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PROTEIN CARBONYL AND LIPID HYDRO PEROXIDES AS PUTATIVE BIOMARKERS IN PATIENTS WITH CERVICAL AND OVARIAN CARCINOMA

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ABSTRACT

The present study was conducted in patients with cervix and ovarian carcinoma to evaluate the status of oxidative stress biomarkers as protein carbonyl and lipid hydro peroxides. Biomarkers of oxidative stress were measured as protein carbonyl (PC) and total lipid hydro peroxides (LOOH). Newly diagnosed women with carcinoma (n=100; 30-65 years of age) and age- matched clinically healthy women (n=50) were included in the present study. Circulating plasma total lipid hydro peroxides and protein carbonyl levels were significantly ($p<0.05$) higher in patients as compared to controls. The application of protein carbonyl and lipid hydro peroxides as biomarkers of oxidative stress has some advantages in comparison with measurement of other oxidation products because of the relative early formation and relative stability. Elevation of protein carbonyl and lipid hydroperoxides reflect oxidative stress in carcinoma patients and might be helpful in management of such patients.

KEY WORDS. **Protein carbonyl, Lipid hydroperoxide, Cervical , Ovarian carcinoma, Oxidative Stress biomarkers.**

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INTRODUCTION

Gynaecological cancers include malignancies affecting female reproductive system, uterus, cervix, ovary etc. It is a major cancer problem in women worldwide. In recent years, researchers have focused on the role of free radicals in a variety of diseases, among which the most important ones are neurodegenerative diseases, aging and cancers. Oxidative stress plays an important role in normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention[1-16]. Free radicals are responsible for DNA, lipid and protein damage and play an important role in the development and progression of many human diseases including cancer [17 - 20]. High levels of oxidative stress result in the peroxidation of cell membrane lipids with the generation of lipid peroxides that can decompose into multiple mutagenic carbonyl products. TBARS and LOOH are well-characterised lipid peroxidation products. They are considered mutagenic and carcinogenic. The level of MDA and LOOH reflect the extent of lipid peroxidation. On the other hand, protein carbonyl (PC) is a product of irreversible non-enzymatic oxidation or carbonylation of proteins and indicators of free radical generation in cells[21]. Carbonylation of protein often leads to a loss of protein function, which is considered a widespread marker of severe oxidative stress, damage and disease-derived protein dysfunction[22.] The usage of protein carbonyl groups as biomarker of oxidative stress has some advantages in comparison with measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated protein. Free radicals or oxidants are already known to damage the body while antioxidants are believed for protection but little known about the mechanism involved. The present study was planned to evaluate oxidative stress in terms of protein carbonyl and lipid hydroperoxides in gynaecological malignancies.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with Obstetrics and Gynaecology Department, King George's Medical University, Lucknow, India**.** This study consisted of 50 cases of cervix cancer and 50 cases of ovarian cancer with age group (30-65). Control group comprised of 50 female volunteers of similar age group (30 -65) without any evidence of malignancy. Patients with a history of tobacco consumption were excluded from this study. Women also suffering from diabetes mellitus, chronic liver disease, rheumatoid arthritis and any other chronic disease like tuberculosis or concurrent second malignancy were excluded from the present study. Patients on prolonged medication of any kind that could have resulted in discrepancy during estimation parameters of oxidative stress were also not included in this study. Ethical clearance for this study was obtained from the Institutional Ethics Committee and was in accordance with the Declaration of Helsinki. Blood samples was taken from patients and controls following an overnight fasting into EDTA vials to avoid the probable influence of nutritional factors on the ROS level. The plasma was separated by centrifugation at 3000 rpm for 15 minutes. Plasma Protein carbonyl (PC) content was measured by spectrophotometric detection of protein hydrazone, which is formed by the reaction between 2, 4-dinitrophenyl hydrazine (DNPH) and PC[23]. The results were expressed as n moles of PC content per milligram of protein by using molar extinction coefficient ε_{370} =22 000 $M^{-1}cm^{-1}$ at 370 nm. The protein content was determined by the Lowry et al. using bovine serum albumin as a standard . Total lipid hydroperoxides (LOOH) concentration was determined using the FOX-2 method with minor modifications. The FOX-2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides present in the plasma samples, in the presence of xylenol orange which produces a coloured ferric-xylenol orange complex whose absorbance can be measured at 560 nm wavelength using a solution of H_2O_2 as standard[24] . The coefficient of variation for individual plasma samples was less than 5%.

STATISTICAL ANALYSIS

The statistical significance of difference between the various groups was determined by using student-newman-keuls multiple comparison tests. Results were expressed as Mean ± SD. The statistical significance of observed differences is the parameters between the various groups was determined by the student-t- test, P > 0.05 = Not significant; P < 0.05* Significant, P<0.0001 *** highly significant, P<0.01 **.high significant

RESULTS

Present study was conducted to assess the protein carbonyl (PC) and lipid hydroperoxide LOOH as putative oxidative biomarkers in carcinoma patients as well as in controls. Plasma protein carbonylation or oxidation was assessed as PC, which was significantly (P<.0001) higher in patients as compared with controls groups (Fig. No.1). The mean ± SD plasma PC levels in carcinoma cervix was 4.10 ± 1.12, ovarian carcinoma was 4.6 ± 1.4 and in controls 2.04 ± 0.80 nmole/mg. Plasma lipid hydroperoxides levels were also found to be significantly elevated in patients as compared with controls. Mean ± SD plasma level of LOOH in carcinoma cervix patients was 0.65 ± 0.35, in ovarian carcinoma patients 0.84 ± 0.49 and in controls 0.39 ±0.18 mM/mL. We also compared different groups with each other. There were significant (p<0.001) differences between each groups (Table 1and2). Comparisons between controls vs. cervix and ovarian were found to be highly significant (p<0.001) while cervix vs. ovarian was significant (p<0.01).

FIGURE NO.1

Status of protein carbonyl with carcinoma cervix and ovarian as well as in controls

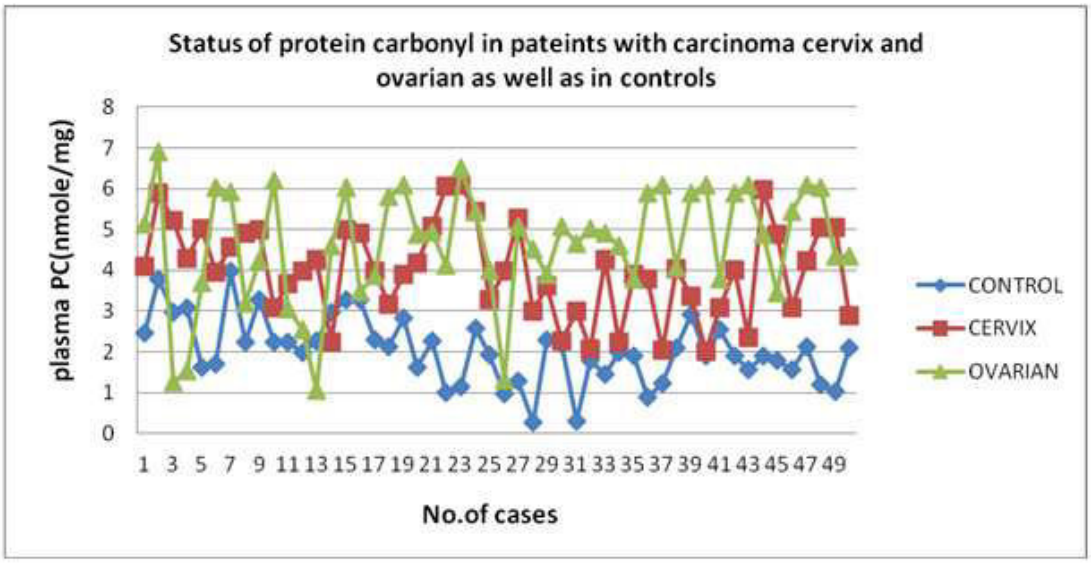

Table no 1

Comparison of changes in plasma protein carbonyl (PC) activity in carcinoma ovary, cervix patients and controls groups.

Comparisons b/w Groups	Mean difference	q	p
Controls vs. Ovarian	-0.2.589	15.858	*** P<0.001
Controls vs. Cervix	-1.968	12.054	*** P<0.001
Cervix vs. Ovarian	-0.6210	3.804	** P<0.01

significant * highly significant

FIGURE NO 2

Status of lipid hydroperoxide with carcinoma cervix and ovarian as well as in controls

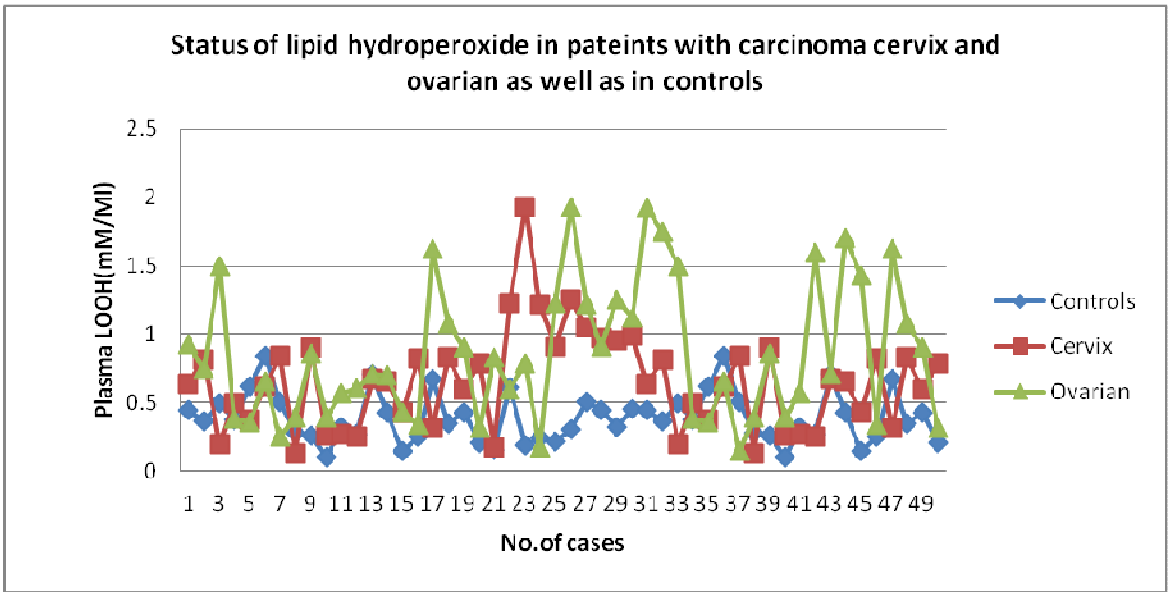

Table no.2

Comparison of changes in plasma lipid hydro peroxide (LOOH) activity in carcinoma ovary, cervix patients and controls groups.

Comparisons b/w Groups	Mean difference	q	p
Controls vs. Ovarian	-0.4541	8.845	*** P<0.001
Controls vs. Cervix	-0.2570	5.007	*** P<0.001
Cervix vs. Ovarian	-0.1970	3.838	** P<0.01

significant * highly significant

DISCUSSION

In spite of scientific advances in understanding of cancer in many parts of the world, still there is alarmingly increase in its incidence. Cancer may increase in almost all parts of the world because of increase in life expectancy, changes in life style and environmental factors. A biomarker of oxidative stress is defined as biological molecule whose chemical structure has been modified by ROS (reactive oxygen species) and which can be used to assess oxidation stress status in animal models as well as in human. To function as suitable biomarker of oxidative modifications in relation to disease, it is critical that such oxidation products are stable, can accumulate to detectable concentrations, reflect specific oxidation pathways, and correlate with disease severity, so that they can be used as diagnostic tools. Biomarkers may yield information on progressive levels of disease outcome as measurable endpoints of damage to bio molecules such as lipids and proteins. Biomarkers can be useful because, they help us understand the pathology of the disease. The cells have the potential to generate these reactive oxidants but we need to know why they generate more reactive oxidants in pathological conditions. Is there a reason to believe oxidants contribute to the symptoms of the disease? Molecular products or metabolic products formed from the reaction between ROS and bio-molecules are generally considered more stable than ROS themselves [25.] These products are too stable to evaluate more easily than direct evaluation of ROS [26.] In previous studies, various metabolic products have been

described as biomarkers for oxidative stress in a numbers of pathologies [26]. Most common metabolic products include products of oxidation of proteins and lipids. PC is an irreversible product of protein oxidation whereas LOOH is the product of lipid oxidation. [26 - 28]. A study on breast cancer provided evidence that protein oxidation might be associated with cancer risk [28.] A significant increased level of LOOH was found in patients with obstructive jaundice and colon cancer [29- 3] . Elevated level of PC and LOOH support the hypothesis that high ROS generation occurs in neoplastic or cancer cells. It could be concluded that plasma PC, LOOH may serve as biomarkers for oxidative stress in patients with cervix and ovarian cancer. There were significant differences between groups. The usefulness of the ideal biomarkers of oxidative damage lies in its ability to provide indications of disease or its progression. A highly structured study with a larger sample size is required to establish the precise role of oxidative stress in pathogenesis of cancer. Such oxidative biomarkers can be used for diagnosis and prognosis of diseases in future.

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