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The Possible Association between Phthalates and Bisphenol A Exposure and Idiopathic Precocious Puberty in Egyptian Girls

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Abstract

BACKGROUND: Bisphenol A (BPA) and phthalates are utilized in large spectrum of plastics, as polyvinyl chloride as well as personal products, medical equipment, and epoxy resins. Phthalate and bisphenol A are the most common endocrine disrupting chemicals that interrupt the endocrine system and cause developmental, reproductive, neurological, and immune disturbances in humans. However, the relation between phthalates and bisphenol A and precocious puberty (PP) in human is still controversial.

AIM: Consequently, the present study aimed to detect and investigate the association between exposure to bisphenol A and monobutyl phthalate (MBP) and precocious puberty in Egyptian girls.

METHODS: Urine samples were collected from 100 young females. The subjects were divided into two major groups, precocious puberty group consisted of 60 young females diagnosed by an endocrine pediatric specialist and controls consisted of 40 normal young females matched in age and demographic characters. In urine, MBP and bisphenol A (BPA) were measured with high-performance liquid chromatography.

RESULTS: The mean concentration of MBP level was 22.758 ± 6.216 for the PP group and 15.283 ± 6.262 for controls with statistical difference between the studied groups ($p < 0.001$). Furthermore, the mean concentration of BPA was 405.02 ± 223.54 for the PP group and 97.95 ± 55 for controls with significant difference between groups ($p < 0.001$).

CONCLUSION: The present study found that idiopathic precocious puberty in young females was associated with high phthalate metabolites and bisphenol A levels in urine.

Introduction

Precocious puberty (PP) in girls is defined as the appearance of secondary sexual traits before the age of 8 or the commencement of menarche before the age of 9 [1]. PP is estimated to affect one out of 5000 children. Girls are more affected with PP than boys [2].

PP has traditionally been divided into central precocious puberty (also known as gonadotropin-dependent precocious puberty or true precocious puberty) results from premature activation of hypothalamic–pituitary–gonadal (HPG) axis, causing premature secondary sexual growth and peripheral precocious puberty (gonadotropin-independent precocious puberty or pseudo-precocious puberty) occurs when the HPG axis is not activated and can be caused by a variety of other factors [3].

During puberty, the estrogen/androgen balance interacts with growth factors to regulate the growth and maturation of all systems and organs which are an important factor in the puberty process's sex-specific tuning. As a result, endocrine disruptors may have a significant impact on the puberty process [4].

Endocrine-disrupting chemicals (EDCs) are a class of hormonally active molecules to which we are frequently exposed through direct or indirect route, and which have been widely utilized in industry for over a century. EDCs influence the hormones (stimulating or suppressing their action) through binding to their receptors and altering the biosynthesis, transport, degradation, and excretion of these hormones [5].

The most common EDCs to which humans are exposed on a daily basis are phthalates and bisphenol A (BPA). These chemicals are found in a wide range of plastics, including polyvinyl chloride (floor carpeting and connectors), as well as epoxy resins, medical devices, tainted food, and personal care goods. As a result, they are everywhere in the environment and our daily lives. Chemicals can be ingested or absorbed through the skin, but they can also be breathed [6].

International biomonitoring studies reveal that BPA is present in >90% of children in Europe, the United States, Australia, and Asia [7], [8], [9], [10]. Urinary BPA concentrations in children have been found to be greater than the general population from the same geographic locations on a worldwide scale, raising concerns about the possible health impacts of exposure during important developmental period in childhood [11], [12]. Human biomonitoring also reveals extensive phthalate exposure from a variety of sources, including food that may be contaminated from its packaging [13] and other food contact materials such as conveyor belts and tubing used in food preparation [14], [15]. Human exposure to phthalates is also increased by building materials and personal care products [16], [17].

These chemicals can have a greater impact on children in their first decade of life, their consequences can be more obvious during pubertal development [18], [19]. Although several studies investigated the phthalates and bisphenol in pediatric population, their association with precocious puberty is still not proven yet. To the best of our knowledge, no previous studies were done in Egypt concerning this association. Therefore, the aim of this study is to detect and investigate the association between exposure to bisphenol A and monobutyl phthalate and precocious puberty in Egyptian girls.

Methods

This case–control study was conducted during the period from September 2019 to January 2021 and included 60 young females with signs of precocious puberty attending to the Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU), at Abu El-Reesh Children Hospital, Cairo University, Egypt. Informed consent was obtained from all guardians before starting the research. The study design was approved by Research Ethical Committee at Faculty of Medicine (Kasr Al-Ainy), Cairo University (IRB MD-139-2019).

Apparently healthy 40 females who matched the study group concerning age and socioeconomic level were included as a control group.

Our study included young females with signs of precocious puberty (breast enlargement, pubic and axillary hair, menarche, and pubertal growth spurt before pubertal age), who were 2–10 years old of age, attending to the children's hospital because of central or peripheral precocious puberty. Patients with liver or kidney diseases, CNS tumors, adrenal or ovarian tumors, and syndromes such as McCune–Albright and Peutz–Jeghers syndromes were excluded from the study.

Data collection

Patients' baseline characteristics were recorded. The clinical variables included the patients' age, residence, parental educational level, their current occupation, birth weight, breast feeding, the onset of pubertal changes and its progression, any endocrine diseases, history of any medications, and body mass index (BMI). Potential exposure to phthalates and bisphenol A and sources, for example, food/water storage, canned food consumption, daily use of creams, lotions and cosmetics, playing with plastic toys. or exposure to household products was obtained by mother self-reported questionnaire [5], [20], [21].

Precocious puberty diagnosis

The study groups were assessed by pediatric endocrinologist. At 9:00–9:30 a.m., a GnRH level was measured. Through an intravenous (IV) cannula, blood samples for basal FSH and LH levels were collected. Blood samples for FSH and LH were taken at the 20, 40, 60, and 90 min after the administration of 100 mg GnRH [22]. Both FSH and LH had a minimum measurable concentration of 0.07 IU/L. In patients with pubertal signs, a cutoff value of stimulated LH of equal or more than 5 IU/L was considered diagnostic for pubertal response [23]. The left hand X-rays were done to assess the bone age.

Measurement of urinary phthalates and bisphenol A

Urine sample

Urine specimens were collected from all included subjects (i.e., cases and control) in clean, glass containers marked with the subject coding number, whereas blood containing samples or turbid samples were excluded from the study. Afterward, the specific gravity was measured by refractometry to detect sample dilution. Then, all specimens were stored at −20°C [24] and then analyzed for the mean bisphenol A (BPA) and mean monobutyl phthalate levels (i.e., the mean value was calculated using three different readings on different occasions) by high-performance liquid chromatography (HPLC).

Bisphenol A

Urine (500μL) was buffered with 30 μL of 2.0 M sodium acetate buffer (pH 5) then hydrolyzed enzymatically with β-glucuronidase at 37°C for 3 h in a shaking water bath. A 100 μL of 2N HCl was added, and the hydrolysate was extracted once with 5 mL of ethyl acetate with 50 ng/ml bisphenol B (internal standard). The supernatant (4 ml) was transferred to a new tube and evaporated after centrifugation. The

residue was dissolved in water with 200 μL of 60% acetonitrile. Autosampler injected 40 μL of sample into HPLC. Acetonitrile, tetrahydrofuran, and water were mixed (35:35:130 and, 70:35:95) in the gradient mode to prepare the mobile phase. Sample detection was done by fluorescent detector at excitation 275 nm and emission 300 nm.

Phthalates

Urine (1 ml) was buffered with 245 μL of 1 M ammonium acetate buffer (pH 5) then hydrolyzed enzymatically with β-glucuronidase at 37°C for 3 h in a shaking water bath.

Solid-phase extraction

Conditioning: With 1 mL of acetonitrile, 1 mL of methanol, and 1 mL of phosphate buffer solution (pH 2) added successively. Loading: Hydrolyzed urine sample (with 10 ul benzyl benzoate as internal standard) was diluted with 1 mL of phosphate buffer solution and then added to the SPE column. Wash: A 2 mL formic acid solution (0.1 M) and 1 mL water were used to wash the cartridges. After that, the cartridges were dried under negative pressure. Elution: A 1 mL of ethyl acetate and 1 mL of acetonitrile were added. The eluent was collected, concentrated, and evaporated. A 1 ml of mobile phase was used to reconstitute the dry residue.

Chromatographic conditions

Mobile phase:

- Mobile phase (1) acetic acid (0.1%) in water and mobile phase (2) acetic acid (0.1%) in HPLC grade acetonitrile.
- The temperature of column was set at 40°C.
- The volume of sample injection was 20 μL, the flow rate was 0.3 mL/min. UV was set from 240 to 280 nm maximum absorbance 254 nm.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA) was used to code and enter the data. For quantitative variables, the mean and standard deviation were used to summarize data, while for categorical variables, frequencies (number of cases) and relative frequencies (percentages) were utilized. The unpaired t-test or analysis of variance (ANOVA) was used to compare the groups [25]. Correlations between quantitative variables were performed using the Spearman coefficient correlation [26]. $p < 0.05$ was considered statistically significant.

Results

Clinical data

The basic clinical characteristics of the studied group are shown in Table 1. The age range was between 2 and 10 years for the PP group and control group, respectively. Age and residence showed no significant difference between the two studied groups. Overall, there was no statistical difference between the studied group regarding the parent educational level and occupation. While concerning anthropometric data of the studied group, there was a significant difference regarding weight, BMI, and waist circumference. The natal history showed no significant difference between the studied group with reference to type of delivery and birth maturity, while birth weight showed statistical difference. In the analysis of hormonal profile, the mean of the basal LH levels was 0.81 ± 0.88 IU/mL in the precocious puberty group and 0.06 ± 0.03 IU/mL in the control group. Furthermore, the mean of the basal FSH levels was 2.92 ± 1.36 IU/mL in the precocious puberty group and 1.77 ± 0.48 IU/mL in the control group.

Table 1: Clinical characteristics of precocious puberty and controls

Variables	Precocious puberty group	Control group	p-value
Age (mean ± SD)	5.47 ± 1.72	5.63 ± 1.82	0.656
Residence			0.546
Urban (N/%)	41 (68.3%)	25 (62.5%)	
Rural (N/%)	19 (31.7%)	15 (37.5%)	
Weight (mean ± SD)	27.37 ± 6.79	24.89 ± 4.34	0.028*
Height (cm) (mean ± SD)	119.91 ± 10.06	120.95 ± 8.09	0.587
Waist circumference (cm) (mean ± SD)	45.95 ± 9.23	28.56 ± 4.49	<0.001*
BMI (mean ± SD)	22.40 ± 4.30	20.46 ± 2.56	0.006*
Natal history			
Type of delivery			
Normal (N/%)	27 (45.0%)	23 (57.5%)	0.221
C-section (N/%)	33 (55.0%)	17 (42.5%)	
Birth weight (kg) (mean ± SD)	2.76 ± 0.56	3.03 ± 0.51	0.013*
Maturity			
Full term (N/%)	46 (76.7%)	36 (90.0%)	0.089
Preterm (N/%)	14 (23.3%)	4 (10.0%)	
Hormonal profile			
LH (IU/L)(basal level) (mean ± SD)	0.81 ± 0.88	0.06 ± 0.03	0.004*
FSH (IU/L)(basal level) (mean ± SD)	2.92 ± 1.36	1.77 ± 0.48	<0.001*

LH: Luteinizing hormone, FSH: Follicular-stimulating hormone. *Significant p value.

Exposure

The potential dietary exposure, there was no significant difference between the groups in terms of milk and type of feeding (breast or bottle feeding) ($p = 0.400$). Furthermore, canned food, fish consumption and dietary containers, and method of food preservation showed no significant difference ($p = 0.514$, % and $p = 0.193$, respectively). Furthermore, the environmental exposure showed no statistical difference as regard to plastic toys and using cosmetics ($p = 0.650$ and $p = 0.019$, respectively). Medical exposures showed significant differences regarding chronic blood transfusion, hospitalization and operative history ($P = 0.011$, $P < 0.001$ and $P = 0.027$, respectively).

Precocious puberty

The precocious puberty data are shown in Table 2. The mean age of pubertal onset in the PP group was 4.85 ± 1.28. Most of the cases have a progressive course (66.7%), no menarche (65%), thelarche tanner Stage 2 (palpable breast bud under the areola) (51.7%), and adrenarche tanner Stage 2 (downy pubic and axillary hair) (73.3%). According to GnRH stimulation test, about 65% of the PP group was central precocious puberty (gonadotropin-dependent precocious puberty) and the remaining was peripheral precocious puberty (gonadotropin-independent precocious puberty). X-ray on the left hand showed that all cases were of advanced age (about 61.6% were over 1 year and 38.4.7% were 1 year or less), and the mean difference from chronological age was 2.56 ± 0.9 years.

Table 2: Precocious puberty data

Variables	Precocious puberty group
Onset of puberty (years old) (mean ± SD)	4.85 ± 1.28
The course of the disease	
Progressive (N/%)	40 (66.7%)
Regressive then progressive (N/%)	2 (3.3%)
Under treatment (N/%)	18 (30%)
Symptoms	
Menarche	
Present	21 (35%)
Not yet	39 (65%)
Adrenarche	
Tanner Stage 1 (N/%)	0 (0.0%)
Tanner Stage 2 (N/%)	44 (73.3%)
Tanner Stage 3 (N/%)	13 (21.7%)
Tanner Stage 4 (N/%)	3 (5%)
Thelarche	
Tanner Stage 1 (N/%)	17 (28.3%)
Tanner Stage 2 (N/%)	31 (51.7%)
Tanner Stage 3 (N/%)	8 (13.3%)
Tanner Stage 4 (N/%)	4 (6.7%)
GNRH stimulation test	
Central precocious puberty (pubertal results) (N/%)	39 (65%)
Peripheral precocious puberty (prepubertal results) (N/%)	21 (35%)
X-ray bone age	
≤- year above chronological age (N/%)	23 (38.4%)
>1 year above chronological age (N/%)	37 (61.6%)

Measurement of phthalates and bisphenol A

The mean level of bisphenol A was 405.02 ± 223.54 for the PP group and 97.95 ± 55 for controls with statistical difference between groups ($p < 0.001$) and the mean level of monobutyl phthalates was 22.758 ± 6.216 PP group and 15.283 ± 6.262 for controls with significant difference between groups ($p < 0.001$), as shown in Figure 1.

Relation between anthropometric measures and bisphenol A and monobutyl phthalates levels in urine in PP group

There was a significant correlation between high bisphenol A levels and increased body weight and waist circumference (Figure 2), while there was no significant correlation between MBP and the anthropometric, as shown in Table 3.

Relation between basal hormonal level and bisphenol A and monobutyl phthalates level in urine in PP group

There was a significant correlation between BPA levels and FSH levels as in Figure 2. There was no significant correlation between MBP and hormonal levels as in Table 3.

Discussion

EDCs are a class of hormonally active which stimulates or inhibits the function of natural hormones. As a result, they have the potential to induce a variety of diseases. Several studies have proven that fetuses, babies, and children are more susceptible to EDCs' harmful effects on health than adults, due to the critical importance of the hormonal homeostasis in both growth and development. Furthermore, they are exposed to these compounds at a higher rate than adults, as seen by the higher levels of EDCs detected in urine and blood [27]. Phthalates and BPA, both well-known environmental chemicals, are examples of EDCs. These EDCs may have a role in the beginning of puberty in females at a young age [5].

In this study, there was a significant difference in body weight between the studied groups. Similarly, Lomenick *et al.* [28] demonstrated that weight was significantly higher in the children with central precocious puberty (CPP) compared with the controls. It was similar to Buluş *et al*. [5] who found that bodyweight standard deviation score was highly increased in both the CPP and peripheral precocious puberty (PPP) groups compared to the control. This was in contrast to Deng *et al.* [29] who reported that at different stage of childhood, there was no significant difference in the peak of weight growth between precocious puberty group and controls. As their study included both male and female children, while the present study included only females, and females are more likely to be obese in comparison to males, due to hormonal differences [30].

Furthermore, in this study, the BMI showed a significant difference between the studied groups. This agreed with Srilanchakon *et al.* [31] who found significant difference between idiopathic precocious puberty, early puberty, and control regarding BMI and Hashemipour *et al.* [32] who found significant difference between central precocious puberty group and control. It was contrary to Park *et al*. [33] who reported that there was no significant difference between the early menarche group and the normal menarche. Also, Buluş *et al*. [5] reported that there was no statistical difference between the CPP group, PPP group, and control regarding BMI.

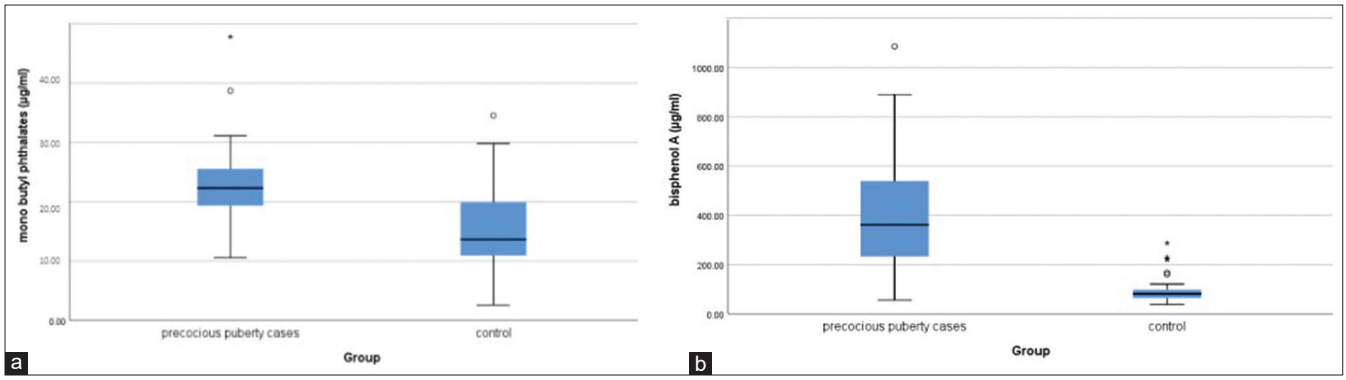

Figure 1: Comparison of bisphenol A and monobutyl phthalates levels between the studied groups. (a) The precocious puberty group showed significant higher levels of monobutyl phthalates compared to the control group (p < 0.001). (b) Bisphenol A was significantly higher in the precocious puberty group in comparison to the controls (p < 0.001)

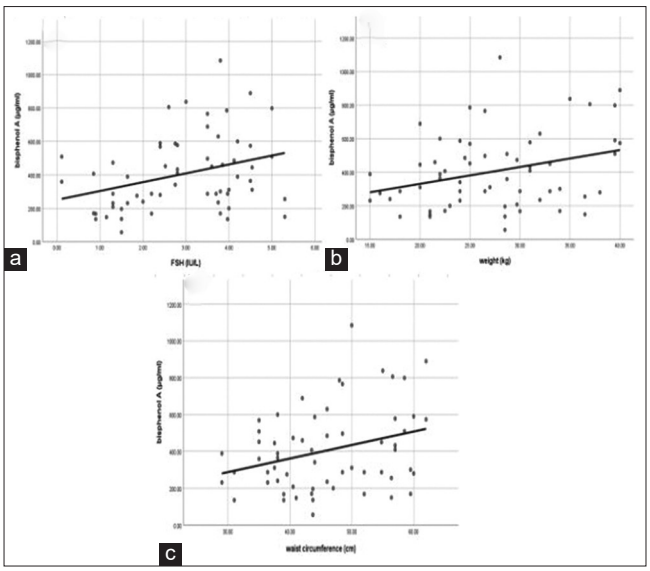

Figure 2: Bisphenol A correlations (a) significant relation between bisphenol A and FSH (p = 0.011), (b) significant correlation between bisphenol A and waist circumference (p = 0.040), and (c) significant correlation between bisphenol A and weight (p = 0.032). FSH: Follicular-stimulating hormone

BMI is a quantitative method of determining body fat males and females of different ages, and it is not only exclusively based on body weight but also on height that differs between population [34]. In addition, we found higher levels of hormones in our study than in the previous studies, which may explain why people in our study had higher body weight.

BPA has been investigated extensively for its estrogen-like properties. Several studies have reported that BPA may play a role in the pathophysiology of many genital and reproductive disorders, including female and male reproductive tract abnormalities, male and female infertility, externalizing behavior in girls, precocious puberty, and so on, as it is structurally similar to 17-oestradiol [27].

In the present study, the mean bisphenol A level was significantly high among precocious puberty group than among controls and this is the first Egyptian study to prove this association. Furthermore, our result is close to that of Qiao *et al.* [35], Durmaz *et al.* [36], Kasper-Sonnenberg *et al*. [6], Miao *et al.* [37], and Supornsilchai *et al.* [38] who found that the precocious puberty group showed a significant higher level of bisphenol A than the control group. Wolff *et al.* [39], Buluş *et al*. [5], and Jung *et al.* [40] detected that there were no significant differences between early pubertal development and BPA level.

Table 3: Correlation between hormonal level and BPA and MBP level

Variables	Bisphenol A (µg/ml)	Monobutyl phthalates (µg/ml)
LH (IU/L)$_{(basal\ level)}$		
Correlation coefficient	0.195	0.036
p value	0.136	0.786
N	60	60
FSH (IU/L)$_{(basal\ level)}$		
Correlation coefficient	0.326	0.240
p value	0.011*	0.064
N	60	60
Weight (kg)		
Correlation coefficient	0.276	0.139
p value	0.032*	0.291
N	60	60
Height (cm)		
Correlation coefficient	0.172	0.101
p value	0.188	0.443
N	60	60
Waist circumference (cm)		
Correlation coefficient	0.266	0.232
p value	0.040*	0.074
N	60	60
BMI		
Correlation coefficient	0.215	0.064
p value	0.100	0.626
N	60	60

LH: Luteinizing hormone, FSH: Follicular-stimulating hormone, BMI: Body mass index. *Significant p-value.

BPA levels in blood are lower than those in urine and diminish rapidly after exposure since it is a non-persistent compound with a half-life of a few hours [41]. There was a substantial day-to-day difference of urinary levels of bisphenol A with significant variability within the same subject [42].

This study detected that the mean level of monobutyl phthalates was higher in PP group than the control group and showed a significant difference. This was compatible with the third round of KoNEHS that was conducted by Park *et al.* [33] to describe the Korean population and assess exposure levels to different chemicals in which they found that the possibility of early menarche was significantly increased in a high concentration of MnBP compared to low concentration. Furthermore, Zhang *et al.* [43] found that phthalate metabolites levels (MMP, MnBP, mono(2-ethyl-hexyl)

phthalate [MEHP], and MEP) were related to increased breast development stage, rapid breast progression, and earlier menarche. On contrary to Jung *et al*. [40] and Hashemipour *et al*. [32] who found that phthalates level higher in the control group than precocious puberty group.

This may be explained as phthalates are eliminated from the body within hours after exposure. The urinary concentrations of DBP, DEHP, DEP, and BBP metabolites in adults and children were in general less than the biomonitoring equivalent values (BE, mg/L, or mg/g creatinine) of these chemicals that evaluated from exposure guidance values. However, in some children's samples, the 95th percentiles of urinary MnBP and MEHP concentrations exceeded the corresponding BE values [44].

In the present study, there was a significant relation between increased waist circumference and weight and high BPA levels in precocious puberty group. Furthermore, Li *et al.* [45] found that there was an association between overweight among girls aged 9–12 and high level of BPA in urine. The explanation is that BPA has an estrogenic effect that may hasten pubertal growth and weight gain in females during this time [45].

Conclusion

The present study found a high level of monobutyl phthalate and bisphenol A in precocious puberty girls when compared to the control. Therefore, these findings suggested that exposure to a high level of monobutyl phthalate and bisphenol A may contribute to idiopathic precocious puberty in girls. However, further studies should be conducted on a larger number of subjects and measuring phthalates in blood and their metabolites in urine to confirm these findings.

Conflicts of Interest

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

Authors' Contributions

All authors contributed to the study conception, design, and data collection. Methodology and data analysis: Rania M. Abdel-Raheem: Conceptualization, methodology, and writing – original draft preparation. Eman A.F. El-Zohairy: Supervision and conceptualization. Mona M. Hassan and Shimaa Atef: Data collection, investigations, supervision, and visualization. Marwa Issak and Sarah Hamed N. Taha: Methodology and validation.

Ethical Approval

The Research Ethical Committee (REC) has reviewed and approved the protocol. The approval is obtained from the Ethical Committee, Faculty of Medicine, Cairo University and IRB code is MD-139-2019.

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