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RESEARCH PAPER

Evaluation of Peroxynitrite and Some Chemical Parameters in Sera of Hospital Cleaning Staffs

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A B S T R A C T:

The long periods of working hours at the hospital, and exposure to residues of harmful substances such as anticancer drugs and others lead to excessive formation of free radicals in the body. The aim of this study was to evaluate peroxynitrite, folic acid, homocysteine, ceruloplasmin, and copper in hospital cleaning worker staff (janitors) exposed to residue of different types of substances such as patients' blood, urine, and tools, as well as anticancer drugs in the work setting. This study was conducted among 40 subjects. Twenty subjects (from hospital cleaning staff) were considered hospital cleaning worker staff subjects, twenty other subjects were included as a healthy control group. The mean levels of peroxynitrite, folic acid, and ceruloplasmin in the hospital cleaning staff were (90.80± 23.93, 3.305±1.388, and 269.9±71.86) and in a healthy control group were (19.7±6.551, 6.232±1.738, and 129.88±37.98) µmol/L, respectively, which are statistically high significant. In contrast, the mean serum levels of homocysteine in two groups were (10.84±4.695 and 8.21±1.31) µmol/L, respectively, which are statistically significant. The average copper content was also found in both groups, although it was statistically insignificant and measured (129.34±.26.51) µg/dL in the hospital cleaning staff group and (133.91±11.25) µg/dL in the control group. These findings suggest that long-term exposure to residues of various types of substances in the work setting may cause an induced oxidative stress that results in a number of diseases including cancer.

KEY WORDS: Peroxynitrite, Folic acid, Homocysteine, Ceruloplasmin, and Copper.
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1. INTRODUCTION

The usage of anticancer drugs or chemotherapy agents has been growing due to the increase in cancer cases around the world. Anticancer medications are extremely hazardous to patients and exposed personnel due to their high toxicity. In addition, workers exposure to the patient's blood, urine, and tool residue left behind patients in the laboratory causes a variety of illnesses. The imbalance between oxidative stress and antioxidant levels in the body leads to elevation of free radicals and (Zhang *et al.*, 2022, Kadhim *et al.*, 2023).

One of the important biomarkers of oxidative stress is peroxynitrite (PN). PN, a powerful oxidant radical that is produced when superoxide anion radicals ($O_2^{\bullet-}$) and nitric oxide (NO) quickly interact together without the use of catalysts in biological systems (Guo *et al.*, 2021, Goswami *et al.*, 2022). Then PN can interact with all biomolecules, including proteins, lipids and others, this could be the reason of dysfunctions of the biomolecules (Ahmad *et al.*, 2019). Nitrotyrosine is produced in the tissue as a result of increased PN synthesis, and its presence indicates that proteins have been damaged (Mahdavinia *et al.*, 2021). Increased production of PN causes a number of metabolic diseases such as Alzheimer's disease, multiple sclerosis,

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cancer, septic stroke, diabetes, dementia, atherosclerosis, and more (Zeng *et al.*, 2023).

Folic acid (FA) or vitamin B9 and its derivatives, generally referred to as folates, are essential micronutrients with significant chemoprotective properties. They belong to the B vitamin complex and serve as one-carbon transporters for the transfer of methyl groups during cellular one-carbon metabolism. The metabolically active form of folic acid is tetrahydrofolate (THF), which may be converted into other structurally similar molecules with different functions that work together to generate a complex network of enzyme activities (Cantarella *et al.*, 2017, Maurya *et al*., 2022). FA plays an important role for cell growth and acts as a component and accelerator for essential biochemical metabolic reactions. FA is also necessary for the formation of amino acids, new blood cells as well as DNA (Medawar *et al*., 2019, Kaldygulova *et al*., 2023).

Homocysteine is a biomarker for all health statuses that can damage blood vessels in a variety of ways (Yang *et al.*, 2023). HCY is an important cofactor in several metabolic pathway (Kositsawat *et al.*, 2023). Increased level of HCY is associated with several diseases such as neurological dysfunction (Lauretta *et al.*, 2022), Parkinson's disease (Sampedro *et al.*, 2022), Alzheimer's disease (Song *et al.*, 2023), dementia (Futschek *et al.*, 2023), and thrombotic diseases (Filip *et al.*, 2022).

Ceruloplasmin (CP) is responsible for 90% of copper transport. CP is an acute response protein with anti-inflammatory properties, and its levels rise in the presence of inflammatory diseases (Xia *et al.*, 2023).

Copper is hypothesized to help maintain immune system functions and plays a role in the elimination of viruses (Pvsn *et al.*, 2023). The deficiency of Cu show an excess susceptibility to infections due to decreased number and function of immune cells including B cells, T cells, macrophages, and neutrophils killer cells in the body. The overload of Cu may catalyze the generation of ROS, resulting in oxidative injury to proteins, lipids, and other molecules (Xu *et al.*, 2022). Therefore, the deficiency or overload in the levels of Cu were associated with many diseases such as anemia, cancer, and tumor development (Ruiz *et al.*, 2021). This study investigates the serum levels of PN, FA, HCY, CP, and Cu, as well as their correlation in the blood of hospital workers who are responsible for cleaning and waste disposal and deal with the residue of different types of substances, particularly anticancer drugs in the work environment.

2. MATERIALS AND METHODS

This study was carried out at Nanakali Hospital in Erbil City/Iraq. The study's subjects were split into 2 groups: Group one (G1) included (n=20) blood samples of normal healthy subjects and Group two included (n=20) blood samples of workers in charge of cleaning and waste disposal in the hospital. About 5 ml of blood samples were collected from 40 individuals of an average age (20-35) years. To separate the serum from the blood, blood samples were centrifuged at 4000 rpm for 15 minutes. Then, the serum was kept in a chiller at (-30^0 C) until analysis. The levels of all biochemical parameters PN, FA, HCY, CP, and Cu were determined from storage samples.

2.1 Evaluation of biochemical parameters

Peroxynitrite determination: The estimation of peroxynitrite radical depended upon the nitration of phenol through PN radical, which leads to the creation of nitrophenol that is absorbed spectrophotometrically at 412 nm (Humaish, 2016).

Folic acid determination: The level of folic acid was assessed by employing a Roche/Hitachi fully automated immunoassay analyzer (Elecsys and Cobase analyzers (Cobas e 411), Germany) and the Roche diagnostics, GmbH REF kit (07559992 190) (Nazki *et al.*, 2014, Mailankody and Landgren, 2016).

Homocysteine and ceruloplasmin determinations: The serum levels of homocysteine and ceruloplasmin were examined by using ELISA Kit (SUN LONG Biotech Co., LTD, China) and Elisa (Biotek, USA).

Copper determination: The serum copper was estimated by using the quantitative colorimetric approach. The copper kit (LTA s.r.l., Italy), and REF kit (CC02150) for the UV-Vis spectrophotometer model 752 (China) was used (Tariq and Mohammed, 2010, Ahmed and Faqi, 2016).

2.2. Statistical Analysis

For the statistical analysis, Graph Pad-Prism (version 9) and Microsoft Excel 2016 were used. The data were presented using the mean, standard error, and probability (P-value). All tests were considered statistically significant if the P value was 0.05 (two-sided) or less.

3. RESULTS AND DISCUSSION

The serum levels of peroxynitrite, folic acid, and homocysteine in two studied groups are illustrated in table 1, and figure 1. The result of table (1) showed that the mean of serum PN levels of G1 was (19.7± 6.551 µmol/L), for G2 was (90.80± 23.93 µmol/L) which was statistically highly significant.

Table 1: The serum mean levels of peroxynitrite, folic acid, and homocysteine in healthy control and hospital cleaning staffs.

Groups	PN (µmol/L) Mean±S.D	P- value	Folic acid (µmol/L) Mean±S.D	P- value	HCY (µmol/L) Mean±S.D	P- value
G1	19.7±6.551		6.232±1.738		8.21±1.31	
G2	90.80± 23.93	<0.0001	3.305±1.388	<0.0001	10.84±4.695	<0.05

G1: healthy control group G2: Hospital cleaning staff group
P- value: probability between G1 with G2.

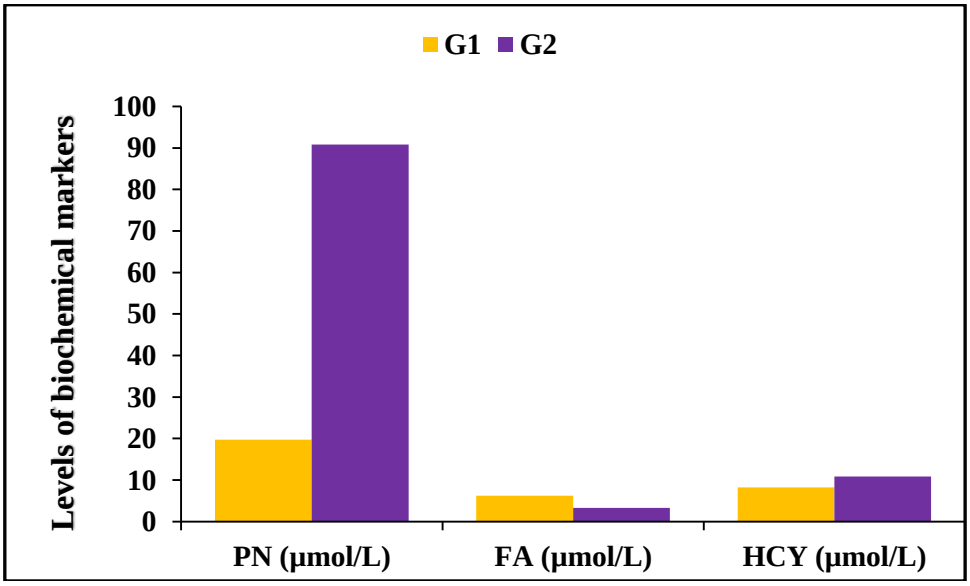

Figure 1: Peroxynitrite, folic acid, and homocysteine concentrations (µmol/L) in two studied groups

There is no doubt that the hospital workers whose work in charge of cleaning and waste disposal in the laboratory and other part departments need to extend their knowledge about the risks of the deal with patients and residues of harmful substances such as anticancer drugs. The long periods of working hours in the hospital, exposure to stressful conditions such as occupational stress, and occupational exposure to residues of harmful substances by touching such as anticancer drug causes to excessive production of free radicals (FRs) or reactive species and lead

to oxidative stress (OS) that contributes to a number of diseases, including liver damage, cardiovascular abnormalities, and neurodegenerative disorders (Sebastià *et al.*, 2020). FRs are produced in plasma membranes, cytosol, lysosomes, mitochondria, and peroxisomes under pathological and physiological conditions (Salem and Ebrahem, 2018). The workplace, and work stress were prevalent among the studied hospital cleaning worker staff as revealed by the high levels of oxidative stress including peroxynitrites (PN). In our study, we analyzed the levels of PN in serum and show high levels in hospital cleaning worker staff when compared to healthy control groups, this is due to the fact that the prolonged occupational exposure and the contamination of cleaning worker staff to a different type of substance residues via their work leads to excessive generation of FRs and accumulates in their body. Also, the high levels of PN due to deficiency of folic acid, this is because FA has been shown to control lipid metabolism and OS, it neutralizes ROS and reduces the activity of enzymes that produce ROS. PN stimulates the activity of antioxidant enzymes such as superoxide dismutase, catalase, and others. It is a powerful scavenger of FRs and has beneficial effects to ameliorate OS status (Asbaghi *et al.,* 2021, Li *et al.,* 2022).

The serum mean level of folic acid for G1 was (6.232±1.738 µmol/L), for G2 was (3.305±1.388 µmol/L), and the serum mean level of homocysteine for G1 was (8.21±1.31 µmol/L), and G2 was (10.84±4.695 µmol/L) which are statistically significant.

Folic acid acts as coenzymes in many metabolic reactions and the deficiency of this vitamin is linked to an increased risk of cancer, Alzheimer's disease, and cardiovascular diseases (Ringling and Rychlik, 2017, Kaldygulova *et al.*, 2023). Particularly, The deficiency of folates can result to the formation of chromosomal abnormalities, which are thought to be a hallmark in leukemia and cancer (Cantarella *et al.,* 2017). Our findings showed that folic acid levels were decreased in hospital working staff this may be in addition to other factors, due to a lack of this vitamin in the diet or poor diet for a long time food.

In a healthy individuals, the level of homocysteine in their blood is low, but the high levels of HCY are the result of impaired metabolism of HCY and now it's thought to be a risk factor for many diseases (Muzurović et al., 2021, Nadir and Ali, 2022, St-Martin et al., 2023). The results of our study show an increased level of HCY due to deficiency of folic acid, and B_{12} (Futschek *et al.,* 2023) because the metabolism process of HCY depends partially on FA and vit. B12 (Sharaf *et al.,* 2022) Also the high level of HCY is due to the increase production of FRs including PN, and this active molecule leads to OS because there is a direct proportion between FRs and HCY. Or the high levels of HCY may be due to oxidative damage that is linked to the oxidation process of the free thiol group of HCY when it binds with different proteins (Moretti *et al.,* 2021).

Table 2, figure 2, and figure 3 show the level of ceruloplasmin and copper in hospital cleaning staff and control groups. The data of serum mean level of CP for G1 was (129.88±37.98 pg/ml), and for G2 was (269.9±71.86 pg/ml), while, the serum mean level of Cu for G1 was (133.91± 11.25 µg/dL), and for G2 was (129.34±26.51 µg/dL). In this study, we observed that there was statistically highly significant increase of CP in hospital cleaning staff group than healthy control group, and statistically non-significant decrease of Cu was found.

Table 2: Ceruloplasmin level (pg/ml), and Copper (µg /dL) of healthy control and hospital cleaning staff groups.

Groups	CP (pg/ml) mean± S. D	P- value	Cu (µg/dL) Mean±S.D	P- value
G1	129.88±37.98		133.91±11.25	
G2	269.9±71.86	<0.005	129.34±.26.51	0.2007

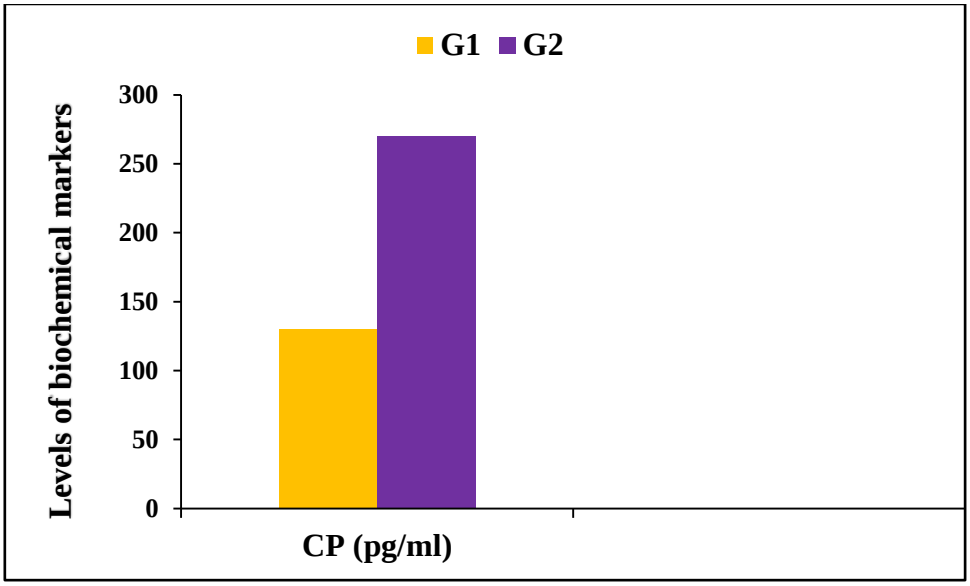

Figure 2: Ceruloplasmin level (pg/ml) of healthy control and hospital cleaning staff groups.

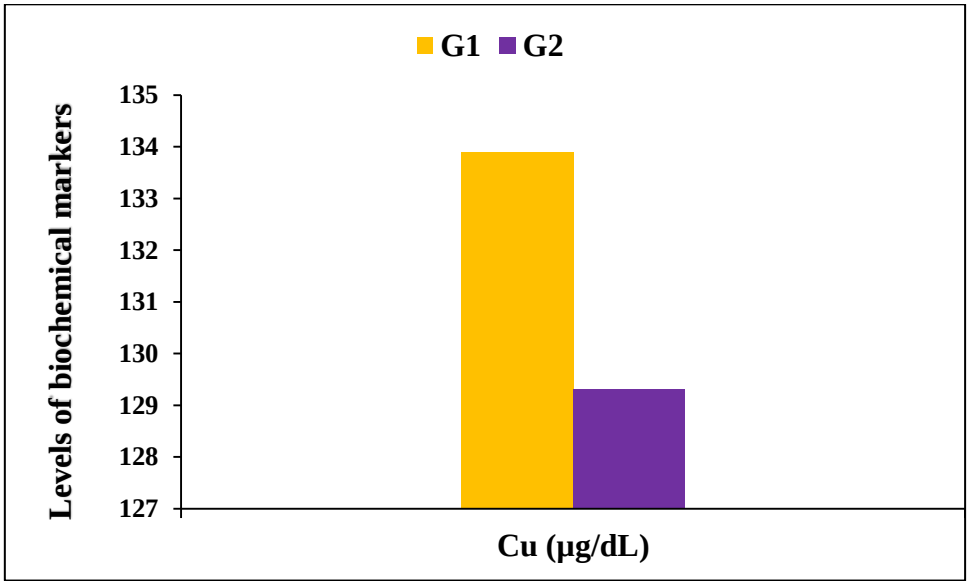

Figure 3: Copper (µg/dL) levels of healthy control and hospital cleaning staff groups.

Ceruloplasmin is a copper carrier glycol-protein present in the plasma (Saed and Ali, 2017). The antioxidant mechanism of CP is the activity of cuprous oxidase, ferrous oxidase, and glutathione peroxidase and its ability to scavenge reactive oxygen species (Liu *et al.*, 2022). CP is important in the control of lipid oxidation in the membrane and is a major defense against ROS in the cells and erythrocytes, and degrades exogenous hydrogen peroxide H_2O_2. The results of this study found high levels of CP in G2 when compared to G1, and this is because CP is considered a protective antioxidant due to its ability to react and scavenge FRs and toxic oxygen species such as PN (Abdel Qawy *et al.*, 2019), so the high generation of these toxic species leads to increase the levels of CPs.

Copper is an important trace elements for human health and its essential for all living cells involved in many biochemical cellular activities (Fan *et al.*, 2022). Cu is a cofactor for many enzymatic antioxidants such as ceruloplasmin (Saed and Ali, 2022, Huang et al., 2023). In our study, the serum concentrations of Cu show non-significant differences between hospital cleaning staff and healthy individual groups this is because this element is widely available in most foods such as legumes, seeds, nuts, whole grains, eggs, meat, so, the level of this element is within normal range (Strand *et al.*, 2017).

Table 3, and figure 4, 5, 6 and 7 show the correlation analysis of PN with FA, HCY, CP, and Cu for hospital cleaning staff. In figure (4), there is statistically significant negative correlation relation between PN level FA (r=-0.4032). While, in figure (5, and 6), there are statistically significant positive correlations between PN with HCY (r=0.4654), and PN with CP (r=0.4566). Figure 7 shows a non-significant negative correlation between PN and Cu (r=0.1972). Significant correlation for PN with HCY, FA, and CP among hospital working staff indicates that increased levels of serum PN may lead to the overproduction of HCY, and CP and eventually lead to hyperhomocysteinemia, and oxidative stress in hospital working staff.

Figures (8, 9. 10, 11 and 12) display the ROC curve (receiver operating characteristic curve) of peroxynitrite, folic acid, homocysteine, ceruloplasmin, and copper. Based on ROC curve analysis, a relatively high AUC (area under the curve) suggests that testing for PN, FA, and CP could help to detect a number of diseases including cancers.

Table 3: Correlation relations between PN and FA, HCY, CP, Cu for hospital cleaning staff's group.

Parameter	Pearson correlation (r)	*p*-value
PN and FA	-0.4032	0.0099
PN and HCY	0.4654	0.0025
PN and CP	0.4566	0.0031
PN and Cu	-0.1972	0.2227

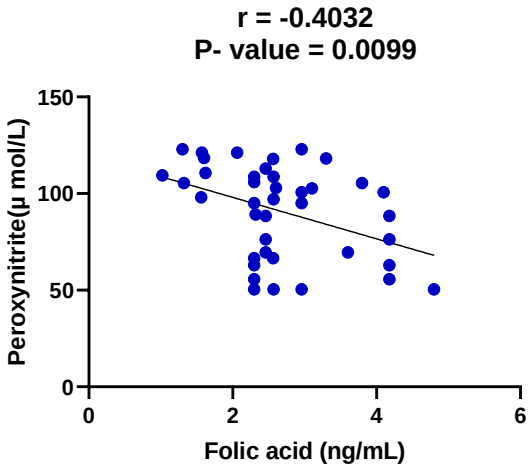

Figure 4: Correlation between PN and FA in hospital cleaning staff's group.

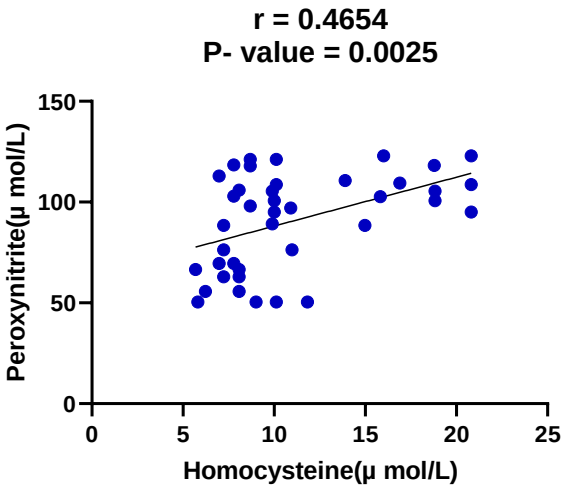

Figure 5: Correlation between PN and HCY in a hospital cleaning staff's group.

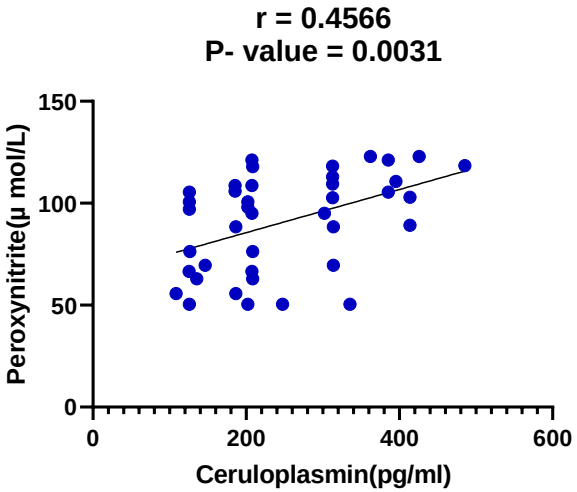

Figure 6: Correlation between PN and CP in a hospital cleaning staff's group.

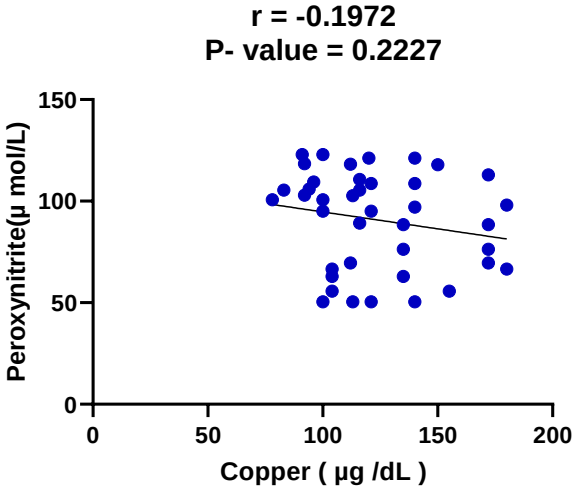

Figure 7: Correlation between PN and Cu in a hospital cleaning staff's group.

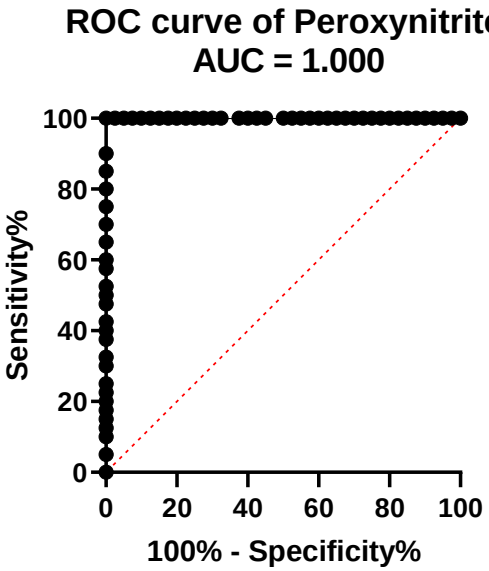

Figure 8: ROC curve illustrates the sensitivity and specificity of Peroxynitrite for the hospital cleaning staff's group.

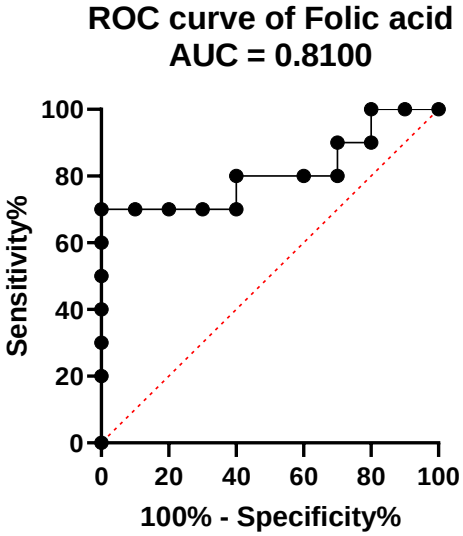

Figure 9: ROC curve illustrates the sensitivity and specificity of Folic acid for hospital cleaning staff's group.

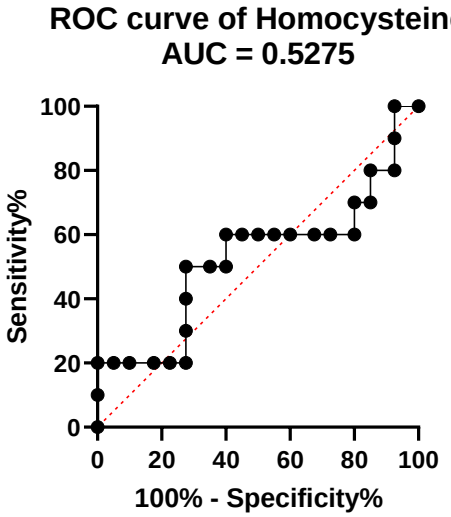

Figure 10: ROC curve illustrates the sensitivity and specificity of Homocysteine for the hospital cleaning staff's group.

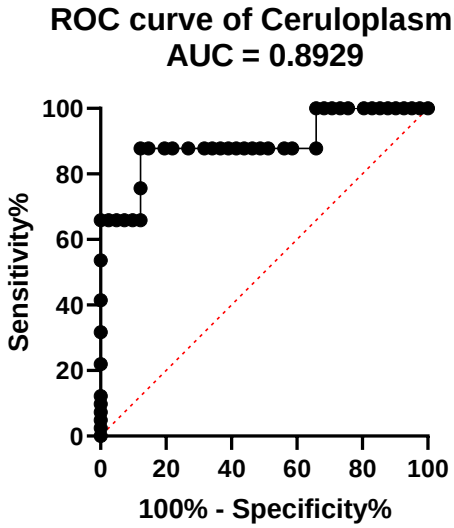

Figure 11: ROC curve illustrates the sensitivity and specificity of Ceruloplasmin for the hospital cleaning staff's group.

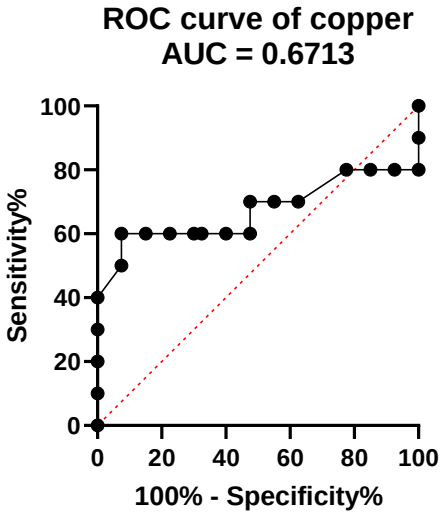

Figure 12: ROC curve illustrates the sensitivity and specificity of copper for the hospital cleaning staff's group.

4. CONCLUSIONS

The present results indicate that long-term exposure to residues of different types of substances in the work setting, could result in an induced oxidative stress such as peroxynitrites that leads to many diseases including cancer. Our recommends undertaking additional research on hospital cleaning staff biological surveillance by assessing drugs, their metabolites, and impacts of exposure. Therefore, we propose that PN, FA, HCY, and CP level measurements can be employed as routine investigations for diagnosing and assessing illnesses.

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