Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 05, pp.68952-68958, May, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF PLASMA ASCORBATE, ERYTHROCYTE PLASMA MEMBRANE REDOX SYSTEM AND ASCORBATE FREE RADICAL REDUCTASE SYSTEM IN DIFFERENT TRIMESTERS OF NORMAL PREGNANCY

Dr. Avishek Sengupta, *Dr. Sangita Biswas, Dr. Soumitra Ghosh and Dr. Sudipta Banerjee

Senior Resident, Department of Biochemistry, Rgkar MCH, Kolkata-004, West Bengal, India

ARTICLE INFO

Article History:
Received 18th February, 2018
Received in revised form
08th March, 2018
Accepted 29th April, 2018
Published online 23rd May, 2018

Key words:
Ascorbate,
Erythrocyte PMRS,
AFRreductase system.

ABSTRACT

Background: Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Normal pregnancy is associated with high metabolic demand and elevated requirement for tissue oxygen. There is increased production of reactive oxygen species and increased oxidative stress. Plasma Ascorbate, Erythrocyte Plasma Membrane Redox System and Ascorbate Free Radical Reductase System are some of the antioxidants. **Objectives:** In our study we wanted to evaluate the change in these antioxidents' levels in different trimester of normal pregnancy in respect to non pregnant women. **Methods:** The methodology includes measurement of PMRS activity by Avron and Savit's procedure; Vitamin C by Teitz and AFR reductase activity by guidline of May et al. **Results:** In respect to control group there was no significant difference in 1st trimester and 2nd trimester, but there was significant difference in 3rd trimester in PMRS values. Vitamin C levels showed no significant difference in 1st trimester but there was significant difference in 2nd and 3rd trimester compared to controls. In respect to control group there was significant difference with 1st,2nd and 3rd trimester in AFR Reductase activity. On correlation study NADH Values (AFR reductase activity was measured in terms of NADH oxidase) had negative correlation with PMRS and positive correlation with Vitamin C in both non pregnant and pregnant women. **Conclusion:** In present study it can be concluded that as the trimesters progress there is increase in PMRS level, AFR activity and decrease in Vitamin C level with increase in oxidative stress.

Citation: Dr. Avishek Sengupta, Dr. Sangita Biswas, Dr. Soumitra Ghosh and Dr. Sudipta Banerjee. 2018. "Evaluation of plasma ascorbate, erythrocyte plasma membrane redox system and ascorbate free radical reductase system in different trimesters of normal pregnancy", *International Journal of Current Research*, 10, (05), 68952-68958.

INTRODUCTION

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. O_2^- (superoxide radical), OH- (hydroxyl radical) and H_2O_2 (hydrogen peroxide) (Chandra Kala, 2015). Normal pregnancy is associated with high metabolic demand and elevated requirement for tissue oxygen. There is increased production of reactive oxygen species. This result in increased oxidative stress (Dr. P. Saikumar, 2013). The property of erythrocyte to reduce membrane impairment anions was reported by Orrienger and Roer (Orringer, 1979). Later researches established the existence of transmembranous NADH dehydrogenases in several other cell type also (Greibing, 1984; Kilberg, 1979). As per A. D. N. J. de Grey the term "plasma membrane redox system "is used to denote the machinery by which cells oxidize electron donors, typically NADH and/or NADPH, and transfer the resulting electrons to extracellular acceptors. This plasma membrane redox system (PMRS) helps the cells to respond to redox changes there by regulating many cellular functions. The PMRS reduces extracellular oxidants by using the reducing power of intracellular antioxidants, making the cell metabolism respond to changes in local redox environment (Eleanor, 2003). Although the exact physiological function of PMRS is not fully understood, proposed function include maintenance of redox state of sulfhydryl residues in membrane proteins neutralization of oxidative stressors outside the cells, stimulation of cell growth, recycling of α tocopherol, reduction

Corresponding author:* **Dr. Sangita Biswas,
Senior Resident, Department of Biochemistry, Rgkar MCH, Kolkata-004, West Bengal, India.

of lipid hydro peroxides, maintainance of extracellular concentration of ascorbic acid and reduction of ferric ions prior to iron by a transferring independent pathway (Rizvi, 2006). One of the main electron donor of PMRS is ascorbate. Vitamin C plays an important role in protecting the cells against oxidative stress. This vitamin readily scavenges reactive oxygen species (ROS) and reactive nitrogen species.(e.g. hydroxyl, peroxyl ,superoxide, peroxynitrite and nitric oxide radicals) as well as singlet oxygen and hypochlorite, thereby preventing cell damage by those substances. Cell metabolism utilizes ascorbic acid essentially as an electron donar, so that ascorbic acid undergoes continous oxidation and reduction. Oxidation products are ascorbic free radical and dehydroascorbic acid. They can be reconverted to ascorbic acid by two enzymes: AFR-reductase and DHA reductase (OresteArrigoni, 1981). In this study change in the level of Vitamin C ,Erythrocyte Plasma Membrane Redox system and Ascorbate free radical reductase system in different trimester of normal healthy pregnancy in respect to age matched non pregnant women were measured. Measurement of PMRS activity was done by ferricyanide reduction test using 2,4-DNPH(as described by Avron and Savit) (Avron, 1963). spectrophotometric measurement of Vitamin C using metaphosphoric acid(Teitz Method) (Tietz, ?) AFR reductase activity was measured by generating AFR in dilute heamolysate incubated at 37 ^{0}centigrade at PH 7 Phosphate buffer saline (PBS) containing 1 m Mascorbate, 100 microlitre of 50U/ml of ascorbate oxidase and 0.1 mM of NADH and amount of NADH oxidized (as described by May et al) (May, 2004). In our study we tried to find out if these antioxidant can be used to reduce oxidative stress in different trimester of normal pregnancy. To full fill this we had set our objectives as follows:

- To assess if there is any increase in oxidative stress in normal pregnancy in respect to non pregnant normal patient by measuring these three parameters.
- To assess whether any interrelationship exists in between these three parameters.
- To assess whether there is any change in these parameters with increase in the trimesters of pregnancy.

MATERIALS AND METHODS

Study design: Cross Sectional Case Control study.

Study area: Department of Biochemistry, in collaboration with, Department of Gynaecology and Obstetrics, in our Medical College and Hospital, Kolkata.

Sample-size: Cases ~ 90 normal pregnant patients attending Gynae and Obstretic-OPD and Biochemistry Department of our Medical College and Hospital. Controls ~ 30 non pregnant age matched women were taken as controls.

Sample design: For selection of study subjects from the population, systematic random sampling done meeting inclusion and exclusion criteria. Each participant was provided with a written informed sheet and blood samples were collected after taking a written informed consent.

Inclusion criteria: Pregnant women of different trimesters during study period.

Exclusion criteria: Pre-eclampsia Pre existing DM, Gestational Diabetes Any chronic inflammatory disease Twin Pregnancy/Molar Pregnancy/Ectopic Pregnancy HIV infected Pregnancy.

Method of data collection: The patients were selected first from the Gynae and Obs. outdoor of our Medical College and Hospital, Kolkata according to the inclusion and exclusion criteria after obtaining consent of the patient in proper consent form. 90 cases were selected randomly from those patients. Detailed history was gathered. After that blood for biochemical investigations was collected. The collected data was then analyzed.

Laboratory parameters and procedures

- RBC PMRS activity by Ferricyanide reduction test using 2,4- DNPH by M. Avron and N. Savit method.
- Plasma Vitamin C estimation by UV- Visual double beam spectrophotometric method using metaphosphoric acid (Teitz's method).
- Measurement of NADH dependant AFR Reductase activity by method used by May et al. AFR reductase activity is reported in terms of μmol of NADH oxidised/mim/ml RBC.
- All reagents are used of AR (Analytical Reagent) or GR (Guaranteed Reagent) grade.

Plan of Statistical Analysis : All recorded data was analyzed using standard statistical methods including standard diagrams and graphs. Statistical software's like IBM SPSS™2020 and Microsoft excel 2013™ were used for this purpose.

RESULTS

In this study it was observed that as per Normality test(Kolmogorv-Smirnov test): study population was normally distributed. The mean, S.D. (Standard Deviation) were calculated. Subsequently relation between different data were analysed by using ANOVA, Least significant differences, pearson correlation co efficient($p<0.05$ considered significant). In our study, we found 75% were pregnant and 25% were non pregnant and 33% in both of 1st and 2nd trimester and 34% in 3rd trimester. There were 42 cases in primigrvida and 48 cases in multigravida and their age, weight and parity wise distribution is shown statistically in Table 1.

Table 1. Age, weight and parity wise distribution of the population

Trimester	Age (years) as mean±SD	Weight (kgs) as mean±SD
1st	22.36 ± 1.06	49.1 ± 0.84
2nd	22.63 ± 1.18	53.9 ± 1.01
3rd	22.9 ± 1.09	57.31 ± 1.26
Control	21.26 ± 1.14	46.06 ± 0.74

AFR reductase activity was measured in terms of NADH. Our study found that as more and more AFR would be used up NADH would be oxidised and value of NADH would decrease (Table 2). This finding can infer that more and more the metabolic demand more will be generation of free radical resulting in increase oxidtive stress as pregnancy goes on oxidative stress will increase.

Table 2. NADH, PMRS and Vitamin C level in different trimesters of cases and controls

	N- sample size	NADH (µmol/min/ml of RBC)	PMRS (µmol/ml of PRBC)	Vitamin C (mg/dl)
1st trimester	30	1.359±0.2020	6.088±0.4271	9.363±0.7841
2nd trimester	30	0.928±0.1807	6.134±0.4223	8.477±0.5425
3rd trimester	30	0.877±0.2389	6.817±0.4978	5.880±0.2999
Controls	30	1.488±0.2582	5.971±0.3463	9.370±0.6331

All values are expressed in mean±SD.

Table 3. Multiple Comparision Study Table

Least Significant Difference			
Dependent variable	Group	Group	Significance
PMRS	Control	1st trimester	0.289
		2nd trimester	0.14
		3rd trimester	0.00
	1st trimester	Control	0.289
		2nd trimester	0.675
		3rd trimester	0.00
	2nd trimester	Control	0.14
		1st trimester	0.675
		3rd trimester	0.00
	3rd trimester	Control	0.00
		1st trimester	0.00
		2nd trimester	0.00
Vitamin C	Control	1st trimester	0.965
		2nd trimester	0.00
		3rd trimester	0.00
	1st trimester	Control	0.965
		2nd trimester	0.00
		3rd trimester	0.00
	2nd trimester	Control	0.00
		1st trimester	0.00
		3rd trimester	0.00
	3rd trimester	Control	0.00
		1st trimester	0.00
		2nd trimester	0.00
NADH	Control	1st trimester	0.026
		2nd trimester	0.00
		3rd trimester	0.00
	1st trimester	Control	0.026
		2nd trimester	0.00
		3rd trimester	0.00
	2nd trimester	Control	0.00
		1st trimester	0.00
		3rd trimester	0.376
	3rd trimester	Control	0.00
		1st trimester	0.00
		2nd trimester	0.376

The Mean difference is significant at the 0.05 level

Next, we wanted to determine from our study that was there any significant differences between groups of these three parameters. For this anova study had been done. It was seen that there was significant difference between the groups (NADH, PMRS, Vitamin C in different trimester of pregnancy) as in all cases p value was less than 0.05 . On Analysis of Multiple Comparision(Table 3) among these parameters in different pregnancy trimesters, our study found out following results -1.

1. PMRS activity

a) In respect to control group there was no significant difference in 1st trimester and 2nd trimester,but there was significant difference in 3rd trimester.
b) In respect to 1st trimester there was no significant difference in 2nd trimester but there was significant difference in 3rd trimester
c) In respect to 2nd trimester there was only significant difference in 3rd trimester
d) In respect to 3rd trimester there was significant difference with control group and 1st and 2nd trimester.

2.Vitamin C level:

a) In respect to control group there was no significant difference in 1st trimester but there was significant difference in 2nd and 3rd trimester
b) In respect to 1st trimester there was significant difference with 2nd and 3rd trimester
c) In respect to 2nd trimester there was significant difference with control group and both 1st and 3rd trimester
d) In respect to 3rd trimester there was significant difference with control group and both 1st and 2nd trimester.

3.NADH dependent AFReductase activity:

a) In respect to control group there was significant difference with 1st,2nd and 3rd trimester
b) In respect to 1st trimester there was significant difference with control group and both 1st and 2nd trimester
c) In respect to 2nd trimester there was significant difference with control group and 1st trimester
d) In respect to 3rd trimester there was significant difference with control group and 1st trimester.

Our study results observed from interrelation between parameters in control group as follows

a) NADH had negative significant correlation with PMRS with a correlation coefficient of 0.780
b) NADH had positive significant correlation with Vitamin C with a correlation coefficient of 0.586
c) PMRS had negative significant correlation with both NADH and Vitamin C with correlation coefficient of 0.780 and 0.707 respectively
d) Vitamin C had negative significant correlation with PMRS with a correlation coefficient of 0.707
e) Vitamin C had positive signficant correlation with NADH with a correlation coefficient of 0.586

In 1st trimester as follows- (Figure 1,2)

a) NADH had negative significant correlation with PMRS with a correlation coefficient of 0.472
b) NADH had positive significant correlation with Vitamin C with a correlation coefficient of 0.666
c) PMRS had significant negative correlation with both NADH and Vitamin C with a correlation coefficient of 0.472 and 0.706 respectively
d) Vitamin C had significant positive correlation with NADH with a correlation coefficient 0.666
e) Vitamin C had negative correlation with PMRS with a correlation coefficient 0.706

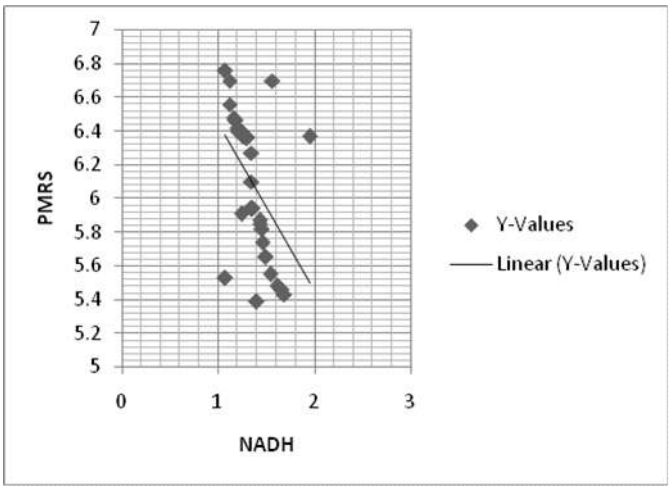

Figure 1. Correlation between NADH and PMRS in 1st trimester

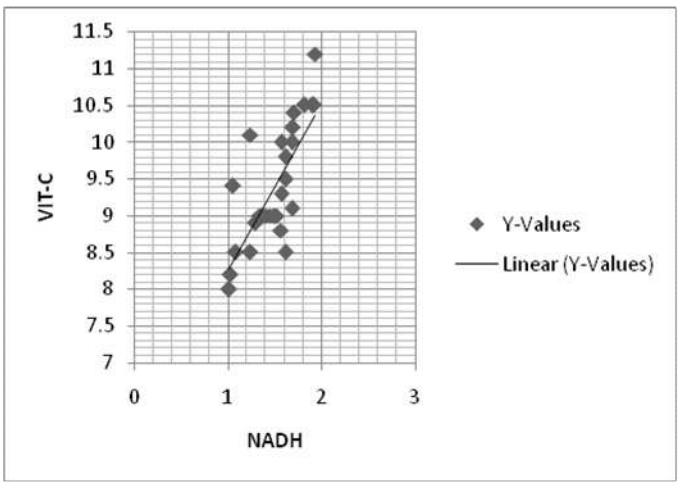

Figure 2. correlation between NADH and Vit C in 1st trimester

In 2nd Trimester as follows- (Figure 3,4)

a) NADH had negative significant correlation with PMRS with a correlation coefficient 0.391
b) NADH had positive significant correlation with Vitamin C with a correlation coefficient 0.636
c) PMRS had negative significant correlation with Vitamin C and NADH with a correlation coefficient 0.617 and 0.391 respectively
d) Vitamin C had positive significant correlation with NADH with a correlation coefficient 0.636
e) Vitamin C had negative significant correlation with PMRS with a correlation coefficient 0.617.

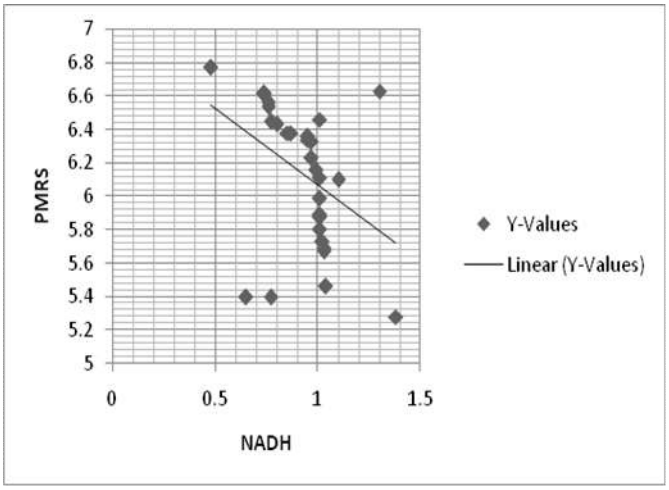

Figure 3. correlation between NADH and PMRS in 2nd trimester

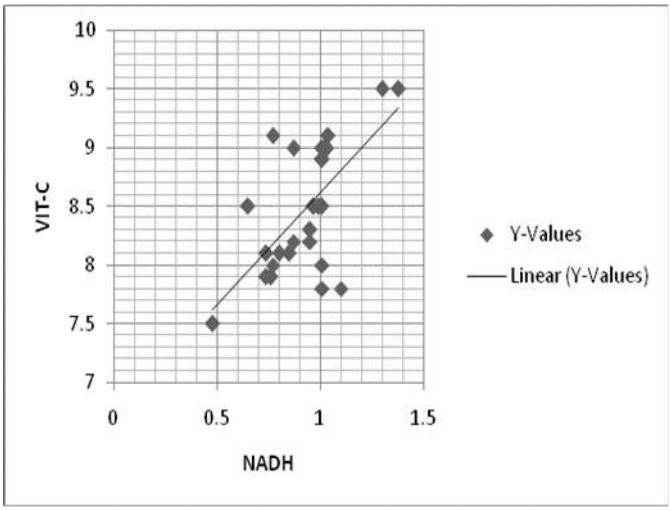

Figure 4. correlation between NADH and Vit C in 2nd trimester

In 3rd trimester as follows- (Figure 5,6)

a) NADH had significant negative correlation with PMRS with a correlation coefficient of 0.719
b) NADH had significant positive correlation with Vitamin C with a correlation coefficient of 0.735
c) PMRS had negative significant correlation with NADH and Vitamin C with a correlation coefficient of 0.719 and 0.539 respectively
d) Vitamin C had positive significant correlation with NADH with a correlation coefficient of 0.735
e) Vitamin C had negative significant correlation with PMRS with a correlation coefficient of 0.539.

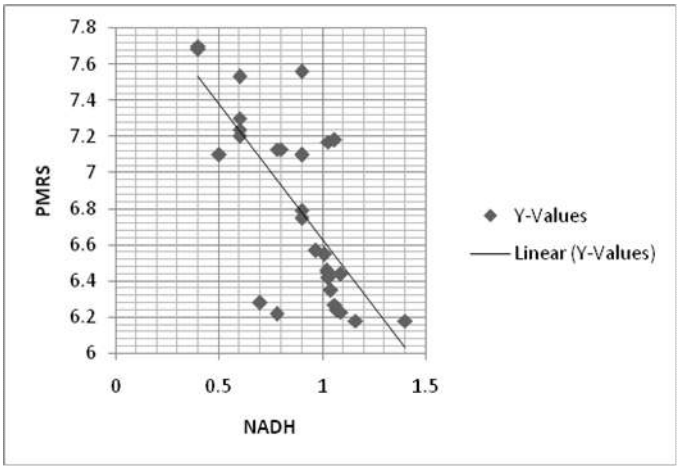

Figure 5. correlation between NADH and PMRS in 3rd trimester

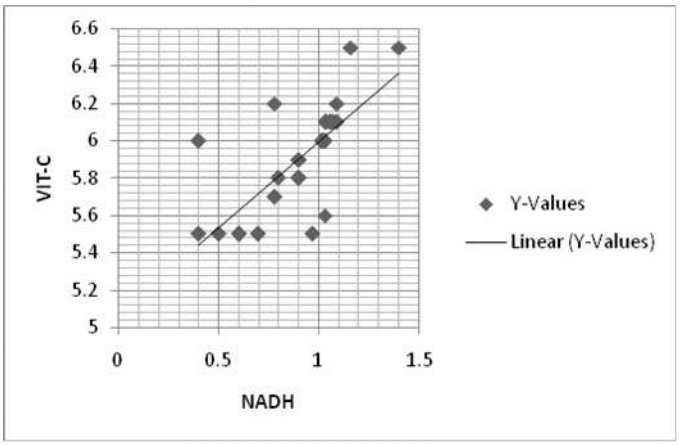

Figure 6. correlation between NADH and Vit C in 3rd trimester

DISCUSSION

This study has tried to evaluate the role of these three antioxidants in oxidative stress. First the level of activity of PMRS is focused, and it was found to be higher than the age matched healthy control subjects, it also increased as trimester of pregnancy increased (Table 2). Larm et al. showed that ρ° cell cultures-mammalian cells divested of their mitochondrial DNA by culturing in ethidium bromide, a potent inhibitor of mitochondrial DNA replication, relied on the plasma membrane redox system for survival, even when cultured in medium containing uridine and pyruvate. This observation prompted these workers to propose that an increase in activity of the PMRS in cells may occur with aging or in some degenerative diseases in response to a reduction of mitochondrial redox control and energy production (GuntterMarsche, 2009). There was a study stating that increased RBC PMRS was evident in Type-2 Diabetes Mellitus patients and 1st degree relatives of these patients (Rizvi, 2002). Transmembrane electron transfer was selectively increased in diabetic nephropathy, where RBC GSH was also depleted. The abnormality was peculiar to the nephropathy group and not contributed by familial or hereditary components because the electron flow was normal in siblings. The close relationship between cytosolic NADH and RBC electron transfer observed in diabetic patients without complications seems to be lost in the microangiopathic patients. Whereas patients with retinopathy alone still had normal activity of the RBC-reducing system (Larm, 1994). Pregnancy is a developmental crisis in women's body. It places a great demand in her body and require adaptation, changes in many of the biochemical function in body during pregnancy leads to high demand for energy and increase oxygen requirement. This lead to increase in intake and utilization of oxygen resulting in increase level of oxidative stress and consequent acceleration in production of reactive oxygen species (Matteucci, 2000). In a study done by Rizvi et al the activity of erythrocyte PMRS was estimated by following the reduction of ferricyanide. The total antioxidant capacity of the plasma was estimated in terms of Ferric Reducing Ability of Plasma (FRAP) values. A significant ($p<0.0001$) positive correlation ($r = 0.7797$) was observed between PMRS activity of erythrocytes and human age. There was an age-dependent decrease in total plasma antioxidant capacity measured in terms of FRAP values. A highly significant correlation was observed between PMRS activity and plasma FRAP values. The authors hypothesize that the increased PMRS in erythrocytes during aging maybe a protective mechanism of the system for efficient extracellular DHA reduction and ascorbate recycling under condition of increased oxidative stress (Garba, 2004).

In our present study it was seen that PMRS activity increases with increase in trimester of pregnancy (i.e. with increase with oxidative stress) though there was only significant difference in third trimester in respect to control group and other two trimester (Table 3), but it may be due to smaller number of subjects taken in every group(30 in number). Secondly in our study change in vitamin C level had been studied. It was seen that as the trimester of pregnancy increases vitamin c level decreases i.e more and more Vitamin C had been used up to reduce the oxidative stress (Table 2). In respect to non pregnant women there was no significant change in Vitamin C level in 1st trimester of pregnancy but there was significant change in 2nd and 3rd trimester of pregnancy (Table 3). A study done by I Garba et al (Garba, 2004), Serum concentration of L ascorbic acid which makes up 80% of Vitamin C activity was assessed in different trimester of pregnancy with aim of determining the effect of pregnancy on vitamin C level and its availability in scavenging reactive oxygen species produced as a result of pregnancy induced oxidative stress, they had seen that there was decrease in Vitamin C level in respect to non pregnant women. In a study done by V.S. Kalaiselvi et al it was seen that plasma vitamin C level was low in all the three trimesters in respect to non pregnant women (Kalaiselvi, 2014).

In a study done by Tamari Y et al, the authors demonstrated that ascorbic acid offset growth defects observed in SOD2-depleted cells and also lowered mitochondrial superoxide to physiological levels in both SOD1- or SOD2-depleted cells. Moreover, depletion of SOD1 or SOD2 resulted in the accumulation of intracellular oxidative stress, and this increased oxidative stress was reduced by ascorbic acid (Tamari, 2013). In a study done by S.Mohanty et al, it was seen that there was a significant fall ($P<0.05$) in the vitamin C levels in primi with mild preeclampsia than in the normal primi. The vitamin C levels in severe preeclamptic patients were lower than the normal primi but the fall was not statistically significant ($P=0.10$). Plasma vitamin C also showed a negative correlation in the control and study group. This observation suggests that in hypertensive disorders of pregnancy there is an imbalance between lipid peroxidation and antioxidant vitamin status because of oxidative stress (Mahanty, 2006).

Increasing levels of MDA, a end product of lipid peroxidation with advancement of gestational age together with decline in plasma vitamin C level in pregnant women as compared to nonpregnant control women revealed that pregnancy poses a body to excessive oxidative stress and ascorbic acid defense system may be helpful in combating the deleterious effects of oxidative stress if sufficient vitamin c is present in plasma (Ghate, 2011). Third parameter that had been taken in our study was Ascorbate free radical reductase activity. It had been measured by amount of NADH oxidized, more and more AFR reductase had been used up more and more NADH oxidized and value of NADH was decreased. In respect to non pregnant women as the trimester of pregnancy increased, NADH decreased in response to increase in oxidativestress (Table 2). In respect to non pregnant women there is significant difference in different trimester of pregnancy (Table 3). Recycling of ascorbic acid from its oxidized forms helps to maintain adequate tissue levels of the vitamin. This recycling is largely intracellular and occurs from both the one- and two-electron-oxidized forms of ascorbate, the ascorbate free radical (AFR) and dehydroascorbic acid (DHA), respectively. The AFR reacts predominantly by dismutation of two AFR molecules, yielding ascorbate and DHA (James, 2004). The target of AFR reductase is to maintain ascorbic acis in its reduced form, so more and more Vitmin C is oxidized in response to increase in oxidative stress more and more AFR reductase is used up to return oxidized form to reduce form thus maintaining the intracellular Vitamin C level. Recently it was reported that in human erythrocytes the activity of PMRS along with the AFR reductase increases with the increase in age. This activation of PMRS and AFR reductase increases the ascorbate recycling in human plasma and reported as a compensatory/ protective mechanism that operates to maintain the ascorbate level in plasma and thereby minimize oxidative stress during aging (Kanti Bhooshan Pandey, 2010).

Conclusion

Therefore this study can conclude that:

1. Increased erythrocyte PMRS activity reflects that there is increased transplasma membrane electron transfer in RBC with increase in trimesters of pregnancy. Further studies are needed to establish the alteration of PMRS activity as a protective factor against the oxidative stress in pregnancy. The effect of PMRS activity in prevention of different complication of pregnancy could be an area of interest.
2. Increased Ascorbate free radical reductase activity as determined by the amount of NADH indicates that there is increased ROS/free radical generation. This is an indirect evidence of increased ROS/free radical generation in body during pregnancy.
3. Plasma vitamin-C / Ascorbic acid level, lower than that of non pregnant women and with increasing trimester of pregnancy, is supposed to be an indirect reflection of antioxidant depletion during oxidative stress during pregnancy.

Funding: Funding was borne by the investigators.

Conflict of interest: No.

REFERENCES

Avron M, Shavit N. 1963. A sensitive and simple method for determination of ferrocyanide. *Anal Biochem.,* 6:549-54.

Chandra Kala, Syed Salman Ali. 2015. Protection Against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costusspeciosus Rhizome Extract. International Journal of Pharmacognosy and Phytochemical Research **7** (2): 383–389.

Dr. P. Saikumar; Dr. B. Joye. Oxidative Stress in Pregnancy. IOSR Journal of Dental and Medical Sciences(IOSP JDMS),e-ISNN:2279-0853,p-ISSN:2279-0861,Volume 3,Issue 6(Jan-Feb,2013)PP 12-13,www.iosrjournals.org

Eleanor C, Kennett and Phipip W. 2003. Kuchel Redox Reactions and Electron Transfer Across the Red Cell Membrane IUBMB Life., 55:375-85.

Garba I, Ubom G. 2004. L-ascorbic Acid Status of pregnant women and its potential role in Pregnancy Induced Stress. The internet journal of nutrtition and wellness, Volume 1, Number 2;print.ispub.com/api10/ispub-article /5703.

Ghate J, Choudhuri AR. 2011. Antioxidant Role of Vitamin C in normal Pregnancy, *Indian Biomedical Research,* 22(1):49-51.

Greibing C, Crane FL, Low H, Hall K. 1984. A transmembranous NADH dehydrogenase in human erythrocyte membranes. *J Bioenerg Biomembr.,* 16:517-33. doi:10.1007/ BF00743243

GuntterMarsche, Sasa Frank. 2009. Plasma Advanced Oxidation Protein Products are potent High Density Lipoprotein Receptor antagonist in vivo;published March 27. http://dx.doi.org/10.1161/CIRCRESAHA:108.193169

James M.May, Zhi-Chao Qu. 2004. Human Erythrocyte Recycling of ascorbic acid, Vol 279,no 15,Issues of April, pp.14975-14982, January 29, DOI 10.1074/ jbc. M312548200

Kalaiselvi, V.S., S. Birundha. 2014. Estimation of Ascorbic Acid status in Normal Pregnancy; World Journal of Medical Sciences 10(2):150-152,2014 ISSN 1817-3055, IDOSI publication, DOI:10.5829/ idosi.wjms.2014. 10.2.82145

Kanti Bhooshan Pandey, Syed Ibrahim Rizvi, 2010. Markers of Oxidative Stress in Erythrocytes and plasma during aging in humans. *Oxid Med Cell Longev*. Jan-Feb:3(1):2-12.doi:10.4161/oxim.3.1.10476

Kilberg MS, Christensen HN. 1979. Electron transferring enzymes in the plasma membrane oh the Erlich ascites tumor cell. *Biochemistry*. 18:1525-30. doi:10.1021/ bi00575a021

Larm JA, Vailant F, Linnane AW, Lawen A. 1994. Up-regulation of the plasma membrane oxidoreductase as a prerequisite for the viability of Namalwa ρ° cells. *J Biol Chem.,* 269:30097–30100.

Mahanty, S., P K Sahu. 2006. Evaluation of Oxidative Stress in pregnancy induced hypertension, I*ndian J Clin Biochem*., mar; 21(1):101-105;doi:10.1007/BF 029/3074

Matteucci E, Giampietro O. 2000. Transmembrane electron transfer in diadetic nephropathy, *Diabetes Care.,* Jul; 23(7):994-99, [PubMed]

May JM,QU ZC. 2004. Human erythrocyte recycling of Ascorbic Acid. *J Bio Chem.,* 279:14975-82.

OresteArrigoni, Silvio Dibierro. 1981. Ascorbate free radical reductase a key enzyme of the Ascorbic Acid System".Instituto di Botawcadell ,Universta di Bari,70126 Bari,Italy,Revised 30 Jan 1981,Volume 125,number 02 (FEBS letters)

Orringer EP, Roer ME. 1979. An ascorbate mediated transmembrane reducing system of human erythrocyte. *J Clin Invest.,* 63:53-58. Doi:10.1172/JCI109277.

Rizvi S I, Jha R, Maurya P K. Erythrocyte plasma membrane Redox System in human aging. *Reju Research.* 2006;9:470-74.

Rizvi SI, Srivastava N. 2010. Erythrocyte plasma membrane redox system in first degree relatives of type-2 diabetic patients. *Int. J Diab Mellitus* 2.2010:119-21. doi:10.1016/ j.ijdm.05.005.

Tamari Y, Nawata H. 2012. Protective roles of ascorbic acid in oxidative stress induced by depletion of Superoxide Dismutase in Vertebrate cells, Free Radic Res,2013 Jan:47(1) :1-7.doi:10.3109/10715762.2012.734916.epub Oct 17

Tietz NW. Fundamentals of Clinical Biochemistry.2nd edi. USA: W B Saunder Company.

****