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وحماية المستهلك

IMPACT OF AIR POLLUTION ON SERUM AND SALIVA NITRIC OXIDE, PEROXY NITRITE AND ISCHEMIA MODIFIED ALBUMIN IN THE WORKERS EXPOSED TO HEAVY FUEL OIL COMBUSTION'S FUMES

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ABSTRACT

The object of this study was to measure the effect of air pollution on the health of the workers exposed to the heavy fuel oil (HFO) combustion fumes. This was achieved by testing the alteration in the levels of saliva and serum nitric oxide (NO), peroxynitrite ($ONOO^-$) and ischemia modified albumin (IMA). The participants enrolled in this study were male workers (N= 59) at heavy fuel oil combustion unit in Dora station of electricity in Iraq. Healthy individuals with matched age and body mass index (N=53) were also enrolled in the study to be used as a control group. The results detected a significant increase in the level of serum nitric oxide (NO) and peroxy nitrite ($ONOO^-$) ($p<0.005$), meanwhile a non- significant alteration of these levels was found in the saliva ($p>0.005$) of the workers group when compared with that of control group. The measured parameters also included measurement of serum and salivary level of ischemic modified albumin (IMA), which showed a highly significant increases ($p<0.001$) in both types of the tested fluids. The influence of different factors such as the age of the workers, mode of working, smoking habit and using the safety equipment on the levels of the measured biochemical parameters were also checked in this study.

Keywords: Nitric Oxide, Peroxynitrite, Ischemia-modified albumin, Air pollution, Heavy fuel oil combustion.

تأثير تلوث الهواء على اوكسيد النتروجين والبروكسي نتريت والالبومين المعدل بنقص التروية في مصل الدم ولعاب العاملين المعرضين لأبخرة احتراق زيت الوقود الثقيل

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الخلاصة

كان الهدف من هذه الدراسة قياس تأثير تلوث الهواء على صحة العاملين المعرضين للبخرة المنبعثة من احتراق زيت الوقود الثقيل. تم ذلك عن طريق فحص التغيرات في مستويات كل من اوكسيد النتروجين والبروكسي نتريت والالبومين المعدل بنقص التروية في مصل الدم و لعاب المشاركين في الدراسة من الذكور العاملين (N= ٥٩) في وحدة احتراق زيت الوقود الثقيل في محطة الدورة الكهربائية. تم تضمين الأفراد الأصحاء (N=٥٣) المتطابقين في العمر ومؤشر كتلة الجسم مع العاملين في الدراسة كمجموعة ضابطة. كشفت النتائج عن زيادة معنوية (P>٠,٠٠٥) في مستوى(NO) و $ONOO^-$) في مصل الدم واللعاب. تم ايضا ضمن القياسات التي اجريت قياس مستوى الالبومين المعدل وكشفت النتائج عن وجود زيادة معنوية عالية (P>٠,٠٠١) في مصل ولعاب العاملين مقارنة مع المجموعة

الضابطة. كذألك تم في هذه الدراسة ايضا فحص تأثير العوامل المختلفة مثل عمر العاملين وطريقة العمل وعادة التدخين واستخدام معدات السلامة على المعايير المقاسة.

الكلمات المفتاحية: أكسيد النيتريك، البيروكسي نتريت، الألبومين المعدل بنقص التروية، تلوث الهواء.

INTRODUCTION

Based on the most recent data from the Environmental Statistics Report, air pollution is viewed as a fundamental environmental health challenge across the world **(Planning, 2020).** Airborne particulates constitute a heterogeneous complex mixture that differ according to their source that varied with time, and the condition of the atmosphere **(Brook *et al.*, 2010)**. A number of factors affect the toxicity of particles, including their size, shape, structure, surface reactivity, solubility and ‘‘leachable’’ components **(Elder *et al.*, 2010)**. The most common used method for characterizing the particulate matter (PM) is dependent on their size. Particulate matter <10 µ*M* (PM_{10}), particulate matter <2.5 µ*M* ($PM_{2.5}$), and those of <0.1 µ*M* ($PM_{0.1,}$ which also called ultrafine particles, UFPs) are the most important sizes-fractions. In Iraq the annual mean concentration of $PM_{2.5}$ is 62 g/m^3, which exceeds the World Health Organization (WHO) Air Quality Guidelines for health–harmful pollution levels of 10 g/m^3. In 2016, about 4.2 million premature deaths worldwide was reported to be resulted from ambient air pollution in both rural areas and cities; this mortality has been reported to be attributed to $PM_{2.5}$ exposure **(WHO Europe, 2016)**. According to the WHO Global Health Estimates database.

Heavy Fuel oil is the oil that make up all the distillation residue, including those obtained by blending of fuel oils. Its kinematic viscosity, at 80 ℃ is above 10 CSt, with a flash point always above 50 ℃ and a density > 0.90 kilogram/liter **(Levy *et al.*, 1971)**. Upon burning of HFO at power plants, it creates emissions of carbon dioxide (CO_2), nitrogen oxides (NOx), sulfur dioxide (SO_2), particulate matter (PM), mercury (Hg), and other pollutants. NO_X and SO_2 emissions involve in the formation of fine PM and ground level ozone. These pollutants emit when the combustion is incomplete. The term complete combustion is applied if all carbon in the fuel burns to carbon dioxide, all hydrogen burns to water and all sulfur (if any) burns to sulfur dioxide. Conversely, combustion is incomplete if the products of combustion contain components such as H, CO, C& OH, or any unburned fuel and the main reason for such incomplete combustion is the presence of insufficient oxygen **(Cato *et al.*, 1997).** Exposure to air pollution were found by many researchers such as (**Cohen *et al*., 2017**) to have adverse health effects and air pollution has been documented to be linked to many types of the illnesses, including cardiovascular disease, respiratory disorders, impaired lung function, renal disease, preterm birth, dementia, autism, male infertility and overall mortality. **(Schraufnagel *et al.*, 2019: Hassani *et al*., 2016).**

Nitric oxide (NO) is an important molecule, in mammals it involves in many physiological and pathological processes. It can be protective, or hazardous for tissues or organs in where it exists (**Zetterquist *et al.*, 1999)**. In human body, it may be produced from both dietary substances and metabolic pathways, followed by its transport via the blood to the salivary glands **(HFO, 2016)**. Furthermore, its harmful effect in the human body is due to its production of peroxynitrite (the ugly free radical) upon its reaction with superoxide **(Zelnickova *et al.*, 2008).** The generation of peroxynitrite, in a moderate flux, over long

periods of time lead to substantial oxidation and potential destruction of the different host cellular constituents, that results in dysfunction of critical cellular processes, cell signaling pathways' disruption, and critical cellular processes' dysfunction, then, through both apoptosis and necrosis, induction of cell death **(Radi *et al.*, 2013)**. Several studies dealing with different types of air pollution measured the levels of blood (NO and $ONOO^-$) in their studied participants (**Sandrah *et al.*, 2015).**

Ischemia-modified albumin (IMA) is a protein formed due to damage of blood albumin final amino acid terminal as a result of presence of free radicals, which damage albumin and reduces its capacity to bind to heavy transition metals (nickel, cobalt) IMA forms about 1%–2% of total serum albumin levels **(Kaefer *et al.*, 2010).** Though IMA is an ischemia-sensitive marker, recent studies have shown that it increases in situations such as late stage renal failure and obesity and diseases such as diabetes and chronic liver disease, hypercholesterolemia, cancer and multiple myeloma **(Ellidag *et al.*, 2013).**

Generally, nowadays, saliva has been reported to be suitable for diagnosis of many local and systemic diseases. It generating considerable interest in term of its economic, easy noninvasive sampling method that needs no experience, or special equipments to be collected compared with that required for the blood collection **(Pink *et al.*, 2009)**. It is worth to mention, no literatures have been found that dealing with the study the variation of the present study measured biochemical parameters in saliva.

In a previous study carried out in our laboratory, oxidative stress was reported to be present among the workers at the HFO combustion unit **(Ahmed & Hasan, 2022)**. The main objective of the current study was to check the impact of the fumes emitted from HFO combustion, at molecular level, on the health of the workers in the electricity Dora station/ Baghdad/ Iraq , by measuring the variations in the level of serum and saliva of some related oxidative stress parameters including the good free radical: nitric oxide (NO), the ugly free radical: peroxy nitrite $(ONOO)^-$ and ischemia modified albumin (IMA), of the workers at the combustion unit of heavy fuel oil in this station.

PARTICIPANTS AND METHODS

Study participants, Ethical approval, **Exclusion criteria**, **Serum and saliva sampling*:***
All details concentering the participants of the present work were the same as mentioned in our previous publication (**Ahmed & Hasan, 2023**).

Participants and methods

Study participants:

This study participants were males (N= 59) who are working at the HFO combustion unit in the electricity Dora station/ Baghdad/ Iraq ,as well as age, gender and body mass index (BMI) matched apparently control healthy individuals (N=53). Biological samples were collected during the period of December 2021 to February 2202.

Ethical approval

The ethics Committee of the College of Science/ University of Baghdad had approved this study protocol.

Exclusion criteria

All workers and healthy individuals who were alcohol drinker, and those presented acute or other chronic diseases such as high pressure, diabetic, cardiac disease, cancer or any immune dysfunction were excluded from the study.

Serum and saliva sampling: -

The initial number of the workers from whom the saliva and serum specimens were collected was 83. Twenty-four of them were excluded. Therefore, the final participants of the present study were 59. In order to perform the required measurements of this study, a volume of 5ml of venous blood was collected from each healthy individual and worker and left for ten-twenty minute at the room temperature, then was centrifuged at 2000xg for 10 minutes, and any hemolyzed sample was discarded. The obtained sera were transferred immediately to test tubes and frozen at – 20 °C for subsequent analysis. Meantime the workers and healthy individuals were asked to rinse their mouths with saline before a volume of about 2 ml of unstimulated whole saliva was collected. The collection time was always between 8.0-10.0 a.m. and the collection period was approximately twenty minutes. The collected saliva was centrifuged at 2000xg for 10 minutes, this was done within one hour after collection to eliminate debris and cellular matter. The resulting supernatant was stored at – 20 °C until used for different parameters assays.

Measurement of the **concentration of nitric oxide**

Method (**Jose *et al.*, 1998**) was used to measure the nitric oxide concentration. This method is based on that cadmium reduces nitrate to nitrite and then the produced nitrite level was determined using Griess reaction. The azo dye formation was detected via its absorbance at the wavelength =540 nm. In order to construct the standard curve, the following different concentrations (0, 50, 100, 150, 200, and 250µM) of sodium nitrite (NaNO2) were prepared from the stock $NaNO_2$ solution and were used instead of the tested samples. The nitric oxide concentration in the serum and saliva samples was calculated using the equation derived from this constructed standard curve.

Measurement of the concentration of peroxynitrite

The [Peroxynitrite] was determined by using to **(Vanuffelen *et al.*, 1998)** procedure. In which the nitration of phenol that is mediated by peroxynitrite results in the formation of nitrophenol, which its absorbance (A) can be measured at λ=412 nm.

Calculation

$$\textbf{Peroxynitrite concentration (mM)} = \frac{\textbf{A test – A blank}}{\textbf{Molar extinction coefficient}(\boldsymbol{\varepsilon})} \times 10^3$$

Where:

A test was referred to the absorbance of the test, **A blank** was referred to the absorbance of blank solution

ε was referred to the nitrophenol molar extinction coefficient which is equal to 4400 M^{-1} cm^{1}**.**

Measurement of the concentration of ischemia modified albumin

Ishemic modified albumin [IMA] was measured using an indirect measurement method (Gurumurthy et al 2014). By using this method, the unbound cobalt to albumin is measured**.**

RESULTS AND DISUSSION

The results of this work was expressed in the form of mean value (M) ± the standard deviation (S.D.). The data were compared using SPSS version 26 (Independent samples T-Test), where the difference ($P < 0.001$) was considered as highly significant, ($P < 0.05$) significant and ($P > 0.05$) as a nonsignificant.

The general detailed characteristics of the used participants in the current study were illustrated in Figure (1)

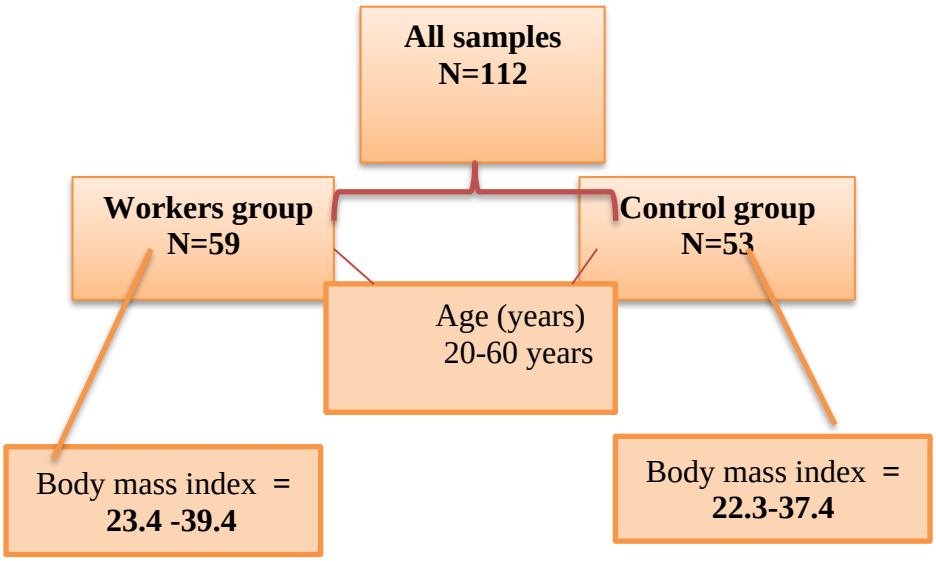


Figure (1): The participant characteristics of the present study.

The above Figure showed that the number of the workers occupationally exposed to the fumes of the fuel's combustion that were enrolled in the present study was 59 and the number of the control group was 53. All of them were males with age ranged between 20-60 years. Their weights and heights were measured, and their body mass index (BMI) was calculated. The workers' group were further divided into sub-groups, according to their ages, the period of the service at the HFO combustion unit and if they were smokers or using safety protection equipment's.

The level of nitric oxide (NO) and peroxy nitrite ($ONOO^-$) were measured in serum and saliva of the exposed workers to the fumes emitted from HFO combustion and control groups and the results were presented in Figure (3).

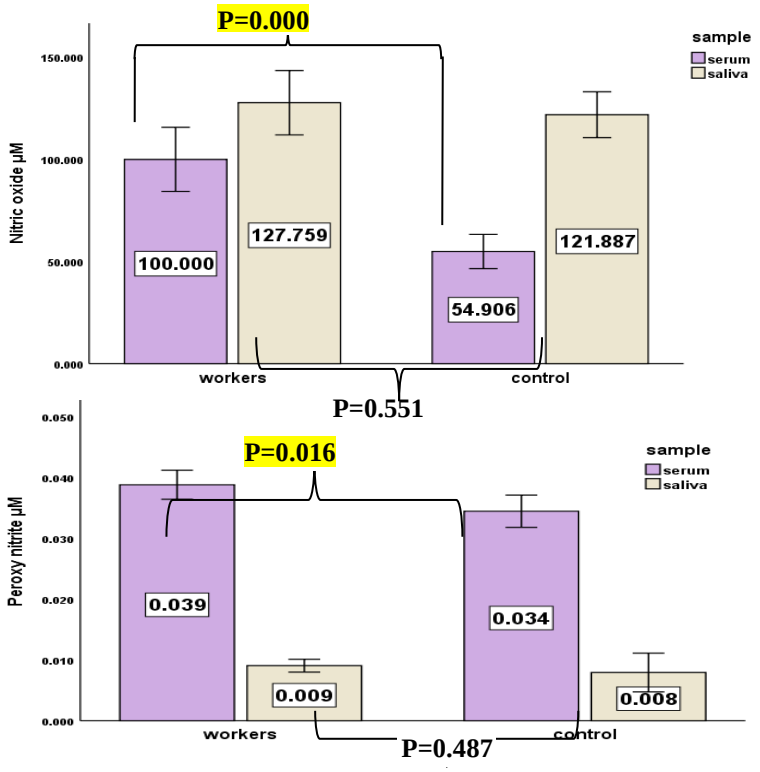


Figure (2): Comparison between levels of nitric oxide, peroxy nitrite in serum and saliva of both the workers and control groups.

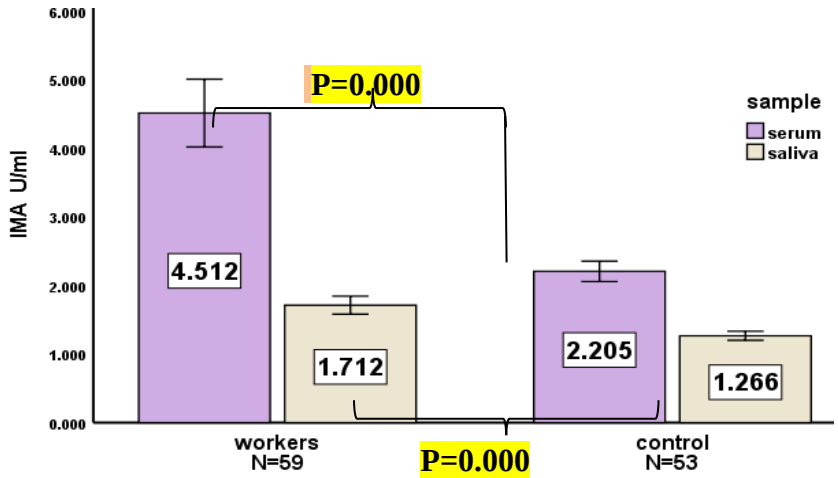
It is clear from the Figure (2) that a highly significant increase in serum level of nitric oxide and peroxynitrite ($p<0.005$) was detected in serum of the workers when compared with that of the control, while a non-significant increase in these levels ($p>0.05$) was measured in the saliva samples of the workers in comparison with that of the control group.

Nitric oxide (NO) is an intercellular messenger, with a short life, that was shown to play important roles in a wide range of biological functions such as many related processes to pathophysiology of pulmonary and cardiovascular systems such as inflammation; host defense, neurotransmission and immune function, (**Bogdan, 2001).** In the lung, neutrophils, macrophages, endothelial cells, epithelial cells, and non-adrenergic noncholinergic neurons are capable of nitric oxide production **(Barnes &Liew, 1995)**. Various isoforms of nitric oxide synthase (NOS) control the endogenous production of this molecule. These isoforms broadly are classified as constitutive and inducible NOS (iNOS). A hypoxia is reported to be a synergistic inducer of iNOS expression that favors the reaction between NO and oxygen, resulting in nitrosative modifications. Therefore, the measured elevation in [nitric oxide] in the workers group (Figure 2) may be a result of cytokines release by inflammatory mediators due to presence of pollution, or may be due to the presence of hypoxia, a condition that was detected to exist in the enrolled workers of the current study (the result not shown), such condition was reported to result in over expression of iNOS synthesis **(Holgate, 2012).** In the current study a significant increase in serum with a non-significant alteration in saliva, was

measured in the [peroxynitrite] of the workers compared to the control group (Figure 2) **(Pryor & Squadrito, 1995).** reported that peroxy nitrite produce upon the reaction of superoxide and nitric oxide, each can modulate the effects of the other and the ratio of their concentrations, is important in systems in which both of them are produced. Superoxide dismutase (SOD) is the usual scavenger of superoxide. This enzyme is a metalo proteins presents in all aerobic metabolizing cells. The reaction of nitric oxide with superoxide anion competes the reaction of scavenging of this ion by SOD, thus the presence of SOD in various compartments of human body enables it to get rid of superoxide radicals immediately as soon as it forms, this protects all the different biomolecules in the cells from its oxidative damage **(Hasan & Rashid, 2004)**. Many studies observed a decrease in this enzyme activity upon exposure to air pollution such as the study by (**Hasan *et al.*, 2001)** who measured a decline in SOD activity and thus suggested that the depression in SOD activity may result in cellular injury by superoxide radical.

This study included the measurement of Ischemia modified albumin (IMA) level. IMA level was reported, would be influenced by the albumin concentration in the used samples (Lee *et al*, 2007), therefore recently another term: (IMA index) albumin-adjusted ischemia-modified albumin index has been introduced as a new marker that compensates the effect of serum albumin and reported to be more accurate and sensitive marker than the conventional IMA value, therefore IMA index was calculated according to the following formula:

Ischemia Index IMA= IMA χ [induvial.[alb.]/median [alb.], furthermore the ratio IMA/Albumin was calculated in the saliva and serum samples of the exposed workers to the fumes emitted from HFO combustion as well as of the control group and the results were presented in Figure (3).



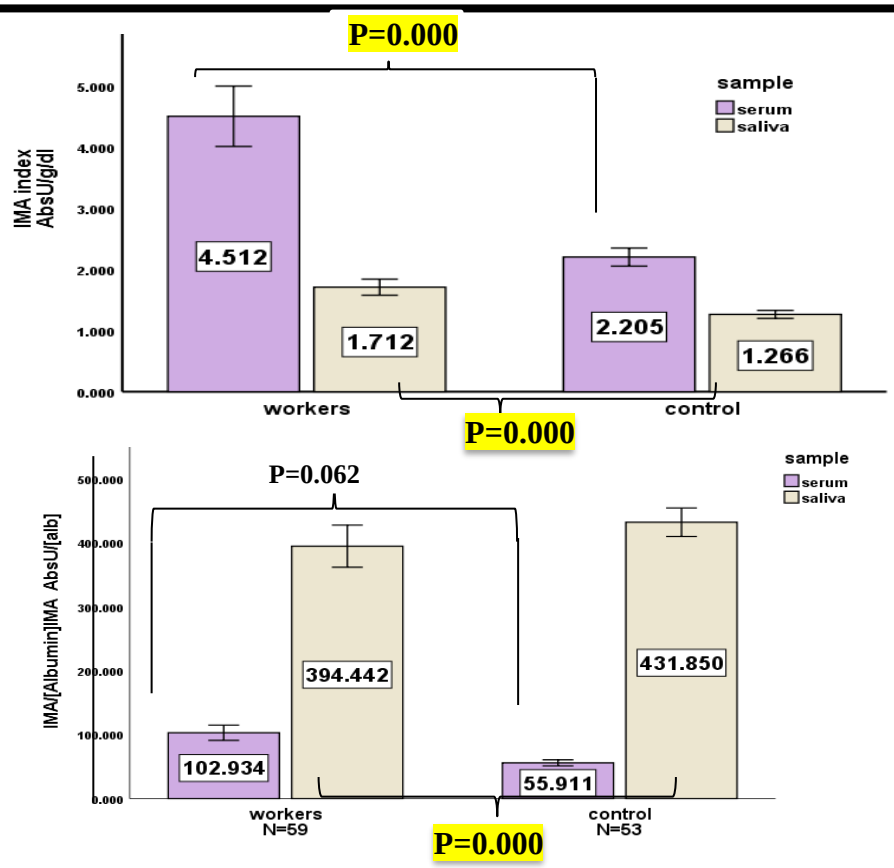


Figure (3): Comparison between levels of Ischemia modified albumin, Ischemia modified albumin index and ratio (IMA/Albumin) in serum and saliva of both the workers and control groups.

It is clear that a high significant increase ($p<0.001$) in IMA, IMA index and the ratio (IMA/Albumin) IMAR was detected in the saliva and serum of the workers group compared with the control group. IMA is a modified form of serum albumin in which its nitrogen-terminal amino acids are modified and become unable to bind to transition metals. IMA is produced as a result of the presence of free radicals where the presence of oxidative stress and acidosis during ischemia are the major determinants of its formation **(Sahin *et al.*, 2018).** The measured increase in [IMA] in the workers group thought to be either due to an increase in its production, or a decrease in its clearance (**Gafsou *et al.*, 2010**). Under normal conditions, the [IMA] was reported to correlate negatively with the [albumin]. The presence of inflammation, hypoxia, the severity and duration of OS with its related oxidative damage, in addition to tissue hypo- perfusion and the accompanying disorders affect the serum level of IMA, all of these has been reported by Sahin *et al* to be involved in the different types of mechanisms that cause an increased [IMA] in serum (**Sahin *et al.*, 2018)**. In a previous study carried on in our laboratory, [albumin] was found to elevate significantly in the same workers group (**Ahmed & Hasan 2023**). This result with the current ones lead us to the suggest that the high OS status and the

hypoxia measured in the workers group are among the causes of the increased [IMA] and its related parameters in the worker included in the present study.

Most individuals who are exposed to harsh working environments are unaware of the impact of long exposure to such environment on their health, therefor in order to study the possible influence of the different time of exposure to pollution on the level nitric oxide and peroxynitrite, The variations in these parameters were checked in saliva and serum of those workers who were working in an alternative mode (seven hours/ day for two successive days, followed by one day off) and those who worked for seven hours/ day for five days weekly. The results were presented in Table: 1. When the influence of the different factors (age, period of working, mode of working, smoking and using safety equipment) were checked on the above observed variations on serum and saliva levels of NO, $ONOO^-$, IMA and its related parameters. The results showed that the only factor that was found to affect these variations was the mode of work (as it was clear from Table 1).These results showed presence of a significant elevation ($p<0.005$) in the level of both (NO) and ($ONOO^-$) in serum of those workers who worked in an alternative mode than those who worked for 7 hours/day for successive 5 days. But such influence was not present, in saliva samples where a non-significant difference was observed between both subgroups. It is worth to mention, that all the above-mentioned studied factors showed no influence on the above variations (the results were not shown).

Table (1): The effect of the working mode on serum and saliva nitric oxide and peroxy nitrite of the workers.

parameters	Mean±SD			
	Serum		Saliva	
	Alternative mode of work (N=24)	Working 7 hours/day for successive 5 days followed by 2 days off. (N=35)	Alternative mode of work (N=24)	Working 7 hours/day for 5 successive days followed by 2 days off. (N=30)
Nitric oxide µM	80.238±31.681	109.428±68.048	130.714±77.302	123.142±48.447
	0.034		0.653	
Peroxy nitrite µM	0.042±0.008	0.036±0.009	0.009±0.004	0.008±0.003
	0.017		0.239	

The non-significant variations that observed in the levels of nitric oxide and peroxy nitrite with the observed alteration in [IMA] may be explained as follows: even though, in the body the saliva is considered as first defense line against active oxygen species (**Zukowski *et al*., 2018**), it seems that the alteration in saliva nitric oxide and peroxynitrite are independent of that of the circulation system, and a different nature of disturbances occur in saliva than that of serum during the exposure to the air pollution.

Inhalation generally was reported to be the main route of exposure to the harmful contaminates present in pollutant air. Previously many studies reported an association between air pollution and chronic and acute respiratory & systematic inflammatory responses **(Odewabi**

***et al.*, 2012)**. How could the measured changes in the present study parameters contribute to the adverse respiratory outcomes associated with HFO fume emissions in the workers group? This may be explained as follows: Lung epithelial dysfunction is considered central to development of many respiratory diseases such as asthma; with insults such as air pollutants serving not simply as triggers for disease exacerbation but also as playing critical roles in the origin and progression of airway and lung pathology **(Fitzpatrick *et al.*, 2012)**. A growing body of literature further implicates impaired antioxidant defenses and disturbances in oxidation/ reduction (redox) balance as risk factors for development of this type of diseases Accumulating *in vitro* and *in vivo* experimental studies have shown that PM exposure is associated with increased lung oxidant burden related to increased ROS such as $O_2^{\bullet-}$ **(Sugimoto *et al.*, 2005).** Other studies of Diesel exhaust particles (DEP):-exposed rodents revealed concomitant increases in NO and ONOO- in BAL fluid cells **(Zhao *et al.*, 2006).**

CONCLUSIONS

The presence of oxidative stress index, which was previously measured in these workers, and the presence of a high concentration of albumin, resulting in an elevation of ischemia modified albumin (IMA) in all its forms, as well as increase in peroxy nitrite($ONOO^-$) as a result of the increase in nitric oxide(NO) and the decrease in superoxide dismutase (SOD) activity, which were recorded in several studies in people exposed to air pollution, this confirm the presence of OSI that was reported to be statistically significant in the working individuals, while it was not significant in their saliva except for IMA and related parameter.

Further studies which included larger samples size are required to further confirm the present study obtained results, especially which obtained with the saliva parameters.

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