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EVALUATION OF OXIDATIVE STRESS BIOMARKERS IN DIFFERENT CANCER TYPES IN PAKISTANI POPULATION

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ABSTRACT

The oxidative stress have been implicated in the pathogenesis of several diseases. It play a pivotal role in the pathophysiology of cancers. The aim of the current study was to explore the oxidative stress biomarkers in patients with different cancers. Two hundred and fifty different cancerous patients and fifty control subjects were recruited for the study after informed consent. Oxidative stress biomarkers including Xanthine Oxidase (XO), Nitric Oxide (NO), Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR) were estimated spectrophotometrically in blood samples. The correlation of oxidative stress biomarkers in different cancer was also analyzed. **S**ignificantly increased plasma levels of XO, MDA and NO and markedly decreased CAT, SOD and GR levels in plasma were observed in cancer patients. A strong positive correlations between MDA and SOD were observed in Multiple Myeloma (MM) patients, MDA and XO in Oral Cancer (OC) Patients. Furthermore in Colorectal cancer (CRC) patients NO and SOD have shown the direct correlation. While strong negative correlation was observed between NO and Catalase in Gastric Cancer (GC) patients. The study provides evidence of the relationship between oxidative stress biomarkers and different cancer, which could be used as diagnostic biomarkers for the individuals who are prone to cancer.

Keywords: ROS, Oxidative stress, Cancer.

ABREVIATIONS: Xanthine Oxidase (XO), Nitric Oxide (NO), Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Deoxyribonucleic acid (DNA), Reduced Glutathione (GSH), Oral Cancer (OC), Gastric Cancer (GC), Colorectal Cancer (CRC), Multiple Myeloma (MM), Ethylenediaminetetraacetic acid (EDTA).

INTRODUCTION

The worldwide cancer burden is alarmingly increasing and worsening a massive public health (Vineis and Wild, 2014). According to the WHO report, about 8.8 million cancer related death were reported in 2015. In Pakistan approximately 1.5 million new cases are diagnosed every year out of which 4.7% was due to esophageal cancer, 3.9% was bowel cancer patient, and 3.5% cases were due to stomach cancer (Stewart and Wild, 2017).

Numerous researches revealed that the oxidative stress plays a vital role in etiopathogenesis of many diseases, such as diabetes mellitus, neurodegenerative diseases (Sairazi *et al*., 2017), chronic inflammation (Kallaur *et al*., 2017), metabolic disorder (Carrier, 2017) and extensively in various cancers (Moe *et al*., 2004).

Oxidative stress-mediated DNA damage and cell proliferation has been reported to intervene with progression of cancer (Ollberding *et al*., 2012). Elevated oxidative stress disturbs the oxidant and antioxidant balance. It also cause the generation of reactive oxygen metabolites, including hydrogen peroxides, superoxide radicals, and peroxides radicals. Several researches demonstrated the worsen effects of reactive oxygen species on membrane lipid peroxidation, tissue damage and damage to deoxyribonucleic acid (DNA) (Shackelford *et al*., 2002; Badid *et al*., 2009).

Altered levels of antioxidant enzymes such as, SOD, catalase, and glutathione reductase and non-enzymatic antioxidants like GSH, Vitamin vitamin C, and thioredoxin as well as changes in the related signal pathways has been reported in various previous studies in many human cancers (Bonnefont-Rousselot *et al*., 2004).

The purpose of the study was to evaluate the specific role antioxidant enzymes and TBARS in different cancer. The study also focus on the role of oxidative stress damage and the compromised antioxidant enzymes and their correlation in different types of cancer in Pakistani population.

MATERIALS AND METHODS

Study Design: The study was designed and approved by institutional review board of University of Karachi. Blood samples were collected from two tertiary hospitals, Jinnah Post graduate Medical Center Karachi (JPMC) and Dar ul Sehat Hospital Karachi. Two hundred and fifty different cancer patients and fifty healthy individuals as controls

were included with written informed consent. Samples were collected from Patients were allocated in groups according to the cancer type. Group 1 served as control, Group 2; was gastric cancer (GC) samples (n = 50), Group 3; was oral cancer (OC) samples (n = 50), Group 4; was colorectal cancer (CRC) samples (n = 50), Group 5; was multiple myeloma (MM) samples (n = 50).

Inclusion/Exclusion Criteria: Group1: Normal healthy individuals with no known history/sign and symptoms of cancer were selected. Group 2 to 5: Cancer patients with known history were selected. All patients would receive standard chemotherapy were excluded. Patients with synchronous cancer in other organs, multiple and distant metastasis were also excluded from the study in advance.

Sample Collection: Blood samples for clinical assays were collected under aseptic conditions using, sterile syringes and EDTA vacutainers. The blood was centrifuge at 3000 rpm for 10 minutes at 4^{o}C. The plasma was separated and stored in different aliquots at -80^{o}C for the biochemical analysis.

Estimation of Xanthine Oxidase

Xanthine oxidase (XO) activity, a marker of oxidative stress was assayed by the method of (Prajda and Weber, 1975). The activity was estimated by the formation of uric acid from xanthine and absorbance was recorded at 292 nm against a blank. Enzyme activity was expressed as 1 unit activity equals 1 micromole of substrate converted to uric acid per min.

Estimation of Nitric oxide

Nitrite/nitrate in plasma sample was estimated by the Griess reaction (Guevara *et al*., 1998) after the reduction of nitrate into nitrite by nitric oxide. The sample was suspended in Tris buffer and incubated with nitrate reductase for 30 minutes at room temperature for the estimation of nitrate. For the nitrite estimation, sample was subjected deproteinization. Supernatant was incubated with reaction mixture of HCl and sulphanilic acid and NED for 30 min at 4°C. The mixture was then centrifuged at 10,000 rpm for 10 min and absorbance was observed spectrophotometrically at 540 nm.

Estimation of TBARS

The malondialdehyde (MDA) level is a measure of lipid peroxidation. The principle of the method is based on determining the thiobarbituric acid reacting substance, by measuring the absorbance at 530 nm according to the method of Okhawa *et al*., 1979).

Estimation of Catalase

Catalase activity was determined by the method of Sinha, 1972).

Estimation of superoxide dismutase

Levels of SOD were calculated by the reduction of nitro blue tetrazolium (NBT), used as an indicator of $O2^{\bullet-}$ production as described by Kono, 1978).

Estimation of Glutathione Reductase

This assay described by (Carlberg and Mannervik, 1985), is based on the oxidation of NADPH to NADP+ catalyzed by glutathione reductase. One GR activity unit is defined as the amount of enzyme catalyzing the reduction of one micromole of GSSG per minute at pH 7.6 and 25°C. Absorbance was recorded at 340 nm for 5 minutes.

Statistical analysis

Results were presented as mean ± standard deviation. Data were analyzed for statistically significant difference by using SPSS statistics version 19. Multiple comparison between the control and disease groups were tested using one way analysis of variance (ANOVA) followed by Tukey test and significance was accepted as $P < 0.05$. Pearson's correlation was estimated to test correlation between different parameters., where (p) and (r) values were recorded.

RESULTS

Assessment of plasma MDA levels in OC, GC, CRC and MM cancer patients

The plasma MDA levels in different cancer patients are shown in Table 1. OC, GC, CRC and MM patients show marked elevation ($P < 0.05$) as compare with control. In addition GC and MM patients also show increase plasma MDA levels ($P < 0.05$) as compared to OC. On comparison with GC, the MM patients have shown significant rise in plasma MDA levels ($P < 0.05$). While CRC patients show significant difference ($P < 0.05$) as compared to MM patients.

Assessment of plasma XO activity in OC, GC, CRC and MM cancer patients

Table 1 shows plasma XO activity in different cancer patient. Upon comparison with control, the GC and MM patients ($P < 0.05$) have shown high XO activity. While GC and MM patients have shown significant increase ($P < 0.05$) in XO activity when compared with OC patients. However CRC patients have shown significant difference with GC patients.

Assessment of plasma NO levels in OC, GC, CRC and MM cancer patients

The comparison of plasma NO levels in different cancer patients are shown in Table 1 OC, GC, CRC and MM patients show significant increase $P < 0.05$ in plasma NO level when compared to controls. Whereas GC and CRC patients show significant $P < 0.05$ rise in plasma NO levels as compared to OC. MM and CRC patients' comparison with GC show significant $P < 0.05$ increase in plasma NO levels. However CRC patients show significant elevation in plasma NO levels as compared to GC patients.

Table 1. Evaluation of oxidants in control and cancer patients.

Parameters	Superoxide dismutase (U/L)	Catalase (µmole/L)	Glutathione reductase (µmole/L)
Control	41.50 ± 4.81	86.13 ± 11.66	33.87 ± 7.60
Oral cancer	31.50 ± 4.64 $^{*\#\&}$	42.36 ± 11.87$^{*\&}$	38.37 ± 8.73 $^{*\&}$
Gastric cancer	25.28 ± 6.43$^{*+\&}$	34.61 ± 4.42$^{*\&}$	33.65 ± 8.80$^{+\&}$
Colorectal cancer	29.00 ± 7.55$^{*+\#}$	43.06 ± 7.28$^{*+\#}$	48.33 ± 7.23 $^{*+\#}$
Multiple myeloma	21.52 ±5.13$^{*+\#\&}$	47.01 ± 7.16$^{*+\#\&}$	44.11± 8.38$^{*\#\&}$

Results are expressed as mean ± S.D; n=50 for each group
(n=number of samples, SD=Standard Deviation, p=Test of significant)
*P<0.05 as compared to control
+P<0.05 as compared with OC
#P<0.05 as compared with GC
&P<0.05 as compared with CRC

Assessment of plasma SOD activity in controls, OC, GC, CRC and MM patients

The plasma SOD activity in **different** cancer patients is shown in Table 2 OC, GC, CRC and MM patients show marked decrease in plasma SOD activity ($P < 0.05$) as compared with controls. Likewise reduced plasma SOD activity is shown by GC patients and MM patients ($P < 0.05$) as compare to OC. On comparison with GC, CRC patients have shown significance difference.

Assessment of plasma catalase activity in controls, OC, GC, CRC and MM patients

Table 2 shows plasma catalase activity in **different** cancer patients. Upon comparison with control, OC, GC, CRC and MM patients show low plasma catalase levels ($P < 0.05$). Moreover GC patients show reduced plasma catalase levels ($P < 0.05$) as compared to OC. CRC patients ($P < 0.05$) and MM patients ($P < 0.05$) have shown significance decrease in plasma catalase activity as compared with GC patients.

Assessment of plasma Glutathione Reductase levels in OC, GC, CRC and MM patients

The comparison of OC, GC, CRC and MM plasma Glutathione Reductase levels in different cancer patients is shown in Table 2. The plasma Glutathione Reductase of OC, CRC and MM patients were significantly increased as compared to controls. While GC patients did not show any significant rise in plasma Glutathione Reductase level when compared to controls. However CRC and MM patients show significant increase in plasma Glutathione Reductase levels as compared to ($P < 0.05$) GC. Furthermore CRC and MM patients show significant difference ($P < 0.05$) with OC, GC patients.

Table 2. Evaluation of antioxidant enzymes in control and cancer patient.

Parameters	Malondialdehyde (µmole/L)	Xanthine Oxidase (U/L)	Nitric oxide (µmole/L)
Control	3.67 ± 1.01	0.46 ± 0.23	76.16 ± 9.62
Oral cancer	9.70 ± 2.65$^{*\#\&}$	0.48 ± 0.23$^{\#\&}$	113.98 ± 10.43$^{*\#\&}$
Gastric cancer	11.60 ± 2.99$^{*+\#}$	0.75 ± 0.17$^{*+\&}$	179.76 ± 12.05$^{*+\&}$
Colorectal cancer	10.81± 2.2$^{*+\#}$	0.58 ± 0.26$^{*+\#}$	97.94 ± 19.74$^{*+\#}$
Multiple myeloma	13.35 ± 2.85$^{*+\#\&}$	0.67 ± 0.23$^{*+\#\&}$	114 ± 13.5$^{*+\#\&}$

Results are expressed as mean ± S.D; n=50 (n=number of samples, SD=Standard Deviation, p=Test of significant)
*P<0.05 as compared to control
+P<0.05 as compared with OC; #P<0.05 as compared with GC; &P<0.05 as compared with CRC

Table 3. Simple Linear correlation analysis between the oxidative stress parameters in control and cancer patients.

	MDA level	XO level	NO level	Catalase level	SOD level	GSH level
Control Samples						
MDA level	1					
XO level	.111	1				
NO level	.223	.110	1			
Catalase level	-.097	-.035	.129	1		
SOD level	-.116	.079	.055	.070	1	
GSH level	.199	-.030	-.028	-.026	-.262	1
Oral Cancer Samples						
MDA level	1					
XO level	.281*	1				
NO level	.088	-.165	1			
Catalase level	-.094	-.132	-.043	1		
SOD level	-.121	.092	-.018	-.128	1	
GSH level	-.079	-.102	-.001	.277	.022	1
Gastric Cancer Samples						
MDA level	1					
XO level	.028	1				
NO level	.001	-.135	1			
Catalase level	.066	.147	-.393**	1		
SOD level	.118	.148	-.053	.021	1	
GSH level	.219	.012	.092	.238	.210	1
Colorectal Cancer Samples						
MDA level	1					
XO level	.126	1				
NO level	-.148	-.142	1			
Catalase level	.187	.021	-.143	1		
SOD level	-.159	.040	.294*	-.126	1	
GSH level	-.027	.229	-.134	-.201	-.087	1
Multiple Myeloma Samples						
MDA level	1					
XO level	.121	1				
NO level	-.016	-.017	1			
Catalase level	.076	-.060	.170	1		
SOD level	.285*	-.138	-.057	-.023	1	
GSH level	-.086	-.146	.163	.242	-.010	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

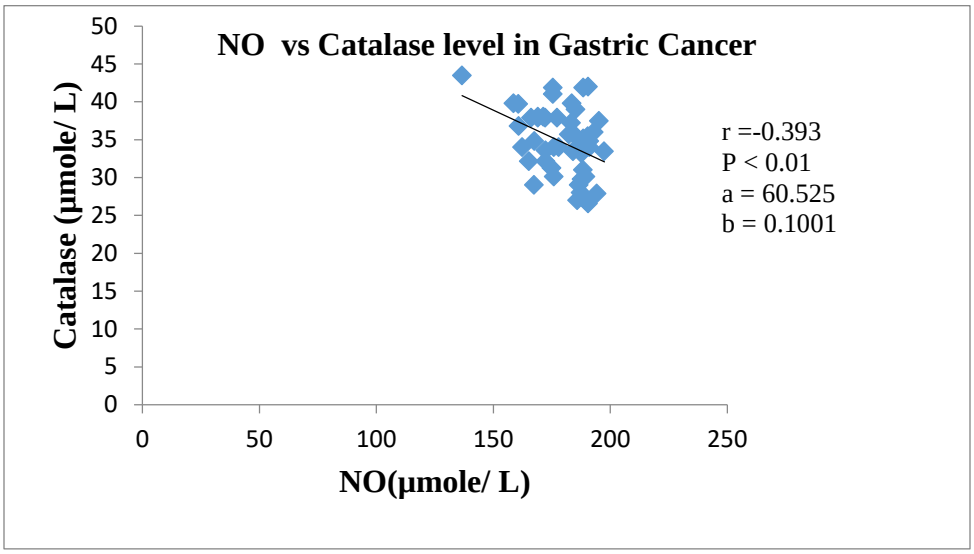

Fig. 1. Relationship between NO and Catalase level in Gastric Cancer Patients.
Linear regression analysis of NO with Catalase level in Gastric cancer Patients.

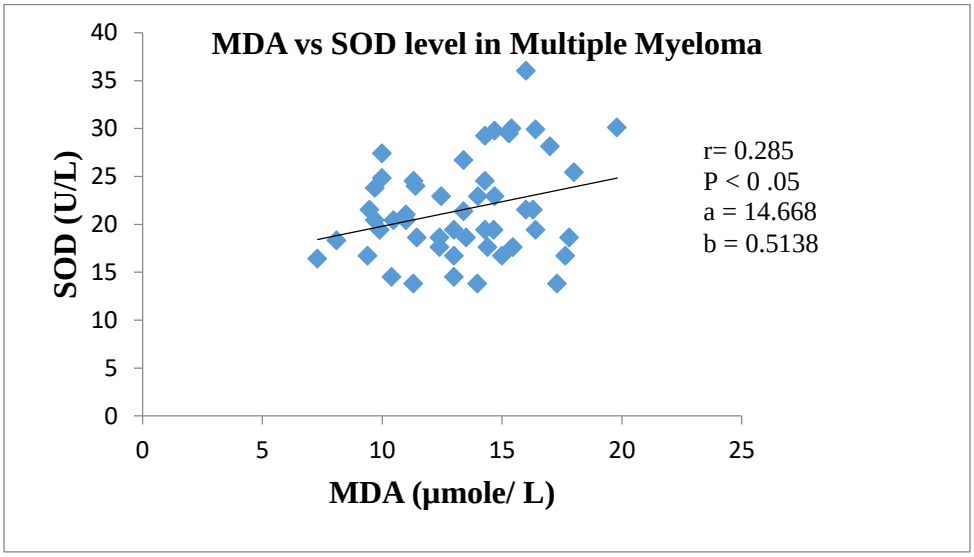

Fig. 2. Relationship between MDA vs SOD level in Multiple Myeloma Patients.
Linear regression analysis of MDA vs SOD level in Multiple Myeloma Patients.

Pearson Correlation

Table 3 shows the significantly inverse correlation of NO and Catalase in GC patients $P < 0.01$ (Figure 1). While significantly positive correlation was found between MDA and SOD in MM patients $P < 0.05$ (Figure 2), MDA and XO $P < 0.05$ (Figure 3) (Table 3) in OC Patients. Furthermore CRC patients NO vs SOD $P < 0.05$ (Figure 4) have shown the direct correlation. However none of other correlations are observed significant.

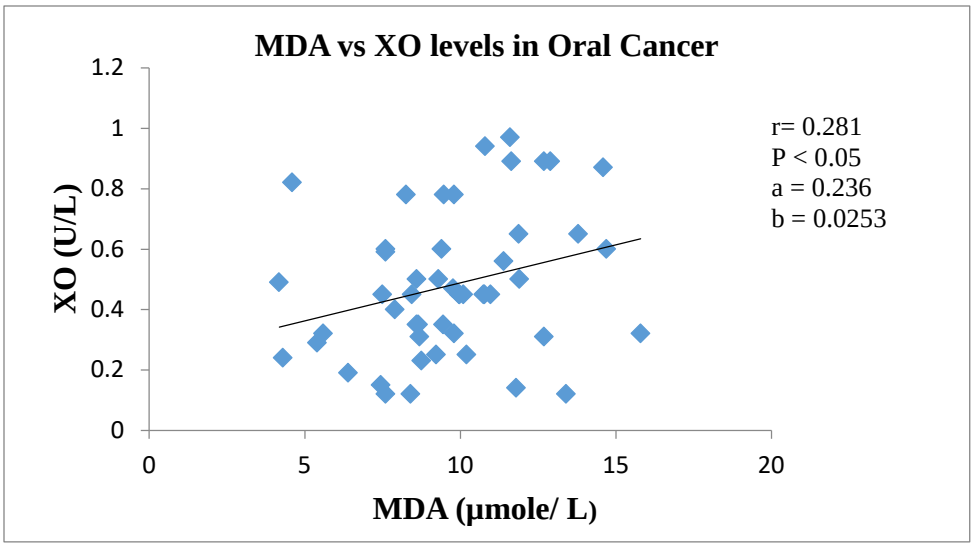

Fig. 3. Relationship between MDA vs XO levels in Oral Cancer Patients.
Linear regression analysis of MDA vs XO levels in Oral Cancer Patients.

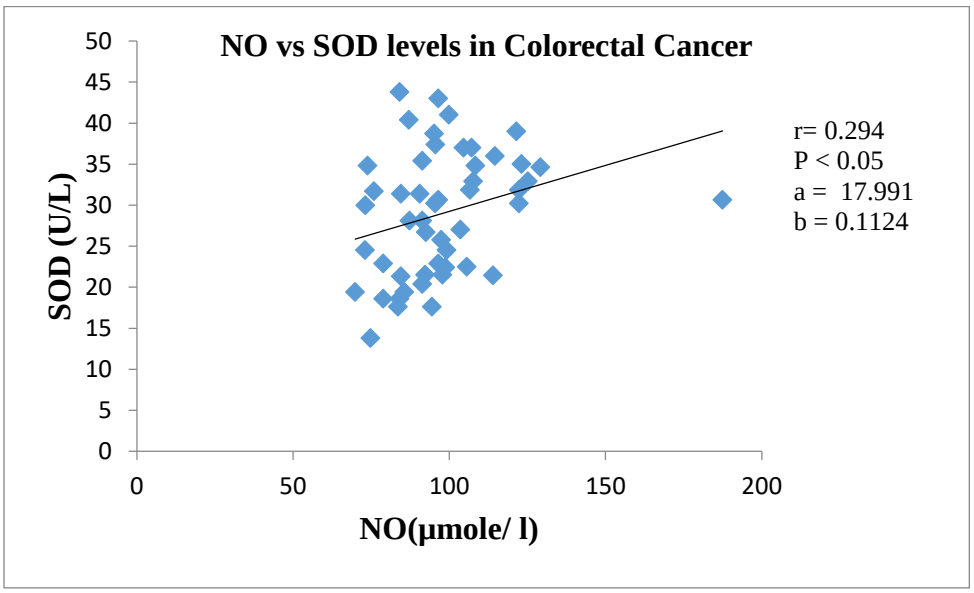

Fig. 4. Relationship between NO with SOD levels in Colorectal Cancer Patients.
Linear regression analysis of NO and SOD levels in Colorectal cancer Patients.

DISCUSSION

Despite the presence of endogenous defense mechanisms against Reactive Oxygen Species (ROS), it has been observed that any disturbance in the cellular antioxidant systems lead to abnormally elevate ROS levels that cause oxidative damage to the cells and considered as a leading cause for several pathological conditions, including Cancer (Sun, 1990).

In the recent years, several researches confirm that the free radicals have role in the mechanisms of initiation and development of neoplastic transformations as well as in the stimulation of specific oncogenes which results in proliferation. Cancer patients are prone to oxidative damage as their antioxidant defense mechanism was disturbed. The results of the present study showed significantly decrease SOD activity, catalase activity and glutathione reductase activity in all types of cancers as compared with control ($P < 0.05$) except the gastric cancer that did not

show significant change in glutathione reductase in comparison with control (Table 1). The results were in accordance with the previously reported studies which revealed that increased production of OH^- and superoxide anion in cancer patients may decreased the catalase activity (Shilpasree *et al*., 2013; Dursun *et al*., 2006; Wang *et al*., 2006) Significant decrease in plasma SOD levels among cancer patients has also reported in numerous studies (Skrzycki *et al*., 2015; Tu *et al*., 2011). A negative correlation between catalase and NO was shown in GC ($r = -0.393$, $P < 0.01$) (Fig. 1).

The results of the present study also demonstrated markedly increase MDA, XO and NO in all type of cancer in comparison with control ($P < 0.05$) except oral cancer that did not show significant change in XO as compared with control (Table 2). An increase in plasma MDA concentrations has been widely reported in gastric, oropharyngeal, renal, breast and lung and colorectal adenomas cancers (Dursun *et al*., 2006; Mehdi *et al*., 2013). The direct correlation between MDA and SOD was observed in MM patients ($r = 0.285$, $P < 0.05$) (Fig. 2, Table 3).

High activity of XO in the plasma was observed in different types of cancer (Battelli *et al*., 2016) as well as in various pathological conditions. Therefore XO is considered as a major cellular source of oxygen radical which contributes to DNA damage Moreover a direct relation between MDA and XO ($r = 0.281$, $P < 0.05$) (Fig. 3) has been observed in OC patients.

Nitric oxide (NO) is considered as pleiotropic regulator, has role in vasodilatation, neurotransmission and macrophage-mediated immunity. Results of the present study show elevated NO levels in cancer patients (Table 1). Similar results have been reported previously in OC (Beevi *et al*., 2004), MM (Fionda *et al*., 2015), GC (Choudhari *et al*., 2013) and CRC (Fransén *et al*., 2005) patients. Additionally the direct correlation among NO and SOD in CRC patients was observed ($r = 0.294$, $P < 0.05$) (Fig. 4).

Diverse results showing the correlations with various cancer types and oxidative stress parameters were observed. These correlations are valuable in terms of diagnosis in cancer patients as our results are indicating low antioxidant profile in cancer patients. Therefore it is established that cancer cells proliferation is mediated by multiple signaling cascades via ROS (Sena and Chandel, 2012).

Thus, the current study showed the antagonistic association with lipid peroxidation and antioxidant enzyme system.

CONCLUSION

This study showed diversified correlations among the cancer types with oxidative stress which could be used as diagnostic tool for the individuals who are prone to cancer, as remarkable rise in oxidative stress markers is one of the factors involve in carcinogenesis. The outcomes of our study in the light of aforementioned results reveal that antioxidant profile could be used as diagnosis for individuals who are at high risk. The alleviation of antioxidants, may contribute to cause cancer, the impediment in oxidants and restoration of antioxidant activity may prompt their defensive role.

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