Age-associated changes in erythrocyte glutathione peroxidase activity: Correlation with total antioxidant potential

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Oxidative stress is believed to play a central role in aging and age-associated diseases. It leads to oxidative changes in human red blood cells (RBCs) in vivo and in vitro. In this study, we evaluated the oxidative damage to the erythrocytes during aging in the humans using RBC as a model, by measuring the cytosolic antioxidant enzyme glutathione peroxidase (GPx) activity. GPx activity was found to be significantly decreased as a function of human age and positively correlated with total antioxidant capacity, while negatively correlated with SOD activity. Thus, results of the present study showed involvement of oxidative stress as one of the risk factors, which can initiate and/or promote human aging.

Keywords: Glutathione peroxidase, Aging, Human, Oxidative stress

It has been suggested that aging could be caused by the deleterious effects of reactive oxygen species (ROS) throughout the life1, but the exact mechanism underlying aging is not well understood. Aerobic cells produce ROS as a byproduct of their metabolic processes. ROS cause oxidative damage to macromolecules under conditions when the antioxidant defense of the body is overwhelmed2,3. A certain amount of oxidative damage takes place even under normal conditions, however, the rate of this damage increases during the aging, as the efficiency of anti-oxidative and repair mechanisms decreases4,5. The oxidative stress theory of aging offers the best mechanistic elucidation of aging. The susceptibility of an individual depends on the antioxidant status of the body. In humans, antioxidant system includes a number of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants such as glutathione (GSH), protein –SH, ascorbic acid, uric acid and dietary antioxidants.

Glutathione peroxidase (GPx; EC 1.11.1.9), an enzyme dependent on selenium (Se) plays a critical role in the reduction of lipids and hydrogen peroxides. It consists of four apparently identical protein subunits, each of which contains one Se atom6. Selenium is present as selenocysteine which is incorporated into peptide backbone of GPx7. There are four subspecies of GPx i.e. GPx 1-4 that catalyze the reduction of hydrogen peroxides8,9, but GPx 1 is ubiquitous and found in cytosol of most cells, including erythrocytes.

The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species10,11, however, data on changes of oxidative stress markers in plasma and erythrocytes of healthy populations during aging are few and sometimes contradictory12-14. Earlier, we reported age-dependent alterations in SOD and CAT15, nitric oxide16 and biomarkers of oxidative stress viz. malonaldehyde (MDA), reduced glutathione (GSH) and membrane –SH group17 and a significant age-dependent decline in plasma antioxidant capacity, measured in terms of ferric reducing ability of plasma (FRAP) values18. Antioxidant capacity of the plasma is related to dietary intake of antioxidants19,20, and enzymatic antioxidants also alter as a function of age. In the present study, we have evaluated the oxidative damage of the erythrocyte during aging by measuring GPx activity and correlation between antioxidant capacity in Indian population.

Materials and Methods

The study was carried out on 53 normal healthy subjects of both sexes between the ages of 18-82 yrs. The subjects were screened for diabetes mellitus, asthma, tuberculosis or any other major illness. None of the subjects were smokers or taking any medication. All persons gave their informed consent for the use of their blood samples for the study. The protocol of study was in conformity with the guidelines of the Institutional Ethical Committee.

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Human venous blood from different healthy volunteers was obtained by venipuncture in heparin and centrifuged at 1800 rpm for 10 min at 4°C. After removal of plasma, buffy coat and upper 15% of the packed red blood cells, the RBCs were washed twice with cold PBS (0.9% NaCl, 10 mM Na₂HPO₄, pH 7.4).

Erythrocyte GPx activity was determined as described previously. Protein was determined by the method of Bradford.

The ferric reducing ability of plasma (FRAP) values were determined as described previously. Working FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 2, 4, 6-tri[2-pyridyl]-s-triazine (10 mM in 40 mM HCl) solution and FeCl₃·6H₂O (20 mmol/L) solution in 10:1:1 ratio respectively. 3 ml of FRAP reagent was mixed with 100 µl of plasma and the content was mixed vigorously. The absorbance was read at 593 nm at the interval of 30 s for 4 min. Aqueous solutions of known Fe²⁺ concentration in the range of 100-1000 µmol/L were used for calibration. Using the regression equation, the FRAP values (µmol Fe(II) per liter) of the plasma were calculated.

SOD activity was determined as described previously.

Statistical analyses were performed using the software PRISM 4. Relationship between various parameters was assessed using Pearson correlation coefficient (r). P<0.05 was considered as statistically significant.

**Results and Discussion**

Detrimental effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species. Inactivation and removal of ROS depend on reactions involving the antioxidative defense system. The capacity of antioxidative defense is determined by the contribution of certain vitamins (A, E, and C), β-carotene, reduced glutathione, and antioxidative enzymes. Wide inter-individual variations may exist regarding antioxidative capacity, thus affecting individual susceptibility against deleterious oxidative reactions. However, very limited information exists concerning the biological variation of anti-oxidative enzymes in representative population samples.

Figure 1a shows the age-dependent decrease in the activity of erythrocyte GPx activity in humans. A significant (p<0.0001) positive correlation (r = -0.850) was observed between erythrocyte GPx and age. To analyze the correlation of erythrocyte GPx activity with plasma antioxidant capacity, measured in terms of FRAP values, we plot a quotient: GPx activity/FRAP as a function of age (Fig. 1b). The plot shows a strong positive correlation (r = 0.725) between GPx and total plasma antioxidant potential. Among various antioxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects cells from ROS. It is also suggested that the activity of antioxidative enzymes may play an important role in determining the life-span of animal species. Our results showed that GPx activity negatively correlated (r = -0.7653) with SOD activity as a function of age Fig. 1c.
The decreased activity of GPx is a manifestation of increased generation of ROS as a function of age\textsuperscript{25} and could be due to increased level of free radicals in older population as compared to young. Our results were consistent with studies in adult population which suggest an age-dependent decrease in the activity of antioxidant enzymes\textsuperscript{26,27}, with hypothesis that increased free radical damage contribute to aging\textsuperscript{28}. Previous population studies have provided conflicting results as to how GPx activity is altered with age\textsuperscript{29}. Some studies have shown that it increases\textsuperscript{4,26}, while other studies indicate that GPx activity decreases as a function of age\textsuperscript{30}. When GPx activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage and activation of nuclear factor–kB–related inflammatory pathways\textsuperscript{31,32}.

In conclusion, the present study demonstrated significant age-related decrease in GPx activity and the correlation of these changes with the age-dependent decline in the total antioxidant capacity of the plasma. These findings emphasized the need to establish age-dependent reference values for oxidative stress markers.

References
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