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EVALUATION OF PLASMA CERULOPLASMIN, PLASMA PROTEIN CARBONYL AND ERYTHROCYTE REDUCED GLUTATHIONE LEVEL IN EPITHELIAL CARCINOMA OF OVARY

Dr. Md Hefjur Rahaman [1] | Dr.Sujit Kumar Dey [2] | Dr. Sandeep Jain [3] | Dr.Bilas Kumar Sarkar [4] | Dr. Soumitra Ghosh [5]

[1] Demonstrator, Departmentof Biochemistry, Murshidabad Medical College, Berhampur, West Bengal, India.

[2] Associate Professor, Department of Biochemistry, R.G.Kar Medical College, Kolkata, West Bengal, India.

[3] Consultant Biochemist, Shanti Wellness Care, Kolkata, West Bengal, India.

[4] MD Biochemistry, Kolkata, West Bengal, India.

[5] MD Biochemistry (Student, PGT), R.G.Kar Medical College, Kolkata, West Bengal, India.

ABSTRACT

Background: *Ovarian cancer involves all the age groups but is commonest among peri and post menopausal women. It has the gravest prognosis among gynaecological malignancies.*

Aims &Objectives: *To study plasma ceruloplasmin, plasma protein carbonyl and erythrocyte reduced glutathione levels in patients with ovarian malignancies and comparing them with age matched controls of similar socioeconomic status, for any statistically significant correlation.*

Results: *The mean and SD of different parameters in the control and test groups were as follows : Protein Carbonyl (1.65± 0.87 & 4.76± 0.17 nanomol/mg of total protein), GSH(8.07± 0.28 & 6.81± 0.59 µmol/g of Hb), Ceruloplasmin (28.33± 4.33 & 62.47 ±12.80 mg/dl), CA 125(23.70 ±5.84 & 718.9± 490.58U/ml). By Mann Whitney U test it was concluded that groups are age matched. Protein Carbonyl, ceruloplasmin and CA 125 are significantly increased and GSH was decreased in the test groups. On Spearman's rho correlation, Protein carbonyl was positively correlated with ceruloplasmin and CA125(p<0.005) and negatively with GSH(P<0.005). On post Hoc test, ceruloplasmin,GSH and protein carbonyl were significantly related to the stages but the rise ofCA 125 was not significant on comparing stage 2&3.But it was significantly increased on comparing stage 3&4 with 2&4.*

Conclusions: *It can be concluded that there is increase in oxidative stress with disease progression as evident by increase in Protein Carbonyl and ceruloplasmin and decrease in GSH. Both rise in protein carbonyl and ceruloplasmin can be used as a biomarker for disease progression. There is depletion of GSH with disease progression; further studies are needed to ascertain whether glutathione supplementation can halt the progression of the disease.*

Keywords: Ceruloplasmin,Protein Carbonyl, Erythrocyte Reduced Glutathione,Epithelial Carcinoma Ofovary.

INTRODUCTION:

In India, 15% of all gynecological cancers are ovarian malignancies (1). It has the highest mortality rate amongst all gynecologic malignancies (2, 3). The majority of cancers of the ovary are thought to originate from a surface epithelial cell perturbed by ovulation (4). Reactive oxidants (hydroxyl radicals, superoxide radicals, hydrogen peroxide, and singlet oxygen) generated during the mechanics of ovulatory follicular rupture, damage the DNA of ovarian surface epithelial cells that are located within limited diffusion radius (5). In malignancies overproduction of free radicals leads to oxidative stress.The “oxidative stress” signature enables to identify ovarian tumors of high grade and advanced stage, which are yet highly sensitive to treatment. The“fibrosis” signature characterizes tumours with high risk of dissemination. The “oxidativestress” signature provides thus a better predictive value than the “fibrosis” signature (6). Ceruloplasmin (Cp) is an acute-phase-responsive oxidase enzyme and also an antioxidative biomarker which controls oxidative stress by segregating copper which otherwise could generate reactive oxygen species (ROS) (7). Determination of the Glutathione (GSSG)/Reduced Glutathione (GSH) seems to be a reliable index to assess thedegree of oxidative stress "in vivo" (8). Protein carbonyl estimation is also used as biomarker of oxidative stress (9). Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells, the redox imbalance thus may be related to oncogenic stimulation. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions (8-OH-G) have been noted in various tumors, strongly implicating such damage in the etiology of cancer. It appears that the DNA damage is predominantly linked with the initiation process (10). Moreover, severe oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes which are initial events in carcinogenesis (11), but can also play an important role in the promotion of multi-step carcinogenesis (12, 13). In the present study, it is aimed to study whether any variation in the biomarkers

of oxidative stress and antioxidants viz. ceruloplasmin, protein carbonyl and erythrocyte reduced glutathione level in ovarian cancer patient alter in comparison to the controls.

Materials and methods:

The present study is a hospital based cross sectional study involving Department of Biochemistry and Department of Gynaecology and Obstetrics, R.G. Kar Medical College and Hospital.

Study Population: cases and controls

For our study we have selected a group of 30 patients with ovarian cancer with TVS and CA125 positivity but excluded histopathologically.30 age matched women who are apparently healthy and free from the specific disease were used as control group.

The study was approved by the Institutional Ethical Committee. Informed consent was taken from every participant or their guardian as applicable.

Inclusion criteria:

Women with CA ovary only (TVS and CA125 positive).

Exclusion criteria:

Patientswith history of acute and chronic diseases apart from CA ovary, prior exposure to ionic radiation, use of antioxidant medication, TVS and CA125 positive but postoperative histopathologically negative patients (these will be removed from collected data)were excluded from study.

Estimation of test parameters:

Estimation of serum CA-125 by ELISA test.

Estimation of serum ceruloplasmin by Ravin's Method using paraphenylindiamine hydrochloride as substrate.

Protein Carbonyl estimation by Levine's method using dinitrophenyl hydrazine (DNPH).

Measuring GSH with the help of Beautler's method using dithiobisnitrobenzoic acid.

Statistical Analysis:

Calculations were performed using SPSS 20 software for Windows.

Results:

This study has been designed to detect Whetherceruloplasmin, protein carbonyl and GSH are altered or not in cases of CA ovary and to assess whether any interrelationship exists in between these three parameters.For this 30 cases and 30 controls were selected on the basis of inclusion and exclusion criteria of the study.

Most of the cases in stage 3 (45%) and then the stage 4 (35%) (Table1).

Protein carbonyl activity level was higher in cases than controls. Reduced glutathione activity level was lower in cases than controls.Ceruloplasmin activity level was more in cases than control. CA125 level was very much elevated in cases than control. All the alterations of mean values were significant satistically(Table2,3,4).

Spearman's rho correlation shows that protein carbonyl is positively correlated with ceruloplasmin (r=0.858) and CA125(r=0.882) and negatively correlated with reduced glutathione(r= -0.811). All the correlations were statistically significant (Table 6).

ROC curve of Reduced Glutathione shows area under the curve is 0.984 that indicates the results will be valid (100% sensitive) at sensitivity of GSH< 7.91.It means, the cut-off value of GSH is 7.91(Figure1).

ROC curve of Ceruloplasmin shows area under the curve is 0.999 that indicates the results will be valid (100% sensitive) at sensitivity of Ceruloplasmin >38 mg/dl .It means, the cut-off value of Ceruloplasmin is 38 mg/dl (Figure2).

ROC curve of Protein Carbonyl shows area under the curve is 1.00 that indicates the results will be valid (100% sensitive and 100% specific) when Protein Carbonyl level is 2.44.It means, the cut-off value of Protein Carbonyl is 2.44(Figure3).

ROC curve of CA 125 shows area under the curve is 1.00 that indicates the results will be valid (100% sensitive and 100% specific) when CA 125 level is 104.5.It means, the cut-off value of CA 125 is 104.5(Figure 4).

Table 1.Stage –wise distribution of cases:

Stage	Percentage (%)
Stage 1	3
Stage 2	17
Stage 3	45
Stage 4	35

Table 2: Table showing mean and S.D. of plasma Protein carbonyl level of cases & control population.

Plasma Protein Carbonyl (nanoMole / mg of Total Protein)			
Group	Mean	S.D.	p-Value
Control	1.65	0.87	<0.005
Case	4.76	0.17	

Table 3: Table showing mean and S.D. of reduced glutathione (GSH) level of cases & control population

Reduced glutathione level (µmol/gm of Hemoglobin)			
Group	***Mean***	***S.D.***	***p-Value***
Control	*8.07*	*0.28*	*<0.005*
Case	*6.81*	*0.59*	

Table 4: Table showing mean and S.D. of plasma ceruloplasmin level of cases & control population.

Ceruloplasmin level (mg/dl)			
Group	***Mean***	***S.D.***	***p-Value***
Control	*28.33*	*4.33*	*<0.005*
Case	*62.47*	*12.80*	

Table 5: Table showing mean and S.D. of serum CA125 level of cases & control population.

CA125 level			
Group	***Mean***	***S.D.***	***p-Value***
Control	*23.70*	*5.84*	*<0.005*
Case	*718.9*	*470.58*	

Table 6: Spearman's rho correlation among protein carbonyl, ceruloplasmin, reduced glutathione and CA125.

		Protein carbonyl	Ceruloplasmin	Reduced glutathione	CA125
Protein carbonyl	Correlation coefficient	1.000	.858	-.811	.882
	Single (1-tailed)	-	.000	.000	.000
Ceruloplasmin	Correlation coefficient	.858	1.000	-.845	.880
	Single (1-tailed)	.000	_	.000	.000
Reduced glutathione	Correlation coefficient	-.811	-.845	1.000	-.846
	Single (1-tailed)	.000	.000	_	.000
CA125	Correlation coefficient	.882	.880	-.846	1.000
	Single (1-tailed)	.000	.000	.000	-

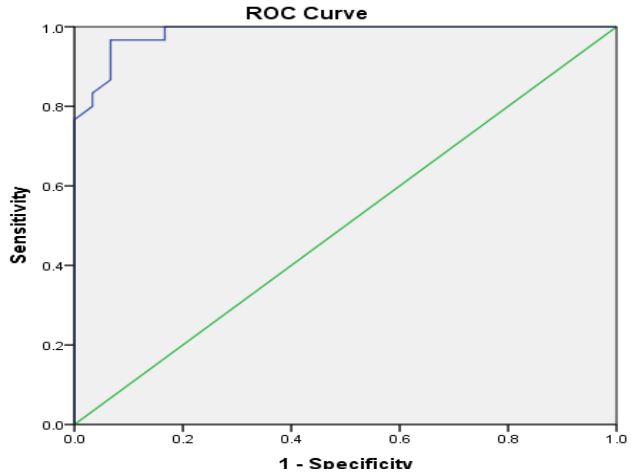

Figure 1: ROC curve of reduced glutathione (GSH)

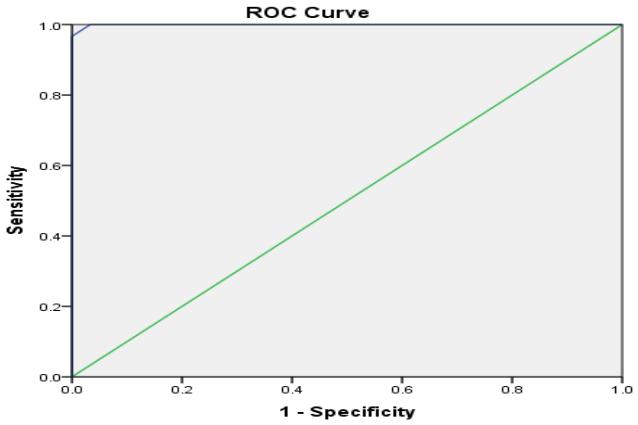

Figure 2: ROC curve of Ceruloplasmin

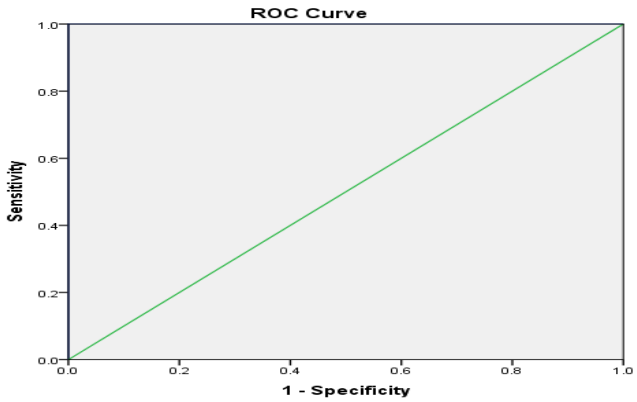

Figure 3: ROC curve of protein carbonyl

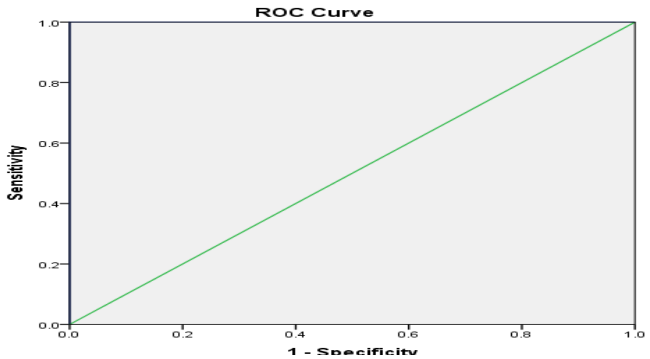

Figure 4: ROC curve of CA125

Table 7: Post Hoc test for stage wise comparison of significance of different parameter

Dependent variable	(I) stage	(J) stage	Significance
CA125	Stage 2	Stage 3	0.233
		Stage 4	0.000
	Stage 3	Stage 2	0.233
		Stage 4	0.000
	Stage 4	Stage 2	0.000
		Stage 3	0.000
GSH	Stage 2	Stage 3	0.000
		Stage 4	0.000
	Stage 3	Stage 2	0.000
		Stage 4	0.000
	Stage 4	Stage 2	0.000
		Stage 3	0.000
Ceruloplasmin	Stage 2	Stage 3	0.000
		Stage 4	0.000
	Stage 3	Stage 2	0.000
		Stage 4	0.000
	Stage 4	Stage 2	0.000
		Stage 3	0.000
Protein Carbonyl	Stage 2	Stage 3	0.000
		Stage 4	0.000
	Stage 3	Stage 2	0.000
		Stage 4	0.000
	Stage 4	Stage 2	0.000
		Stage 3	0.000

Discussions:

In India, 15% of all gynecological cancers are ovarian malignancies (1). It has the highest mortality rate amongst all gynecologic malignancies (2, 3). Epithelial cancers are the most common ovarian malignancies. They are usually asymptomatic until they have metastasized. Patients have advanced disease at diagnosis in more than two thirds of the cases. This cancer is hard to detect at an early stage because of the non-specific symptoms and misdiagnosis as other disease. There is no definitive tumor marker to diagnose in early stage. The high mortality associated ovarian cancer is due to delayed diagnosis after metastasis to other organs (14). Ovarian cancer represents a major surgical challenge, requires intensive and often complex therapies. There are no effective screening tests for ovarian cancer and few notable early symptoms. Aggressive debulking surgery, followed by platinum-based chemotherapy, usually results in clinical remission. 80% women will develop a relapse that leads to disease progression and death.

CA125 is not produced by normal ovarian epithelium, but may be produced by both benign and malignant ovarian tumors. CA125 is synthesized within affected ovarian epithelial cells and often secreted into cysts. In benign tumors, excess antigen is released into and may accumulate within cyst fluid. Hypothetically, abnormal tissue architecture associated with malignant tumors allows antigen release into the vascular circulation (Verheijen, 1999) (15).

There is no proof that routine screening with serum markers, sonography, or pelvic examinations decreases mortality rates (American College of Obstetricians and Gynecologists, 2009; Morgan, 2011; Schorge, 2010a) (16, 17, 18).

Hundreds of possible markers have been identified, yet no test currently available approaches sufficient levels of accuracy (American College of Obstetricians and Gynecologists, 2011) (19).

In malignancies overproduction of free radicals leads to oxidative stress.The “oxidative stress” signature enables to identify ovarian tumours of high grade and advanced stage, which are yet highly sensitive to treatment. The“fibrosis” signature characterizes tumours with high risk of dissemination. The “oxidativestress” signature provides thus a better predictive value than the “fibrosis” signature (6).

Severe oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes which are initial events in carcinogenesis (11), but can also play an important role in the promotion of multi-step carcinogenesis (12, 13).

Ceruloplasmin (Cp) is an acute-phase-responsive oxidase enzyme and also an antioxidative biomarker which controls *oxidative stress* by segregating copper which otherwise could generate reactive oxygen species (ROS) (7). Determination of the Glutathione (GSSG)/Reduced Glutathione (GSH) seems to be a reliable index to assess the degree of oxidative stress "in vivo" (8). Protein carbonyl estimation is also used as biomarker of oxidative stress (9).

In a study, the copper to ceruloplasmin ratio was moderately increased ($P<0.05$) but the copper and ceruloplasmin levels were significantly increased in ovarian cancer patients as compared to controls.(20).

Ceruloplasmin promoter activation is specifically and efficiently enhanced in ovarian cancer (21). Elevation of serum Ceruloplasmin has been reported to be useful in diagnosis and prognosis of other malignancies (22)

Immunohistochemical analyses of human ovarian cancer specimens showed a direct correlation between expression levels of c-jun and ceruloplasmin. The ceruloplasmin promoter activity is significantly enhanced in ovarian cancer and therefore may be exploited as a promising cancer-specific promoter in developing new

gene therapy strategies for ovarian cancer (23). Ceruloplasmin promoter activation is specifically and efficiently enhanced in ovarian cancer (21).

In the present study, Ceruloplasmin level is significantly increased in ovarian cancer cases than normal healthy patients . Moreover, Ceruloplasmin has positive correlation with Plasma Protein Carbonyl and CA125 and a negative correlation with Reduced Glutathione which are the other parameters of thisstudy . Besides, Ceruloplasmin has strong correlation between stages of Ovarian Carcinoma . There is no conflict with previous researches in these contexts.

Plasma protein carbonyls were associated with an increase in breast cancer risk although not in a dose-dependent manner. It has been documented that, oxidative damage is a risk factor for breast cancer in high-risk women (24).

In a study, it was found that Plasma protein carbonyls were associated with an increase in breast cancer risk (25).

In the present study, a significant increase in Plasma protein carbonyl activity is observed in carcinoma of ovary patients in respect to age and sex matched healthy control subjects. It also correlates with stages of the disease. In addition, Plasma protein carbonyl follows positive correlation with Ceruloplasmin and CA125 and a negative correlation with Reduced Glutathione . All these information obtained from the current study supports previous ones.

In a study, blood glutathione was estimated in fifty patients of head and neck cancer in the age group of 18-76 years and the results were compared with a group of normal healthy controls. Mean blood glutathione level was found to be significantly lowered in patients than the controls. The decreased levels of GSH in-patients with head and neck cancer, observed in that study, may be due to its increased utilization by the cells. The results suggest that patients with head and neck cancer have increased oxidative stress (26).

Reduced Glutathione decreases drastically under various carcinogenic conditions. In this study, GSH is decreased in ovarian cancer patients . Besides, Reduced Glutathione shows a negative correlation with the other parameters tested in this study, viz, Ceruloplasmin, Plasma Protein Carbonyl and CA125 . It is also found in this present study that, GSH follows a very significant correlation with stages of ovarian carcinoma .

Conclusion:

Increased plasma Protein carbonyl formation indicates that there is increased ROS/free radical mediated damage of protein. Plasma protein carbonyl level may be used as biomarker of disease severity or as a prognostic marker. As plasma protein carbonyl , plasma ceruloplasmin also can be used as a biomarker of disease severity. Erythrocyte Reduced Glutathione (GSH) level, lower than that of healthy control subjects, is supposed to be a reflection of low antioxidant defense status in ovarian cancer patients. GSH level has negative correlation with stages of ovarian cancer patients indicates that higher the stage lower the antioxidant defense by GSH. Further study can be done to assess whether GSH supplementation can slow disease progression or not.

REFERENCES

1. Chhabra S, Sonak M, Prem V, et al. Gynaecological Malignancies in a Rural Institute in India. J ObstetGynaecol 2002; 22(4):426-9.

2. O'Rourke J, Mahon SM, et al. A Comprehensive Look at the Early Detection of Ovarian Cancer.Clin. J. OncolNurs. 2003; 7 (1):41-7.

3. Edwards BK. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 116:544-73.

4. William J Murdoch and James F Martinchick. Oxidative Damage to DNA of Ovarian Surface Epithelial Cells Affected by Ovulation: Carcinogenic Implication and Chemoprevention. Experimental Biol-ogy and Medicine, 2004; 229; 546-552

5. Cancer Risk Factors. Ovulation Stress might Cause Ovarian Cancer. Cancer Weekly 2005

6. Bogdan M, Luciana B, Melissa C. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress Response. Nature Medicine, November 20, 2011, online.

7. Altgelt., Helen LY., Michael McCulloch. Pine street foundation-ovarian cancer,chemotherapy& antioxidant by Johanna

8. Kang DH. Oxidative Stress, DNA Damage, and Breast Cancer. AACN Clin Issues 2002; 13:540-9.

9. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress.ClinChimActa. 2003; 329(1-2):23-38.

10. Valko M, Rhodes CJ, Monocol J. Free radicals, Metals and Antioxidants in Oxidative Stress-induced Cancer. ChemBiol Interact, 2006; 160: 1-40.

11. Kang DH. Oxidative Stress, DNA Damage, and Breast Cancer.AACN Clin Issues.2002; 13:540-9.

12. Ahmed MI, Fayed ST, Hossein H, et al. Lipid Peroxidation and Antioxidant Status in Human Cervical Carcinoma. Dis Markers. 1999; 15(4): 283-91.

13. Ahmed A D, Hye-Yeon C, Jung-Hyun K. Review Role of Oxidative Stress in Stem, Cancer, and Cancer Stem Cells. Cancers. 2010; 2(2):859-884.

14. Visintin I, Feng Z, Longton G, et al: Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. 2008; 14:1065–1072.

15. Verheijen RH, Mensdorff -Pouilly S, van Kamp GJ, et al: CA-125: fundamental and clinical aspects. Semin Cancer Biol 9:117, 1999

16. American College of Obstetricians and Gynecologists: Hereditary breast and ovarian cancer syndrome. Practice Bulletin No. 103, April 2009

17. Morgan RJ Jr, Alvarez RD, Armstrong DK, et al: NCCN Clinical Practice Guidelines in Oncology. Ovarian cancer, including fallopian tube cancer and primary peritoneal cancer.Version 2. 2011. www.nccn.org. Accessed May 12, 2011

18. Schorge JO, Modesitt SC, Coleman RL, et al: SGO White Paper on ovarian cancer: etiology, screening and surveillance. GynecolOncol 119:7, 2010a

19. American College of Obstetricians and Gynecologists: The role of the generalist obstetrician-gynecologist in the early detection of ovarian cancer. Committee Opinion No. 477, March 2011

20. Nayak SB, Bhat VR, Mayya SS. Serum copper, ceruloplasmin and thiobarbituric acid reactive substance status in patients with ovarian cancer.Indian J PhysiolPharmacol. 2004; 48(4):486-8.

21. Lee CM, Lo HW, Shao RP, et al: Selective activation of ceruloplasmin promoter in ovarian tumors: potential use for gene therapy. Cancer Res. 2004; 64:1788–1793.

22.Nayak S.B., Yashwanth S., Sneha M., Serum Copper,Ceruloplasmin ,ProteinThiols and thiobarbituric acid reactive substance status in Liver Cancer Associated with Elevated levels of Alfa-fetoprotein. Indian J PhysiolPharmacol. 2005; 49 (3): 341–344.

23. Lee CM, Lo HW, Shao RP, Wang SC, Xia W, Gershenson DM, Hung MC. Selective activation of ceruloplasmin promoter in ovarian tumors: potential use for gene therapy. Cancer Res. 2004; 64(5):1788-93

24. Cancer Res 2009;69 (7):2966–72.

25. Jennifer Z, Mary B T, Yuyan L. Plasma Protein Carbonyls and Breast Cancer Risk in Sisters Discordant for Breast Cancer from the New York Site of the Breast Cancer Family Registry.Cancer Res, 2009 69; 2966.

26. Singh Y P., Sachdeva O P., Aggarwal S K., Chugh K. and Lal H. Blood Glutathione levels in Head and Neck Malignancies. Indian Journal of Clinical Biochemistry, 2008 ; 23 (3) 290-292.