Concentrations of urinary parabens and reproductive hormones in Iranian women: Exposure and risk assessment

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ABSTRACT
Parabens are antimicrobial preservatives in a variety of processed foods and beverages, cosmetics, pharmaceuticals, and personal care products. Parabens may be associated with reproductive and endocrine disorders among women of reproductive age. The first objective of this study was to examine the association between urinary parabens concentrations and follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin disorders. The second objective of this study was to calculate the Hazard Quotient (HQ) and Margin of Exposure (MOE) to assess the potential risk for endocrine disrupts for each woman based on urinary paraben concentrations. To address these two objectives, a cross-sectional study was designed in the Imam Reza Hospital in Kermanshah. The association between early morning urinary paraben concentrations and the serum of fasting blood specimens was analyzed using multivariable linear regressions adjusted for confounding variables (i.e., creatinine, age, body mass index, and time spent on physical activity). Among the 96 women who participated in the study, those with BMI greater than 25 and aged 18–40 years showed higher levels of total urinary parabens. The highest sum of urinary parabens (54,955.16 µg/L) was observed among the women who were using toothpaste several times per day. Almost all the parabens such as methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), butyl paraben (BuP) had a positive and negative significant association with serum concentrations of FSH and LH (p-value < 0.05). However, no significant association was found between any of the four parabens and serum prolactin hormone (p-value > 0.05). The Margin of Exposure (MOE) approach calculated for all parabens (-10,000) showed a potential risk in the studied population. The results suggested that parabens could adversely affect reproductive and endocrine systems in women. Further studies relying on long-term exposure to parabens are necessary to better understand the potential risk of the association between urinary paraben concentrations with reproductive hormones.

1. Introduction
Parabens (alkyl or aryl esters of p-hydroxybenzoic acid) are a category of antimicrobial preservatives that are added to a number of processed foods and beverages, cosmetics, pharmaceuticals and personal care products since 1920 [36,53]. The widespread use of parabens...
in various products is the reason for their contaminating prevalence in both the environment and human specimens, such as urine, milk and serum ([18,36,37]). More than 50% of personal care products and around 90% of processed foods and beverages contain parabens [5,14,32]. The estimated daily human exposure to parabens is in the range of 76–142 mg, and due to their susceptibility to metabolic capture and excretions, they are nearly detectable in all urine specimens [5]. Exposure to parabens, as a result of their estrogenic properties, can reduce serum testosterone levels and diminish sperm count [4,6,34,44]. Para-

2. Materials and methods
2.1. Study design and sample collection

Of the 300 women who were referred to the Educational and Medical Imam Reza Hospital in Kermanshah city, only 96 women exhibited abnormal pubertal and breast development [48,55,54,8,19]. Moreover, a number of studies have shown that paraben exposure can exhibit a number of reproductive hormone disorders, especially anti-androgenic manifestations [15,23,48,51]. Mechanistic and epidemiological studies have provided a causal or correlative interaction between paraben contamination and increases in luteinizing hormone (LH), decreasing plasma corticosterone levels, increased follicle-stimulating hormone (FSH) [25,24,27,28,45,51].

The goal of this study was to characterize the relationship between concentrations of parabens compounds and reproductive-axis hormones such as FSH, LH, and prolactin in the Iranian women living in Kermanshah, Iran. These parameters were used to calculate a Hazard Quotient (HQ) and Margin of Exposure (MOE) measurements to ascertain the potential risk exposure for women based on their urinary paraben concentrations.

2.2. Sampling and measurement of urinary parabens

Because parabens have short biological half-lives (13–24 h) [1] and first morning urine samples may reduce exposure misclassification. For this reason, first morning urinary discharge samples of participants were collected in a sterile container, and then stored in the Imam Reza Hos-

pital laboratory at −20 °C until analysis in the laboratory in Isfahan University of Medical Sciences [22]. The urine creatinine was measured by the Jeff method using Hitachi 704 auto-analysis. All standards, including methyl paraben, ethyl paraben, propyl paraben, and butyl paraben, β-glucuronidase/sulfatase and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma–Aldrich. Chlorobenzene, methanol, and acetone solvents were obtained from Merck (Darmstadt, Germany). A standardized solution-mix of the four parabens were prepared in methanol and kept at −4 °C until analysis. Urinary paraben extraction was conducted by dispersion and liquid-liquid microextraction (DLLME) method and analyzed using the GC/MS. In order to determine the total of urinary parabens (free and conjugated), 10 μL of the β-glucuronidase/sulfatase enzyme was injected to the bottle and incubated for 24 h at 37 °C. Then, 0.1 g of sodium chloride was added to the solution, and centrifuged for 5 min at 4000 rpm. 10-ml from obtained supernatant was injected into another falcon tube and extracted by the DLLME method. For extraction, 500 μL of acetone and 30 μL of chlorobenzene were injected to the falcon tube containing supernatant to form a cloudy solution. The cloudy solution was centrifuged at 5000 rpm for 5 min. A 50-μL aliquot of the clarified phase was transferred into a GC vial, and gently dried by nitrogen gas. Moreover, 20 μL of MSTFA reagent was injected to the dry deposit and centrifuged. Finally, 2 μL from the derivatized solution was injected into the GC/MS device for parabens analysis. Setting and optimizing the analysis condition were conducted according to the previous studies [16]. The calibration curve was generated using four parabens compounds (0.01–1000 ng/mL) in synthetic urine.

2.3. Measurement of serum reproductive hormones

The serum of fasting blood specimens was analyzed for FSH, LH, and prolactin concentrations using a clinical immunochemistry analyzer in Imam Reza Hospital in Kermanshah [15].

2.4. Validation of the analytical method

To ensure the accuracy of the analytical method, quality control (QC) samples along with blank samples and spiked urine samples were analyzed. The average recovery of four parabens was calculated in the range of 89–93%, and the regression coefficients (R²) were in the range of 0.992–0.998 for four parabens. The limit of detection (LOD) and the limit of quantification (LOQ) were determined based on the signal-to-noise (S/N) ratio being 3:1 and 10:1, respectively. LOD and LOQ calculated for all urinary parabens were in the range of 0.015–0.05 μg/L and 0.014–0.049 μg/L, respectively. Paraben analysis by GC/MS device was performed according to the previous study conducted in Isfahan University of Medical Sciences [17].

2.5. Exposure and risk assessment

Exposure and risk assessment of four parabens was performed based on their urinary levels in the studied women. HQ, as a risk measurement tool, was calculated using the estimated daily intake (EDI) [26]. The EDI was calculated according to Eq. (1).

$$EDI = \frac{C \times R}{F \times BW}$$

Whereas, EDI (mg/kg bw/day) is the estimated daily intake of a paraben compound; C (μg/L) is the urinary concentration of parabens; C is the
95th percentile for each compound (Table 2); R (L/day) is the total volume of an adult urine per day; BW (kg) is the bodyweight, and F (no dimension) is the urinary paraben excretion factor. According to previous studies, R was considered as 2 L and the F values for MeP, EtP, PrP, and BuP were considered as 17.4, 13.7, 10.2, and 5.6, respectively. The mean body weight of the participants was considered as 60 kg [17,21,38].

The risk assessment was performed depending on the reference values available for each compound. The expert panel from EFSA established an acceptable daily intake (ADI) of 0–10 mg kg-BW/day for the sum of MeP and EtP. Consequently, the risk assessment for the sum of MeP and EtP was done using the HQ as a risk (Eq. 2) [50].

\[
HQ = \frac{\sum EDI}{ADI}
\]  

(2)

The calculated EDI was considered safe if HQ < 1 [26]. In addition to HQ, MOE can be used to estimate the health risk of a substance. MOE is the quotient derived from the toxicological of No Observed Adverse Effect Level (NOAEL) value and the EDI.

\[
MOE = \frac{NOAEL}{EDI}
\]  

(3)

An MOE of over 10,000 denotes that the substance can be considered as of low concern from the public health point of view [50]. NOAEL of MeP and EtP was considered as 1000 mg kg-BW/day. However, NOAEL for BuP and PrP was considered as 2 mg kg-BW/day [46].

2.6. Statistical analysis

All the analyses were done via SPSS Version 22. A descriptive test was used to assess the frequency of the participants’ characteristics. The associations among individual urinary parabens, Σ parabens, demographic characteristics and reproductive hormones (FSH, LH, and prolactin) were also examined using the multivariable linear regression analysis. Based on this regression, parabens were considered as dependent and independent variables in Tables 3 and 4, respectively. Multiple logistic regression models were used to remove the probably hidden bias created by creatinine, age, BMI and time spend on physical activity on the association between the hormones and tertile parabens concentrations (Table 5). Models II and III were adjusted for a set of some confounders (Model I: crude/unadjusted; Model II: adjusted on creatinine; and Model III: adjusted on creatinine, age, BMI, and time spend on physical activity).

3. Results

3.1. Description of the studied population, urinary parabens, and serum reproductive hormone concentrations

The obtained results indicated that 47.82% and 50% of the women could be labeled as overweight and normal in terms of BMI, respectively. Higher concentrations of the sum of urinary parabens were observed in the 18–40 years-old and women with BMI > 25. Only 7.61% of the women answered yes to the question of spend time on physical activity. The highest sum of urinary parabens (54955.16 µg/L) was observed among the women using toothpaste several times per day (Table 1). PrP (9441 µg/L) and MeP (8556 µg/L) had the maximum concentrations in the studied population, respectively. The concentrations of FSH, LH and Prolactin were shown in Table 2.

3.2. The association between change in urinary paraben concentrations and the participants’ characteristics

In this study, the association between paraben concentrations and the general characteristics and lifestyles of the participants was calculated by the multiple linear regression (Table 3). The results indicated that not using the antiperspirant products was associated with 0.298 lower urinary PrP concentration (p-value = 0.049) compared to using the antiperspirant products. Moreover, urinary MeP concentration was 0.293 lower in the women who were not using hair color (p-value = 0.016), and urinary PrP concentration was 0.348 higher in the women who were using toothpaste several times per day (p-value = 0.003), compared to other women (Table 3).

3.3. Associations between urinary parabens concentrations and reproductive hormone levels

The association between urinary parabens and serum reproductive hormones in the women was studied using the multivariable regression analysis (Table 4). A significant association was observed among MeP (p-value < 0.001), PrP (p-value = 0.035), BuP (p-value = 0.033) and FSH

Table 1
Demographic factors of the participants and the means of their urinary Σ paraben concentrations (n = 92).

| Characteristics of the participant | n (%) | Σ Parabens (µg/L) |
|-----------------------------------|-------|------------------|
| **Age (year)**                     |       |                  |
| < 18                              | 2 (2.18) | 10625.06        |
| 18–40                             | 55     | 32279.00        |
| > 40                              | 35     | 3114.07         |
| **BMI (kg/m²)**                    |       |                  |
| < 18                              | 2 (2.18) | 33467.08        |
| 18.5–24.9                         | 46     | 28326.41        |
| > 25                              | 44     | 34424.32        |
| **Education level**                |       |                  |
| Academic                          | 51     | 33355.12        |
| Non-academic                      | 41     | 28866.61        |
| **Time spent on physical activity**|       |                  |
| Yes                               | 7 (7.61) | 23894.01        |
| No                                | 85     | 31969.21        |
| **Using daily health skin products**|      |                  |
| Yes                               | 80     | 32006.00        |
| No                                | 12     | 27008.36        |
| **Using daily body lotion**        |       |                  |
| Yes                               | 65     | 3330.032        |
| No                                | 27     | 26665.01        |
| **Using daily antiperspirant products**|   |                  |
| Yes                               | 73     | 39390.62        |
| No                                | 19     | 21460.12        |
| **Using daily sunscreen cream**    |       |                  |
| Yes                               | 77 (83.7) | 31509.75        |
| No                                | 15     | 30559.21        |
| **Using daily bath products**      |       |                  |
| High                              | 84     | 31464.03        |
| Low                               | 8 (86.9) | 30206.06        |
| **Using daily liquid soap**        |       |                  |
| Low                               | 84     | 30499.13        |
| High                              | 8 (86.9) | 40341.15        |
| **Using daily hair spray**         |       |                  |
| Yes                               | 44     | 29177.07        |
| No                                | 48     | 33350.01        |
| **Using daily hair color**         |       |                  |
| Yes                               | 70     | 28118.59        |
| No                                | 22     | 41653.05        |
| **Using daily toothpaste**         |       |                  |
| One time per day                  | 88     | 30282.43        |
| Several times per day             | 4 (4.34) | 54955.16        |
| **Using daily tap water for drinking**| |                  |
| Yes                               | 86     | 31413.00        |
| No                                | 6 (6.52) | 30514.57        |
concentrations. Moreover, a significant association was detected among all the parabens (MeP, EtP, PrP, and BuP) and serum concentration of LH (p-value < 0.05). Higher urinary MeP and BuP concentrations were generally associated with significantly lower serum concentrations of FSH and LH (p-value < 0.05), whereas higher concentrations of EtP and PrP were associated with higher serum concentrations of FSH and LH (p-value < 0.05). The highest decrease in hormone concentration due to MeP was related to LH (CI%, 0.00, 0.0) (p-value = 0.012), and the highest increase in hormone concentration due to PrP belonged to LH (CI%, 0.0, 0.0) (p-value = 0.008). According to Table 4, no significant association was observed between parabens and prolactin concentrations (p-value > 0.05).

3.4. Association between change in all reproductive hormone concentrations and tertiles of urinary paraben concentrations

Table 5 indicates the association between the paraben concentration tertiles and the studied reproductive hormone disorders. The results
were shown based on the logistic regression (with 95% confidence interval) in the three tertiles of parabens and odds ratio (OR) in crude and adjusted forms in the three models (Model 1: crude/undefined; Model 2: adjusted for creatinine; and Model 3: adjusted for creatinine, age, BMI, and time spend on physical activity. Based on Models II and III, 2nd vs. 3rd tertiles of MeP were significantly associated with odds ratios of paraben concentrations was as follows: MeP and time spend on physical activity. Based on Models II and III, 2nd vs. 3rd tertiles of MeP were significantly associated with odds ratios of 0.323 (0.111–0.941) and 0.255 (0.079–0.818) for change in reproductive hormones, respectively (p-value < 0.05). Also, based on Model II, the 2nd vs. 3rd tertile of PrP was significantly associated with an odds ratio of 0.922 (0.859–0.990) (p-value = 0.025) for change in reproductive hormones (Table 5).

3.5. Exposure to parabens and risk assessment

Exposures and risk assessments of MeP, EtP, PrP, and BuP in the studied women are presented in Table 6. The HQ calculated for the sum of MeP and EtP was 0.090, suggesting that these compounds could be regarded as a low priority in risk assessment. As can be seen in Table 6, the MOEs related to all parabens are lower than 10,000, implying that they are unsafe.

4. Discussion

Detection of parabens in urine specimens revealed that exposure to these compounds is prevalent among this demographic of Iranian women. The highest and the lowest mean concentrations belonged to PrP (9441 µg/L) and BuP (4961 µg/L), respectively. Similarly, Hajizadeh et al. [17] indicated that, among the Iranian women living in Isfahan city, the mean concentrations of parabens followed an order of MeP > PrP > EtP > BuP. In another study, Amin et al. [3] reported that in both tissues (malignant and normal tissues) of the breast, the order of paraben concentrations was as follows: MeP > EtP > PrP > BuP.

Many studies have demonstrated the association of urinary concentrations of parabens with the usage of cosmetic and personal care products [13,17,29,30,35,42,47,7]. In the present study, it was also shown that using both toothpaste and antiperspirant products had a positive association with the concentrations of urinary PrP (p-value < 0.05), whereas using hair color was associated with the concentrations of urinary MeP (p-value < 0.05) (Table 3). Previous studies indicated that parabens were associated with reproductive hormone disorders in women [2,23,48,51]. These findings are consistent with the results of the present study, showing that lower LH and FSH concentrations in serum were associated with higher concentrations of urinary MeP and BuP and higher LH and FSH concentrations in serum. Similarly, Guth et al. [15] found that lower LH and FSH concentrations in serum were associated with higher concentrations of the urinary parabens. Another study revealed that a 2-fold increase in the concentration of urinary paraben was associated with lower FSH (95% CI, −7.9, −0.3) and LH (95% CI, −17.4, −3.7) in the 6–17 years-old Canadian girls [15]. In contrast, Lee et al. [31] showed that parabens concentrations have a significant association with increasing the FSH levels and decreasing the total follicles counts. The results of the present study were in agreement with the findings of Smith et al. [51] and Jurewicz et al. [25,24] who reported that a higher concentration of urinary PrP was associated with a higher serum concentration of FSH. An identical relationship between PrP and diminished ovarian reserve in both human and animal data represents the estrogenicity of parabens and, therefore, a greater potential of PrP for re-productive toxicity compared to MeP [49,52,9]. As described in Table 6, the EDI sum of MeP and EtP (0.90 + 1.49) was 2.39 mg/kg.bw/day and the HQ of them was below 1, therefore, they were considered safe. However, the MOEs calculated for all parabens (below 10,000) indicated that they were unsafe and should be considered a high priority in risk management actions. Sanchis et al. [50], in their study, showed that the EDIs calculated for urinary parabens ranged from 0.00042 mg/kg.bw/day for PrP to 0.0434 mg/kg.bw/day for MeP and EtP. They also reported that the HQs calculated for the sum of MeP and EtP showed no risk in the breastfeeding mothers, though this conclusion could not be validly deduced from this exercise. The greatest caveat of this study was the small sample size that may influence the outcomes and results of the research.

5. Conclusion

Four parabens (MeP, EtP, PrP, and BuP) were detected in the urine of a subset of Iranian women living in Kermanshah. The results indicated that MeP and PrP had a higher mean concentration compared to EtP and BuP. The application of antiperspirant products, hair color, and toothpaste were found as the potentially predominant sources of paraben exposure. Positive associations were observed between the use of antiperspirant products, hair color, and toothpaste with the urinary MeP and PrP concentrations. Moreover, the parabens exposure was associated with significant changes in serum concentration of several reproductive hormones among the studied women. The biomonitoring data were interpreted in a risk assessment context. Since the MOE values for all parabens in the studied population were below 10,000 (unsafe), they were labeled to be treated as high-priority threats for risk management
actions. Future studies on the potential paraben-related to changes in the reproductive system and risk assessment should rely on a comprehensive design during development of the female reproductive system and their fertility.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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