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Original Research Article

A study of urinary Bisphenol A levels in endocrine disorders

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ABSTRACT

Background: Bisphenol A (BPA) is a very common endocrine disruptor. Traditionally high doses of BPA showed adverse effects with respect to organ failure and cancer. However, evidence now shows that doses well below the traditional toxicological threshold have metabolic effects. This observational study was aimed to measure the urinary levels of BPA among patients with endocrine disorders namely type 2 diabetes mellitus, hypothyroidism, non-obstructive azoospermia, polycystic ovarian syndrome (PCOS) and simple obesity, and to correlate urinary BPA levels with different clinical, biochemical and hormonal parameters.
Methods: 30 newly diagnosed cases each of Type 2 diabetes mellitus, primary hypothyroidism, polycystic ovarian syndrome (PCOS), non-obstructive azoospermia and simple obesity were selected for study (single disorder in one patient). Age and sex matched healthy relatives of patients (n=30) were recruited as controls. All cases and controls were subjected to spot urinary BPA level estimation.
Results: There were significant differences obtained in the median values of BPA in urine between cases of azoospermia, and simple obesity as compared to controls whereas no correlation was obtained between urinary BPA levels and BMI or waist hip ratio, in patients of type 2 DM and primary hypothyroidism. Urinary BPA was significantly lower than controls in cases of PCOS.
Conclusions: Urinary levels of BPA are an indicator of its toxic effects especially in patients of non-obstructive azoospermia and simple obesity. The values of BPA in urine were widely distributed showing variability of exposure from the environment.

Keywords: Bisphenol A (BPA), Hypothyroidism, Non-obstructive azoospermia, PCOS, Simple obesity, Type 2 diabetes mellitus

INTRODUCTION

An endocrine-disrupting substance is a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment (WHO Report 2012). These heterogeneous compounds include synthetic chemicals used as industrial solvents/lubricants and their by-products [polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], plastics [bisphenol A (BPA)], plasticizers (phthalates), pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)], fungicides (vinclozolin), and pharmaceutical agents [diethylstilbestrol (DES)]. Natural chemicals found in human and animal food (e.g., phytoestrogens, including genistein and coumestrol) also act as endocrine

disruptor.[1] BPA is the most abundant, as it is used in almost all household articles and disposal in garbage ensures continuous exposure in human beings through diet and water. The effect of BPA on biological system has evoked controversy, and studies have refuted and proved toxic effects. Traditional toxicological approach using extremely high doses of BPA showed adverse effects, but doses below a certain threshold were safe with respect to organ failure and cancer. However, evidence now shows that doses well below the traditional toxicological threshold have development and metabolic effects.[2]

While there are a number of studies from Europe, USA, Canada and China on the possible and probable effects of Bisphenol exposure and the effects on human development and metabolism, there are no studies from India. The present study was aimed to measure the urinary levels of BPA among patients with endocrine disorders namely type 2 diabetes mellitus, hypothyroidism, non-obstructive azoospermia, polycystic ovarian syndrome (PCOS) and simple obesity, and to correlate urinary BPA levels with different clinical, biochemical and hormonal parameters.

METHODS

The study was conducted in the Department of Endocrinology and Metabolism, IMS, BHU, Varanasi from March 2012 till September 2013. The study subjects comprised of newly diagnosed cases of Type 2 diabetes mellitus, primary hypothyroidism, polycystic ovarian syndrome (PCOS), non-obstructive azoospermia and simple obesity (single disorder in one patient; n = 30 for each disease). Age and sex matched healthy relatives of patients (n = 30) were recruited as controls. The study was approved by the Institute's Ethics Committee. Informed consent was obtained from all study participants.

Inclusion criteria

- Newly diagnosed cases of Type 2 Diabetes Mellitus (ADA 2011 criteria)
- Newly diagnosed cases of Primary hypothyroidism. (Low T_3/T_4, TSH>15 uIU/L)
- Newly diagnosed cases of PCOS females (Rotterdam criteria 2003)
- Non-obstructive azoospermia in males.
- Simple Obesity (BMI$\geq$30 kg/m^2).

Exclusion criteria

- Patients not satisfying inclusion criteria
- The patients who were found to be taking any drug treatment (including indigenous preparations) for any disease were excluded from the study.

The study population was evaluated clinically (including anthropometry) and biochemically (plasma glucose fasting and 2 hours post oral glucose challenge, fasting serum lipid profile, serum total T3, serum total T4 and serum TSH, serum LH, serum FSH and serum Testosterone). Serum T_3, T_4, TSH, LH, FSH and testosterone were measured by chemiluminescent immunoassay (Immulite 1000, Siemens, India). In cases of PCOS the estimation was done on the second day of the spontaneous/induced menstrual periods.

The spot urine samples from patients and controls for BPA levels and creatinine were collected in high grade plastic test tubes and immediately stored at -20^0 C to prevent leaching of BPA from test tubes. The urine samples were analysed after thawing for BPA levels by ELISA (Detroit R&D Inc., Detroit, USA) as per protocol for total BPA. Urinary spot creatinine was done as per manufacturer's protocol (Crest Biosystems Goa, India).

Urinary BPA level so obtained was corrected for spot urinary creatinine and values were obtained in pg/mg of creatinine.

RESULTS

The study was conducted on 150 cases and 30 healthy controls. Table 1 shows clinical characteristics. The table 2 shows urinary BPA levels.

Table 1: Clinical characteristics in different endocrine disorders.

Subject	Age Mean±SD (Years)	Sex M/F	BMI Mean ± SD (Kg/m^2)	Waist/Hip ratio
Control				
Type 2 Diabetes mellitus	40.8±5.6	19/11	25.6±3.3	1.2±0.1
Primary hypothyroidism	37.9±10.0	03/27	24.7±2.1	1.1±0.1
Polycystic ovary syndrome	27±3.1	-/30	26.5±2.3	1.1±0.1
Non-obstructive azoospermia	30.1±4.8	30/-	30.1±2.2	0.9±0.1
Simple obesity	38.3±3.4	16/14	34.2±2.2	1.4±0.1

In type 2 diabetes mellitus, no correlations were obtained between urinary BPA levels and BMI or waist-hip ratio, and between components of lipid profile and urinary BPA level. In primary hypothyroidism, no correlation was found between urinary BPA levels and BMI or waist/hip ratio.

In PCOS, there was significant lower urinary BPA levels than controls. No correlation was obtained between urinary BPA level and BMI or waist-hip ratio and with serum LH and FSH levels. In cases of non-obstructive azoospermia, there was significant difference between urinary BPA levels and controls. In simple obesity, there was significant difference between urinary BPA levels and controls. However even in these patients no correlation was observed between BMI or waist-hip ratio.

Table 2: Urinary BPA levels in different endocrine disorders.

Subject	Urinary BPA pg/mg creatinine			Mean urinary BPA (pg/mg creatinine)	Median urinary BPA (pg/mg creatinine)
	<500	500-1000	>1000		
control (N=30)	0	11	19	1139.66±266.60	1209.72
Type 2 Diabetes mellitus (N = 30)	2	9	19	1225.01±550.71	1214.33
Primary hypothyroidism (N = 30)	2	15	13	1225.40 ±1497.85	987.77
Polycystic Ovary Syndrome (N = 30)	5	8	17	941.94 ±428.04	1054.83
Non-obstructive Azoospermia (N = 30)	6	11	13	864.96±365.55	953.43
Simple Obesity (N = 30)	12	5	13	802.43±554.52	954.01

DISCUSSION

Bisphenol A, a biphenolic compound, is used in plastic industry as a hardening agent in consumer plastics (polycarbonate plastic), in dental sealants and fillings, CDs/DVDs, and household electronics, in epoxy resins used for lining food and beverage cans, and in the plastic baby bottles. The increased production and prevalent use has made BPA a ubiquitous chemical in the environment. BPA interferes with transcription of proteins and hormones. The population is directly and chronically exposed to BPA, as it leaches when plastic is exposed to heat or acidic environments.[3]

The BPA shows estrogenic property similar to its successor diethylstilbestrol (DES). DES was later proved as carcinogen and could lead to reproductive defects in girls born to mothers taking DES. Thus, a chemical with similar chemical structure and properties such as BPA could have similar toxic effects.[4,5] There is a large data on exposure in human beings from the industrial countries in the world. Most studies in humans lack linear dose-dependent effects of BPA making it difficult to extrapolate the action(s) of BPA. While the debate remains open, it is clear that BPA can act as an endocrine disruptor that can possibly disrupt a number of metabolic processes.[6]

The study participants were young males and females from areas of north India surrounding Varanasi and had various metabolic and reproductive disorders. All patients (N=150) and controls (N=30) had detectable levels of BPA in urine using ELISA Kit which had the lowest level of detection of 1 pg/ml. Studies from US, Canada and China have found a prevalence of more than 93-98% for presence of detectable levels of BPA in urine.[7] The mean urinary BPA in controls was 1139.665±266.605 pg/mg creatinine while the median value of urinary BPA was 1209.720 pg/mg creatinine. This value is higher than the values of controls in other studies which have reported values from 600-800 pg/ml in the urine samples.[8] Bisphenol A is metabolized through glucoronide conjugation and excreted in urine over a period of 24 hours.

However, the presence of continuous exposure from the food and water consumption from plastic containers made from polycarbonate plastics or with an inner lining made from epoxy resins causes the levels to rise and persist at higher levels in the blood; this allows BPA enough time to interact with various hormone receptors and behave as endocrine disruptor. The high values in control subjects can be explained by incidental higher exposure over previous 24 hours. These subjects were the healthy relatives or attendants of the patients coming to the out-patient department who agreed to give urine samples for analysis. Exposure history of Bisphenol A was not obtained from any patient or control subjects in this study. This high value obtained for controls may not be representative of exposure level in the population because of the small sample size and wide variability of exposure.

Nineteen patients (63%) in the type 2 diabetes mellitus group, 13 patients (43.3%) in the primary hypothyroidism group, 17 patients (56%) in the PCOS group, 13 patients (43.3%) in the non-obstructive azoospermia group and 13 patients (43.3 %) in the obesity group had urinary BPA levels of more than 1000 pg/mg creatinine. Nineteen subjects in the control group (63.3%) also had values of more than 1000 pg/mg of creatinine. Other environmental exposure studies on BPA have reported values from 0.4 µg/L to 4.0µg/L (NHANES database 2003-2006). The values in this study lie in the middle of this range probably because these studies have been reported from the most industrial countries in the world namely USA, Canada and China as against this study which was carried

out at Varanasi and the patients and controls hailed from Urban areas and rural areas in and around Varanasi. Also, Varanasi is non-metro city, we may find higher values in metro cities in India.

The values of BPA in urine obtained in our study demonstrate that the population is exposed to high levels of this endocrine disruptor although the absolute levels are lower than the industrialized countries. Higher levels could be obtained if levels are measured in industrial cities in India.

The urinary BPA levels in type 2 diabetic patients did not show significant difference as compared to controls, however, the high standard deviation shows wide variability of exposure to BPA from food and water. The patients and control subjects sometimes travelled to Varanasi from distant places and were exposed to plastics in the form of water bottles and packaging materials for eatables.

In Center for Disease Control and Prevention's (USA) biennial health survey (NHANES), the association between urinary BPA and diabetes was significant in pooled data (2003-06) and 2003-04 data but did not reach significance in 2005-06.[9] All of these studies like our study were cross-sectional studies and did not include multiple exposure measurements or disease development over time.

A study from Shanghai, China showed similar results when interquartile analysis was performed for urinary BPA levels.[10] Prevalence of diabetes was higher in subjects with urinary BPA concentration between 1800-2600 pg/ml. Another study from Shanghai, China found serum BPA (HPLC) levels from 0.3 µg/L-2.6 µg/L; interquartile analysis with respect to prevalence of diabetes mellitus revealed higher prevalence of diabetes Mellitus in the third and fourth quartiles of serum BPA levels.[9] In both studies the number of subjects analysed were more than 3000. Small sample size in this study might have precluded similar results but is in consonance with recent US data.

Among the diabetes mellitus patients there was no correlation obtained between urinary BPA levels and LDL, HDL, total cholesterol and triglycerides. On applying Spearman's Rho with respect to every component of lipid profile no significant correlation could be obtained with respect to urinary BPA levels. No comparable data was found in available literature.

In primary hypothyroidism, the difference urinary BPA as compared to controls was not statistically significant. The high standard deviation shows the wide difference in exposure in this group as well. Human studies viz. Hugo et al 2008 (US), also did not show significant difference in urinary BPA levels between hypothyroidism and healthy subjects using NHANES database. In PCOS, the mean urinary BPA value was 941.94±428.04 pg/mg creatinine. The median value of urinary BPA level was 1054.83 pg/mg creatinine. A significant relationship of Serum BPA levels has been reported in cases of PCOS as against controls (1.05±0.56 versus 0.72±0.37ng/ml, P <0.001).[11] The mean value of urinary BPA (pg/mg creatinine) among cases of PCOS in this study was lower than that reported from western Europe and USA. The mean values of urinary BPA obtained in the control samples in this study was higher than that reported from Europe, this might be the reason for not obtaining a significant correlation between cases of PCOS and controls. The control subjects in our study were healthy relatives of those with endocrine disorders and shared the same exposure from the environment. No correlations were obtained between urinary BPA levels and LH, FSH levels in PCOS. This fact has not been studied in any clinical study. There are some studies which have reported an increase in LH levels on exposure to estrogenic compounds and environmentally relevant doses of BPA in sheep.

In non-obstructive azoospermia, the mean urinary BPA level was not significant different as compared to controls. There are no clinical studies to find an association between BPA exposure and prevalence of azoospermia in a population. In this study we studied cases of non-obstructive azoospermia with normal pituitary gonadal axis and did not find high levels of urinary BPA levels in azoospermia group as against healthy control group. However, when median values were analysed using Mann Whitney test of significance, there was a significant difference observed. This could be because of wide variation in exposure levels among cases and control subjects.

In simple obesity, the mean value of urinary BPA was 802.431±545.195 pg/mg creatinine. The median value of urinary BPA was 954.013 pg/mg creatinine. A study on US (Caucasian) obese children showed higher levels of urinary BPA had higher rates of obesity. In US adults, higher urinary BPA was associated with a higher BMI and waist circumference in both genders and in all ethnic groups. Higher BPA levels have been linked to higher body mass index (BMI) in Chinese school children.[8] Chinese adults also show higher rates of obesity if they have higher concentrations of BPA in their urine.[8] At age 9, BPA levels in Californian children were associated with higher BMI, obesity/ overweight, waist circumference, and fat mass. All these studies were large cross-sectional studies with more than 2000 subjects. Lesser number of patients in our study and higher values obtained for controls are probably the reasons for not getting a correlation between BMI and waist hip ratio with respect to urinary BPA levels.

In this study there was no correlation obtained between urinary BPA levels and BMI and waist hip ratio in cases of diabetes mellitus, primary hypothyroidism, obesity, azoospermia and PCOS. Although some studies have shown a relationship between urinary BPA levels and

Waist hip ratio, others have failed to show a relationship. This result may not show the true relationship existing in our population as the sample size of 30 patients in each endocrine disorder was very small to derive any statistically significant associations.

No correlations could be found between clinical parameters and any of the endocrine disorders studied. All the patients were exposed to BPA had detectable levels in the urine which shows for the first time in India that the population is continuously being exposed to Bisphenol A from the environment. Lack of correlation could be because of the small sample size. Further large studies for prevalence of diseases with measurement of BPA exposures in urine or serum may show the relationships.

In this study exposure history was not evaluated from patients or controls. The history of exposure in the form of using plastic bottles for drinking water or beverages and use of packaged foods over previous 24 hours could be used in future studies to evaluate the correlations between prevalence of endocrine disorders and urinary BPA levels.

CONCLUSION

The present study shows Indian population is exposed to Bisphenol A. The urinary levels of BPA were more than 1000 pg/mg of creatinine in more than 50% of the patients with endocrine disorders as well as control subjects. The values of BPA in urine were widely distributed showing variability of exposure from the environment.

There were significant differences obtained in the median values of BPA in urine between cases of azoospermia, PCOS and obesity as compared to controls.

In the present study, no correlation was found between urinary BPA values and BMI, waist-hip ratio, LH, FSH, LDL, HDL, total cholesterol and triglycerides in respective endocrine disorder(s).

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