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Age associated oxidative damage in RBC and serum of humans

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ABSTRACT

Objective: To evaluate the level of lipid-protein damage and antioxidant status in red blood corpuscle (RBC) and serum of healthy individuals to correlate between oxidative damage with the aging process. **Methods:** Twenty healthy individuals of each age group (11-20; 21-30; 31-40; 41-50; and 51-60 years old) were selected randomly. Blood samples were drawn by medical practitioner and serum was separated and RBC was isolated from blood samples. Malondialdehyde (MDA), protein carbonyls (PC) level were evaluated to determine the lipid and protein damage in RBC and serum. superoxide dismutase (SOD), catalase (CAT), glutathione and glutathione dependent enzymes were estimated to evaluate the antioxidant status in RBC and serum. **Results:** Increased MDA and PC levels strongly supported the increased oxidative damage in elderly subject than young subjects. The results indicated that, balance of oxidant and antioxidant systems in RBC and serum shifts in favor of accelerated oxidative damage during aging. **Conclusions:** Oxidative stress in RBC and serum may particular interest in aging and may play important role in immunosenescence.

1. Introduction

During the last decades, many researches have been directed towards establishing correlations between oxidative damage, antioxidant defense systems and aging. Aging a complex phenomenon is an inevitable biological process that is generally characterized by the declining ability to respond to stress, increasing homeostatic imbalance and increase risk of disease. Because of this death is an ultimate consequence of aging. The aging process includes the accumulation of changes with time and a decline of the organism response to these changes. It is well known that with aging, even during healthy aging there is a decrease of the immune response, this is termed as immunosenescence. A general feature of the aging or senescence process is a progressive physiological deterioration with time leading to an impairment of the homeostasis, vulnerability to disease and ultimately to death of the organism[1]. The free radical theory of aging proposes that age-dependent deterioration in cell function is related to accumulation molecular oxidative damage, caused by reactive oxygen. An increase in production of reactive radicals, particularly as a result of mitochondrial dysfunction has been recognized as a major cause of oxidative stress. How ever age-associated alterations in the antioxidant status could also contribute to increased oxidative stress in aging. Reactive oxygen species (ROS) are implicated in aging and in various degenerative disorders[1,2]. ROS such as hydrogen peroxide, superoxide anion, hydroxyl radical, and nitric oxide, are formed in the body as a consequence of aerobic metabolism, damaging all intracellular components, including nucleic acids, proteins, and lipids. To attenuate such a rampant attack, appropriate anti-oxidant defenses arise to protect against damage from ROS. The first line anti-oxidant system includes enzymes, such as superoxide dismutase (SOD),

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glutathione (GSH), peroxidase and catalase, which are re-enforced by nonenzymatic anti-oxidants, GSH, protein-SH, vitamin C, E, β-carotene, and uric acid. During normal oxygen metabolism, the concentration of free radicals is controlled by various antioxidants and a balance exists between prooxidant and antioxidant process[2]. Changes in antioxidant capacities like hydrophilic radical scavengers (ascorbate, urate and glutathione), lipophilic radical scavengers (tocopherols, carotenoids), metal chelators and antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase activities have been demonstrated in aging[3].Several studies have explored the effects of aging on total antioxidant capacity of plasma and tissues. So, in the present study, we attempted to document the effects of aging on the oxidative damage on red blood cells (RBC) and serum by quantifying lipid peroxidation, protein oxidation and different antioxidant status in healthy individuals.

2. Materials and methods

2.1. Selection of human subjects

This study was carried out in 100 healthy subjects who were divided according to five different age groups: Group A (11-20 years old), Group B (21-30 years old), Group C (31-40 years old), Group D (41-50 years old) and Group E (51-60 years old). All subjects enrolled in this study were asymptomatic and none of them had abnormality on physical examinations and routine laboratory tests. Groups had similar socioeconomic status and dietary habits. All the subjects were from same geographical area and same economic status, non-smokers and non-alcoholic and having same food habit. These subjects received no medication, including vitamin E and vitamin C. All subjects gave informed consent. The selection excluded not only individuals with acute infections or chronic diseases, but also excluded healthy individuals undergoing supplementation with antioxidative substances. The study protocol was in accordance with the declaration of Helsinki, and was approved by the ethical committee of Vidyasagar University.

2.2. Chemicals and reagents

Sodium dodecyl sulfate, DTNB, standard reduced glutathione, glutathione reductase, NADPH Na_4, oxidized glutathione were purchased from Sigma Chemical Co., USA. Sodium chloride, ethylene diamine tetra acetate, sodium azide, were purchased from Himedia, India. Tris-Hcl, Tris buffer, KH_2PO_4, K_2HPO_4, sodium hydroxide, alcohol and other chemicals were procured from Merck Ltd., SRL Pvt. Ltd., Mumbai, India. All other chemicals were from Merck Ltd., SRL Pvt., Ltd., Mumbai and were of the highest grade available.

2.3. Collection of blood samples and separation of serum and RBC

Fasting blood samples were collected from all groups of individuals satisfying the Helsinki protocol. Serum was obtained by centrifugation at 1 500×*g* for 15 min of blood samples taken without anticoagulant. Serum was kept at -86 ℃ for the biochemical estimation of different parameters. RBC was separated followed by the separation of lymphocytes and neutrophils by sequential sedimentation (Histopaque 1077, Sigma Chemical Co.) in 1:2 ratio and in Dextran T-500 (Pharmacia LKB, Biotechnology, Piscataway, NJ) in 0.9% sodium chloride, centrifugation on a Fycoll-Hypaque cushion (specific gravity 1077, Pharmacia) and hypotonic lysis of erythrocytes, as described in our previous laboratory report[1,4]. The preparation was more than 98% pure and 98% of the cells were viable judged by Trypan blue exclusion.

2.4. Biochemical assays

2.4.1. Determination of lipid peroxidation

Lipid peroxidation of RBC and serum was estimated by the method of Gautam *et al*[4]. Briefly, the reaction mixture contained Tris-HCl buffer (50 mmol, pH 7.4), tetra-butyl hydroperoxide (BHP) (500 μmol in ethanol) and 1 mmol $FeSO_4$. After incubating the samples at 37 ℃ for 90 min, the reaction was stopped by adding 0.2 mL of 8% sodium dodecyl sulfate (SDS) followed by 1.5 mL of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 mL of 0.8% TBA and further heating the mixture at 95 ℃ for 45 min. After cooling, samples were centrifuged, and the TBA reactive substances (TBARS) were measured in supernatants at 532 nm by using (1.53×10^5) $M^{-1}cm^{-1}$ as extinction coefficient. The levels of lipid peroxidation were expressed in terms of nmol/mg protein.

2.4.2. Determination of protein oxidation (PC)

RBC and serum PC levels were measured based on spectrophotometric detection of the reaction of 2, 4 dinitrophenylhydrazine with protein carbonyl to form protein hydrazones[4]. Briefly, after precipitation of protein with an equal volume of 1% trichloroacetic acid (TCA), the pellet was resuspended in 10 mmol DNPH in 2 N Hcl. Next, after the washing procedure with 1:1 ethanol/ethyl acetate, the final pellet was dissolved in 6 mol Guanidine. The carbonyl group was determined from the absorbance at 370 nm. The results were expressed as μmoles of carbonyl groups per milligram of protein with molar extinction coefficient of 22 000 M • cm.

2.4.3. Determination of reduced glutathione (GSH) level

Reduced glutathione estimation in RBC and serum was performed by the method of Gautam *et al*[4]. The required amount of sample was mixed with 25% of trichloroacetic acid and centrifuged at 2000 ×*g* for 15 min to settle the precipitated proteins. The supernatant was aspirated and diluted to 1 mL with 0.2 mol sodium phosphate buffer (pH 8.0). Later, 2 mL of 0.6 mmol DTNB was added. After 10 min the optical density of

the yellow-colored complex formed by the reaction of GSH and DTNB (Ellman's reagent) was measured at 405 nm. A standard curve was obtained with standard reduced glutathione. The levels of GSH were expressed as nmol of GSH/mg protein.

2.4.4. Determination of oxidized glutathione (GSSG) level

The oxidized glutathione level in RBC and serum was measured after derevatization of GSH with 2-vinylpyridine according to the method of Gautam *et al*[4]. In brief, with 0.5 mL sample, 2 μL 2-vinylpyridine was added and incubates for 1 h at 37 ℃. Then the mixture was deprotenized with 4% sulfosalicylic acid and centrifuged at 1000 ×*g* for 10 min to settle the precipitated proteins. The supernatant was aspirated and GSSG level was estimated with the reaction of DTNB at 412 nm in spectrophotometer and calculated with standard GSSG curve.

2.4.5. Determination of glutathione peroxidase (GPx) activity

The GPx activity of RBC and serum was measured by the method of Gautam *et al*[4]. The reaction mixture contained 50 mmol potassium phosphate buffer (pH 7.0), 1 mmol EDTA, 1 mmol sodium azide, 0.2 mmol NADPH, 1 U glutathione reductase and 1 mmol reduced glutathione. The sample, after its addition, was allowed to equilibrate for 5 min at 25 ℃. The reaction was initiated by adding 0.1 mL of 2.5 mmol H_2O_2. Absorbance at 340 nm was recorded for 5 min. Values were expressed as n mol of NADPH oxidized to NADP by using the extinction coefficient of $(6.2 \times 10^3)\ M^{-1}\ cm^{-1}$ at 340 nm. The activity of GPx was expressed in terms of n mol NADPH consumed/min/mg protein.

2.4.6. Determination of glutathione reductase (GR) activity

The GR activity of RBC and serum was measured by the method of Gautam *et al*[4]. The tubes for enzyme assay were incubated at 37 ℃ and contained 2.0 mL of 9 mmol GSSG, 0.02 mL of 12 mmol NADPH, Na_4, 2.68 mL of 1/15 mol phosphate buffer (pH 6.6) and 0.1 mL of sample. The activity of this enzyme was determined by monitoring the decrease in absorbance at 340 nm. The activity of GR was expressed in terms of n mol NADPH consumed/min/mg protein.

2.4.7. Determination of glutathione-s-transferase (GST) activity

The GST activity of RBC and serum was measured by the method of Gautam *et al*[4]. The tubes of enzyme assay were incubated at 25 ℃ and contained 2.85 mL of 0.1 mol potassium phosphate (pH 6.5) containing 1 mmol of GSH, 0.05 mL of 60 mmol 1-chloro-2, 4-dinitrobengene and 0.1 mL of sample. The activity of this enzyme was determined by monitoring the increase in absorbance at 340 nm. The activity of GST was expressed in terms of nmol/min/mg protein.

2.4.8. Determination of catalase (CAT) activity

Catalase activity of RBC and serum was measured by the method of Gautam *et al*[4]. The final reaction volume of 3 mL contained 0.05 mol Tris-buffer, 5 mmol EDTA (pH 7.0), and 10 mmol H_2O_2 (in 0.1 mol potassium phosphate buffer, pH 7.0). About 50 μL of sample was added to the above mixture. The rate of change of absorbance per min at 240 nm was recorded. Catalase activity was calculated by using the molar extinction coefficient of 43.6 $Mc^{-1}\ m^{-1}$ for H_2O_2. The level of CAT was expressed in terms of nmol/min/mg protein.

2.4.9. Determination of super oxide dismutase (SOD) activity

SOD activity of RBC and serum was determined from its ability to inhibit the auto-oxidation of pyrogalol[4]. The reaction mixture considered of 50 mmol Tris (hydroxymethyl) amino methane (pH 8.2), 1 mmol diethylenetriamine penta acetic acid, and 20-50 μL of sample. The reaction was initiated by addition of 0.2 mmol pyrogalol, and the absorbance measured kinetically at 420 nm at 25 ℃ for 3 min. SOD activity was expressed as unit/ mg protein.

2.4.10. Protein estimation

Protein content was estimated using BSA as the standard[4].

2.5. Statistical analysis

The data are reported as mean±SEM. The difference between Groups A-E were analyzed with Student's *t*-test. $P<0.05$ was considered statistically significant.

3. Results

In the present study, we investigated the oxidative damage occurs in RBC and serum of healthy individual of different age groups. The antioxidant status in RBC and serum was assessed by measuring the enzymatic and non enzymatic antioxidants activity.

3.1. Lipid peroxidation and protein oxidation level.

Lipid peroxidation and protein oxidation are the two important determinants to assess the cellular damage. Lipid peroxidation and protein oxidation in RBC and serum was measured in terms of MDA and PC, respectively. MDA levels also shows significantly increased in RBC and serum of Group B: (9.72±0.31) nmol/mg protein and (5.130±0.279) nmol/mg protein, $P<0.05$; Group C: (13.31±0.37) nmol/mg protein and (7.371±0.310) nmol/mg protein, $P<0.05$; Group D: (17.47 ±0.30) nmol/mg protein and (9.110±0.311) nmol/mg protein, $P<0.05$; Group E: (21.05±0.32) nmol/mg protein and (11.317± 0.360) nmol/mg protein, $P<0.05$, respectively, as compared to Group A: (7.01±0.25) nmol/mg protein and (2.50±0.159) nmol/mg protein, $P<0.05$ (Figure 1).

Increased PC levels were found in RBC and serum of Group B: (8.01±0.27) nmol/mg protein and (5.510±0.199) nmol/mg protein, $P<0.05$; Group C: (9.31±0.31) nmol/mg protein and (7.071 ±0.21) nmol/mg protein, $P<0.05$; Group D: (12.31±0.37) nmol/

mg protein and (9.371±0.241) nmol/mg protein, P<0.05; Group E: (15.171±0.390) nmol/mg protein and (10.312±0.311) nmol/mg protein, P<0.05, respectively, as compared to Group A (5.81± 0.21) nmol/mg protein and (3.180±0.189) nmol/mg protein, P<0.05 (Figure 2).

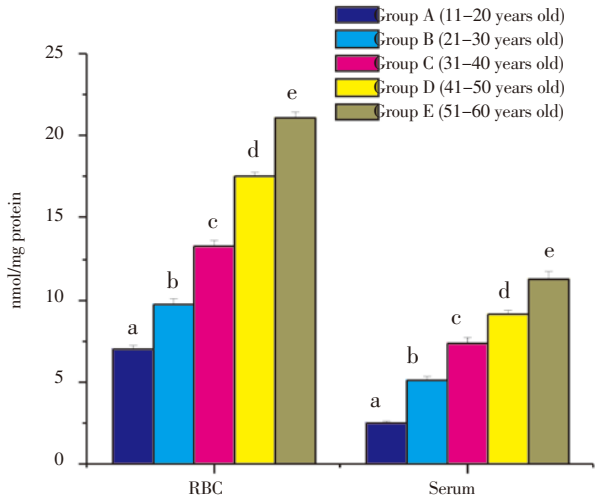

Figure 1. MDA levels in RBC and serum of dif ferent age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of P<0.05.

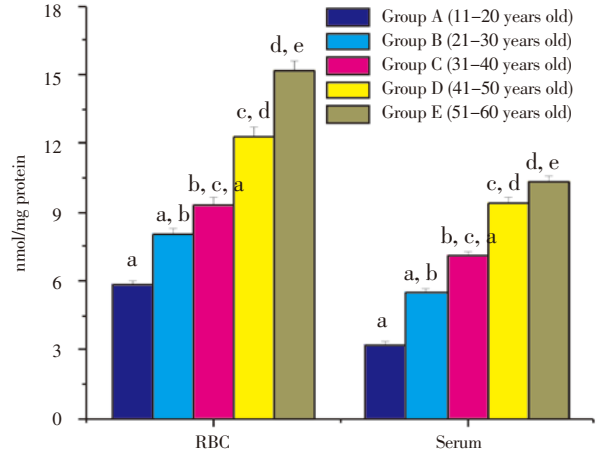

Figure 2. PC levels in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of P<0.05.

3.2. Glutathione level

Glutathione is an important antioxidant in cellular system. So, to understand glutathione level, we have measured reduced glutathione (GSH) and oxidized glutathione (GSSG) level. Age related decline was observed in the GSH level, the results were shown in Figure 3. There was significant decreased in GSH value for Group B: (15.31±0.39) nmol/mg protein and (3.99 ±0.21) nmol/mg protein, P<0.05; for Group C: (11.09±0.31) nmol/mg protein and (3.010±0.191) nmol/mg protein, P<0.05; for Group D: (9.31±0.29) nmol/mg protein and (2.550±0.175) nmol/mg protein, P<0.05; and for Group E: (6.371±0.250) nmol/mg protein and (1.990±0.145) nmol/mg protein, P<0.05; in comparison with Group A: (18.51±0.41) nmol/mg protein and (5.150±0.237) nmol/mg protein, P<0.05, respectively. GSSG levels in RBC and serum was also decreased significantly for Group B: (7.130± 0.217) nmol/mg protein and (3.410±0.165) nmol/mg protein, P<0.05; for Group C: (5.31±0.19) nmol/mg protein and (3.11± 0.157) nmol/mg protein, P<0.05; for Group D: (3.970± 0.165) nmol/mg protein and (2.980±0.145) nmol/mg protein, P<0.05; and for Group E: (2.50±0.15) nmol/mg protein and (2.500 ±0.111) nmol/mg protein, P<0.05, respectively, in comparison with Group A: (9.710±0.241) nmol/mg protein and (4.170± 0.171) nmol/mg protein, P<0.05 (Figure 4).

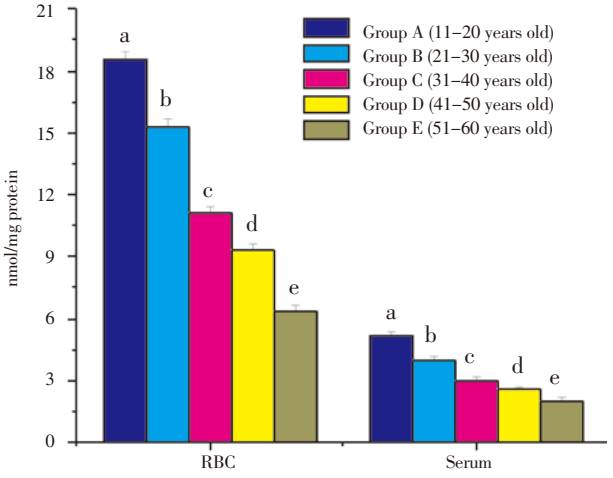

Figure 3. GSH levels in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of P<0.05.

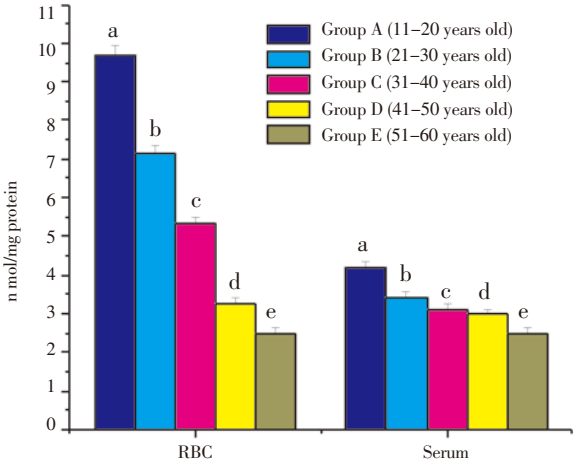

Figure 4. GSSG levels in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of P<0.05.

3.3. Antioxidant enzymes status

The glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), SOD and CAT activity were measured to understand the antioxidant status of different group of RBC and serum.

GPx activities were observed in all the different age groups of RBC and serum. The results were shown in the Figure 5. The GPx activity decreased significantly for Group B: (14.71 ±0.71) nmol/mg protein and (9.71±0.51) nmol/mg protein, $P<0.05$; for Group C: (12.37±0.69) nmol/mg protein and (7.73±0.49) nmol/mg protein, $P<0.05$; for Group D: (10.13 ±0.61) nmol/mg protein and (5.31±0.41) nmol/mg protein, $P<0.05$; and for Group E: (8.57±0.59) nmol/mg protein and (3.750±0.390) nmol/mg protein, $P<0.05$, respectively, in comparison with Group A: (17.13±0.89) nmol/mg protein and (12.31±0.69) nmol/mg protein, $P<0.05$, respectively. GR activity was significantly decreased in RBC and serum of Group B: (7.71±0.61) nmol/mg protein and (3.710±0.313) nmol/mg protein, $P<0.05$; in Group C: (5.37±0.55) nmol/mg protein and (2.730± 0.231) nmol/mg protein, $P<0.05$; in Group D: (3.13±0.41) nmol/ mg protein and (1.310±0.175) nmol/mg protein, $P<0.05$; and in Group E: (1.57±0.27 nmol/mg protein and (1.15±0.165) nmol/mg protein, $P<0.05$, respectively, as compared to Group A: (10.13± 0.72) nmol/mg protein and (5.31±0.431) nmol/mg protein, $P<0.05$ (Figure 6). GST activity was significantly decreased in RBC and serum of Group B: (9.17±0.63) nmol/mg protein and (5.710± 0.431) nmol/mg protein, $P<0.05$; in Group C: (7.73±0.58 nmol/mg protein and (4.370±0.313) nmol/mg protein, $P<0.05$; in Group D: (5.13±0.47) nmol/mg protein and (3.130±0.275) nmol/mg protein, $P<0.05$; and in Group E: (3.75±0.29) nmol/mg protein and (2.950 ±0.205) nmol/mg protein, $P<0.05$, respectively, as compared to Group A: (12.31±0.75) nmol/mg protein and (7.310±0.413) nmol/mg protein, $P<0.05$ (Figure 7).

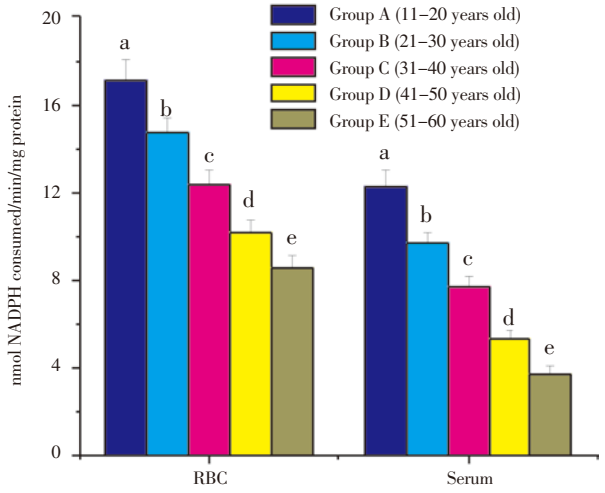

Figure 5. GPx activities in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of $P<0.05$

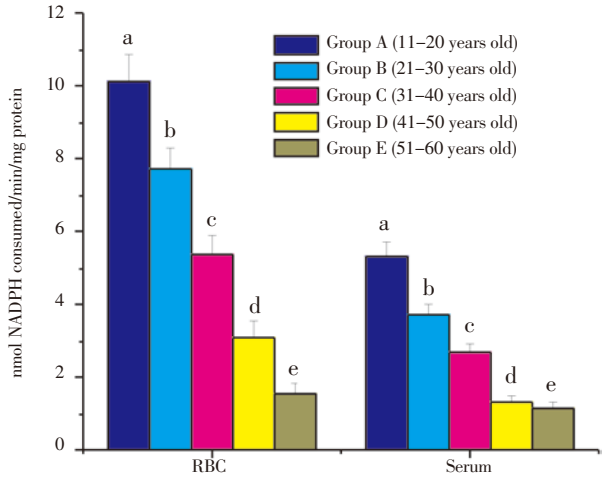

Figure 6. GR activity in RBC and serum of different age group of human subjects was estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of $P<0.05$.

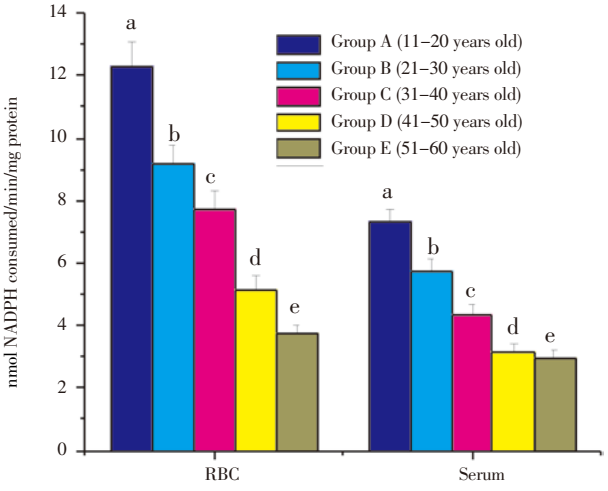

Figure 7. GST activity in RBC and serum of different age group of human subjects was estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A–E were analyzed with Student's t-test. Values not sharing a common superscript letter (a–e) are statistically significant at the level of $P<0.05$.

CAT activity was observed in all the different age groups of RBC and serum. The results were shown in the Figure 8. The CAT activity decreased significantly for Group B: (38.47± 1.67) nmol/mg protein and (18.47±0.67) nmol/mg protein, $P<0.05$; for Group C: (24.37±1.17) nmol/mg protein and (14.37± 0.47) nmol/mg protein, $P<0.05$; for Group D (15.01±0.99) nmol/ mg protein and (10.73±0.37) nmol/mg protein, $P<0.05$; and for Group E: (11.37±0.69) nmol/mg protein and (7.130±0.269) nmol/ mg protein, $P<0.05$, respectively, in comparison with Group A: (47.31±1.89) nmol/mg protein and (27.31±0.89) nmol/mg protein, $P<0.05$. SOD activity was observed in all the different age groups of RBC and serum. The results were shown in the Figure 9. The SOD activity decreased significantly for Group B: (10.370±0.313) nmol/mg protein and (6.371±0.213) nmol/mg protein, $P<0.05$; for Group C: (9.890±0.299) nmol/mg protein

and (5.890±0.199) nmol/mg protein, $P<0.05$; for Group D: (9.37±0.28) nmol/mg protein and (5.37±0.18) nmol/mg protein, $P<0.05$; and for Group E: (8.87±0.25) nmol/mg protein and (4.87 ±0.15) nmol/mg protein, $P<0.05$, respecitvely, in comparison with Group A: (12.010±0.379) nmol/mg protein and (8.010± 0.279) nmol/mg protein, $P<0.05$.

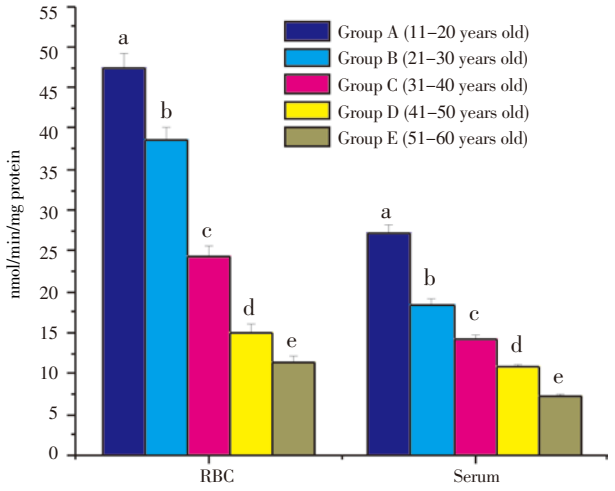

Figure 8. Catalase activities in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A-E were analyzed with Student's t-test. Values not sharing a common superscript letter (a-e) are statistically significant at the level of $P<0.05$.

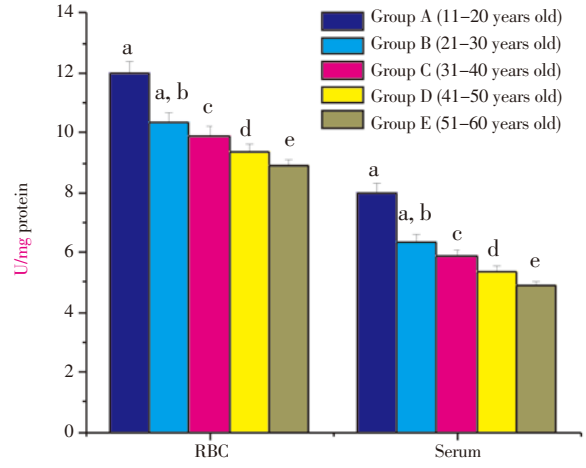

Figure 9. SOD activities in RBC and serum of different age group of human subjects were estimated using UV-Vis Spectrophotometer.
Data are expressed as mean±SEM, n=20. The differences between Group A-E were analyzed with Student's t-test. Values not sharing a common superscript letter (a-e) are statistically significant at the level of $P<0.05$.

4. Discussion

When a disturbance in the pro-oxidant antioxidant balance (redox status) occurs in favor of the former, the potential for tissue and cell damage due to oxidative stress ensues[5]. Aging and age-related diseases are associated with oxidative stress from the over-production of ROS. In the present study, we investigated the redox status of serum and RBC during aging. Our study found age-related decline in activities of antioxidant enzymes in serum and RBC from five different age groups. Decreased content of GSH and decline in activities of catalase, SOD, GR, GPx and GST enzymes were the prominent alterations observed in the present investigation with aging. The destruction of reactive oxygen intermediates and of free radicals involves the activities of SOD, catalase, GR, GPx and GSH as well as supply of NADPH. Our results depict a parallel decline in GST activity and GSH content with aging. A major factor that affects glutathione homeostasis is its utilization by conjugation primarily via GST. The ability of GST to alter levels of cellular glutathione in response to production of reactive oxygen species has been implicated in protection of cells from reactive oxygen species inducing agent[6]. There are several conflicting reports on GR activity with aging. Some studies reported a decline in GR activity that accompanies the age-related decrease in GSH content, others have indicated that GR activity either does not change or even increase with age[7,8]. Glutathione is an important cellular reductant, which offers protections against free radicals, peroxide and toxic compounds. It is reformed from GSSG by donation of hydrogen from NADPH, the reaction being catalyzed by glutathione reductase (GR)[8]. Adequate concentrations of GSH are required for a variety of immune functions, it has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immuno deficiency. The present study reports a decline in GSH content in serum and RBC from elder group like Group C, D and E as compared to younger Groups like Group A, B. Depletion of intracellular GSH inhibits T cell function[9]. Our findings indicate that total thiol levels in elderly group like C, D and E are significantly lower than those observed in young. Decreased glutathione levels and glutathione peroxidase activity are coupled to increased oxidative damage to DNA, lipids and proteins. If the level of reduced GSH is high, the oxidative stress is low and the level of lipid peroxidation is low, but with age the level of GSH is falling down and this is seen as raising amounts of MDA. Free radicals can also react with proteins and DNA, in addition to lipids. Lipid peroxidation is one of the important phenomena and has been implicated in a number of deleterious effects such as increased osmotic fragility, decreased membrane rigidity and cellular deformation[1], thus, we have measured the lipid peroxidation in all groups and it has been observed that the LP level increased significantly with age. Current studies on cellular injury implicate peroxidation of polyunsaturated fatty acids (PUFA), leading to the degradation of phospholipids as an index of cellular deterioration[10]. Our results show a significant increase of MDA levels in serum and RBC with aging. Recent report by our laboratory suggested that, increased lipid peroxidation and decreased antioxidant enzyme status can be indicator of disease progression of oral cavity cancer patient. PC formation has been proposed to be an early marker for protein oxidation.

However, available knowledge on PC formation as a function of age is limited in humans with aging. Some investigators have reported that PC content increased in neutrophilss, muscle, fibroblasts and eye lens of elderly subjects[1,4]. In this study, we detected a significant increase of PC levels in serum and RBC in elderly subjects from Group B, C, D and E, as previously reported. The highest level is in group E. Oxidative modification of proteins may lead to the structural alteration and functional inactivation of many enzyme proteins, as evidenced by the decreased activity of different antioxidant enzymes like SOD, CAT, GPx, GR and GST. The results strongly suggest that a shift in the redox balance occurs between pro-oxidant and anti-oxidant in favor of the former during aging. To further support this possibility, we assessed glutathione system levels, a major contributing factor to the maintenance of the redox state, particularly in the leukocytes. Aerobic cells contain various amounts of two main antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). SODs rapidly dismutate superoxide anion ($O_2^{-\bullet}$) to less dangerous H_2O_2, which is further degraded by CAT and glutathione peroxidase (GPx) to water and oxygen[1]. Decreased CAT activity may compromise the overall antioxidant enzyme defense system. The results of the present study showed a significant fall in SOD activities, in the elderly groups like Group B, C, D and E. The depletion in SOD activity was may be due to dispose off the free radicals, produced due to aging induced oxidative stress. H_2O_2 produced by dismutation of superoxide anion, may have been efficiently converted to O_2 by CAT but the enzyme activities showed a marked reduction[11,12]. The depletion of antioxidant enzyme activity was may be due to inactivation of the enzyme proteins by ROS generation with aging, depletion of the enzyme substrates, and/or down-regulation of transcription and translation processes. In summary, the present experiments demonstrate that the redox status undergoes increased oxidative stress with age, reflected by a decreased anti-oxidant capacity and an associated increased of biomolecule oxidation. Our results strongly support the presence of increased oxidative stress in elderly subjects.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgments

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