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Title: OXIDATIVE STRESS IN AGED PREGNANCY (OSAP)

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ABSTRACT

Advanced maternal age is one of the causes of maternal and fetal mortality and morbidity. Advanced pregnancy is associated with oxidative stress (OS), in maternal circulation. Our aim was to evaluate and compare the levels of OS and antioxidant enzymes (AOE) in Human Umbilical Cord Blood (HUCB) samples and normal blood samples ofIndian women from the age group of 19 to 31.Human Umbilical Cord Blood (HUCB) was obtained from 2 concerned hospitals-Lakshmi Maternity Home and Narayana Super-Specialty Hospital with prior consent of the donor. First pregnancy between the age group of 19-31 were considered (N=5). The subjects were pre-evaluated in terms of gestational complications such as diabetic, hypertension, thyroid etc. and were not included in the study. Human Umbilical Cord Blood (HUCB) collected from both caesarian and normal delivery was considered. Biochemical analysis of Antioxidant enzymes (AOE) - SOD, CAT, Protein oxidation(PO)- P-SH, AOPP and Lipid peroxidation (LPO) was analyzed and Human Umbilical Cord Blood (HUCB) methemoglobin and hemoglobin was determined.

The present study revealed that pregnancy with advancing age exerts a moderate oxidative stress in Human Umbilical Cord Blood (HUCB) but is characterized by compensatory up regulation of antioxidant enzymes. Hence, a less pronounced lipid peroxidation and protein oxidation were noticed especially due to the up-regulation of SOD activity in aged mothers when compared to their younger counterparts.

RELEVANCE OF FINDINGS: Our findings indicate a good potential for therapeutic approaches in advanced pregnancy to minimize the oxidative stress complication in both mother and the fetus. Future investigation focusing on adequate antioxidant therapy, after confirmation of these findings through intervention studies, may decrease the indices of oxidative stress

related complications in advanced maternal age and reducing the chances of complicated pregnancy during later age for carrier oriented women.

Key Words- Oxidative stress (OS), Antioxidant Enzymes (AOE), Thiobarbutiric acid reactive substances (TBARS), Protein sulphydryl (P-SH), advanced oxidation protein products (AOPP), Methemoglobinemia (MetHb)

INTRODUCTION

Recent living trend demands a career oriented advanced lifestyle, thereby delaying the onset of bearing children and a family. Hence modern times the gestational age has been postponed owing to many physiological consequences, affecting both mother and child. Many researchers are now taking up this issue seriously and are working hard to minimize the age- associated complications with advanced pregnancy. The current project is an effort to get an insight into the OS status of pregnant women's above the age of 25 years.

Oxidative stress (OS) is an imbalance between reactive oxygen species (ROS) and antioxidants (AOE). ROS are highly reactive molecules with unpaired electrons, leaked from mitochondrial membrane during electron transport chain and they tend to become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates, there by destroying the normal cells. The free radicals (FR) thus formed as a by-product of oxygen metabolism, are also called oxidants. These are formed as intermediates in the formation of water from molecular oxygen by losing electrons (Helmut S, 1997).

AOE are the molecules that are present in the cells to prevent the action of FR by donating an electron, without destabilizing themselves. There are two types of antioxidants in the human body: enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants are also known as natural antioxidants; they neutralize excessive ROS and prevent it from damaging the cellular structure. They include Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione reductase (GR). Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements such as vitamin C, vitamin E, selenium, zinc, etc.

Antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are components of an organism's mechanisms for combating oxidative stress which is generated in normal metabolism and which may also be a reaction in response to external stimuli. SOD converts superoxide to hydrogen peroxide and oxygen. Small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, and glutathione also play important roles as cellular antioxidants (Biswas S *et al*, 2014)

Oxidative stress and pregnancy

ROS affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy. It has been suggested that OS modulates the age-related decline in fertility. It plays a role during pregnancy and normal parturition and in initiation of preterm labor. Pregnancy, mostly because of the mitochondria-rich placenta, is a condition that favors OS. Transitional metals, especially iron, which are particularly abundant in the placenta, are important in the production of FRs. Protective mechanisms against FR generation and damage increase throughout pregnancy and protect the fetus, which, however, is subjected to a varying degree of oxidative stress.

Pregnancy or gestation is a physiological condition that occurs in women, in which they are prone to oxidative stress, because pregnancy is associated with high metabolic demand and elevated oxygen utilization by the tissue (Saikumar P *et. al*, 2013). The placenta is rich in mitochondria and contains the transition metals like iron in abundance, which is responsible for the production of ROS (Casanueva and Viteri, 2003).It has been suggested that the age-related decline in fertility is modulated by OS. It also plays a role during normal parturition and in initiation of preterm labor. (Agarwal A *et al*, 2005).

Oxidative stress peaks by the second trimester of pregnancy, ending what appears to be a vulnerable period for fetal health and gestational progress. Conditions restricted to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit exaggerated indications of free radical damage. Antioxidants as well as avoidance of iron in excess ameliorate maternal and early fetal damage (Casanueva and Viteri, 2003).

As pregnancy progresses, different mechanisms enhance the transfer of iron to the placenta and the fetus therefore the oxidative stress level increases with the gestational period. The studies have indicated that the levels of antioxidants enzymes like SOD found to be lower in pregnancy whereas product of lipid peroxidation (LPO) caused by FR increases. Defense mechanisms

against free radical damage are also enhances as pregnancy progresses.Studies show that, the foetus is protected by placental ROS, mainly during last week of gestation. (Casanueva and Viteri, 2003).

Oxidative stress also causes methemoglobinemia.Methemoglobinemia is a condition in which hemoglobin is oxidized to the ferric form and is unable to transport oxygen to tissues, therefore causing hypoxia. The physiologic level of methemoglobin is 1% in peripheral blood, and it may increase because of a variety of genetic, dietary, idiopathic, toxic, and other Factors. Methemoglobinemia primarily occurs when erythrocytes are affected by xenobiotics and pharmaceutical compounds with toxicological properties, such as volatile organic compounds, oxidants, nitrogen oxides, peroxynitrites, phenacetin, and sulfonamides(Francini K, 2011). The evaluation of the methemoglobin concentration is an important marker of biological processes of oxidative damage. Methemoglobinemia can cause the deterioration of maternal and fetal hypoxia, which can indirectly explain how the failure to notice high levels of fetal methemoglobin that can cause sudden fetal death and stillbirth that is frequently referred to as unexplained. However, current research data are insufficient to confirm methemoglobin as a biomarker of the adverse effects of oxidative stress and its pro-oxidative properties.

The term “advanced maternal age” is commonly used by health care providers to describe pregnancies in women aged 32 and older. There is an increase in oxidative stress and high magnitude suppression/decrease in antioxidant enzymes activities with increase in age of pregnant women. This indicates an increased risk with maternal age could be due to an increase in oxidative stress. (Haque S K, *et al*, 2010)

Pregnancy in women over 35 can trigger high blood pressure and diabetes, and the risk of preeclampsia (pregnancy-induced hypertension) may also increase. The risk of miscarriage and stillbirth goes up with age as well, possibly due to chromosomal abnormalities or uterine fibroids (benign tumors found in nearly one-quarter of women over 35), which may interfere with fetal development. The incidence of twin births also increases and can cause preterm labor, which occurs in almost half of all multiple pregnancies. Older women have a higher rate of cesarean sections as well, which involve a longer recovery.

There is growing literature on the effects of OS in female reproduction with involvement in the pathophysiology of preeclampsia, free radical-induced birth defects and other situations such as abortions. Late pregnancy is also associated with the formation of susceptible, oxidisable

particles (high LDL score) and an increase in oxidative damage. These biochemical changes may be relevant for the long-term cardiovascular health of women, especially those of high parity or those who are at high risk for cardiovascular disease.

OBJECTIVE AND HYPOTHESIS

The objective of this prospective study was to determine if a correlation could be established between advanced age and pregnancy in terms of oxidative stress in umbilical cord blood (UCB). Umbilical cord blood remains in the placenta and is attached to the umbilical cord after childbirth. Cord blood until recently considered as medical waste, has potential applications as it contains stem cells, which could be used to treat hematopoietic and genetic disorders. It also provides information about fetomaternal status at the time of birth (Billert H *et al*, 2007).

In the view of the mentioned facts, we put forth the hypothesis that,

- Increased OS may be a debilitating consequence of physiological changes during pregnancy.
- Endogenous antioxidant enzymes impart a beneficiary effect in pregnancy
- Advancing age attenuates the oxidative stress in pregnancy

The objective of the present study was designed to meet the afore-mentioned hypothesis which included and detailed analysis of the following parameters.

OS biomarkers such as,

- Endogenous AOE, catalase, and super oxide dismutase
- Evaluation of thiobarbutaric acid reactive species (TBARS) as a marker of lipid peroxidation.
- Protein oxidation was studied through protein thiol content (-SH-), and advanced oxidation protein products (AOPP) as oxidative protein modifications.
- Determination of UCB methemoglobin and hemoglobin content.

All of the aforesaid objectives have been studied in the umbilical cord blood collected from healthy pregnant women of different age.

MATERIALS & METHODS

CHEMICALS

All the chemicals used were of laboratory /analytical grade- Acetic acid, Acrylamide, Ammonium per sulphate, Bis acryl amide, ethyl alcohol, Glycine, Hydrogen peroxide, Hydrocholric acid. Ortho phosphoric acid, Potassium iodide, Sodium chloride, sodium hydrogen carbonate, sodium dihydrogen ortho phosphate,sodium dodecyl sulphate, sodium EDTA, Sodium hydroxide, Sodium nitrate, Tricholoro acetic acid(TCA), TEMED, Tris-EDTA, Tris-HCl, Triton-X-100.Fine chemicals were purchased from Sigma.

SAMPLE COLLECTION

Human umbilical cord blood samples were obtained from 2 concerned hospitals-Lakshmi Maternity Home and Narayana Super Specialty Hospital with prior consent of the donor. First pregnancy between the age group of 19-31 were considered (N=5).

The subjects were pre-evaluated in terms of gestational complications such as diabetic, hypertension, thyroid etc. and not included in the studies.

UCB from both caesarian and normal delivery were considered.

BIOCHEMICAL ANALYSIS

I.ANTIOXIDANT ENZYMES

I.A. SUPER OXIDE DISMUTASE ACTIVITY (SOD E.C.1.15.1.1)

SOD activity was determined by the method of Mishra and Fridovich (1972). To 100µl of sample 880µlof carbonate buffer was added.20µl of epinephrine was added to the mixture in the cuvette & the change in activity was measured at 480nm for 5mins. Activity was expressed as the amount of enzyme that inhibited the oxidation of epinephrine by 50%, which is equivalent to one unit and is expressed in terms of unit/mg protein.

I. B. CATALASE ACTIVITY (CAT E.C 2.3.1.6)

CAT activity was measured by the method of Aebi H (1984). To 100 µl of sample, 10µl of absolute ethanol was added and incubated at 0^o for 30minutes followed by room temperature for 10minutes. 10µl of triton X 100 was added and treated this as sample. 40µl of this sample is mixed with 960µl of phosphate buffer and 1ml of 0.066 M H_2O_2 solution. The decrease in activity was measured at 240nm for 1minute against suitable blank.

II.PROTEIN OXIDATION (PO)

II.A. PROTEIN SULFHYDRYL CONTENT (P-SH)

Concentration of P-SH was measured by the method of Habeeb (1972). To 1.5ml of buffer containing 0.08mol/l sodium phosphate, 0.5mg/ml of sod-EDTA & 2% SDS was added to each assay tube followed by 0.2ml of sample. After vortexing, 0.1ml of 5, 5 dithiobis-2 dinitrobenzoic acid was added. The solutes were vortexed again. Color was allowed to develop for 15 mins at room temperature & absorbance was measured at 412nm against equivalent concentration of sample but without DTNB. P-SH concentration was calculated at the net absorbance & molar absorptivity of 13,600mol/l/cm. The results were expressed as n mol/mg protein.

II.B.ADVANCED OXIDATION PRODUCT (AOPP)

AOPP was determined by the method of Witco et al. (1992). To 1.2ml of sample, 100 µl of 1.16mol/l of potassium iodide was added & 2 mins later 200µl of acetic acid was added. The absorbance of the reaction mixture was immediately read at 340nm against a blank containing

1.2ml of PBS, 100µl of KI & 200µl of acetic acid. Concentration of AOPP was calculated by using the extinction coefficient of 26Mm $^{-1}$cm$^{-1.}$ The final result was expressed as n mol/mg protein.

III.PROTEIN CONCENTRATION

Protein concentrations of samples were determined by the method of Bradford et al, 1976. 100µl of sample was added to 5ml of protein reagent, mixed well and absorbance was read at 595nm after 2 minutes in a double beam bio-spectrophotometer and expressed as g/ml.

The concentration of protein in sample was determined from standard curve of 1000mg bovine Serum Albumin Stock. Standard graph was constructed with concentration of 20-100µg.

IV. LIPID PEROXIDATION (LPO)

THIOBARBUTARICACID REACTIVE SUBSTANCES (TBARS)

TBARS was determined by the method of Ohkawa et al.,(1979). To 40 µl of sample, 0.9% of Sodium chloride and 40µl of distilled water was added. This was incubated at 37^{o}C for 20 min. 600µl of 0.8 M hydrochloric acid containing 12.5% Trichloro acetic acid and 780µl of 1% Thiobarbutaric acid was added. The mixture was boiled for 20 min and cooled at 4^{o}C for 1hr. This reaction mixture was then centrifuged at 3000rpm for 20 min and absorbance was read at 532 nm against the blank (0.9% sodium chloride).the results were expressed as µg/mg protein.

V. A. METHEMOGLOBIN CONTENT

Methaemoglobin was measured using the method of Evelyn and Malloy. MetHb blood samples were prepared by treating normal blood with $NaNO_2$. 0.2ml of this treated blood was mixed with 10 ml of MetHb stabilizing solution containing phosphate buffer and non-ionic detergent. Absorbance was measured at 630nm against a suitable blank. The methemoglobin content was calculated by subtracting the treated blood value with non-treated blood samples and expressed in percent.

V. B. HEMOGLOBIN CONTENT

This was done using haemoglobin test kit method. The 10 µl sample was mixed with Drab kin's reagent and allowed to stand for 5minutes. Absorbance was measured at 546 nm against a suitable blank containing reagent without sample. The hemoglobin content was expressed in gm%

VI. RESULTS

STATISTICAL ANALYSIS

All the data were expressed as Mean ± SEM of five blood samples of same age. The changes were analyzed by One-way ANOVA and was considered significant at $p<0.005$. Two-Way ANOVA was performed between the age and oxidative stress parameters, significance was considered at $p<0.005$.

I. ANTIOXIDANT ENZYMES

I.A.SUPER OXIDE DISMUTASE ACTIVITY (SOD)

SOD activity remains unchanged in all the age group of pregnant women ($p<0.05$). **(Fig No. 1).** Increased SOD activity was seen with lesser TBARS in all the age group. ($p<0.001$) **(Fig No. 3).**

Our results showed decreased TBARS in the older UCB samples when compared to the UCB from their younger counterparts. However, TBARS from UCB of 26years old showed a 4% increase when compared to 19, 23 and 24years old samples and 11% increase when compared to 28, 30 and 31 years respectively. **(Fig No. 2).**

I.B.CATALASE (CAT)

CAT activity increased with advancing age in pregnancy, CAT activity showed 25% increase in 30years old UCB samples in comparison with 19years old samples. Whereas a increase of 23% in 23years, 26% in 24, 30% in 26, 40% in 28 and 31% in 31years old samples were noticed when compared with 19year UCB sample respectively, (**Fig No. 4).** TBARS were decreased in the older women when compared to their younger counterparts, ($p<0.05$) **(Fig No. 2).**However from our results its seen that, increased TBARS and unchanged CAT activity in the UCB samples of all the age group. ($p<0.0001$) **(Fig No. 5).**

II.PROTEIN OXIDATION (PO)

II.A. PROTEIN SULFHYDRYL CONTENT (P-SH)

Protein thiol content was measured in terms of P-SH. The increased level of P-SH is an indicator of excess OS. All the age group except for 23years had decreased level of P-SH. 19 year showed a 46% increase, where 24,26,28,30 and 31years showed 53%, 50%, 46%, 38% and 33% increase respectively in comparison with 23 yrs UCB sample. (**Fig No. 6).** However, a significant difference was observed in comparison with SOD ($p<0.001$) as analyzed by Two-way ANOVA, where elevated activity of SOD was seen with lowered level of P-SH. (**Fig No. 7).**

II.B.ADVANCED OXIDATION PRODUCT (AOPP)

AOPP did not show any marked changes in 23, 24, 26, 28, 30 and 31year old samples, but 19 years showed an 80% decrease in comparison with the other older groups. (**Fig No. 8).** However, a significant difference was observed in comparison with SOD ($p<0.001$) as analyzed in Two-way ANOVA.Catalase enzyme did not have any marked effect on P-SH content. (**Fig No. 9)**

V.METHEMOGLOBIN AND HEMOGLOBIN CONTENT

19 year,23 year and 24 years showed normal range of UCB Hb content**(Fig No. 10)** as well as MetHb, **(Fig No 11)** where as in 28years both Hb and MetHb was increased and in 31years both Hb and MetHb was decreased ($p<0.05$)**.**Two-way ANOVA analysis revealed, Hb and MetHb did not show significant difference in the any of the age group.($p<0.001$).**(Fig No 12).**

DISCUSSION

Advanced maternal age has a risk factor for low birth weight, preterm delivery and other complications**.** There is growing literature on the effects of OS in female reproduction with involvement in the pathophysiology of preeclampsia, hydatidiform mole, free radical-induced birth defects and other situations such as abortions. Numerous studies have shown that OS plays a role in the pathophysiology of infertility and assisted fertility. Cells have developed a wide range of antioxidants systems to limit production of ROS, inactivate them and repair cell. OS influences the entire reproductive span of women's life and even thereafter (i.e. menopause). It has been suggested that the age-related decline in fertility is modulated by OS. It plays a role during pregnancy and normal parturition and in initiation of preterm labor. The pathological effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of ATP (Agarwal *et al*, 2005).

From our results, increased AOE activity is also seen irrespective of age. In 31years the SOD activity was decreased when compared to younger age indicating that SOD level decreases in advanced pregnancy. This trend is similar to the studies that have indicated that the levels of antioxidants enzymes like SOD found to be lower in pregnancy whereas product of lipid peroxidation (LPO) increased.

Normal pregnancy is associated with OS. Superoxide dismutase activity will be significantly higher in the ectopic endometrium than in eutopic endometrium, during the pregnancy. (Agarwal. A *et. al,* 2005). The anti-oxidant enzyme SOD activity increases throughout pregnancy. This occurs in response to normal oxidative stress due to pregnancy (Nakai *et al*., 2000).

SOD activity in erythrocytes and plasma thiol levels were found to be lower during pregnancy than in non-pregnant women, suggesting an oxidative environment and stress (Wisdom, S. J *et. al*, 1991; Ilouno L. E *et. al*, 1996) but there also exists a defense mechanisms against free radical damage are also enhanced as pregnancy progresses. Extracellular SOD activity have also been

found to increase progressively throughout gestation up to the third trimester, possibly as a response to increased presence of superoxide. (Uotila, J *et al*, 1991; Tamura, T *et. al*, 2001)

Catalase is a ubiquitous enzyme associated with the microbodies in all aerobic cells (Deisseroth and Dounce, 1970; Duve D, 1983). It is one of the three enzymes that interact with ROS. Catalase specially degrades hydrogen peroxide (H_2O_2) to H_2O and O_2 (Schonbaum and Chance, 1976). Our results show that there is an increased SOD activity, accelerate the production of H_2O_2, thereby enhancing the CAT activity as seen from our results. This probably suggests that elevated CAT activity in older pregnant women in an compensatory mechanism to check the excess production of FR. This finding is in line with the studies,whereinErythrocyticcatalaseactivity was decreased less significantly compared with values for non pregnant women. (Wisdom, S. J *et. al*, 1991; Ilouno L. E *et. al*, 1996)

Protein-carbonyls also result from the interaction of free radicals with amino acid residues (especially arginine, histidine, lysine and proline) oxidating sulfhydryl groups and hydroxylating tyrosine and phenylalanine. Our results showed no significant ($p<0.05$) changes in the PO products among the age groups, suggesting that PO is a consequence of OS in pregnancy irrespective of age.

P-SH and AOPP showed a significant difference with respect to SOD activity indicating that increase in SOD activity in response to increase PO. No significance was seen between P-SH and CAT activity and AOPP and CAT activity respectively.

But the decrease in AOPP and P-SH concentration in the 19, 23years old respectively, may be attributed to the psychological or other factors such as type of delivery i.e., normal or caesarian.

Increased lipid levels in pregnancy may increase the susceptibility of polyunsaturated fatty acids (PUFA) to peroxidation damage by free radicals that may lead to increased production of malondialdehyde (MDA), a marker for lipid peroxidation (Ciragil*et al.*, 2005).

Normal pregnancy is associated with oxidative stress causing increase in lipid peroxidation products, but this peroxidation is balanced by adequate anti oxidative responses (Chaudhari *et al*., 2003). The TBARS are formed as a byproduct of lipid peroxidation. Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. It is shown that TBARS level was increased with decrease antioxidant enzyme in normal pregnant women. (Poranen AK, e al, 1996).

The current study showed an increase in the LPO in terms of TBARS in the UCB of 19, 23, 24 and 26years old group in comparison to the aged pregnant women. This was also accomplished with an increase in the SOD activity, probably indicating elevation of LPO in pregnancy in association with elevated AOE.(Poranen AK, e al, 1996). The decrease in TBAR content in 30years old UCB samples be as seen from the results probably could be because of increased CAT activity in this age group. This finding suggests that in advanced pregnancy there is an up regulation of AOE.

Methemoglobinemia (MetHb) is a clinical syndrome caused by an increase in the blood levels of methemoglobin (MetHb) ,secondary to both congenital (chronic) changes in hemoglobin (Hb) synthesis or metabolism, or acute imbalances in reduction and oxidation reactions (redox imbalance) induced by the exposure to several chemical agents . (Tatiana and Souza do Nascimento, 2008)

The concentration of fetal hemoglobin is relatively high in the early months of life, and fetal hemoglobin forms oxyhemoglobin more readily than adult hemoglobin does. This means that fetal hemoglobin may be susceptible to oxidation to form methemoglobin. The level of methemoglobin in erythrocytes under normal conditions is lower than 1% of the total hemoglobin. Nitrogen compounds are strong oxidants that can reversibly oxidize oxyhemoglobin (Fe II) to give methemoglobin (Fe III), which is incapable of binding oxygen, thus contributing to a decline in tissue oxygenation.When the high levels of methemoglobin become irreversible, the deficiency of antioxidants persists, and oxidative stress continues, attacking the vascular endothelium of the kidneys, the brain and other vital organs and tissues of the mother. (LucijanMohorovic et al, 2010). Cord RBCs are unique cells that differ from adult RBCs in membrane composition and biophysical properties (Brugnara C and Platt O.S ,2003; Matovcik L.M and Mentzer W.C ,1985)], hemoglobin (Hb) structure, metabolism, and enzymatic profile (Oski F.A, 1973) One of the most important physiological differences is the high concentration of fetal hemoglobin (HbF) in cord RBCs. This is practically absent in adult RBCs (normal adult HbF is <1%) (Ludvigsen B.F,1997). HbF has a higher affinity for oxygen compared to adult hemoglobin (HbA). This allows HbF to bind oxygen more easily, with a left shift of the oxygen dissociation curve and the release of less oxygen to the tissues (Ludvigsen B.F, 1997).

Perinatal maternal and fetal complications have been found to increase exponentially under extreme conditions such as decrease in Hb values decrease below 90 g/L. These values are

observed almost exclusively in populations where there are chronic blood losses, malaria or other hemolytic conditions. The other extremes, those of high Hb and ferritin levels have also a pronounced negative effect on the course and product of pregnancy.

From our studies, Hb content was increased in 24, 26, 28 and 30 years old UCB samples, while 19 and 31 years showed a normal reference range, whereas, an increased level of MetHb was noticed in UCB from all the age groups. The current results is of suggestion of elevated OS in pregnancy, thereby the changes of methemoglobinemia is high in late as well as early pregnancies. (LucijanMohorovic*et al*, 2010).

CONCLUDING REMARKS

In conclusion, the present study shows that pregnancy with advancing age exerts a maximal oxidative stress in cord blood but is characterized by compensatory upregulation of antioxidant enzymes in terms of SOD.

This results in less pronounced lipid peroxidation and protein oxidation in aged mothers in comparison their younger counterparts.

However OS has recognized to be crucial for oxygen transportation to the fetus and is reflected in terms of Methemoglobinemia. Our finding indicates that with advancing maternal age, methemoglobin concentration also have been increased with a decrease in hemoglobin content.

In summary, oxidative stress is associated with pregnancy, more so in the aged pregnancy, but at present there is little data convincing the role of oxidative stress in pregnancy with advancing age. The breadth of the strategies attempted so far has not been extensive, and there is good evidence that well conducted, adequately powered trials involving other approaches would be of value. However, safety and ethical considerations must remain a predominant issue in any move toward other approach.

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REFERENCES

1. Aebi H (1984); Catalase.***Methods Enzymol***. 105:121-126.

2. Ashok Agarwal, Sajal Gupta and Rakesh K. Sharma (2005); Review, Role of oxidative stress in female reproduction.***Reproductive Biology and Endocrinology.***;BioMed Central.

3. Bayani Uttara, Ajay V. Singh, Paolo Zamboni,and R.T Mahajan (2009); Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options.***Curr Neuropharmacol.*** 7(1): 65–74.

4. Bradford, M.M (1976); A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding; ***Anal. Biochem***.72, 248-254.

5. Brugnara.C and Platt. O.S (2003).; "The neonatal erythrocyte and its disorders," in Hematology of Infancy and Childhood, D. G. Nathan, S. H. Orkin, D. Ginsburg, and A. T. Look, Eds., pp. 19–55, Saunders, 6th edition.

6. Casanueva E, Viteri FE (2009).; Iron and oxidative stress in pregnancy. J. Nutr. 133: 1700S–1708S, 2003; ***Curr Neuropharmacol.*** Mar 2009; 7(1): 65–74. PMCID: PMC2724665.

7. Chaudhari L, Tandon OP, Vaney N, Agarwal N (2003); Lipid peroxidation and antioxidant enzymes in gestational diabetes. ***Indian Journal of Physiology and Pharmacology.***; 47 (4) 441-46.

8. Ciragil P, Kurutus EB, Gul M, Kilinc M, Aral M, Guven A (2005).; The effects of oxidative stress in urinary tract infections during pregnancy; ***Mediators of Inflammation*** (5) 309-311.

9. Deisseroth A, Dounce AL (1970); Catalase: physical and chemical properties, mechanism of catalysis, and physiological role.***Physiological Reviews.*** 50, 319-75

10. Habeeb A, F.S.A (1972); Reaction of protein sulphydryl groups with Ellman's reagent. ***Methods enzymol.*** 34, 457-464.

11. Haque S K, Mashiat U., Siddiqui, Najmul Islam, and Shagufta Moin. , (2010); Erythrocyte Markers of Oxidative Stress in Higher Age-Group Preeclamptic and Normal Pregnant Mothers. Vol. 29, No. 1, Pages 69-81

12. Ilouno, L. E., Shu, E. N. &Igbokwe, G. E (1996); An improved technique for the assay of red blood cell superoxide dismutase (SOD) activity. ***Clin.Chim.Acta*** 247: 1–6.

13. Jennifer L. Martindale And Nikki J. Holbrook (2002); Cellular Response to Oxidative Stress: Signaling for Suicide and Survival. ***Journal of Cellular physiology*** 192:1–15.
14. KelyFranciniRechetzki, RailsonHenneberg, Paulo Henrique da Silva, Aguinaldo José do.(2011); Reference values for methemoglobin concentrations in children, Brazil.
15. LeventeLázár.; Review, The Role of Oxidative Stress in Female Reproduction and Pregnancy; 1st Departmet of Obstetrics and Gynecology, Semmelweis University Budapest, Hungary
16. LucijanMohorovic 1, Oleg Petrovic , Herman Haller and Vladimir Micovic (2010); Article Pregnancy Loss and Maternal Methemoglobin Levels: An Indirect Explanation of the Association of Environmental Toxics and Their Adverse Effects on the Mother and the Foetus; ***Int. J. Environ. Res. Public Health*** , 7, 4203-4212.
17. Lucilla Poston, Natalia Igosheva, Hiten D Mistry, et.al.(2013); Role of oxidative stress and antioxidant supplementation in pregnancy disorders. ***IOSR Journal of Dental and Medical Sciences*** (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 3, Issue 6, PP 12-13
18. Ludvigsen. B. F (1997).; "Hemoglobin synthesis and function," in Clinical Hematology: Principles, Procedures, Correlations, E. A. Stiene-Martin, C. A. Lotspeich-Steininger, and J. A. Koepke, Eds., pp. 73–86, Lippincott, 2nd edition.
19. Matovcik .L.M and .Mentzer W.C (1985); "Themembraneofthehu- man neonatal red cell," Clinics in Haematology, vol. 14, no. 1, pp. 203–221.
20. Michael Moretti, Michael Phillips, Ahmed Abouzeid, et.al (2004); increased breath markers of oxidative stress in normalpregnancy and in preeclampsia; New York.
21. Misra, H.P & Fridovich, I, (1972); The role of super oxide anionin the autooxidation of epinephrine and a simple assay for Superoxide Dismutase. ***The journal of Biological Chemistry,*** Vol. 247, No. 10, pp. 3170-3175.
22. Nakai A, Oya A, Kobe H.; (2000); Changes in maternal lipid peroxidation levels and antioxidant enzymatic activities before and after delivery.***Journal of Nippon Medical School;*** 67 (6) 434-43.
23. Nascimento, T.S.D.; Pereira R.A.O.; Mello H.L.D.; Costa. J (2008); Methemoglobinemia: from Diagnosis to Treatment; Vol. 58, No 6, : 651-664.

24. Ohkawa, H.; Ohishi, N. & Yagi, K (1979); Assay for lipid peroxides in Animal tissues by Thiobarbaric Acid Reaction. Analytical Biochemistry, Vol. 95, No. 2. University Press, New York.
25. Oski.F. A (1973); "The unique fetal red cell and its function.***Pediatrics,*** Vol.51, no.3, pp.494–500.
26. Poranen AK, Ekbald U, Uotila P, Ahotupa M, (1996); Lipid peroxidation and antioxidants in normal and pre-eclampticpregnancies;***Placenta***Sep;17(7):401-5
27. Saikumar, Jaya. B, Renuka devi. M.R; Oxidative Stress in Pregnancy.Dept of Physiology, SreeBalaji Medical College &Hospital,Chromepet, Bharath University, India
28. Schonbaum GR, Chance B.; (1976); Catalase in the Enzymes, Vol. 13, 3rd edn (Boyer PD, ed). New York: Academic Press, pp 363-408, part C
29. Soumika Biswas, Swati. B. Hattacharyya, Arghya Basu, ;(2014); Assessment of oxidative stress and antioxidant status among newborns in relation to mode of delivery. ***Int J Cur Res***.; India
30. Tamura, T., Olin, K. L., Goldenberg, R. L., Johnson, K. E., DuBard, M. B..; (2001); Plasma extracellular superoxide dismutase activity in healthy pregnant women is not influenced by zinc supplementation. ***Biol. Trace Elem. Res***. 80: 107–114.
31. Uotila, J., Tuimala, R., Aarnio, T., Pyykko, K. &Ahotupa, M.; (1991); Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. ***Eur. J. Obstet. Gynecol. Reprod. Biol***. 42: 95–100.
32. Wisdom, S. J., Wilson, R., McKillop, J. H. & Walker, J. J.; (1991); Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension.***Am. J. Obstet. Gynecol.*** 165: 170–174.
33. Yoshikawa .T and Naito Y.; (2002); What is oxidative stress ;***JMAJ*** 45(7): 271–276.

ABBREVIATIONS

AOE	Antioxidants
AOPP	Advanced oxidative protein products
CAT	Catalase
FR	Free radicals
Hb	Hemoglobin
HbA	Adult hemoglobin
HbF	Fetal hemoglobin
MetHb	Methemoglobin
O^2	Super oxide species
OH^-	Hydroxyl ion
OS	Oxidative stress
P-SH	Protein sulphydryl content
ROS	Reactive oxygen species
SOD	Super oxide dismutase
TBARS	Thiobarbutiric acid
UCB	Umbilical cord blood

LEGENDS

Figure. No.1: SOD activity in human umbilical cord blood. Values are mean ± SEM of N=5 The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.2: TBARS in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.3: SOD and TBARS activity in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by Two-way ANOVA and significance was considered at $p<0.0001$. The ** indicates significance at $p<0.0001$, * indicates significance at $p<0.05$.

Figure. No.4: CAT activity in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.5:CAT and TBARS activity in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by Two-way ANOVA and significance were considered at $p<0.005$. The ** indicates significance at $p<0.0001$, * indicates significance at $p<0.05$.

Figure. No.6: Amount of P-SH in human umbilical cord blood. Values are mean ± SEM of N=5 The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.7: CAT activity and P-SH concentration in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

CAT is expressed inH_2O_2 degraded/min/mg protein and P-SH is expressed in moles/l/cm/mg protein.

Figure. No.8: Concentration of AOPP in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.9: CAT activity and AOPP in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

CAT is expressed in H_2O_2 degraded/min/mg protein and AOPP is expressed in nmoles/l/cm/mg protein.

Figure. No.10: Percentage of hemoglobin concentration in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.11: Percentage of Methemoglobin concentration in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

Figure. No.12: Percentage of Methemoglobin and hemoglobin concentration in human umbilical cord blood. Values are mean ± SEM of N=5. The changes were analyzed by One-way ANOVA and significance were considered at $p<0.05$.

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Figure.No.1

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Figure. No. 2

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Figure.No.3

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Figure.No.4

Figure.No.5

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Figure.no.6

Figure.no.7

Figure.no.8

Figure.no.9

Figure.no.10

Figure.no.11

Figure.no.12

Normal range Hb: 11-16% and MetHb: 3.60-4%