Obesity Medicine 14 (2019) 100090

Contents lists available at ScienceDirect

Obesity Medicine

journal homepage: www.elsevier.com/locate/obmed

Bisphenol A (BPA) acts as an endocrine disruptor in women with Polycystic Ovary Syndrome: Hormonal and metabolic evaluation

Iram Ashaq Kawa[a], Akbar Masood[a], Mohd Ashraf Ganie[d], Qudsia Fatima[a], Humira Jeelani[a], Saika Manzoor[a], Syeed Masuma Rizvi[b], Mohd. Muzamil[c], Fouzia Rashid[a,*]

[a] Department of Biochemistry/Clinical Biochemistry, University of Kashmir, Srinagar, India
[b] Department of Obstetrics and Gynecology, Government Medical College, Srinagar, India
[c] Department of Psychology, University of Kashmir, Srinagar, India
[d] Sheri Kashmir Institute of Medical Sciences, Srinagar, India

ARTICLE INFO

Keywords:
Bisphenol A
Polycystic ovary syndrome
Hematology

ABSTRACT

Aim: To assess the serum Bisphenol A levels in women with Polycystic Ovary Syndrome (PCOS) and its possible association with their hormonal, metabolic and hematological parameters.

Methods: A total of 49 women with PCOS and 39 healthy controls were included in the study. The diagnosis of PCOS was done using the Rotterdam criteria 2003. Anthropometric and clinical baseline profile of all the study subjects was done. Serum BPA levels were estimated and its correlation with hormonal, metabolic and hematological parameters was investigated.

Results: The women with PCOS demonstrated higher levels of BPA as compared to healthy women (26.4 ± 14.9 versus 18.95 ± 8.88 ng/ml; $p = 0.0046$). A significant association of clinical features like BMI ($r = 0.296$, $p = 0.039$), Waist circumference ($r = 0.315$, $p = 0.027$) and waist-hip ratio ($r = 0.402$, $p = 0.004$) with BPA was found. The BPA levels are also strongly associated with testosterone levels and with the biochemical abnormalities describing the syndrome (Blood glucose-Fasting ($r = 0.478$, $p = 0.001$), Blood glucose-1 hour ($r = 0.307$, $p = 0.032$), Blood glucose-2 hour ($r = 0.393$, $p = 0.005$), total cholesterol ($r = 0.361$, $p = 0.011$), triglycerides ($r = 0.362$, $p = 0.011$), Insulin fasting ($r = 0.426$, $p = 0.002$), HOMA-IR ($r = 0.543$, $p = < 0.0001$), QUICKI ($r = -0.459$, $p = 0.0009$). BPA was also positively correlated with erythrocyte parameters like HCT ($r = 0.284$, $p = 0.048$) and MCV ($r = 0.360$, $p = 0.011$).

Conclusion: The present study suggests that BPA an endocrine disruptor plays an important role in the pathogenesis of PCOS and contributes in the development and phenotype of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common and heterogeneous endocrinopathy in women of reproductive age and is characterized by hyperandrogenic chronic anovulation (Futterweit, 1999). The prevalence of PCOS is reported to vary from 6% to 20% in women of reproductive age, which is based on the diagnostic criteria used to define the syndrome (Fauser et al., 2012; March et al., 2010). The condition seems to be on rise in Kashmir valley although systemic studies on the subject are underway. The disorder manifests as hirsutism, male-pattern baldness, acne vulgaris, menstrual disturbances, obesity, an-ovulation, infertility, recurrent abortions and psychological and psychosexual morbidity (Tsilchorozidou et al., 2004). It is the most important cause of infertility and hirsutism in our population (Zargar et al., 1997, 2002). Besides the above mentioned manifestations of PCOS, women afflicted by this disease are at increased risk of developing Hypertension, Abdominal Obesity, Metabolic syndrome, Type 2 Diabetes Mellitus, Dyslipidemia and Cardiovascular disease (CVD) as compared to healthy women and the long-term presence of cardiovascular risk profile in these women may result in premature atherosclerosis (Jeelani et al., 2017). PCOS being a collection of multiple variables as regards its pathophysiology, as yet it is thought to involve the interaction of genetic and environmental components synergistically contributing to the variation in clinical picture of this syndrome. The role of environmental factors in the pathogenesis of PCOS is under thorough investigation and has recently been expanded to include a

* Corresponding author.
E-mail address: rashid.fouzia@gmail.com (F. Rashid).

https://doi.org/10.1016/j.obmed.2019.100090
Received 27 August 2018; Received in revised form 8 April 2019; Accepted 13 April 2019

broad category of industrial chemicals known as endocrine disrupting chemicals, that are widespread in the atmosphere and have the ability to interfere with all hormone-sensitive systems either by interfering with the synthesis or excretion of hormones, by mimicking their actions or by blocking their effects (Diamanti-Kandarakis et al., 2009). Bisphenol A (BPA) is one of the most abundant chemicals produced worldwide and a potent endocrine disrupting chemical. It is used in the synthesis of polycarbonate plastics and epoxy resins which are used in various products of daily lives like water containers and bottles, resin linings of food and beverage cans, electronic equipments, dental sealants and so on. Also upon heating it is able to migrate into water and food. Human exposure to BPA is considered widespread and continuous and the possible link between BPA exposure and adverse health outcomes has been investigated by a number of studies.

Owing to estrogen like activity of BPA, its most sensitive target happens to be ovarian cells as previous reports have already testified for its effects on ovarian morphology, steroidogenesis and folliculogenesis (Markey et al., 2003; Newbold et al., 2007). Neonatal or perinatal exposure to BPA has been shown to cause significant changes in the reproductive tract (Newbold et al., 2007, 2009), alteration of estrous cyclicity (Rubin et al., 2001), decreased reproductive capacity (Cabaton et al., 2011) and changes in hormonal levels (Rubin et al., 2001; Fernandez et al., 2009) later in adult life. The association of BPA exposure with lower antral follicle counts (Souter et al., 2013), follicle loss (Souter et al., 2013) and decreased oocyte survival is reported (Brieno-Enriquez et al., 2011). Further, Ehrlich et al. in their study showed the association of BPA with impaired oocyte maturation, yield and fertilization which critically affects the success of IVF treatment (Ehrlich et al., 2012). Caserta et al. found that the infertile women as compared to fertile controls are significantly more likely to have detectable serum BPA, while other exposures like (di-(2-ethylhexyl) phthalate (DEHP), perfluorooctanoic acid (PFOA), perfluoctane sulfonate (PFOS) and mono-ethylhexyl phthalate (MEHP)) are not different between the two groups (Caserta et al., 2013). In women, the BPA exposure has also been associated with obesity, abnormal karyotypes, endometrial hyperplasia and recurrent miscarriages (Vandenberg et al., 2010). Further, it has been demonstrated from recent data of experimental animals that exposure to BPA at neonatal stage results in PCOS development (Fernandez et al., 2010) and impairment in the regulation of glucose metabolism/insulin secretion (Alonso-Magdalena et al., 2006). The BPA levels are reported to be higher in women with PCOS compared to healthy individuals and could possibly be involved in one of many underlying causes of this disorder (Kandaraki et al., 2011).

PCOS which affects reproductive aged women is not only a gynecological condition but, is also associated with lifelong risk of type 2 diabetes, metabolic syndrome and cardiovascular disease. Increased insulin resistance appears a crucial mechanism behind this association. Recent studies have demonstrated the close relationship between various hematological parameters like white blood cells (WBC) count and red blood cell (RBC) count and components of metabolic syndrome. Various hematological parameters have been found to be associated with prothrombotic and proinflammatory state also. There are reports that PCOS women show alteration in such parameters like WBC, Platelets, Mean platelet volume (MPV) and Red cell distribution width (RDW). These could serve as markers for the detection of various associated diseases like CVD (linked to the systemic inflammation) at an early stage (Kebapcilar et al., 2009; Papalou et al., 2015; ÃzrMehmet et al., 2014).

Therefore, this study was undertaken to analyze the serum BPA levels in women with PCOS versus healthy individuals to explore a possible association between BPA exposure and hormonal/biochemical abnormalities describing the syndrome. Also we assessed the correlation between BPA with hematological parameters (RBC, WBC, RDW, MPV) in PCOS women that will provide an evidence whether increased BPA levels effects Hematological parameters, which may in turn link the higher BPA exposure with CVD risk factors.

2. Materials and methods

The study was approved by Institutional Board of Research Studies and was carried in the Department of Clinical Biochemistry, University of Kashmir, Srinagar, J&K, India. A total of 250 subjects were screened according to the considered inclusion and the exclusion criteria from a period of 2014–2016. Among them a total of 100 PCOS women and 80 controls were initially enrolled in the study out of which 49 patients of PCOS and 39 controls were included in the present study.

2.1. Subjects

Patient group: This group included 49 PCOS women which were recruited from various hospitals (Endocrinology and Gynecology OPD's under guidance of specialists). The diagnosis was based on Rotterdam criteria 2003 which is defined by the occurrence of two of the following three criteria: menstrual dysfunction (intermenstrual periods > 35 days or < 21 days); clinical and/or biochemical hyperandrogenism (hirsutism and/or an elevated total testosterone) and the ultrasonographic presence of Polycystic Ovary morphology (> 12 follicles in either or both ovaries measuring 2–9 mm in diameter). Women with etiologies like congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, hyperprolactinemia and androgen-secreting tumors plus women who had any medication in the past three months like oral contraceptives, insulin-sensitizing drugs, antiandrogens, aspirin, statins, warfarin, antidepressant medication, Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, Gonadotropin-relaesing hormone (GnRH) antagonists or agonists were excluded. Furthermore, patients with hypertension, a history of angina or myocardial infarction, known coagulation abnormalities, spontaneous abortion, anorexia, infection or inflammatory diseases were also excluded from the study.

Control group: 39 healthy volunteers recruited from various screening camps conducted at university and various colleges with regular menstrual cycles, no evidence of hirsutism, acne or hyperandrogenemia served as control group and were studied and analysed during the follicular phase. Subjects with findings of Polycystic Ovaries on ultrasonographic examination were excluded from the study.

2.2. Methods

All the study Subjects were informed about the study and those who agreed to participate in the study were asked to sign an informed consent. All women were interviewed in detail regarding their menstrual history (includes regularity, duration, age of menarche, flow, number of menstrual cycles per year and dysmenorrhea), hair growth (extent and duration), acne, weight gain, family history of infertility, menstrual disorders, hirsutism, glucose intolerance, coronary artery disease, diabetes mellitus or obesity at least in three generations. All women underwent anthropometric assessments like measurement of weight, height, waist-hip circumference, blood pressure recording, and detailed systemic examination. Body mass index (BMI) which is the ratio of individual's weight and square of the height (kg/m^2) was calculated. Ferriman-Gallwey scoring system was used to assess hirsutism in which nine specified body areas are evaluated. Each area is given a score of 1–4 and a score of 8 out of total 36 was taken as significant. All the patients were graded for acne vulgaris and grade III and above plus the extent of male pattern baldness was taken as clinical feature of hyperandrogenemia. The transabdominal ultrasonography (USG) was done for all the subjects by a single observer to record the ovarian morphology (peripheral cysts, ovarian volume).

The samples were collected on 2nd to 7th day of early follicular phase of either progesterone induced or spontaneous menstrual cycle after an overnight fast. The samples were collected at 0, 1 h and 2 h after 75 g oral load of anhydrous glucose to perform an Oral Glucose Tolerance Test (OGTT). The samples were centrifuged immediately for

biochemical investigations and serum was stored at −80 °C until assayed for hormonal parameters and BPA. On the same day blood samples were analysed for investigating their hematological parameters such as RBC, WBC, Platelets within 1 h of venipuncture using a Sysmex KX- 21 hematology auto analyzer. Hormonal assays were done by Chemiluminescence Immunoassay using commercial kits according to supplier protocol. Biochemical investigations which included glucose and lipid (total cholesterol, triglycerides) levels, kidney and liver function tests were estimated on semi autoanalyser (ERBACHEM 7) by using standard commercially available kits. Insulin levels were estimated on BIORAD analyzer using commercially available ELISA kit (Ray biotech). The serum BPA levels were measured using commercially available ELISA kit (SHANGHAI YEHUA Biological Technology Co., Ltd) considered for the purpose of quantitative determination of BPA in human plasma or serum samples, Urine, Saliva, and other related tissue fluids. The procedure for the measurement of samples and standards was carried out according to the manufacturer's instructions. The kit is based on biotin double antibody sandwich technology and the measurement range of kit is 0.3 ng/ml to 90 ng/ml BPA.

2.3. Calculations

Insulin resistance was assessed by means of homeostasis model assessment insulin resistance index (HOMA-IR) calculated as [fasting insulin (μIU/mL) x fasting glucose (mg/dL)]/405, quantitative insulin sensitivity check index (QUICKI) calculated as 1/[log fasting insulin (μIU/mL) + log fasting glucose (mg/dL)] and fasting glucose to insulin ratio (FGIR). The FGIR values were calculated as fasting glucose (mg/dL)/fasting insulin (μIU/mL). High HOMA-IR, low QUICKI and low FGIR scores denote insulin resistance (low insulin sensitivity).

2.4. Statistical analysis

All the statistical analysis was done on SPSS 16.0 version. An unpaired two-tailed *t*-test was used for comparing variables between two groups (PCOS and healthy controls) and data is presented as mean ± SD. To assess the relation of BPA with the variables of PCOS Pearson's correlation coefficient and linear/multiple regression analysis was applied. The PCOS related baseline parameter was set as the response variable (y) while using BPA as the independent variable (X) while introducing other confounders (X′) to check for confounding analysis. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Baseline characteristics of the subjects

Comparisons of demographic and clinical characteristics of subjects are shown in Table 1. The women with PCOS showed significant differences in all base line clinical, anthropometric and biochemical parameters, except age, age of menarche, BMI, waist hip ratio, fasting glucose, cholesterol, urea and creatinine compared to the control women. Ferriman-Gallwey score was significantly higher in PCOS women compared to healthy controls (10.94 ± 4.20 versus 5.74 ± 1.12; p = < 0.0001). PCOS women had significantly higher anthropometric parameters like weight (58.17 ± 10.57 versus 53.73 ± 6.61 kg; p = 0.0183) and Waist circumference (86.04 ± 9.43 versus 81.25 ± 5.47 cm; p = 0.0039). Metabolic parameters like 2 h glucose was significantly higher in PCOS women as compared to control women (104.46 ± 26.41 versus 94.8 ± 12.7 mg/dl; p = 0.0279) as was triglycerides (105.1 ± 42.4 versus 85.4 ± 42.0 mg/dl; p = 0.033), uric acid (4.75 ± 1.07 versus 3.72 ± 0.89 mg/dl; p = < 0.0001), Serum glutamic oxaloacetic transaminase: SGOT (20.21 ± 6.28 versus 17.77 ± 4.48 mg/dl; p = 0.047) and Serum glutamic pyruvic transaminase: SGPT (18.02 ± 5.83 versus 14.72 ± 4.10 mg/dl; p = 0.0041), fasting Insulin levels (13.49 ± 3.03 versus 9.26 ± 1.70; p = < 0.0001), HOMA-IR (3.09 ± 1.04 versus 1.98 ± 0.43; p = < 0.0001), QUICKI (0.33 ± 0.01 versus 0.34 ± 0.01; p = < 0.0001), FGIR (7.07 ± 1.80 versus 9.66 ± 1.90; p = < 0.0001). Hormonal parameters like testosterone was significantly higher in PCOS women as compared to controls (51.5 ± 16.7 versus 29.2 ± 13.0 ng/dl; p = < 0.0001) as was Luteinizing hormone: LH (8.99 ± 6.03 versus 5.45 ± 3.53 IU/L; p = 0.0029) and Follicle-stimulating hormone: FSH (7.19 ± 2.85 versus 5.90 ± 1.87 IU/L; p = 0.024). Hematological parameters like platelets and Mean corpuscular hemoglobin concentration: MCHC were significantly higher in PCOS women as compared to controls (190.8 ± 60.6 versus 160.1 ± 56.9 10^3/ml and 32.97 ± 1.73 versus 32.12 ± 1.14 g/dl; $p = 0.017$, $p = 0.0067$) respectively.

Table 1
Baseline characteristics and BPA levels in PCOS women versus controls.

Parameter	Cases n = 49	Controls n = 39	p Value
Age (years)	23.72 ± 4.50	22.21 ± 2.97	0.067
Age of Menarche (years)	13.07 ± 1.15	13.38 ± 0.93	0.17
FG-score	10.94 ± 4.20	5.74 ± 1.12	< 0.0001
Weight (kg)	58.17 ± 10.57	53.73 ± 6.61	0.0183
Waist Circumference (cm)	86.04 ± 9.43	81.25 ± 5.47	0.0039
W/H	0.93 ± 0.05	0.92 ± 0.03	0.17
BMI (kg/m^2)	23.14 ± 3.48	21.87 ± 2.75	0.06
LH (IU/L)	8.99 ± 6.03	5.45 ± 3.53	0.0029
FSH (IU/L)	7.19 ± 2.85	5.90 ± 1.87	0.024
LH/FSH ratio	1.40 ± 0.94	0.95 ± 0.51	0.014
Testosterone (ng/dl)	51.5 ± 16.7	29.2 ± 13.0	< 0.0001
Blood glucose-Fasting (mg/dl)	91.4 ± 14.5	86.83 ± 9.75	0.08
Blood glucose-1 hour (mg/dl)	124.28 ± 26.47	103.1 ± 12.4	< 0.0001
Blood glucose-2 hour (mg/dl)	104.46 ± 26.41	94.8 ± 12.7	0.0279
Serum Total cholesterol (mg/dl)	148.6 ± 40.1	139.5 ± 33.6	0.25
Serum Triglycerides (mg/dl)	105.1 ± 42.4	85.4 ± 42.0	0.033
Urea (mg/dl)	24.55 ± 5.79	22.82 ± 7.07	0.23
Creatinine (mg/dl)	0.80 ± 0.19	0.75 ± 0.24	0.25
Uric Acid (mg/dl)	4.75 ± 1.07	3.72 ± 0.89	< 0.0001
SGOT (AST) (IU/L)	20.21 ± 6.28	17.77 ± 4.48	0.047
SGPT (ALT) (IU/L)	18.02 ± 5.83	14.72 ± 4.10	0.0041
Fasting Insulin (μIU/mL)	13.49 ± 3.03	9.26 ± 1.70	< 0.0001
HOMA-IR	3.09 ± 1.04	1.98 ± 0.43	< 0.0001
QUICKI	0.33 ± 0.01	0.34 ± 0.01	< 0.0001
FGIR	7.07 ± 1.80	9.66 ± 1.90	< 0.0001
RBC 10^6/μL	4.62 ± 0.52	4.47 ± 0.34	0.11
Hemoglobin g/L	11.46 ± 1.62	10.93 ± 1.25	0.085
HCT %	37.89 ± 4.08	36.94 ± 3.14	0.22
MCV fl	83.51 ± 6.80	83.35 ± 4.59	0.90
MCH pg	27.80 ± 2.80	26.88 ± 2.35	0.098
MCHC g/dl	32.97 ± 1.73	32.12 ± 1.14	0.0067
RDW fl	44.72 ± 6.82	44.38 ± 3.00	0.76
WBC 10^3/μL	6.86 ± 1.82	6.16 ± 1.90	0.086
Platelets 10^3/ml	190.8 ± 60.6	160.1 ± 56.9	0.017
MPV fl	12.40 ± 1.56	11.55 ± 2.24	0.14
BPA (ng/ml)	26.4 ± 14.9	18.95 ± 8.88	0.0046

Note: Values are mean ± SD. $p < 0.05$ is considered significant. FG = Ferriman Gallwey score; W/H = Waist-Hip ratio; BMI = Body Mass Index; LH = Luteinizing hormone; FSH = Follicle-stimulating hormone; LH/FSH = LF FSH ratio; SGOT = Serum glutamic oxaloacetic transaminase; AST = Aspartate aminotransferase; SGPT = Serum glutamic pyruvic transaminase; ALT = Alanine aminotransferase; HOMA-IR = Homeostasis Model Assessment Insulin Resistance Index; QUICKI = Quantitative Insulin Sensitivity Check Index; FGIR = Fasting Glucose to Insulin Ratio; RBC = Red blood cells; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular Hemoglobin; MCHC = Mean corpuscular Hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cells; MPV = Mean platelet volume; BPA = Bisphenol A.

3.2. Serum BPA levels and its correlation with various components of PCOS

The PCOS women showed statistically significant increase in serum

Table 2
Correlation of BPA levels with clinical, hormonal, biochemical and hematological parameters.

Parameters	r	p
FG score	0.127	0.384
Waist Circumference	0.315	0.027
W/H	0.402	0.004
BMI	0.296	0.039
Testosterone	0.443	0.001
LH	0.60	0.717
FSH	0.067	0.680
Blood glucose-Fasting	0.478	0.001
Blood glucose-1 hour	0.307	0.032
Blood glucose-2 hour	0.393	0.005
Serum total cholesterol	0.361	0.011
Triglycerides	0.362	0.011
Insulin Fasting	0.426	0.002
HOMA-IR	0.543	0.000
QUICKI	−0.459	0.0009
RBC	−0.056	0.703
Hemoglobin	0.232	0.108
HCT	0.284	0.048
MCV	0.360	0.011
MCH	0.253	0.079
MCHC	0.031	0.831
RDW	0.162	0.270
WBC	−0.057	0.698
Platelets	−0.131	0.370
MPV	0.10	0.963

Note: p < 0.05 is considered significant. FG = Ferriman Gallwey score; W/H = Waist-Hip ratio; BMI = Body Mass Index; LH = Luteinizing hormone; FSH = Follicle-stimulating hormone; LH/FSH = LF FSH ratio; SGOT = Serum glutamic oxaloacetic transaminase; AST = Aspartate aminotransferase; SGPT = Serum glutamic pyruvic transaminase; ALT = Alanine aminotransferase; HOMA-IR = Homeostasis Model Assessment Insulin Resistance Index; QUICKI = Quantitative Insulin Sensitivity Check Index; RBC = Red blood cells; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular Hemoglobin; MCHC = Mean corpuscular Hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cells; MPV = Mean platelet volume; BPA = Bisphenol A.

BPA levels as compared to healthy women (26.4 ± 14.9 versus 18.95 ± 8.88 ng/ml; p = 0.0046) as depicted in Table 1. BPA is significantly associated with the clinical, hormonal, metabolic and hematological parameters as follows - Waist circumference (r = 0.315, p = 0.027) W/H (r = 0.402, p = 0.004), BMI (r = 0.296, p = 0.039), testosterone (r = 0.443, p = 0.001), Blood glucose-Fasting (r = 0.478, p = 0.001), Blood glucose-1 hour (r = 0.307, p = 0.032), Blood glucose-2 hour (r = 0.393, p = 0.005), Serum total cholesterol (r = 0.361, p = 0.011), triglycerides (r = 0.362, p = 0.011), Insulin fasting (r = 0.426, p = 0.002), HOMA-IR (r = 0.543, p = < 0.0001), QUICKI (r = −0.459, p = 0.0009), HCT (r = 0.284, p = 0.048) and MCV (r = 0.360, p = 0.011) depicted in Table 2. The Inter correlation matrix is further represented as Table 3.

3.3. Linear regression analysis

To further analyze the impact of BPA we used the linear regression models depicted in Table 4. All the clinical variables were included as dependent variables in separate models and BPA as independent variable. The regression models for clinical parameters (BMI: R^2 = 0.088; Beta = 0.296; p = 0.039, WC: R^2 = 0.099; Beta = 0.315; p = 0.027, Waist-Hip ratio: R^2 = 0.161; Beta = 0.402; p = 0.004) indicated that 8.8% variation in BMI, 9.9% variation in WC and 16.4% variation in Waist-Hip ratio was explained by positive association with BPA. A regression model for testosterone levels (R^2 = 0.197; Beta = 0.443; p = 0.001) demonstrated that 19.7% variation in testosterone levels was explained by positive association with BPA. The regression models

Table 3
Inter correlation matrix.

	BPA	HCT	MCV	BMI	WC	WHR	Testosterone	Glucose Fasting	Glucose 1-h	Glucose 2-h	CHO	TG	Insulin	HOMA IR	QUICKI
BPA	1	0.284*	0.360*	0.296*	0.315*	0.402	0.443**	0.478**	0.307*	0.393**	0.361*	0.362*	0.426**	0.543**	−0.459**
HCT	0.284*	1	0.445**	0.038	0.055	0.216	0.145	0.101	0.164	0.174	0.299*	0.12	−0.059	0.008	0.011
MCV	0.360*	0.445**	1	0.050	0.112	0.214	0.067	0.005	0.092	0.096	0.148	0.077	0.063	0.057	−0.03
BMI	0.296*	0.038	0.05	1	0.834**	0.449**	0.182	0.020	0.083	−0.014	0.173	0.115	0.045	0.065	−0.005
WC	0.315*	0.055	0.112	0.834**	1	0.654**	0.202	0.174*	0.067	0.062	0.168	0.077	0.155	0.208	−0.17
WHR	0.402**	0.216	0.214	0.449**	0.654**	1	0.283*	0.333	0.260	0.288*	0.213	0.107	0.245	0.345*	−0.28
testosterone	0.443**	0.145	0.067	0.182	0.202	0.283*	1	0.382**	0.290*	0.165	0.295*	0.147	0.201	0.337*	−0.276
Glucose fasting	0.478**	0.101	0.005	0.020	0.174	0.333*	0.382**	1	0.419**	0.454**	0.096	−0.165	0.493**	0.823**	−0.753**
Glucose 1-h	0.307*	0.164	0.092	0.083	0.067	0.26	0.292*	0.419**	1	0.244	0.074	−0.177	0.179	0.323*	−0.292*
Glucose 2-h	0.393**	0.174	0.096	−0.014	0.062	0.288*	0.165	0.454**	0.244	1	−0.051	−0.056	0.355*	0.460**	−0.425**
CHO	0.361*	0.299*	0.148	0.173	0.168	0.213	0.295*	0.096	0.074	−0.051	1	0.444**	−0.048	0.002	0.011
TG	0.362*	0.120	0.077	0.115	0.077	0.107	0.147	−0.165	−0.177	−0.056	0.444**	1	−0.067	−0.105	0.144
Insulin	0.426**	−0.059	0.063	0.045	0.155	0.245	0.201	0.493**	0.179	0.355*	−0.048	−0.067	1	0.893**	−0.926**
HOMA-IR	0.543**	0.008	0.057	0.065	0.208	0.345*	0.337*	0.823**	0.323*	0.460**	0.002	−0.105	0.893**	1	−0.967**
QUICKI	−0.459**	0.011	−0.03	−0.005	−0.170	−0.280	−0.276	−0.753**	−0.292*	−0.425**	0.011	0.144	−0.926**	−0.967**	1

Note: p < 0.05 is considered significant. FG = Ferriman Gallwey score; W/H = Waist-Hip ratio; BMI = Body Mass Index; LH = Luteinizing hormone; FSH = Follicle-stimulating hormone; LH/FSH = LF FSH ratio; SGOT = Serum glutamic oxaloacetic transaminase; AST = Aspartate aminotransferase; SGPT = Serum glutamic pyruvic transaminase; ALT = Alanine aminotransferase; HOMA-IR = Homeostasis Model Assessment Insulin Resistance Index; QUICKI = Quantitative Insulin Sensitivity Check Index; RBC = Red blood cells; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular Hemoglobin; MCHC = Mean corpuscular Hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cells; MPV = Mean platelet volume; BPA = Bisphenol A.

Table 4
Linear Regression Analysis with BPA as an independent variable.

	R Square	Beta	p value	95% Confidence Interval for B	
				Lower bound	Upper bound
BMI	0.088	0.296	0.039	0.004	0.135
Waist	0.099	0.315	0.027	0.023	0.377
W/H	0.161	0.402	0.004	0.001	0.003
Testosterone	0.197	0.443	0.001	0.202	0.792
Glucose Fasting	0.229	0.478	0.001	0.216	0.719
Glucose 1 h	0.094	0.307	0.032	0.049	1.043
Glucose 2 h	0.154	0.393	0.005	0.219	1.178
Cholesterol	0.130	0.361	0.011	0.235	1.712
Triglycerides	0.131	0.362	0.011	0.251	1.813
Insulin Fasting	0.181	0.426	0.002	0.033	0.141
HOMA-IR	0.295	0.543	0.000	0.021	0.056
QUICKI	0.210	−0.459	0.001	0.000	0.000
HCT	0.080	0.284	0.048	0.001	0.155
MCV	0.129	0.360	0.011	0.039	0.290

Note: $p < 0.05$ is considered significant. HCT = Hematocrit; MCV = Mean Corpuscular Volume; BMI = Body Mass Index; HOMA-IR = Homeostasis Model Assessment Insulin Resistance Index; QUICKI = Quantitative Insulin Sensitivity Check Index; BPA = Bisphenol A.

for metabolic parameters (Glucose fasting: $R^2 = 0.229$; Beta = 0.478; $p = 0.001$, Glucose 1 h: $R^2 = 0.094$; Beta = 0.307; $p = 0.032$, Glucose 2 h: $R^2 = 0.154$; Beta = 0.393; $p = 0.005$) demonstrated that 22.9% variation in fasting glucose levels, 9.4% variation in 1 h glucose levels and 15.4% variation in 2 h glucose levels was explained by positive association with BPA. Finally, the regression models for fasting insulin levels ($R^2 = 0.181$; Beta = 0.426; $p = 0.002$), HOMA-IR ($R^2 = 0.295$; Beta = 0.543; $p = 0.000$) and QUICKI ($R^2 = 0.210$; Beta = −0.459; $p = 0.001$) demonstrated that 18.1% variation in fasting insulin levels, 29.5% variation in HOMA-IR was explained by positive association with BPA and 21% variation in QUICKI was explained by negative association of BPA. The regression models for hematological parameters (HCT: $R^2 = 0.080$; Beta = 0.284; $p = 0.048$, MCV: $R^2 = 0.129$; Beta = 0.360; $p = 0.039$) demonstrated that 8% variation in HCT and 12.9% variation in MCV was explained by positive association with BPA.

3.4. Confounding analysis

In order to check for confounding effects of other variables, multiple linear regression analysis was done using separate models. Initially the BPA was studied in relation to one dependent variable and then the potential confounders were added one by one backed by theoretical evidences and change in beta value was noticed. The effect (beta-coefficient) changed considerably with various variables but only the significant ones are mentioned in the Table 5. The potential confounders associated with BPA in relation to clinical, hormonal, metabolic and hematological parameters are as follows: for waist hip ratio→FG score, insulin; BMI→Glucose 2-h, triglycerides; testosterone→glucose fasting, HOMA-IR, QUICKI; glucose fasting→BMI, triglycerides, Insulin; glucose 2-h→triglycerides, Insulin; cholesterol→triglycerides, insulin, HOMA-IR, QUICKI; triglycerides→glucose fasting, glucose 2-h, insulin, HOMA-IR, QUICKI; HCT→ QUICKI; MCV→LH/FSH, glucose fasting, glucose 2-h, triglycerides, HOMA-IR, QUICKI. This analysis clearly depicts that the confounder effect is visible among various clinical parameters and it further implies that PCOS is a multispectral disorder involving complex interactions among various variables and thus needs to be further explored with large sample numbers for better understanding of its pathogenesis.

4. Discussion

PCOS is a multifactorial heterogeneous condition and is present most frequently with complaints of menstrual irregularity, hirsutism, acne or alopecia, infertility, pregnancy complications alongside various metabolic disturbances such as dyslipidemia, insulin resistance and hyperinsulinism, type 2 diabetes and possibly cardiovascular disease (Lath et al., 2015). As regards the baseline parameters in PCOS diagnosis, the present study showed a statistically significant increase in anthropometric parameters like weight, waist circumference and a statistically insignificant, small increase in waist hip ratio and BMI. There was no significant difference between the two groups in terms of age and age of menarche. As expected, in our study, we observed a significant increase in FG score, testosterone levels and LH/FSH ratio which are consistent with earlier reports (Dogan et al., 2014). Approximately 70% of PCOS women are thought to have abnormalities in serum lipid levels and such abnormalities continue to exist even after weight adjustment (Vrbikova et al., 2003). In our study we found a significant increase in the lipid profile (total triglycerides and cholesterol) of PCOS women as compared to the healthy controls and the cause is multifactorial. Insulin resistance seems to have an important role partly mediated by the stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase (Wild et al., 1985).

Further, a significantly higher prevalence rate of abnormal glucose tolerance is reported in PCOS women as compared to the healthy controls (Celik et al., 2013). A study by Ganie et al. concluded that young Indian PCOS women have high Abnormal Glucose Tolerance (AGT) which is not predicted by family history of type 2 DM and the detection rate of AGT is significantly improved by OGTT (Ganie et al., 2016). In our study we found that fasting serum glucose was comparable between PCOS women and healthy controls and the 2 h glucose level was found to be significantly higher in PCOS women compared to healthy controls. Insulin resistance is clinically defined as the inability of cells to respond normally to the hormone insulin and fails to increase the glucose uptake and utilization in an individual than it does in a normal population. It can be assessed by means of Homeostasis Model Assessment Insulin Resistance index (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI) and Fasting Glucose to Insulin Ratio (FGIR). In present study, a significantly higher mean fasting insulin levels, HOMA-IR and significantly lower QUICKI and FGIR was seen in women with PCOS compared to healthy women and these accords well with the results of other studies. Therefore, the results from our study suggest that PCOS women are at increased risk of developing metabolic syndrome and type 2 diabetes mellitus in future in comparison to healthy individuals. The PCOS women should be routinely screened for their metabolic profile so that the risk tendencies could be managed well in time.

The potential causal association between PCOS and Cardiovascular disease has been reported by many studies. The association between PCOS and vascular dysfunction (platelet dysfunction) markers of subclinical atherosclerosis and hypertension is suggested by several studies (Kelly et al., 2002; Lo et al., 2006; Yildiz et al., 2002). There is a tendency for thrombosis in patients with PCOS and this hyper coagulation can be related to increased platelet count. Further, there are reports that presence of risk factors related to atherosclerosis like hyperglycemia, hyperlipidemia and hypertension activates the vascular endothelial cells and promotes the increased synthesis and release of chemokines and cytokines into circulation. The resulting increased pro-inflammatory state further augments the activation of WBCs and endothelial cells and in doing so promotes the aggregation of platelets and formation of thrombus (Ross, 1999). On the other hand, there is the hypothesis that activation of leukocytes and platelets, increased platelet number and cell mass promotes the platelet leukocyte aggregate formation and contribute to increased risk for atherosclerotic disorders (Harrison, 2005). Further, serum androgen levels have been shown to affect platelet aggregation (Pilo et al., 1981). Therefore, the

Table 5
Confounding analysis.

Parameters	$\Delta_{WC} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{WHR} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{BMI} = I_{Beta}F_{Beta}$	$\Delta_{Testosterone} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{Glucose\ fasting\ g} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{Glucose\ 2\text{-}h} = I_{Beta}\text{-}F_{Beta}$
Age	0.315–0.344 = 0.029	0.402–0.405 = 0.003	0.296–0.330 = 0.034	0.443–0.446 = 0.003	0.478–0.487 = 0.009	0.393–0.396 = 0.003
WC	–	–	–	0.443–0.422 = 0.021	0.478–0.530 = 0.052	0.393–0.450 = 0.057
WHR	–	–	–	0.443–0.394 = 0.049	0.478–0.488 = 0.010	0.393–0.386 = 0.007
BMI	–	–	–	0.443–0.428 = 0.015	0.478–0.562 = 0.184	0.393–0.461 = 0.068
FG score	–	0.402–0.291 = 0.111	–	–	0.478–0.510 = 0.032	0.393–0.380 = 0.013
Testosterone	–	0.402–0.344 = 0.058	–	–	0.478–0.384 = 0.094	0.393–0.398 = 0.005
LH	–	0.402–0.342 = 0.060	–	0.443–0.429 = 0.014	0.478–0.404 = 0.074	0.393–0.420 = 0.027
LH/FSH	–	0.402–0.347 = 0.055	–	0.443–0.451 = 0.008	0.478–0.429 = 0.049	0.393–0.447 = 0.054
Glucose fasting	–	0.402–0.314 = 0.088	0.296–0.371 = 0.075	0.443–0.338 = 0.105	–	–
Glucose 2-h	–	–	0.296–0.398 = 0.102	0.443–0.358 = 0.085	–	–
Cholesterol	–	–	0.296–0.379 = 0.083	–	0.478–0.510 = 0.032	0.393–0.473 = 0.080
Triglycerides	–	–	0.296–0.426 0.130	–	0.478–0.605 = 0.127	0.393–0.512 = 0.112
Insulin	–	0.402–0.219 = 0.183	0.296–0.338 0.042	0.443–0.434 = 0.006	0.478–0.448 = 0.150	0.393–0.296 = 0.097
HOMA-IR	–	–	0.296–0.373 0.077	0.443–0.336 = 0.220	–	
QUICKI	–	–	–	0.443–0.332 = 0.221	–	

Parameters	$\Delta_{CHO} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{TG} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{Insulin} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{HOMA\text{-}IR} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{QUICKI} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{HCT} = I_{Beta}\text{-}F_{Beta}$	$\Delta_{MCV} = I_{Beta}\text{-}F_{Beta}$
Age	0.361–0.363 = 0.002	0.362–0.356 = 0.006	0.426–0.427 = 0.001	0.543–0.549 = 0.006	−0.459-(-0.464) = 0.005	–	0.360–0.343 = 0.017
WC	0.361–0.337 = 0.024	0.362–0.351 = 0.011	0.426–0.443 = 0.017	0.543–0.574 = 0.031	−0.459-(-0.499) = 0.040	–	0.360–0.317 = 0.043
WHR	0.361–0.319 = 0.042	0.362–0.357 = 0.005	0.426–0.428 = 0.002	0.543–0.542 = 0.001	−0.459-(-0.480) = 0.021	–	–
BMI	0.361–0.300 = 0.024	0.362–0.340 = 0.022	0.426–0.466 = 0.040	0.543–0.604 = 0.061	−0.459-(-0.534) = 0.075	–	0.360–0.330 = 0.030
FG score	–	0.362–0.355 = 0.007	0.426–0.464 = 0.038	0.543–0.580 = 0.037	−0.459-(-0.516) = 0.057	–	–
Testosterone	–	0.362–0.369 = 0.007	0.426–0.419 = 0.007	0.543–0.490 = 0.053	−0.459-(-0.419) = 0.040	–	0.360–0.410 = 0.050
LH	–	–	0.426–0.007	0.543–0.508 = 0.035	−0.459-(-0.435) = 0.024	–	0.360–0.397 = 0.037
LH/FSH	–	0.362–0.335 = 0.027	0.426–0.479 = 0.053	0.543–0.550 = 0.007	−0.459-(-0.482) = 0.023	–	0.360–0.474 = 0.114
Glucose fasting	0.361–0.408 = 0.047	0.362–0.571 = 0.209	–	–	–	–	0.360–0.463 = 0.103

(*continued on next page*)

Table 5 (*continued*)

Parameters	$\Delta_{CHO} = I_{Beta}.F_{Beta}$	$\Delta_{TG} = I_{Beta}.F_{Beta}$	$\Delta_{Insulin} = I_{Beta}.F_{Beta}$	$\Delta_{HOMA\text{-}IR} = I_{Beta}.F_{Beta}$	$\Delta_{QUICKI} = I_{Beta}.F_{Beta}$	$\Delta_{HCT} = I_{Beta}.F_{Beta}$	$\Delta_{MCV} = I_{Beta}.F_{Beta}$
Glucose 2-h	0.361–0.459 = 0.098	0.362–0.596 = 0.234	–	–	–	–	0.360–0.460 = 0.100
Cholesterol	–	0.362–0.450 = 0.088	–	–	–	–	0.360–0.457 = 0.097
Triglycerides	0.361–0.240 = 0.121	–	–	–	–	–	0.360–0.547 = 0.187
Insulin	0.361–0.466 = 0.105	0.362–0.476 = 0.114	–	–	–	0.284–0.377 = 0.093	0.360–0.407 = 0.047
HOMA-IR	0.361–0.501 = 0.140	0.362–0.621 = 0.295	–	–	–	0.284–0.379 = 0.095	0.360–0.483 = 0.123
QUICKI	0.361–0.543 = 0.182	0.362–0.586 = 0.224	–	–	–	0.284–0.411 = 0.127	0.360–0.473 = 0.113

Note: Δ = Change in beta value of BPA due to inclusion of potential confounders. I_{Beta} = Initial Beta value, F_{Beta} = beta value after inclusion of confounder.$p < 0.05$ is considered significant. FG = Ferriman Gallwey score; W/H = Waist-Hip ratio; BMI = Body Mass Index; LH = Luteinizing hormone; FSH = Follicle-stimulating hormone; LH/FSH = LF FSH ratio; SGOT = Serum glutamic oxaloacetic transaminase; AST = Aspartate aminotransferase; SGPT = Serum glutamic pyruvic transaminase; ALT = Alanine aminotransferase; HOMA-IR = Homeostasis Model Assesment Insulin Resistance Index; QUICKI = Quantitative Insulin Sensitivity Check Index; RBC = Red blood cells; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular Hemoglobin; MCHC = Mean corpuscular Hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cells; MPV = Mean platelet volume; BPA = Bisphenol A.

hyperaggregability of the platelets in women with PCOS might also be dependent on the hyper androgenic state. In our study, a significant increase in platelets was observed in PCOS women as compared to healthy controls and we conclude that increased platelet count might put the PCOS women at increased risk of clot forming tendency. In addition, Mean Corpuscular Hemoglobin Concentration (MCHC) was found to be significantly higher in PCOS women as compared to healthy controls. MCHC is defined as the average concentration of hemoglobin in a given volume of red cells. The other hematological parameters were found to be comparable between the two groups. Our results are similar to the observation documented by Aleem et al., who reported a significant increase in platelets and MCHC (Aleem et al., 2013). In contrast, kebapcilar et al. showed a significant increase in WBC and MPV levels in PCOS patients as compared to healthy controls (Kebapcilar et al., 2009). A study by Yilmaz et al., in their study reported a significant increase of RDW in PCOS women compared to control women (ĀzrMehmet et al., 2014).

The present study showed a significant increase of serum BPA levels in PCOS women as compared to control women. A significant positive association between BPA and testosterone levels but not with LH and FSH was seen in our study, a finding in agreement with previous studies which have shown a significant association between BPA and androgen levels (Kandaraki et al., 2011). The bidirectional interaction between BPA and androgens is supported by a number of studies. Specifically, it has been reported that BPA acts as a potent binder for sex hormone-binding globulin and when present in higher concentrations displaces androgens (Hanioka et al., 1998) and likely the free androgen index in circulation gets increased (Kandaraki et al., 2011). Further, BPA is reported to decrease the hepatic hydroxylase activity related to androgen resulting in reduced degradation of testosterone and thus there is an expected increase in hormone levels (Hanioka et al., 1998). In addition, the metabolism of BPA is known to be influenced by androgens. An increased androgen concentration down regulates the activity and transcripts of a liver enzyme known as uridine diphosphate-glucuronosyl transferase (UGT) which has a role in clearance of BPA from the circulation. As a result of this down regulation there is decreased detoxification and clearance of BPA creating a vicious cycle between BPA and androgens (Fernandez et al., 2010; Takeuchi et al., 2006). BPA has also been shown to stimulate hyperandrogenemia in the ovary and the possible mechanism appears to be the high mRNA expression of some vital enzymes involved in steroidogenic pathway, including steroidogenic acute regulatory protein, cholesterol side chain cleavage enzyme and 17-α hydroxylase (Zhou et al., 2008). Hence, it could be proposed that an elevated levels of BPA presented in PCOS women in our study along with their inherit enzymatic defect in ovarian androgen production as has been suggested elsewhere, leads to further increase in the overall androgen production.

Additionally, a significant positive association of elevated BPA levels with BMI, Waist circumference and waist-hip ratio was seen in PCOS women in our study and hence supports the role of BPA in increasing visceral adiposity. Our data is confirmatory of earlier literatures which have shown the association between BPA and BMI (Wang et al., 2012; Zhao et al., 2012). Further, data from various In Vivo/Vitro studies clearly showed the potential involvement of BPA for the non-genomic activation of adipogenic transcription factors in 3T3-L1 pre-adipocytes (Phrakonkham et al., 2008), up-regulation of adipogenic genes (Somm et al., 2009), lipid accumulation, increase in adipocyte differentiation (Wada et al., 2007; Masuno et al., 2005) and alteration of glucose homeostasis (Alonso-Magdalena et al., 2006) which ultimately results in hyperinsulinemia and T2DM. A study by Silvia et al., showed a significant correlation of BPA with markers of glucose homeostasis (fasting plasma glucose, insulin and HOMA-IR), Waist circumference and triglycerides (Silvia et al., 2015). Our data showed similar observation of significant positive association of BPA with fasting blood glucose, blood glucose-1 hour, blood glucose-2 hour, triglycerides cholesterol, fasting Insulin and HOMA-IR in PCOS women.

This association let us to speculate the potential involvement of this endocrine disrupting chemical in the metabolic aberration observed in PCOS women.

To our knowledge we are the first to investigate the association between BPA and hematological parameters in PCOS women. In our study, a significant positive association of BPA was found with the RBC indices (HCT and MCV) in PCOS women. MCV is defined as average size of the red blood cells and Hematocrit (HCT) is the ratio of the volume of red cells to the volume of whole blood. This linkage might be explained by insulin resistance which has an essential role in PCOS pathogenesis. The insulin receptor (INS-R) present during the entire period of development in human erythropoietic cells implies that insulin acts as a co-factor in erythropoiesis. The most widely accepted theory elucidating the role of insulin in erythropoiesis is that the tyrosine kinase activation in INS-R could be important for the growth-promoting action of insulin (Azziz, 2006). Also, IR could stimulate proliferation of erythroid progenitors thereby resulting in increased R.B.C. mass (Aleem et al., 2013). Elevated HCT could increase blood viscosity and peripheral resistance to blood flow, and further contribute to IR. Furthermore, the association of BPA and insulin resistance has been demonstrated by various studies like a single dose of BPA, in an adult mice, resulted in an immediate decrease in glycemia, an increase in insulin, and lowered metabolism, while a prolonged exposure increases beta-cell insulin concentrations, and results in chronic hyperinsulinemia and insulin resistance (Alonso-Magdalena et al., 2006; Batista et al., 2012; Ropero et al., 2008).

In conclusion, our study demonstrated that PCOS women have elevated BPA levels as compared to control women and are strongly related to the testosterone levels and the metabolic aberrations associated with the syndrome. To the best of our knowledge we are the first to describe the positive association of BPA with the erythrocyte parameters (HCT and MCV) along with the positive association with the fasting blood glucose levels, blood glucose 2 h, total cholesterol, triglycerides, fasting insulin and HOMA-IR and negative association with QUICKI. These findings suggest the potential role of this abundant chemical compound in the pathogenesis of PCOS and further studies are needed on a large sample size to clarify the possible clinical implications of these findings.

Author contributions

I.A.K, F.R and A.M participated in study design. I.A.K acquired data and wrote manuscript which was revised by F.R. I.A.K, F.R ; M.M. analysed the data and I.A.K, F.R drafted the result. S.M.R as gynecologist provided cases for the study. Q. F, H.J and S.M helped in data collection. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

The authors would like to thank the Department of Biochemistry and Clinical Biochemistry for providing facilities and funds to carry out this research work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.obmed.2019.100090.

References

Aleem, J., Showkat, H.I., Ganai, A., Atta, A., Duhath, Jan I., Showkat, H.I., 2013. Haematological related perturbations in polycystic ovarian syndrome: a tertiary centre experience. Glob. Res. Anal. 2, 2277–8160.

Alonso-Magdalena, P., Morimoto, S., Ripoll, C., Fuentes, E., Nadal, A., 2006. The estrogenic effect of bisphenol A disrupts pancreatic Ĩ²-cell function in vivo and induces insulin resistance. Environ. Health Perspect. 114, 106–112.

Ãzr, Yilmaz, Mehmet, C., Kelekci, S., Temur, M., 2014. Association between red blood cell distribution width and polycystic ovary syndrome. Endocr. Res. 40, 181–187.

Azziz, R., 2006. Diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. J. Clin. Endocrinol. Metab. 91, 781–785.

Batista, T.M., Alonso-Magdalena, P., Vieira, E., Amaral, M.E.C., Cederroth, C.R., Nef, S., et al., 2012. Short-term treatment with bisphenol-A leads to metabolic abnormalities in adult male mice. PLoS One 7, e33814.

Brieno-Enriquez, M.A., Robles, P., Camats-Tarruella, N., Garcia-Cruz, R., Roig, I., Cabero, L., 2011. Human meiotic progression and recombination are affected by Bisphenol A exposure during in vitro human oocyte development. Hum. Reprod. 26, 2807–2818.

Cabaton, N.J., Wadia, P.R., Rubin, B.S., Zalko, D., Schaeberle, C.M., Askenase, M.H., 2011. Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environ. Health Perspect. 119, 547–552.

Caserta, D., Bordi, G., Ciardo, F., Marci, R., La Rocca, C., Tait, S., et al., 2013. The influence of endocrine disruptors in a selected population of infertile women. Gynecol. Endocrinol. 29, 444–447.

Celik, C., Abali, R., Bastu, E., Tasdemir, N., Tasdemir, U.G., Gul, A., 2013. Assessment of impaired glucose tolerance prevalence with hemoglobin A1c and oral glucose tolerance test in 252 Turkish women with polycystic ovary syndrome: a prospective, controlled study. Hum. Reprod. 28, 1062–1068.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. 30, 293–342.

Dogan, B.A., Arduc, A., Tuna, M.M., KarakÄ ± lÄ ± c, E., Dagdelen, I., Tutuncu, Y., 2014. Association of mean platelet volume with androgens and insulin resistance in non-obese patients with polycystic ovary syndrome. Int. J. Endocrinol. Metab. 12, e18642.

Ehrlich, S., Williams, P.L., Missmer, S.A., Flaws, J.A., Berry, K.F., Calafat, A.M., et al., 2012. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. Environ. Health Perspect. 120, 978–983.

Fauser, B., Tarlatzis, B.C., Rebar, R.W., Legro, R.S., Balen, A.H., Lobo, R., et al., 2012. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum. Reprod. 27, 14–24.

Fernandez, M., Bianchi, M., Lux-Lantos, V., Libertun, C., 2009. Neonatal exposure to bisphenol a alters reproductive parameters and gonadotropin releasing hormone signaling in female rats. Environ. Health Perspect. 117, 757–762.

Fernandez, M., Bourguignon, N., Lux-Lantos, V., Libertun, C., 2010. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environ. Health Perspect. 118, 1217–1222.

Futterweit, W., 1999. Polycystic ovary syndrome: clinical perspectives and management. Obstet. Gynecol. Surv. 54, 403–413.

Ganie, M.A., Dhingra, A., Nisar, S., Sreenivas, V., Shah, Z.A., Rashid, A., 2016. Oral glucose tolerance test significantly impacts the prevalence of abnormal glucose tolerance among Indian women with polycystic ovary syndrome: lessons from a large database of two tertiary care centers on the Indian subcontinent. Fertil. Steril. 105, 194–201.

Hanioka, N., Jinno, H., Nishimura, T., Ando, M., 1998. Suppression of male-specific cytochrome P450 isoforms by bisphenol A in rat liver. Arch. Toxicol. 72, 387–394.

Harrison, C.N., 2005. Platelets and thrombosis in myeloproliferative diseases. Hematol. Am. Soc. Hematol. Educ. Prog. 409–415.

Jeelani, H., Ganie, A.G., Amin, Shajrul, Kawa, I.A., Fatima, Q., Drabu, Z., Rashid, F., 2017. Polycystic ovary syndrome (PCOS) and related diseases. Pmj 2, 23–29.

Kandaraki, E., Chatzigeorgiou, A., Livadas, S., Palioura, E., Economou, F., Koutsilieris, M., et al., 2011. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. J. Clin. Endocrinol. Metab. 96, E480–E484.

Kebapcilar, L., Taner, C.E., Kebapcilar, A.G., Sari, I., 2009. High mean platelet volume, low-grade systemic coagulation and fibrinolytic activation are associated with androgen and insulin levels in polycystic ovary syndrome. Arch. Gynecol. Obstet. 280, 187–193.

Kelly, C.J.G., Speirs, A., Gould, G.W., Petrie, J.R., Lyall, H., Connell, J.M.C., 2002. Altered vascular function in young women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 87, 742–746.

Lath, R., Shendye, R., Jibhkate, A., 2015. Insulin resistance and lipid profile in polycystic ovary syndrome. Asian J. Biomed. Pharm. Sci. 5, 30–35.

Lo, J.C., Feigenbaum, S.L., Yang, J., Pressman, A.R., Selby, J.V., Go, A.S., 2006. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 91, 1357–1363 2006.

March, W.A., Moore, V.M., Willson, K.J., Phillips, D.I.W., Norman, R.J., Davies, M.J., 2010. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum. Reprod. 25, 544–551.

Markey, C.M., Coombs, M.A., Sonnenschein, C., Soto, A.M., 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol. Dev. 5, 67–75.

Masuno, H., Iwanami, J., Kidani, T., Sakayama, K., Honda, K., 2005. Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. Toxicol. Sci. 84, 319–327.

Newbold, R.R., Jefferson, W.N., Padilla-Banks, E., 2007. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod. Toxicol. 24, 253–258.

Newbold, R.R., Jefferson, W.N., Padilla-Banks, E., 2009. Prenatal exposure to bisphenol a

at environmentally relevant doses adversely affects the murine female reproductive tract later in life. Environ. Health Perspect. 117, 879–885.
Papalou, O., Livadas, S., Karachalios, A., Tolia, N., Kokkoris, P., Tripolitakis, K., 2015. White blood cells levels and PCOS: direct and indirect relationship with obesity and insulin resistance, but not with hyperandogenemia. Hormones 14, 91–99.
Phrakonkham, P., Viengchareun, S., Belloir, C., Lombes, M., Artur, Y., Canivenc-Lavier, M.C., 2008. Dietary xenoestrogens differentially impair 3T3-L1 preadipocyte differentiation and persistently affect leptin synthesis. J. Steroid Biochem. Mol. Biol. 110, 95–103.
Pilo, R., Aharony, D., Raz, A., 1981. Testosterone potentiation of ionophore and ADP induced platelet aggregation: relationship to arachidonic acid metabolism. Thromb. Haemostasis 46, 538–542.
Ropero, A.B., Alonso-Magdalena, P., Garcia-Garcia, E., Ripoll, C., Fuentes, E., Nadal, A., 2008. Bisphenol-A disruption of the endocrine pancreas and blood glucose homeostasis. Int. J. Androl. 31, 194–200.
Ross, R., 1999. Atherosclerosis–an inflammatory disease. N. Engl. J. Med. 340, 115–126.
Rubin, B.S., Murray, M.K., Damassa, D.A., King, J.C., Soto, A.M., 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ. Health Perspect. 109, 675–680.
Silvia, S., Giovanni, T., Vittorio, D., Federica, P., Serena, C., Antonietta, L., et al., 2015. Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: a cross-sectional study on adult male population. J. Transl. Med. 13, 169.
Somm, E., Schwitzgebel, VrM., Toulotte, A., Cederroth, C.R., Combescure, C., Nef, S., et al., 2009. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ. Health Perspect. 117, 1549–1555.
Souter, I., Smith, K.W., Dimitriadis, I., Ehrlich, S., Williams, P.L., Calafat, A.M., et al., 2013. The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. Reprod. Toxicol. 42, 224–231.
Takeuchi, T., Tsutsumi, O., Ikezuki, Y., Kamei, Y., Osuga, Y., Fujiwara, T., et al., 2006. Elevated serum bisphenol A levels under hyperandrogenic conditions may be caused by decreased UDP-glucuronosyltransferase activity. Endocr. J. 53, 485–491.
Tsilchorozidou, T., Overton, C., Conway, G.S., 2004. The pathophysiology of polycystic ovary syndrome. Clin. Endocrinol. 60, 1–17.
Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–1070.
Vrbikova, J., Cifkova, R., Jirkovska, A., Lanska, V., Platilova, H., Zamrazil, V., et al., 2003. Cardiovascular risk factors in young Czech females with polycystic ovary syndrome. Hum. Reprod. 18, 980–984.
Wada, K., Sakamoto, H., Nishikawa, K., Sakuma, S., Nakajima, A., Fujimoto, Y., et al., 2007. Life style-related diseases of the digestive system: endocrine disruptors stimulate lipid accumulation in target cells related to metabolic syndrome. J. Pharmacol. Sci. 105, 133–137.
Wang, T., Li, M., Chen, B., Xu, M., Xu, Y., Huang, Y., et al., 2012. Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. J. Clin. Endocrinol. Metab. 97, E223–E227.
Wild, R.A., Painter, P.C., Coulson, P.B., Carruth, K.B., Ranney, G.B., 1985. Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 61, 946–951.
Yildiz, B.O., Haznedaroglu, I.C., Kirazli, S., Bayraktar, M., 2002. Global fibrinolytic capacity is decreased in polycystic ovary syndrome, suggesting a prothrombotic state. J. Clin. Endocrinol. Metab. 87, 3871–3875.
Zargar, A.H., Wani, A.I., Masoodi, S.R., Laway, B.A., Salahuddin, M., 1997. Epidemiologic and etiologic aspects of primary infertility in the Kashmir region of India. Fertil. Steril. 68, 637–643.
Zargar, A.H., Wani, A.I., Masoodi, S.R., Laway, B.A., Bashir, M.I., Salahuddin, M., 2002. Epidemiologic and etiologic aspects of hirsutism in Kashmiri women in the Indian subcontinent. Fertil. Steril. 77, 674–678.
Zhao, Hy, Bi, Yf, Ma, Ly, Zhao, L., Wang, Tg., Zhang, Lz, et al., 2012. The effects of bisphenol A (BPA) exposure on fat mass and serum leptin concentrations have no impact on bone mineral densities in non-obese premenopausal women. Clin. Biochem. 45, 1602–1606.
Zhou, W., Liu, J., Liao, L., Han, S., Liu, J., 2008. Effect of Bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol. Cell. Endocrinol. 283, 12–18.