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Assessment of the Effect of Exposure to Plastics on Some Biochemical Parameters among Workers in Plastic Industries in Port Harcourt, Nigeria

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Abstract **Original Research Article**

Plastics have a widespread use globally. The chemicals referred to as additives used in its manufacturing are capable of exerting a number of toxicological effects on biological tissues especially the endocrine system. This cross sectional study was aimed at assessing the effect of exposure to plastics on some biochemical parameters among workers in plastic industries in Port Harcourt, Nigeria. The study population comprised of 80 subjects divided into two groups: the exposed group consisting of 40 workers, occupationally exposed to plastic additives and an age-matched control of 40 workers who were not occupationally exposed. Serum samples were obtained from the participants and laboratory investigations such as total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides, superoxide dismutase, reduced glutathione, malondialdehyde, testosterone and estrogen levels were determined using standard procedures. Statistical analysis was done using Graph Pad Prism version 5.03 and variation in means was considered significant at $p<0.05$. The results show a significantly decreased values in triglycerides and malonaldehyde, and increased values in testosterone, estrogen, testosterone/estrogen ratio when compared with the control group at $p<0.05$.There was, however, no significant difference among the groups in total cholesterol, high density lipoprotein, low density lipoprotein, atherogenic indices, superoxide Dimutase (SOD) and reduced glutathione. In conclusion, the exposed subjects exhibited a change in lipid profile, atherogenic indices, oxidative stress markers and reproductive hormone levels which may predispose them to cardiovascular diseases.

Keywords: Plastics, lipoprotein, testosterone, estrogen, liver enzymes.

INTRODUCTION

The plastic industry is one of the most profitable and significant industries worldwide with massive production each year. Plastic workers are occupationally exposed to variety of chemicals used in the production of plastics such as styrene, bisphenol A (BPA), phthalate, brominated flame retardants and heavy metals [1]. The main route of exposure to these chemicals in the industry is through inhalation; however, exposure can also occur because of ingestion or skin absorption [2]. Some individual chemicals or a mixture of them can contribute to the development of cancer (breast and prostate), metabolic disorders and reproductive problems [3]. This is because some of these chemicals are endocrine disrupting chemicals capable of distorting the regulated hormonal system of the body [4] and they tend to be ubiquitous in the industry. Significant adverse health effects by action at the endocrine level can be produced at concentrations thousands of times lower than presumably safe levels [5, 6].

Our everyday lives have been changed by the increasing use of plastic made products. The production in plastic industries started flourishing between the 1940s and 1950s and the annual global production of plastics in the last fifteen years has doubled to approximately 299 million tonnes in 2013. The global demand of plastics is, however, governed by thermoplastic types with polypropylene having 21%, low and linear low density polyethylene-18%, polyvinyl chloride-17%, high density polyethylene-15%, expandable PS-8%, polyethylene terephthalate-7% and the thermosetting plastic polyurethane [7].

One of the most significant industries in the globe is the plastic industry due to the widespread usage of its products. Workers employed in such industries are potentially exposed to the chemicals used in production referred to as plastic additives examples are bisphenols,

phthalates, polybrominated diphenyl ether, flame retardants and styrene [1]. Plastic polymers are used to make synthetic fibers, coatings, sealants used in many application, foams and consumer products. Almost all sectors of life, involve the use of plastics such as in transportation, telecommunications, clothing, footwear, packaging materials facilitating the transportation of food and drinks and other goods, also as innovative materials used as engineered tissues, prosthetics, absorbable sutures and other medical applications [8]. Nevertheless, huge amounts of plastic wastes are generated mainly due to diverse consumption and short life span of plastic products, these waste cause severe environmental hazard and management problems [9].

Inhalation is the basic route of exposure of these additives in the industry however; exposure can occur through ingestion or skin absorption [2]. Some individual additives or a mixture of them have been reported to cause adverse health effect such as breast and prostate cancers, metabolic disorders and reproductive problems probably due to the endocrine disrupting effects some of them possess [3]. The exact mechanism of these health effects is not fully understood but it is known that occupational exposure to some chemicals induce oxidative stress and DNA damages. Despite the proliferation of plastic industries in Port Harcourt, reports of studies on the implication of plastic products on the life of residents and or workers in these industries is rare. This study is thus designed to assess some biochemical parameters such as lipid profile, oxidative stress parameters and some reproductive hormones among workers in some plastic industries in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Study subjects

This study was conducted on eighty (80) subjects, forty (40) of which were employees of plastic industries along the Trans Amadi Industrial Layout in Port Harcourt, Nigeria and the other forty (40) were age matched unexposed subjects used as controls.

Inclusion criteria

Male workers exposed to plastic additives for at least one (1) year, who were non-smokers, do not consume alcohol and were not using medication or supplements were included in the study. Informed consent of participants was also obtained.

Exclusion criteria

Those who have worked in the industry for less than one (1) year, smokers and alcohol consumers, workers with metabolic diseases, chronic diseases, cardiovascular diseases with other illness and complications before employment to the factory and those on medication or multivitamin supplementation were excluded.

Ethical Approval

Ethical approval was obtained from the Rivers State Hospital Management Board before the commencement of the study. Questionnaires were used to collect information such as age, alcohol consumption, smoking status, employment history (including years of working in the industry and use of personal protective clothing), past history of disease (such as hypertension, diabetes mellitus).

Anthropometric and blood pressure measurement

The following anthropometric measurements of weight were done to the nearest 0.1kg and height to the nearest centimeter (cm) according to the method of [10]. Body mass index (BMI) which is defined as the ratio of weight (kg) by the square of the height (m^2) was measured in accordance with [11]. The blood pressure was recorded using a standard mercury sphygmomanometer and stethoscope. All blood pressure measurements were performed with the participants in a seated position for at least five minutes resting their feet on the floor comfortably. It was measured twice on the left arm on each subject and measurements were spaced 20 minutes apart and this was performed before collecting blood samples. The average of the two measurements was used for all analyses [12].

Sample Collection

About 5mls of venous blood after at least ten (10) hours of fasting was withdrawn (between 8:00am – 10:00am) by sterile syringe through venipuncture of the antecubital vein and put into plain tubes (no anticoagulant) and left to clot and then centrifuged at 1500 rpm for 10 minutes and stored in dry plain plastic screw-capped containers and samples were stored frozen at -20^0C until required for analysis.

Laboratory Analysis

Serum total cholesterol and HDL-C were analysed enzymatically by method described by [13]. Triglycerides were assayed using method described by [14]. LDL was calculated using Friedewald *et al.* [15] equation. Castelli Risk Index 1 & 11 (CRI- 1 & 11) and atherogenic index were calculated using established formulas as described by Akpinar *et al*. [16]. Atherogenic Index of Plasma (AIP) was calculated using base 10 logarithm of the ratio triglycerides to high density lipoprotein as described by Dobiasova & Frohlich, [17]. Superoxide Dismutase (SOD) activity was determined by a method described by Xin *et al*. [18]. Reduced glutathione (GSH) was determined according to the method of King & Wooton [19]. Malondialdehyde (MDA) was measured by the method described by Wallin *et al*. [20].Testosterone was determined by enzyme linked immunosorbent assay (ELISA) method described by Elder & Lewis, [21] and estrogen was determined by a method described by Manickum *et al*. [22]. Estrogen to Testosterone ratio was calculated as T/E2 as described by Gong *et al.* [23].

STATISTICAL ANALYSIS

All values were expressed as mean and standard deviation (SD) and the differences in more than two groups were determined by performing the one-way analysis of variance (ANOVA) while variation between two was done using the Student's t-test. The collected data were analyzed using Graph Pad Prism version 5.03 and the statistical significant difference was considered at $p < 0.05$.

RESULTS

Table 1 shows the demographic characteristics of the study population. Eighty (80) male participants were divided into exposed and control groups. Forty (40) subjects each in a group were enlisted into the study. Majority of the subjects were in age group 20-29 years in exposed group (47.5%) and 50% in control group followed by the age group 30-39 year with 37.5% in the exposed and 30% in the control group and then 15% in age group 40 and above in the exposed group and 20% in the control. Seventeen (17) subjects were found to be underweight representing 12.5% in exposed group and 30% in control group while 42.5% were overweight in the exposed group and 10% in the control group, however, 15% and 7.5% were obese in the exposed and control groups respectively. The time of occupational exposure was not less than 1 year.

Table-1: Demographic Characteristics of the Study Population

Parameters	Exposed	Control
Sex		
Male	40 (100%)	40 (100%)
Age (yrs)		
20-29	19(47.5%)	20(50%)
30-39	15 (37.5%)	12(30%)
40 & Above	6 (15%)	8(20%)
BMI groups (kg/m^2)		
<18.5	5 (12.5%)	12 (30%)
18.5-24.9	11(27.5%)	21 (52.5%)
25.0-29.9	17 (42.5%)	4 (10%)
> 30	6(15%)	3 (7.5%)
Time of exposure (yrs)		
1-3	25 (62.5%)	0
4 & Above	15 (37.5%)	0

BMI: Body Mass Index

In table 2, the average age (years) of the exposed and control groups was 30.98±7.26 years and 31.33 ±8.16 years respectively which was not statistically significant ($p>0.05$). The BMI of the exposed group was significantly higher than that of the control group ($p<0.05$). The average BMI of the exposed and control groups were 25.53 ± 5.82 kg/m^2 (borderline of overweight) and 21.79 ± 5.56 kg/m^2 respectively. The systolic pressure of the exposed group was 123.7±15.9 mmHg and it was significantly ($p<0.05$) increased than the value in the control group which was 114.6±13.5 mmHg depicting borderline hypertension. Also, the diastolic pressure was observed to be significantly increased in the exposed group (86.9±15.2 mmHg) than the control group (77.3±9.8mmHg) at $p< 0.05$.

Table -2: Comparison of Age, BMI and Blood Pressure of Exposed and Control Groups

Subjects	Age (yrs)	BMI (kg/m^2)	SBP (mm/Hg)	DBP (mm/Hg)
Exposed N = 40	30.98 ± 7.26	25.53 ± 5.82	123.7 ± 15.9	86.9 ± 15.2
Control N = 40	31.33 ± 8.16	21.79 ± 5.56	114.6 ± 13.5	77.3 ± 9.8
p-value	0.8399	0.0043	0.0068	0.0013
Summary	NS	S	S	S

Key: N – Number of subjects, NS – not significant, S – significant, BMI-Body Mass Index, SBP- systolic blood pressure, DBP- diastolic blood pressure

It was observed in table 3 that there were significant ($p<0.05$) increase in triglycerides level between exposed group (1.09±0.26 mmol/L) and control group (1.30±0.55mmol/L). No significant ($p>0.05$) differences were observed in TC, HDL-C and LDL-C although the level of HDL-C was higher in the control than in the exposed groups. LDL-C was, however, insignificantly ($p>0.05$) higher in the exposed than in the control groups.

Table-3: Lipid Profile among the Exposed and Control Groups

Subjects	TC (mmol/L)	Trigs (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Exposed N = 40	4.75 ± 1.04	1.09 ± 0.26	1.26 ± 0.23	3.00 ± 0.86
Control N = 40	4.68 ± 0.91	1.30 ± 0.55	1.30 ± 0.23	2.79 ± 0.92
p-value	0.7321	0.0315	0.4587	0.2970
Summary	NS	S	NS	NS

Key: N = Number of subjects, NS = not significant, S = significant, TC=total cholesterol, Trigs = Triglycerides, HDL-C = high density lipoprotein cholesterol, LDL- low density lipoprotein cholesterol.

Table-4: Comparison of Lipid Ratios between the Exposed and Control Groups

Subjects	TC/HDL	LDL/HDL	Non-HDL/HDL	AIP
Exposed N = 40	3.82 ± 0.74	2.42 ± 0.71	2.82 ± 0.74	0.09 ± 0.01
Control N = 40	3.74 ± 1.01	2.26 ± 0.89	2.74 ± 1.01	0.08 ± 0.01
Risk level*	>3.5	>3.3	>3.37	>0.1

Key: N = Number of subjects, NS = not significant, S = significant, TC= Total Cholesterol, Trigs = triglycerides, HDL = high density lipoprotein cholesterol, LDL= low density lipoprotein cholesterol, AIP- Atherogenic Index of Plasma. *Milan *et al*. [24]

The Castelli Risk index I shows moderate risk for both the exposeed and control groups while Castelli Risk Index II and non-HDL –C level was normal for both populations. AIP computed for both populations was also normal. These ratios were computed based the recommendation as reported by Milan *et al*. [24].

Table-5: Oxidative Stress Parameters in Exposed and Control Groups

Subjects	SOD (IU/L)	GSH (mg/dL)	MDA (nmol/L)
Exposed N = 40	36.46 ± 3.39	2.59 ± 0.71	5.43 ± 2.14
Control N = 40	37.33 ± 2.52	2.43 ± 0.31	7.36 ± 2.04
p-value	0.1963	0.1857	< 0.0001
Summary	NS	NS	S

Key: N – Number of subjects, NS – not significant, S – significant, SOD- Superoxide Dismutase, GSH- reduced glutathione, MDA-Malondialdehyde

In the exposed group, MDA level was significantly reduced (5.43±2.14nmol/l) when compare to the control group (7.36±2.04nmol/l) at $p<0.05$ while SOD and GSH showed no significant ($p>0.05$) difference in both groups (Table 5).

Furthermore, table 6 shows a significantly higher testosterone concentration in the exposed group (6.73±1.64 ng/ml) than in the control group (4.22±0.65ng/ml) at $p<0.05$. Similarly in the concentration of estrogen, a significant increase was observed in the exposed group having 18.46± 4.84 pg/ml when compared to the control group (15.95±2.61 pg/ml) at $p<0.05$. The values obtained for the exposed group in testosterone/estrogen ratio was 0.38±0.10 and control group 0.27±0.05 respectively and the value was significantly higher in the exposed group at $p< 0.001$. However, the ratio is less than the reference risk limit of 1.7 ±0.12 Fejes *et al*. [25].

Table-6: Comparison of Testosterone, Estrogen, Testosterone/Estrogen Ratio in Exposed and Control Groups

Subjects	Testosterone (ng/mL)	Estrogen (pg/mL)	Testosterone/Estrogen ratio
Exposed N = 40	6.73 ± 1.64	18.455 ± 4.84	0.38 ± 0.10
Control N = 40	4.22 ± 0.65	15.95 ± 2.61	0.27 ± 0.05
p-value	< 0.0001	0.0052	*1.7 ± 0,12
Summary	S	S	S

N – Number of subjects, NS – not significant, S – significant, *Normal value: Fejes *et al*. [25].

Table 7 compares testosterone, estrogen and testosterone/estrogen ratio among different age groups in the exposed group. Subjects within the age group of 30-39 years had significantly ($p<0.05$) higher testosterone concentration of 7.71±1.29 ng/ml than subjects within the age range of 20 – 29 years (6.40 ± 1.70 ng/mL) and 40 years and above (5.33 ± 0.53 ng/mL). Estrogen level across the age ranges did not show any significant variation ($p>0.05$). The testosterone/estrogen ratio within the age ranges studied was within the normal reference value.

Table-7: Comparison of the effect of Age on Testosterone, Estrogen and Testosterone-Estrogen Ratio among Different Age Groups in the Exposed Group (Mean ±SD)

Age Groups	Testosterone (ng/mL)	Estrogen (pg/mL)	Testosterone/ Estrogen ratio
20 – 29 Yrs N = 19	6.40 ± 1.70[a]	18.68 ± 5.25	0.36 ± 0.11
30 – 39 Yrs N = 15	7.71 ± 1.29[b]	18.93 ± 4.80	0.42 ± 0.08
40 Yrs and above N = 6	5.33 ± 0.53[ac]	16.50 ± 3.62	0.34 ± 0.07
p-value	0.0030	0.5692	-
F-value	6.823	0.5723	-
Summary	S	NS	-

Key: N – Number of subjects, NS – not significant, S – significant, mean ± SD with the same superscripts are not statistically different.

Table 8 presents the effect of duration of exposure of the workers in years on the levels of the reproductive hormones and its ratios. The number of years of exposure to plastic products within the population studied did not significantly ($p>0.05$) affect the levels of testosterone, estrogen and testosterone /estrogen ratio.

Table-8: Effect of Work Duration on Testosterone, Estrogen and Testosterone-Estrogen Ratio

Work Duration	Testosterone (ng/mL)	Estrogen (pg/mL)	Testosterone Estrogen ratio
1 – 3 Yrs N = 25	6.74 ± 1.65	18.20 ± 5.01	0.39 ± 0.10
4 Yrs and above N = 15	6.71 ± 1.68	18.87 ± 4.67	0.36 ± 0.09
p-value	0.9552	0.6786	0.4780
Summary	NS	NS	NS

N – Number of subjects, NS – not significant, S - significant

DISCUSSION

The presence of plastic materials can be found everywhere in the Nigerian environment causing the human body to be bombarded daily by it. In this study, it was found that BMI, diastolic and systolic blood pressures were significantly ($p<0.05$) elevated in workers occupationally exposed to plastics than the controls. This finding agrees with the report of Mungreiphy *et al*. [26] who stated that individuals who work in plastic industries develops high BMI with resultant increase in their blood pressures. The study of Balakumar *et al*.[27] had reported that plastic additives are associated with cardiovascular diseases by causing an increase in the systolic and diastolic blood pressures, a finding that was also observed in this study.

Our data showed that in the majority of the exposed group, total cholesterol, HDL-C and LDL-C levels were not significantly ($p>0.05$) different from the controls. A significantly ($p<0.05$) decreased level of triglycerides though within the normal range was observed in the exposed group. This agrees with the findings which reported that smaller doses of exposure to plastic additives such as poly brominated diphenyl

ether (PBDE) suppresses the uptake of lipid by the liver resulting in the accumulation of serum triglycerides, however, higher doses induce the uptake and accumulation of lipid by the liver resulting in low serum triglycerides concentration [28]. The values for lipid ratios reveal that both the exposed and control groups were at moderate risk of cardiovascular disease when Castelli Risk Index I was used to evaluate the atherogenic potential of the studied population. Castelli Risk Index II, AIP and non-HDL levels were normal within both the exposed and control populations indicating that the likelihood of development of cardiovascular disease may be low in the studied subjects.

MDA is one of the aldehydes which are formed as secondary products of lipid peroxidation and appears to be the most mutagenic products of lipid peroxidation [29 30]. The concentration MDA in the exposed population was observed to be significantly ($p<0.05$) decreased indicating the absence of lipid peroxidation. This could be attributed to the elevated though not significant concentration of GSH and SOD elucidating a cell defence response or the presence of a stimulant effect of chronic exposure resulting in enhanced resistance to oxidative stress in occupationally exposed individuals as reported by Eken *et al*. [31].

It has been reported that plastic additives have endocrine disrupting effects resulting in elevated levels of testosterone, estrogen as well as the testosterone/estrogen ratio [32]. The present study shows significantly ($p<0.05$) elevated concentrations of these hormones in the exposed group. Elevated concentrations of this hormone in individuals exposed to plastic additives have been linked to an increased risk for heart disease and certain cancers and can as well affect the motility of sperm by lowering its percentage [33]. Mann, [34] had earlier reported that men exposed to high levels of plastic additives such as bisphenol A (BPA) show a small but significantly increased testosterone concentration still within the normal range suggestive of the fact that BPA being anti-androgenic could block the normal action of testosterone in the body thereby stimulating the body to produce more testosterone to overcome it. It could exert its anti-androgenic activity indirectly through the regulation of aromatase enzyme to convert androgens to estrogens [35]. Furthermore, BPA is known to have a high potency on estradiol even at low concentrations [36].

In the present study, decline in testosterone and estrogen concentrations after age 40 years was observed. This finding agrees with the report of other investigators who in their studies explained that this observation may represent a combination of factors such as insulin resistance or depression, decrease in function, number or responsiveness of secretory cells of the testicle [37, 38]. It was observed in this study that duration of exposure had no effect on hormonal levels. This observation may probably be due to the fact that these plastic additives especially BPA have a long term adverse effect. Being that it is lipid soluble, a fraction that is absorbed is distributed to sites of storage in the body followed by a slow release into the blood stream resulting in a low dose continuous exposure as reported by Frenandez *et al*. [39] or it may also be that the years of exposure and concentration of the additives to which the subjects were exposed were not long and high enough to initiate adverse effects.

LIMITATION

The results were obtained on a limited number of subjects hence it deserves further investigations on a larger population of workers which could be a tool useful in biomonitoring of workers occupationally exposed to hazardous substances.

CONCLUSION

It is evident that the slight change in lipid profile, lipid ratios and reproductive hormones observed in this study indicates that workers in plastic industries could chronically be occupationally exposed to substances that could pose an effective risk on their health which could manifest as cardiovascular disease, sexual dysfunction and infertility since plastics additives are potential endocrine disruptors.

REFERENCES

1. Halden R. Plastics and health risks. Annual Review of Public Health. 2010; 31: 179–194.
2. Ormond G, Nieuwenhunjsen MJ, Nelson P, Elliot P. Endocrine disruptions in the workplace, hairspray, folate supplement, and risk of hypospadias: case control study. Environmental Health Perspectives. 2009; 117: 303-307.
3. Eskenazi B, Warner M, Samuels S, Young J, Gerthoux PM. Serum dioxin concentrations and risk of uterine leiomyoma in the Seveso Women's Health Study. American Journal of Epidemiology. 2007; 166: 79–87.
4. Wolstenholme JT, Rissman EF, Connely JJ. The Role of Bisphenol A in shaping the brain, epigenome and behaviour. Hormonal Behaviour. 2010; 10(2): 13-20.
5. VomSaal FS, Hughes C. An extensive new literature concerning low-dose affects of bisphenol A shows the need for a new risk assessment. Environmental Health Perspective. 2005; 113: 926–933.
6. Welshons WV, Nagel SC, Vom Saal FS. Large effects from small exposures. III. Endocrinemechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. 2006; 147: 56–69.
7. Market Research Statistic Group Plastics Europe. Business Data and Carts 2007.StatusSeptember

2008. Plastics Europe Association of Plastics Manufacturers, 2008
8. Andrady AL, Neal, MA. Applications and societal benefits of plastics.Philosophical Transactions Royal Society B. London. Biological Sciences. 2009; 364: 1977–1984
9. Achilias DS, Roupakias C, Megalokonomos P, Lappas AA, Antonakou EV. Chemical recycling of plastic wastes made from polyethylene (LDPE and HDPE) and polypropylene (PP). Journal of Hazardous Materials. 2007; 149: 536–542.
10. Hill A, Roberts J. Body mass index: A comparison between self-reported and measured height and weight. Journal of Public Health. 1998; 20: 206-210.
11. World Health Organisation (WHO). The world health report: Reducing risks, promoting healthy lifestyles. Geneva, 2002.
12. Famodu AA, Osilesi O, Makinde YO, Osonuga OA. Blood pressure and blood lipids levels among vegetarian, semi vegetarian and non-vegetarian native Africans. Clinical Biochemistry. 1998; .317: 545-549
13. Allain CC, Poon CS, Chan CG, Richmond W, Fu FC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974; 20: 470-475.
14. Friedman M, Rosenman RH. Association of specific overt behavior pattern with blood and cardiovascular findings. Journal of America Medical Association. 1959; 169: 1286-1296.
15. Friedewald WT, Levy RI, Fredickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972; 18: 499-502.
16. Akpinar O, Bozkurt A, Acarturk E, Seydaoglu G. A new index (CHOLINDEX) in detecting coronary artery disease risk. Anadolu Kadiyol Derg. 2013; 13(3): 5-9
17. Dobiasovo M, Frolich J. The plasma parameter log(TG/HDL) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apo B lipoprotein depleted plasma. Clinical Biochemistry. 2001; 34(7): 583-585
18. Xin Z, Waterman DF, Henken RM, Harmine RJ. Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in stress. Journal of Diary Science. 1991; 74: 3078 – 3082.
19. King KJ, Wooton IP. Special constituents and drugs. In: Microanalysis in Medical Biochemistry, 5th efition, Churchill Livingstone, Edinburgh. 1974; pp 129-152.
20. Wallin B, Rosengren B, Shertzer HG, Cameyo G. Lipoprotein oxidation and measurement of TBARS formation in a single microlitrepeate; its use for evaluation of antioxidants. Annual Review Medicine. 1993; 208: 10-15.
21. Elder PA, Lewis JG. An enzyme-linked immunosorbent assay (ELISA) for plasma testosterone. Journal of Steroid Biochemistry. 1985; 22(5): 635-638.
22. Manickum T, John W. The current preference for the immuno-analytical method for quantification of steroid hormones (endocrine disruptor compounds) in waste water in South Africa. Analytical and Bioanalytical Chemistry. 2015; 407(17): 4949-4970.
23. Gong Y, Xiao H, Li C, Bai J, Cheng X, Jin M. Elevated T/E_2 ratio is associated with an increased risk of cerebrovascular disease in elderly men. PloS ONE. 2013; 8(4)
24. Milan J, Pinto X, Munoz A, Zuniga M, Rubies-Prat J, Pallardo LF, Masana L. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. Vascular Health and Risk Management. 2009; 5: 757-765
25. Fejes I, Koloszar S, Kawaczki Z, Daru J, Szollossi J, Pal A. Effect of body weight on testosterone/ estrogen ratio in oligozoospermic patients. Archives of Andrology. 2006; 52(2): 97-102
26. Mungreiphy NK, Kapoor S, Sinha R. Association between BMI, Blood pressure and Age: study among Tangkhul Naga tribal males of North East India. Journal of Anthropology. 2011; 748147: 1-6.
27. Balakumar M, Raji L, Prabhu D, Sathishkumar C, Prabu P, Mohan V, Balasubramanyam M. High-fructose diet is as detrimental as high-fat diet in the induction of insulin resistance and diabetes mediated by hepatic/pancreatic endoplasmic reticulum (ER) stress. Molecular and Cellular Biochemistry. 2016; 423(1-2): 93-104.
28. Ahmed K, Sebnem EC, Stephanie H, Sridurgadevi K, Monika AR, Alexander S. Developmental exposure to $2,2^1,4,4^1$- tetrabromodiphenyl ether permanently alters blood-liver balance of lipids in male mice. Frontiers of Endocrinology. 2018; 9: 548 – 552.
29. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malondialdehyde, and 4-hydroxynonenal. Methods in Enzymology. 1990; 186: 407-421
30. Esterbauer H, Eckl P, Ortner A. Possible mutagens derived from lipids, and lipid precursors: Mutation Research. 1990; 238(3): 223-233.
31. Eken A, Aydin A, Erdem O, Akay C. Induced antioxidant activity in hospital staff occupationally exposed to ionizing radiation. International Journal of Radiation. 2012; 88(9): 648-653.
32. Takeuchi T, Tsutsumi O. Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels. Biochemical Biophysiology Research Communique. 2002; 291: 76–78.
33. Lassen TH, Frederiksen H, Jensen T K, Peterson JH, Joensen UN, Main KM, Anderson AM. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. Environmental Health Perspectives. 2014; 122: 478-484.

34. Mann D. BPA linked to higher testosterone levels. Environmental Health Perspectives. 2010; 117: 720-728.
35. Kok-yong C, Kok-lun P, Wun-fui ML. A review on the effects of Bisphenol A and its derivatives on skeletal health. International Journal of Medical Science. 2018; 15(10): 1043-1050.
36. Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM. In vitro molecular mechanisms of bisphenol A action. Reproductive Toxicology. 2007; 24 (2): 178-198.
37. Wu M. Multiparameter metabolic analysis reveals a close link between attenuated mitochondrialbioenergetic function and enhanced glycolysis dependencyin human tumor cells. American Journal of Physiology and Cell Physiology. 2007; 292: 125-136.
38. Bhasin S, Bremmer WJ. Emerging issues in androgen replacement therapy. Clinical review. Journal of Clinical Endocrinology and Metabolism. 1997; 82: 3-8.
39. Fernandez MF, Arebola JP, Taoufiki, J, Nafalon A, Ballestews O, Pulgar R,Vilchez J L, Olea N. Bisphenol-A and chlorinated derivatives in adipose tissue of women. Reproductive Toxicology. 2007; 16: 117-122.