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The Effects of Air Pollution Exposure on The Different Serum and Saliva Proteins Levels: A Pilot Study on The Workers at HFO Combustion Unit in AL- Dora Electricity Station/ Baghdad, Iraq

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ABSTRACT

Background: Many studies reported that exposure to hazardous air conditions such as chemicals, fumes, diesel exhaust affects human health and become a major concern globally. Body proteins play central roles in cell structure and functions. **Aim:** Although exposure to such exposure at the workplace has negative effects on health. This pilot study aimed to follow the changes in the total protein [T], albumin [A], globulin [G], and [A/ G] ratio in the sera and saliva samples of workers who are exposed to the fume emitted from heavy fuel oil (HFO) combustion unit.

Participants and Methods: Workers (N=59) at the heavy fuel oil combustion unit in electricity station of Al- Dora station/ Baghdad/ Iraq and 53 unexposed age and gender matched healthy individuals were the participants of this study. The levels of serum and salivary total proteins and albumin were measured, and the level of globulins and albumin / globulin ratio were calculated.

Results: There were variations in both serum and salivary total protein [TP], albumin [A], globulin [G] and [A]/ [G] ratio between that of the workers group and the control healthy group. The impact of the fume combustion on the studied biochemical parameters were further analyzed based on the workers age, period of their service, smoking habit and using of safety equipment.

Conclusion: The results of this pilot study showed that the impact of the air pollution resulted from the HFO combustion on salivary [TP], [A], [G] and [A]/ [G] ratio was more than that on the serum of the workers' group.

Keywords: total protein, albumin, globulin, albumin/globulin ratio.

INTRODUCTION

Occupational exposure to harsh work environment such as dusty, noisy, extreme climate, and that pollutant with different chemicals in the workplace influence human health. And this becomes one of the major public health concern worldwide resulting in multiple chronic disease which associate air pollution [1, 2]. Heavy fuel oil (HFO), which is referred to as bunker fuel, or residual fuel oil, consists of remnants of petroleum sources which is produced upon the extraction of higher hydrocarbons quality using thermal and catalytic cracking [3]. Thus, HFO composition is highly variable, since it is often blended, or mixed streams including carbon numbers ranges from 20- more than 50. As such this type of fuel is usually contaminated with different types of particles and compounds such as aromatic, asphaltenes and olefins, in addition to particulates that contain S, N, and metallic molecules and compounds [4, 5].

Proteins are the major components of all cells and play a central role in cell structure and functions, they are present in all body fluids and serum contains a mixture of proteins that differ in origin and function. The amount of protein in the vascular compartment depends on the balance between the rate of its synthesis and the rate of its catabolism, or loss [6]. Li *et al*. reported that the alteration in serum protein may be used as a biomarker to predict the occupational hazard risk [7].

In recent years, attempts have been made to replace blood test by other body fluids. Serum constituents are reported to be found in whole saliva, as a result of gingival crevicular fluid (GCF) outflow and most of these constituents which are not part of normal salivary components can reach saliva via intracellular (by passive transfer) and extracellular (by ultra-filtration) routes [8].

Since there has a need for clearly elucidated the underlying mechanism inducing the harmful effect of air pollution on human health and to evaluate the outcome of chronic exposure as early as possible, therefore this pilot study aimed to look for the alteration in the concentrations of total proteins (TP), albumin (A), globulins (G) and the A / G ratio in serum and saliva of individuals who are exposed to the fume emitted from HFO combustion. Meantime looked for the possibility of using saliva instead of blood serum to check up such alteration, since saliva, is easier and doesn't need special care and experience to be collected.

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

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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, and 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.

Biochemical measurements:

Measurement of total protein concentration:

The concentration of total serum and saliva protein [TP] was determined using the modified Lowry method by Hartree [(9)] and was expressed in g/dl. Different concentrations of bovine serum albumin (BSA) were used as standard to construct the protein standard curve and the derived straight line equation of this curve was used to calculate TP in both types of tested fluid samples.

Measurement of albumin concentration:

The concentration of albumin [A] in the serum and saliva samples was determined by the dye-binding method [(10)] using the kit manufactured by Bio-Merieux. The measurement was based on albumin quantitative binding at pH 4.2 with bromocresol green (BCG) to form a blue-green complex which was measured at **λ** =630 nm.

Calculation of globulin concentration:

The concentration of globulin [G] in the sera and saliva samples of healthy individuals and workers was calculated, using the following equation:

$$\text{Globulin}_{(g/dl)} = \text{Total proteins}_{(g/dl)} - \text{Albumins}_{(g/dl)}$$

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 $P > 0.05$.

The general detailed participant characteristics in the current study were presented in Table (1).

Table (1): The participant characteristics of the present study

Characteristics	Total control	Total workers
Number	53	59
Gender	male	Male
Body index	22.3-37.4	23.4 -39.4
Age (year) 20-39 year 40-60 year	20-60 51% 49%	20-60 60% 40%
Periods of service (year) 1-10 year 11-20 year <20 years	-------	1-≤20 [years] 49% 34% 17%
Workers with alternative mode Seven hour working workers	------- -------	40.6% 59.4%
Smoking and nonsmoking Smokers Non-smokers	53 % Smoking and 47 % nonsmoking	51% Smoking and 49% nonsmoking
Workers using safety equipment **Workers without safety equipment**	-----	47% 53%
Medical history	None	None

The above table showed that the final number of workers included in the present study was 59 and the number of the control group was 53. All of them were males. Their weights and lengths were measured, and their body mass index (BMI) was calculated. Their ages ranged between 20-60 years. The workers' group was divided into sub-groups, as shown in the above table according to their ages, the period of the service at the HFO combustion unit and if they were smokers or using safety protection equipments.

In this study total protein (TP) and albumin (A) were measured in serum and saliva of the exposed worker group to the fumes emitted from HFO combustion and control group. Meanwhile the globulin (G) and A / B ratio were calculated and the results were shown in Figure (1).

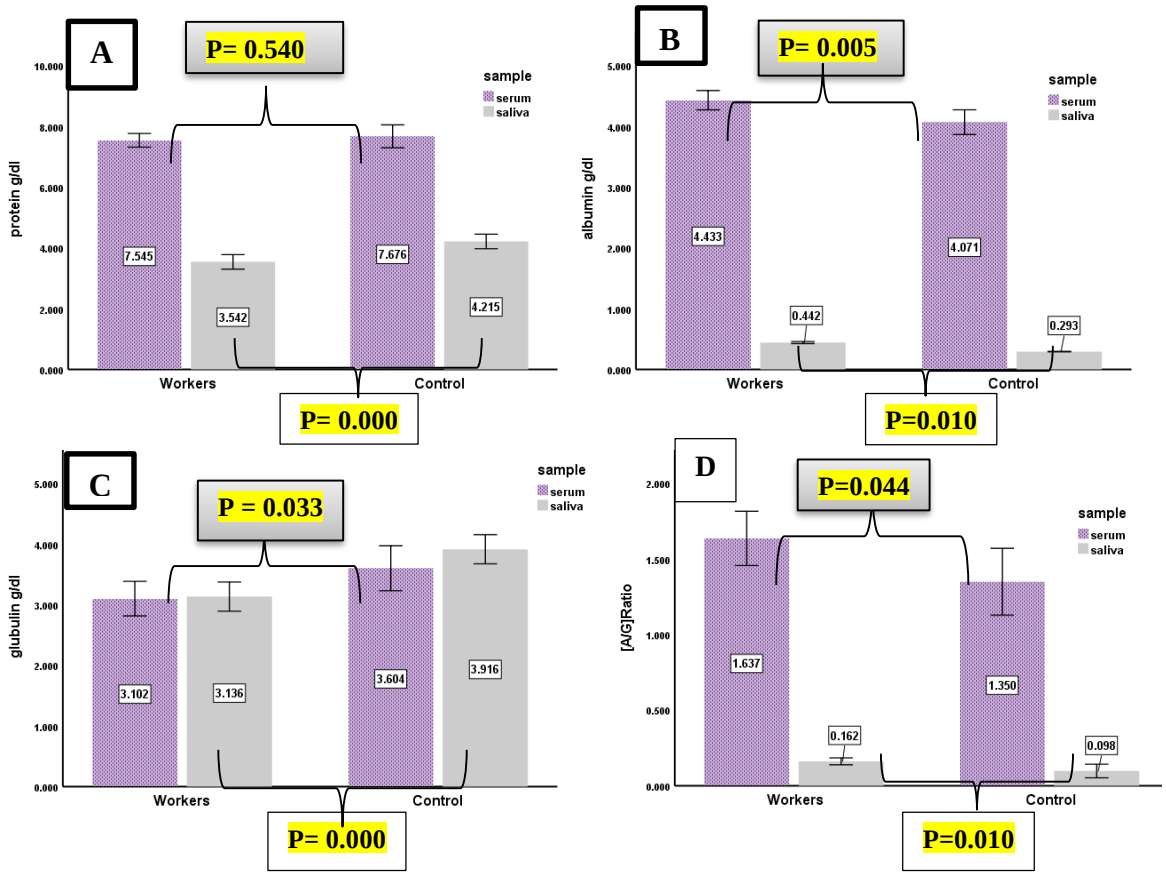

Figure (1): Comparison between levels of (A) Total protein, (B) albumin, (C) globulin and (D) the A/ B ratio in serum and saliva of both the workers and control groups.

The TP results in Figure 1 (A), indicated the presence of a nonsignificant decrease (P> 0.05) in the serum of the workers' group compared with that of the control group, while there was a highly significant decrease in salivary TP of the workers group (P,0.001). Additionally, the results in Figure 1: B, C and D showed a significant increase in serum albumin, globulin and the A/G ratio of the workers' group when compared with that of the control group (P< 0.005), while a highly significant increase in salivary albumin, globulin and A/G ratio of the workers' group in comparison to that of the control group (P< 0.001).

The nonsignificant variation observed in serum total protein between the exposed workers' group and the control group agreed with that of Hasan *et al.* [11] who recorded nonsignificant variation in total plasma protein concentration when compared to that of the workers in factory manufacturing lead storage batteries with that of the control group. Also, it agreed with that recorded in the results of the study on the effect of tetra-ethyl lead on several biochemical parameters among which was total proteins of Iraqi workers at oil refinery station [12].

Most individuals who are exposed to harsh working environments are unaware of the impact of long exposure to such environment on their health, and thus to study the possible influence of different time of exposure to pollution on total protein, albumin, globulin and A/B ratio, this was achieved by measuring and calculating these parameters in 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 Figure 2.

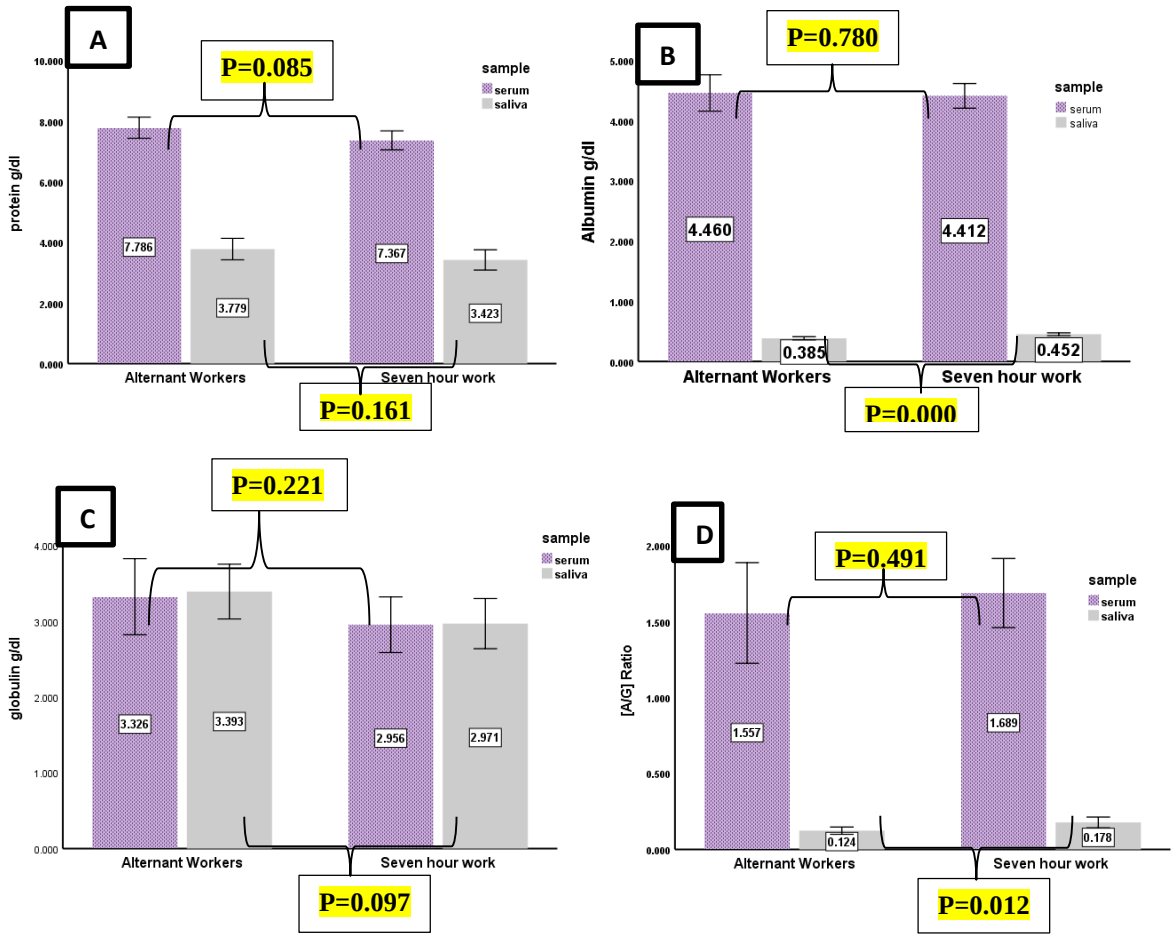

Figure (2): The effect of the working mode on serum and saliva (A) Total protein, (B) albumin, (C) globulin and (D) albumin/ globulin ratio of the workers.

The results in the above Figure illustrated that there was a nonsignificant increase (p-value> 0.05) in the serum level of TP, A, G and nonsignificant decrease in A / G ratio, with a nonsignificant increase (P> 0.05) in TP and G of the saliva samples of those who worked in alternative mode. While a highly significant decrease (P< 0.001) was found in salivary albumin concentration (Figure 2 B), with a significant increase in the salivary ratio of A/G of those who worked seven hours/ week for successive days (Figure 2 D).

The other way used in this study to check the effect of chronic exposure to the fumes emitted from HFO combustion was by studying the effect of service period at the combustion unit on the saliva and serum of the workers at the different assayed parameters included in the present study.

The results in Table (2) showed the variations in each of salivary and serum TP, A, G and A/ G ratio according to the service period.

Table 2: The impact of the service period on the serum and saliva levels of total protein, albumin, globulin and the albumin/ globulin ratio of the workers at HFO combustion unit

Serum						
Group	**Working period 1-10 years (N=29) (1)**	**Working period 11-20 years (N=20) (2)**	**Working period ≥20 years (N=10)) (3)**	**$P_{1.2}$ Value**	**$P_{2.3}$ Value**	**$P_{1.3}$ Value**
[Total protein] g/dl	**7.635 ± 0.907**	**7.587 ± 0.921**	**7.416 ± 0.615**	**0.858**	**0.527**	**0.634**
[Albumin] g/dl	**4.402 ± 0.641**	**4.410 ± 0.547**	**4.464 ± 0.730**	**0.963**	**0.817**	**0.833**
[Globulin] g/dl	**3.23 ± 0.181**	**3.110 ± 0.013**	**2.952 ± 0.898**	**0.710**	**0.053**	**0.703**
[A/G]Ratio	**1.542 ± 0.276**	**1.645 ± 00.130**	**1.711 ± 0.806**	**0.587**	**0.509**	**0.837**

Saliva						
Group	**Working period (1-10) years (N=29) (1)**	**Working period 11-20 years (N=20) (2)**	**Working period ≥20 years (N=10) (3)**	**$P_{1.2}$ value**	**$P_{2.3}$- Value**	**$P_{1.3}$ value**
[Total protein] g/dl	**3.625±0.826**	**3.510±0.872**	**3.255±0.299**	**0.645**	**0.539**	**0.320**
[Albumin] g/dl	**0.413±0.030**	**0.435± 0.139**	**0.448±0.043**	**0.423**	**0.793**	**0.011**
[Globulin] g/dl	**3.211±0.832**	**3.152±0.783**	**2.807±0.326**	**0.804**	**0.386**	**0.283**
[A]/ [G] Ratio	**0.139±0.047**	**0.159±0.039**	**0.229±0.082**	**0.123**	**0.287**	**0.021**

Table 2 illustrated the comparison between the measured parameters in the saliva and the serum between the workers with different service period at the combustion unit of HFO, where the results showed that the variation among the workers T, A, G and A/G ratio was nonsignificant (p>0.005) in serum samples. Meanwhile a nonsignificant (p>0.005) variations in both TP and G in the saliva samples of those who worked for 1-10 years and those worked for 11-20 years, or > 20 years. While the variations (an increase) in salivary A and the ration of A / G was significant between those with a service period of 1-10 years and those with ≥ 20 years of service.

The results in Figure (3) showed the effect of using safety equipment's on the level of total protein, albumin, globulin and A/ G ratio in serum and saliva of the workers at the HFO combustion unit.

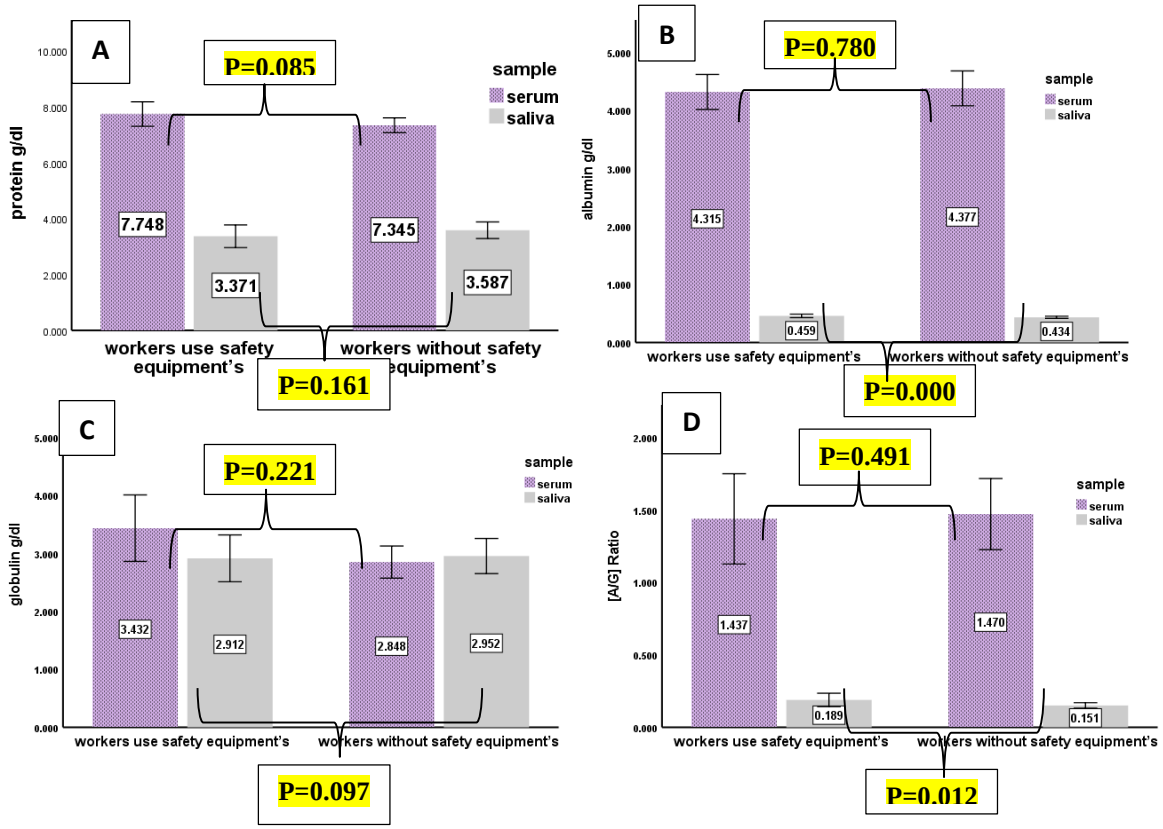

Figure 3: The effects of using safety equipment's on the level of total protein, albumin, globulin and A/ G ratio in serum and saliva of the workers at the HFO combustion unit.

The results in Figure 3 showed that there was a nonsignificant variation (p>0.05) in the measured parameters in the serum of the workers who were using safety equipment groups compared to those who were without safety group.

As is clear from these results, the A and the A/ G ratio were lower in serum of those workers using safety equipment compared with those who did not use them and the difference was non-significant (P>0.05), with a non-significant increase in both TP and G (P< 0.05). The results of salivary parameters indicated that TP and G levels decreased non- significantly (P.0.05) and that there was a highly significant increase (p< 0.001) in salivary albumin, with a significant increase (P< 0.05) in A/ G ratio of the workers groups who were using safety equipment compared to those workers who did not use them.

In order to check if the fumes emitted during the HOF combustion had more effect on the total protein, albumin, globulin and A/ G ratio. 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 other subgroup were those with age of 40-60 years and Table (3) presented the obtained results.

Table (3): Comparison between the levels of total protein, albumin, globulin and albumin/ globulin ratio in serum and saliva of the study participants according to their age.

	Serum			Saliva		
Age Years	**Workers =20-39 N=35**	**Workers =40-60 N=24**	**p-value**	**Workers =20-39 N=35**	**Workers =40-60 N=24**	**P-value**
[Total protein] g/dl	**7.864±0.856**	**7.057±0.657**	**0.000**	**3.640±0.762**	**3.382±0.127**	**0.303**
[Albumin] g/dl	**4.400±0.656**	**4.494±0.554**	**0.575**	**0.441±0.069**	**0.442±0.054**	**0.957**
[Globulin] g/dl	**3.463±0.123**	**2.563±0.761**	**0.001**	**3.198±0.750**	**2.940±0.132**	**0.300**
[A/ G] Ratio	**1.439±0.08**	**1.930±0.186**	**0.006**	**0.146±0.045**	**0.188±0.024**	**0.067**

The results in Table (3), illustrated that there is a highly significant decrease (P< 0.001) in serum TP and G with a significant increase (P<0.05) in the ratio of A/ G of the workers at age ranged between 20- 39 years, meanwhile a non-significant variations in salivary TP, A, G and A/ G ratio. The air pollution at the HFO combustion unit appeared to affect the serum measured parameters except albumin [A], with nonsignificant variations in all of them in the saliva samples as the age of the workers increase.

The participants of the current study were sub-grouped into the smoker and nonsmoker subgroups to look at the effect of the emitted pollutants from HFO combustion and from tobacco smoking on serum and saliva total protein, albumin, globulin and A/ G ratio of the workers, and the results were presented in Table (4).

Table 4: The effects of the fumes emitted from combustion of heavy oil fuel (HFO) and smoking on the level of Total protein, Albumin, Globulin and albumin/ globulin ratio in serum and saliva of the workers at the HFO combustion unit in the Dora electricity station

	Serum			Saliva		
Group	**Workers nonsmoker N= 30**	**Workers Smoker N=29**	**p-value**	**Workers nonsmoker N= 30**	**Workers Smoker N=29**	**p-value**
[Total protein] g/dl	**7.562±1.034**	**7.516±0.667**	**0.839**	**3.450±0.116**	**3.273±0.816**	**0.530**
[Albumin] g/dl	**4.430±0.672**	**4.368±0.939**	**0.771**	**0.487±0.068**	**0.400±0.014**	**0.000**
[Globulin] g/dl	**3.204±0.223**	**3.082±0.895**	**0.662**	**2.962±0.123**	**2.873±0.822**	**0.744**
[A/G]Ratio	**1.584±0.697**	**1.409±0.168**	**0.331**	**0.205±0.030**	**0.152±0.052**	**0.050**

As is clear from the above results in Table 4, the serum total protein, albumin, globulin and A/ G ratio was lower in the smoker workers than in the nonsmokers workers and the difference was nonsignificant (P> 0.05). Meanwhile the smoking seemed to have more effect on salivary albumin and A/ G ratio as a highly significant (P< 0.001) and a significant reduction (P< 0.05) occurred respectively in the smoker worker group in comparison to that of non-smoker worker group.

DISCUSSION

The measured variations in the levels of total protein, albumin, globulin and A/G ratio among the worker groups and the healthy control may be explained as follows:

Generally, in the medical practice, the concentration of total proteins is referred to the total levels of albumin and globulins (which are sub grouped into: α_1-, α_2 -, β- and γ- globulin) in blood serum and plasma [13].

The concentration of serum, or plasma proteins reflects metabolism and immunity status of the body, therefore it has a great potential and used as biomarkers for different health conditions [14].

The most abundant proteins in serum is albumin (A), which is synthesized mostly in the liver [15].

Serum albumin [A] constitutes about 55% -60% of blood proteins, it serves many physiological properties [16], and is a major transport of different hydrophobic molecules such as lipids and steroid hormones, it is responsible of microvascular integrity, regulate metabolic and vascular function, support antioxidant activities, and anticoagulant effects [17].

It is known as "sponge" of the circulation and this is due to its structure flexibility which adapts it readily to ligand since its 3 essential domains design provides a variety of binding sites [18].

This albumin may be subjected to different types of modification such as oxidative modification due to increase oxidative stress, which was reported to be present in the workers at the same combustion unit [19], such modification will lead to decrease native albumin and thus perturb albumin normal functions resulting in some diseases.

Lodovici and Bigagli, 2011 [20] showed that inflammation and oxidative stress are known to be the main biological mechanisms by which air pollution induces health effects, which might be translated to an inflammation cascade, and oxidation stress process in the lung, vascular, or heart tissue together with dysfunction of vascular endothelium [21].

On the other hand, globulins make up 38% of blood proteins assisting in immune function [18]. Meantime saliva serves as the primary non-specific immunity barrier which protects the oral cavity against any foreign molecule enter the human body and it is considered as the first protection site in the body [22, 23].

The ratio A/ G has been considered to be an inflammatory indicator and it was suggested to predict the prognosis of various diseases [24]**.** The observed increase in this ratio in both serum and saliva of the workers' group compared to the healthy control group is due to a differential alteration in both albumin and globulin.

From the results of Figure (2) and Table (2), it was clear that the measured total protein 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. IgA is the predominate isotype of immunoglobin of saliva, which is capable of neutralizing the viruses, bacteria and toxins. It inhibits microorganisms' binding to the oral mucosa by binding their surface [21].

This protein is reported to be affected by environmental pollution and the environment changes [22]. Long term exposure to particulate air pollution was reported to have a stronger effect as compared to short term exposure [23].This is in line with the present study results (Figure 2 and Table 2). This long term effect of pollution can be explained by the cumulative effects of tissue damage with inflammation over a longer duration.

The effect of the fume emitted from fuel combustion on the worker group, according to the age observed in the current study (Table 3) may be because that the elderly individuals are more susceptible to the health impacts of air pollution as suggested by Kim *et al*. [25] and the reason for this is due to a substantial burden regarding the onset of chronic disease, impairment in their immune system function and activity status changes in addition to insufficient physical activity. Some earlier studies detected the presence of a relationship between smoking and decreased serum protein and albumin concentration. However, the biological mechanisms leading to such decreased levels of serum protein and albumin by smoking have not been studied [26].

Tobacco smoking is associated with increased oxidative stress [27]. The protein depression may be due to reduce protein synthesis or increased proteolytic activity due to cigarette smoke exposure or *via* release of high levels of cellular oxidative free radicals which result in increased proteolytic activity [28].

Also the significantly decreased level of salivary albumin in the smokers' workers in comparison to that of non-smokers may be due to the role of albumin as one of the extracellular antioxidants [18].

Furthermore, smoking affects systemic inflammation by activating and releasing inflammatory cells into the circulation and increasing circulating inflammatory mediators such as pro-inflammatory cytokines and acute phase proteins [28].

In a previous study, it was illustrated that during inflammation, the concentration of some acute phase proteins increased (positive acute phase protein), while others decreased referred to as negative acute phase proteins (e.g. albumin, transferrin, etc.) [12].

Thus, smoking seems to have an additive effect on health sequelae of air pollution and this agree with Chamber *et al*. results [29].

In general, it has been reported that the particulate molecules with a diameter of less than 10μm which are predominantly present in the HFO combustion fumes (usually referred to as inhalable particles) have the ability to penetrate into the lung and thus leads to different respiratory disease especially in

early ages individuals and elderly ones [30], followed by induction of inflammatory and cancer [31].

The human body is exposed to pollutant air containing multi pollutants rather than an isolated single pollutant [28]. And using the safety equipment such as the mask can aid in the deconstruction of the complex mixture of air pollutants, which result in simplifying the exposure matrix [32]. Doing research as the current study, one should take into consideration that the circulating proteins level in plasma is considered as the outcome, and some previous studies examined the level of inflammatory mediators' protein by stimulated immune cells and not their circulating level. Moreover the plasma level of protein is outcome, therefore it needs more time to investigate any exogenous factor impact on its level more than that require to measure, for example protein in particular cells since a longer time is required to synthesize and secrete this particular protein into serum.

Conflict of interest: The declaration of no conflict of interest by the authors is stated clearly.

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