cuvette and the measurement was made against air. The procedure is as given in table 11.

Table 11: Procedure for GPx estimation in UV-VIS spectrophotometer

Reagents to pipette into cuvete	Reagent Blank	Diluted Sample
Diluted sample	--	0.05mL
Distilled water	0.05mL	--
Reagent R1	2.50mL	2.50mL
Cumene R2	0.10mL	0.10mL

Mixed well and initial absorbance of reagent blank read after one minute and timer was started simultaneously and the absorbance values at 1 minute and 2 minutes were read and noted. The same procedure was repeated for sample also i.e. absorbance after 1 minute the timer was started then again readings were noted at 1 minute and 2 minutes.

CALCULATION

TO DETERMINE THE CHANGE IN ABSORBANCE Δ340nm/min

The 3 Absorbance values were plotted as a function of time i.e. X axis Time in minutes and Y axis Absorbance at 340nm. Then the slope rate of change "m" of linear portion of the curve was calculated using the formula

(Slope) $m = (y_2-y_1)/(x_2-x_1)$

Thus the "slope" value for reagent blank and samples were calculated and then subtracted to get ΔA 340nm/min

Glutathione peroxidase concentration was calculated with the formula

U/L of Hemolysate= 8412 x ΔA 340nm/min

This gave the value of diluted sample

Dilution factor calculation

Vf/Vi = final volume/initial volume = 2.05/0.05=41

Glutathione peroxidase concentration in whole blood (U/L) =8412 x ΔA 340nm/min x 41

REFERENCE RANGE

4171-10881 U/L

URIC ACID

METHOD

Uricase/POD

REAGENTS AND THEIR PREPARATION AND STABILITY

Uric acid enzyme reagent and Uric acid standard with value 6mg/dL were provided in the kit and were ready to be used and when stored at 2-8℃ were stable till their expiry date.

SAMPLE PREPARATION

Heparinized plasma was the sample used.

PRINCIPLE

Uric acid when acted upon by Uricase enzyme gets converted into Allantoin and hydrogen peroxide. The released hydrogen peroxide when made to further react with phenolic compound and 4 aminoantipyrine in the presence of peroxidase enzyme forms a red colouredquinoneimine dye complex. The red colour intensity formed is measured and is found to be directly proportional to the concentration of Uric acid present in the sample.

PROCEDURE (table 12)

This is an end point reaction measured at wavelength 505nm and temperature 37?C

Table 12: Procedure for Uric acid estimation

Reagents to be added	Blank	Standard	Test
Enzyme reagent	1mL	1mL	1mL
Standard		25µL	
sample			25µL

Mixed well and then kept in incubator which was set to temperature 37?C for 5 minutes.

Then the absorbance values of standard and sample were measured at 505nm.

CALCULATION

URIC ACID (mG/dL)= Abs (test)/Abs (standard) x 6

REFERENCE RANGE

Male: 4.0-7.2mG/dL

Female: 2.7-6.5mG/dL

COENZYME Q_{10} ASSAY

METHOD

Plasma total Coenzyme Q_{10} assay by single dilution method[97] using High-performance liquid Chromatography.

REAGENTS AND THEIR PREPARATION AND STABILITY

MOBILE PHASE

Ethanol – Methanol 65%-35% HPLC grade

EXTRACTION OF COENZYME Q_{10}

Para – benzoquinone solution prepared by mixing 2mg in 1mL of acetonitrile.

STANDARD PREPARATION REAGENT:

N Propanol:water 5:1 HPLC grade- Solvent

Pure Coenzyme Q_{10} HPLC grade >99.9% purity (Sigma-aldrich)

All reagents stored at -20?C in light protected bottles in dark

SAMPLE PREPARATION

Fresh venous sample was taken in light protected heparinized tubes.

The sample was centrifuged at 4000g at 4?C for 15 minutes

Estimation was done in fresh plasma. In some samples when estimation was not possible immediately it was stored at -80?C for next day estimation. But analyte was found to be stable up to 3 months when stored in light protected tubes at -80?C.

PRINCIPLE

The Coenzyme Q_{10} present in the sample is oxidized with para- benzoquinone and then extracted using 1-propanol. The extracted supernatant is then injected into the HPLC and 20µL volume is taken up by the system for analysis. On reaction with 1,4 para benzoquinone the CoQ_{10} present in the sample gets completely oxidized because para benzoquinone has a higher redox potential value when compared to CoQ_{10}. Following this process extraction with 1 propanol, which is an alcohol, causes precipitation of protein and extraction of oxidized CoQ_{10} that is the total CoQ_{10} present in the sample.

EXTRACTION OF COENZYME Q_{10}

200 µL of fresh plasma taken and then 50 µL of 1,4-benzoquinone solution was added into a light protected tube. Then the tube was vortexed for 10 seconds. After 10 minutes standing 1 mL of n Propanol was added. Again the tube was vortexed for 10 seconds. Then centrifuged at 10000 rpm for 2 minutes so that the protein precipitate gets settled down. The resultant supernatant was removed.All the

processing was done in an ice box in a dark room.The supernatant was stable for maximum 3 days when kept at 20?C.

STANDARD PREPARATION

Pure coenzyme Q_{10} >99% purity HPLC standard bought from Sigma Aldrich was used to prepare standard stock solution. The diluting solution used was a mix of 5ml of propanol and 1ml of water. Stock solution was prepared with 24mg of CoQ10 standard with 6ml of diluting solution i.e. 4mg/ml. The direct dilutions from standard stock solution were made to get 0.4mG/L, 0.8mG/L, 1.2mG/L, 1.6mG/L, 2.0mG/L and 2.4mG/L with addition of diluting solution to aliquots of stock solution. Standards were prepared in dark room without light exposure to prevent oxidation of standard. Standards were prepared fresh everyday.

PROCEDURE

HPLC apparatus was LC100 MS make with an injector capacity uptake of 20µLvolume.C18 column(4.6mm ID x 25mm)was used to analyse in reverse phase.

Wavelength was set at 275nm.Flow rate 1ml/min

Mobile phase was run and removal of air bubbles was done if present.

The working condition of the apparatus ensured to be good.

The machine was set at external standard mode.

Standard was run and the peak at 275nm after 12 minutes elution time noted.

Direct dilutions of the standard stock were run to calibrate the apparatus. The dilutions were 0.4mG/L, 0.8mG/L, 1.2mG/L, 1.6mG/L, 2.0mG/L and 2.4mG/L.

And using the external standard method of calculation the values of standards run were cross checked every day before the running of the samples.

Following the standardization sample processing was done:

Mobile phase was run for 10minutes. Then 20 µL of supernatant derived from the test subject sample was injected into the injector. Then the system was switched to run mode for 12 minute.

The peak at 275nm after 12 minutes elution time noted.

Merging of the peaks of standard and sample and noting the uniformity in elution time between the standard and sample confirmed the peak specific for the test substrate.

Spiking the sample with external standard and predicting the change in peak area in software analysis further confirmed the test peak.

Then the peaks with peak area was analysed by using peak area analysis software of Cyberlab.

From the linear plot of standard the concentration of sample was determined.

CALCULATION

External standard calculation method

Response factor = Peak area/ standard amount

Amount of analyte = peak area/ response factor = sample amount

REFERENCE RANGE

TOTAL COENZYME Q_{10} - 433-1532 μG/L

Statistical analysis:

For this cross sectional observational study the statistical analysis was done using SPSS Inc. version 17.0 IBM software. The mean median 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 up to < 0.05 was taken as significant. Frequency distribution of the lifestyle factors of the study population was studied using descriptive statistics. Inter relationship between the four antioxidants were also studied using Pearson correlation and scatter plot was drawn for significant associations. Gender wise alteration between the lifestyle factors and antioxidants were also studied.

RESULT

In this cross-sectional observational 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, median and standard deviation values for the age, BMI, and the four antioxidants studied are given in table 13.

Table 13: Mean, median and standard deviation of numerical parameters in this study.

PARAMETER	VALUES Mean± S.D, Median
Age (in years)	25.30±3.214, 25
BMI (kg/m^2)	22.5±4.65, 22
Superoxide dismutase (U/mL)	181.78±17.9, 180
Glutathione peroxidase (U/L)	8607.36±1237.2, 8900
Coenzyme Q_{10}(µg/L)	484.12±59.65, 472
Uric acid (mG/dL)	4.92±1.27, 5.35

The lifestyle factors were studied by means of questionnaires and divided into various categories. The distribution frequency of the lifestyle categories in the study sample is given in table 14.

Table 14: Frequency distribution of lifestyle categories among the study population selected.

Lifestyle factors and their categories	Frequency
Gender – Males	51
Females	53
Socio economic score- Upper high	8
High	38
Upper middle	24
Lower middle	30
Poor	4
Very poor or below poverty line	0
IPAQ- highly active	14
Moderately active	43
Lowly active	47
FACET- < 5 a day	79
= 5 a day	25
Stress – no risk	28
Moderate	76
High risk	0

Smoking – 1 or more pack years	8
Not smoking now	16
Not smoked at all or <1 pack year	80
Alcohol- never had alcohol	68
Low consumption	34
High consumption	2
BMI- normal	45
Underweight	11
Overweight	34
Pre-obese	14
Extreme obesity	0

Pearson correlation studies between the lifestyle factors and serum SOD, GPx, CoQ_{10} and Uric acid were done. The summary of the findings is shown in table 15.

Superoxide dismutase

SOD had a positive relationship with increased dietary intake of fruits and vegetables ($p<0.01$). It also increased with increased smoking with level of significance being < 0.05. It showed a negative relationship with age ($p<0.05$), socio-economic score ($p<0.05$), psychological stress ($p<0.01$), BMI ($p<0.05$).

this study SOD showed no significant relationship with physical activity and alcohol consumption. Though it showed increased values as physical activity increases and negative trend of values with alcohol consumption.

Glutathione peroxidase

GPx hada positive correlation with age and alcohol ($p<0.01$), whereas it had a negative correlation with physical activity and smoking ($p<0.01$). GpX had no significant correlation with socioeconomic condition, diet, psychological stress and BMI. But it had increasing values with higher socioeconomic class and increased stress and BMI. And the values tended to be lower with increased dietary intake of fruits and vegetables.

Coenzyme Q_{10}

CoQ_{10} had a significant positive association with smoking and dietary intake of fruits and vegetables ($p<0.01$), and an inverse relationship with socioeconomic class and BMI ($p<0.01$). With increasing age, psychological stress and alcohol consumption the CoQ_{10}value seems to decrease and with increasing physical activity the CoQ_{10}value tends to increase but the association was not significant.

Uric acid

The serum uric acid values were positively correlated with age ($p<0.01$), alcohol consumption ($p<0.01$), and BMI ($p<0.01$). And it had a negative relationship with socioeconomic class ($p<0.01$), physical activity ($p<0.01$), and smoking ($p<0.01$). And though it had no significant association it had an increasing trend of values with increased stress and decreased dietary intake of fruits and vegetables.

Table 15: Pearson correlation study between serum antioxidants and the lifestyle factors.

ioxidant	Age	SE score	IPAQ score	FACET score	STRESS score	Smoking score	Alcohol score	BMI score
eroxide nutase rson 2 tailed	-.204* .038	-.198* .044	.191 .052	.284** .004	-.331** .001	.216* .028	-.162 .101	-.207* .035
tathione oxidase rson 2 tailed	.266** .006	.059 .549	-.857** .000	-.103 .300	.155 .117	-.594** .000	.765** .000	.039 .691
nzyme Q_{10} rson 2 tailed	-.092 .354	-.309** .001	.068 .492	.358** .000	-.093 .348	.258** .008	-.110 .267	-.259** .008
acid rson 2 tailed	.340** .000	-.273** .005	-.344** .000	-.016 .876	.043 .661	-.381** .000	.436** .000	.733** .000

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

The antioxidants also showed significant relationship with each other as shown in table 16 and in scatter plot diagrams 1 and 2. SOD and GPx are negatively associated ($p<0.05$); SOD and CoQ10 are positively associated ($p<0.01$), GPx and uric acid are positively associated ($p<0.01$).

Table 16: Pearson correlation study between the antioxidants.

Antioxidants	Superoxide dismutase	Glutathione peroxidase	Coenzyme Q_{10}	Uric acid
Superoxide dismutase Pearson Sig 2 tailed	1	-	-	-
Glutathione peroxidase Pearson Sig 2 tailed	-.210* .032	1	-	-
Coenzyme Q_{10} Pearson Sig 2 tailed	.526** .000	-.078 .433	1	-
Uric acid Pearson Sig 2 tailed	-.155 .116	.330** .001	-.136 .168	1

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

Diagram 1: scatter plot diagram showing the relationship between SOD and CoQ_{10}

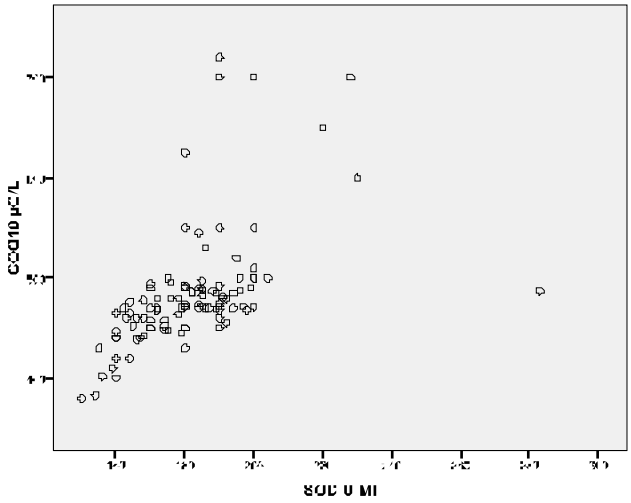


Diagram 2: scatter plot to show relationship between SOD and GPx

For association between gender and antioxidant levels Spearman's Rho test was performed. Table 17 shows the results.

Table 17: Spearman rho test for association between gender and antioxidant levels.

Antioxidant	Gender
Superoxide dismutase Correlation coefficient Sig 2 tailed	.084 .396
Glutathione peroxidase Correlation coefficient Sig 2 tailed	-.487** .000
Coenzyme Q_{10} Correlation coefficient Sig 2 tailed	.006 .951
Uric acid Correlation coefficient Sig 2 tailed	-.433** .000

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

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 18 and table 19.

Males

Among males SOD had a positive association with dietary intake of fruits and vegetables ($p<0.01$), psychological stress ($p<0.01$), smoking ($p<0.01$) and alcohol consumption ($p<0.05$), whereas the levels decreased with age ($p<0.01$).

GPx had a positive association with physical activity ($p<0.01$) and alcohol consumption ($p<0.01$). And negative association with smoking ($p<0.01$).

Coenzyme Q_{10}had a negative relationship with age ($p<0.05$), socioeconomic class ($p<0.01$), alcohol consumption ($p<0.05$) and BMI ($p<0.01$). it had a positive relationship with dietary intake of fruits and vegetables ($p<0.01$) and smoking ($p<0.01$).

Uric acid had a negative association with physical activity ($p<0.05$) and smoking ($p<0.05$), and a positive relationship with alcohol consumption ($p<0.05$) and BMI ($p<0.01$).

Females

Among females SOD had a positive relationship with physical activity ($p<0.05$) and dietary intake of fruits and vegetables ($p<0.05$).

GPx had a positive association with physical activity ($p<0.01$).

Coenzyme Q_{10} had a positive correlation with dietary intake of fruits and vegetables ($p<0.05$).

Uric acid had a negative association with socioeconomic class ($p<0.01$) and positive association with BMI ($p<0.01$).

Table 18: Pearson correlation study to determine the influence of lifestyle factors on serum antioxidants in males

ioxidant	Age	SE score	IPAQ score	FACET score	STRESS score	Smoking score	Alcohol score	BMI score
eroxide	-.380*	-.239	.112	.291*	.454**	.366**	.310*	-.275*
nutase	.006	.091	.434	.038	.001	.008	.027	.051
:son								
2 tailed								
tathione	.051	.129	.850**	-.173	.036	-.599**	.884**	.185
oxidase	.722	.369	.000	.226	.800	.000	.000	.193
:son								
2 tailed								
nzyme Q_{10}	-.296*	-.445**	.132	.438**	-.104	.431**	-.280*	-.361**
:son	.035	.001	.356	.001	.467	.002	.047	.009
2 tailed								
: acid	.129	-.026	-.352*	-.214	.134	-.311*	.333**	.750**
:son	.366	.854	.011	.131	.350	.026	.017	.000
2 tailed								

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

Table 19: 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 dismutase Pearson Sig 2 tailed	.064 .650	-.172 .217	.296* .031	.275* .047	-.238 .086	-.081 .564	.075 .596	-.145 .299
Glutathione peroxidase Pearson Sig 2 tailed	.150 .284	.045 .749	.805** .000	.007 .962	.196 .160	-.206 .138	.224 .107	-.213 .125
Coenzyme Q_{10} Pearson Sig 2 tailed	.062 .661	.168 .228	.031 .827	.286* .038	-.096 .493	.029 .838	.069 .634	-.141 .314
Uric acid Pearson Sig 2 tailed	.229 .100	-.467** .000	-.053 .704	.164 .240	-.107 .446	-.116 .408	.049 .729	.866** .000

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

Interrelationship between antioxidants among males and females was also seen separately. They are shown in table 20 and 21.

Table 20: Relationship within the antioxidants in males

Antioxidants	Superoxide dismutase	Glutathione peroxidase	Coenzyme Q_{10}	Uric acid
Superoxide dismutase Pearson Sig 2 tailed	1	-	-	-
Glutathione peroxidase Pearson Sig 2 tailed	.248 .080	1	-	-
Coenzyme Q_{10} Pearson Sig 2 tailed	.712** .000	-.256 .070	1	-
Uric acid Pearson Sig 2 tailed	-.422** .002	.415** .002	-.407** .003	1

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

Table 21: Relationship within the antioxidants in females

Antioxidants	Superoxide dismutase	Glutathione peroxidase	Coenzyme Q_{10}	Uric acid
Superoxide dismutase Pearson Sig 2 tailed	1	-	-	-
Glutathione peroxidase Pearson Sig 2 tailed	-.179 .200	1	-	-
Coenzyme Q_{10} Pearson Sig 2 tailed	.375** .006	.082 .561	1	-
Uric acid Pearson Sig 2 tailed	.021 .880	-.089 .526	.002 .990	1

* Significant at 0.05 level (2 tailed)

** Significant at 0.01 level (2 tailed)

The statistical analyses performed separately in males and females showed the change in trend of influence of lifestyle factors on antioxidant levels. And the inter gender variability of antioxidant levels were also seen to show the role of gender difference in altering the antioxidants and their association with lifestyle factors.

DISCUSSION

This study was conducted among normal individuals to determine whether these apparently "healthy" individuals were actually healthy or they through their lifestyle choices have unknowingly initiated the process of internal metabolic instability that may lead to occurrence of NCD in the future. The individuals were studied as a whole and in terms oftheir inter gender variability, as among Indian population lifestyle choices are more gender centric when compared to other countries. The influence of each lifestyle choice on the antioxidants in general and among males and females separately with comparisons to previously conducted research is enumerated below:

AGE

Age though a non-modifiable risk factor plays a great role in influencing internal metabolism. In this study its role cannot be fully emphasized because of the restricted age group under research. But within this restricted age group itself significant association have been seen. When compared to the sample population in general the influence of age on SOD seems to be the same in males but it becomes statistically insignificant in females and a trend of increasing values with increasing age is seen rather than an inverse relationship as seen in males and the sample population in total in this study.Age has no significant association with

GPx among males and females but the whole sample population showed positive relationship. These findings for SOD and GPx are consistent with the studies conducted by Alejandro et al[28] and PP Singh et al[31]. Among males age shows a negative influence on CoQ_{10} but no significant association among females. Kaikonnen et al have shown positive influence of age on CoQ_{10} and a study conducted among only healthy female showed no association between age and CoQ_{10}. As in this study the selected age group has peak of CoQ_{10} it could be the reason for insignificant result in females. No significant influence of age was seen on uric acid among males and females but a positive association was seen in the whole sample,which is consistent with the previous conducted studies[83].

DIET

Dietary intake of fruits and vegetables can theoretically be said to increase the antioxidants, as they are one of their richest sources. Indian population has great diversity in their dietary habits. We have different dietary pattern when compared to Mediterranean diet or western diet. Hence the influence of dietary fruits and vegetables were alone taken into account in this study. Overall they have positive influence on SOD and CoQ10 levels which is consistent with other studies conducted. Among males and females when considered separately also this significance is maintained.Toaldo et al had similar results with SOD and uric acid and they also reported increase of GPx with fruit juice intake[37].

Gutierrez-Marisca et al reported about the importance of dietary intake of fruits and vegetables and CoQ10 in preventing oxidative DNA damage[75].

SOCIOECONOMIC CLASS

In this study we have evaluated the socioeconomic class of the individuals based on a unique pre-validated questionnaire pertaining to urban and rural Indian population. It showed that with increasing socioeconomic betterment there was a statistically significant decrease of SOD, CoQ_{10} and uric acid. When seen according to gender males shoed 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[30].

PHYSICAL ACTIVITY

In India physical activity is a result of our daily living activity and work activity only in most cases, especially in this sample population negligible number of individuals only exercises, the rest are ignorant or have no time to indulge in exercise. Based on this physical activity has been shown to negatively influence GPx and uric acid in general. And when considered among males alone the results were controversial i.e. positive association with GPx and negative with uric acid.

And in females alone the physical activity had positive significant influence on SOD and GPx. Mena et al have proven positive influence of physical activity on SOD and GPx but this has been proven to be true only in case of females in this study. Sarmiento et al showed positive influence of physical activity on CoQ_{10} levels[71].

STRESS

Psychological stress is highly prevalent unknowingly among our population. Only on evaluation it is known that at least 50% of the population under study have moderate risk for developing stress related diseases. It is shown that stress has a negative influence with SOD levels alone and when considered in males and females separately males had a positive association between stress and SOD which was consistent with previous studies conducted to evaluate the effect of stress on SOD levels in seminal plasma in males[39]. More studies are required as stress seems to cause many non-communicable diseases and the way to prevent the pathogenesis is still proving to be elusive.

SMOKING

Smoking is more prevalent among male population than in female population in India but this level is slowly rising among female population. In our population under study only three female smokers were present. Overall smoking had a

positive influence on SOD, CoQ10 and negative with GPx and uric acid. The influence on SOD and GPx were consistent with previously conducted studies but the negative association of uric acid with smoking is inconsistent and hence the presence of other confounding factors has to be evaluated. Among males alone positive association was seen between SOD and CoQ10 and negative with GPx and uric acid.No significance was found in females. Alejandro et al have shown increased levels of SOD with smoking[28].Conenet al[81] no showed no correlation between uric acid and smoking. Niklowitz et al showed male smokers had increased levels of CoQ_{10} than female smokers[73].

ALCOHOL

Similar to smoking alcohol consumption in females were negligible and hence the overall effect though showed positive association with GPx and uric acid only when seen among males alcohol had positive association with SOD, GPx and uric acid and showed negative association with CoQ_{10}. Females showed no significant association. This finding was consistent with the studies conducted in case of GPx and uric acid but in case of SOD studies have shown no association. The increased levels of GPx in alcohol consumers seem to be protective against oxidative liver injury.Kaikonnenet al[66] showed negative association of alcohol and CoQ_{10}.Chinenet al[84]showed positive association of alcohol on uric acid Lieber et

al[85] also proved the influence of alcohol on uric acid. Mulholland et al showed no relationship between alcohol and SOD and GPx[30].

BODY MASS INDEX

Increased BMI being one of the risk factors for many NCD has been widely evaluated in patients with NCD. In this study among healthy population BMI has a negative influence on SOD and CoQ10 and positive with uric acid. Among males BMI has negative influence on CoQ10 and positive with uric acid and in females BMI had positive influence on uric acid. Marklund et al have also shown decreasing levels of SOD with increasing BMI[38].Kaikonnenet al[66] have increasing levels of BMI with increasing CoQ_{10}.Conen et al[81] and Chinen et al[84]have shown positive association of uric acid with BMI.

Thus the findings in our study and the comparison with other studies show that most findings are consistent but certain discrepancies exist, which may need further detailed evaluation. The difference in ethnicity could not be seen as the study population significance was to belong to the same ethnicity.

CONCLUSION

In our population this study has shown the influence of the lifestyle factors on the serum antioxidant levels. From these findings we can safely come to the conclusion about the good and bad lifestyle choices. The bad lifestyle choices have been proven to initiate the process of internal metabolic alterations and in some cases there is an adaptive increase in antioxidant levels. The good lifestyle choices with healthy antioxidant levels seem to be consistent among the population. Individuals with good dietary intake of fruits and vegetables, showing moderate to high physical activity, in middle and above social class with no risk of stress related diseases, no smoking, low to nil alcohol consumption and normal BMI have good healthy antioxidant levels. Hence Health of an individual also depends on our lifestyle choices and not just on our routine biochemical parameters tested in our laboratories. This elevation of antioxidant may be protective initially but with further years and persistent insult to our metabolism we may go into a state oxidant antioxidant defense system failure which may initiate the onset of Non communicable diseases. In our study with a sample population of 104 itself shows the increased frequency of bad lifestyle risk factors prevalent. This may be the reason for increased incidence of NCD and shortening of the age of onset among south Indian population.

RESEARCH PROSPECTS

This study can be taken further by follow up of this study population in regular intervals of 5 or 10 years to see the alterations in their lifestyle choices and the relationship with antioxidant levels. They can also be studied to see the onset of NCD and the age of onset of NCD and whether they have any association with their lifestyle choices in their earlier age and their serial antioxidant levels over the years can be used to predict the risk of defense system failure and onset of NCD like diabetes mellitus, hypertension, cancer, cardiovascular diseases, neurodegenerative disorders etc.

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Siqueira,EmeliAraújo,Iracy L. S. Torres,

CarmemGottfried,CarlosAlexandreNetto,and Carlos-Alberto GonçalvesB YśĊ◘┼ś ■┘

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ANNEXURES

INSTITUTIONAL ETHICAL COMMITTEE

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTRE

MADURANTHAGAM-603 308.

21.01.2015

CERTIFICATE FOR APPROVAL

The Institutional Ethical Committee of Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam reviewed and discussed the application for approval **"EFFECT OF LIFESTYLE FACTORS ON SERUM ANTIOXIDANT LEVELS IN APPARENTLY HEALTHY INDIVIDUALS"** by **Dr. R. HARINI,** Post Graduate Student, Department of Biochemistry, Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam.

The proposal is **APPROVED**

The Institutional Ethics Committee expects to be informed about the progress of the study, and Adverse Drug Reaction occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTRE
21 JAN 2015

CHAIRPERSON

S.NO.	AGE	GENDER	SE SCORE	IPAQ SCORE	EACET SCORE	STREE SCORE	SMOKING SCORE	ALCOHOL SCORE	BMI SCORE	SOD U/Ml	GP U/L	COQ10 µG/L	URIC ACID mG/dl	AGATT SCO
1	25	m	4	2	1	2	1	2	3	160	10000	400	7.4	0
2	29	f	2	3	1	2	3	1	4	154	6000	383	6.5	0
3	27	m	4	1	1	2	1	2	1	180	10005	430	6.5	0
4	26	f	4	3	1	2	3	1	1	190	7000	700	3.8	0
5	22	f	4	3	1	1	3	1	1	200	8000	550	3.5	0
6	21	m	3	2	2	1	3	1	1	230	9200	600	4.5	0
7	21	m	3	2	1	2	3	1	1	190	9000	450	5	0
8	22	f	4	3	1	2	3	1	4	150	5200	380	6.8	0
9	20	f	3	2	1	2	3	1	1	180	9000	450	3.7	0
10	27	f	3	2	1	2	3	1	2	160	9000	440	3.2	0
11	20	f	4	3	1	2	3	1	2	155	8000	430	3.1	0
12	20	f	4	2	1	2	3	1	1	170	9000	450	3.3	0
13	20	f	3	2	1	2	3	1	1	179	9000	445	3.4	0
14	20	f	4	3	1	2	3	1	1	174	8010	452	3.2	0
15	20	f	4	2	1	2	3	1	2	167	8500	440	3.2	0
16	22	f	4	2	1	2	3	1	1	175	9050	448	3.3	0
17	23	m	2	1	2	2	3	2	1	180	10100	625	5.1	0
18	23	f	2	2	2	2	3	1	3	190	9000	550	6	0
19	23	f	4	2	1	2	3	1	1	174	9100	458	3.4	0
20	23	m	3	2	1	2	3	2	1	166	9800	460	5.6	0
21	24	f	3	2	1	2	3	1	1	190	8600	468	3.8	0
22	24	f	3	2	2	1	3	1	3	168	8700	478	5.8	0
23	21	f	4	2	2	2	3	1	2	160	8900	465	3.2	0
24	20	f	4	2	1	2	3	1	3	164	8900	420	5.9	0

25	23 f	2	2	2	2	3	1	3	190	9100	720	5.9	0
26	27 m	4	1	1	2	1	3	1	170	10800	470	4.2	1
27	21 m	4	2	1	2	1	2	4	180	9900	471	6.2	0
28	27 f	2	3	1	1	3	1	3	200	7200	500	5.8	0
29	25 f	4	3	1	2	3	1	1	190	7300	472	3.4	0
30	29 m	4	1	1	1	2	2	1	197	10100	472	4.9	0
31	28 m	4	1	1	2	2	2	3	198	10002	468	6.2	0
32	24 f	4	3	1	2	3	1	2	189	8000	469	3.8	0
33	23 f	4	3	1	2	3	1	1	187	7900	470	3.4	0
34	22 f	4	3	1	1	3	1	1	200	7400	471	3.2	0
35	23 f	4	3	1	2	3	1	1	190	7600	475	3.3	0
36	21 f	4	3	1	2	3	1	1	191	7700	480	3.4	0
37	22 f	1	3	1	2	3	1	3	186	7400	470	5.9	0
38	20 f	1	3	1	1	3	1	4	180	7200	474	6.3	0
39	22 f	2	3	1	1	3	1	1	204	7210	500	3.4	0
40	23 f	2	3	1	1	3	1	3	180	7300	472	3.6	0
41	28 m	2	2	2	2	3	2	1	184	9000	490	4.4	0
42	28 m	1	2	2	2	2	2	3	179	9500	470	5.8	0
43	28 m	2	1	2	2	1	2	3	178	10000	464	5.9	0
44	28 m	2	3	1	1	3	1	1	200	7400	500	4.1	0
45	28 f	2	2	1	1	2	2	3	194	9500	470	5.4	0
46	27 m	2	3	1	2	3	1	4	184	7200	474	6.3	0
47	26 f	2	3	2	1	3	1	4	185	7200	472	5.9	0
48	23 m	2	2	1	2	3	1	1	200	9000	700	4.4	0
49	23 f	2	2	1	2	3	2	1	180	10000	550	5.5	0
50	23 m	2	2	1	2	3	2	1	184	9900	545	5.4	0

51	25 m	2	2	2	2	3	2	1	220	9200	650	4.9	0
52	29 f	3	2	1	1	3	1	3	196	9100	500	5.8	0
53	28 f	3	2	2	2	3	1	3	184	9500	470	5.7	0
54	28 f	2	2	1	2	3	1	3	164	9800	465	5.9	0
55	28 f	2	2	2	1	3	1	1	178	8900	480	3.9	0
56	27 f	1	2	1	2	3	2	1	186	8800	530	3.9	0
57	28 f	2	2	1	2	3	1	3	162	9700	470	5.9	0
58	29 f	3	2	1	2	3	1	1	180	9000	490	3.6	0
59	29 m	3	1	1	2	3	2	4	156	11000	402	7.3	0
60	29 m	2	1	1	1	2	2	3	160	10800	420	6.8	0
61	29 m	1	2	1	2	3	2	3	176	9800	480	6.5	0
62	30 m	1	3	2	1	3	1	1	228	7000	700	4.5	0
63	30 m	2	3	1	2	3	1	3	164	7500	476	5.9	0
64	25 m	4	3	1	1	3	1	1	190	8000	460	4.3	0
65	21 m	4	3	1	1	3	1	1	192	7900	455	4.4	0
66	28 m	5	3	1	2	3	1	1	170	7000	450	4.1	0
67	29 m	4	2	1	2	2	2	3	159	9900	410	6.3	0
68	28 f	3	3	1	2	3	1	2	172	8800	468	3.1	0
69	29 f	5	2	1	2	3	1	2	160	7400	440	3	0
70	30 m	4	3	1	2	2	2	3	180	9900	450	5.3	0
71	29 m	4	3	1	2	2	2	1	174	9200	448	5.5	0
72	29 m	3	1	1	2	1	2	3	165	10020	452	6.5	0
73	28 m	2	1	1	2	1	2	1	160	10100	447	5.5	0
74	29 m	2	2	1	2	2	2	1	170	9800	458	4.9	0
75	30 m	5	1	1	2	1	3	1	168	10900	460	5.1	1
76	30 f	5	3	1	1	3	1	2	172	7500	480	3.2	0

77	30 m	4	2	1	1	2	2	3	168	9800	442	5.8	0
78	29 m	3	2	1	2	2	2	3	166	9870	439	5.8	0
79	30 m	4	2	2	2	2	2	4	163	9900	460	6.5	0
80	29 m	2	3	2	2	3	1	3	170	7700	490	6	0
81	29 m	2	3	2	2	3	1	3	175	7800	500	5.9	0
82	28 m	2	2	1	2	2	2	3	172	9820	470	5.8	0
83	28 f	2	3	1	2	3	1	4	172	8000	468	6.6	0
84	29 m	3	1	1	2	2	2	3	190	10000	492	5.9	0
85	28 m	2	2	1	2	2	2	4	189	9900	485	6.1	0
86	23 f	3	3	2	1	3	1	1	195	7400	520	3.3	0
87	23 f	2	3	1	1	3	1	1	196	7300	488	3.2	0
88	23 f	1	3	2	1	3	1	1	199	7800	490	3.4	0
89	24 f	2	3	2	1	3	1	3	188	7800	487	5.5	0
90	23 f	2	3	2	2	3	1	2	180	7200	492	3	0
91	25 m	3	3	1	2	3	1	2	170	6900	494	6.4	0
92	25 m	3	3	1	2	3	1	3	176	7000	496	5.9	0
93	26 m	3	3	1	2	3	1	1	185	7300	497	3.1	0
94	27 m	2	3	2	2	3	1	1	180	7400	491	3.1	0
95	26 m	3	2	1	2	3	2	3	185	9000	488	5.9	0
96	25 m	3	3	1	2	3	1	3	182	7500	486	5.8	0
97	24 f	2	3	2	2	3	1	3	182	6900	486	4.9	0
98	24 f	2	3	1	2	3	1	2	185	7600	489	3.2	0
99	24 f	3	3	2	2	3	1	3	283	6870	487	5.9	0
100	23 m	2	1	1	1	2	2	4	194	10100	485	6.4	0
101	22 m	2	1	1	1	3	2	4	192	10008	479	6.2	0
102	23 m	2	2	1	1	3	2	4	191	9900	482	6.3	0
103	21 f	1	3	1	1	3	1	4	185	6500	483	5.9	0
104	21 f	3	3	2	2	3	1	1	200	7500	510	3.2	0

INFORMATION SHEET

Your blood sample has been accepted.

We are conducting a study on apparently healthy individuals attending Karpaga Vinayaga Institute of Medical Sciences,Kanchipuram Distric-603308 and for that your blood sample may be valuable to us.

The purpose of this study is to identify ***EFFECT OF LIFESTYLE FACTORS ON SERUM LEVELS OF ANTIOXIDANTS IN APPARENTLY HEALTHY INDIVIDUALS*** with the help of certain special tests.

We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

PATIENT CONSENT FORM

Title of the study ***:EFFECT OF LIFESTYLE FACTORS ON SERUM ANTIOXIDANT LEVELS IN APPARENTLY HEALTHY INDIVIDUALS***

Name : Date :

Age : OP No :

Sex : Individual ID No:
:

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study.

signature

PROFORMA FOR PATIENT DETAILS

. ! - %Ù ***! ' %Ù***
GENDER:

OP.NO.: ***INDIVIDUAL ID. NO:***

ADDRESS:

SOCIOECONOMIC STATUS SCORE:

HISTORY:

SMOKING CATEGORY:

ALCOHOL CONSUMPTION CATEGORY:

FACET QUESTIONNAIRE SCORE:

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE SCORE:

STRESS QUESTIONNAIRE SCORE:

GENERAL EXAMINATION:

HEIGHT:

WEIGHT:

BMI:

BLOOD PRESSURE: ***PULSE:***

SYSTEMIC EXAMINATION:

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM:

ABDOMEN:

NERVOUS SYSTEM:

INVESTIGATION:

Fasting serum glucose:

Total cholesterol:

Triglycerides:

Hemoglobin:

Superoxide dismutase:

Glutathione peroxidise:

Uric acid:

Coenzyme Q_{10} :

DIAGNOSIS:

PART 1

For each question, please indicate the answer (or answers) by crossing the relevant box(es)

Try to make sure the crosses are clearly in the box they refer to, like this ☒, not like this ☒

Please use black or blue biro

If you make a mistake, just blank out the mistake like this ₱ and carry on

Q.1 Please write in today's date.

Day	Month	Year
		2003

Q.2 Have you eaten any of the following foods in the last 24 hours ?

PLEASE "X" THE NUMBER OF PORTIONS OF FOODS EATEN FOR <u>EVERY</u> ROW

FOR EXAMPLE:

	0	1	2	3	4+
Fruit as a dessert	☐	☒	☐	☐	☐

NUMBER OF PORTIONS

	0	1	2	3	4+
Breakfast cereal	☐	☐	☐	☐	☐
Fruit for breakfast, e.g. on cereal	☐	☐	☐	☐	☐
Crisps	☐	☐	☐	☐	☐
Fruit as a between meal snack	☐	☐	☐	☐	☐
A glass of pure, unsweetened fruit juice (not squashes or fruit drink)	☐	☐	☐	☐	☐
Fruit as a starter to a meal	☐	☐	☐	☐	☐
A baked potato	☐	☐	☐	☐	☐
A bowlful of home-made style vegetable soup	☐	☐	☐	☐	☐
Portions of vegetables with main meals (include baked beans and pulses as vegetables but not potatoes)	☐	☐	☐	☐	☐
Any type of meat	☐	☐	☐	☐	☐
A vegetable based meal	☐	☐	☐	☐	☐
Any type of fish	☐	☐	☐	☐	☐
A bowlful of salad	☐	☐	☐	☐	☐
Fruit as a dessert	☐	☐	☐	☐	☐

PART 2

Please read each question carefully and "X" the answer that most accurately reflects your circumstances or views. In some questions you will be asked for your opinion on a topic, please write your answers in the box provided.

Q.1 How many portions of a combination of fruit and vegetables do you think health experts would recommend eating every day ?

PLEASE "X" ONE BOX ONLY

None	☐ 1		Five	☐ 6	
One	☐ 2		Six	☐ 7	
Two	☐ 3		Seven or more	☐ 8	
Three	☐ 4		Don't know	☐ 9	
Four	☐ 5				

Q.2 How many portions of fruits and vegetables do each of the following provide ?

PLEASE "X" ONE BOX ONLY IN EACH ROW

	0	1	2	3	4	Don't know
A small glass (150 mls) of unsweetened orange juice	☐	☐	☐	☐	☐	☐
One glass of orange squash (diluted)	☐	☐	☐	☐	☐	☐
A thin slice of tomato	☐	☐	☐	☐	☐	☐
Three heaped tablespoons of carrots	☐	☐	☐	☐	☐	☐
One medium-sized apple	☐	☐	☐	☐	☐	☐
One small raspberry flavoured yoghurt	☐	☐	☐	☐	☐	☐

Q.3 How important are the following to you in deciding how much fruit and vegetables that <u>you eat</u> ?

PLEASE "X" ONE BOX ONLY IN EACH ROW

	Very unim-portant		Unim-portant		Neither unim-portant nor important		Important		Very important		Don't know	
The money I have available to spend on fruit and vegetables	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
Price of fruit and vegetables	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
My knowledge about ways to prepare fruit and vegetables	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
The time I have available to prepare fruit and vegetables	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
How easy it is for me to get the shops*	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
How heavy my shopping is to carry	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
Likes and dislikes of my household for fruit and vegetables	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6
The quality of fruit and vegetables available	☐	1	☐	2	☐	3	☐	4	☐	5	☐	6

*any shops within walking distance

Q.4 Do you think you will <u>increase</u> the amount of fruit and vegetables you eat in the next year ?

PLEASE "X" ONE BOX ONLY

No, definitely not	No, probably not	Possibly	Yes, probably	Yes, definitely	Don't know
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Q.5 By eating more fruit and vegetables, I think that people can reduce their chances of getting….

PLEASE "X" ONE BOX ONLY IN EACH ROW

	Agree strongly	Agree slightly	Neither agree nor disagree	Disagree slightly	Disagree strongly	Don't know
Stroke	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Cancer	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Back pain	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Hearing problems	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Heart disease	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

PART 3

To help us in analysing this survey, please provide the following information

Q.1 Your date of birth

Day	Month	Year

Q.2 Sex

PLEASE "X" ONE BOX ONLY

Male ☐ 1

Female ☐ 2

Q.3 Which of these apply to you?

PLEASE "X" ONE BOX ONLY

Current smoker ☐ 1

Ex smoker ☐ 2

Never smoked ☐ 3

Indian Journal of Community Medicine Vol. 30, No. 4, October-December, 2005

Annexure

Note : The present proforma has largely included family paramters and very few are based on head of the family. The proforma has been developed for all sections of the society.

Definition of a family :

It includes nuclear or joint family. Married couple with unmarried children or without children. Head of the family will be either husband/wife. Dependent father/mother/brother/sister does not become head of the family unless he/she is earning and one kitchen with pooled income is managed by him/her.

Q 1. Monthly per capita income from all sources (total monthly income /no. of family members)

1. >50000 7
2. 20000-49999 6
3. 10000-19999 5
4. 5000-9999 4
5. 2500-4999 3
6. 1000-2499 2
7. <1000 1

Q 2. Education of either husband or wife who is more educated among them

1. Professional qualification with technical degrees or diplomas e.g. Doctor, Eng. CA, MBA, etc. 7
2. Postgraduation (non-technical incl. Ph.D) 6
3. Graduation 5
4. 10th class pass but <Graduation 4
5. Primary pass but <10th 3
6. <Primary but attended school for at least one year 2
7. Just literate but no schooling 1
8. Illiterate 0

Q 3. Occupation of husband, otherwise wife.

1. Service in central/State/Public undertakings or Owner of a company employing >20 persons or self employed professional viz Doctors, CAs, Eng. Etc. 5
2. Service in Private sector or independent business employing 2-20 persons 4
3. Service at shops, home, transport, own cultivation of land 3
4. Self employed e.g. shops, Rehdies or petty business with income >5000 2
5. Self employed with income <5000 (labourer, house wife) 1
6. None of the family member is employed 0

Q 4.F amily possessions (presence of each item given below will carry score of '1') 10

1. Refrigerator 2. TV 3. Radio/Transistor/Music system 4. AC 5. Washing Machine 6. Telephone 7. Mobile Tel 8. Credit card 9. Sanitary lat. 10. Any newspaper subscribed throughout the month

Q 5. Living in a type of house

1. Own house with 5 or more rooms 7
2. Own house with 3-4 rooms 6
3. Rented/Govt. house with 5 or more rooms 6
4. Own house with 1-2 rooms 5
5. Rented/Govt. house with 3-4 rooms 5
6. Rented/Govt. house with 1-2 rooms 4
7. Own jhuggi 3
8. Rented jhuggi 2
9. No place to live, pavement, mobile cart 1

Q 6.P ossession of a vehicle or equivalent

1. 2 or more cars/Tractors/Trucks 4
2. 1 Car /Tractor/Truck 3
3. 1 or more scooter(s)/Bullock cart (s) 2
4. 1 or more cycles (not baby cycle) 1
5. None of the above 0

Q. 7 No. of earning members in the family (Nuclear/Joint)

1. 3 or more members earning and income pooled 3
2. 2 or both husband and wife earning 2
3. Only 1 family member earning 1
4. No earning member 0

Q 8. No. of children head of the family has/had

1. 0-1 5
2. 2 4
3. 3 3
4. 4 2
5. 5 1
6. >6 0

Q 9.F acility of some essentials in the family

1. Both tap water supply and electricity 2
2. Only one of above two is present 1
3. None is present 0

Q10. Education of children (in relation to head of the family)

Note : Exclude under 5 children for this item. A child applicable here is one who is 5 yrs or above.

1. All children going/ever gone to school/college 3
2. >50% children ever gone/going to school/college 2
3. $\leq$ 50% children ever gone/going to school/college 1
4. No child ever gone/going to school/college 0

Q 11. Employment of a domestic servant at home

1. Employed $\geq$2 full time servants on salary for domestic work 4
2. Employed only 1 full time servant on salary for domestic work 3
3. Employed $\geq$ 3 part time servants on salary for domestic work 2
4. Employed 1-2 part time servants on salary for domestic work 1
5. Employed no servants for domestic work 0

Q 12.Type of locality the family is residing

1. Living in urban locality 5
2. Living in rural locality 4
3. Living in resettlement colony 3
4. Living in slums/jhuggis 2
5. No fixed living and mobile 1

Q 13.Caste of the family
1. Upper caste 4
2. OBC 3
3. Dalits 2
4. Tribals 1

Q. 14. Members of family gone abroad in last three years (officil or personal)
1. Whole family 3
2. Only husband and wife 2
3. Only 1 family member 1
4. None 0

Q 15. Possession of agricultural land for cultivation
1. Own agricultural land >100 acres 5
2. Own agricultural land 51-100 acres 4
3. Own agricultural land 21-50 acres 3
4. Own agricultural land 6-20 acres 2
5. Own agricultural land 1-5 acres 1
6. No agricultural land 0

Q 16. Possession of non-agriculatural land/land for housing or other type of land
1. Own non-agricultural land/land for housing>1000 Sq Yards 3
2. Own non-agricultural land/land for housing 501-1000 Sq. Yards 2
3. Own non-agricultural land/land for housing 25-500 Sq. Yards 1
4. Own non-agricultural land/land for housing <25 Sq. Yards -OR- 0

Does not own non-agricultural land/land for housing at all

Q 17.Presence of milch cattles in the family for business or non-business purposes
1. Own 4 or more milch cattles 3
2. Own 1-3 milch cattles 2
3. Own 1 milch cattle 1
4. Does not own any milch cattle 0

Q 18. Presence of non milch cattles or pet animals in the family
1. Own 2 or more 2
2. Own 1 1

Q 19. Besides the house in which the family is living, the family owns other house or shop or shed etc. of any size whether given on rent or not
1. Owns 3 or more 3
2. Owns 2 or more 2
3. Owns 1 1
4. Does not own any 0

Q 20.Positions held (besides the positions as employee) by any one member in the family
1. Holding position of 3 or more official or non-official organizations viz. president/chairman/Secretary/ Treasurer etc. 4
2. Holding position of 1-2 official or not-official organizations viz. president/chairman/Secretary/ Treasurer etc. 3
3. Holding position as member only of executive or other committees of official or non-offical organizations. 2
4. Does not hold any such position 1

Q 21. Parental support in the form of non-movable property
1. >50 acres of agricultural land -OR -a house/plot >1000 sq yards -OR -Both 4
2. 21-50 acres of agricultural land -OR-a house/plot 501-1000 sq yards -OR - Both 3
3. 1-20 acres of agricultural land -OR -a house /plot 100-500 sq yards -OR-Both 2
4. No agricultural land -BUT - a house/plot 25-100 sq yards 1
5. No parental property 0

Q22.Total amount of income tax paid by the family (include all the earnign members IT)
1. >10 lacs 7
2. 1-10 lacs 6
3. >50000 but <1 lac 5
4. >20000 - <50000 4
5. >10000-<20000 3
6. >5000-<10000 2
7. <5000 1
8. Nil 0

TOTAL SCORE OF THIS FAMILY ☐☐☐

SCORING SYSTEM

	Social Status	Score
1.	Upper High	≥76
2.	High	61-75
3.	Upper Middle	46-60
4.	Lower Middle	31-45
5.	Poor	16-30
6.	Very Poor or Below Poverty Line	≤15

Stress Questionnaire

Because everyone reacts to stress in his or her own way, no one stress test can give you a complete diagnosis of your stress levels. This stress test is intended to give you an **overview** only. Please see a Stress Management Consultant for a more in depth analysis.

Answer **all** the questions but just tick one box that applies to you, either yes or no. Answer yes, *even if only part of a question applies to you*. Take your time, but please be completely honest with your answers:

		Yes	No
1	I frequently bring work home at night		
2	Not enough hours in the day to do all the things that I must do		
3	I deny or ignore problems in the hope that they will go away		
4	I do the jobs myself to ensure they are done properly		
5	I underestimate how long it takes to do things		
6	I feel that there are too many deadlines in my work / life that are difficult to meet		
7	My self confidence / self esteem is lower than I would like it to be		
8	I frequently have guilty feelings if I relax and do nothing		
9	I find myself thinking about problems even when I am supposed to be relaxing		
10	I feel fatigued or tired even when I wake after an adequate sleep		
11	I often nod or finish other peoples sentences for them when they speak slowly		
12	I have a tendency to eat, talk, walk and drive quickly		
13	My appetite has changed, have either a desire to binge or have a loss of appetite / may skip meals		
14	I feel irritated or angry if the car or traffic in front seems to be going too slowly/ I become very frustrated at having to wait in a queue		
15	If something or someone really annoys me I will bottle up my feelings		
16	When I play sport or games, I really try to win whoever I play		
17	I experience mood swings, difficulty making decisions, concentration and memory is impaired		
18	I find fault and criticize others rather than praising, even if it is deserved		
19	I seem to be listening even though I am preoccupied with my own thoughts		
20	My sex drive is lower, can experience changes to menstrual cycle		
21	I find myself grinding my teeth		
22	Increase in muscular aches and pains especially in the neck, head, lower back, shoulders		
23	I am unable to perform tasks as well as I used to, my judgment is clouded or not as good as it was		
24	I find I have a greater dependency on alcohol, caffeine, nicotine or drugs		
25	I find that I don't have time for many interests / hobbies outside of work		
A **yes** answer score = I (one), and a **no** answer score = 0 (zero).		**TOTALS**	

1a. During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling,?

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____ days per week ð

or

none

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities*?*

_____ hours _____ minutes

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week ð

or

none

2b. How much time in total did you usually spend on one of those days doing moderate physical activities*?*

_____ hours _____ minutes

3a. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ days per week ð

or

none

3b. How much time in total did you usually spend walking on one of those days*?*

_____ hours _____ minutes

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend *sitting* on a **week day?**

____ hours _____ minutes

This is the end of questionnaire, thank you for participating.

The Alcohol Use Disorders Identification Test: Interview Version
Read questions as written.

1. How often do you have a drink containing alcohol?

0 points - Never
1 point - Monthly or less
2 points - 2 to 4 times a MONTH
3 points - 2 to 3 times a WEEK
4 points - 4 or more times a week

Questioner may skip to Questions 9 and 10 if reply to Question 1 is never, or if both answers to Q 2 and 3 are 0.

2. How many units of alcohol do you drink on a typical day when you are d

0 points - 1 or 2 drinks
1 point - 3 or 4 drinks
2 points - 5 or 6 drinks
3 points - 7 or 8 or 9 drinks
4 points - 10 or more drinks

3. How often have you had 6 or more units if female, or 8 or more if male, occasion in the last year?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

AUDIT-C Sc[ ]/12 (complete full questionnaire if score is 3 or more)

4. How often during the last year have you found that you were not able to once you had started?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

5. How often during the last year have you failed to do what was normally from you because of drinking?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

6. How often during the last year have you needed an alcoholic drink in th get yourself going after a heavy drinking session?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remors drinking?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

8. How often during the last year have you been unable to remember wha the night before because you had been drinking?

0 points - Never
1 point - Less than monthly
2 points - Monthly
3 points - Weekly
4 points - Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?

0 points - No, never
2 points - Yes, but not in the last year
4 points - Yes, during the last year

10. Has a relative or friend or a doctor or another health worker been conc your drinking or suggested you cut down?

0 points - No, never
2 points - Yes, but not in the last year
4 points - Yes, during the last year

The Alcohol Use Disorders Identification Test (AUDI[ ]re/40

Scores of 8 or more are considered an indicator of hazardous and harmful alcohol use.