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Research Paper

Urinary Bisphenol-A Identification in an Iraqi Population

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ABSTRACT

Background: Investigation into environmental chemicals and their impact on human health is a topic of great interest. It is essential to understand the relationship between the quantity of chemical substances involved and the prevalence of particular diseases. Bisphenol-A (BPA) is a disrupting agent to human endocrine health. Human exposure to BPA is on the rise due to the widespread use of this agent, particularly in manufacturing industries. This study aimed to investigate the concentrations of BPA in the Iraqi population.

Methods: This study aimed to determine the amount of BPA in the urine samples of 100 adults residing in the three cities of Diyalah, Kirkuk, and Solaymania in northeastern Iraq. After the subjects' urine samples were collected, the overall, free, and conjugated BPA contents of the samples were measured using high-performance liquid chromatography and mass spectroscopy (HPLC/MS). The data were adjusted based on the precalculated creatinine levels in the subjects.

Results: Urine samples were collected from 100 individuals residing in three different cities in Iraq, comprising 47 men and 53 women. The concentrations of BPA in all participants' urine samples ranged from greater than the limit of quantification to 80.24 µg/g creatinine, with a calculated geometric mean of 4.17 µg/g creatinine.

Conclusion: The findings of this preliminary study indicated that the Iraqi population's level of BPA exposure falls within an acceptable range. To provide a more accurate estimate of BPA exposure nationwide, further investigations on larger sample sizes are required across a wider range of age and gender distributions.

Keywords: BPA-conjugate, Endocrine disruptor, Iraqi population, Urinary Bisphenol-A

Introduction

Environmental toxicology is the study of the health effects associated with exposure to hazardous substances found in workplaces and residential settings. This field of study examines the detrimental effects of various chemicals and biological substances on living organisms [1].

Environmental Pollutants: Hazardous materials that contaminate the environment, such as tainted water, soil, or air, can have a major impact on the populace. Contaminants are chemicals that are present in the environment in greater amounts than they should be by nature. Human exposure to such pollutants can arise from a variety of sources, including industrial, commercial, and residential areas or naturally occurring agents [2].

Bisphenol-A (BPA) is an organic compound initially introduced in 1891 by the scientist Alexander Dianin [3]. However, it was Theodor Zincke who first recorded the BPA production in 1905 [4]. BPA is produced on a large scale and is also used in food and water storage. Initially, its production was limited to the synthesis of epoxy resin for applications on metals, the structure of which is presented in Figure 1 [5].

Polycarbonates that are generated from BPA have significant features, such as strong heat resistance, rigidity, endurance, and resistance to breaking. Furthermore, they are known for their good electrical insulation properties and increased weather and radiation resistance. Polycarbonates are widely used in mobile phones and motorcycle riders' helmets [6].

BPA is also a hormone disruptor, with the potential to interfere with the function of human endogenous hormones. Hormone disruptors, such as BPA, interfere with the production, discharge, transmission, metabolism, binding, and withdrawal of the body's natural hormones [7]. Exposure to BPA can cause obesity [8], reproductive health problems [9], cardiovascular abnormalities [10], and hepatic complications [11]. BPA can also interfere with estrogen response elements (ERE) and transcriptional

activation through its interaction with human estrogen receptors, both alpha (ERα) and beta (ERβ). Indeed, BPA binds to ERβ 6 to 10 times stronger than the hormone itself. On the other hand, its affinity for estrogen receptors is far lower than that of estradiol, that is, approximately 300-fold lower affinity for ERβ and 2000-fold lower affinity for ERα [12].

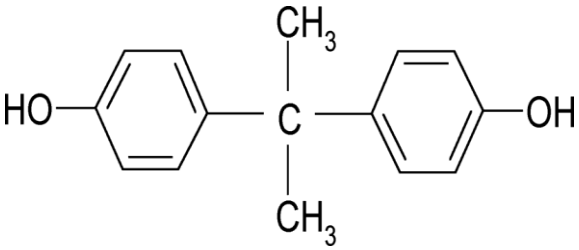

Figure 1. Bisphenol-A chemical structure [5]

The metabolic process of BPA is critical for the determination of the compound's toxicity profile at the cellular and molecular levels and for predicting its bioavailability and biological characteristics. Since BPA's water solubility is low due to its lipid solubility, it requires a considerable metabolic process to become water soluble. Findings from numerous studies have revealed that BPA's metabolic routes are diverse [13]. However, phase II conjugation processes in the digestive tract and liver have been identified as the primary sites of BPA metabolism.

Glucuronosyl transferases catalyze the conversion of lipophilic substances such as BPA to more water-soluble compounds. Specifically, BPA-glucuronide (BPA-gluc) is produced through the rapid conjugation by uridine diphosphate glucuronosyl transferase (UGT) in the second metabolic phase. This metabolic phase is also significant because of its chemical processes. Another compound in this metabolic route is the sulfotransferase enzyme, which forms BPA-sulfate, in both human and rat cells [14]. Notably, due to immature glucuronidation and sulfation processes, significant quantities of free BPA may be found in newborns. Additionally, BPA metabolism includes minor metabolites, such as BPA-3 and 4-quinone, in addition to the glucuronide and sulfate forms of BPA [15].

Due to the widespread use of BPA in the packaging of beverages and foods, the oral ingestion of this molecule is regarded as the primary source of BPA exposure in humans. Furthermore, BPA may be used in the production of paper products; therefore, its transdermal entry into the human body might be envisioned [16]. Additionally, BPA may be found in pollens and furnishings; therefore, its inhalation route may also be considered [17]. Depending on the type of research, the estimated human daily consumption of BPA, based on the data from urine sample analyses, might range from 0 to 2 µg/kg/day [18]. However, there is limited data available on the toxicokinetics of BPA in human models, which comprises a single oral delivery of 5 mg BPA. Based on that study's findings, BPA's peak plasma concentration reached around 1.5 h post-treatment, and its clearance in the urine took 30 h in both genders. It was also shown that the half-life of BPA in the blood was 5.4 h. Lastly, BPA-gluc, one of BPA's metabolites, has been found in both human urine and blood samples [19].

Aim of the Study: The aim of this study was to determine the extent of BPA exposure in a sample of the Iraqi population. This study was carried out as a preliminary analysis of BPA in the urine samples of an Iraqi population living in in urban and rural areas in three different cities.

Materials and Methods

To ensure the reliability of the findings, all reagents used in the experiments were of the purest grade available. BPA creatinine was purchased from Sigma Aldrich (Taufkirchen, Germany). The internal standards for BPA, that is, creatinine-d3 and BPA-gluc, were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Methanol and acetonitrile supplies for HPLC-grade were obtained from KGaA (Darmstadt, Germany). The required deionized water was prepared through filtration with the Millipore Milli-Q water pureness system purchased from Barnstead Intl. (*Dubuque, IA, USA*) and was utilized in aqueous solutions.

The North Cyprus Clinical Research Ethics Committees at the Near East University, Nicosia, North Cyprus, reviewed and approved the study protocol, including the informed consent form that governed the collection of research data and urine samples. The sampling period lasted seven months, from November 2020 to May 2021. Throughout the study period, 100 urine samples were collected from adult volunteer participants of both genders in three cities: Diyala, Kirkuk, and Sulaymaniyah in northeastern Iraq (Figure 2). Each participant was given a copy of the study's questionnaire to review and complete, which contained eight questions related to demographic characteristics and educational achievement as listed in Table 1.

A single urine specimen was collected from each participant in a 125 mL container lined with poly-tetrafluoro-ethylene that was rinsed in advance with hexane. Following the collection, each urine sample was divided into aliquots. To incorporate the two BPA samples (BPA-gluc and pure BPA), the urine samples were collected early in the morning before breakfast. The samples were stored in a fridge at 4°C, packed with dry ice, and frozen at -20°C until they were used for further analyses.

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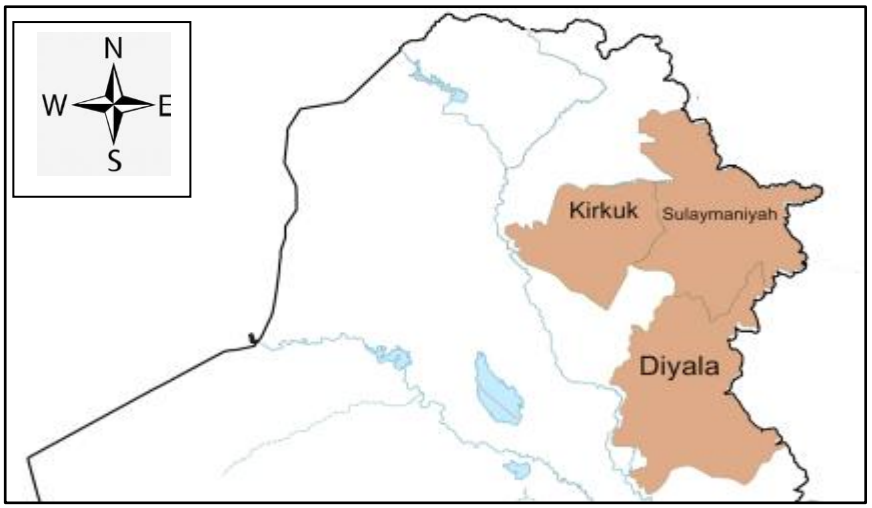

Figure 2. Northeastern Iraqi cities where the study samples were collected.

Table 1. Participant features, where "n" is the total number of participants in the study.

Gender (n/No.)	Age Mean ± SD	Urban(n=50) N	%	Rural(n=50) N	%
Male (47/100)	39.1±13.95	23	46.0	24	48.0
Female (53/100)	35.8 ± 12.68	27	54.0	26	52.0
Smoker		14	28.0	8	16.0
Alcoholic		3	6.0	-	-
	No Education	0	0	4	8.0
Education	Primary to College	12	24.0	18	36.0
Level	University	30	60.0	26	52.0
	MSc to PDH	8	16.0	2	4.0

To eliminate contamination in the laboratory environment, glass test-tubes and other glassware were used as much as feasible. Each participant was provided with a consent form to review and sign prior to sample collection. The informed consent form contained information about the specific experimental processes, the study's main sequences, and a line for the participant's signature.

Sample Preparation: The materials were combined and aliquoted in glass vials and pre-cleaned with a hexane solution. One aliquot was left unspiked, while others were added with either BPA or BPA-gluc (0.01 g/mL, each). C_{12}-BPA was used as the internal standard during the sample preparation process. The urine specimens (0.5 mL) were marked as either pure BPA or BPA-gluc. We used 0.5 mL acetonitrile solvent and 0.5 mL of the internal standard. Following the protein precipitation process, all urine samples were centrifuged at 2250 rpm and 25°C for 10 min. The BPA and BPA-gluc contents were measured in the supernatants on a high-performance liquid chromatography and mass spectroscopy (HPLC/MS) unit.

Instrumentation and Analytical Conditions: Mass spectrometry with an atmospheric pressure electrospray ionization source and the Mass Hunter software (version 4) were used to quantify and identify free BPA and conjugate-BPA, respectively. The HPLC system was carried out based on the method of Battal *et al.* [20].

When the BPA and BPA-gluc analyses were completed, the mobile phases A and B were consistent between water and methanol. The gradient flow rate was 0.500 mL/min, which started at 20% (MEOH) and was gradually raised to 70% after four minutes. The gradient was then raised to 90% methanol within 4-9 min. At 9-10 min, the gradient was reduced to 20% methanol. Tandem mass spectrometry was carried out using negative electrospray ionization in the selected reaction monitoring (SRM) mode. Nitrogen served as both the cover and the explosion gas. The working electrospray ionization condition was set as follows: the sheath gas and auxiliary gas were nitrogen, and the column temperature was set at 35°C. Applying the Mass Hunter software, the peak area of the examined chemical and its internal standard were set, with the correlation coefficients being $R2>0.99$ for BPA and $R2\geq0.99$ for BPA-gluc. The relevant detection level for BPA-gluc was 0.1 ng/mL, while for BPA, it was 0.03 ng/mL.

Creatinine Content: The amounts of BPA-gluc and free BPA found in the urine samples were corrected for creatinine content throughout the study. The urine creatinine content was assessed using a valid modified method [21], which involved adding an internal standard of creatinine-d3 to the urine samples and diluting them with methanol by ×100. The chromatographic parameters were the same for the creatinine, BPA, and BPA-gluc analyses. The calibration curve construction using creatinine dissolved in methanol was linear, with $R2>0.99$ for creatinine.

Prediction of Estimated Daily Intake: The

prediction of daily BPA exposure was calculated depending on the adults' urine removal volume of 1.7 L over 24 h. The 24-h BPA dosage for each person was determined using the equation presented in Figure 3 [22].

$$\text{EDI } (\mu g/kg\ bw/day) = \frac{\text{Urinary BPA concentration } (\mu g/L) \times \text{Urinary output } (L/day)}{\text{Body weight } (kg)}$$

Figure 3. Equation for the estimation of daily BPA consumption [22]

Statistical Analyses: The data analyses were carried out using the Statistical Package for the Social Sciences (SPSS) software (version 23). For each participant, the data were processed as described later. The Mann-Whitney U test was used to compare the differences between the data sets in nominal, qualitative, and non-normally distributed variables. Further, the Kruskal-Wallis test was used for non-normally distributed and ordinal qualitative variables, with the significance level set at $P \leq 0.05$.

Results

Free BPA and BPA-gluc were measured in the 100 urine samples, and the overall BPA was found in 52 samples. Based on the statistical analyses, the participants had been exposed to high levels of BPA. The creatinine measurement in the samples was carried out similarly (creatinine g/mL). Of all the urine samples collected, 52% contained BPA, with levels ranging from 0.25 to 80.24 µg/g creatinine. The total BPA content measured in the samples ranged from 0.30 to 80.24 µg/g creatinine for men and from 0.25 to 59.53 µg/g creatinine for women as shown in the table 2.

Daily Bisphenol-A Intakes: The measurement of urine BPA is a crucial method for assessing its daily consumption and serves as a helpful reference for its excretion. The measured BPA was consistently associated with the same volume of urine produced during a 24-h period, allowed for measuring the daily BPA elimination (ng/day), and reflected the estimated daily ingestion for each participant [23]. Based on the findings, the Iraqi population consumed BPA between 0.04-3.91 µg/kg bw/day, as summarized in Table 3. The estimated daily consumption of BPA in this population had a mean of 0.072 µg/kg bw.

Table 2. The urinary levels of total BPA in the Iraqi population µg/g creatinine, n is number of participants. AM is an arithmetic mean. GM is geometric mean. No. is number of samples above limit of detection, * first and third quartile values.

	Adjusted Total BPA µg/g Creatinine					
	n	**AM**	**GM**	**No.**	**Range**	**[%25-%75] ***
Male	47	16.61	6.89	27	0.30-80.24	3.4-23
Female	53	6.97	2.42	25	0.25-59.53	0.4-7.08
Total	100	11.97	4.17	52	0.25-80.24	0.66-16.96

The urinary levels of total BPA (µg/g creatinine); n=Number of participants; AM=Arithmetic mean; GM=Geometric mean; No=number of samples above the detection limit; *=First and third quartile values

Table 3. The estimated daily intake of bisphenol-A in the Iraqi population in µg /kg bw/day. GM is geometric mean, * first and third quartile values.

	GM	**[25%-75%] ***	**95% Confidence limits**
Total Population	0.072	0.011-0.590	0.103-0.191
Urban	0.062	0.007-0.299	0.086-0.185
Rural	0.091	0.064-0.286	0.077-0.287
Male	0.167	0.041-0.447	0.111-0.229
Female	0.042	0.023-0.122	0.05-0.190

The estimated daily intake of Bisphenol-A was measured in µg/kg/day. GM=Geometric mean; *=First and third quartile values

Discussion

Endocrine disruptors, such as BPA, alter the level of hormones over time. Chronic exposure to these chemicals, which only slightly affect the function of hormones, can have detrimental biological repercussions for humans [24]. The use of urine specimens for monitoring BPA offers an overview of exposure from non-food sources [19]. Additionally, due to its excretion process, urine collection is less interfering with the body than taking blood samples and reflects the data for the recent 24 h. Urine samples can thus be considered a practical method of choice for measuring BPA exposure in humans. Indeed, urine has a practical half-life of less than six hours for the human body, making it a popular biological sampling method for estimating BPA exposure in humans [19].

Analyzing BPA exposure reveals that human fluids and tissues, including plasma, urine, semen, placenta, blood, serum, and breast milk, are likely to contain detectable levels of this compound and its metabolites. BPA has also been found in a variety of human biological fluids by numerous monitoring experiments, including blood, milk, urine, and amniotic fluid [25-29]. When assessing BPA exposure in humans, it is crucial to monitor total rather than individual BPA exposure. According to recent theories, the unconjugated form of BPA, that is, the free form, is physiologically active. Consequently, measuring the free BPA concentration is more realistic for assessing the potential health risks to humans [30].

In the current study, both the overall BPA and free BPA values were accounted for. In conducting

monitoring studies, a variety of target populations have been considered, including infants, toddlers, children, teenagers, pregnant women, men vs. women, and normal adults vs. those with diabetes or obesity [25-36]. In this study, we also found that those who resided in rural and urban areas had essentially comparable BPA levels in their urine.

A number of analytical methods for tracking BPA have been employed recently. Using powerful and accurate HPLC/MS, the human urine contents of both BPA and its glucuronide type can be assessed experimentally [37]. In the current research, the experimental limits of detection of BPA and BPA-gluc were 0.1 and 0.03 ng/mL, respectively. We obtained highly sensitive data compared to those reported by earlier studies. Specifically, according to two earlier studies [18, 38], the limits of detection for BPA have been between 2.0 and 2.6 ng/mL [18].

The first monitoring research on urine BPA in the US was carried out by Brock *et al.* in 2001 [39]. The results reported by other studies have shown a wide range of BPA distributions in the urine, from 44% to 100% in the United States. For instance, the extent to which BPA has been detected in adults varies from 91% to 95% [39]. The reported distributions in Germany and Canada were 91% [40], in Kuwait, 81% [41], and in India, Japan, and China, 100% [41]. Of these studies, the ones conducted in the United States and Canada attracted the most attention since they were both large-scale research projects. Based on the current study, 52% of the people in the Iraqi sample had BPA detected in their urine.

The findings of the current study are quite significant since this is the first study to assess the BPA level in an Iraqi population. They provide baseline information on the prevalence of BPA in the Iraqi population, both in urban and rural areas. The comparison of our reported mean with comparable studies conducted in various nations showed that our mean was greater than that of Germany (2.4 µg/g) but lower than that of the United States (5.49 µg/g) [42, 43].

The mean and span for both free and overall BPA contents were higher in the specimens for men than in those for women. This finding was consistent with those of previous studies [44], which compared BPA levels between men and women, discovering that men had higher levels of BPA and other chemicals than women.

With respect to the regional variations, the quantity of BPA was typically higher in the residents of city centers (12.72 µg/g creatinine) and lower in those living in rural areas (9.94 µg/g creatinine). This finding was consistent with the data reported by previous research, in which the researchers attributed lower levels of BPA in urine specimens from rural areas to their consumption of their own production [20].

Further, a high extent of using BPA-containing products was noted among the city dwellers based on the data obtained from questionnaires prior to collecting urine samples. Therefore, increased intake of unhealthy foods and beverages can be linked to greater levels of BPA exposure in metropolitan settings. In order to determine the quantity of BPA exposure during the past 20 years, a number of monitoring studies have been conducted. Based on studies conducted to date, the estimated daily worldwide consumption of BPA is about 30 ng/kg bw [45].

Iraqi adults are estimated to consume a level of BPA that is comparable to the documented levels for the same substance in other nations. The daily mean for Iraqi adults who belonged to the same population was 0.072 µg/kg bw. However, compared to the documented BPA exposure for Belgian (0.040 µg/kg) and Canadian (0.031 µg/kg) populations, the Iraqi predicted daily consumption of BPA was nearly twice as much [45].

Conclusions

Industrial compounds, such as BPA, are used in manufacturing polycarbonate goods and epoxy resins. Toxicological studies tracking human exposure to chemicals have attracted worldwide attention due to their potential to disrupt the human endocrine system. Numerous scholarly articles have addressed the detrimental effects of BPA on human health. The results of the current study, being the first of its kind, offer a preliminary understanding of the Iraqi population's exposure to BPA. Large-scale monitoring studies on larger population samples from multiple provinces in Iraq should be conducted in the future to accurately determine the actual BPA exposure. Moreover, in order to determine the extent of BPA exposure, it is critical to conduct follow-up studies in the same provinces involving children and other vulnerable populations. The findings of the current study are believed to enhance awareness among Iraqi healthcare practitioners considerably with respect to the BPA's detrimental impacts on human health.

Conflict of Interests

The authors declare no conflicts of interest.

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Compliance with Ethical Guidelines

This study received ethical approval (Code: YDU/2020/83-1163) from the Scientific Research Ethics Committee, Near East University, Nicosia, North Cyprus, on September 23, 2020.

Authors' Contributions

Conceptualization, study design, laboratory tests, sample collection, data analysis, and writing the article were done by DSHM. Study supervision, design, and

proofreading were done by SS. Both authors have critically reviewed and approved the final draft of the manuscript and have checked the accuracy of the content of the manuscript.

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