Urinary Bisphenol A and Menarche in Female Adolescents

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Abstract

Menarche is a result of the complex interaction between the hypothalamus, pituitary gland, and ovaries. Its timing is influenced by a combination of genetic predisposition and environmental factors, along with their interactions. Bisphenol A (BPA), a chemical compound known as an endocrine disruptor due to its estrogenic properties can impact the onset of menarche. Therefore, research needs to be carried out to evaluate BPA exposure and the onset of menarche in female adolescents. **Methods:** A descriptive study of female adolescents aged 9-14 years old at Darul Arafah Raya Islamic Boarding School, from January 2022 to January 2023 were recruited for this study. Thirty female adolescents were included in the study. Samples were collected using a simple random sampling method. The data was obtained from interviews and examination of urinary bisphenol A using High-Performance Liquid Chromatography (HPLC). **Results:** Thirty subjects participated in this study. BPA was detected in 3 (10%) of the children, all of whom experienced menarche at 11 years old. Meanwhile, among the 27 children with undetectable BPA levels in their urine, the average age of menarche was 11.15 years old. **Conclusion:** Based on the results of the urine examination, it was determined that BPA was detected in 3 children (10%). Conversely, the urine of the majority of sample, totaling 27 (90%), showed no detectable BPA levels.

Keywords: Bisphenol A, menarche

Chemical compounds play a crucial role in the human body. Among them are compounds known as endocrine disruptor chemicals, which can interfere with the body's hormonal system, leading to long-term effects. One of the most prevalent compounds to which humans are exposed is bisphenol A (BPA), known for its potential to alter the timing of menarche in female adolescents. Despite its known effects, BPA continues to be extensively used in everyday products like food storage containers and plastic bottles, where exposure can occur through food, water, air, and skin [1].

Research conducted in the United States has revealed an association between BPA exposure and delayed menarche in female adolescents. Those with moderate urinary BPA levels experienced a delay in menarche compared to those with lower values [2]. Furthermore, another study identified an association between 2,5-DCP and BPA exposure with an earlier onset of menarche [3].

The BPA compound can influence reproductive development and the timing of puberty due to its estrogenic characteristics. This influence can occur directly by binding to estrogen receptors, increasing aromatase enzyme activity, and ultimately enhancing estrogen sensitivity. Alternatively, it can indirectly impact the reproduction of endogenous estrogen by affecting Gonadotropin-Releasing Hormone (GnRH) [4, 5]. Moreover, BPA has been shown to increase the number of antral follicles, potentially triggering early menarche [6, 7]. Research on BPA and menarche has not been conducted in Indonesia. Therefore, research needs to be carried out to evaluate BPA exposure and the onset of menarche in female adolescents.

1. Methods

This study was a descriptive study to determine the urinary BPA exposure and the onset of menarche in female adolescents. Conducted at the Darul Arafah Raya Islamic Boarding School in Kutalimabru, Deli Serdang District, North Sumatera, Indonesia, the study spanned from January 2022 to January 2023 which achieved the required sample size according to the sample size formula.

Adolescent girls attending Darul Arafah Raya Islamic Boarding School were recruited for this study. The inclusion criteria for the patients to be included were: (1) aged 9-14 years old, (2) have experienced menarche, and (3) parental willingness to participate in the research, confirmed by signing the informed consent form. Female adolescents with a history of kidney disease were excluded. Samples were collected using a simple random sampling method. Thirty female adolescents were included in the study.

Demographic and clinical data were collected from all participants, including age, anthropometric measurements (weight, height), grade level, age of menarche, age of mother's menarche, parents' occupation, parents' income, and history of previous illnesses. The data were collected through direct interviews or during clinical assessments. Additionally, a physical examination was conducted. Before obtaining consent, all participants received an explanation regarding the study's purpose and procedures, including urine testing.

Urine samples were taken to examine for BPA using High-Performance Liquid Chromatography (HPLC), which is a separation technique using both stationary and mobile phases. BPA levels were assessed at the Mutifa Medan laboratory using an HPLC instrument, with standard BPA then dissolved with methanol in a measuring flask until reaching a specific concentration. The BPA levels were calibrated against the value of the BPA standard acquired from an American chemical company, Sigma-Aldrich. BPA levels in the Limit of Detection (LOD) were measured. BPA was detected if the LOD was found to be 5 pg/mL, while it was classified as not detected if the LOD was not identified in the HPLC machine. The data collected was processed and analyzed using the help of the Statistical Package for Social Science (SPSS) computer software version 23.0.

2. Results

A total of 30 female adolescents were included in this study, and their demographic characteristics are presented in **Table 1.** Among them, twenty-one (70%) subjects were in grade VI. The mean age of the subjects was 11.63 years old. The median age of menarche for mothers was 12 years old, with the youngest onset at 11 years old and the oldest at 14 years old. For female adolescents, the median age of menarche was 11 years old, ranging from 10 to 12 years old. The median body weight was 38.4 kg, with an average BMI of 19.96 kg/cm2. The majority of fathers worked as teachers, which were 10 individuals (33.3%), followed by those employed in the private sector, totaling 11 individuals (36.7%). Most mothers were housewives, which were 16 individuals

(53.3%), followed by 8 individuals (26.7%) working as teachers. Regarding income, the majority of parents earned between Rp. 2 million and less than IDR 3 million, which constituted 11 individuals (36.7%).

Based on the results of the urine examination, it was determined that BPA was detected in 3 children (10%). Conversely, the urine of the majority of sample, totaling 27 (90%), showed no detectable BPA levels, as indicated in **Table 2.**

Table 1. Demographic characteristics of participants

Characteristics	n (%)
Grade, n (%)	
IV	2 (6.7)
V	7 (23.3)
VI	21 (70)
Age (years)	
Mean (SD)	11.63 (0.7)
Age of mother's menarche (years)	
Median (Min-Max)	12 (11-14)
Age of girl's menarche (years)	
Median (Min-Max)	11 (10-12)
Body weight (kg)	
Median (Min-Max)	38.4 (20-57)
Body height (cm)	
Mean (SD)	140.77 (6.88)
BMI (kg/m^2)	
Mean (SD)	19.96 (5.08)
Father's occupation, n (%)	
Teacher	10 (33.3)
Private sector employees	12 (40)
Nurse	1 (3.3)
Farmer	2 (6.7)
Self-employed	5 (16.7)
Mother's occupation, n (%)	
Midwife	1 (3.3)
Teacher	8 (26.7)
Housewives	16 (53.3)
Private sector employees	3 (10)
Nurse	1 (3.3)
Self-employed	1 (3.3)
Parents' income	
< IDR 2 million	1 (3.3)
IDR 2 - <3 million	11 (36.7)
IDR 3 - <4 million	8 (26.7)
IDR 4 - <5 million	6 (20)
≥ IDR 5 million	4 (13.3)

Table 2. Frequency Distribution of BPA in Urine

Presence of BPA	n = 30 (%)
Detected	3 (10)
Not detected	27 (90)
Total	30 (100)

3. Discussion

The compound BPA is an endocrine-disrupting chemical similar to estrogen, commonly found in food storage containers, baby bottles, plastic bottles, can linings, and thermal paper [8, 9]. This chemical compound can influence the onset of menarche due to its estrogenic properties [4, 5]. Upon oral ingestion, BPA enters the body and rapidly binds with glychronic acid to form BPA glucuronide (BPA-G). This metabolic process, known as glucuronidation, is facilitated by enzymes such as UDP-glucuronosyltransferases (UGT) and Sulfotransferases (SULTs) in the liver and intestines, eventually leading to excretion in urine [10, 11].

BPA compounds can be detected in human urine, serum, breast milk, and other biological samples, and they are capable of stimulating human cellular responses at exposure levels ranging from low picograms to low nanograms per milliliter. The high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method can accurately, selectively, and sensitively quantify BPA in human urine, with a quantitation limit of 50 pg/mL and a detection limit of 5 pg/mL [10].

Urine sample collection was chosen due to its non-invasive examination and provides several advantages compared to blood sampling. These include lower protein/lipid content, which can affect the solubilization of unconjugated BPA, as well as the ability to collect larger volumes without requiring sterile sampling techniques [12].

In women populations, elevated serum concentrations of BPA (ranging from 1.53 to 2.22 µg/L) can lead to alterations in estradiol levels, disrupting hormonal balance and causing metabolic abnormalities such as precocious puberty. This disruption arises from the estrogen-like activity of BPA, which can initiate positive feedback mechanisms, enhancing the activity of the GnRH pulsatile generator. Consequently, this leads to an increase in central secretion of LH and FSH [4, 13]. BPA compounds also affect the hypothalamic-pituitary-gonadal axis, resulting in heightened GnRH secretion and LH surge induced by estradiol. Additionally, numerous human studies have reported a correlation between BPA exposure and early menarche, as well as delayed thelarche in female adolescents [2, 13].

Menarche signifies the onset of menstruation during early adolescence, marking the transition into the reproductive phase. It is a pivotal milestone indicating the maturation of sexual organs, typically characterized by the first menstrual period. Menarche is a significant indicator for women, signaling the initiation of hormone production by the hypothalamus, which is subsequently transmitted to the ovaries and uterus [14].

The age of menarche is influenced by genetic and environmental factors, as well as their interactions. However, trends indicate that environmental factors primarily contribute to the rise in early menarche. These factors include nutrition (such as high protein intake and high leptin levels), growth parameters (height, weight, and arm circumference), and the influence of pollutants that can alter the timing of menarche [6, 15].

Previous study has shown that 5% of the population experiences menarche before the age of 10 years old or after 15 years old [16]. Research conducted by Kim et al. in Korea revealed a significant decrease in the

average age of menarche over the past 50 years. Presently, the prevalence of early menarche stands at 22.3% [15]. Indonesia itself ranks 15th out of 67 countries in terms of the decline in the age of menarche, which has decreased by 0.145 years per decade. The 2010 Basic Health Research (Riskesdas) report indicates that 20.9% of Indonesian female adolescents experience menarche before 12 years old. Research by Febrianti in 2017 at West Sumatra found that approximately 43% of females experienced menarche before 11 years old, while 37% experienced it between 11 and 12 years old, and 20% after 12 years old [14].

This study exclusively collected urine samples from 30 female adolescents at the Darul Arafah Islamic Boarding School in North Sumatra. A previous study by McGuinn et al. also investigated BPA levels in urine among 987 female adolescents [2], which is consistent with research by Buttke in 2012 which utilized 461 female adolescents as samples [4].

In this study, subjects were 9 to 14 years old, with the majority being over 11 years old. This could be attributed to the fact that the majority of Indonesian female adolescents experience menarche at 12 years old [17]. Research by Buttke et al. indicated that the subjects were in the range of 12 to 15 years old [2], while research conducted by Miao et al. in China included samples of 9 to 18 years old [9].

Regarding the mothers' menarche age in this study, it ranged from 11 to 14 years old, and the entire sample did not fall into either the early or late menarche categories. The age of menarche in mothers can influence the rate of their daughters' body growth, consequently impacting the timing of menarche. This association is linked to the lobe that regulates estrogen [14]. Other studies have shown that daughters of mothers who experienced early menarche exhibit a significant association with early menarche. However, it remains unclear whether this association reflects genetic or environmental influences [18]. Research conducted in Korea revealed that daughters of mothers with an earlier average age of menarche (less than 13 years old) were more likely to experience early menarche ($P<0.05$) [15].

An earlier age of menarche has been found to correlate with higher fat intake and BMI [6]. Environmental factors such as lifestyle changes, dietary shifts, and increased exposure to endocrine-disrupting chemicals (EDCs) also contribute to the propensity for developing obesity [19]. Research conducted by Kaplowitz in 2008 revealed that obesity, as measured by Body Mass Index (BMI), can influence urine BPA concentrations and the age of menarche, as obesity serves as a significant endogenous hormonal risk factor for pubertal development [2]. This finding is supported by a study conducted by Lassek and Gaulian in 2007, which demonstrated that menarche is closely associated with fat distribution and correlated with leptin levels [6].

The average BMI value of the samples in this study was 19.96 and was below the 95th percentile on the growth curve, thereby categorizing them as normal or not obese. This trend was consistent across all samples where BPA was detected in urine. Obesity can impact hormones involved in sexual development, including progesterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Leptin levels influence gonadotropin-releasing hormone (GnRH), thereby altering the secretion levels of GnRH and LH. Moreover, leptin exerts an effect on oocyte maturation, stimulating the maturation of ova produced by the ovaries [14].

In this study, all samples that tested positive for BPA originated from parents in the low-income group (IDR 2 million - less than IDR 3 million). This finding contrasts with the study by McGuinn et al., where BPA was detected predominantly in the high-income groups [2]. Another study conducted in the United States revealed a correlation between parents' income and the timing of menarche, with female adolescents experiencing early menarche (before 11 years old) showing an increase in prevalence over 50 years within low socioeconomic status families. Conversely, a recent study in the United Kingdom spanning 85 years found a

reduction in the age of menarche across all socioeconomic groups. Therefore, it is unlikely that socioeconomic status alone can explain changes in the timing of menarche over time [6].

In this study, all three children who tested positive for BPA in their urine experienced normal menarche at 11 years old. Conversely, among the 27 children with undetectable BPA in their urine, the average age of menarche was 11.15 years old, with the youngest onset at 10 years old and the oldest at 12 years old. This finding contradicts the research by McGuinn et al., where patients with detected BPA in their urine exhibited an earlier onset of menarche [9].

Female adolescents who have abnormal ages of menarche are known to have a greater risk of encountering psychosocial and behavioral problems. They also tend to achieve shorter final heights and are more likely to be obese [15].

4. Conclusion

The age of menarche in the sample group ranged from 10 to 12 years old, with no participants categorized as having abnormal menarche. Only 3 children (10%) had BPA detected in their urine and all of them had a normal onset of menarche.

5. References

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