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Menstrual Irregularities in Adolescent Girls and Correlation with the Extent of Exposure to Plastics: A Case-Control Study

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ABSTRACT

Introduction: Menstrual irregularities in adolescent girls are rapidly increasing worldwide. Exposure to endocrine-disrupting chemicals such as plastics including Bisphenol A (BPA), Phthalates and Polyvinyl chloride (PVC) are potential environmental toxins contributing to the hormonal imbalance and irregular menstrual cycles.

Aim/Objectives: We examined the association between plastic usage and menstrual cycle characteristics among Adolescent girls aged 15–19 years who were participating in a questionnaire-based cohort study under the program on reproductive health awareness in adolescent girls.

Materials and Methods: A case-control study of 360 girls was conducted in 2016 and 2019 in a school in Rajasthan, India. We used self-reported information through a questionnaire to assess the association of irregular menstrual cycles (≤25 days and ≥35 days) with extent of plastic usage categorized here as medium and high.

Results: The study cohort had a high prevalence of irregular menstrual cycles (40.8%). Heavy plastic exposures increased the chances of getting irregular periods significantly. Girls with irregular menstrual cycles had more prevalence of hair fall, acne, *acanthosis nigricans*, and abnormal hair growth/hirsutism. Girls with family history of diabetes and CVD were more susceptible to develop irregular menstrual after heavy usage of plastics. Both medium and high exposures to plastics were associated with abnormal hair growth/hirsutism ($p < 0.026$) indicating a role of androgen imbalance in these girls. Most girls with irregular cycles had no history of obesity or diabetes and CVD, indicating the association of plastics use with menstrual irregularities in adolescent girls.

Conclusion: These results suggest that exposure to plastics increases the chances of having irregular menstrual cycle and the characteristics of PCOS. Due to its endocrine disruptive actions, plastics may influence the neuroendocrine functions during preadolescent period and cause imbalance in the levels of endogenous hormones essential for optimal menstrual function.

Key Words: Plastics, BPA, Phthalates, PVC, Irregular, Menstrual cycle

INTRODUCTION

Adolescence is an important phase transition from childhood to physical and psychological maturity in human development.[1] Normal menstrual cycle in females is synonymous to their good reproductive health however, its regularity is affected by environmental factors such as obesity, endocrine disruptors and stress.[2] Irregular menstrual cycles have become a common complaint among adolescents in the last decade. Menstrual irregularity percentage in adolescent girls is on the rise in different countries and it ranges approximately from 15% to 40%.[3,4,5] However, there are different theories about the aetiology of menstrual irregularities however, the most popular is the impact of endocrine disruptors in the environment.[6]

The endocrine disruption in young children and adults can have long lasting effects on health including reproductive dysfunction and cancer.[6,7-9] Additionally, due to the different susceptibilities of each person to these compounds, a population may have an overall increase in the disease burden. These environmental substances include plastics and plasticizers such as polyvinyl chloride (PVC), bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), polytetrafluoroethylene (PTFE) or Teflon, perfluoro-octanoic (PFOA or C8), and polychlorinated biphenyls (PCBs),

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which are established to be endocrine disrupter in animals (5-9). Plastics are present in water due to their use for water storage tanks made of PVC, which is a stiff material, however it can be made flexible by the addition of plasticizers like phthalates. By weight, phthalates constitute 40% of intravenous medical bags and up to 80% of medical tubing. In addition to the plastics, softened PVC is present in toys, car interiors, shower curtains, and flooring and they initially release chemical gases into the air which may contribute to health complications.

Endocrine disrupting chemical like Bisphenol A (BPA) has structure and activity similar to naturally occurring steroid hormone estrogen.[10] It binds to estrogen receptors and alter the homeostasis of the endocrine system by mimicking or blocking the estrogenic responses. It binds to estrogen receptors (ER α and ER β) thereby inhibiting the activity of endogenous estrogen.[11,12] It also binds to GPR30, a non-classical transmembrane ER and the aryl hydrocarbon receptor (AhR).[6,11,12] It binds an orphan nuclear receptor called estrogen-related receptor-gamma (ERR-g) and inhibits hepatic insulin signaling through phosphatidic acid phosphatase and LIPIN1.[13] BPA being hydrophobic compound gets accumulated in the adipose tissue of animals and humans. In addition, it has been detected in biological fluids such as sera, urine, follicular fluid, amniotic fluid, and breast milk and their levels are high in biological fluids of women with polycystic ovary syndrome (PCOS). BPA affects animal reproductive health which results in anovulation and reduced fertility. BPA and phthalates are released into water or eatables when heated at high temperatures. BPA has partial estrogenic and adipogenic activities and may have adverse effects on human health especially the reproductive health.[14-16] It is well established that the ovary is sensitive to increased BPA exposure.[15] Exposure to estrogenic compounds during the critical time period of follicle development can change or modify the normal follicle dynamics in the adult ovary, which may lead to anovulation and polycystic ovaries.[14-16] Therefore, BPA exposure may lead to menstrual irregularity in adolescent girls and make them more susceptible to PCOS.

There are several gaps in the understanding of adolescent health research in India; one among them is daily plastic/plasticizer exposure and its health consequences in adolescent and reproductive age. Here we hypothesized that everyday plastic/plasticizer exposure in adolescent girls may lead to menstrual irregularities (oligomenorrhea). This research aimed to study the correlation between the extent of exposure to plastic/plasticizer and oligomenorrhea in a cohort of adolescent girls living in north Indian states.

MATERIALS AND METHODS

Study design and participants

A school based descriptive cross-sectional study was conducted using a questionnaire distributed to female students (Total No. 1055). This study was conducted in schools in Rajasthan, Uttar Pradesh and Delhi from 2016 to 2019. The participants of the study were female adolescent students in resident schools. Exclusion based on missing data was done and 536 records were not included in the study. The age of the girls was critically scrutinized and 159 girls could not qualify to be in adolescent age. A total of 360 girls, who provided complete information and fit the selection criteria, were included in the study.

Criteria for selection

A total of 360 female students aged 14 to 19 years who completed the questionnaire were included in the study and those who had not provided consent and submitted incomplete questionnaires were excluded from the study. The study was based on the sources of plastic exposure, abnormalities in the menstrual cycle and family history of different diseases.

Data collection

A predesigned, pre-tested structured self-administered questionnaire was used for data collection. The questionnaire was administered in English and was structured in three parts, the first part included 10 questions regarding the ways of exposure to plasticizers (Plasticizer exposure risks included, use of plastic containers for food, use of microwave utensils, type of utensils used for cooking, type of pipe used for water supply and type of water storage tank used), the second part was based on abnormalities in the menstrual cycle and its related problems and the third part was regarding family history of PCOS, Diabetes, Cancer, Obesity, Blood pressure, CVD and infertility related problems. The questionnaire was explained and one researcher was present in the classroom to facilitate accurate responses by the subjects. The completion of the questionnaire lasted between 12 and 20 minutes. The questionnaires were collected immediately after completion to prevent interpersonal communication and influence of peers on individual responses among girls. In addition, after collecting data, an educational session related to the subject was conducted for the girls

Assessment of menstrual dysfunction

Menstrual history was assessed retrospectively by self-reporting. At baseline (after run-in, and just before randomization), the following questions were asked: (1) "Have you had your first period?

If yes, how old was you when your period began? and (3) How many periods have you had in the past 6 months?" Regular periods were defined as a response of "yes" to the

question about having a period at baseline, having had five periods or more in the past 6 months, and having had the first period at least 1 year before baseline. The timeframe of 1 year was chosen on the basis of evidence that most girls achieve a regular menstrual cycle within 1 year of menarche. Irregular menses were defined as a response of "yes" to the question about having had a period at least 1 year before baseline and having had three or fewer periods in the past 6 months. Anthropometric measurement

Body weight (kg) and height (cm) were provided by the students on the questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m2).

Data analysis

Data analysis was performed using RStudio (Version 4.0.4) and the Fisher Exact Test was used between two nominal (categorical) variables. The Pearson's chi-square value was used as the significance level. $p<0.05$ was considered statistically significant.

RESULTS

Exposure to eight predominant plastics, phthalates, PVC containing household items like water bottles, non-stick ware, plastic use in microwave; menstrual irregularities, acne, black puffy skin, hirsutism and hair fall were self-reported by the girls. In addition, information on family histories of type II diabetes, cardiovascular disease, infertility, obesity and cancer was also given by them and analyzed for the correlation with menstrual irregularity. The study cohort included Indian girls of mean age of 15.91± 0.99 and normal body weight (BMI=20.68 ± 3.19). The age of menarche was 9 to 10 years for 13 girls, 11 to 12.2 years for 74 girls, 12.3-13 years for 191 girls and 14-15 years for 77 girls (Table 1). Irregular menstruation was reported by 40.8% girls. Based on the usage of plastics, the girls were divided into two groups, with medium plastic usage (61) and high plastic usage (299) (Table 2). Heavy plastic exposure significantly increased the likelihood of irregular periods (Table 3. 43.81%Vs 26.22%, $p<0.05$). Both medium and high exposure to plastics were associated with heavy hair fall which correlated with abnormal hair growth/hirsutism ($p<0.026$) indicating a role for androgen imbalance in these girls (Table 3). Girls with irregular menstrual cycles had a higher prevalence of hair falls, acne, acanthosis nigricans, and abnormal hair growth/hirsutism (Table 4). Girls with a history of diabetes and CVD were more susceptible to irregular menstruation after heavy plastics usage (Table 4). Most girls with irregular cycles had no history of obesity or type II diabetes and CVD, confirming the adverse effects of plastics on their menstrual health. Adolescent girls with both medium and heavy use of plastics had no family history of infertility or cancer indicating a clear role of plastic use in causing menstrual irregularities (Table 5). Limitation of the study was that there were no adolescence girls who had zero exposure to plastics.

DISCUSSION

This study demonstrates an increased incidence of endocrine defects and menstrual irregularity in adolescent girls in north India which was associated with the higher plastics usage. Here, data indicates that the exposure to plastics during childhood or preadolescent years may contribute to the development of irregular menstruation in young women with no family history of infertility, obesity or CVD. However, most girls enrolled in this study with or without irregular menstruation had family history of type II diabetes which indicates a significant prevalence of this disease in this area of study and a systematic study is required to understand its causes. Evidence of the correlation between plastic usage and reproductive health issues in Indian adolescent girls has not yet been reported. Several studies have shown an increased incidence of menstrual irregularities however, the reason for the increase in these health issues has not been clear. This is the first report of the association of menstrual irregularities with plastic exposure.

Recently, there has been interest in whether EDCs in the environment, particularly BPA contributes to reproductive disorders, irregular menstruation and PCOS. Here, we report that plastic use contributes to the development of hormonal imbalance leading to observable outcomes such as acne and hirsutism. BPA inhibits the activity of endogenous estrogen and/or disrupts estrogen receptor action. Several studies have indicated the role of BPA in impairing the balance of androgens like testosterone, androstenedione and dehydroepiandrosterone sulfate in women.

BPA modulates the enzyme 17β-hydroxylase in the theca-interstitial cells and decreases the level of steroid binding globulin, SHBG and thereby increases the level of free testosterone in girls and that is one of the significant defects known in women with PCOS.[17-21] In particular, BPA has been reported to be associated with alterations in hypothalamic-pituitary axis, imbalance of hormones, reduced oocyte quality due to perinatal and/or adult exposure, and defective uterine receptivity resulting in reproductive disorders and infertility.[10, 18-21] The untimely estrogenic properties of BPA increase the insulin levels and contribute to the development of metabolic disorders such as insulin resistance, type II diabetes, and PCOS.[22]

Women with poor ovarian response had higher urinary BPA levels during IVF therapy and BPA levels were negatively associated with peak estradiol levels and the number of oocytes retrieved.[23,24] BPA may have non-monotonic, tissue-specific

damaging effects on the reproductive system at concentrations lower than the LOAEL (50µg/kg body wt./d).[25,26] Several studies have implicated BPA in metabolic defects and development of ovarian cysts.[27] BPA affects the adrenal gland and increases the conversion of cortisone to cortisol through the 11beta-hydroxysteroid dehydrogenase type 1 (11bHSD1) enzyme in adipose tissue of children.[28] Maternal BPA levels are associated with increased cortisol levels in female infants.[29]

Most women first learn about PCOS while seeking consultations for their either irregular menstruation, cosmetic problems or infertility. The increasing number of young Indian women report to the clinic with reproductive disorders such as polycystic ovary syndrome (PCOS) and subfertility and this has posed both economic and psychosocial pressures on society. PCOS is a genetically complex, heterogeneous endocrine disorder of unknown aetiology in women, worldwide. The syndrome is characterized by irregular or absent menstruation, hyperandrogenism, and cystic ovaries. The symptoms of PCOS can be worsened by obesity and insulin resistance.

Women with PCOS have higher risk of developing diabetes mellitus, cardiovascular disease and endometrial cancer in women with PCOS. [9, 30,31] Our observations reported here support the role of plastics as the environmental factors in the development of menstrual problems and androgen excess and therefore, it directly or indirectly contributes in the aetiology of PCOS. The expected exposure to BPA is higher in women in tropical countries like India than those in western countries. This is due to the increased leeching of BPA from plastics at warm temperatures. Based on our findings, we hypothesize that increased incidence of hyperandrogenism and PCOS in adolescent girls may be due to increased exposure to plastics during gestation, childhood and/or adolescence. However, further studies are required to establish the role of plastics in the development of PCOS/PCOD in young Indian girls with menstrual irregularities and androgen excess.

CONCLUSION

The findings here indicate that exposure to plastics increase the chances of adolescent girls to have irregular menstrual cycle and symptoms of androgen excess. Our data demonstrates for the first time that there is increased percentage of adolescent girls having irregular period and hyperandrogenism in north Indian states. Due to its endocrine disruptive actions, plastics may influence the neuroendocrine functions during preadolescent period and cause imbalance in the levels of endogenous hormones essential for optimal menstrual function. Further investigation on clinical samples and ultrasonography reports of adolescent would be required to understand the role of chronic exposure to plastics/BPA in reproductive health issues and PCOS.

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Conflicts of interest

There are no conflicts of interest.

Author's contributions

RS: Conceptualization, designing the study, literature review, methodology, analysis, and writing. SY: Literature review, data collection, analysis, writing, and SB writing and editing the paper: IA: Data analysis

Ethical statement

Ethical Clearance has been obtained from IHEC, Department of Zoology, University of Delhi, Delhi. In addition, all the participants were assured that their anonymity and confidentiality would be maintained and they were entitled to drop out of the study at any time.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Abbreviations: Bisphenol A (BPA), Phthalates, Polyvinyl chloride (PVC), E2 Estrogen,

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Table 1: Participant Demographics and menstrual irregularities in School girls

Characteristics	Mean (SD) or n (%)
Age, Yr. range 15-19	15.91± 0.99
BMI	20.68 ± 3.19
Race, Indian	360 (100%)
Age at menarche (Years)	
9-10	13 (3.61)
11-12.2	74 (20.84)
12.3-13	191 (53.80)
14-15	77 (21.69)
Regular Menstruation	213 (59.16)
Irregular Menstruation	147 (40.8)
Medium plastic usage	61 (16.94)
High plastic usage	299 (83.05)

Table 2: Questions wise records of menstrual irregularities in adolescent girls with medium and high plastic usage.

Parameters	Medium Plastic usage (61)		High Plastic usage (299)	
Age (Mean ± SD)	**15.98±1.05**		**15.90±0.99**	
BMI (Mean ± SD)	**20.33±3.02**		**20.76±3.23**	
	Prevalence of Outcome (%)			
Parameters to assess the plastic use	**No. of Girls (No. excluding plastic users)**	**No. of girls with irregular menstruation (%) (n=16)**	**No. of Girls (Number excluding plastic users)**	**No. of Girls with irregular menstruation (%) (n=131)**
1. PVC Water Pipes	0	0	264	110 (41.66)
2. Plastic Water Storage Tank	0	0	181	81 (44.75)
3. Drinking Water Source*				
Plastic Water Storage Tank/through Filter/RO	0	0	44	14 (41.0)
Fresh water direct supply	9	0	34	12 (35.3)
Fresh water/through Filter/RO	46	15** (32.6)	210	98# (46.6)
No answer selected	6	-	11	-
4. Reuse of Plastic Bottles for storage	40	10 (25.0)	132	55 (41.66)
5. Utensil used in Microwave				
Plastic	12	2 (16.6)	60	26 (43.3)
Glass and others	42 (40)	7 (17.5)	232 (177)	81 (45.7)
6. Utensils used for Cooking				
Non-stick Cookware	26	8 (30.7)	140	64 (45.7)
Steel and Iron	50 (33)	7 (21.2)	194 (147)	60 (40.8)
7. Utensils used for Storage Food				
Plastic	11	3 (27.2)	55	21 (38.8)
Steel and Glass	53 (38)	8 (18.4)	271 (170)	73 (42.9)
8. Utensils used for Packed Lunch				
Plastic	31	10 (32.2)	163	71 (43.55)
Steel and Glass	31 (29)	6 (20.8)	142 (124)	55 (44.35)

Table 3: Correlates of high plastic usage compared with medium plastic usage among adolescent girls.

	Prevalence of Outcome (%) (n= 360)		
Outcome Variable	Medium plastic usage (n=61)	High Plastic usage (n=299)	p-Value
Irregular Menstrual cycle	26.22	43.81	0.015
Hair-fall	45.90	45.15	1.000
Acne	3.27	20.27	0.0006
Black puffy skin	16.94	19.86	0.718
Abnormal hair growth	27.86	28.76	1.000
Family history of Diabetes	24.59	39.13	0.040
Family history of CVD	30.00	31.10	1.000
Family history of Infertility	01.63	05.46	0.325
Family history of Obesity	18.33	22.07	0.607
Family history of Cancer	20.00	14.42	0.325

Table 4: Correlates of irregular Menstrual cycles among adolescent girls.

	Prevalence of Outcome (%) (n= 360)		
Age (Mean ± SD)	15.96±0.96	15.84±1.05	p=0.263
BMI (Mean ± SD)	20.43±3.15	21.05±3.24	p=0.070
Outcome Variable	Regular Menstruation (n=213)	Irregular menstruation (n=147)	p-Value
Heavy Hair-fall	40.28	55.31	0.017
Acne	13.74	22.60	0.033
Black puffy skin	13.67	27.78	0.001
Family history of Obesity	19.81	23.80	0.364
Family history of Diabetes	30.52	45.58	0.004
Family history of CVD	25.00	39.45	0.005
Family history of Infertility	02.87	06.94	0.115
Family history of Cancer	15.63	14.96	0.883

Table 5: Family history and menstrual irregularities in adolescent girls with high and medium plastic usage.

		All women <20, (n= 147)		
Plastic Usage	Type of Family History	Prevalence of Outcome (%) in girls with Irregular Menstruation		
		Family History (n)	Family History (%)	No Family History (%)
Medium (n=16)	Family history of Diabetes	4	25.0	75.0
	Family history of CVD	0	0.00	100.0
	Family history of Diabetes & CVD	3	18.75	81.25
High (n=131)	Family history of Diabetes	27	20.80	79.20
	Family history of CVD	22	16.79	83.21
	Family history of Diabetes & CVD	33	25.19	74.81