How to Cite:
Ahmed, A. M., & Hasan, H. R. (2022). Study the oxidative stress parameters in serum and saliva of the workers at the heavy fuel oil combustion unite. *International Journal of Health Sciences*, *6*(S6), 8104–8117. https://doi.org/10.53730/ijhs.v6nS6.12226

Study the oxidative stress parameters in serum and saliva of the workers at the heavy fuel oil combustion unite

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***Abstract*---**The goal of this study was to investigate the effect of the pollutants emitted from the combustion of heavy fuel oil (HFO) on the workers' health who are working at this unit. This was done by checking the changes in serum and saliva total oxidant status (TOS), total antioxidant capacity (TAC) and the oxidative stress index (OSI).The participants of the study were male workers (N= 83) who are working at the combustion of HFO unit in the electricity Dora station. They were divided into subgroups according to: their age, smoking habit, and the service period. Age and gender matched healthy individuals, (N=53) were also included in the study as a control group. Serum and saliva total oxidant status (TOS) and total antioxidant capacity (TAC) were measured using Erel's methods and OSI was calculated. Serum (TOS) and (OSI) levels were found to be higher (p-value <0.000) in the workers than in controls with a reduced levels of antioxidants (p-value <0.05) in these workers. When the changes in these parameters were examined in the workers group according to their age, significant increase (p-value <0.05) in serum and saliva TOS and OSI compared with that found in the control group, while the measured TAC indicated that there was a reduction in serum (p-value <0.05), but only in saliva in-significant decrease (p-value <0.05) was found. The service period and the mode of working both seemed to have effects on TOS and OSI (p-value <0.05) while there was non-significant decrease (p-value >0.05) in the level of TAC in these workers. When the effect of smoking was checked on TOS, OSI and TAC, the result showed that even-though the level of TOS increased significantly in the smoker workers, this increase was accompanied with the increased in OSI in the serum and this was due to the highly significant variation in the antioxidants level (p= 0.000), While the

situation was different in the saliva where even- though there was a significant increase in TOS level and a significant decrease in the level of TAC, the OSI increased non significantly (p= 0.099).

Keywords---air pollutants, fuel combustion, age, working mode, service period, smoking.

Introduction

Air is an important component of the environment that aids various life processes and the subsistence on earth [1]. Air pollution among the different pollution types is considered to be the most dangerous one and this is because of its effect on the environment and the health of all living organisms including human [1, 2]. It can be defined as the alterations of the atmosphere natural composition as a result of introducing contaminated materials, such as particulate matter, different chemicals, different toxic gases and radioactive materials, into the atmosphere which can cause damage to human health [3,4].These pollutants arise from industrial processes ,unpaved and paved demolition site and building [3,5].Various environmental components including, air, water and soil are damaged by various air in dust real activities [6].

Common toxic gaseous such as carbon monoxide (CO), ammonia (NH_3), ethylbenzene (C6H5C2H5), hydrogen sulfide (H_2S), nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and ozone (O_3), in addition to particulate pollutants present in smoke ,mist ,dust etc., , at high exposure, are very dangerous to human health and are so to other animals as well as to the plants [7,8]. Humans suffer from many air pollution related disease such as anemia, pulmonary tuberculosis long-term lung injury, acute respiratory problems, , birth defects, cancer, neurological and optical damage, risk of stroke, cardiovascular disease, premature death, and morbidity [1]. Recent studies on the impacts of air pollution have confirmed that there is a linear relationship between infection and death rates of patients suffering respiratory-related diseases in highly polluted environments [9, 10] .World health organization (WHO) recorded that about 7 million death/ year worldwide caused from the excessive exposure to air pollution [11].

Heavy Fuel Oil (HFO) also known as a residual fuel oil, or bunker fuel is a category of fuel oils of a tar-like consistency. This type of fuels are blended to achieve certain viscosity and flow characteristics for a given use. The chemical composition of HFO is highly variable and this is due to the fact that HFO is often blended, or mixed with cleaner fuels, using blending streams that can include carbon numbers from C20 - greater than C50 [12]. Since this type of fuel being the final remnant of the petroleum cracking process therefore , it is contaminated with several different compounds including olefins, aromatics, and as well as molecules containing sulfur nitrogen and metals, making emission upon its combustion, more pollutants in comparison to other type of fuel oils. [13].

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals, shift tissue redox homeostasis in different tissue towards pro

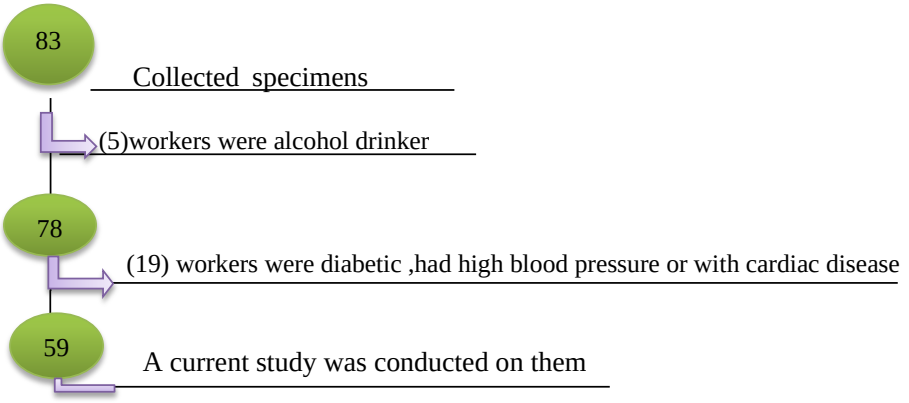
oxidation that lead to the existence of what is known oxidative stress (OSI). Generally it is known that ROS play adaptive roles in cell growth and differentiation, gene regulation, replicative senescence, protection against invading pathogens, cell signaling and regulation of apoptosis and cell survival. The involvement of oxidative stress in disease etiology and pathogenesis is increasingly recognized [14].

Several investigators have demonstrated corresponding patterns of oxidative stress between serum and saliva, suggesting that saliva contains oxidation biomarkers akin to those in blood (15). Meanwhile saliva is considered to be the first body defense fluid of the oral cavity [16]. Many studies carried on in Iraq repented that there is an association between the differents type of pollutions and oxidative stress[17,18]. The aim of the present work is to investigate the impact, at the molecular level, of the emitted pollutants from HFO combustion on the health of the individuals who work at the HFO combustion unit in the electricity Dora station.

Material and Methods

Study participants

This study participants were(N= 83) male workers at the HFO combustion unit in the electricity Dora station ,as well as age, gender and BMI matched apparently healthy individuals (N=53).The period of samples collection were between December 2021 to February 2202. All workers and healthy individuals who were alcohol drinker, and those presented acute or chronic disease such as parasitosis, high pressure, diabetic, cardiac disease, cancer or any immune dysfunction were excluded from the study. The ethics Committee of the College of Science/ University of Baghdad had approved this study protocol. The following scheme illustrated the total collected number of the specimens and the final used one in the present study.



Scheme: Illustrated the number of the specimens breakdown in the present study

Collection of serum and saliva samples :- A volume of ten milliliters of venous blood was collected from each individual and workers and left for ten minute ten at the room temperature for 10 minutes, then was centrifuged at (2000xg) for 10 minutes. The obtained sera were transferred immediately to other test tubes and frozen at – 20 °C for subsequent analysis. Whereas hemolized samples were discarded. Meantime about ten milliliters of unstimulated whole saliva were collected. The workers and healthy individuals were asked to rinse their mouth with saline before the saliva collection into a wide test tube. 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 (2000 xg) 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.

Biochemical measurements
Measurement of total oxidant status [TOS]

Erel's method [19] was followed in order to measure [TOS] in the saliva and serum samples of the participants. The assay was calibrated with hydrogen peroxide standard solution. And the [TOS] in the samples was quantified by using the straight line equation derived from the standard curve, which was obtained by plotting the absorbance of the H2O2 standard solutions at λ=560 nm against the concentration of these standards.

Determination of total antioxidant capacity [TAC]

The total antioxidant status value was determined using Erel's method [20]. The assay was calibrated with a standard solutions of H2O2. In order to construct the standard plot the measured absorbance's at a wave length of λ=444 nm was plotted against the concentrations of the standard solution. The straight line equation derived from this standard curve was used to quantify [TAC] in the studied saliva and serum samples.

Calculation of oxidative stress index [OSI]

In order to calculate the oxidative stress index the following equation was used

OSI (arbitrary unit) = TOS (μmol H2O2/L)/TAC (μmol glutathione/L).

Data analysis

The data throughout this work was reported in the form of (mean value ± the standard deviation).The data were compared by SPSS version 26 (Independent samples T-Test), where the difference was considered as highly significant when ($P < 0.001$), significant when ($P < 0.05$) and nonsignificant when ($P > 0.05$)

Results and Discussion

The general detailed characteristic of the participants in the current study were presented in Table (1).

Table1
The characteristic of the participants of the present study

Characteristic		Total workers	Total control
Number		59	53
Gender		male	Male
Body index		23.4 -39.4	22.3-37.4
Age (year)		20-60	20-60
	20-39 year	60%	51%
	40-60 year	40%	49%
Period of service (year)		1-≤20 years	-------
	1-10 year	49%	
	11-20 year	34%	
	<20 years	17%	
Smoking and Nonsmoking			
	Smokers	51% Smoking and	53% Smoking and
	Non-smokers	49% nonsmoking	47% nonsmoking
Medical History		None	None

As it is obvious from the above table, the final number of workers included in the present study was 59 and the number of the used control group was 53. All of them were males. Their weights and length were measured and their body mass index (BMI) were calculated. Their ages ranged between (20-60) years. The workers group were divided into sub-groups, as shown in the above table according to; their ages, period of the service at the HFO combustion unit and if they were smoker or not. Total Oxidant Status (TOS) and antioxidant statue(TAC) were measured in serum and saliva of all the participants of the present study .Meanwhile the oxidative stress indies (OSI) which can be expressed as ratio of TOS/TAC were calculated and the results were shown in Table (2).

Table 2
Comparison between levels of TOS, TAC and OSI in the serum and saliva of the total number of the workers and control groups

parameters	Mean ±SD			
	Serum		Saliva	
	Total Workers N= 59	Total Control N= 53	Total Workers N= 59	Total Control N= 53
[TOS] (µmol H_2O_2 Eq/L)	420.661±206.785	48.529±21.686	261.644±186.413	100.192±36.420
	P-value = 0.000		P-value = 0.000	
[TAC] (µmol	1.837± 0.268	2.107± 0.160	2.135 ±0 .152	2.102 ±0.109
	P-value = 0.000		P-value = 0.190	

H_2O_2 Eq/L)				
OSI	233.719±129.101	22.963±10.785	130.848±103.768	46.251 ± 19.564
	P-value = 0.000		P-value = 0.000	

Total Oxidant Status (TOS) {referred to a shift developing in an oxidative/anti oxidative ratio in favor of the oxidative side, was measured, meanwhile the most accurate method to express the presence of oxidative stress is known as Oxidative Stress Index (OSI) [21], was calculated in the collected serum and saliva samples As it was clear that there is a statistically significant difference in the in [TOS] and [OSI] levels between the workers group and the control (p=0.000). But the TAC level showed a highly significant decrease in the workers serum (p=0.000) with a nonsignificant decrease in their saliva (p=0.190). The reason for this rise in level of TOS and OSI among the workers group can be attributed to the continuous effort in the exposure to the fuel combustion fumes which the workers were exposed to during their work, this causes shortness of breath and ultimately extreme fatigue, especially with the existence of a reduction in (TAC) [22]. In order to check if the fumes emitted during the HFO combustion had more effect on the oxidative stress parameters in the worker group as they were getting older, the study workers were sub-grouped into two sub-groups, the first one with age ranged between 20-39 years and the others subgroup were those with age of 40-60 years and Table (3) presented the obtained results

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Table 3
Comparison between the levels of TOS, TAC and OSI of in serum and saliva of the study participants sub-groups according to their age

	Age Years	Mean± SD		
		[TOS] (µmol H_2O_2 Eq/L	[TAC] (µmol H_2O_2 Eq/L	OSI
Serum	Workers=20-39 N=35	363.428 ±220.786	1.895 ±0.246	194.866 ±120.917
	Control=20-39 N=27	44.074 ± 22.362	2.120 ± 0.160	20.931 ± 10.642
		P-value = 0.000	0.000	0.000
	Workers=40-60 N=24	493.291±206.192	1.801±0.281	276.618±134.003
	Control=40-60 N=26	51.923 ± 20.788	2.094 ±0.161	25.073 ± 10.726
		P-value = 0.000	0.000	0.000
	Workers with Workers	P-value = 0.026	0.179	0.018
	Control with Control	P-value =0.191	0.554	0.164
Saliva	Workers=20-39 N=35	226.470±172.150	2.117 ±0.156	109.259 ± 90.480
	Control=20-39 N=27	92.407 ±40.628	2.128 ± 0.090	41.737 ±20.983
		P-value = 0.000	0.728	0.000
	Workers=40-60 N=24	272.291 ± 179.225	2.159± 0.142	130.017 ± 89.542
	Control=40-60	105.192 ± 33.984	2.080 ±0.125	50.808 ±17.161

	N=26	P-value = 0.000	0.043	0.000
	Workers with Workers	P-value =0.331	0.300	0.391
	Control with Control	P-value =0.221	0.116	0.091

The showed results indicated that there was a significant increase in serum and saliva TOS level and OSI (p=0.000) in both age subgroups compared to their corresponding control subgroups. While TAC level showed a highly significant decrease in serum (p=0.000) with a nonsignificant decrease saliva (p=0.728) in comparison to control of the same age. The difference between both age subgroups of the workers was nonsignificant (p>0.05). This reflected that the pollutants emitted from HOF combustion affect the younger workers more than the order one. As it can conclude from the result in table (3), the observed change of the measured parameters in serum and saliva was due to the pollutants effect rather than advancing in age since the same change were observed in the control subgroups.

At the combustion unit, some of the workers were working seven hours/day for 5 day /week, while others were working in an alternative mode (work for two successive days for 7 hours/ day, then taking the following day as a rest). Therefore the oxidative parameters were compared between these two subgroups to check the effect of the mode of working on them, and the results were presented in (Table 4).

Table 4
The effect of the working mode on serum and saliva TOA, TAC and OSI of the workers

parameters	Mean±SD			
	Serum		Saliva	
	Alternant Workers N=24	Seven hour work N=35	Alternant Workers N=24	Seven hour work N=35
[TOS] (µmol H_2O_2 Eq/L)	461.950±220.002	398.142±196.105	295.333±211.932	222.894±132.112
	P-value = 0.272		P-value = 0.002	
[TAC] (µmol H_2O_2 Eq/L)	1.843± 0.231	1.824± 0.297	2.153± 0.139	2.139± 0.138
	P-value = 0.034		P-value = 0.585	
OSI	263.161±149.327	219.204±116.694	150.846±120.810	102.979±62.636
	P-value = 0.261		P-value = 0.001	

The result in above table illustrated that there was a nonsignificant difference in the serum TOS and OSI, but in saliva a significant difference was found in each of TOS, OSI (P value<0.05). Meanwhile TAC level showed a significant decrease in

serum (p= 0.034) with a nonsignificant decrease saliva (p=0.585). The results in table (5) showed the effect of the years of the working period at the HOF combustion unit on the measured oxidative stress workers in serum and saliva of the workers.

Table 5
The impact of the service period on the level of TOS, TAC and OSI in serum and saliva of workers sup groups

Parameters	Group		
	Workers (1-10) N=29 (1)	Workers (11-20) N=20 (2)	Workers (20≥) N=10 (3)
[TOS] serum (µmol H_2O_2 /L)	385.862±201.773	401.2000±235.827	529.444±251.115
	$P_{1.2}$value = 0.808	$P_{1.3}$value = 0.033	$P_{2.3}$-value = 0.195
[TAC] serum (µmol H_2O_2 /L)	1.869 ±0.266	1.881± 0.243	1.759±0.299
	$P_{1.2}$value = 0.871	$P_{1.3}$value = 0.301	$P_{2.3}$-value = 0.255
OSI serum	211.214±115.771	217.294±131.552	297.346±173.226
	$P_{1.2}$value = 0.868	$P_{1.3}$value = 0.093	$P_{2.3}$-value = 0.181
[TOS] saliva (µmol H_2O_2 /L	222.749±152.526	302.000±233.244	361.363±228.353
	$P_{1.2}$-value=0.013	$P_{1.3}$-value=0.023	$P_{2.3}$-value = 0.433
[TAC] saliva (µmol H_2O_2 /L	2.134± 0.178	2.153±0.162	2.159 ±0.124
	$P_{1.2}$-value=0.044	$P_{1.3}$-value=0.057	$P_{2.3}$-value =0.131
OSI saliva	107.063±65.598	142.296±111.347	165.878±108.354
	$P_{1.2}$-value=0.002	$P_{1.3}$-value=0.004	$P_{2.3}$-value = 0.500

The above (table 5) results illustrated the comparison between the measured parameters between the workers who worked (1-10) years with those who worked (10-20) years. These results illustrated and that the variation among the workers (TOS,TAC and OSI) was a nonsignificant in serum samples, with a significant variations in the saliva samples were significant especially between those who worked for (1-10)years and those worked for≥) 20) years. From the results of tables (4 and 5), it was clear that the measured oxidative stress parameters in the present work studied workers seemed to be affected by the period of working at the HFO combustion unit. This was more obvious in the measured parameters in the saliva than in the serum samples. The obtained results here of serum was in line with that reported in the workers at petroleum station in Mosul city/ Iraq measured previously by[23].

The cigarette smoke is one of the major source of free radicals and many oxidants and pro-oxidant were reported to be present in the tobacco smoke [24]. Therefore to look at the influence of the smoking on the measured oxidative stress parameters in the workers at the HFO combustion unit. The participants of the current study were sub-grouped into smoker and nonsmoking subgroups to look at the effect of the emitted pollutants from HOF combustion and from tobacco combustion on the level TOS, TAC and OSI on the serum and saliva of the workers, and the results were presented in table (6).

Table 6
The effects of air pollution and smoking on the level of TOS, TAC and OSI in serum and saliva of the workers at the HOF combustion unit

	Group	Mean±SD		
		[TOS] (µmol H_2O_2 /L	[TAC] (µmol H_2O_2 /L	OSI
Serum	Workers non smoker N= 30	348.928±201.417	1.858 ±0.213	188.440±106.058
	Control non smoker N= 25	46.800 ± 22.026	2.128±0.173	22.2478 ± 10.758
		P-value = 0.000	0.000	0.000
	Workers smoker N= 29	477.966 ± 226.652	1.862 ± 0.309	263.556± 144.116
	Control smoker N= 28	48.928 ± 21.873	2.089 ± 0.148	23.602±10.965
		P-value = 0.000	0.001	0.000
	Nonsmoker workers with Smoker workers	P-value= 0.026	0.946	0.0029
Saliva	Nonsmoker workers N= 30	205.806 ± 152.310	2.073± 0.154	101.121 ± 82.228
	Control (non-smoker) N= 25	97.678 ± 44.2108	2.107 ± 0.1238	44.850 ± 23.217
		P-value = 0.000	0.206	0.000
	Smoker workers N= 29	299.137 ± 198.499	2.203 ±0.120	139.762± 96.056
	Smoker control	102.600 ± 28.654	2.090 ± 0.1188	49.371 ± 14.552
		P-value =0.000	0.004	0.000
	Nonsmoker workers with smoker workers	P-value= 0.045	0.001	0.099

The result in (table 6) showed that there was a highly significant increase (p=0.000) in the measured parameters serum and saliva of the workers groups compared to their corresponding control group. As is clear from these results, the TOS level and OSI was higher in the smoker workers than in the nonsmokers workers and the difference was significant. While the decrease in TAC was more obvious in the smoker workers than in the nonsmoker workers. The results about the effect of smoking on TOS level and on OSI is in line with the result of Ozbay and Dulgers results that pointed to the presence of a significant increase in lipid peroxidation [25] and with Morrow *et al.* Ref [26]as well as with Shermatov *et. al.*, [27], who reported that the determined (TOS) level was significantly higher in children exposed to secondhand cigarette smoke than that of the group who not exposed to cigarette smoke [28]. While disagreed with Charalabopoulos *et. al.*, who reported a non- significant change in salivary antioxidants defence mechanisms [29].

Generally it is known that tabacco contains many components that produce free radicals upon its burning [30], such free radicals caused the observed extra increase in the OS observed in the smoker workers in addition to that caused as a result of exposure to air pollution, as a results of HOF combustion. Upon the comparison of the results of the nonsmoker workers with smoker workers a significant increase (p value < 0.05) in the level of TOS and OSI in serum as well in saliva with a nonsignificant difference (P-value > 0.05) in level of serum TAC. It has been reported that smoking is an important factor influencing the salivary levels parameters of oxidative stress, it influenced the oxidative balance in the whole body through stimulation the oxidative burst of neutrophils [31]. Free radicals formation and presence, is naturally controlled by antioxidants, which are capable of deactivating, or stabilizing free radicals before they attack the different components of the cells (32). The results here agreed with that of Kurku *et. al.*, study which reported an increase in serum and salivary OSI value in Turkish cigarette smokers (33).

The observed increase that measured OS in the workers at the combustion unit of HFO many explained as follows:- Even though each air pollutant has its own toxicity mechanism, for example: Co causes decreased blood oxygen carrying capacity, moreover it causes visual problem, while SO_2 poses a heavily damage upon exposure for long time, it is absorbed in the upper respiratory tract by the mucus membrane [34], on the other hand the particulate materials (PM2.5). Nitrogen oxides, iron and other transition elements are efficient oxidants, Furthermore the ozone which is formed by a series of reactions in which the sunlight acts on the atmosphere that contain hydrocarbons and nitrogen oxides, is a strong oxidizing agent with potential effect on oxidative damage of DNA. PM 2.5 are reported to adsorb the heavy metals on their pours and surfaces [35] and these various transition metals are considered as free radical producer through Fenton reaction, moreover the PM 2.5 themselves are able to generate hydroxyl radicals in large amounts[36]. All of these different types of air pollutants are capable of producing free radicals and thus promote the presence of oxidative stress that has been reported to be the most important mechanism responsible of the toxic effect of these pollutant [36].

Due to the presence of oxidative stress, redox sensitive pathways will be triggered which cause inflammations and cell death but the susceptibility of the target cells and organs to the oxidative damage depends upon their ability to accelerate the body protective scavenging system [37]. Inflammation is initially acts as protective mechanism which removes the injurious stimuli. Thus in the early phase of inflammation, the presence of the oxidants does not cause any cell damage since it induces the stress defense genes transcription including the transcription of the antioxidant genes. Such effect of these oxidants (oxygen reactive species), will enhance the resistance against the future inflammation caused by the presence of the oxidative stress and promote the initiation of the process of tissue repair. Meanwhile the additional release of the cells contents will amplifies further the inflammatory reactions and as a result can induce tissue injury through oxidative damage of the different biomolecules including lipids, nucleic acids, and proteins [38] [39].

The systemic OS was reported to be induced by acute air pollution and this depends on the chemical composition of the pollutants present in the air the existence of co-pollutants in the air as well as host susceptibility. On the other hand the magnitude of antioxidant response is influenced by several factors as particular molecular concentration duration of susceptibility to air pollution. For example in the early phase of response i.e.during the presence of low oxidative stress, the body antioxidant increase to reduce the oxidative damage, while upon chronic exposure to the pollution and in the presence of highly toxic pollutants, the endogenous antioxidant will be exhausted and not enough to limit the oxidative damage [40].

As a conclusion, the oxidative stress is present in the workers at the combustion unit of HFO as a result of exposure to the different pollutants emitted from this combustion as well as to the reduction of the body antioxidants defense system. Therefore these workers are advised to take continues antioxidants supplements Furthermore the saliva acts in different way from the serum due to that it has its own defense mechanisms that are not exactly similar to what is present in the serum.

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