

Original Article

STATUS OF LIPID PEROXIDATION AND TOTAL ANTIOXIDANT CAPACITY IN REGULAR VOLUNTARY BLOOD DONORS

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Abstract :

Oxygen free radicals and products of oxidative cell damage are known as biomarkers that escalate in the pathophysiology of many diseases. The aim of this study is to evaluate association between blood donation and total antioxidant capacity and lipid peroxidation product malondialdehyde(MDA). The levels of MDA and total antioxidant capacity(TAC) were studied in serum of 90 male voluntary blood donors aged from 20 to 45 years attending K. S. Hegde charitable hospital, Blood bank, for determination of lipid peroxidation and antioxidant status, respectively. The donors were divided into nine groups depending on number of blood donation. With increase in number of blood donation the serum MDA level were significantly reduced ($p < 0.01$) but total antioxidant activity was significantly increased ($p < 0.01$). High frequency of blood donors had evidence of decreased lipid peroxidation and enhanced activity of total antioxidants when compared with low frequency blood donors. which can be considered as one of the fringe benefits of regular blood donation.

Keywords : Blood donation, lipid peroxidation, malondiadehyde, total antioxidant capacity

Introduction :

Free radicals are highly reactive molecules generated by biochemical redox reactions which occur as a part of normal cell metabolism and in the course of free radical mediated diseases such as cancer, diabetes mellitus cardiovascular and renal diseases.¹ Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Free radicals may cause lipid peroxidation, the level of lipid peroxidation expressed as malondialdehyde, that damage all components of the cell, including proteins, lipids, and DNA. Malondiadehyde is a metabolic product of peroxidative reactions (auto-oxidative)of lipids exposed to oxygen.² It serves as a reliable marker of lipid peroxidation.³ Free radicals are eliminated from the body by their interaction with non enzymic antioxidants such as uric acid, albumin, bilirubin, vitamin A,C,E, glutathione, peroxidase, superoxide dismutase and catalase.^{4,4}

Blood is permanently exposed to oxidative stress and therefore it has high antioxidant capacity.^{1, 5} Significantly

decreased antioxidants and markedly increased MDA can cause metabolic disorders, increase oxidative damage and oxidative stress in human body.^{2,6}

The present study aims to determine the alteration of serum lipid peroxidation (by measuring the level of MDA) and total antioxidant capacity in regular blood donors.

Materials and methods :

The reference population consisted of 90 male volunteer blood donors aged from 25 to 50 years attending K.S.Hegde charitable hospital, Blood Bank. The ethical committee of our institution approved the trial. We obtained informed consent from all participants.

The inclusion criteria included no history of minor surgery during the previous 72 hours, no major surgery within 6 months, no current infectious or non infectious disease, notably cardiovascular disease, diabetes, polycyaethemia vera, no addiction to drugs and/or alcohol, no history of tattoo in the previous 6 months, minimum weight of 50kgs, systolic and diastolic blood pressure between

100-120mmHg and 60-80mmHg, respectively. Subjects were divided into nine groups depending on number of blood donations; i.e 0, 3, 4, 5, 6, 7, 8, 9, 10 and 10 persons in each group, respectively. Questionnaire was prepared for data collection.

Just before blood donation 2ml of venous blood was taken. Serum was separated by centrifugation. The serum was recovered and transferred to fresh tube for determination of MDA level and total antioxidant capacity(TAC).

Determination of MDA level :

Lipid peroxidation was estimated by measurement of thiobarbutric acid by the method of Buege and Aust(1978).Serum-100 μ L was diluted to 500 μ L with distilled water. To the diluted sample 1mL of TCA-TBA-HCl (Trichloroacetic acid- thiobarbutric acid-hydrochloric acid) reagent was added. The samples were kept in boiling water bath for 15 minutes. The reaction mixture was cooled and centrifuged. The supernatant was taken and the optical density of the pink colour formed was read at 535nm. A blank was also maintained simultaneously by taking 500 μ L of water instead of sample in the reaction mixture. The concentration of malondialdehyde in the sample was determined by plotting the obtained absorbance against the standard graph. The optical density of the pink colour formed is directly proportional to the concentration of malondialdehyde in the given sample.

Enzymatic determination of total antioxidant capacity :

Total antioxidant capacity was determined by the method of Prieto et.al.,1999.Exactly 100 μ L of serum sample was pipetted out into a clean test tube and 100 μ L of 5%TCA (Trichloroacetic acid) was added to it to precipitate out the proteins in the sample, the mixture was then allowed to stand for about five minutes and centrifuged. 100 μ L of the clear supernatant was transferred into a clean test tube and 1mL of total antioxidant capacity(TAC) reagent was added to it and the mixture was then incubated in water bath at 90°C for 90 minutes. A blank was also maintained simultaneously by substituting 100 μ L of water instead of sample in the reaction mixture. Following the incubation the reaction mixture was cooled and the optical density of

the greenish to bluish colour formed was read at 695nm against blank. The total antioxidant capacity in the sample is obtained by plotting the obtained absorbance against the standard graph.

Data analysis :

Results were expressed as mean \pm standard error of mean (SEM). Correlations were evaluated using Pearson's correlation coefficient. The data were subjected to one way ANOVA. The predetermined upper limit of significance throughout this investigation was $p < 0.05$. all analyses were done using SPSS 11.5.

Results :

In present study we determined the serum level of malondialdehyde and total antioxidant status. Mean and standard error of mean for each group was calculated (Table I). There is a significant decrease in serum malondialdehyde level (Graph I)and significant increase in total antioxidant capacity (Graph II) with increase in number of blood donation ($p < 0.01$). MDA showed significant correlation with total antioxidant capacity ($r = -0.880$, $p < 0.001$) in group II with blood donors having donated three times. (Table I)(Diagram I)

Discussion :

The current findings demonstrate evidence of increase activity of antioxidants, decrease oxidative stress and decrease lipid peroxidation in high frequency blood donors. Lipid peroxidation is a free radical generating process which occurs on every membranous structure of the cell.² Free radicals are known to be involved in pathologies including atherosclerosis, cancer and hypertension.⁷ Our finding of decreased MDA in high frequency donors is consistent with the hypothesis that high frequency blood donation is associated with decrease oxidative stress. To best of our knowledge, only one study,has been conducted on blood donors to find association between blood donation frequency, antioxidant enzymes and lipid peroxidation, results suggested evidence of reduction of body iron stores, reduced oxidative stress and enhanced activity of antioxidant enzymes like superoxide dismutase, in

voluntary blood donors.² Few studies have examined the association of plasma iron levels and plasma MDA in human population. Results from study in patients with coronary artery disease, showed an association of high serum ferritin with increase lipid peroxidation.⁸ Number of studies were conducted to know the association between repeated blood donation and iron status.^{9,10,11}

To our knowledge, this is the second study to determine association between blood donation, lipid peroxidation

and antioxidant capacity in regular blood donors.

Conclusion :

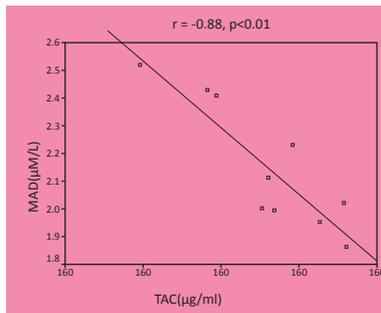
Increase in number of blood donation was associated with evidence of reduced oxidative stress and enhanced activity of antioxidants in regular voluntary blood donors, which can reduce the risk of certain diseases. Additional studies are required to evaluate the effect of blood donation on lipid peroxidation and antioxidant status in larger population by dividing groups according to the frequency of blood donation per year in different age groups.

Table I. Serum malondialdehyde and total antioxidant capacity in subjects based on number of blood donation and its correlation

| Groups (n=10) | MDA Mean ± SEM | TAC Mean ± SEM | Pearson's correlation 'r' | p value |
|-----------------|----------------|----------------|---------------------------|----------|
| I (0 time) | 2.68 ± 0.079 | 183.74 ± 1.94 | 0.229 | 0.525 |
| II (3 times) | 2.15 ± 0.07 | 185.92 ± 2.64 | - 0.880 ** | 0.001 ** |
| III (4 times) | 2.16 ± 0.07 | 191.44 ± 1.05 | - 0.401 | 0.251 |
| IV (5 times) | 2.45 ± 0.06 | 203.40 ± 1.35 | 0.269 | 0.452 |
| V (6 times) | 1.90 ± 0.10 | 226.56 ± 3.14 | - 0.277 | 0.438 |
| VI (7 times) | 2.24 ± 0.10 | 224.85 ± 3.78 | 0.592 | 0.071 |
| VII (8 times) | 2.08 ± 0.06 | 237.73 ± 4.03 | -0.611 | 0.061 |
| VIII (9 times) | 2.08 ± 0.05 | 235.33 ± 5.50 | -0.144 | 0.692 |
| IX (10 times) | 2.08 ± 0.11 | 270.40 ± 12.47 | - 0.154 | 0.672 |

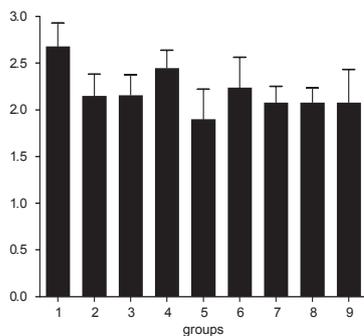
Abbreviations: MDA, malondialdehyde; TAC, total antioxidant capacity
 ** Correlation is significant at 0.01 level (2 tailed)

Diagram 1. Scatter plot of serum malondialdehyde and total antioxidant capacity

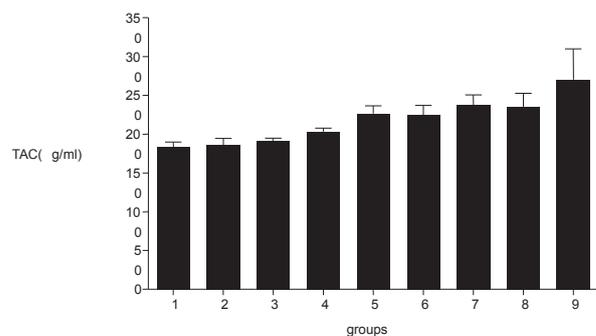


Abbreviations : MDA, malondialdehyde; TAC total antioxidant capacity

Graph I. Error bar graph for serum malondialdehyde (MDA) with mean and standard deviation



Graph II. Error bar graph for total antioxidant capacity (TAC) with mean and standard deviation



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