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Effect of Chronic Exposure to Gas Flaring on Sex Hormones and Some Antioxidant Parameters of Men from Ebocha, Niger Delta Area of Nigeria

I. P. Mokelu[1*], G. O. C. Onyeze[1], L. A. Nwaogu[1] and C. U. Igwe[1]

[1]*Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The effect of pollution due to gas flaring on men native to Ebocha, in the Niger Delta Area of Nigeria was investigated.

Materials and Methods: Blood specimens from twenty approximately healthy men from Ebocha community within the age groups 30-34, 35-39, 40-44, 45-49, 50-54 years were screened. Control blood specimens were obtained from twenty approximately healthy men from Uzi, Owerri (a location with no history of petroleum hydrocarbon pollution) with the same age bracket. Standard analytical procedures were used to determine the concentrations of male sex hormones; testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Some antioxidant parameters; glutathione and ascorbic acid (vitamin C) were also determined. The activity of lactate dehydrogenase (LDH) was also assayed.

Results: Results obtained revealed that there was a significant ($p < 0.05$) decrease in testosterone concentrations of men from Ebocha within the age groups of 35-39, 45-49 and 50-54 in contrast to those within the age groups of 30-34 and 40-44 when compared to the control. There was a significant ($p < 0.05$) increase in the concentration of LH of only men from Ebocha within the age group 45-49 when compared to those from Owerri. There was no significant ($p < 0.05$) difference in

**Corresponding author: E-mail: ifeomamokelu@gmail.com, augustineairaodion@yahoo.com;*

the concentration of FSH of men within the age groups from both sites. However, there was a significant ($p < 0.05$) decrease in the concentrations of GSH for all age groups in men from Ebocha when compared to those from the control sites. The same trend was observed for ascorbic acid concentrations in men from Ebocha for all the age groups from 30-54 years old. There was a significant ($p < 0.05$) increase in LDH activity of men from Ebocha among all the age groups when compared to those from Owerri.

Conclusion: These findings show that testosterone, glutathione, ascorbic acid concentrations and lactate dehydrogenase activity in the blood specimens of men from Ebocha were affected and it is possibly due to the chronic exposure to gas flaring in the environment.

Keywords: Antioxidants; chronic exposure; gas flaring; Niger Delta; sex hormones.

1. INTRODUCTION

Crude oil exploration has been on in Nigeria since 1956 when crude oil was discovered in commercial quality as Oloibiri in the Niger Delta region, by Shell Darcy. The earnings from the petroleum industry in the country grow in 1960 and became high in the early 1970s, during the oil boom [1]. Conversely, petroleum exploration and exploitation in Niger Delta areas over the years have resulted in number of environmental, socio-economic and political problems in the region. Oil spillage and gas flaring have caused severe environmental changes, loss of plants, animals and human lives and loss of revenue to both the oil producing companies and the government [2].

The Niger Delta is home to about 20 million people and 40 different ethnic groups and is located in the Southern area of the Southern region of Nigeria [3] and is surrounded by towns and villages with fishing and farming s the prime industries that supports the regional economy. But the effects of natural gas flaring have tainted the fish supply with toxins as well as the fruits and vegetables that are invested and these have taken a toll on the health and wellbeing of the residents too [4].

Ebocha Egbema is an area in the Niger Delta where oil and gas activities have gone on for over fifty years and organisms grown in the area are exposed to the pollution in the environment. The pollutants include spilled crude oil and or its refined products, effluents with traces of heavy metals, particulates and toxicants from gas flaring and greenhouse gases [5]. The energy solution conference estimated that the Niger Delta region has about 123 gas flaring sites [6] and about 45.8 billion kilowatts of heat is released into the atmosphere from 1.8 billion cubic feet of gas burnt daily in the Niger Delta region, leading to temperatures that render large areas non- habitable [7]. These pollutants (crude oil and their products) are considered recalcitrant to biodegradation and persist in the ecosystem due to their hydrophobicity and low volatility [8].

On visiting the Ebocha-Egbema area in Niger Delta, one cannot help but notice the glaring evidence of the devastating impact of gas flares on the environment, people, animals, plants in the area. There is the presence of cloud saturated with thick smoke coming from gas stations accompanied with intense heat as one approaches them, this gives rise to the occurrence of acid rain in this areas, there is also soil and water pollution by oil spills, damaging wild life and vegetation [9]. Residents complain of corrugated roofs, corroded by the composition of the rain due to flared gas and residents also complain of health issues such as asthma associated with the flaring.

The incessant flaring of gas in Ebocha-Egbema in the Niger Delta for over five decades associated with pollution of soil, water and air results in negative consequences both to plants and animals [10]. This has led to many studies being carried out in the area. It has been shown that pollution in Ebocha-Egbema has adverse effects on some biochemical parameters of native fowl (*Gallus domestica*) [11] and the fruit juice of citrus plants (*Citrus Sinesis*) native to that environment in the Niger Delta area [12]. Although there has been studies and results on the effects of gas flaring on animals such as rats and other plants, little study on gas flaring associated with its impacts on human reproductive health and antioxidants has been carried out, hence the necessity for this study.

2. MATERIALS AND METHODS

2.1 Study Area

Ebocha-Egbema in Niger-Delta, an area with gas flaring activities for over five decades, was used

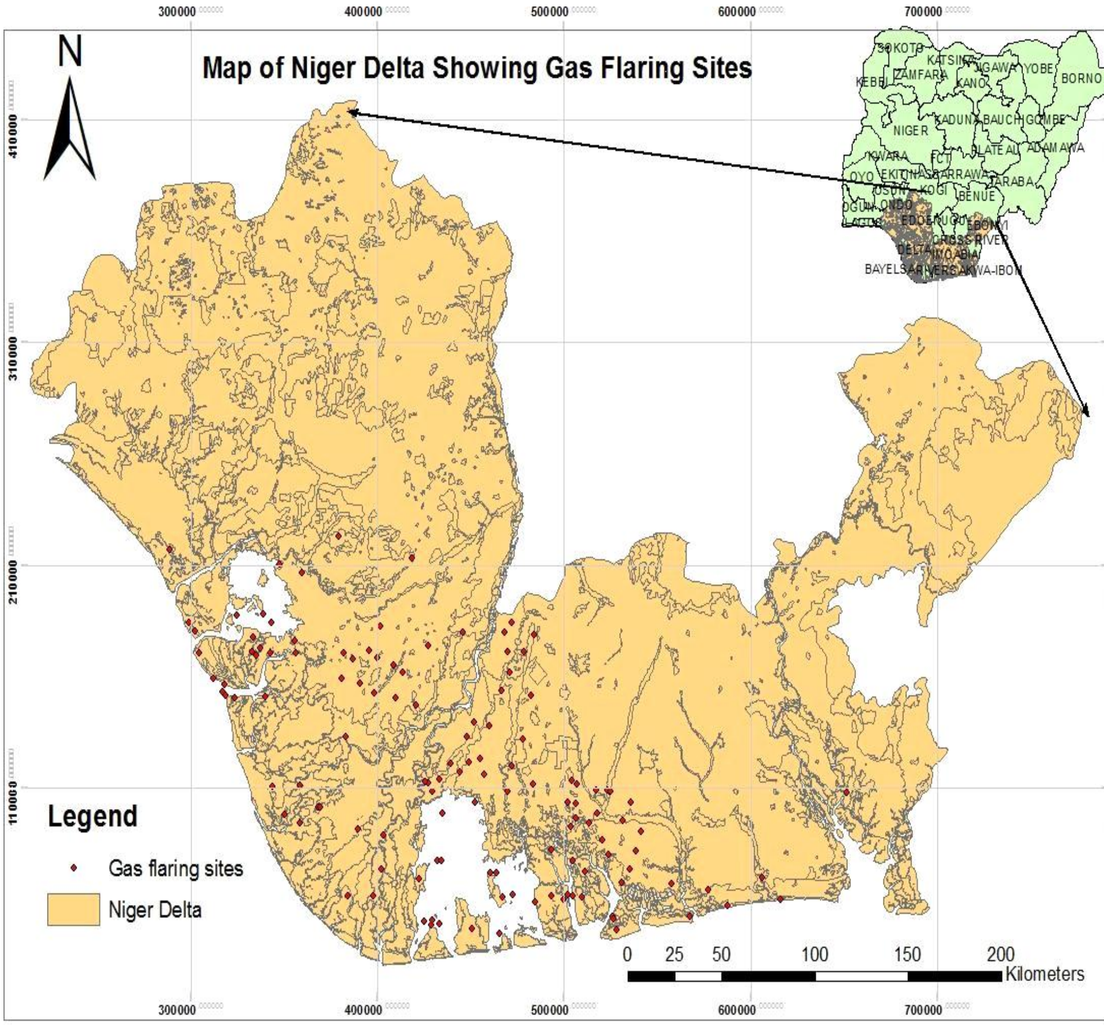

Fig. 1. Map of Niger Delta showing Gas Flaring sites

as the test site, while Uzi in Owerri, Imo State a non-gas flaring area was used as the control site (Fig. 1).

2.2 Criteria for Sample Collection

2.2.1 Inclusion criteria

Venous blood sample was taken from men between the ages of 30-50 each from both the test and control sites. For the test site they were indigenes or foreigners who have lived there for at least 20 years making them eligible as subjects for chronic toxicity study.

2.2.2 Exclusion criteria include

Men with sexually transmitted disease or infection, nonresidents, men less than the age of 30 were excluded from the study.

2.3 Sample Size

Populations of 40 men, 20 each from both the test and control sites were mobilized for blood sample collections.

2.4 Sample Collection and Analysis

Six milliliters of venous blood samples were collected from each subject and put into a set of plain bottles and Ethylene diamine tetra-acetic acid (EDTA); sample bottles for the different tests, blood samples placed in the plain bottles were allowed to clot and used for the hormonal test and, while blood samples placed in the EDTA bottles were used for the antioxidant test. The clotted samples were spun in a bench top centrifuge to obtain sera. The serum samples were separated into another set of plain sample tubes. The separated samples were stored in a refrigerator until they were required for use for hormonal concentration test. The serum samples were analyzed for Testosterone, Follicle stimulating hormone (FSH), and Lutenizing hormone (LH) using micro plate enzymes immunoassay methods. The respective immunoassay kits were obtained from Diagnostic Automation and microplate reader used in taking absorbance. Calculations of concentration of the hormones were made according to the method given in the kit handbook. While the samples placed in EDTA that contained the plasma samples were assayed for lactate dehydrogenase activity, and determined glutathione and ascorbic acid concentrations respectively.

2.5 Determination of Antioxidants

Lactate dehydrogenase (LDH) activities was assayed using Randox commercial Enzyme kits according to the method of Reitman and Frankel [13], Glutathione concentration was determined according to the methods previously described by Airaodion et al. [14], while vitamin C concentration was determined by the method of Benderitter et al. [15].

2.6 Determination of Sex Hormone Concentrations

Testosterone concentration was determined by the method of Tietz [16], luteinizing hormone concentration was determined by the method of Kosasa [17] and follicle - stimulating hormone concentration was determined by Marshall [18].

2.7 Study Limitation

This study did not indicate cause-effect relationship.

2.8 Statistical Analysis

Data generated was analyzed using Analysis of Variance (ANOVA) with the aid of Statistical Package for Social Science (SPSS) running on windows PC to determine any significant difference between the results from the test and control sites. Data from each parameter was expressed as mean value ± standard deviation.

3. RESULTS

The results of the effect of chronic exposure to gas flaring on antioxidant and sex hormones of men from Ebocha are presented in Figs. 2-7. The results obtained revealed that there was a significant ($p < 0.05$) decrease in testosterone concentrations of men from Ebocha within the age groups of 35-39, 45-49, and 50-54 in contrast to those within the age groups of 30-34 and 40-44 when compared to the control. There was a significant ($p < 0.05$) increase in the concentration of LH of only men from Ebocha within the age group 45-49 when compared to those from Owerri.

4. DISCUSSION

The liver function test is a very important index in accessing the level of oxidative stress as a result of pollution in the cell of organism due to its relevance in the survival of an organism [19]. Oxidative stress occurs when there is an overload of free radicals, exceeding the cells ability to protect it against them; these free radicals are produced as a result of chemical reactions from an oxidative process occurring in the cells, leading to chain reactions that may cause cell damage.

In this study, the enzymatic activity of lactate dehydrogenase (LDH) in the serum of men from Ebocha exposed to gas flares in all the age groups were compared to that of the men from Owerri, the serum activities of LDH of men exposed to gas flares in Ebocha was observed to be significantly ($p < 0.05$) higher than those from Owerri, who are not exposed, (336.55 ± 0.07 and 244.45 ± 5.59 respectively). This corresponds to the findings of Airaodion et al. [20] who reported a significant increase in the activity of LDH when animals were fed with crude oil-treated diet. Elevation in the activity of LDH sequel to hydrocarbon exposure might be an indication that hydrocarbon induced oxidative stress. Lactate dehydrogenase is an index of cell damage including hepatotoxicity and the endothelial disruption in blood vessel. The significant increase observed in the activity of LDH due to hydrocarbon exposure might be suggestive of the beginning of cytolysis, which is a possible indication of membrane damage including the endothelial membranes of blood vessels. This disruption of endothelial membrane, directly or indirectly includes the generation of reactive oxygen species in endothelial cells [21]. Free radicals attack unsaturated fatty acids in the membranes resulting in membrane lipid peroxidation which decreases membrane fluidity, leakage of enzyme

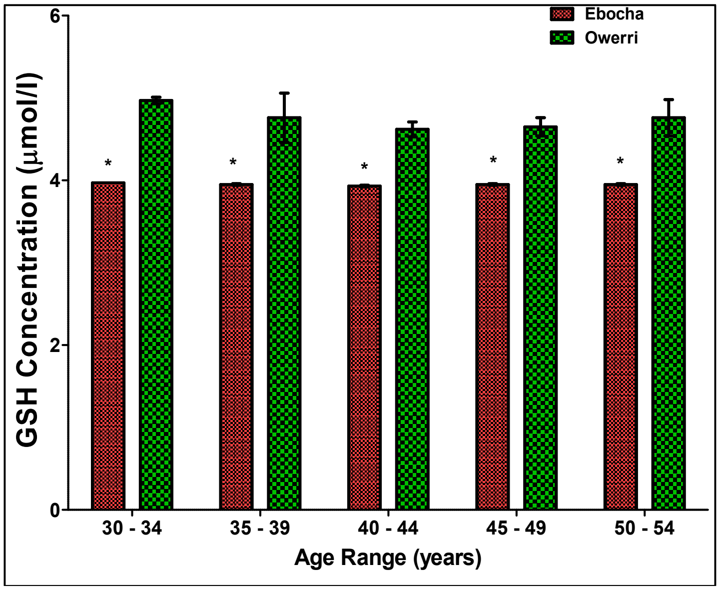

Fig. 2. Serum glutathione concentration by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean± standard deviation. Bars bearing * are significantly (p<0.05) different compared with their corresponding control values*

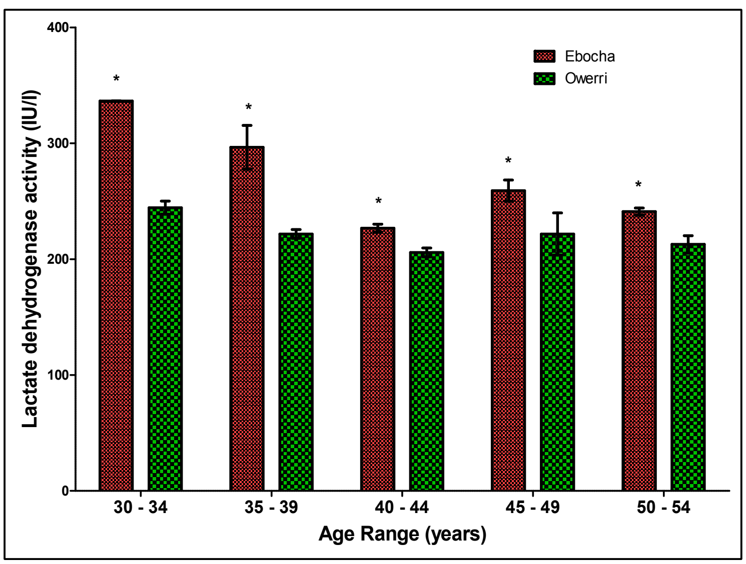

Fig. 3. Serum lactate dehydrogenase activity by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean± standard deviation. Bars bearing * are significantly (p<0.05) different compared with their corresponding control values*

and loss of receptor activity as well as damage membrane proteins leading to cell inactivation [22]. As lipid peroxidation progressively increase, antioxidant defense system decrease equivalently resulting in oxidative stress. This suggests that the exposure to hydrocarbon might have weakened the liver membrane with subsequent penetration and elevation of the hepatic biomarker enzymes.

In a situation where cells have a high production of reactive oxygen species, than the cellular antioxidants can defend, the cell's attempts to remove these toxic compounds results in oxidative stress, glutathione has been known to be involved in reducing these oxidative stress. In this study, there was also a significant ($p < 0.05$) decrease in serum glutathione of men from Ebocha in all age groups when compared with those of men from Owerri. This is similar to the significant decrease in GSH reported by Airaodion et al. [20] when they investigated the hepato-protective efficiency of ethanolic leaf extract of *Moringa oleifera* against hydrocarbon exposure. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism [23]. Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation [14]. Studies have shown that in a situation where the ratio between reduced glutathione (GSH) concentration to oxidized glutathione (GSSG) concentration in the cell is decreased, it is indicative of oxidative stress, although work on oxidized glutathione was not carried out in this study, the decrease in reduced glutathione concentration agrees with findings of Mehdi, *et al*. [24] in their report, serum and tissue concentrations of reduced glutathione in cancer patients were observed to be decreased compared to noncancerous patients, the justification for the results obtained was due to oxidative stress in cancer cell and the association of the content of reduced glutathione (GSH) with higher levels of GSH-related enzymes such as γ-gluthamyl-transpeptidase (GGT), γ-gluthamylcysteine ligase (CL).

Ascorbic acid is an effective quencher of singlet oxygen and other radicals. It has a vitamin E sparing antioxidant action, coupling lipophilic and *hydrophilic reactions* [25]. In this study, it was observed that the serum ascorbic acid concentrations in men from Ebocha who are exposed to gas flares had a significant decrease in their concentrations when compared with those in men from Owerri. Ascorbic acid is known to scavenge free radicals by reducing the already oxidized and unstable free radicals by donating a hydrogen atom to make them stable, oxidized ascorbic acids have no antioxidant activity which needs to be converted back into ascorbic acid by a donation of its two electrons (H^+), in a case where the free radical exceeds the ascorbic acid concentration, the resultant decrease occurs. An investigation by Fiaschi et al. [26] showed that plasma concentration of ascorbic acid were significantly ($p<0.05$) decreased in patients with oral cancer compared to the plasma of the control groups that were not cancerous.

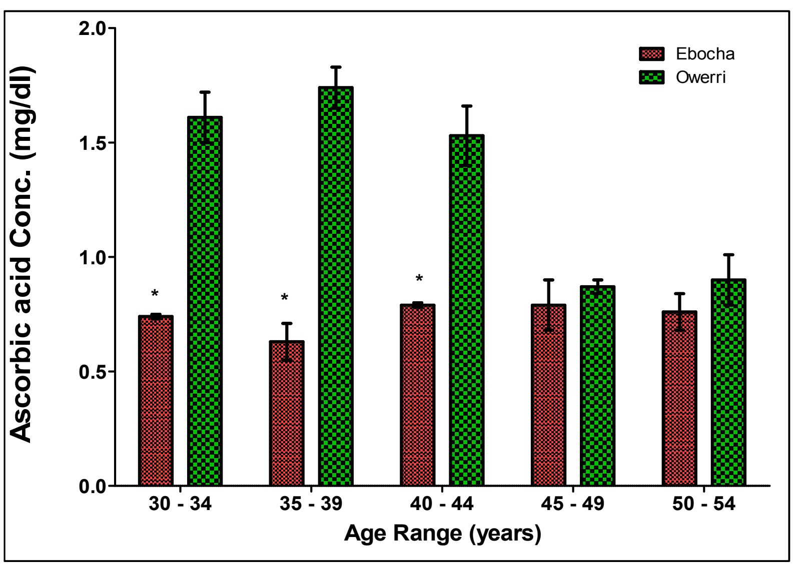

Fig. 4. Serum ascorbic acid concentration by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean± standard deviation. Bars bearing * are significantly (p < 0.05) different compared with their corresponding control values*

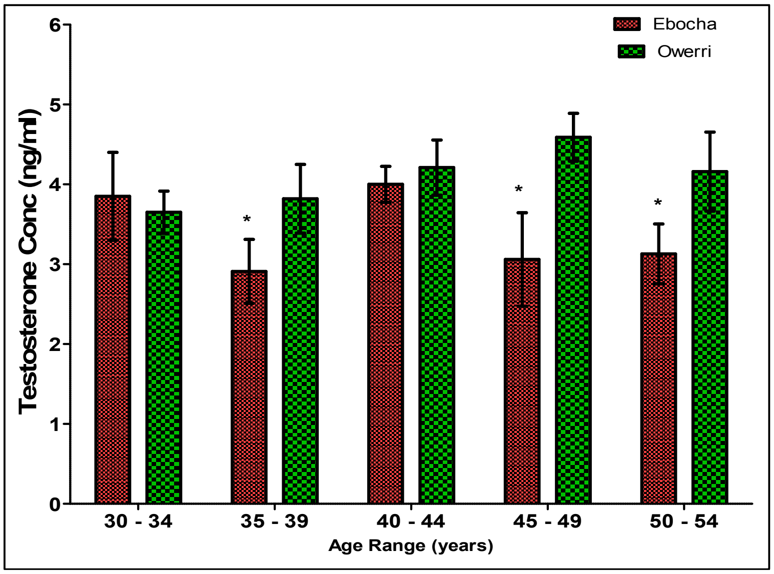

Fig. 5. Testosterone concentration by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean± standard deviation. Bars bearing * are significantly (p<0.05) different compared with their corresponding control values*

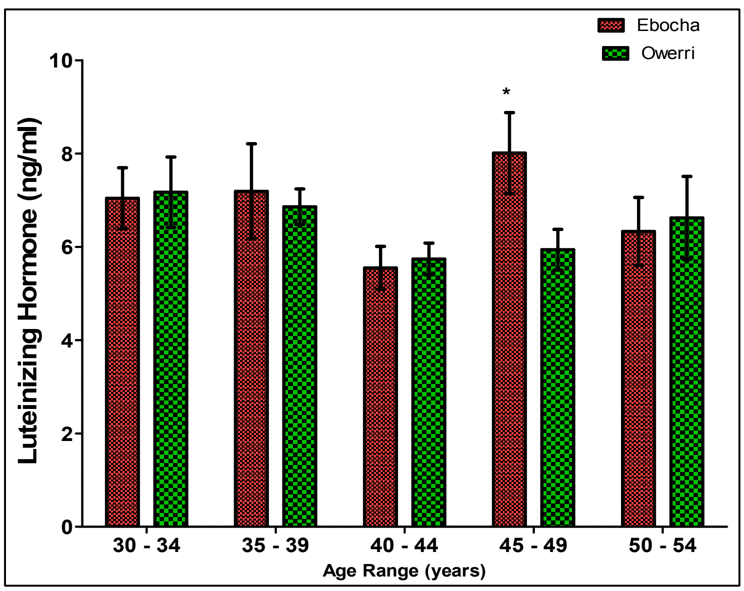

Fig. 6. Luteinizing hormone concentration by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean ± standard deviation. Bars bearing * are significantly (p < 0.05) different compared with their corresponding control values*

The reproductive functions and characteristics in males are regulated by the sex hormones [27]. The major sex hormones in males are the testosterone, luteinizing hormone and the follicle stimulating hormone; thus, to assess the reproductive integrity or fertility of a male (both animals and humans) these serum sex hormones profile are measured. There have been several factors known to be responsible for the alterations in sex hormone profiles which includes; aging, sedentary life styles, genetic factors, and in some studies, exposure to certain chemicals [28]. The male sex hormone; testosterone is primarily produced in the gonads under the influence of the follicle stimulating hormone and the luteinizing hormone which regulates it, an increase in the concentration of testosterone, exerts a positive feedback to the pituitary gland where the regulation of the secretions take place.

The results obtained in this study, showed that chronic exposure to gas flaring resulted in a significant ($p < 0.05$) decrease in the serum testosterone concentration of men from Ebocha of age groups 35-39, 45-49 and 50-54 who are exposed when compared to those in men from Owerri who are not exposed (3.85 ± 1.10 and 3.65 ± 0.53 respectively). The results obtained for serum testosterone which is the prevalent male sex hormone, agrees with the findings of Darbre, [29], *who* reported that air pollution can have serious hormonal effects, as many environmental pollutant chemicals have been shown to possess the ability to interfere in the functioning of the endocrine system and have been termed endocrine disrupting chemicals. The result of this present study is also consistent with the findings of Airaodion et al. [30] who reported a significant decrease in testosterone level in animals exposed to insecticides. Though the exact mechanism on how these hormones are affected by pollutants remain poorly understood, but it can be similar to that of the ubiquitous chemical bisphenol. A which looks and acts like sex hormones thereby binding to the receptors of the sex hormones causing a disruption in the functioning of the endocrine system and resulting in an alteration in sex hormone concentration.

Darbandi et al. [31] has reported that the pivotal hormonal regulators of male reproductive functions can be affected by the disruption of the balance between reactive oxygen species production and the antioxidant defense mechanism in the male reproductive system. Uncontrolled generation of reactive oxygen species may directly damage reproductive tissues or can interfere with the normal regulatory mechanisms of the hypothalamic-pituitary gonadal axis and its crosstalk with other endocrine axis, to adversely affect male reproductive functioning, thereby inducing male infertility [31]. From their study, following the generation of reactive oxygen species, the hypothalamic pituitary axis becomes activated and releases cortisol (in humans) in response to stress. These stress hormones, through the cross-talk between the hypothalamic-pituitary gonadal and hypothalamic-pituitary axis, negatively affect luteinizing hormone secretion from the anterior pituitary.

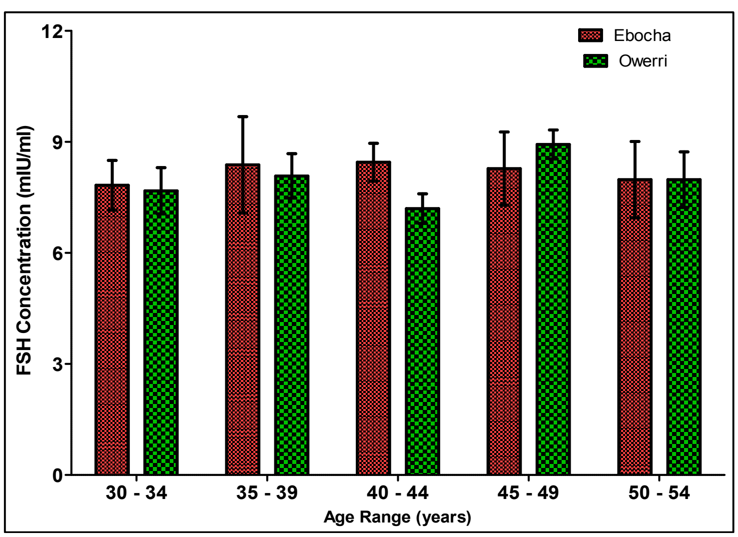

Fig. 7. Follicle stimulating hormone concentration by age of subjects from Ebocha (Test area) and Owerri (control area)

*Bars are mean ± standard deviation. Bars bearing * are significantly (p<0.05) different compared with their corresponding control values*

Decreased luteinizing hormone fails to stimulate Leydig cells to produce enough testosterone. Decreased follicle stimulating hormone diminishes the release of androgen-binding protein (ABP) from the Sertoli cells, and thus, an overall decline in circulating testosterone occurs during severe oxidative stress [32]. Increased oxidative stress also decreases the secretion of insulin from the pancreas which further negatively affects T_3 release from the thyroid gland and thus testosterone biosynthesis. Therefore, through its actions on an individual hormonal axis and/or by disrupting the cross-talk among different endocrine systems, reactive oxygen species can lead to decreased testosterone production as the outcome of endocrine disruption. Decreased testosterone fails to regulate spermatogenesis properly to produce enough mature spermatozoa [30]. It also fails to maintain the normal growth of accessory reproductive organs which play crucial roles in sperm maturation. As a prime regulator of male reproductive behavior, testosterone deficiency may lead to suppressed sexual behavior among men. Thus, by disrupting the endocrine reproductive functions, reactive oxygen species may result in male infertility.

The result obtained for luteinizing hormone concentration showed that there was a significant increase in age group 45-49 only, while the other age groups (30-34, 35-39, 40-44, 50-54) showed no significant ($p < 0.05$) difference, when compared with those in men from Owerri.

5. CONCLUSION

The results of this study showed that testosterone, glutathione, ascorbic acid concentrations and lactate dehydrogenase activity in the blood specimens of men from Ebocha were affected and it is possibly due to the chronic exposure to gas flaring in the environment. Further studies at molecular level can be confirmed.

CONSENT

As per international standard informed and written participant consent has been collected and preserved by the authors

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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