World Journal of Methodology

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DOI: 10.5662/wjm.v5.i4.216

World J Methodol 2015 December 26; 5(4): 216-222
ISSN 2222-0682 (online)

Biomarkers of oxidative stress in erythrocytes as a function of human age

Pawan Kumar Maurya, Prabhanshu Kumar, Pranjal Chandra

Pawan Kumar Maurya, Interdisciplinary Laboratory of Clinical Neuroscience (LINC), Department of Psychiatry, Federal University of São Paulo, São Paulo 04039-032, Brazil

Pawan Kumar Maurya, Prabhanshu Kumar, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida 201303, India

Pranjal Chandra, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

Author contributions: Maurya PK had conceived the idea; Maurya PK and Chandra P set up the frame of the review article and wrote up manuscript; Kumar P edited the entire manuscript.

Supported by Fellowship (to Pawan Kumar Maurya) (Science without Borders-Level A) from coordination of Improvement of Higher Education Personnel (CAPES), National Counsel of Technological and Scientific Development (CNPq), Brazil.

Conflict-of-interest statement: The authors report no conflicts of interest.

Correspondence to: Dr. Pranjal Chandra, MTech, PhD, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Kamrup, Guwahati 781039, Assam, India. pchandra13@iitg.ernet.in
Telephone: +91-361-2583207
Fax: +91-361-2582249

Received: April 8, 2015
Peer-review started: April 9, 2015
First decision: June 24, 2015
Revised: September 1, 2015
Accepted: September 16, 2015
Article in press: September 18, 2015
Published online: December 26, 2015

Abstract

Despite more than 300 theories to explain the aging process, oxidative stress theory offers the best mechanism to explain aging and age related disorders. Several studies has shown the importance of oxidative stress during aging. PubMed, Science Direct and Springer online data bases are taken into consideration to write this mini-review. Human erythrocytes are most abundant and specialized cells in the body. Erythrocytes were extensively studied due to their metabolism and gas transport functions. Recent studies on erythrocytes have provided us detailed information of cell membrane and its structural organization that may help in studying the aging and age associated changes. The susceptibility of an organism is associated with the antioxidant potential of the body. Erythrocytes have potent antioxidant protection consisting of enzymatic and non-enzymatic pathways that counteract with reactive oxygen species, thus maintaining the redox regulation in the body. The non-enzymatic and enzymatic antioxidants and other biomarkers associated with erythrocyte membrane transport functions are the main content of this review. Biomarkers of oxidative stress in erythrocytes and its membrane were taken into the consideration during human aging that will be the main subject of this mini-review.

Key words: Biomarkers; Humans; Aging; Oxidative stress; Erythrocytes; Erythrocyte membrane

Core tip: The aim of present review is to summarize important oxidative stress biomarkers in erythrocytes during human aging. Erythrocyte membrane is rich in

lipids and proteins which are easy targets of reactive oxygen species. Erythrocytes are also equipped with antioxidant defense system. Studies on biomarkers of oxidative stress are important in the establishment of reference values in different populations and in studies involving their role in different disease conditions.

Maurya PK, Kumar P, Chandra P. Biomarkers of oxidative stress in erythrocytes as a function of human age. *World J Methodol* 2015; 5(4): 216-222 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/216.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.216

INTRODUCTION

Aging is a characterized by alterations that takes place in a single cell or in the whole organ system. The exact process of aging is still not well understood but many evidences support that it is associated with excess production of free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) throughout life[1]. During aerobic respiration, ROS/RNS are produced from electron transport chain present inside mitochondria (Table 1). Excess ROS/RNS damages proteins, lipids and nucleic acids, when enzymatic and non-enzymatic antioxidants of the body are unable to scavenge free radicals[2]. Even under normal metabolic conditions, certain amount of oxidative damage to cell and its membrane takes place, but its rate increases with the increase of OS during aging, as the antioxidant variation machinery gets diminished[3,4]. Besides many recent studies at molecular level like telomere shortening contributes to the accumulation of DNA damage during cellular aging[5], erythrocytes cell as a whole and its membrane has its own importance in aging and age associated diseases.

Human erythrocytes or red blood cells (RBCs) are in the circulatory system for 120 d[6], which transport oxygen from the lungs to all other tissues of the body and carbon dioxide (CO_2) from the body tissues back to the lungs. These erythrocytes are produced in the bone marrow by differentiation process and hematopoietic stem cells differentiate to form nucleate erythrocytes. After degradation of endoplasmic reticulum and formation of nuclei, reticulocytes appear in the circulation. An erythrocyte is a disc shaped, 8 μm biconcave structures bounded by a plasma membrane. The protein and lipid bilayer to erythrocytes changes throughout the whole life. This can be particularly seen at the stage of its plasma membrane[7], since it is made up of protein-lipid bilayer. Erythrocyte contains a conjugate protein in the form of hemoglobin. The main function of hemoglobin is the binding and releasing oxygen and carbon dioxide, for this reason the membrane of RBC is extremely important. The plasma membrane is a two dimensional meshwork of protein called as spectrin membrane skeleton. It helps in maintenance of structure of erythrocytes. Because of the above mentioned facts of erythrocyte and the plasma membrane, this cell type has been studied extensively.

Table 1 Reactive oxygen species

Oxygen centered radicals	Oxygen centered non-radicals
$O_2\cdot$	H_2O_2
$\cdot OH$	
$HOO\cdot$	$^1O_2$
$ROO\cdot$	

RBCs as oxygen carriers are continuously exposed to high oxygen tension. Oxidative stress, which decreases the antioxidant capacity, irreversibly damages erythrocytes, resulting in their eventual damage by hemolysis and their removal by circulation. Because mature RBCs are cells without nucleus and other cell organelles, they have no capacity to repair the damaged components. Celedón *et al*[8], showed that biochemical alteration which takes place during acute hypobaric hypoxia, make erythrocyte susceptible to oxidative stress. During erythrocyte life span there are many changes in size and deformability, lipid and protein content in the membrane, ion exchange and action of enzyme. This review will also address the effect of OS in erythrocytes during normal aging based on the structural alteration in the erythrocytes and its membrane. The widely studied clinical biomarkers of oxidative stress and its mechanism in human erythrocytes has been represented in Figure 1.

BIOMARKERS RELATED OF OXIDATIVE STRESS

Lipid peroxidation-malonaldehyde

Malonaldehyde, is a byproduct of lipid per-oxidation. It reacts with amino acids, membrane proteins, phosphatides, DNA and RNA of the cell, which leads to structural and functional modification. Excess level of malonaldehyde (MDA) levels has been reported when it undergoes cell damage or in several diseased conditions. Red blood cell membrane is made up of 60% of phosphatides. Cholesterol (non-esterifies) represents about 30% of the lipidic erythrocyte composition, and the last 10% are carbohydrate containing lipids[9]. Due to presence of polyunsaturated fatty acids (PUFA), cell membrane becomes more susceptible to free radicals and its oxidation takes place that breaks double bonds of PUFA and generate malonaldehyde. Previously, we have reported elevated level of erythrocyte MDA in normal elderly population[10]. Increased MDA level was supported by other findings, such as, decreased membrane sulfhydryl (-SH) groups and total antioxidant potential in older individuals compared to younger individuals[11]. The increases in the MDA level are found in diabetes, hypertension, inflammation, coronary heart and liver disease[12]. The free radical chain reaction is the mechanism involved in the process of lipid peroxidation. It increased with the increased production of ROS/RNS.

The erythrocyte membrane is rich in phosphatides

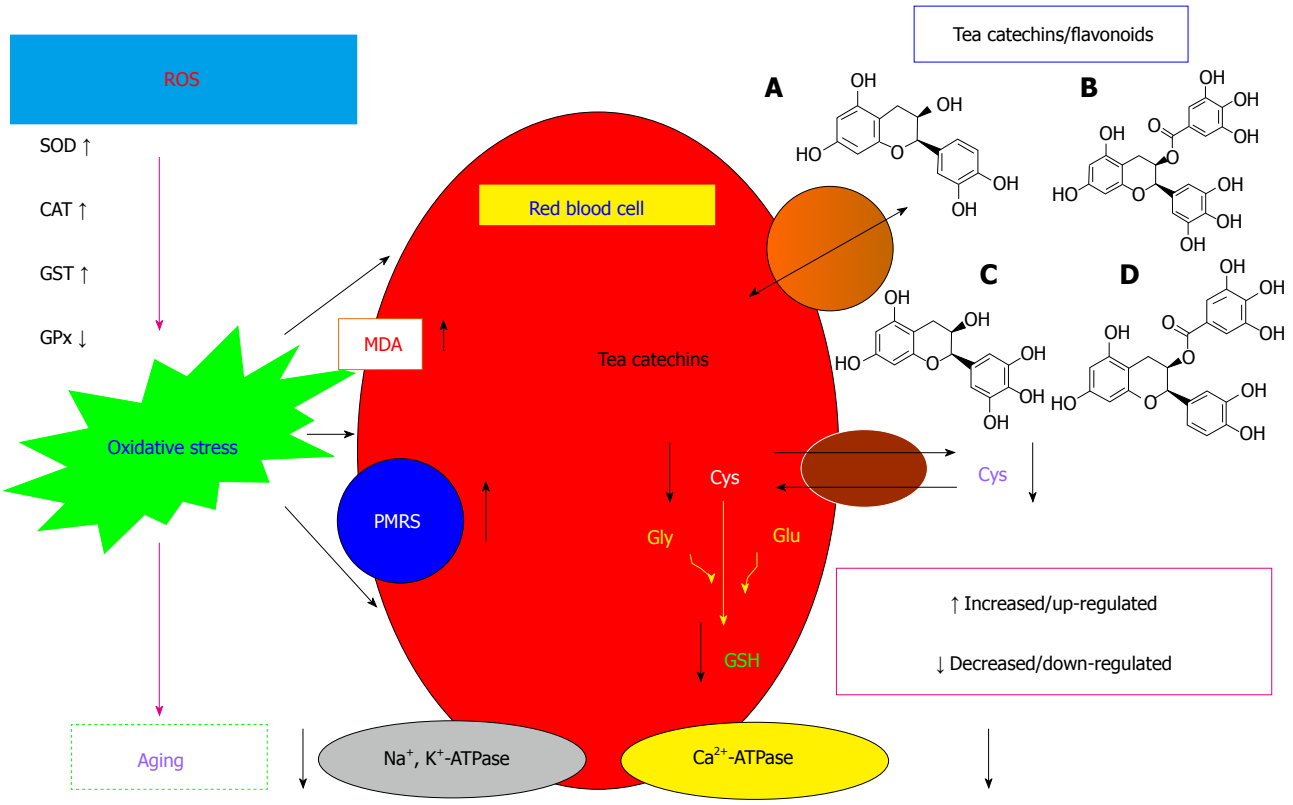

Figure 1 Aerobic cell produce reactive oxygen species as a byproduct of cellular respiration. Erythrocytes are equipped with antioxidant defense system for overcome excess production of ROS. The activity of SOD, CAT, GST and PMRS is upregulated while GPx activity is down regulated during human aging. Increased oxidative stress during aging results in elevated MDA levels and decreased GSH levels. Cysteine influx and efflux decreases during aging, which is an important amino acid for GSH biosynthesis. Red blood cell membrane bound enzymes (Na^+, K^+-ATPase; Ca^{2+}-ATPase) activity decreases as a function of human age. Dietary flavonoids like tea catechins modulate various biomarkers of oxidative stress and are protective in nature. Chemical structures of epicatechin (A), epigallocatechin gallate (B), epigallocatechin (C), and epicatechin gallate (D). Cys: Cysteine; Glu: Glutamic acid; Gly: Glycine; GSH: Reduced gluathione; GSSG: Oxidised glutathione; MDA: Malonaldehyde; SOD: Superoxide dismutase; CAT: Catalase; GST: Glutathione-S-transferase; GPx: Glutathione peroxidase; PMRS: Plasma membrane redox system; ROS: Reactive oxygen species.

containing proteins present outside the membrane. The proteins present in the outer membrane are easy target for free radicals which results in the formation of MDA. Increased MDA level results in alterations of the cell membrane polarity, charge sharing across lipid phase surface and oligomer formation. Further elevation in lipid peroxidation, results in decline resistance towards denaturation process, decreased number of membrane-SH groups and altered mobility of lipids. Several studies reported the importance of peroxidation of lipids in caloric restriction and longevity in different populations[13]. It has been observed that animals and avians have long life because they contains less number of unsaturated fatty acids in its plasma membranes, so they are not easy targets for ROS/RNS and have lower degree of MDA formation and less modification in proteins in long life of these species. Results clearly indicate that the animals/species having low degree of unsaturated fatty acids/unsaturation have longer life span.

Reduced glutathione

Reduced glutathione (GSH) is a primary antioxidant of erythrocytes. It is a tripeptide containing there amino acids and is an intracellular non-protein sulphydryl (-SH) compound. A significant decrease in erythrocyte GSH level has been reported in human erythrocytes[10]. Erythrocyte GSH level negatively correlated between decline GSH and age has been observed, this decrease is also correlates with total antioxidant potential of plasma[14]. Erythrocytes contain most important hydrophilic antioxidant in form of GSH[15]. Reduced glutathione contains sulphur containing amino acid cysteine which is the rate liming amino acid in GSH biosynthesis. Due to presence of cysteine amino acid, GSH helps in maintaining reduced status of SH group of cell membrane, including many other biological functions. OS results in the oxidized form of SH groups, which will results in many cellular and functional dysfunctions. A study carried out in European subjects also demonstrate a significant decline in erythrocyte GSH level during aging[16], while other reports on the same study did not show significant alterations in intracellular glutathione[17]. Oxidized form of glutathione (GSSG) is not favourable for body. A recent study on age associated change in the glutathione level in brain of rat shows decline in glutathione in almost all regions of the brain. The conversion of reduced glutathione to oxidized glutathione was further increased and the ratio of these two (GSH/GSSG) was

also reported to decrease, which measures the oxidation/reduction status of the cell[18]. The GSH/GSSG ratio has many biological functions including oxidation/reduction signaling and in the protection of antioxidants. The ratio also helps in providing a link to influence of environment with elderly population[19].

Membrane-SH group

The human RBCs are rich in membrane SH groups. The -SH group play a major role in maintenance of oxidation-reduction status of the cell[20]. The OS caused by ROS/RNS in erythrocytes effect cell membrane and its mechanical characteristics. Any oxidative damage to the plasma membrane-SH group of erythrocyte will induce the alterations in micro-elasticity under pathological and physiological state of OS[21]. Alterations in the oxidation/reduction balance during normal aging can modify several enzyme activates and proteins within the cell. Most of the protein molecules contain sulphur containing amino acids, methionine and cysteine which are subject to reox changes. We reported as related decrease in erythrocyte membranes-SH group during human aging[10]. Enzymes and proteins having -SH group are easy target for ROS/RNS. Age related changes in protein oxidation have been documented in erythrocytes and plasma[22]. Nitric oxide is highly reactive having half life of few seconds yet it can diffuse cell membrane freely which make NO ideal for a transient signal molecule. It is known to be involved in aging process[23].

ENZYMATIC ANTIOXIDANTS

RBCs are exposed permanently with potentially damaging level of ROS/RNS, but their metabolic processes are capable of reversing this oxidative damage under normal conditions. However, it is well known that variety of physiological and pathological factors may increase ROS/RNS which induces the oxidative stress. In addition, hemoglobin is known to stimulate lipid peroxidation[24]. Erythrocytes are equipped by antioxidant defense system in form of enzymatic and non-enzymatic antioxidants[25]. This protective system in form of enzymatic antioxidants includes superoxide dismutase (SOD)[26], which detoxify the effect of superoxide radical ($O_2^{\cdot-}$) catalase (CAT)[27], which is involved in the conversion of H_2O_2 to H_2O, and other enzymatic antioxidants like glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferases (GSTs)[28,29]. Two enzymes are shared in H_2O_2 detoxification: CAT and GPx but their relative significance in H_2O_2 scavenging is still not clear[30]. The reduced glutathione (GSH) is a non-enzymatic antioxidant.

In human subjects there is a considerable disagreement in age-related changes of erythrocyte SOD and CAT activity[31,32]. Lower activities of CAT and SOD were shown in premature infants during first 72 h of their life in comparison with full-term infants and even during aging[33,34]. Less than 10% of normal erythrocyte CAT activity was observed in homozygous carrier of inherited CAT deficiency-acatalasemia[35], and less than 50% in heterozygous subject's hypocatalasemia[36]. An elevated SOD and CAT activities during aging in human erythrocytes has been reported[33]. Increased OS during normal aging was compensated by elevation in the activities of these enzymes. Elevated CAT and SOD activities may be a manifestation of more production of ROS/RNS during aging in humans. Several contradictory results have been shown in published reports for SOD and CAT role in normal aging process, the reason for which is hard to explain[37,38]. As far as pathological processes are concerned; decreased CAT activities were found in erythrocytes from human patients of different ages with several types of brain disorders including dementia, stroke and Parkinson disease[39,40].

Oxidative stress with alterations in profile of antioxidant enzymes in erythrocytes is also related to many others specific pathologies[41,42]. Cell requires certain enzymes to detoxify the toxicants. GSTs are group of enzymes that play a very important role in the detoxification of dangerous compounds to less toxic compounds[43]. Age associated changes in GST has been reported[44,45]. GSTs also play a significant role in drug resistant development in tumor cells, Alzheimer's and Parkinson's disease, atherosclerosis[46,47]. GST are involved in many biological functions in mammals which includes detoxification of toxicants, catalysis of several biological processes, several functions associated with metabolism, resistance towards drugs and inhibiting age associated disorders[48,49].

Age associated changes in the activity of GPx has been shown in human erythrocytes and correlated with total antioxidant capacity[50]. Several studies have been reported having conflicting data as to how GPx activity is changes with age[51]. Increased GPx activity have been shown in smaller population studies, while decreased activity has been reported in most large studies as a function of age[52]. GPx activity decreases in presence of more H_2O_2, which ultimately leads to direct cell/tissue damage and activation of nuclear factor-κB - related inflammatory pathways[53,54].

ALTERATIONS IN ERYTHROCYTE MEMBRANE TRANSPORT FUNCTIONS

In most of the eukaryotic cell membrane, the lipids are distributed in asymmetric manner across the bilayer plan. This kind of structural organization is referred as trans asymmetry. This trans-asymmetric distribution play very important role in structural and functional aspect of cell membrane. Homeostasis is very important for the cell. Transport of ion across the cell membrane are regulated by various kind of cell membrane enzymes such as Na^+, K^+-ATPase and Ca^{2+}-ATPase[55]. A recent study reports a significant decreased activity of these two enzymes during human aging[56]. Transport of dietary flavonoids across cell membrane is well documented[57]. Recently, we reported the beneficial effect of tea catechins in erythrocytes during human aging[58,59]. L-cysteine is a

sulphur containing amino acid that has free functional -SH group which is important in oxidation/reduction reactions. Free -SH group in erythrocyte membrane will help in the regulation and maintenance of intracellular redox status of erythrocytes and other cell types. Human erythrocyte does not have any machinery to synthesize protein inside the cell. Synthesis of GSH take place inside erythrocyte and it require L-cysteine, a semi-essential amino acid. GSH is a tripeptide containing glutamic acid, cysteine and glycine joined together with the help of peptide bonds. All above said amino acids are required for the biosynthesis of GSH, but the rate depends on only the availability of L-cysteine. We report L-cysteine influx and efflux across human erythrocytes during aging[60,61]. L-cysteine is the amino acid which provides free functional -SH group to GSH which play a very important role in antioxidant defense system. GSH is a soluble antioxidant which protect cell from ROS/RNS caused oxidative damage. Recently, there are substantial evidences in literature which supports that normal aging is accompanied with higher level of OS. Incorporation of flavonoids and cysteine in diet has been shown to alter several OS biomarkers which are known to be associated with aging and age related disorders[62]. We report a significant decreased efflux of L-cysteine in erythrocytes during aging[63]. Since, GSH biosynthesis in erythrocytes is dependent on L-cysteine bioavailability; the decreased influx and efflux may explain the low GSH level reported in erythrocytes as a function of age[10]. L-cysteine transported into erythrocytes only when there is properly reduced membrane lipid and protein thiol. Aging is associated with reduced antioxidant capacity which results in induction of conformational and structural changes in the cell membrane and in the amino acid transporters that finally results in L-cysteine influx and efflux.

CONCLUSION

Red blood cells are the main cell types present in blood. It is reviewed in this mini-review that erythrocytes have a very particular membrane structure and composition which alters during aging and that support their features and functions to study human aging. It has been shown that RBCs have ability to encounter various oxidative stressors to prevent oxidative stress. They are good model to study various plant products to evaluate their anti-aging properties. These findings of biomarkers of OS during normal aging will help in the establishment of reference values for biomarkers of OS in elderly peoples and several other parameters. These reference values will help in studies that involve the role of biomarkers in various other diseased conditions.

ACKNOWLEDGMENTS

Pranjal Chandra thanks Indian Institute of Technology, Guwahati for providing necessary facility for completion of this research work.

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P- Reviewer: Haidara M, Mechri A **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

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