Original article

Age related study of serum lipid peroxidation and antioxidant status

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Background : Ageing is a common phenomenan throughout the globe which is characterized by an impaired ability to maintain homeostasis under the condition of physiological stress. Some parameters in serum like malondialdehyde(MDA) , â-carotene, vita-E etc. are useful marker for chronological ageing. To validate the usefulness of the above parameters as an indicators for chronological ageing in Indian subjects.

Materials and Methods: Blood samples were randomly collected from healthy 50 male and 50 female volunteers ranging in age 20-70 years who were coming for routine checkup to OPD in IGIMS, Patna. Fasting blood sample (10 ml) was collected from each individual for estimation of fasting blood sugar(FBS), malondialdehyde(MDA), Vita-E and â-carotene.

Results: Serum MDA levels of these subjects were increased and lowering trend was observed in women. However, Vita-E levels were marginally decreased with age but no significant change in FBS levels were observed. An interesting observation was seen in â–carotene levels decreases markedly after attending the age of 40 years and the lowering tendency remain 70 years of age.

Conclusion : A significant increased levels of MDA and decreased levels of â-carotene were useful marker for the study of chronological ageing in Indian subjects.

Key words: Serum lipid peroxidation, ageing, malondialdehyde (MDA)

Introduction

Ageing is characterized by an impaired ability to maintain homeostasis under the condition of physiological stress and this failure is associated with a decrease in viability and increased vulnerability of the individual morbidity and mortality [1,2]. To elucidate the mechanism of ageing, group of scientists have proposed a number of theories [3] Among these theories the most popular ones are glycation and free radical theories [4,5] The end product of glycation process are known as advanced glycation end products (AGE),which have been linked to stiffening connective tissue, hardening arteries and progressive decline the kidney functions [6].High blood glucose level are correlated with increased diabetes mellitus in human due to high magnitudeof glycation with haemoglobin [7], collegen [8] and albumin [9] .

In recent years the free radical theory of ageing has gained a great deal of support [10]. Free radicals are highly reactive atoms or molecules capable of individual existence due to an unpaired electron in their outer orbit. Free radicals reaction associated with lipid per-oxidation are considered as important tissue damage which may lead to several diseases[11]. These radicals can damage some biological molecules like carbohydrates, lipids, proteins and nucleic acids. Plasma membrane are rich source of polyunsaturated fatty acids(PUFA),readily attacked by oxidizing radicals. Oxygen derived free radicals level increases with age and can causes injury to cell membrane either by initiation of PUFA's per-oxidation, inactivation of membrane bound enzymes , receptors or protein cross-linking and fragmentation. known to cause tissue damage and such injures can lead to several diseases [12] .

Oxidative tissue damage is the most common term used during ageing in which pro-oxidant predominate over antioxidants [13] ,it may be due to either increased production of reactive oxygen species(ROS) or decreased level of antioxidants (enzymatic and non enzymatic) or both . Reactive oxygen species such as superoxide anion (O^-_2) , hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) ,and malondialdhyde(MAD) are directly or indirectly involved in disease processes. Antioxidants like vitamins- A, C, E ,â-carotene, Se, Zn etc. are compounds the scavenge free radicals and protect against ROS toxicity. . The plasma MDA level has been used as an indirect indicator of membrane lipid per-oxidation and general oxidant stress.

Works are reported that the characteristic differences between Indians and Western subjects of comparable age such as body weight , body mass index and nutritional deficiency status [14] .It would therefore be important to validate whether serum MDA and serum antioxidant vita-E and â-carotene levels are useful marker for chronological ageing in Indian subjects .Blood glucose is another parameter to used as indicator for diabetes which has a good correlation with age. Hence , we have conducted a pilot study in healthy adult human subjects of different age groups (20-70)years to validate the usefulness of the above parameters as an indicators for chronological ageing.

Materials and Methods

Subjects : Blood samples were randomly collected from 100 apparently good health subjects(50 male and 50 female)ranging in age from 20-70 years . The volunteers (students ,staffs , non-professional blood donors) selected who were coming for routine check up to out patient department (OPD) in Indira Gandhi Institute of Medical Sciences(IGIMS) , Patna . The age was assessed in literate subjects based on the date of birth and in illiterate subjects it was done by verification of the subjects age at menarche , marriage , birth of the first child etc. A questionnaire on ageing and morbidity was administered to each subject to assess his / her health status and BMI . Subjects who were normal and not suffering any chronic diseases were only recruited for the study . Any past history or present illness of diabetes mellitus , hypertension or other chronic diseases were excluded from the study . All the subjects were distributed in five groups with 20 males 20 females comprised of I-group(20-30 years), II-groups(31-40 years , III-groups (41-50 years), IV-groups (51-60 years) and V –groups(61-70 years).

Chemicals : Glucose standard,â - carotene and vita-E were collected from E-Merck, India. Malondialdehyde (MDA) standard was taken from Sigma , USA. Others reagents and chemicals were used in Analytical Reagents (AR) grade, collected from E-Merck , India.

Blood Collection ; About 10 ml overnight fasting venous blood sample was collected under aseptic condition from each individual . 0.5 ml blood was taken in sodium fluoride vial for estimation of fasting blood sugar . Remaining blood was allowed to clot without anticoagulant for estimation of MDA. â -carotene and Vita-E .

Estimation;

Fasting blood glucose;-Fasting blood glucose level in blood was estimated by the following method of Nelson and Somogyi with minor modification [15] . Values were expressed as mg/dl in blood**.**

Malondialdehyde (MDA) : It was estimated in serum as described K Satoh [16] .Values were expressed as n mole/L in serum.

â- carotene : â-carotene was estimated in serum according to the method of Barker and Frank [17]. Values were expressed as ì mole/L of serum.

Vitamin –E : Vita-E was estimated in serum by the method of Bieri et al [18] . Values were expressed as ì mole/L of serum.

Statistical analysis: All results were expressed in mean ±SD. One-way analysis of variance (ANOVA) was used to test the significance of difference.

Results

The age of the subject enrolled in the OPD in IGIMS, Patna, ranged from 20-70 years and they were divided into five groups with age interval of 10 years. Nutritional status of the subject was assayed by using WHO recommendation [19] ., indicates the nutritional status of all studied groups were sufficient. BMI of all subject were verified by considering National Health and Nutritional Examination Survey [20]. The BMI values of all subject ranged between 19 to 25 indicating the normal nutritional status.

Fasting blood sugar levels in blood of all subject of different age groups are given in Fig-I . Sugar level in blood increased progressively with age but levels remained within normal range. There were no significant differences observed between the sex at any age groups .

Fig. 1: Age and sex dependent changes in fasting blood sugar levels in humans.

Serum MDA levels in subjects of different age groups are depicted in Fig-II . Mean serum levels of MDA were gradually increases with age. But significant value was noticed in 60 – 70 years ($p< 0.05$) aged subjects . On closer look reveals that MDA levels in both male and female were approximately same in five groups. .We have found that there was a positive correlation in all male subjects ($r = 0.534$, $p < 0.01$) and in case of females ($r= 0.437$, $p<0.05$).

Fig. 2: Mean serum MDA levels of different age groups and different sex subjects.

Vita - E level in serum (Fig-III) decreases in different age groups with different sex. However marked decrease value was noticed in 60 – 70 years age of male and female subjects. No significant difference level of vita-E was found between the sex. There was a negative correlation in all groups for male ($r = 0.800$, $P< 0.01$) and for female ($r= -0.911$, $p < 0.01$)

Fig. 3: Serum Vitamin –E level in different age and sex groups

â- carotene level in serum in Fig –IV of all studied groups were seen to be decreases significantly after 40 years of age and it will continue to 70 years of age. There was a slight difference value in between male and female. A negative correlation between the age of all subjects for male ($r=-0.809$, $p<0.01$) and female ($r=- 0.704$, $p<0.05$).

Fig. 4: Serum â- carotene ±SD level in all studied groups.

Discussion

Fasting blood glucose levels were assessed in the present study and the data did not show any significant increase glucose levels in subjects of different age groups. Hence, blood glucose level may not be used as marker of chronological ageing.

MDA, a by-product of lipid per-oxidation is said to be involved in nucleic acid adduct formation which are believed to be responsible for carcinogenesis and many diseases [21]

Present data indicates an age dependent increase in MDA levels in subject of 60-70 year age ,which is corroborate with published work [22] .Interestingly, serum MDA levels observed in the present study are higher than Japanese subjects with similar age [3] . This finding probably suggest that Indians are grater susceptible than Japanese . Lower MDA levels in serum in women than men due to known protective role of female reproductive hormone against lipid per-oxidation [23].

Serum vitamin-E levels were marginally decrease with age but the change with sex were not statistically significant. Vita-E is thought to be an important chain breaking antioxidant which can directly scavenge ROS. It is a major lipid soluble antioxidant in cellular membrane , which protects against LPO. Low serum concentration of Vita-E was associated with increased risk of cataract [24] .

â- carotene protect the biological system by deactivating singlet 0_2 which largely depend on physical status. Our present data indicates the serum â– carotene levels markedly decrease after the age of 40 year and levels remain almost same upto 70 year of age. Some worker established a correlation between serum carotenoid with higher incidence of cataract [25] .‘

In conclusion, the present study indicates that a significant increase in MDA level was seen in subject of 60 year of age confirm the higher oxidation stress with advance age. Based on our study , we may concluded a significant correlation exist between age and serum MDA and â-carotene. These parameters may be useful as marker for chronological ageing in Indian subjects.

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