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ORIGINAL PAPER

Erythrocyte Plasma Membrane Redox System, Plasma Vitamin C and Protein Carbonyl in Carcinoma Cervix Patients

Banerjee Ivy[1], Mukherjee Amrita[2], Lahiri Surajit[3],
Rout Jayanta Kumar[4], Ghosh Debdutta[5], Sarkar Chandan[6]

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ABSTRACT

***Introduction:** Though cervical cancer is a multi-factorial disease, the importance of oxidative stress in the pathogenesis cannot be underestimated. **Methods:** 40 cervical cancer patients (10 patients of each stage) and 40 age matched female controls were recruited for the study. Plasmaascorbate, protein carbonyl and erythrocyte plasma membrane redox system (PMRS) were studied in each subject. **Results:** Compared to controls plasma protein carbonyl and erythrocyte plasma membrane redox system are significantly higher and plasma ascorbate is significantly lower in cervical cancer. **Conclusion:** For the first time we have showed significant increase of erythrocyte PMRS activity in cervical cancer to mitigate oxidative stress in the body.*

***Keywords:** Carcinoma cervix, protein carbonyl, PMRS activity,*

INTRODUCTION

Carcinoma cervix is a very common and dangerous clinical entity affecting women in the developing countries, though it is multi-factorial disease, the importance of oxidative stress in the pathogenesis of disease cannot be undermined.[1] Distant metastasis involving lung, liver, bone, and supraclavicular lymph nodes is the most common feature of patients suffering from carcinoma cervix though the incidence of cancer cervix has reduced in India during the last two decades.[2]

The reactive oxygen species (ROS) damage cellular architectures. Carbonyl groups formed as a result of protein oxidation by ROS. Protein carbonyl level in tissues and plasma is relatively stable marker of oxidative damage.[3] Collectively, these ROS can lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, oxidation of the protein backbone resulting in protein fragmentation and peptide bond cleavages. As per A. D. N. J. de Grey the term "plasma membrane redox system" is used to denote the machinery by which cells oxidise electron donors, typically NADH and/or NADPH, and transfer the resulting electrons to extracellular acceptors.[4] The basic structure of PMRS includes the following entities–The intracellular electron donor species, electron carrier proteins & oxido-reductases, & extracellular electron acceptor.[5] The PMRS reduces extracellular oxidants by using the reducing power of intracellular antioxidants, making the cell metabolism respond to changes in the local redox environment.[6] One of the main electron donor species of PMRS is ascorbate.

Vitamin-C plays an important role in protecting the cells against oxidative stress and readily scavenges ROS, RNS, singlet oxygen and hypochlorite. Different stages of cancer cervix can be associated with a decrease in Vitamin-C.[7]

Keeping the facts in mind, this study aims to evaluate the erythrocyte PMRS activity, Plasma protein carbonyl, and plasma ascorbate in carcinoma cervix patients.

Address for Correspondence:
[1]Post Graguate Trainee of Biochemistry
[2]Asst. Prof. of Biochemistry (**Corresponding Author**)
Email: amrita.m.rc@gmail.com
Mobile: +919433370133
R G Kar Medical College, Kolkata-004, West Bengal, India
[3]Asst. Prof. of Community Medicine,
ICARE Institute of Medical Science and Research,
Haldia, Purba Medinipur- 721631, West Bengal, India
[4]Asst. Prof. of Biochemistry, [5]Professor of Gynaecology and Obstetrics, [6]Professor and Head of Biochemistry
R G Kar Medical College
Kolkata-004, West Bengal, India

MATERIALS AND METHODS

***Cases*:** All carcinoma cervix patients (prior to surgery, chemotherapy and/or radiotherapy) attending G&O-OPD and RT-OPD of R.G.Kar Medical College & Hospital in the specified period of 1yr were recruited. All patients meeting at least one of the following criteria were excluded from the study population- patients unwilling to sign consent form, pregnancy & lactation, malignancy other than carcinoma cervix, Diabetes Mellitus, major chronic illness like- TB, nephrotic syndrome, uremia especially treated with hemodialysis (not related to carcinoma cervix), other conditons where high oxidative stress are evident – infertility, abortion, fibroid, infection etc.

***Control population*:** Equal number of age& sex matched control subjects were also taken.

Chemicals: Metaphosphoric acid and Diphenylphenanthrolinesulphate (DPPS) are purchased from Sigma. Rests of the chemicals are from Sisco research Ltd. or Merck India Ltd. All chemicals are of AR grade. *Blood Collection Protocols*- 5ml of heparinised blood (10U heparin/ml) was collected aseptically after venepuncturefrom 40 carcinoma cervix patients and 40 age & sex matched control subjects. Heparinised blood was centrifuged at 3000 rpm for 5 minutes. Plasma was collected separately for Vitamin-C and protein carbonyl estimation. Blood cells were washed thrice with 0.9% NaCl solution and buffy coat was removed.

Methods: Plasma protein carbonyl was measured as per Levine's method (8) based on the spectrophotometric detection of the reaction between 2, 4-dinitrophenol hydrazine (DNPH) with Protein Carbonyl to form protein hydrazone. Plasma Vitamin-C was measured spectrophotometrically.[8] In this assay - ascorbic acid in plasma is oxidized by Cu++ to form dehydroascobic acid, which reacts with acidic 2,4-DNPH to form a red bis-hydrazone. This bis-hydrazone is measured at 520nm wavelength.[9] The methodology of measurement of PMRS activity wasferricyanidereduction test using DPPS (as described by Avron&Savit).[10] as used by Rizvi & Maurya.[11] Total plasma protein level was assessed by automated analyser (ERBA XL-600) using commercially available kit from ERBA (TransasiaBiomedicals Ltd., using Biurate method).

Statistical Analyses: The quantitative data of erythrocyte PMRS, plasma Vitamin-C & protein carbonyl were evaluated whether they followed the normal distribution or not. For parametric data, unpaired t-test for independent variables was performed between case & control. Pearson's correlation co-efficient was applied for assessment of interrelationship between RBC PMRS, plasma protein carbonyl & Vitamin-C level. All p-values were two-sided and less than 0.05 was considered a statistically significant difference. SPSS-20 software was used.

RESULT

RBC PMRS activity (µ mole Ferrocyanide/ml Packed RBC/30min.), plasma protein carbonyl (nanomole/mg of total protein), and plasma Vitamin-C level (mg/ml) of cases & controls are shown in **table 1**.

Table 1 Table showing mean and S.D. of RBC PMRS activity, Plasma protein carbonyl & Plasma Vitamin-C of cases & control population

Parameter	Cases	Controls	p-value
RBC PMRS activity(µ mole Ferrocyanide/ml Packed RBC/ 30min.)	6.19 ± 0.98	2.75 ± 0.69	< 0.001
Plasma protein carbonyl (nanomole/mg of total protein)	5.25 ± 1.56	1.54 ± 0.30	< 0.001
Plasma Vitamin-C level (mg/ml)	0.24 ± 0.04	0.33 ± 0.02	< 0.001

The table shows all three tests parameters vary significantly in cases of carcinoma cervix &age matched controls. Correlation between PMRS activity of RBC and other two variables (plasma Vitamin-C & protein carbonyl) were non-significant in carcinoma cervix. However, statistically significant negative correlation between protein carbonyl and Vitamin-C level was noticed (Pearson's correlation coefficient r-value is -0.941).

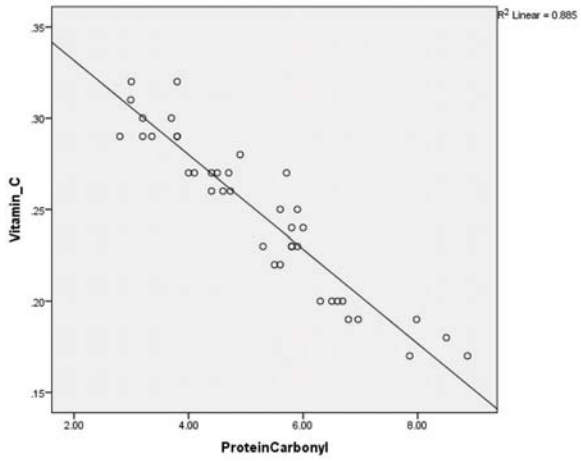

Figure 1 Scattered diagram correlation between Protein carbonyl and Vitamin-C in cases

DISCUSSION

The property of erythrocytes to reduce membrane impermeant anions was reported by Orrienger & Roer.[11] This plasma membrane redox system (PMRS) helps the cells to respond to the redox changes, thereby regulating many cellular functions – cell metabolism, ion channels, growth & death. But perhaps its most important role, especially in the nucleus- free mature erythrocyt is that it acts as a redox sensor. The PMRS reduces extracellular oxidants by using the reducing power of intracellular antioxidants, making the cell metabolism respond to changes in the local redox environment. Although the exact physiological functions of PMRS is not fully understood, proposed functions include maintenance of redox state of sulfhydryl residues in membrane proteins, neutralization of oxidative stressors outside the cells, stimulation of cell growth, recycling of alpha tocopherol, reduction of lipid hydro-peroxides, the maintenance of the extra

cellular concentration of ascorbic acid, and reduction of ferric ions prior to iron uptake by a transferrin-independent pathway.[12] Besides this, RBCs encounter a large load of oxidants throughout their life span. Recent studies show that- PMRS plays important role in protection against oxidative stress in human ageing, type-2 Diabetes Mellitus, uraemia, chronic haemodialysis etc.[5, 12-15] In our study we also obtained significant PMRS activity of RBC in carcinoma cervix patients compared to controls. This indicates presence of oxidative stress.

Protein carbonylation is a key determinant of oxidative stress.Primary modifications occur in metal-catalyzed oxidation.[16] Direct oxidation of Lysine, Histidine, Cysteine, Methionine.[17] side chain residues of protein backbone occurs. Secondary modifications occur when proteins are modified by molecules generated by oxidation of other molecules. Carbonyl groups in tissues and plasma is relatively a stable marker of oxidative damage. Many diseases areassociated with increased protein carbonylation including- acute/adult respiratory distress syndrome, amyotrophic lateral sclerosis, Alzheimer's disease, Diabetes mellitus, cystic fibrosis, dementia with Lewy body, Parkinson's disease, psoriasis, rheumatoid arthritis, systemic amyloidosis etc.[18] Protein carbonylation recently has been linked to epigenetic processes via carbonylation of lysine groups on histones and via carbonylation of class I and II histone deacetylases. Both types of modifications may affect gene expression.[19]

Ascorbate is an important water soluble, chain-breaking antioxidant.Some studies also concluded that antioxidants like Vitamin – C level of plasma in most of the Ca-Cx patients were lower than normal. Antioxidant supplementation showed to be effective in reducing oxidative stress in proteins.[20] In table-1, results of our studied parameters strongly indicate presence of oxidative stress. In figure-1, correlation between ascorbate& protein carbonyl showed inverse relationship. This suggested that oxidative stress led to increased consumption of ascorbate and protein carbonyl concentration increased. However, PMRS activity did not correlate significantly with plasma protein carbonyl or ascorbate. This may be due to the following factors-small sample size, low ascorbate level in carcinoma cervix (ascorbate is one of the main factor for optimum PMRS activity), and PMRS activity is also a function of age.

CONCLUSION

For the first time we have shown that PMRS activity of human RBC increases in carcinoma of cervix & statistically significant negative correlation exists between plasma protein carbonyl & plasma ascorbate.Considering the fact that erythrocytes face oxidative stress continuously this study aims to throw light on this very important aspect of protein carbonylation in the aetiopathogenesis of various diseases.Carbonyl groups being stable markers of oxidative damage they can serve as candidates of pharmacological targets in future.In background of protein carbonyls being involvedin epigenetic processes this can have a possible role in prevention of serious debilitating diseases.Again ascorbate being a very important parameter in maintainance of redox milieau of human physiological system,this study can prove to be relevant in reduction of oxidative stess and its consequent damages.Involvement of a larger numbe of cases in a longer time frame is required to establish the aim of the study on a broader perspective.

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Research involving human participants and/or animals: Human participant were taken

Consent of participation: All the participants were informed about the details of the study and written consent was taken from each of them.

Ethical clearance: Taken.

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