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Biomedical studies on lipid peroxidation and erythrocyte fragility during the process of aging

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ABSTRACT

Objective: To investigate oxidative stress, hemoglobin percentage and erythrocyte osmotic fragility in various aging groups. **Methods:** A total of 200 healthy volunteers of both genders between age group 20–65 years were selected by random method. Determination of hemoglobin percentage was done employing modified cyanide method of Dacie and Lewis. The erythrocyte lysis was observed in hypotonic solution of buffered saline at varying concentrations and optical density was measured at 540 nm. The extent of lipid peroxidation in form of malondialdehyde was measured by thiobarbituric acid method. **Results:** The study found a significant decrease in hemoglobin percentage, increase in erythrocyte osmotic fragility and increased lipid peroxidation in form of malondialdehyde with increasing age. **Conclusions:** Supplementation of antioxidants may prevent the oxidative injury in elderly group of subjects.

1. Introduction

Free radicals contain one or more unpaired electrons. They play an important role in the pathogenesis of tissue damage in many clinical disorders[1]. Aging is one of the normal physiological phenomena affected by free radicals. It is an irreversible process which can be defined as the survival of a growing number of people who have completed the traditional adult roles. Aging is an inevitable consequence of fertility decline. Various theories have been put forward to explain the process of aging in human, but the interest still continues in the role played by free radicals in aging, which could cause oxidative stress due to generation of oxygen free radicals (OFRs). Scientists increasingly believe that OFRs play a significant role in causing many ailments and in aging. Aging process is due to increased generation of reactive oxygen species and reactive nitrogen species. Normally there is a balance between tissue oxidant and antioxidant activity[2]. Aging progresses are due to increased generation of free radicals in oxidative stress and one of the victims of free radicals is erythrocytes cell membrane integrity. The present study was designed to evaluate the effects of free radical generation in the form of lipid peroxides on erythrocytes fragility and hemoglobin percentage during the process of aging.

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2. Materials and methods

2.1. Participants

This study was a population based study. A total of 200 healthy volunteers of both genders between age group 20–65 years were randomly selected. An informed consent was taken. The study was conducted for a period of 3 years from September 2005 to August 2008. The volunteers were classified according to their ages as group I, group II and group III which included healthy volunteers between age groups 35–45 years, 45–55 years and 55–65 years, respectively. Control group included healthy volunteers between ages of 20–30 years. Those who have any serious diseases, use of vitamin E, β-carotene or vitamin A supplements, hypertensive, smokers, and diabetics were excluded from the study.

2.2. Determination of hemoglobin, erythrocyte lysis and lipid peroxidation

Determination of hemoglobin percentage was done employing modified cyanide method of Dacie and Lewis[3]. The hemoglobin percentage was expressed as g% and optical density was read against blank at 540 nm. The erythrocyte fragility was measured by the method described by Dacie and Lewis[4]. The erythrocyte lysis was observed in hypotonic solution of buffered saline at varying concentrations and optical density was measured at 540 nm. The extent of lipid peroxidation in form of malonyldialdehyde (MDA) was measured by thiobarbituric acid method (TBA)[5]. Total amount of lipid peroxidation

product present in the plasma was determined using TBA method which measures MDA reactive products. A total of 0.5 mL of plasma, 0.5 mL of normal saline and 1.0 mL of 24% trichloroacetic acid were added. From this mixture 1.0 mL of protein free supernatant was taken after centrifugation at 2 000 rpm for 20 minutes. To this protein free supernatant, 0.25 mL of 0.33% of TBA was added and boiled at 95 ℃ for one hour. After cooling, the TBA reactive product was extracted in 1.0 mL butanol and intensity of pink color obtained was read at 532 nm against blank.

2.3. Statistical analysis

Data were entered in Microsoft Excel for windows 2003. The mean±SD was obtained using excel software. The two-sample-*t*-test value was obtained between the various groups and the control. The distribution of '*t*'- probability was calculated depending on '*n*' and significance of test was obtained. *P* value <0.05 was considered as significant.

3. Results

Figure 1 showed the hemoglobin percentage in various age groups. Hemoglobin percentage was significantly decreased as aging ($P<0.05$). Table 1 showed changes in erythrocyte fragility in various groups. Figure 2 showed the generation of MDA due to enhanced lipid peroxidation with increasing age. The 50% mean erythrocyte fragility and MDA was significantly increased as aging ($P<0.05$).

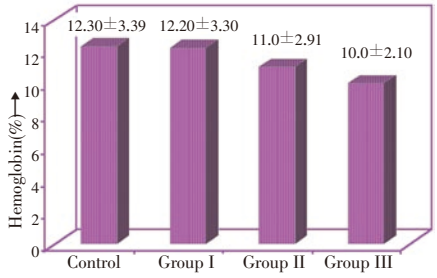

Figure 1. Hemoglobin (%) in controls and various age groups.

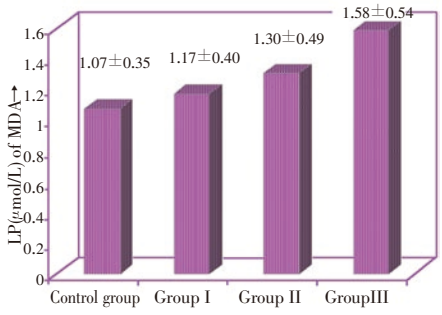

Figure 2. Effect of generation of free radicals on lipid peroxidation.

Table 1
Mean erythrocyte fragility in various age groups (Mean±SD).

Variables	Control group	Group I	Group II*	Group III**
50% mean erythrocyte fragility	0.70±0.09	0.73±0.01	0.75±0.01	0.77±0.02
% Increase	-	4.2	7.1	10

*: $P<0.01$, **$P<0.001$.

4. Discussion

Aging is associated with decreased antioxidants levels and increased OFRs formation. The increased free radical generations has an influence on erythrocyte membrane integrity which makes the cells more fragile and labile to damage. If we retain the levels of antioxidants in our cells by increasing antioxidants intake in diet then we could delay the process of aging by decreasing the effects of toxic radicals produced during aging.

Involvement of OFRs in the physiology of aging in a number of organs and tissues have been reported[6,7]. Indirect evidence of OFR generation in aging has been observed by measuring the lipid peroxidation and erythrocyte fragility as the fragility is affected due to increased accumulation of toxic peroxides within erythrocytes[8,9]. The activities of cellular defense mechanisms especially superoxide dismutase, glutathione peroxidase and catalase have been reported to decrease in human and other species in aging in many studies[10-12]. In the present study, increased erythrocytes fragility and lipid peroxidation are observed in various aging groups compared with young adults (control). Increased erythrocyte fragility may be associated with enhanced generation of OFRs and decreased levels of antioxidant enzymes which succumb to oxidative stress in aging as observed in the present study. Future researches including measurement of parameters of oxidative stress and antioxidant enzymes in human at certain interval of time are still needed to explore the role of lipid peroxidation in erythrocyte membrane fragility in aging.

Conflict of interest statement

We declare that we have no conflict of interest.

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