A STUDY ON THE EFFECT OF ANTIOXIDANTS ON RBC CELL MEMBRANE & SPERM QUALITY OF MALE INDIVIDUALS.

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ABSTRACT

Cell is the most basic functional unit that consists of various components and of various types and helps to sustain life. It. The most vital cell is the RBC which is used to carry oxygen throughout the body through a network of vessels. At present several research's come out at this existing problem and many of them have discovered the harmful effects on the red blood cell membrane by consumption of antioxidants. It has been found that antioxidants can hamper the cell membrane of red blood cell and can fail to deliver oxygen as well as increase the age of the cell as it creates cellular oxidative stress.Oxidative stress is characterized by an imbalance between the quantity of chemically reactive oxidants and the ability of a biological system to detoxify them, or to repair the resulting damage. It is known that oxidative stress plays a significant role in a wide range of chronic and degenerative pathologies such as cancer, diabetes, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases. Similarly, antioxidants have harmful effects on sperm by deteriorating its quality. In severe cases, it can cause male infertility. Oxidative stress can decrease their number, hinders motility and development of normal sperm morphology, and impairs its function.Also, antioxidants have the potential to damage the DNA structure which ultimately leads to apoptosis of the cell.This study is done to know various effects of antioxidants on the membrane of red blood cells as well as the quality of sperms in males.

KEYWORDS: Red blood cell, sperm, stress, antioxidants, membrane, oxidative etc

INTRODUCTION

Antioxidants are artificial or natural substances that can prevent or delay some types of damages by delaying the formation of free radicals. Free radicals are substances or electromagnetic fields that damage to the cells by impairing the immune system. Antioxidants are found in various food and vegetables and are also available as dietary supplements in the market. Some the examples of antioxidants available are ascorbic acid, glutathione, α tocopherol, β-carotene, Lycopene, Vitamin A, Vitamin C, Vitamin E, Selenium etc. Antioxidants function in the body depends on how they are utilised and absorbed.

The amount of antioxidant variesfrom one substance to another substances (fruits and vegetables)i.e. a half cup of dried red kidney beans have about 13,727 total antioxidant capacity whereas 1 cup strawberry have about 5,938 total antioxidant capacity. If antioxidants are taken in high dose then it is proved to be fatal as they are linked to higher risks. For instance, a high dose of beta-carotene increases the risk of lung cancer in smokers andalso high dose of vitamin Eincreases the risk of prostate cancer.

The main reason of consumption of antioxidants is to curb free radicals generated inside the body as they have the potential to cause a number of deleterious events. These free radicals are generated due to various factors such as exposure to the ionising radiations, UV radiations, air pollution, smoking, inflammation and stress. Excess ofReactive Oxygen Species (ROS) are formed which is described a number of reactive molecules and free radicals derived from molecular oxygen. These molecules, produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation. The key role of antioxidant molecule is to neutralise the free radical by donating an electron to it and make it stable to stop the free radical chain. To control the production of ROS antioxidants are generally consumed in higher amounts than the usual.

EFFECT ON MEMBRANE OFRED BLOOD CELL

Antioxidant's effect can be determined by using sulphydryl compounds, bioflavonoids, vitamin A, B, C and E. The effect of damage of the oxidative respiration is caused by ACES complex with 3 amino acids which results in glutathione. Some disorders related to RBC such as Sickle Cell Anemia, antioxidants are used in which oxidative stress is the main feature. Sickle cell disease (SCD) is basically a red blood cell (RBC) disorder characterised

by sickling and haemolysis, but platelets and polymorphonuclear neutrophils (PMN) are also involved[1]. Red Blood Cells from patients of SCA (containing HbSS genotype) SCA contains the abnormal hemoglobin (Hb) HbS. On comparison with normal hemoglobin Hb, HbA, HbS results from the single base substitution in the seventh codon of the β globin which leads to the replacement of glutamic acid with valine. It was hypothesized that antioxidants can also have beneficial effects on the abnormal membrane permeability of sickle cells. Increased cation permeability of these cells encourages HbS polymerization by causing RBC dehydration and also leads to externalization of the prothrombotic amino-phospholipid phosphatidylserine (PS). Three antioxidants with different mechanisms of action were investigated – dithiothreitol, *N*-acetylcysteine, and quercetin. All three were found to inhibit the main cation pathways responsible for dehydration – the deoxygenation-induced cation conductance (or P_{sickle}), the Ca^{2+}-activated K^+ channel (or Gardos channel), and the K^+-Cl^- cotransporter[2]. They also reduced Ca^{2+}-induced PS exposure and hemolysis. Findings provide evidence for additional beneficial actions of antioxidants in maintenance of rheology and reducing vascular adhesion and further inform the rationale for their clinical use.

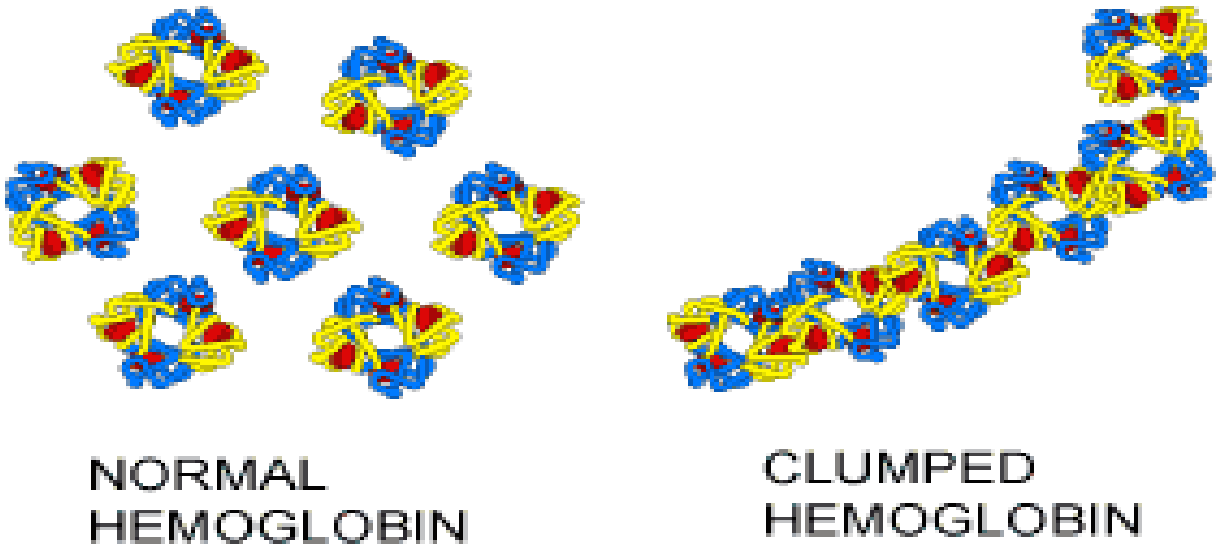

Figure no-1; comparative images of normal haemoglobin and the clumped haemoglobin.

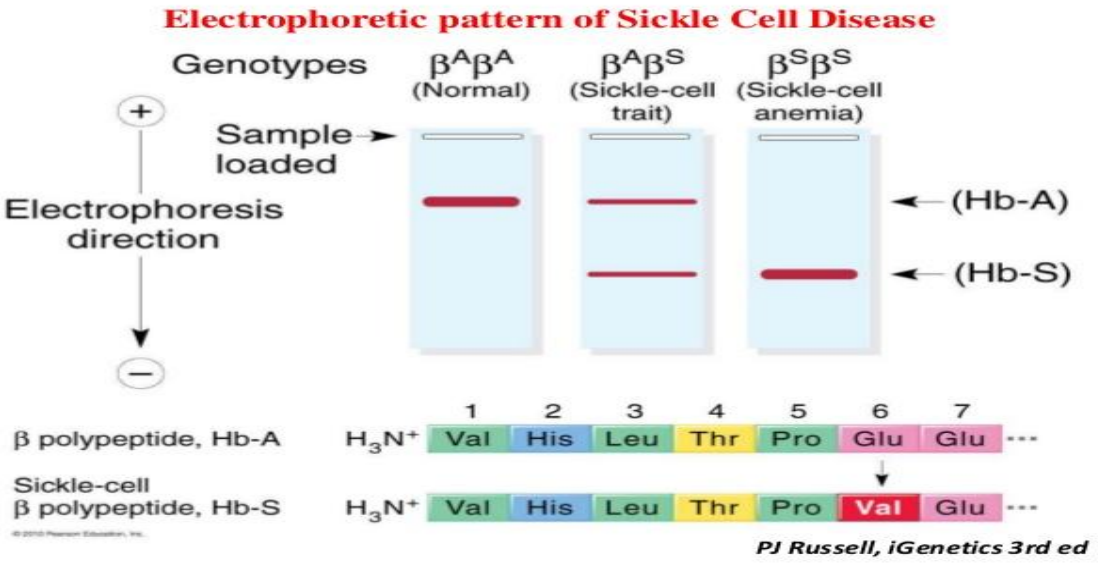

Figure no 2; electrophoretic pattern of stickle cell disease and antioxidant.

Other beneficial effects of antioxidants include to have protection against RBC lipid peroxidation and increasing levels of reduced glutathione (GSH) while reducing levels of reactive oxygen species. The unexplored area consists of RBC membrane permeability. We postulate that antioxidant treatment may have important alternative beneficial effects on RBC function by reducing the permeability of the transport pathways mediating dehydration and by reducing PS exposure[3].

LIPID MEMBRANE

Red cells are the formed through different process that begins in the bone marrow where hematopoietic stem cells are differentiated into nucleated RBCs. Its shape is determined by various membrane proteins such as present in the lipid bilayer. RBCs can maintain their discoid shape and allow cytoskeletal rearrangements that allow them to pass through capillaries and then become normal in their shape without cell fragmentation. They are unable to generate ATP using molecular oxygen because of lack of mitochondrial organelles. Therefore, glycolysis is the only source of ATP generation in matured RBCs. It has been reported that under normal conditions about 90% of total glucose is used to generate ATP through glycolysis and rest 10% of glucose is directed to the hexose monophosphate shunt[4]. Cells often use this alternative pathway to generate NADPH which are used by RBCs to reduce glutathione.

RBCs are largely exposed to several numbers of stressful situations. On an average, RBCs pass once every minute through the lungs where it faces oxidative stress. More than once an hour, it travels through the medulla of kidney where it is exposed to osmotic shock[5]. Due to their size, they have to squeeze through capillaries which are smaller than the cells. Both types of proteins, intrinsic as well as extrinsic, and membrane lipids are susceptible to oxidative modifications. The matured red cellsare restricted in its ability to respond to oxidative stress and it cannot synthesize new protein or replace irreversibly damaged cellular components[6]. The resultant preservation of membrane structure and function is essential for maintaining membrane fluidity and flexibility as well as ionic balance between the intracellular and extracellular compartments. Manystudies have shown that several RBCs parameters are negatively affected by increased oxidative stress including inactivation of membrane bound receptors and enzymes, ionic parameters, increase in oxidation of glutathione (GSH), proteins and lipids.

HEMOGLOBIN

It is the major protein in RBCs, densely packed in cytoplasm and constitutes about 90% of the weight of the red cell. The ferrous ion of hemoglobin is exposed to high concentrations of oxygen and undergoes slow oxidation to methemoglobin (metHb). MetHb does not bind or carry oxygen molecules. Under nominal conditions, metHblevel in RBCs is maintained at <1% of total hemoglobin. However, in higher stress conditions, it is increased by greater folds.

The oxidation of hemoglobincauses the formation of disulfide cross-links between adjacent globin chains which distorts the primary structure of hemoglobin and forms precipitates called Heinz bodies. At limited oxidative result, these aggregates of membrane bound to denatured protein but at higher degrees of oxidation, results in hemolysis of RBCs[7].

POLYUNSATURATED FATTY ACID

RBCs plasma membrane is rich in polyunsaturated fatty acid (PUFA) chains. They are highly susceptible to oxidation. The RBC membrane consists of two domains, cytoskeleton and lipid bilayer. The phospholipids are dispersed asymmetrically in the bilayer whereas cholesterol is distributed evenly throughout the lipid domain. The cholesterol helps in flexibility and provides stability to the membrane. The cell membrane also contains proteins and

glycoproteins inthe lipid bilayer. Lipids help in the maintenance of the RBCs shape. Even certain small changes in the surface area can lead to morphological and functional abnormalities. Red cell membrane fluidity is also important for proper red cell function. ROS attack that causes lipid peroxidation and formation of an array of unwanted products. Malondialdehyde (MDA) is a major lipid peroxidation product[8].

Oxidized lipids are able to produce MDA as a decomposition product and the mechanism involves formation of prostaglandins, like endoperoxides from PUFA with two or more double bonds. In 1990sEsterbauer and Cheeseman suggested an alternative mechanism for the generation of MDA, based on successive hydroperoxide formation and β cleavage of PUFA which is the main source of MDA generation in vivo. The other minor sources of MDA formation also exist such as byproducts of free radical generation by ionizing radiation and biosynthesis of prostaglandins. One can measurethiobarbituric acid reactive substances (TBARS) for the assessment of the extent of lipid-peroxidation in the cell membrane. High levels of cytoplasmic antioxidants both enzymatic and non-enzymatic works against ROS in order to protect the RBCs from the deleterious effects of oxidative stress. Reduced glutathione (GSH), ascorbic acid (ASC), α-tocopherol and other thiols groups are the major endogenous non-enzymatic antioxidants. α-Tocopherol serves as potent scavenger of peroxyl radicals to protect PUFA present in red cellmembranes against peroxidation[9].

Low level of α-tocopherol has been reported in many clinical conditions involving oxidative stress. ASC is the primary cellular antioxidant as it protects the membrane and other hydrophobic compartments from any oxidative damage by regenerating the antioxidant form of α-tocopherol. GSH provides primary protection against oxidants in cells and is considered a molecule with different functions. GSH levels in cells reflect the dynamic equilibrium between its synthesis and utilization. The key role of GSH in RBCs is to maintain hemoglobin in its native form in cells at higher concentrations. Peroxidation of the RBCs membrane is known to cause impaired membrane integrity. Besides a direct role in protection against oxidative stress, GSH is also functions as cofactor for a number of protective enzymes, such as GSH peroxidase and GSH-Stransferases. ROS induced oxidative stress causes GSH depletion as a result of which the overall redox system of the cell is altered. Under oxidative conditions, GSH is reversibly oxidized to glutathione disulfide (GSSG) which can pass through red cell membrane due to oxidative stress induced membrane damage. This mechanism may be responsible for the decreased red cell GSH levels in

oxidative stress condition. It is assumed that the capacity of GSH to neutralize oxidants is due to the nucleophilicity of the thiol group and its high reaction rate with oxidants. Low levels of GSH are more sensitive to the effects of irradiation and stress than cells with normal levels of GSH. Depleted levels of GSH have been reported in a number of pathological conditions such as Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, AIDS, cancer, heart attack, stroke, diabetes and aging.

EFFECT ONQUALITY OF SPERMS IN MALE INDIVIDUALS

Human semen contains various cells including mature and immature spermatozoa, round-shaped cells of differentstages of spermatogenesis, epithelial cells and leucocytes. Leucocytes and spermatocytes are the major sources of ROS. Excess residual cytoplasm has the relative connectivity between increased ROS production and poor sperm quality. According to a study, cytoplasmic droplets are main sourcesof ROSdue to defects in spermiogenesis. During spermatogenesis, a defective cytoplasmic process results in the release ofspermatozoa from germinal epithelium accompanied by residual cytoplasm. The releasedspermatozoa are mainly immature and defective. Two enzyme systems have been proposedto be responsible for ROS production. They are mitochondrial oxidoreductase and the oxidase in spermplasma membrane. Both enzymes are dependent on sperm-specific NADPH. ROS inhuman ejaculates emanate from either immature, morphologically abnormal spermatozoa or seminalleucocytes. A regular supply of energy is needed by the spermatozoa for motility and hence their richnessin mitochondria. Consequently, dysfunctional mitochondria stimulate increased ROS production with a resultant negative effect on its metabolic functions. This may be caused by ROS damage tothe membrane while the weak mitochondrial membrane stimulates increased ROS production.Studies have shown that ROS levels in fertile men are lower than in semi-fertile men.The correlation between oxidative stress and rising leucocyte count had been observed. It can therefore be concluded that the presence of leucocyte is connected with oxidative stressand may impair fertility.

CONCLUSION

To cope with the ROS in the RBCs, they possess effective antioxidant enzyme systems that neutralize the reactive oxidants into non/less reactive species such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) and many more.They give protection by scavenging superoxide radicals and hydrogen

peroxidemolecules. Superoxide dismutase is one of the best enzymatic antioxidant. This enzyme is located in the cytoplasm of the cell and catalyzes the dismutation of superoxide radical (•O2) in to hydrogen peroxide.

Catalase breaks H_2O_2 to water and molecular oxygen. However glutathione peroxidases reduce H_2O_2 to water by oxidizing two molecules of glutathione into GSSG. CAT has ability to degrade H_2O_2 without consuming NADPH which is an energy efficient way of removing H_2O_2. This mechanism of action results in a net gain of reducing equivalents. Many experimental reports shows that the increase of oxidative stress during imbalance in redox hemodynamics leading to the development of a number of pathological changes in RBCs. Altered activities of SOD and CAT have been reported in aging populations. A significant decrease in the activities of enzymatic antioxidant defense system has also been reported in diabetic patients. There are several vital functions of plasma membrane redox system PMRS including maintenance of homeostasis and recycling of ascorbic acid. Since ascorbic acid is a primary antioxidant in the body and humans cannot biosynthesize it, due to lack of functional enzyme, L-gulonolactone oxidase, the role of PMRS becomes vital. It has been reported that PMRS is a compensatory/ protective mechanism that operates to maintain the ascorbate level in plasma and thereby minimize oxidative stress. An altered PMRS status has been found in the RBCs during condition of oxidative stress. The activity of PMRS has been shown to be elevated in RBCs from patients with diabetic nephropathy, type 2 diabetes mellitus and during aging in humans.

Also in the case of sperm cells,superoxide dismutase (SOD) or superoxide oxidoreductase catalyzes dismutation reaction of the superoxide anions. They exist in both extra- and intracellular forms. The first intracellular form contains copper and zinc (SOD-1) in the active center and is localized mostly in the cytoplasm while the second form found in the mitochondria with manganese in the active center is known as SOD-2. The extracellular form of SOD functions in the extracellular space (SOD-3). It is associated with the surface polysaccharide although it can be found in a free form. High activity of SOD has been reported in seminal plasma with 75% of its action being associated with SOD-1 while 25% of activity has been related to SOD-3. Glutathione peroxidase (GPx) also plays its catalytic role by reducing hydrogen peroxide and organic peroxides which include peroxides of phospholipids. Selenium is found in the active site of GPx in the form of selenocysteine. It is

localized in the mitochondria matrix of the sperm but recently a nuclear form of GPx has been associated with sperm DNA protection from oxidative damage.

Another enzyme of antioxidant system is catalase which decomposes hydrogen peroxide to oxygen and water. It has a structure of heme system with iron atom in the center. Its activity has been reported in different organelles such as peroxisomes, endoplasmic reticulum, mitochondria and the cytosol in various types of cells. Catalase protects the cell from nitric oxide induced oxidative stress by stimulating sperm cell capacitation through a complex mechanism using hydrogen peroxide.

CONFLICT OF INTEREST: NA

ETHICAL CONSIDERATION: NA

SOURCE OF FUNDING: NA

REFERENCES

1. Mohanty, J. G., Nagababu, E., & Rifkind, J. M. (2014, February 28). Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging.
2. Cavallini, G., Dachà, M., Potenza, L., Ranieri, A., Scattino, C., Castagna, A., &Bergamini, E. (2014, June). Use of red blood cell membranes to evaluate the antioxidant potential of plant extracts.
3. Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state.
4. Mandal, A. (2017, August 03). What is Oxidative Stress?
5. Mora, A. R., Meniri, M., Gning, O., Glauser, G., Vallat, A., &Helfenstein, F. (2017). Antioxidant allocation modulates sperm quality across changing social environments.
6. B., D., M., & I. (2013, April 29). Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants.
7. An Introduction to Reactive Oxygen Species - Measurement of ROS in Cells | January 26, 2014. (n.d.).
8. Effects of electromagnetic fields exposure on the antioxidant defense system. (2017, August 02).
9. Adewoyin, M., Ibrahim, M., Roszaman, R., Isa, M. L., Alewi, N. A., Rafa, A. A., &Anuar, M. N. (2017, March 01). Male Infertility: The Effect of Natural Antioxidants and Phytocompounds on Seminal Oxidative Stress.