Correlation between Urinary Bisphenol A (BPA) Levels and Male Reproductive Functions among Sample of Egyptian Population

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Abstract

BPA as a xenoestrogen negatively influences male reproductive functions as well as sperm quality. This study aimed at evaluating the correlation between urinary BPA concentrations and male fertility by assessing semen parameters as well as hormone profile. It included (150) males divided into two groups; infertile cases (n= 100) and fertile controls (n= 50). Results showed highly significant difference in mean urinary BPA concentrations between both groups being higher among infertile group (P-value < 0.001). Besides that, there was statistically significant correlation between the mean urinary BPA concentrations and deterioration of sperm concentration and motility among cases with P-values (< 0.001). Finally, there was statistically significant correlation between mean urinary BPA and hormone levels FSH, LH, T and E2 levels with P-values 0.002, 0.033, 0.001 and < 0.001 respectively.

Keywords: *Bisphenol A (BPA); Endocrine disruptors; Male infertility; Sperm functions; Xenoestrogens*

Introduction

Infertility is the inability of sexually active and non-contracepting couples to achieve spontaneous conception within one year duration with overall incidence about 10–15% of couples worldwide [1]. Male factor infertility can be classified to pre-testicular causes (at the level of hypothalamus or pituitary gland), testicular (primary testicular failure) or post-testicular (obstructive or coital) [2]. Exposure to gonadotoxins (either environmental or occupational) as; heavy metals, xenoestrogens, pesticides and organic solvents may negatively influence the Hypothalamo-Pituitary-Gonadal (HPG) axis , sperm functions and DNA integrity [3].

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BPA is an endocrine disruptors of xenoestrogen family (diphenylmethane derivatives) that possess a chemical structure [$(CH_3)_2C(C_6H_4OH)_2$] similar to estrogens, thus interacting with estrogen receptors as agonist or antagonist via specific signaling pathways [4]. It is widely used in plastic industries to make them shiny, flexible and durable [5]. It may enter the body via several routes as; contaminated food or drinks, environmental (from polluted air and water), domestic (household products and cosmetics), medical (from contaminated equipment and devices), and occupational sources (inhalation, dermal contact, and ingestion during manufacturing processes or industrial use) [6].

Several studies have reported the potential risk of BPA exposure and various health hazards among animals and humans including; infertility, precocious puberty, and polycystic ovary syndrome, hormone dependent tumors (breast, ovarian and prostate cancer) and various metabolic disorders including diabetes mellitus and thyroid dysfunction [7].

Many mechanisms have been suggested its effect on male fertility including; disturbance in HPG axis with subsequent dysfunction of steroidogenesis

and spermatogenesis. Additionally, BPA may affect sperm functions and integrity through increased DNA damage, epigenetic methylation, oxidative stress, lipid peroxidation and mitochondrial dysfunctions [8-10]. Up till now, ongoing researches are being conducted to verify the exact effect of BPA on the male fertility potential and sperm functions.

Material and Method

Total of (150) participants were enrolled in this prospective study being age matched from 20 to 50 years old. All were recruited from the outpatient clinic of Andrology - Cairo University from February 2018 to August 2019. Approval of the local ethical committee was obtained after getting in writing informed consent from the participants about the purpose of the study conforming to Helsinki declaration. Notably, cases were assorted according to certain inclusion and exclusion criteria, which were:

Inclusion criteria

All cases (n=100) were males complaining of infertility (either primary or secondary) for at least one year duration with no obvious cause of female factor of infertility. On the other hand, the Control group included (50) fertile males with history of previous conceptions in the last year confirmed by normal semen parameters.

Exclusion criteria

All subjects with uncontrolled medical disorders, chronic heavy smoker (smoking index ≥ 400), and history of drug abuse, radiotherapy and/or chemotherapy, or abdomino-pelvic surgery were ruled out. Furthermore, any subjects with obesity (BMI ≥30), clinical varicocele, genetic infertility (e.g. Klienfelter's syndrome and Y-chromosome microdeletions), or Leukocytozoospermia were also excluded.

Notably, all participants were assessed for urinary BPA concentration, semen analysis as well as hormone profile (FSH, LH, testosterone, E2 and PRL).

Urinary BPA

Urine specimens were collected from all included subjects in clean, glass containers labeled with the subject identification number, whereas turbid samples or those containing blood were excluded. Afterwards, all specimens were stored frozen below 20ºC, and then analyzed for the mean BPA (i.e. the mean value was calculated using 3 different readings in different occasions) by High Performance liquid chromatograph (**HPLC**).

Conventional Semen analysis

The patients were instructed to abstain for (2-7 days), and bring the sample by masturbation using no lubricants in a sterile container. The samples were then incubated for 1 hour at 37ºC for liquefaction and were examined using a light microscope [**Olympus Co., BH-2 (BHTU), Japan**] with an objective optical magnification (**40 X**). the standard criteria for evaluation were according to (**WHO**) criteria of normal semen parameters [Volume >1.5ml, Concentration >15x10^6 sperm/ml, Total sperm count >39x10^6/Ejaculate, Vitality >58%, Total motility >40%, Progressive motility >32%, Abnormal forms ≤ 96%, Pus cells < 1 million / ml].

Hormone profile

All studied subjects were subjected to withdrawal of venous blood sample between (8:00-11:00 am) for measurements of FSH, LH, testosterone (total), prolactin and Estradiol (E2) using an electro chemiluminesence immunoassay analyzer [***Roche Co., Cobas e 602, Japan***]. The normal reference values were as follow; FSH = 1.5 - 12.4 mIU/ml, LH= 1.2 -7.8 mIU/ml, testosterone (total) = 2.5 - 8.4 pg/ml, prolactin = 2-18 ng/ml, and E2 less than 40 pg/ml).

Statistical methods

By the end of the study, all collected Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test [11]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [12]. Correlations between quantitative variables were done using Spearman correlation coefficient [13]. P-values less than 0.05 were considered as statistically significant.

Findings

In this prospective controlled study, a total number of 100 infertile males (*either primary or secondary*

infertility) were included and then compared to 50 normal fertile controls. The mean age of the infertile patients was 33.53 ± 5.98, while the mean age of fertile controls was 35.20 ± 8.17; and there was no significant difference between the studied groups regarding the age (p = 0.3). Both groups were assessed for the urinary BPA levels, semen analysis as well as hormone levels (FSH, LH, testosterone, E2 and PRL) as shown in ***table (1)***.

Table (1): The mean urinary BPA level, semen parameters and hormone profile among cases and controls

Parameters	Cases (n=100)		Control (n=50)		P-value
	Mean	SD	Mean	SD	
Urinary BPA level	10.07	9.98	1.69	1.32	< 0.001
Sperm concentration	27.93	25.75	45.34	22.90	< 0.001
Motility	37.01	22.04	61.40	13.78	< 0.001
Abnormal forms %	55.59	23.47	59.50	13.75	0.315
FSH	8.28	5.63	4.23	1.00	< 0.001
LH	6.38	2.96	3.36	0.84	< 0.001
Testosterone	4.34	1.40	4.74	1.10	0.064
E2	33.60	11.26	27.56	8.05	< 0.001
PRL	12.26	4.09	8.99	3.45	< 0.001

Concerning the mean urinary BPA levels, there was a highly statistical significant difference between both groups; whereas the mean levels among cases was 10.07 +/- 9.98 compared to controls that was 1.69 +/- 1.32 with P-value <0.001.

On the other hand, the semen analysis parameters among both groups showed a highly significant statistical difference concerning sperm concentration and sperm motility (p < 0.001), whereas mean sperm concentration and motility among cases were 27.93 +/- 25.75 and 37.01 +/- 22.04 respectively, compared to controls that were 45.34 +/- 22.90 and 61.40 +/- 13.78 respectively. However, there was no significant difference concerning abnormal forms among both groups (P= 0.315).

Furthermore, there was a highly significant statistical difference between cases and controls concerning FSH, LH, PRL and E2 levels (p < 0.001), whereas the mean levels were 8.28 +/- 5.63, 6.38 +/- 2.96, 12.26 +/- 4.09 and 33.60 +/- 11.26 respectively, compared to controls that were 4.23 +/- 1.00, 3.36 +/- 0.84, 8.99 +/- 3.45 and 27.56 +/- 8.05. On contrary, there was no significant difference between both groups as regard to testosterone level (P= 0.064).

Regarding the correlation between mean urinary BPA level with semen parameters as well as hormone profile among the infertile group as shown in ***table (2)***; our results have shown that there was a highly statistically significant negative correlation between sperm concentration and mean urinary BPA level (p <0.001). Furthermore, there was statistically significant negative correlation concerning sperm motility and the mean BPA level (p= 0.003). However, there was no significant correlation concerning abnormal form of sperm and mean BPA level (p= 0.178).

In another perspective, there was statistically significant positive correlation concerning FSH, LH level and BPA level, whereas the p-values were 0.002 and 0.003 respectively. Eventually, there was a highly significant negative correlation between testosterone and E2 levels and BPA level with p-values 0.001 and <0.001 respectively. On contrary, there was no significant correlation concerning prolactin level and BPA level (p=

0.275).

Table (2): Correlation between the mean urinary BPA level with semen parameters and hormone profile among infertile group.

Parameters	**Mean urinary BPA (micro/l)**	
Sperm concentration	Correlation Coefficient	-0.310
	P- value	< 0.001
Motility	Correlation Coefficient	-0.240
	P- value	< 0.001
Abnormal forms %	Correlation Coefficient	0.110
	P- value	0.178
FSH	Correlation Coefficient	0.255
	P- value	0.002
LH	Correlation Coefficient	0.174
	P- value	0.033
Testosterone	Correlation Coefficient	-0.265
	P- value	0.001
E2	Correlation Coefficient	0.294
	P- value	< 0.001
PRL	Correlation Coefficient	0.090
	P- value	0.275

Discussion

Urinary metabolites of BPA represent exposures to chemicals from all routes of exposure including oral, dermal, inhalation and ingestion. As a non-persistent chemical with an elimination half-life of a few hours, the BPA concentrations in blood are lower than those in urine and decrease quickly after the exposure [14]. There was substantial day-to-day variation of urinary levels of BPA with considerable variability with in the same subject. Therefore, the assessment of BPA exposure on the base of multiple urine measurement was recommended [15]. In addition, consistent individual time activity patterns may lead to stable concentrations over long periods of time, so the assessment of BPA exposure on the base of multiple urine measurement. Also, one semen sample may be representative of semen quality over several weeks in epidemiological studies [16]. Therefore, in our study we have obtained three urinary samples for the same subject in different occasions and we have calculated the mean urinary BPA levels.

Concerning urinary bisphenol (BPA), the mean levels was 10.07 +/- 9.98 for cases and 1.69 +/- 1.32 for controls denoting a highly significant difference between both groups ($p < 0.001$) and this agreed with **Lassen et al., (2014**) who found a clear match between individual exposure to biphenol A and male reproductive dysfunction [17].

Our results showed a highly statistically significant correlation between sperm concentration and mean BPA level ($p < 0.001$). Besides that, there was significant correlation between sperm motility and the mean BPA

level (p= 0.003). These results agreed with **Li et al., (2011)** who found a significant correlation between the urinary BPA concentrations and the poor semen quality. However, there was no significant correlation between sperm abnormality and mean BPA level **(p= 0.178).** These results agreed with ***Li et al., (2011)*** who found no significant correlation between the urinary BPA concentrations and poor sperm morphology [18]. However, ***Meeker et al., (2010)*** found significant correlation between higher urinary BPA concentrations and poor sperm morphology [19].

On the other hand, there was a highly significant statistical difference between cases and controls concerning FSH, LH, PRL and E2 levels **(p < 0.001).** However, there was no significant difference concerning testosterone level **(P= 0.064).** These results agreed with ***Liu et al., (2015)*** who found a significant correlation between the urinary BPA concentrations and the reproductive hormones [20]. Additionally, there was statistically significant correlation concerning FSH, LH level and BPA level, whereas the p-values were 0.002 and 0.003 respectively. Eventually, there was a highly statistically significant correlation between testosterone and E2 levels and BPA level with p-values **0.001** and **<0.001** respectively.

In correlation of BPA level and hormonal assay among cases, there was significant correlation with FSH, LH, Testosterone and E2, and this agrees with ***Liu et al., (2015***) who had observed the same findings [20]. On contrary, there was no significant correlation concerning prolactin and BPA among cases (p= 0.275), and this agrees with ***Meeker et al., (2010)*** [19]. However, this goes against ***Liu et al., (2015***), who stated that increased urine BPA level was associated with increased prolactin [20].

Finally, there were some differences in results and this may be attributed to differences in races, age, sample size, environmental circumstances, and requirements of product labeling in different countries and also due to different combinations of the studied parameters that may affect the results. Additionally, it must be taken into consideration that these results are only presented as a preliminary study, thus increasing the sample size is recommended.

Conclusions

BPA is an endocrinal disruptor that negatively influences male fertility. The current study showed higher levels of urinary BPA among cases compared to controls. Furthermore, it demonstrated negative effects of BPA on semen parameters and hormone profile among the infertile group. Notably, those findings need to be confirmed in future large scale and multi-centric studies.

Ethical Clearance: Ethical committee approval was obtained from the department of clinical toxicology at the faculty of medicine – Cairo University.

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Conflict of Interest: Nil.

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