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Treatment With Diagnostic Radiation Field And Stress: A Biochemical Study To Evaluate Cortisol And Oxidative Stress Parameters In Sera Of Handlers In The Sections Of Diagnostic Radiation In Hospitals

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ABSTRACT

Diagnostic radiation still one of the most important way in the diagnosis of several diseases, in spite of the possible passive effects of their product radiations. In order to test the hypothesis that radiations may perturb in the body functions (that including the function of nervous, sexual, immune and gastrointestinal tract systems) of the handlers in diagnostic radiation field, the present study was designed to determination of oxidative stress parameters (MAD and NO as a lipid peroxidation agents, and SOD in addition to CP levels, as protective agents against lipid peroxidation) in addition to cortisol level as a reflection parameters to the bearable effect in sera of 25 workers in Al- Sadder Medical City, and 30 non work controls. Elevation of MDA NO and cortisol levels were recorded ($p<0.005$, $p<0.05$, and $p<0.05$; respectively) in sera sample of workers in the diagnostic radiation fields, on the other hand, significant decreases ($p<0.005$ and $p<0.001$; respectively)in levels of antioxidation parameters CP and SOD. Current work can present a simple and sensitive tool to evaluate the radiations effect in this field workers, oxidation and oxidation parameters can illustrate a good imagination about the workers health. This work can be applied in other fields which recorded radiance activities, in addition to that it can develop to involve estimation of several oxidative stress and antioxidation parameters and compare their results to present work.

Keywords: Radiation, Oxidative stress, Cortisol, Trace elements, Handlers.

INTRODUCTION

X- ray is one of the most important diagnostic tool to detect several diseases in our hospitals, it is belong to the indirectly ionizing electromagnetic group of radiations, and it has a very high penetrating power because of it low linear energy transfer [1]. Exposure of eukaryotic cells to ionizing radiation results in the immediate formation of free radicals. A free radical is any molecule with an odd number of electrons, and can occur as both organic and inorganic molecules, they are highly reactive and therefore highly unstable and short lived species. For instance, the half-life of lipid peroxyl radical ($ROO^{\cdot}$) is 7 seconds, and that of hydroxyl radical ($HO^{\cdot}$)is 10^{-9} seconds. They are produced naturally in vivo as byproducts from normal metabolism [2, 3]. Over production of free radicals can cause oxidative stress through the radiolysis of body water which is often referred to as the indirect effect of radiation

[1]. This coupled with the oxygen effect enhances tissue injury through the process of lipid peroxidation, then this process can become autocatalytic after initiation, leading to the production of lipid peroxide, lipid alcohol, aldehydes and other chemical species, or cross linking of membrane lipids and intracellular compounds, thus leading to cell aging and death. Although this is part of the normal aging process of cells, the presence of increased oxidative stress is thought to lead to premature cell aging. Both these effects are more pronounced for low linear energy transfer radiations, accounting for more than 70% of molecular damage. Previous studies illustrated that free radical damage induced by low dose ionizing radiation was no greater than the free radical damage caused during routine metabolic chemistry itself [4, 5].

Researchers generally agree that many human diseases are attributable, at least partly, to free radical mediated reactions [6-9]. Oxidative stress in this work has been studied by estimating the levels of malondialdehyde (MDA) and nitric oxide (NO) in addition to antioxidation agents like super oxide dismutase (SOD) and ceruloplasmin activity(CP) in sera of medical radiographers and comparing them with those of controls, then try to find a dialectical relationship among oxidative and antioxidation parameters to the levels of cortisol. Cortisol is one of the most important stress hormones in humans[10], it is synthesized by the adrenal cortex. Cortisol is involved in regulating protein and carbohydrate metabolism by promoting protein degradation and the conversion of amino acids into glucose[11]. A reciprocal control, the hypothalamic-pituitary-adrenal axis, exists between the brain and glucocorticoid hormones. Under stressful conditions, the brain promotes adrenocortical function via hypothalamic corticotrophin releasing hormone. On the other hand, glucocorticoids act at specific receptors in the hypothalamus, thus producing negative feedback mechanisms[10], so this study designed to investigate an alteration in the cortisol level simultaneity with the attendant injury to exposure of the radiation stress.

The basis of current work is to form harmonics opinion between argument and the paucity of data studying the effects of low dose ionizing radiation in humans when compared to literature dealing with the different aspects of non ionizing and high dose ionizing radiations. Studies such as this are important because in the countries like ours, where biological security controls are not strict and extended work days are common, this type of monitoring may be useful as an indicator to detect early damage in order to demand more controls in radiation protection.

MATERIALS AND METHODS

Population of the Study: Fifty five individuals were enrolled in the present study, they are classified into radiographers (25 individuals, with working period of 1-25 years, mean ± S.D. of working period was 12.40 **±8.196 years**). Basically; the study focused on x-ray technicians as a work subjects, but some radiographers were also involved in other radio diagnostic procedures like angiogram, CT scan and MRI. The study involved workers from both ganders with the ratio of 3:1 male to female. Control individuals group involved workers in other hospital sections (emergency, CCU, laboratory, and management), in addition to persons not working in hospital, but they are residing in the same geographical area, and they were in the same socioeconomic status and similar diet habits. The age information of the study groups were showed in table 1.

Table1: Age Information of The Diagnostic Radiation Field Worker and Control Groups

		Radiographers	Controls
Age	No.	25	30
	(Mean ± S.D.)	37.560±9.399	37.360±9.464
	Min-Max	23-54	23-54
	Median	37	34

Full information about the working duration were summarized in figure 1.

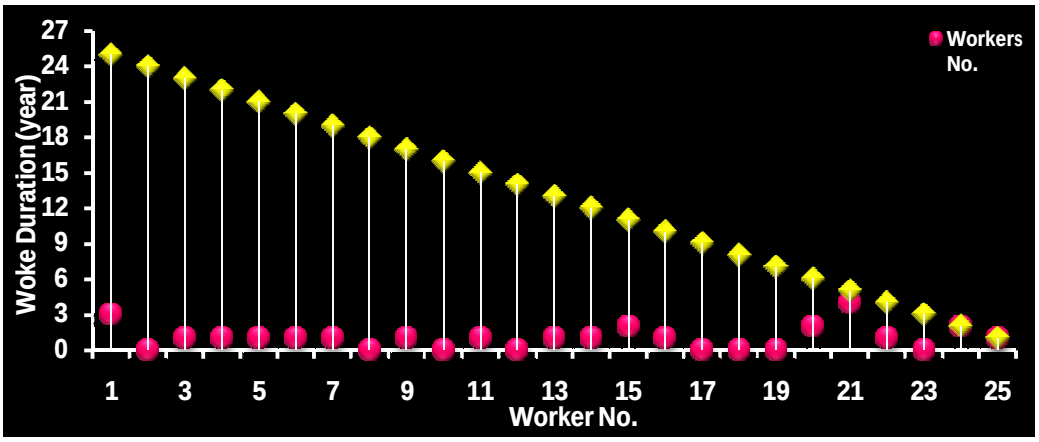


Fig. 1: Distribution of Workers to The Work Duration(Year)

Samples Collection: Approximately at 9 AM in the morning, after about 10 h fasting, five to ten milliliters of blood were obtained from each radiographers and from control persons, then serum was collected in tubes without anticoagulant and were left for 30 minutes at room temperature after coagulation. The blood samples were centrifuged at 5000 x g for 5 minutes. Clear sera were separate and stored at -20 °C until time of use.

Estimation of Serum Cortisol: Serum cortisol was estimated by ELISA. The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK **.**

Measurement of Serum Superoxide Dismutase Activity (SOD): Serum superoxide dismutase activity of diagnostic radiation field workers and control subjects was determined according to the method of Sun [12]. The principle of the method is based on inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate, while the specific SOD activity was expressed as U mg^{-1} protein.

$$\textbf{Specific SOD Activity (U/mg)} = \frac{\textbf{SOD Activity (U/dL)}}{\textbf{Protein Concentration (mg/dL)}}$$

Determination of Total Serum Proteins Levels: A total serum protein was estimated using Biuret method [13]. Bovine serum albumin was used as a standard protein.

Measurement of Serum Nitric Oxide Level: In the study samples (workers and controls), serum nitric oxide was measured in terms of its products, nitrite and nitrate, by the method of Griess modified by Fiddler**.** The principle of this method is based on a two-step process, the first is the conversion of nitrate to nitrite using tin metal powder and the second is the addition of sulphanilamide and (N -naphthyl) ethylenediamine, this converts nitrite into a deep purple azo compound, which was measured at 540 nm[14].

Measurement of Serum Malondialdehyde (MDA) Level: Malondialdehyde level is measured by the thiobarbituric acid-reacting substances (TBARS) assay [15]. Abridgment, 150 µl of the serum sample was mixed with 1 ml of trichloro acetic acid (TCA) (17.5 %) and 1ml of thiobarbituric acid (0.6 %). Using the vortex, the final mixture was mix, the reaction mixture was then heated at 100°C for 15 minutes in the water bath. After the mixture was cooled with tap water, it was extracted with 1 ml TCA (70 %), the mixture was stand for 20 minutes at 25°C, and centrifuged at 3000 xg for 15 minutes. The organic phase was measured by use of a spectrophotometer with a wavelength of 534 nm.

Determination of Ceruloplasmin Oxidase activity: The activity of ceruloplasmin oxidase was determined in serum using the modified Rice method [16]. The procedure included two glass tubes, test (A) and blank (B), 1ml of substrate buffer (prepared by: dissolving of 0.1g of crystal p-phenylenediamine-2HCl in 100ml of acetate buffer "0.4M pH 5.2, containing 0.4 µM EDTA") was added to each tube, then incubated at 37ºC for 5 min. A 100µl of serum sample was added to tube A then incubated at 37ºC for 15 min. A volume of 3 ml of cold working inhibition solution (this solution was prepared by diluting 3 ml of

stock inhibition solution 0.1 M of sodium azide and 0.5 M of sodium chloride) to 100 ml with deionized water) was added to all of A and B tubes; at last 100µl of deionized water was added to tube B. The absorbance was measured at λ=540 nm.

Ceruloplasmin Oxidase Activity = The absorbance of A–B tubes×349.04

Ceruloplasmin oxidase concentration was determined by measuring the absorbance of A and B tubes at wavelength = 605 nm

Ceruloplasmin oxidase concentration = The absorbance of A–B tubes×87.5

Statistical Analysis: The findings were expressed as the mean ± standard deviation (S.D.). The data were analyzed with Student's independent *t*-test. Statistical significance was set at $p<0.05$. All statistical analysis were performed with the program Statistical Package for the Social Science (SPSS) (SPSS Inc., Chicago, USA) Windows, Version 17.0. Associations between serum cortisol levels and measurement oxidative and antioxidative parameters in addition to the period of working were examined through Pearson's correlation coefficient and linear regression.

RESULTS AND DISCUSSION

The present study involved 55 persons. They were categorized into two groups. The first one is 25 individuals worked in different hospital sections treated with radiation fields. Out of them, 11individuals worked in the x-ray section for 1-25 years, remaining number were distributed to other sections. The second one is 30 individuals (Control group). Out of them 18 were distributed to 18 hospital's (workers in laboratory, physical treatment, emergency, and management sections). The other 12 individuals not working in the hospital before.

Table 2: Effects of Radiation Expose on The Oxidation and Antioxidation Parameters In Sera of Workers in The Radiation Field Comparison to Control Subjects (Mean±S.D.)

Parameters	Mean ± S.D.	Min-Max	Range	Median	p-value
Cortisol (µg/dL)					
Workers	15.332±2.239	6.880-18.900	12.020	12.890	
Controls	12.421±3.611	6.145-17.572	11.427	11.859	**<0.05**
MDA (µmol/L)					
Workers	18.029±6.252	9.100-30.800	21.700	17.300	
Controls	11.066±2.982	6.600-18.500	11.900	11.100	**<0.005**
NO (µmol/L)					
Workers	0.631±0.084	0.498-0.832	0.334	0.621	
Controls	0.472±0.048	0.409-0.581	0.172	0.472	**<0.05**
CP Activity (U/L)					
Workers	20.327±6.553	11.043-35.491	24.448	19.570	
Controls	35.709±7.442	15.705-48.860	33.155	35.249	**<0.005**
SOD Activity (U/dL)					
Workers	0.952±0.188	0.722-1.511	0.789	0.891	
Controls	1.883±0.267	1.570-2.340	0.770	1.860	**<0.001**

Using Pearson's correlation, the relations among the study parameters were illustrated. Highlight of the figure 2 shows that there was a negative relation between the oxidative stress (MDA and NO) and antioxidation (CP and SOD) parameters, the highest statically result ($r = -0.871$ at $p<0.001$) was recorded when the relationship was exam between NO and CP, as shown in figure 2B. with same manner (as demonstrated in figure 2A), the relation of MDA to CP was significantly negative correlated ($r = -0.698$ at $p<0.005$). generally, the correlation result of NO to SOD (figure 2D) was less than previous cases ($r = -0.588$ at $p<0.005$),while; no significant variation was observed when the relation was carried out between MDA and SOD, as established in figure 2C.

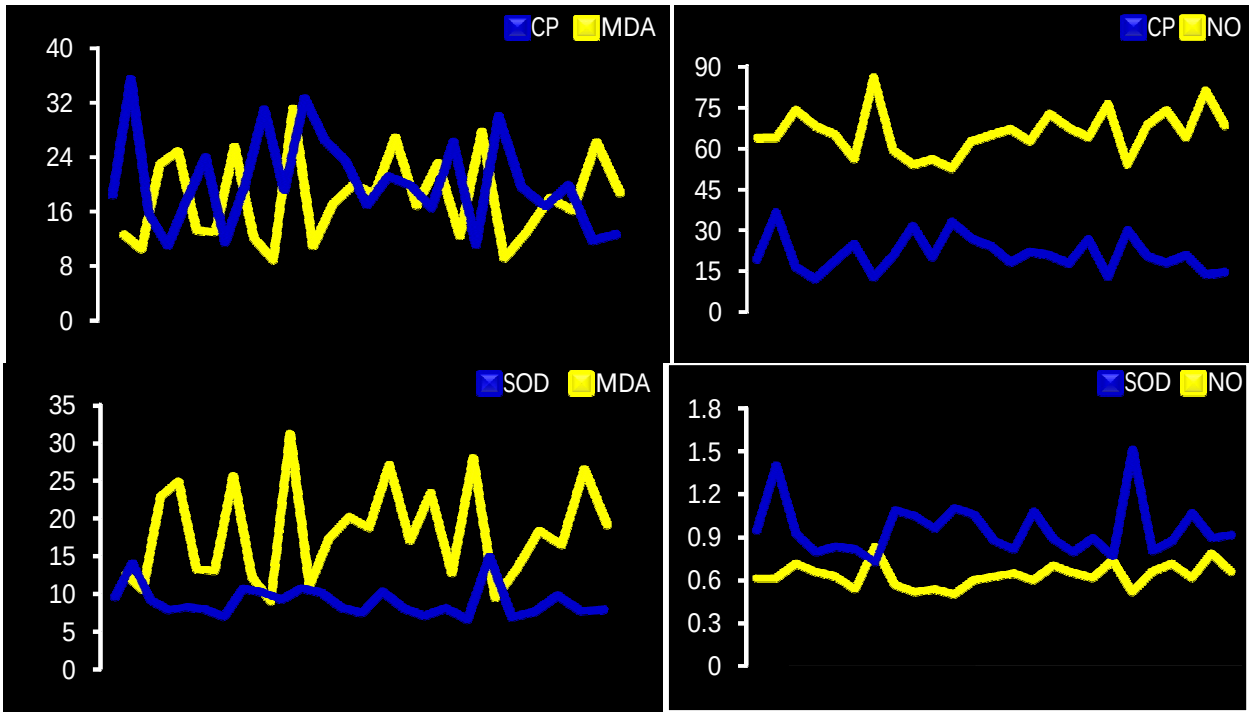
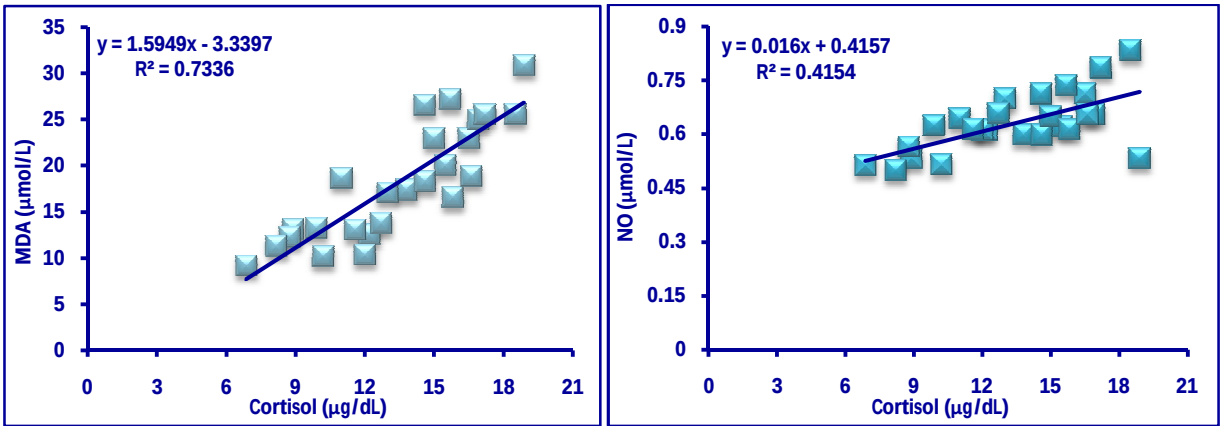


Fig. 2: Relation of Oxidation to Antioxidation Parameters (A): MDA to CP, (B): NO to CP, (C): MDA to SOD, and (D):NO to SOD

In order to explore the probable relation between the present study's examined oxidation-antioxidation parameters to the cortisol level, Person's correlation was employed. Strong positive correlation was recorded when the relationship was done between cortisol and MDA levels ($r = 0.857$ at $p<0.001$), with the same manner, moderate positive correlation ($r = 0.645$ at $p<0.05$) was noted between cortisol to the NO level (figure 3A and 3B). On the other hand, a significant negative correlation ($r = -0.659$ at $p<0.005$) was recorded when the relation carried between serum cortisol level to the ceruloplasmin activity in the samples of the radiation field workers (as shown in figure 3C), while no such correlation was illustrated when the same factor (serum cortisol level) was correlated to the super oxide dismutase activity ($r = -0.359$ at $p> 0.05$), this data appeared clearly in figure 3D.



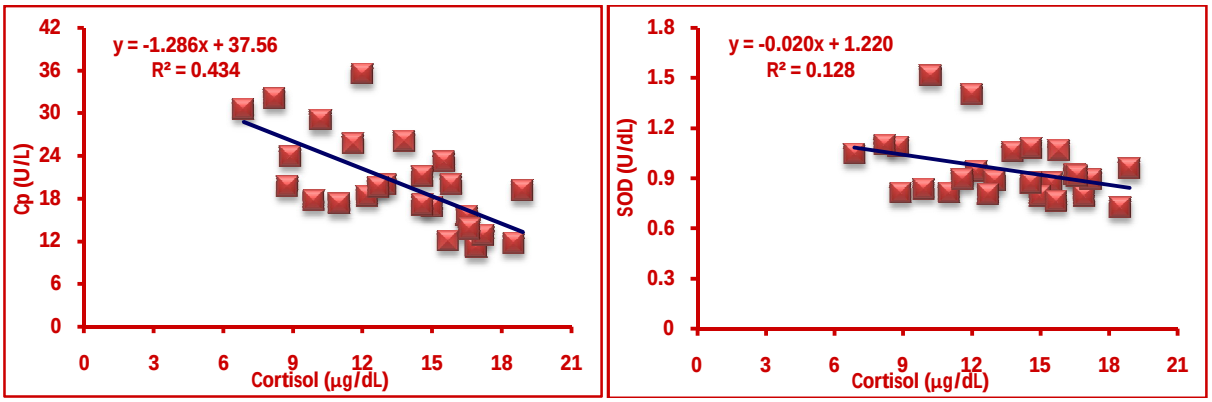
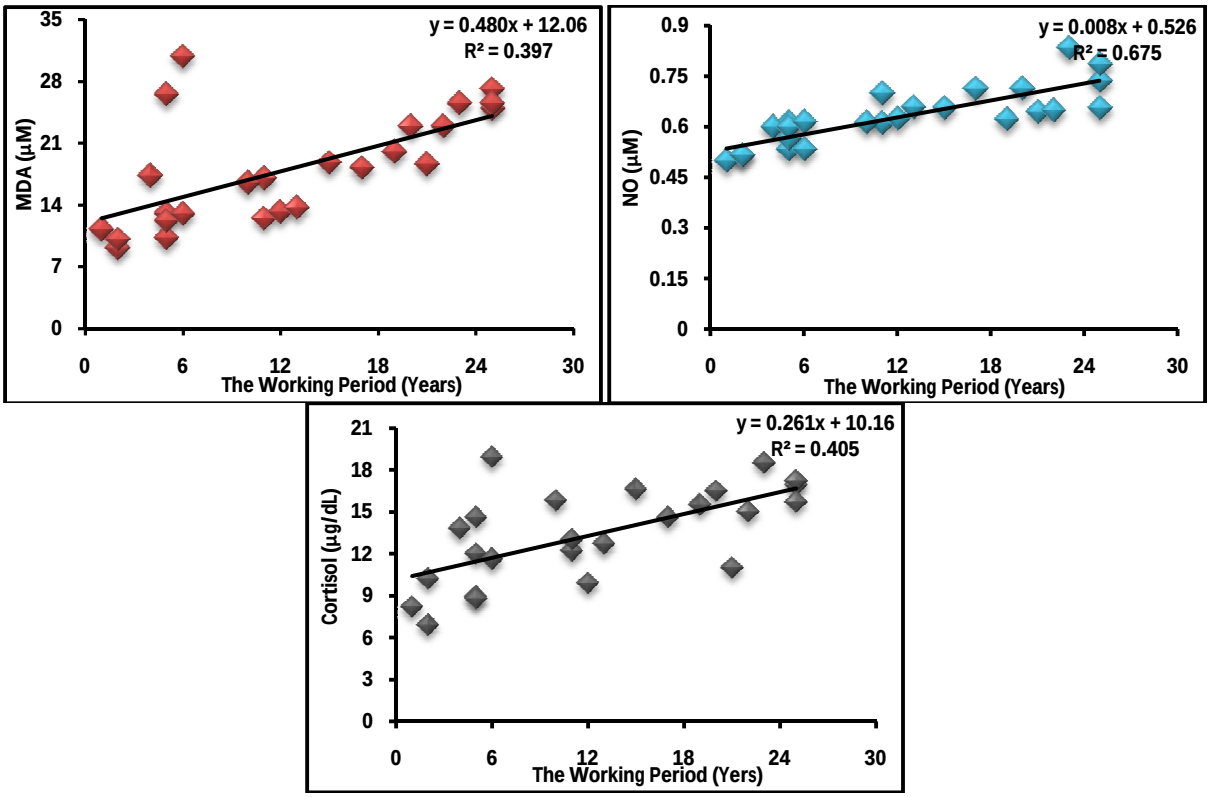


Fig. 3: Relation of Serum Oxidation (A: MDA, B: NO) and Antioxidation (C:Cp, D: SOD)Parameters to Cortisol levels

Primarily, the present work was designed to explore the effect of radiation incurrence on the oxidation, antioxidation and cortisol parameters, to prove this hypothesis, the relationships of the tested parameters to the period of working was carried out. As shown in figure 4A, 4 B, and4C, positive correlations were recorded ($r = 0.630$ at $p< 0.005$, $r = 0.822$ at $p< 0.001$, and $r = 0.637$ at $p<0.005$) MDA, NO, and Cortisol levels respectively to the period of working. According to the same reference, the relationship of the work period to the antioxidation parameters (Cp and SOD activities) was examined, significant negative correlations were observed in relations of Cp activity ($r = -0.820$ at $p< 0.001$) and SOD activity ($r = -0.677$ at $p< 0.01$) to the period of working; respectively (data were summarized in figure 4D and 4E). Focused look on the outresults of the current data illustrates the period of working in the radiation field was the most effectiveness on the NO levels among the tested parameters followed by Cp activity, as shown in figure 4B and 4D.



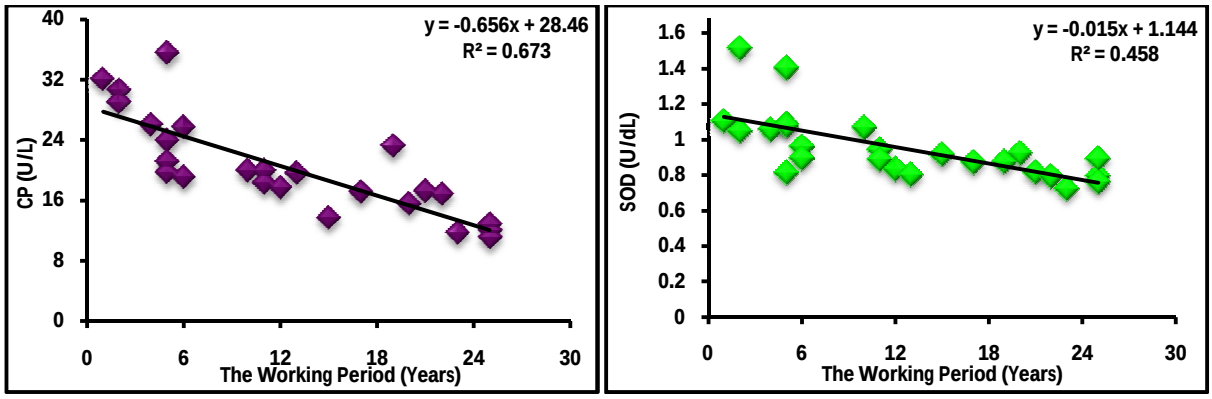


Fig. 4: Relation of Oxidation and Antioxidation Parameters and Cortisol to the Period of Working.

Generally, the stress causes an increase in glucocorticoid levels, through the hypothesis mechanism: during stress exerts a stimulatory influence at the hypothalamic level, by increasing the corticotropin releasing factor (CRF) mRNA levels in the paraventricular nucleus of the hypothalamus. Increased CRF release contributes to the dysregulation of corticosterone secretion [17]. Chronic stress also causes an increase in the ACTH secreting mechanism and hence an increase in cortisol levels. Although it is expected that corticosteroid feedback reduces CRF mRNA levels have opined that this occurs in a dose dependent manner, that both physical and psychological stressors produce a robust and readily reproducible increase in CRF mRNA and that these responses cannot be prevented by changes in circulating corticosteroids. It can be contended that chronic restraint stress in this study activated the hypothalamic, pituitary and anderal (HPA) axis, thus resulting in increased cortisol levels and possibly by above mentioned mechanisms. In the same time, high levels of glucocorticoid hormones are known to decrease blood reduced antioxidant agents like glutathione and superoxide dismutase activity [18-20 and 9]. Abundant literatures recorded that chronic stress causes a decrease in the activities of both manganese SOD and Cu, Zn-SOD isoforms in various tissues. MDA and NO are products of ROS mediated damage to the polyunsaturated fatty acids, ROS are known to cause oxidative damage to macromolecules. Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as prooxidants. On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation or opposing their actions are called as antioxidants[21-25]. Some of the important antioxidants include SOD, Cp, in addition to Glutathione-SH and glutathione peroxidase. In a normal cell, there is an appropriate prooxidant:antioxidant balance. However, this balance can be shifted towards the prooxidant when production of ROS is increased or when levels of antioxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged. It is well known that Cu, Zn-SOD is inactivated by free radical fragments [5]. Thus, it can be said that inefficient removal of active oxygen species in workers sera samples due to decreased of reduced species especially GSH level may be causing inactivation of SOD.

APPLICATIONS

The present research, levels of oxidative stress parameters suggest that continued exposure to low dose of ionizing radiation during working in the diagnosis radiation fields may induce a higher level of oxidative stress.

CONCLUSIONS

Serum cortisol levels were a complementary and incremental ionizing pollution risk predictor in radiographic field workers, and best tool to diagnose over radiation dose event prediction based on cortisol levels was influenced by oxidative stress.

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