

OCCUPATIONAL EXPOSURE TO BISPHENOL A (BPA) AND ITS RELATIONSHIP WITH SERUM TESTOSTERONE, ADIPONECTIN, ESTRADIOL AND INSULIN RESISTANCE

ABSTRACT

Studies on occupational exposures to Bisphenol A (BPA) and its health effects are insufficient, because most studies on the health hazards of BPA have been on the general public. This study aimed to determine the relationship between workplace exposure to BPA, sex hormones and some metabolic parameters. Fasting blood samples were obtained from 46 occupationally exposed and 23 non-occupationally exposed adults. The levels of serum BPA, estrogen, testosterone, adiponectin, cortisol and insulin were analysed using enzyme linked immunosorbent assay (ELISA), plasma glucose, total protein and insulin resistance (HOMA-IR) were also determined. The results revealed that only 78% (n=36) of the occupationally exposed and 65% (n=15) of the non-occupationally exposed individuals had detectable serum BPA level. The results also showed that the occupationally exposed individuals had a statistically non-significant ($P=0.2$), greater serum BPA level ($3.30\pm 1.46\text{ng/ml}$) than the non-occupationally exposed subjects ($0.05\pm 0.01\text{ng/ml}$). However compared with the non-occupationally exposed group, the subjects who are occupationally exposed to BPA had a significantly higher level of serum testosterone ($P=0.0003$) and adiponectin ($P=0.03$). There were no significant differences in the serum level of estradiol ($P=0.07$), cortisol ($P=0.28$), insulin ($P=0.85$), plasma glucose ($P=0.26$), total protein ($P=0.97$) and insulin resistance (HOMA-IR) ($P=0.46$) between the two groups. In conclusion this study revealed that occupational exposure to BPA results in higher serum BPA level, testosterone and adiponectin in Humans.

KEYWORDS: Occupational exposure, Bisphenol A, Serum BPA, Serum testosterone, Adiponectin, Metabolic Parameters

1.0 INTRODUCTION

Bisphenol A (BPA) is one of the endocrine disrupting chemical to which humans are constantly exposed. Human exposure to BPA results from its use in the manufacturing of a variety of products, including polycarbonate plastics, food and beverage packaging, flame retardants, adhesives, electronic components, printer ink, automobile and optical lenses, carbonless papers, dental sealants and composites and paper coatings [1; 2]. Studies showed that these products leached measurable amounts of BPA [3; 4; 5] hence human exposure to BPA is direct and chronic due to its presence in the environments as well as in food and drink products. The capacity for human exposure to BPA was supported by a study conducted by Calafat *et al.* [6], in the USA that found BPA in urine samples of almost 95% of the volunteers.

Exposure to BPA has been associated with a number of negative health effects such as oxidative stress [7], polycystic ovary syndrome in women [8], inflammation in postmenopausal women [9], infertility in men [10], recurrent miscarriage [11] and developmental anomalies [12]. However most of these studies are reports from non-occupational exposure to BPA. This is founded on the belief that food sources are primary source of human exposure to BPA. Although several studies has revealed that oral ingestion accounts for the majority of human exposure to BPA especially via ingestion of food contaminated with BPA [8; 13; 14], there are other studies reporting that exposure to BPA by healthy individuals can be through non-oral means [15; 16; 17], and only a small number of research [8; 17] have investigated these additional workplace exposure to BPA or have linked occupational exposures to adverse health effects. Therefore investigations assessing the impact of occupational exposure to BPA on human health using serum BPA levels are required. This study sought to evaluate the association between occupational exposure to BPA, serum sex hormones and some metabolic parameters in occupationally exposed subjects from South-Western Nigeria.

2.0 MATERIALS AND METHODS

2.1. Study Population

The study subjects consisted of 46 volunteers (male or female, between the ages 18 and 60 years), working at a plastic producing factory in Ibadan, Oyo State, Nigeria, who had spent over 10 years or less in the industry, and 23 age-matched individuals who does not work in a plastic factory or who are not occupationally exposed to BPA were used as control. A questionnaire with clear instruction and an informed consent form were read, filled and signed by each subjects. Only individuals who responded to the survey were included in the study. A fixed response format type of questionnaire was used to obtain information from the subjects. The investigation was presented to all participating plastic producing factory staff as a health study on general occupational hazards. All subjects were oblivious of the specific hypothesis of the study. The questionnaire included; Demographic data, department and duration of work years at the industry, occupational safety measures taken, questions on reproductive health as well as any other health challenges.

2.2. Case definition:

The subjects were divided into two main groups of those who are exposed to BPA in the work place (occupationally exposed group) and those who are not occupationally exposed to BPA but are exposed outside the workplace. The occupationally exposed group were further sub divided to those who have been working in the plastic company for 10 years or over 10 years (Occupationally exposed group ≥ 10 years) and those who had worked for less than 10 years (Occupationally exposed group < 10 years).

2.3. Consent: an informed, signed consent form was obtained from all the subjects before the commencement of the study, confidentiality was assured.

2.4. Ethical Approval

According to the University standard, Ethical clearance for the study was obtained from committee of research ethics of the Federal Medical Centre (FMC) Owo, Ondo State, Nigeria. We authors hereby declare that this research was approved by the ethics committee of Federal Medical Centre Owo, Ondo state Nigeria before it was carried out.

2.5. Sample collection

Fasting blood (5ml) samples were obtained from the subjects through the vein, under aseptic condition between 8 a.m. to 11 a.m. The samples were dispensed into fluoride oxalate bottle for the determination of plasma glucose and into plain bottles for hormones analysis and analysis of other clinical parameters. The samples were centrifuged at 3000rpm for 5 minutes and separated within 30 minutes and stored at -10°C until when needed.

2.6. Biochemical Analysis

2.6.1. Analysis of Serum BPA concentrations of participants

The serum BPA levels of subjects were determined using Enzyme linked immunosorbent assay (ELISA). The ELISA kit (with product code Cat # BPA 1) was purchased from Detroit R and D, Incorporation, Metro Centre for High Technology Bldg. (MCHT) Detroit, USA.

2.6.2. Serum hormone analysis

Serum testosterone of Participants was determined using Accu-Bind ELISA kit (with a product code: 3725-300) obtained from Monobind Inc. Lake Forest, CA 92630, USA. Serum estradiol level of subjects was determined using Bio-inteco Enzyme Immunoassay (EIA) test kit (with catalog number: 10009 (96 Tests)) from inteco diagnostics UK Ltd, Unit B1, 62 Beechwood Road, E8 3DY England. Serum cortisol was determined using Bio-inteco ELISA kit (with catalog number: 10017C (96 Tests)) from inteco diagnostics UK Ltd, Unit B1, 62 Beechwood Road, E8 3DY England. Serum adiponectin was determined using AssayMax Human Adiponectin ELISA kit (with catalog number EA2500-1 and Lot number. 04011503) which was obtained from Assaypro LLC, 3400 Harry S Truman Blvd St. Charles, MO, USA,. Serum insulin of subjects was determined using Accu-Bind ELISA kit (with product code: 2425-300) obtained from Monobind Inc. Lake Forest, CA 92630, USA. All assays were carried out following to the instructions on the manufacturer's manual.

2.6.3. Determination of fasting Plasma Glucose and Total Protein (mg/dl)

The plasma glucose concentrations of subjects were analysed using the methods of Trinder [18]. The serum total protein was analysed according to the method described by Watanabe *et al.*[19]. Using Randox kit obtained from Randox laboratory LTD, UK).

2.6.4. Determination of Insulin Resistance

As described by Keskin *et al.* [20], the homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine the level of Insulin resistance in subjects.

2.7. Data analysis

The Data analyses were performed using one-way analysis of variance (ANOVA) and Duncan multiple range test for comparison between means of different groups. Values were expressed as the mean \pm SEM. All statistical analyses were performed using SPSS statistical package (SPSS Inc.). The level of significance (*P*) was set at <0.05

3.0. RESULTS

Among the 69 subjects who participated in the study only 51 (73%) had detectable serum BPA level. Among the 46 occupationally exposed subjects, only 36 (78%) samples had detectable BPA levels with a mean serum BPA level of 3.30ng/ml. while among the 23 non-occupationally exposed subjects only 15 (65%) had detectable serum BPA level with a mean concentration of 0.05ng/ml BPA. Compared with those measured in the non-occupationally exposed subjects, the mean serum BPA level in the occupationally exposed subjects was 66

folds higher. However the mean serum BPA concentrations between these group was statistically non-significant ($P=0.2$). As shown in table 2, when the results were adjusted for the length of occupational exposure to BPA, the results revealed that those who had worked at the plastic manufacturing factory for more than 10 years had a (statistically non-significant ($P=0.8$)) greater serum BPA level compared those who had worked there for less than 10 years.

Table 1: Serum BPA concentration in occupationally and non-occupationally exposed subjects

Variables	Occupationally exposed subjects (n=36)	Non-occupationally exposed subjects(n=15)
Serum BPA(ng/ml)	3.30±1.46	0.05±0.01
P value	0.20	

Results are shown as mean ±SEM. * $P < 0.05$ indicates statistical significance.

Table 2: Occupational exposure duration and serum BPA level

Variables	Duration of occupational exposure (years)	
	<10(n=18)	≥10(n=18)
Mean BPA(ng/ml)	2.62±1.53	4.33±2.47
P value	0.80	

Results are shown as mean ±SEM. * $P < 0.05$ indicates statistical significance.

Serum hormones concentration of subjects

The association between exposure to BPA (occupationally and non-occupationally) and some hormone levels in volunteers are summarised in Table 3. The findings revealed that as compared with the non-occupationally exposed group, the occupationally exposed group had statistically higher serum levels of testosterone and adiponectin ($P < 0.05$). The result revealed that there is a positive association between higher serum BPA and serum testosterone and adiponectin in the occupationally exposed individuals. When compared with the non-occupationally exposed group, the serum estradiol level in the occupationally exposed was marginally ($P < 0.07$) higher. The serum cortisol concentration of the non-occupationally exposed and the exposed group were not significantly different $P = 0.28$. The relationship between the duration of workplace exposure to BPA and the serum hormone level of the occupationally exposed group were presented in table 4. The serum levels of testosterone, adiponectin, estradiol and cortisol of workers who have been exposed to BPA for more than ten years did not change significantly from those who has worked for less years (>10 years).

Table 3: Serum estradiol, testosterone, cortisol and adiponectin of the occupationally exposed and non-occupationally exposed participants

	Occupationally exposed participants (n=36)	Non-occupationally exposed participants (n=15)	P value
Testosterone ng/ml	11.10±0.87	5.05±0.87	0.0003*
Estradiol pg/ml	61.51±5.89	44.94±5.82	0.07
Adiponectin µg/ml	31.69±2.95	20.00±3.75	0.03*

Cortisol ng/ml	114.26±14.52	88.82±10.32	0.28
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Results are shown as mean ±SEM. **P* < 0.05 indicates statistical significance.

Table 4: Serum estradiol, testosterone, cortisol and adiponectin of occupationally exposed participants (for <10 years (n=18) and ≥10 years (n=18)) and non-occupationally exposed subjects (n=15)

	Occupationally exposed group <10 years (n=18)	Occupationally exposed group ≥10 years(n=18)	Non-occupationally exposed (n=15)
Testosterone ng/ml	12.12±1.35	10.09±1.08	5.05±0.87 ^{ab}
Estradiol pg/ml	66.36±6.94	55.67±7.77	44.94±5.82 ^a
Adiponectin µg/ml	27.87±3.81	35.99±4.06	20.00±3.75 ^b
Cortisol ng/ml	136.16±27.76	93.57±5.58	88.82±10.32

Results are shown as mean ±SEM. Superscript a, b and c indicates significantly different from occupationally exposed subjects for < 10 years, ≥ 10 years and non-occupationally exposed respectively.

The relationship between occupational exposure to BPA and serum insulin, plasma glucose, plasma protein and insulin resistance index (HOMA-IR) of the occupationally exposed and the non-occupationally exposed group are shown in table 5. There were no significant (*P*>0.05) differences in the serum insulin, plasma glucose, serum total protein and insulin resistance index (HOMA-IR) of the occupationally exposed individuals compared with the non-occupationally exposed group. Table 6 shows there were no significant differences between those who are occupationally exposed for less than 10 years or above for serum insulin, plasma glucose and insulin resistance index (HOMA-IR)

Table 5: Serum insulin, plasma glucose, total protein and HOMA-IR of occupationally exposed participants and non-occupationally exposed group

	Occupationally exposed group (n=36)	Non occupationally exposed group (n=15)	P value
Fasting serum Insulin µIU/ml	12.12±1.75	12.73±2.47	0.85
Fasting plasma glucose mg/dl	62.18±2.47	66.76±3.10	0.26
Total protein mg/dl	4.70±0.14	4.70±0.09	0.97

HOMA-IR	1.76±0.27	2.15±0.40	0.46
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Results are shown as mean ± SEM. **P*< 0.05 indicates statistical significant

Table 6: Fasting serum insulin, plasma glucose, total protein and HOMA-IR of occupationally exposed participants (for <10 years (n=18) and ≥10 years (n=18)) and non-occupationally exposed individuals (n=15)

	Occupationally exposed group <10years (n=18)	Occupationally exposed group ≥10years (n=18)	Non occupationally exposed group (n=15)
Serum Insulin μIU/ml	15.56±2.77	9.26±2.25 ^a	12.73±2.47
Plasma glucose mg/dl	59.84±2.97	63.55±4.03	66.76±3.10
Total protein mg/dl	4.44±0.19	4.91±0.09	4.70±0.09
HOMA-IR	2.04±0.42	1.75±0.43	2.15±0.40

Results are shown as mean ±SEM. Superscript a, b and c indicates significantly different from occupationally exposed group for < 10 years, ≥ 10 years and Non-occupationally exposed respectively.

4.0 DISCUSSION

Out of the 69 subjects who participated in this study, total BPA was detected in the serum samples of only 75% (n=51) of the volunteers at levels ranging from 0.05 to 3.30 ng/ml. The serum BPA levels obtained in this present study are similar to those obtained from several previous studies [11; 21; 22; 23; 24; 25; 26], which employed a variety of different analytical methods. These earlier investigations found unconjugated serum BPA levels in both men and women from various nations and ages, ranging from 0.2 to 20 ng/ml serum. While some research found greater levels of circulating BPA, some reported lower levels; the average circulating BPA concentrations determined by ELISA for these investigations was 12.0 ng/ml.

Serum total BPA was only detected in 78% of the 46 occupationally exposed subjects who supplied blood samples, with a mean value of 3.30ng/ml, and in only 65% of the serum samples from non-occupationally exposed subjects, with a mean concentration of 0.05ng/ml.

The fact that serum BPA was not detected in all the samples (from both occupationally and non-occupationally exposed individuals) obtained, may be as a result of the fact that BPA is metabolised rapidly as soon as is ingested [27], or due to variations in the rate of BPA metabolism among subjects. BPA does persist in the body; it is metabolized rapidly, therefore the serum concentrations of BPA found in the subjects may be affected by other factors such as presence and availability of secondary metabolites, routes of exposure, sex, age, BMI, genetics, serum levels of other hormones, duration and frequency of exposure, haematological parameters and nutritional status. Therefore a single serum sample analysis may only reveal very recent exposure in individuals [28].

In the current study the serum BPA (0.05ng/ml) level detected among those who are not occupationally exposed is a reflection of a well-established fact that oral route is the most frequent means of exposure to BPA in humans. Compared with occupationally exposed individuals, the lower serum BPA level found in this group may be as a result of the rapid

metabolism of BPA following oral ingestion. Additional exposure to BPA in humans occurs in the workplace via other routes, such as inhalation, dermal and ingestion of BPA in the air and dust [8; 17]. More than a few studies which measured BPA in the air and dust [29; 30; 31] have emphasised the pharmacological significance of these other routes of exposures which bypass the hepatic metabolism associated with oral exposure. As shown in table 1. Those who are exposed to BPA in the workplace had a 66 folds higher mean serum BPA (3.30 ng/ml) than the mean serum BPA level of non-occupationally exposed individuals (0.05ng/ml). However the non-significant ($P=0.20$) differences between the mean serum BPA level of the two groups (occupationally exposed and non-occupationally exposed individuals) maybe a reflection of the wide range of serum BPA concentrations measured among the those who had work place exposure to BPA.

In this study the duration of occupational exposure to BPA has no significant impact on the serum level of BPA. The fact that BPA is rapidly metabolised in humans through hepatic glucuronidation and sulfation can be used to explain the lack of a meaningful correlation between duration of occupational exposure to BPA and serum BPA level as shown in table 2. In addition metabolic studies revealed that the major BPA metabolite (BPA-glucuronide) is water soluble and is rapidly excreted via the kidneys with a half-life of roughly 6 hours and a complete urinary excretion is achieved within 24 hours [27]. Hence, a low-dose continuous exposure to BPA may be the reasons for the long-term effects linked with BPA [32].

Having higher serum androgen level may result from a genetic condition [33] or excessive fat [34]. According to recent speculations, androgen synthesis may be stimulated by endocrine disrupting chemicals such as BPA [22]. The result from the present study showed that occupationally exposed participants had significantly ($P=0.0003$) higher ($11.10\pm 0.84\text{ng/ml}$) serum testosterone than the non-occupationally exposed participants ($5.05\pm 0.87\text{ng/ml}$), according to the findings, the occupationally exposed group's higher serum testosterone levels correlated with higher serum BPA levels. The higher serum testosterone level in the occupationally exposed group could have resulted from overstimulation of the androgen producing cells due to long-term persistent exposure to BPA. The results of the current study thus offer crucial proof that there is increased risk of male sexual dysfunction for those who are occupationally exposed to BPA. This is in accordance with results obtained from other studies. For examples two human studies on BPA exposure and hormone levels indicated statistically significant positive relationships between serum BPA levels and circulating total and free testosterone levels in both men and women [21; 22]. It was proposed by Lee *et al.* [35] that elevated free androgen levels results from the binding of BPA to androgen receptors, which subsequently leads to a partial disruption of the binding of androgens to the androgen receptor (AR). Meanwhile a study carried out by Takeuchi and Tsutsumi [22], suggested that the high levels of BPA reported is as a result of androgens interference with the activity of BPA clearance enzymes. Hence this current study is in support of these studies suggesting that BPA interfere with testosterone metabolism and thus interfere with male sexual functions.

Results of the current study, shows that there were significant positive correlations between higher serum BPA levels and serum adiponection level in the occupationally exposed group as shown in table 3. The higher serum adiponectin level of the occupationally exposed individuals may be the result of a direct relationship between higher serum testosterone and adiponectin. It is thought that body fat mediates the inverse relationship between testosterone and insulin resistance, thus both circulating adiponectin and testosterone levels are important indices of type II diabetes especially in men. It is well established that testosterone

contributes to the development type 2 diabetes. Serum testosterone levels are lower in men with type 2 diabetes, while higher in women with this condition. It is believed that testosterone lowers type 2 diabetes risks in men, but increases it in women [36]. Thus there is a correlation between increased serum levels of BPA, testosterone and adiponectin among the occupationally exposed individuals. In addition continuous low-dose overstimulation of the adipokines or suppression of the adiponectin receptors which results in elevated circulating level of adiponectin may be another mechanism through which BPA exerts its endocrine disrupting effects. For instance, Bisphenol A has been shown to improve the transport of glucose in adipocytes which may in turn facilitate lipogenesis [37]. In addition BPA stimulates adipogenesis in 3T3-L1 adipocytes according to other studies [38; 39] Conversely a previous study by Hugo *et al*, reported that BPA suppressed the release of adiponectin in breast, isolated mature adipocytes obtained from more than 20 patients as well as subcutaneous and visceral adipose tissue explants, it should be noted that the study was an *ex vivo* study [40].

Studies published as early as 1936 demonstrated that BPA has estrogenic properties [41]. In the present study, the mean serum estradiol levels (61.51 ± 5.89 pg/ml) in the occupationally exposed workers was non-significantly ($P=0.07$) higher than the serum estradiol level (44.94 ± 5.82 pg/ml) in the non-occupationally exposed control groups. The result thus revealed that those who are exposed to BPA at work had higher serum levels of BPA and elevated serum level of estradiol, this finding is consistent with other studies. For instance, Steinmetz *et al*. [42] reported that Bisphenol A stimulates prolactin release *in vitro* and *in vivo*. Therefore, it is expected that continuous exposure of men to BPA and subsequent disruption of estradiol homeostasis may be responsible for the reproductive challenges associated with occupational exposure to BPA as reported in a previous study by [35].

Associations between serum BPA concentrations and some metabolic parameters of occupationally exposed and the non-occupationally exposed individuals were presented in table 5, as revealed in the table, the difference in the serum insulin, plasma glucose and serum total protein in the occupationally exposed individuals and non-occupationally exposed group was non-statistically different ($P>0.05$). Despite the fact that there was no statistically significant difference in the insulin resistance index (HOMA-IR) values between the occupationally exposed individuals, it should be noted that the occupationally exposed group had a lower mean HOMA-IR value than the non-occupationally exposed individuals. The lower level may have resulted from the effect of circulating testosterone and adiponectin on insulin as well as glucose homeostasis in the occupationally exposed group. Serum testosterone level and insulin resistance are inversely correlated according to several studies [42; 43; 45].

In conclusion, this study showed that employees in the plastic manufacturing industries, who are occupationally exposed to BPA, have higher level of serum BPA levels than the non-occupationally exposed individuals, and the higher serum BPA is linked to higher levels of serum testosterone, estradiol and adiponectin. The current findings strengthened the evidences that BPA disrupts to the endocrine system and by extension the hormone homeostasis of humans. Hence there should be a routine monitoring of BPA manufacturing companies as well as those companies using BPA as an additive to limit the additional exposure of workers to BPA in the workplaces.

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