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OXIDATIVE STRESS BY THE FOX2 METHOD IN FILLING STATION ATTENDANTS IN TERESINA, PIAUÍ

Ayres Fran da Silva e Silva[1*], Regina Célia de Assis[2†], Maria do Carmo de Carvalho e Martins[3], Eveline Brandão Madeira[4], Mariana Batista Gonçalves[4], Aline de Almeida Sousa e Silva[4], Raphaela Martins Ferroz dos Santos[4] and Maximiliano de Souza Zierer[5]

[1]PhD in Biotechnology, Professor at the Federal Rural University of Amazonia, Campus Parauapebas, Pará, Brazil
[2]PhD in Biochemistry, Professor at the Federal University of Piauí, Department of Biochemistry and Pharmacology, Teresina, Piauí, Brazil. [†]In memoriam
[3]PhD in Biological Sciences, Professor at the Federal University of Piauí, Department of Biophysics and Physiology, Teresina, Piauí, Brazil
[4]Undergraduate student in medicine, Federal University of Piauí, Teresina, Piauí, Brazil
[5]PhD in Biochemistry, Professor at the Federal University of Piauí, Department of Biochemistry and Pharmacology, Teresina, Piauí, Brazil

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ABSTRACT

Occupational exposures to toxic substances make up an important public health problem. In the literature, several studies have revealed that gasoline can cause functional changes in people who are often exposed to it. Due to the potentially negative impact of the exposure to fuel in the health of workers, we investigated the level of oxidative stress in filling station attendants of Teresina, Piauí state capital. We found high concentrations of lipidic hydroperoxide in the blood plasma of the filling station attendants when compared to the low levels of lipid hydroperoxide in the control group. The hydrogen peroxide concentration of the gas station attendants was about 7 to 12 times higher than the control group, depending on the age group considered. In addition, high concentrations of lipid hydroperoxide in the gas station attendants have a tendency to increase with increasing exposure time to gasoline. Regarding the lipid profile of the filling station attendants, we found a significant positive correlation with the increased levels of total cholesterol and LDL cholesterol levels and lipid hydroperoxide levels above the reference classified as normal. The FOX assay we employ can be used as a sensitive and reliable tool to check the levels of oxidative stress of those who work exposed to fuels, especially gasoline.

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INTRODUCTION

The environment of the fuel stations is a contamination site that reaches a considerable number of workers (D'Alascio *et al.* 2014). This contamination also affects the vicinity of fuel stations, and the main contaminant is gasoline, derived from petroleum, used widely as fuel in Brazil and in the world (Leite & Leal 2007, Drumm *et al.* 2014). The automotive gasoline exiting the refinery consists of a complex and balanced mixture of volatile and flammable hydrocarbons to meet commercial specifications (Carvalho & Filho 2014).

***Corresponding author:* Ayres Fran da Silva e Silva,**
[1]PhD in Biotechnology, Professor at the Federal Rural University of Amazonia, Campus Parauapebas, Pará, Brazil.

Several studies have looked at the composition of gasoline to ensure better performance and reduce pollution, for example, the addition of oxygenated compounds. Among these oxygenated compounds are methyl tert-butyl ether, ethyl tert-butyl ether, tert-amyl methyl ether, methanol or ethanol (Westerholm *et al.* 1988, Abrantes *et al.* 2009, Azevedo *et al.* 2013). One of the concerns is the use of aromatic hydrocarbons, in particular benzene, toluene, ethylbenzene and xylene isomers (BTEX), for these compounds are among the sources of gasoline contamination. These studies also point out that monoaromatic compounds are powerful depressants of the central nervous system (Machado *et al.* 2011), and therefore there is great concern about their contamination. Routinely people are exposed to gasoline, and among them the workers

of refineries and, especially, the attendants of gas stations (Foo 1991, Backer *et al.* 1997, Dib *et al.* 2007, Costa *et al.* 2011). Studies showed that gasoline can cause several functional changes in people who are overly exposed to it: excessive irritability, psychomotor retardation, dry mouth, sleep disturbance, tear reduction, headache, nausea, vertigo, eye and nasal irritation (Pereira & Andrade 1998; Danni-Oliveira 2008). A number of organizations, such as the International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA), point out that benzene, one of the constituents of gasoline, is a carcinogen in humans. Among the organs affected by benzene are those that participate in the metabolism of this agent, such as the liver and bone marrow. In addition, benzene may cause chromosomal, protein and immune system changes (D'Alascio *et al.* 2014). All abnormal states cited above caused by gasoline exposure involve cellular processes governed by redox balance. It should be noted that the redox balance of a given healthy population is influenced by genetic (ethnic characteristics), environmental (e.g., air pollution), nutritional (meat and vegetable consumption) and cultural influences (e.g., intense physical activity habits, alcoholism, or smoking). Changes in the redox balance of biological systems can cause oxidative stress. The intensity and pathogenicity of these imbalances will depend, of course, on local concentrations of pro and anti-oxidant species, reaction rate constants with target molecules and cellular compartmentalization of these processes, where solubility and diffusibility factors are determinants (Vasconcelos *et al.* 2007).

The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), among other reactive species, is an integral part of human metabolism and is observed in several physiological conditions (Júnior *et al.* 2001). ROS and RNS have important biological function, as in phagocytosis, a phenomenon in which these species are produced to eliminate the aggressor agent. On the other hand, when its production is exacerbated, the organism has an efficient antioxidant system that can control and restore equilibrium (Desikan *et al.* 2001; Schafer & Buettner 2001). The oxidative stress results from the imbalance between the pro and the antioxidant system with predominance of the oxidants, with consequent damage (Vasconcelos *et al.* 2007). Studies have shown that cell membranes constitute one of the focuses of ROS, RNS and other reactive species, and their function is vital for the cell (Lee *et al.* 2017; Naidoo *et al.* 2017, Romek *et al.* 2017). In addition to the membrane surrounding the cell, the membranes of intracellular organelles, such as mitochondria, endoplasmic reticulum and nucleus have a bilipid structure and a variety of proteins and sugars. The cell damage results basically from ROS and RNS attack on macromolecules such as sugars (Neto *et al.* 2017), DNA (Aitken 2017), proteins (Park *et al.* 2017) and lipids (Lee *et al.* 2017). There is strong evidence that oxidative stress is implicated in aging processes (Ahmad *et al.* 2017), and cell alterations and death (Kim *et al.* 2017, Zou *et al.* 2017), with direct effects on the pathogenesis of various diseases, such as cancer (Kosala *et al.* 2007), seropositive patients (HIV+) (Chaves *et al.* 2003, Rastad & Green 2016), autoimmune diseases (Schallreuter 2008, Schallreuter *et al.*, 2008), heart diseases, lung diseases (Mossman 2003, Wang *et al.* 2007), xenobiotic intoxication (Klotz & Steinbrenner 2017), among others. On the other hand, ROS and RNS exert physiological roles in blood pressure control (Oyagbemi *et al.* 2017, Togliatto *et al.* 2017), cell signaling (Feng *et al.* 2017), apoptosis (Guon & Chung 2017), fecundation (Kazama *et al.* 2014) and fruit ripening (Huan *et al.* 2016). Due to the potentially negative impact of exposure to fuels on workers health, this study sought to evaluate oxidative stress in filling station attendants of the city of Teresina, capital of the State of Piauí.

MATERIALS AND METHODS

This article is based on a cross-sectional study, the choice of which fuels stations occurred in a convenient way, being carried out in the municipality of Teresina, Piauí, in the period from 2011 to 2012, based on the cadastre of the gas stations of the municipality, which at the time comprised 347 establishments in operation. There were 90 gas stations visited, which were randomly drawn. The criteria for the selection were the accessibility of the gas station, its consent to participate in the study, its regulation, and, above all, the willingness of the participants to participate in the research. The sample represents 95% confidence with sample error of 8.91% per establishment. The survey covered students and workers of both sexes, aged over 18 years. The study sample consisted of 180 individuals between the ages of 20 and 64 years, of whom 172 were males and 8 were females.

Of the 180 individuals who agreed to participate in the survey, 90 were fuel station workers (filling station attendants) and 90 were non-occupationally exposed to gasoline (students and workers from another area). In order to minimize the confounding factors, the groups were categorized by two groups: Group I (control) and Group II (filling station attendants). All the owners of the fuel stations were contacted in advance, allowed to search and signed the necessary terms of agreement. The volunteers signed the informed consent form. A questionnaire was applied individually, with open and closed questions regarding age, working time at the gas station, alcohol consumption and smoking. The existence of chronic diseases (diabetes, hypertension, hypercholesterolemia, chronic renal failure and arthritis) was used as exclusion criterion. The application of the questionnaire, the blood samples and the necessary measurements were carried out in the workplace, and whenever possible in a private environment, in order to minimize possible influences in data collection.

Obtaining blood samples for biochemical analysis: Total blood samples were collected in EDTA (ethylenediaminetetraacetic acid) vacuum tubes to perform the lipid profile - total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides - and in tubes with heparin for FOX2 assays. The collected samples were transported in a suitably refrigerated container to the analysis site, Biochemistry Laboratory, Department of Biochemistry and Pharmacology, Federal University of Piauí (UFPI).

Evaluation of the lipid profile: Biochemical measurements of lipid levels were determined using colorimetric enzyme kits: Total Cholesterol Labtest, HDL Cholesterol LE Labtest and Labtest Triglycerides. The LDL cholesterol value was estimated using the Friedewald formula [LDL = CT- (HDL-TG / 5)]. Spectrophotometric measurements were performed on a Hitachi U-3000 spectrophotometer. The lipid profile was evaluated according to the criteria established by the IV Brazilian Guidelines for Dyslipidemias and the glycemic

levels according to the criteria of the Brazilian Consensus of Diabetes (Oliveira *et al.* 2013).

FOX2 test: The FOX2 reagent was prepared as described by Nouroozzadeh (1999). After preparation, it was used to obtain the calibration curve with hydrogen peroxide concentrations at 560 nm, which showed an equation (y = 0.04704X + 0.00953) (r = 0.99975). The assay was performed and monitored at a wavelength of 560 nm, as recommended by Nouroozzadeh (1999). Spectrophotometric measurements were obtained on the Hitachi U-3000 spectrophotometer.

Statistical analysis: In the descriptive analysis, the mean and standard deviations for age were calculated and the independent Student's t test was applied, with a 95% confidence interval in both Group I and Group II. In addition, the percentage distribution frequency of the categorical variables (sex, age, alcohol consumption, smoking habits and working time at the fuel station) was calculated. In order to compare the averages of the plasma hydroperoxide concentration in the study participants, both in Group I and Group II, with the related categorical variables (age, alcohol consumption, smoking habits and working time at the fuel station), independent Student's t-test was used, with a 95% confidence interval. The same independent Student's t-test was applied to verify the lipid hydroperoxide plasma lipid concentration in the studied groups. The data were analyzed using SPSS for Windows software version 15.0.

RESULTS

The mean age of Group I (control) was 36.47 ± 12.40 years, whereas in Group II (filling station attendants) the mean age was 33.37 ± 8.252 years. The independent T-test of the groups revealed no significant difference between the mean ages at p = 0.05 (Table 1). From Group I, 80.00% (n = 72) consumed alcoholic beverages, while in Group II 76.4% (n = 69) consumed alcoholic beverages. Smokers were 23.30% (n = 21) in Group I and 13.30% (n = 12) in Group II. No use of any personal or collective protective equipment during the working day, including during the receipt of fuel, has been reported or recorded during the interview and blood collection. Group I and II data regarding age, consumption of alcoholic beverages, smoking habits and working time as filling station attendants are presented in Table 1. The results of the FOX2 assay for Groups I and II are shown in Table 2. The blood plasma lipid hydroperoxide concentration values showed significant differences ($p < 0.05$) between the two groups. The results of the analyzes regarding individual factors such as age, alcohol consumption and smoking also showed differences between groups.

Figure 1 shows the linear regression model adjusted between the concentration of lipid hydroperoxide (hydrogen peroxide) in plasma measured by the FOX2 method and the working time variable as a filling station attendant (Group II) obtained by the stepwise method. When evaluating the correlations, we found that this variable correlated positively, with a "Pearson Correlation" r = 0.635, showing a strong correlation, being significant with $p < 0.001$. Table 3 shows correlations (Pearson's coefficient) between the Group I and Group II hydrogen peroxide concentration variable (HPC) and the concentrations of total cholesterol (TC), LDL cholesterol, HDL cholesterol and triglycerides (TG) in the fasted state. For Group I (control) the hydrogen peroxide concentration did not correlate with TC, LDL and HDL, but there was a positive correlation with triglycerides. For Group II (filling station attendants), the concentrations of hydrogen peroxide had no correlation with HDL and triglycerides, but for both TC and LDL there was a positive correlation.

DISCUSSION

Nouroozzadeh *et al.* described the application of the FOX2 method with determination of hydroperoxides in the plasma. They validated the method by extracting total plasma lipids using ethyl acetate prior to the FOX2 reagent assay. The plasma of 23 normal subjects contained hydroperoxide in the range of 0.22 to 7.8 μM (Nouroozzadeh *et al.* 1994). In a hydroperoxide assay with the orange ferric xylenol complex, they showed that the apparent molar absorption coefficients of H2O2 and t-butyl, cumene, bovine serum albumin and linoleate hydroperoxides were measured using known concentrations of hydroperoxide independently determined by a iodometric test. The values of apparent molar absorption coefficients differed significantly and depended on the hydroperoxide, the solvent and the source of the xylenol orange. Furthermore, it was possible to determine the numbers of Fe3+ ions formed by a series of hydroperoxides in different solvents, showing that H2O2 provided the hydroperoxides 2,5, t-butyl and cumene 5 and the other hydroperoxides 2 ions Fe3+ per -OH group. This general finding allows the determination of approximate concentrations of hydroperoxide even in chemically complex systems, such as plasma (Gay *et al.* 1999).

In the determination of 5-lipoxygenase, which is a key enzyme involved in the synthesis of leukotrienes and whose inadequate regulation is implicated in several inflammatory diseases, the FOX trial with some modifications was employed for the high throughput assay for the 5- lipoxygenase in the primary screening stage (Cho *et al.* 2006). Our study investigated the risk of occupational exposure to fuels at gasoline sales stations, in order to verify the possible effects of increased peroxide concentration in blood plasma on gas station attendants. For this, the FOX2 test was used to determine the concentration of hydrogen peroxide in individuals exposed and not exposed to gasoline, verifying aspects such as age, consumption of alcoholic beverages, smoking habits, time of exposure (working time) and the lipid profile. Occupational exposures to toxic substances make up a relevant public health problem and this experience indicates the possibility to identify risk factors as an instrument to distinguish the problem, as well as to increase the precision and reach of active controls to protect the health of workers in this sector of the oil demand market.

Individuals not normally exposed to gasoline (group I, control) had low concentrations of plasma hydrogen peroxide (Table 2), and for that group there was an increase in the concentration of hydrogen peroxide associated with alcohol consumption and smoking. On the other hand, individuals who are usually exposed to gasoline (group II, gas station attendants) exhibited high concentrations of plasma hydrogen peroxide, and there is also an increase in the concentration of hydrogen peroxide related to alcohol consumption and smoking (Table 2). In the age group of 20 to 35 years of age, the concentration of hydrogen peroxide in the plasma of the filling station attendants was about 9 times higher than that of the control group. In the age group of 36 to 50 years of age, the concentration of hydrogen peroxide was about 12 times higher

Table 1. Attributes of the groups surveyed regarding age, alcohol consumption, smoking habits and working time.

Criteria		Group I n (%)	II n (%)
Age group (years)	20 – 35	52 (57,8)	61 (67,8)
	36 – 50	20 (22,2)	26 (28,9)
	>50	18 (20,0)	3 (3,3)
Consumption of alcoholic beverage	No	9 (10,0)	13 (14,4)
	Yes	72 (80,0)	69 (76,7)
	Ex consumer	9 (10,0)	8 (8,9)
Smoking habit	No	52 (57,8)	70 (77,8)
	Yes	21 (23,3)	12 (13,3)
	Ex-smoker	17 (18,9)	8 (8,9)
Working time (years)	<5	–	41 (45,6)
	5 – 10	–	32 (35,6)
	>10	–	17 (18,9)

Table 2. Concentration of hydrogen peroxide by the FOX2 test for groups I and II.

Criteria		Group I	Group II	
		(n) [H_2O_2] (µM)	(n) [H_2O_2] (µM)	P
Age group (years)	20 – 35	(52) 0,0676	(61) 0,6029	0,00
	36 – 50	(20) 0,0564	(26) 0,6652	0,00
	>50	(18) 0,0856	(3) 0,5750	0,01
Consumption of alcoholic beverage	No	(9) 0,0191	(13) 0,5288	0,00
	Yes	(72) 0,0803	(69) 0,6651	0,00
	Ex consumer	(9) 0,0643	(8) 0,6182	0,01
Smoking habit	No	(52) 0,0015	(70) 0,3946	0,00
	Yes	(21) 0,1072	(12) 0,6567	0,00
	Ex-smoker	(17) 0,0375	(8) 0,6041	0,00

Table 3 - Correlation between the Group I and Group II hydrogen peroxide concentration (HPC) variables and the concentrations of total cholesterol (TC), LDL cholesterol (LDL), HDL cholesterol (HDL) and triglycerides (TG) in the fasted state

Group I	TC	LDL	HDL	TG
HPC	0,187 (NS)	0,126 (NS)	0,066 (NS)	0,270*
Group II				
HPC	0,388*	0,328*	-0,184 (NS)	0,075 (NS)

NS: not significant *: $p < 0,05$.

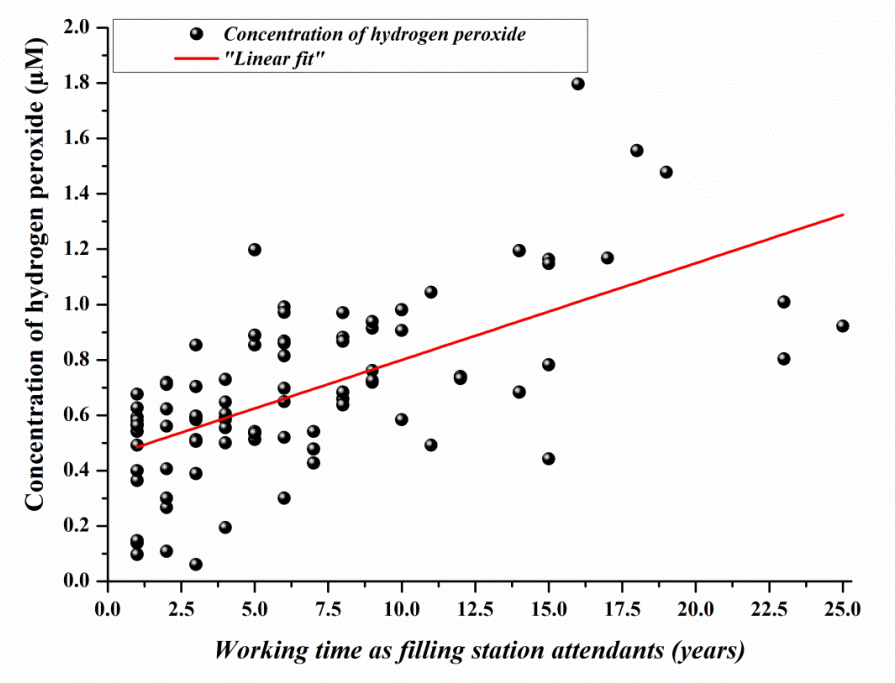

Figure 1. Linear regression analysis between the measures of concentration of hydrogen peroxide by the FOX2 method with the working time as a filling station attendants

than that of the control group. And for the age group above 50 years of age, the concentration of hydrogen peroxide was about 7 times higher than that of the control group (Table 2).
The FOX2 trial was successfully used to study circulating levels of lipid hydroperoxides in apparently healthy patients, as well as in a large number of clinical situations close to oxidative stress. Three studies with 23, 23 and 41 healthy patients indicated the mean lipid hydroperoxide content as being 3.02 ± 1.85 μM, 3.76 ± 2.48 μM, and 4.1 ± 2.2 μM, respectively (Nouroozzadeh *et al.* 1995, Nouroozzadeh *et al.* 1996, Nouroozzadeh *et al.* 1997). In two separate studies in type II diabetic patients, for 22 and 81 samples, concentration values were obtained 9.04 ± 4.3 μM and 9.4 ± 2.48 μM simultaneously (Nouroozzadeh *et al.* 1995, Nouroozzadeh *et al.* 1997). In addition, 67 patients with chronic renal failure had a mean level of lipid hydroperoxide concentration of 5.95 ± 3.04 μM. The mean plasma lipid hydroperoxide level in 52 hypercholesterolemic patients was 4.4 ± 2.1 μM. It is important to report that there is no influence of the patient's condition being fasted or not. The coefficient of variation for individual plasma is less than 10% for trials using FOX2 (Nouroozzadeh *et al.* 1994; Nouroozzadeh *et al.* 1995, Nouroozzadeh *et al.* 1996, Nouroozzadeh *et al.* 1997).

In another study, measurements were made of four important oxidative stress parameters in plasma from 23 healthy subjects, and then measurements were repeated on the same samples held at -70°C after different time intervals. Hydroperoxides and total antioxidant capacity were determined by oxidation of ferrous ions in the presence of xylenol orange and ferric antioxidant power, respectively. The sulfhydryl and carbonyls were measured spectrophotometrically. In fresh samples, oxidative stress appeared to increase with age and relatively good correlations were found between different parameters. The extent of changes in hydroperoxide concentrations varied considerably from one subject to another, even after 1 day. Similar phenomena were observed in the total antioxidant capacity, but after 7 days, suggesting that measurements of hydroperoxides in fresh samples should be carried out within a maximum of one week from the collection of the samples (Firuzi *et al.* 2006).

Oxidative stress was also evaluated during hemodialysis with bicarbonate, and the results were consistent with (1) the hypothesis that superoxide generated during hemodialysis reacts with bicarbonate to form toxic carbonate and formic radicals and (2) patients undergoing dialysis with bicarbonate (but not lactate) had increased plasma concentrations after hemodialysis. Thus, the results suggested an increase in total plasma concentrations of glutathione and hydroperoxide as a result of lipid peroxidation by reactive species (Epperlein *et al.* 1998). In streptozotocin-induced diabetic rats for 9 weeks, plasma lipid hydroperoxides were measured by ferrous oxidation with xylenol orange II (FOX method) and red blood cell membrane malondialdehyde and related aldehydes as thiobarbituric acid reactive substances. Plasma lipid hydroperoxide was higher in induced diabetic rats versus control rats (Ihm *et al.* 1999). Urinary concentrations of hydrogen peroxide in healthy people and in cancer patients showed significant differences, and may be indicated as a marker of oxidative stress in cancer patients (Banerjee *et al.* 2003). In a more recent study, 30 patients diagnosed with osteomyelitis and 30 healthy volunteers were evaluated. Serum concentrations of lipid hydroperoxide were measured by ferrous oxidation with xylenol orange (FOX) assay.

Serum lipid hydroperoxide concentrations were significantly higher in patients with osteomyelitis than in controls, and may be related to increased oxidative stress and inflammatory conditions present in these patients, which may cause a much more severe disease (Venturini *et al.* 2012). In patients with chronic pancreatitis which were compared with healthy controls, there was an increase in lipid hydroperoxide levels, and consequently an increase in the consumption of antioxidants in the pancreatic tissue. Nevertheless, this study showed a lack of significant difference in serum lipid hydroperoxide levels in patients with chronic pancreatitis versus controls, suggesting that oxidative stress is limited to the pancreatic tissue, and is therefore indicative of an organ-specific pathology, confirmed by lipid hydroperoxides and conjugated dienes and pancreatic inflammation indexes (amylase and lipase) (Santini *et al.* 2003).

Analysis of plasma total peroxide levels in patients with preeclampsia, which is one of the most serious complications of pregnancy, has shown that total plasma peroxide levels are significantly elevated (Harma *et al.* 2005). The increase in oxidative stress measured is usually associated with inflammatory processes (Araújo *et al.* 2009). Pinho *et al.* (2010) points out that factors such as atherosclerosis, smoking, diabetes mellitus, hypertension, hypercholesterolemia and others, are related to increased free radical formation. The formation of excess ERO promotes the direct formation of cytotoxic species and the inactivation of nitric oxide (NO). This inactivation leads to the loss of the beneficial effects of NO, such as regulation of arterial tone, inhibition of local inflammation and coagulation, as well as cell proliferation. The exposure time of the gasoline increases the levels of hydroperoxide lipid concentration in the blood plasma, a fact corroborated by the linear regression model applied (Figure 1). The exposure time is pre-potently significant for raising the levels of lipid hydroperoxide concentration for the researched gas station attendants. In studies of occupational exposure to benzene in fuel workers, the Cometa Test was used as a genotoxicity biomarker. The biomarker of benzene exposure, trans-muconic acid, allowed to verify that the exposed population studied was in contact with the substance and that the increased levels of this biomarker for fuel fronts characterized occupational exposure (Campos *et al.* 2017, Rabelo *et al.* 2017, Valente *et al.* 2017). In the city of Bangalore, India, there was an evaluation of an 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine biomarker in the urine of the gas station attendants that can characterize oxidative DNA damage. In this research, the relation of the increase of the biomarker concentration with the chemical exposure was verified (Beerappa *et al.* 2013).

Excessive exposure to gasoline may cause early hematological and immunological changes, such as inhibition of ALA-D activity, inhibition of CD80 and CD86 expression in monocytes, and elevation of IL-8 levels. Measures taken together may contribute to the early detection of benzene-induced changes in the immune system following occupational exposures. It is necessary to promote the development of preventive health measures for exposed workers, which will ultimately improve their quality of life (Moro *et al.* 2015). One measure to improve the quality of life of workers exposed to gasoline was the promotion of a healthy diet with a diet rich in fruits and vegetables, which demonstrated an inverse association with levels of oxidative stress markers, suggesting a protective role of ingestion of antioxidant foods in workers

exposed to oxidizing agents (Costa *et al.* 2016). Blood samples that were used for biochemical determinations, both lipid and FOX2, were used to show correlation with the lipid profile (Table 3). Group I (control) showed a positive correlation between the triglycerides and the lipid hydroperoxide levels presented, and the triglyceride levels are within the reference values considered normal. In relation to Group II (gas station attendants) there was a significant positive correlation with the increased levels of CT and LDL, the levels of lipid hydroperoxide, where these lipid profile parameters were above the reference levels classified as normal. Nouroozzadeh *et al.* (1996) reported that hydroperoxides accumulate primarily (greater than 65%) in LDL, as assessed by hydroperoxide content by amount of protein or cholesterol, or expressed as a ratio of total hydroperoxide in plasma, while HDL appeared to be relatively resistant to hydroperoxide accumulation (Nouroozzadeh *et al.* 1996). Data suggest that oxidative stress is increased in patients with familial hypercholesterolemia and is particularly pronounced in those patients with vascular disease. It is possible that increased oxidative stress may precede the development of vascular disease (Nouroozzadeh *et al.* 2001). Compared with controls, subjects with untreated growth hormone deficiency had higher levels of LDL cholesterol, higher triglyceride levels, elevated hydroperoxide levels, but lower HDL cholesterol (Smith *et al.* 2002).

Oxidative stress in overweight individuals (BMI between 25 and 29.9 kg/m2) showed higher concentrations of hydroperoxide measured by chemiluminescence compared to the control group, higher concentrations of hydroperoxide and hydrogen peroxide determined by iron-xylenol orange oxidation compared to overweight and normal weight subjects (Venturini *et al.* 2012). In an investigation in patients with active disease and apparently in reasonably modest therapeutic regimens, oxidative stress was assessed by reduced plasma glutathione activity. The lipid peroxidation was expressed by malondialdehyde (MDA) and lipid hydroperoxide (ferrous xylenol orange oxidation [FOX]) in patients with pulmonary arterial hypertension and healthy non-smokers. The measured concentrations of MDA and lipid hydroperoxide FOX presented higher values in patients with pulmonary arterial hypertension, when compared to control. Thus, oxidative stress and inflammation are present in patients with pulmonary arterial hypertension, which was confirmed by the increase of lipid peroxidation, the reduction of GSH and the low concentrations of vitamin E. (Reis *et al.* 2013).

An important factor to be taken into consideration is the fact that Group II (gas station attendants) has a tendency to have higher rates due to the effects of ROS. Enzyme-linked immunosorbent assays may also be performed to check plasma oxidative stress and heat shock protein (HSP) levels in fuel workers. It has been shown that HSP levels are inversely correlated with SOD (superoxide dismutase) activities, showing a positive correlation between plasma HSP levels and plasma malondialdehyde (MDA) levels, indicating that plasma HSP levels can be used as a sensitive biomarker for workers exposed to gasoline (Xia *et al.* 2017). The use of enzymatic parameters of oxidative stress in fuel workers by the determination of enzyme activities revealed a decrease in the antioxidant test capacity with superoxide dismutase (SOD), reduced glutathione (GSH) (Onunkwor *et al.* 2004), catalase (CAT) and glutathione S-transferase (GST), which correspond to protective factors for oxidative stress (Moro *et al.* 2013), CAT acts on the degradation of reactive oxygen species (ROS) and GST, Phase II biotransformation, conjugates the glutathione in the benzene molecule by the connection with the thiol group, favoring the process of elimination of benzene by the organism. Other assays have demonstrated the activity of the CAT enzyme in whole blood using H2O2 as a substrate by the spectrophotometric method. The results of the biological analyzes, when compared between the groups by gender, found statistically significant differences for CAT results. These differences between the sexes, however, were not observed for the activity of the GST enzyme (Amaral *et al.* 2017).

Conclusion

Based on the data obtained, this study proved to be important for the region studied, since it provided previously non-existent data on the filling station attendants exposure to fuels, mainly gasoline, in the city of Teresina, capital of the state of Piauí. In addition, we showed the relationship of lipid hydroperoxide levels with some living habits of the studied gas station attendants. The results also point out that gasoline is a determinant of oxidative stress in gas station attendants, as evidenced by the comparative study of group I and group II. In this way, our study expects, mainly, to cooperate to increase future research in the area of occupational exposure in our State, in the Northeast region and in Brazil. It is important to point out that the results of high levels of lipid hydroperoxide observed during this research should be considered as indicative and not conclusive, since a greater monitoring is necessary in order that definitive conclusions can be elaborated that can favor the fuel station attendants , with the application of technical solutions to reduce the risks of exposure of fuel dispensers in the supply operations at the resale points, aiming at greater protection of the health of these workers.

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