Evaluation of Blood Bisphenol A Contents: A Case Study

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ABSTRACT

Objective: *It has been recently reported that Bisphenol A (BPA) may leach out into food, beverages and water samples from the plastic ware in which it is stored. Serious health hazards have been reported from BPA. The purpose of this study is to assess the BPA contents in blood and to assess the risk of cancer.*

Method: *A total of 100 individuals were selected for study according to the following five age groups: 5−10, 11−20, 21−30, 31−40 and 41−50 years. They were then further divided into normal and diseased. Age, gender, education, source of drinking water, type of food, smoking habit, any exposure to chemicals and history of cancer were elicited during interview. Blood samples were collected and processed for analysis using reversed phase-high performance liquid chromatography (rp-HPLC) in isocratic mode. The mobile phase consisted of acetonitrile and water (1:1) at a flow-rate of 1 ml* min^{-1}.

Results: *Bisphenol A contents found in blood samples of all age groups ranged from 1.53−3.98 (mean = 2.94, SD = 0.9).* P-*values, for the exposed people and those having a history of cancer, were < 0.05 showing a significant relationship between BPA and cancer. The United States Environmental Protection Agency (US EPA) has established a reference dose of 50 µg/L. Odd ratios and relative risk for smoking habit were < 1 while for all others they were > 1.*

Conclusion: *It was concluded from the study that people using bottled water, packaged food, having a history of cancer and who had been exposed to any type of chemicals are at higher risk of disease.*

Keywords: Bisphenol A (BPA), BPA health effect, BPA in blood, cancer

Evaluación de las Concentraciones de Bisfenol A en la Sangre: Estudio de Caso

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RESUMEN

Objetivo: *Se ha reportado recientemente que el bisfenol A (BPA) puede filtrarse a alimentos, bebidas y agua, a partir de los recipientes plásticos en que aquellos se almacenan. En tal sentido, se han reportado serios casos de riesgo para la salud a causa del BPA. El propósito de este estudio es evaluar la concentración de BPA en sangre, y el consiguiente riesgo de enfermedades cancerosas.*

Método: *Un total de 100 individuos fueron seleccionados para el estudio, de acuerdo con los siguientes cinco grupos etarios: 5–10, 11–20, 21–30, 31–40 y 41–50 años. Dichos grupos fueron divididos entonces sobre la base de sujetos normales frente a enfermos. En la entrevista se tomó nota de la edad, el género, la educación, la fuente de agua potable, el tipo de comida, el hábito de fumar, cualquier exposición a productos químicos, así como la historia de cáncer. Las muestras de sangre fueron recogidas y procesadas para realizar análisis, utilizando cromatografía líquida de alta eficacia de fase reversa (rp-HPLC) en modo isocrático. La fase móvil consistió en acetonitrilo y agua (1:1) con una tasa de flujo de 1 ml* min^{-1}.

Resultados: *Las concentraciones de bisfenol-A halladas en las muestras de sangre de todos los grupos etarios, oscilaron de 1.53 – 3.98 (M = 2.94, SD = 0.9). Los valores P para las personas expuestas y con una historia de cáncer, fueron < 0.05, indicando una relación directa entre el BPA y el cáncer. La*

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Agencia de Protección Ambiental de los Estados Unidos (US EPA) ha establecido una dosis de referencia de 50 µg/L. El cociente de probabilidades (odd ratios) y el riesgo relativo con respecto al hábito de fumar fueron < 1 mientras que para todos los otros casos otros fueron >1.
Conclusión: *A partir del estudio se concluye que las personas que usan agua embotellada, alimentos empaquetados, así como las personas que poseen una historia de cáncer, y los individuos que habían estado expuestos a cualquier tipo de productos químicos, presentan un mayor riesgo de enfermedad.*

Palabras claves: Bisfenol A, BPA en sangre, efecto del BPA en la salud, cáncer

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INTRODUCTION

Bisphenol A (BPA) is a widely used industrial chemical with many applications. Bisphenol A has been reported to be weakly oestrogenic and, due to possible low-dose effects of unknown toxicological relevance, is the subject of many investigations assessing its potential toxicity and human exposures for a more conclusive risk assessment (1−6). Human exposure to BPA is estimated to be mainly by food consumption and, according to worst-case scenarios, may reach 40 µg/kg bodyweight (7).

Bisphenol A was first recognized in the 1930s to be a potential synthetic oestrogen (8). Most studies on the health effects of BPA have focussed on well-documented oestrogenic activity (9) but reports have highlighted additional modes of action (10) including liver damage (11−15), disrupted pancreatic β-cell function (16), thyroid hormone disruption (17) and obesity-promoting effects (18). The potential for low-dose effects (19) has added to the controversy about possible hazards and whether currently recommended exposure thresholds require revision (4, 20−22). The purpose of this study is to analyse BPA in human blood samples and then calculate the risk for cancer. Research has been carried out regarding BPA toxicity in developed countries but before this no research has been carried out in Pakistan. So there is a need to assess the risk of BPA in a developing country. The aim of the work was to access exposure of the Lahore population to BPA.

SUBJECTS AND METHODS

Bisphenol A (minimum purity 97%) was obtained from Sigma Aldrich. High performance liquid chromatography (HPLC) grade dichloromethane, methanol, and acetonitrile were obtained from Merck. A standard solution containing 100 mg/L of BPA and a substock containing 10 mg/L of BPA were prepared in methanol and kept in the refrigerator. Solutions of the required concentrations were prepared daily by dilution.

A total of 100 individuals were selected for study and were divided into five age groups (5−10, 11−20, 21−30, 31−40 and 41−50 years). Further divisions were made into normal and diseased (Table 1). Age, gender, education, source of drinking water, type of food, smoking habit, any exposure to chemicals and history of cancer were recorded during interview. Of 100 test subjects, 69 individuals were apparently normal while 31 were suffering from different diseases (diabetes, hepatitis, blood infection and liver problem). This division was made after the interview. Individuals having any type of disease were grouped as diseased. Source of drinking water of all individuals was fresh water and only 19.35% of diseased individuals had history of cancer. Of all normal and diseased subjects, only 2.89% were exposed to chemicals [different individuals were exposed to different chemicals according to their workplace] (Table 1).

Blood samples were collected in vacuum glass tubes (obtained with an anti-clotting agent from a medical laboratory) and were homogenized by inverting the bottle five to

Table 1: Characteristics of human volunteers participating in the study

Characteristic	Category	Normal (n = 69[1]) (%)	Diseased (n = 31[1]) (%)
Age (years)	> 10	16 (23)	4 (13)
	11−20	14 (20)	6 (19)
	21−30	14 (20)	6 (20)
	31−40	13 (19)	7 (22)
	41−50	12 (18)	8 (26)
Education	Middle	33 (48)	20 (64)
	High school	23 (34)	11 (36)
	College	13 (18)	00 (0)
	University	01 (1)	
Food	Fresh	59 (86)	30 (97)
	Packaged	10 (14)	01 (3)
Drinking water	Fresh	60 (87)	30 (97)
	Bottled	09 (13)	01 (3)
History of cancer	Yes	09 (13)	06 (19)
	No	60 (87)	25 (81)
Ever exposed to solvents and chemicals[2]	Yes	08 (12)	01 (3)
	No	61 (88)	30 (97)
Current smoker	Yes	12 (18)	02 (6)
	No	57 (82)	29 (94)

[1]Number of individuals in study
[2]May include organic solvents and pesticides *etc*

six times. These were then taken to the research laboratory and were processed for HPLC analysis without further storage.

Statistical analysis was carried out using Microsoft Excel and SPSS version 16.

All samples were extracted under similar conditions. To one millilitre of each sample, dichloromethane (20 ml) was added with stirring and then 100 ml acetonitrile was mixed slowly while stirring constantly. A precipitate was formed; the suspension was centrifuged at 3000 rpm for 10 minutes. Supernatant fluid was separated and concentrated under reduced pressure at 40°C to about 2 ml. The concentrate and 8 ml acetonitrile were shifted to a volumetric flask and adjusted to 20 ml with distilled water. An appropriate amount was filtered and analysed by reverse phase-HPLC.

The HPLC system (Waters 1500) consisted of a pump, a UV detector (2487) and a C18 column (250 x 4.6 mm, 5 mm particle sizes). The samples were qualitatively analysed in isocratic mode, with acetonitrile/water (1:1) at a flow-rate of 1 ml min^{-1}. The injection volume was 10 ul and the elute was monitored at 217 nm. The sonicated and filtered extracts (0.5 microns) of all water samples were injected under these conditions and compared with an authentic standard of BPA, injected under similar conditions. All samples were analysed by the reverse phase-HPLC.

The instrument was calibrated using calibration standards. Calibration standards were prepared from stock solution of BPA to obtain a calibration curve.

Experiments were carried out in triplicate for maximum reliability of results and to assure precision and accuracy. When starting analysis, stability of baseline and response linearity of the detector was examined. Detector was able to detect BPA at a signal to noise ratio of 3:1. The same operating conditions of the HPLC system were maintained throughout the analysis of all water samples. Each solution was injected at least in duplicate. Samples were kept in a refrigerator at + 4°C in closed glass bottles and excluded from light.

Triplicate analysis was carried out in order to ensure the accuracy and precision. Recognizing that calibration of the working standards was important for accurate analysis, care was taken to ensure that the calibration curve had a correlation as close to one as possible. The calibration curve was obtained by plotting the areas under the peaks of the standard *versus* the absolute amounts. Bisphenol A in the samples was identified by comparing its liquid chromatography retention time with that of the authentic standard of BPA with distilled water taken as blank (Figure).

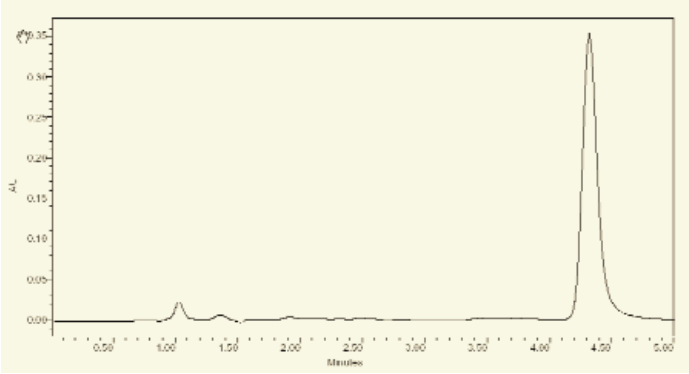

Figure: High performance liquid chromatogram of bisphenol A at room temperature and pressure.

RESULTS

Detectable levels of BPA in blood samples of all age groups ranged from 1.53–3.98 µg/L (mean = 2.94, SD = 0.9). There was a wide range of BPA for different age groups. In the case of diseased individuals it was observed that BPA contents increased with age.

Median and geometrical means were calculated along with percentiles (Table 2). Mean BPA contents in diseased individuals was 3.02 ± 0.9 µg/L while in normal individuals it was 2.80 ± 0.8 µg/L. Percentiles (50^{th} and 25^{th} – 75^{th}) were also calculated to show the distribution of BPA contents (Table 3). In females (n = 56), the mean value of BPA was

Table 2: Exposure of Lahore population to bisphenol A

Age groups	Samples (n)	Detectable level of BPA + STD	Median	Geometric mean	Percentile 50^{th}	Percentile 25^{th} – 75^{th}
5–10 years	20	1.53 ± 0.2	1.49	1.51	1.49	1.33 – 1.73
11–20	20	2.48 ± 0.3	2.48	2.45	2.48	2.32 – 2.79
21–30	20	3.16 ± 0.3	3.24	3.13	3.24	2.83 – 3.48
31–40	20	3.55 ± 0.6	3.78	3.48	3.73	3.15 – 4.05
41–50	20	3.98 ± 0.3	4.05	3.96	4.12	3.69 – 4.26

Table 3: Distributions of bisphenol A levels between normal and diseased persons

Title	Samples (n)	Mean ± STD	Median	Geometrical mean	Percentile 50^{th}	Percentile 25^{th} – 75^{th}
Diseased	31	3.02 ± 0.9	2.97	2.84	2.97	2.51 – 3.96
Normal	69	2.80 ± 0.8	2.79	2.65	2.97	2.25 – 3.47

2.99 ± 0.9 µg/L while in males (n = 44), contents were 2.87 ± 0.9 µg/L with median value 2.97 and 2.78, respectively (Table 4).

Table 4: Distributions of bisphenol A levels between male and female

Title	Samples (n)	Mean ± STD	Median	Geometrical mean	Percentile 50th	Percentile 25th – 75th
Male	44	2.87 ± 0.9	2.78	2.71	2.78	2.25–3.71
Female	56	2.99 ± 0.9	2.97	2.80	2.97	2.34–3.70

DISCUSSION

It was observed that in the case of normal individuals, there was no relationship between increase in age and BPA contents in blood. Variation in values may be due to different levels of exposure and different use of products containing BPA because it has been reported that most of BPA may leave the body through excretion (23).

The United States Environmental Protection Agency (US EPA) has the same "safe" level as the European Union (EU). The EPA has established a maximum acceptable or "reference" dose for BPA of 0.05 mg/kg bodyweight/day (= 50 µg/kg bodyweight/day). This was based on a test for cancer (24).

Odd ratios and relative risks were calculated to assess the risk of cancer in males and females, on the basis of source of drinking water, type of food, smoking habit, any exposure to chemicals (workplace exposure *ie* people working in plastic and chemical industries have different levels of exposure to different types of chemicals) and history of cancer. The results of this study indicate a direct relation between BPA and cancer ($p < 0.05$). Odd ratios and relative risk for smoking were < 1 (0.6) which means that this group is 40% less likely to have cancer; for all other characteristics, odds ratios were > 1 showing that first group in each case is at a higher risk of cancer (Table 5). Individuals who were exposed to organic solvents or pesticides *etc* were at the highest risk. If one considers the exposure to BPA from all sources (dietary, through air, water *etc*) then there would be a high risk of BPA-related diseases.

CONCLUSION

In the present study, it has been observed that diseased individuals have high BPA in their blood. It was clear from this study that people using bottled water, packaged food, having a history of cancer and individuals who had been exposed to any type of chemicals were at higher risk of disease. Odd ratios showed that males were at higher risk of cancer than females. If we consider the diseased subjects, they are at higher risk due to higher BPA contents so they may reach the reference dose earlier than normal individuals. Because BPA has been detected in blood samples of all test subjects, proper biomonitoring is required to assess the risk from BPA exposure.

Table 5: Exposure to bisphenol A and risk of cancer

Title		Normal (n = 69[1]) (%)	Diseased (n = 31[1]) (%)	OR[1] (95% CI)	Relative risk (95% CI)	Trend test *p*-value
Sex	Male	30 (43.47)	14 (45.16)	1.3 (0.58–3.11)	1.1 (0.78–1.6)	0.4
	Female	39 (56.52)	17 (54.83)			
Food	Fresh	59 (85.50)	30 (96.77)	2.03 (0.21–19.0)	1.96 (0.23–16.0)	0.5
	Packaged	10 (14.79)	01 (03.22)			
Drinking water	Fresh	60 (86.95)	30 (96.77)	1.5 (0.15–15)	1.4 (0.16–13)	0.7
	Bottled	09 (14)	01 (3.22)			
Ever exposed to solvents and chemicals[2]	Yes	08 (12)	01 (3.22)	10 (0.7–146)	1.2 (0.2–2.2)	0.04
	No	61 (88.40)	03 (96.77)			
Current smoker	Yes	12 (17.40)	02 (06.45)	0.6 (0.12–3.45)	0.96 (0.85–1.09)	0.4
	No	57 (82.60)	29 (93.54)			

[1]Number of individuals in study; [2]May include organic solvents and pesticides *etc;* OR[1] = crude; 95% C1 = 95% confidence interval

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