



Bisphenol-A Levels in Obese and Lean Polycystic Ovarian Syndrome (PCOS) Patients

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Introduction

Endocrine disrupting chemicals and human health

Endocrine disrupting chemicals, are known as “endocrine disruptors” EDs, are defined by the Environmental Protection Agency as “exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action or elimination of natural blood borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental processes”^[1,2].

The concern with human exposure to EDs derives from:

- i. Evidence regarding molecular mechanisms correlated to effects at very low doses;
- ii. In vitro effects in animal models resultant from doses within the range of human exposure; and
- iii. Extensive and prevalent human exposure to EDs at concentrations that have been

documented to endorse adverse effects in animals

The assessment of potential risks to human health resultant of exposure to EDs and mixtures is a key and foremost important subject for consumer safety^[2].

Considering human exposure patterns through oral intake, EDCs can be separated in four major categories:

- EDs with bioaccumulation ability (e.g., polychlorinated biphenyls (PCBs), poly-brominated flame retardants, per fluorinated chemicals);
- Compounds utilized in food production (e.g., pesticides);
- Chemicals present in food due to contact materials, processing aids, etc. (e.g., BPA); and
- Endocrine active substances naturally present in food (e.g., genistein).

Bisphenol A is widely used in the manufacture of resins such as polycarbonate plastic products and

epoxy resins. The most common contaminators are BPA, polychlorinated biphenyls, phthalates and dioxins^[3].

The immunological activities of BPA may be mediated through estrogen receptor signalling, aryl hydrocarbon receptor, and the peroxisome proliferator-activated receptor family of nuclear receptors^[4].

PCOS

Few studies have been undertaken to assess the possible effects of BPA on the reproductive hormone balance in animals or humans with often contradictory results^{[5], [6]}.

PCOS Indian Data

Based on community-based study that was undertaken in Mumbai, India, Polycystic ovarian syndrome is one of the most common reproductive endocrinological disorders with a broad spectrum of clinical manifestations affecting about 6-8% of women of reproductive years^[7]. Community based studies using Rotterdam criteria among reproductive age group women have demonstrated varied prevalence figures in few Asian countries ranging from 2% to 7.5% in China to 6.3% in Srilanka^{[8], [9], [10]}.

Most of the young population do not visit health facilities until they have late sequel of the problem. Most prevalence studies in India are in hospital set ups and and only a few studies among adolescents in schools are there which, report prevalence of PCOS as 9.13% to 36%^{[11], [12]}.

It is appropriately pointed by Gainie and Kalra^[13] that the health budget of India is unlikely to meet the costs posed to tackling the associated multiple consequences of PCOS.

Aims and Objectives

To evaluate BPA an endocrine disruptor in population of

- Lean, obese PCOS patients
- In comparison to age and BMI matched normals (control).

Materials and Methods

Source of data: The data were collected from the patients of Ramaiah Medical College Hospitals, Bengaluru.

Study period: The study includes PCOS patients, presenting Endocrinology OPD at MS Ramaiah Hospital from March 2016- December 2017

Study design: Cross sectional study

Procedure: The diagnosis of PCOS was based on Rotterdam criteria. Blood samples were collected from all the subjects in empty stomach after overnight fast and were analysed for serum Bisphenol A, fasting blood sugar, fasting Insulin, serum testosterone. Bisphenol –A was processed with ELISA kit (Sincere Biotech), fasting blood glucose levels, insulin, testosterone were processed with ELISA kit (DRG international).

Inclusion criteria

- PCOS patients age in between 18 to 40 years as per Rotterdam criteria.

Exclusion criteria

- Age more than 40 years,
- known cardiovascular disease,
- neoplasms,
- current smoking,
- diabetes mellitus,
- renal impairment (serum creatinine more than 1.3 mg/dl),
- hypertension,
- Oral contraceptives or other drugs involved in carbohydrate metabolism like steroids, (if administered, were discontinued for at least 3 months before the study),
- Cushings syndrome,
- Congenital adrenal hyperplasia,
- Other causes of hyperandrogenism.

Results

All PCOS patients will be divided in to 3 groups- viz; lean PCOS, Obese PCOS and controls as normal healthy subjects

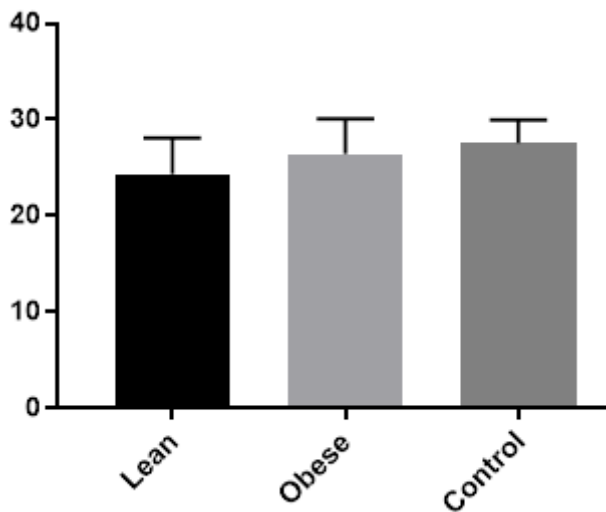
There were 32 patients in each of the groups.

The parameters identified were segregated into,

1. Demographics
 - a. Age
 - b. BMI
2. Evaluation parameters for PCOS
 - a. FSH (miu/ml)
 - b. LH (miu/ml)
 - c. Fasting Blood Sugar (mg/dl)
 - d. Insulin (miu/ml)
 - e. HOMA IR
 - f. Testosterone (ng/ml)
 - g. Bisphenol (ng/ml)

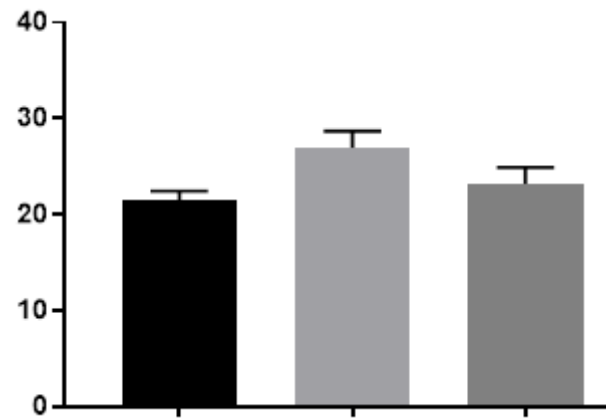
1. a. The mean age in lean, obese and control group (Figure 3)

The mean age in lean, obese and control group was 24 years (min 18, max 32, SD 3.7, SEM ± 0.66, 95% CI 23-26), 26 years (min 20, max 34, SD 3.7, SEM ± 0.65, 95% CI 25-28) and 28 years (min 23, max 32, SD 2.4, SEM ± 0.43, 95% CI 27-28) respectively.



1. b. The mean BMI in lean, obese and control group (Figure4)

The mean BMI in lean, obese and control group was 21 kg/m² (min 20, max 23, SD 1, SEM ± 0.18, 95% CI 21-22), 27 kg/m² (min 25, max 32, SD 1.7, SEM ± 0.31, 95% CI 26-28) and 23 kg/m² (min 20, max 27, SD 1.7, SEM ± 0.31, 95% CI 23-24) respectively.



2. a. The FSH and LH levels in Lean, Obese and the control group were compared.

The minimum levels of FSH (miu/ml) in lean, obese and control group was 2.5, 2.1 and 1.6 respectively.

The maximum levels of FSH (miu/ml) in lean, obese and control group was 5.5, 7.3 and 7.8 respectively.

The mean levels of FSH (miu/ml) in lean, obese and control group was 4.1 (miu/ml) (SD 0.85, SEM ± 0.15, 95% CI 3.7-4.4), 4.2 (SD 1.1, SEM ± 0.19, 95% CI 3.8-4.6) and 3.3 (SD 1.4, SEM ± 0.24, 95% CI 2.8-3.8) respectively.

The minimum levels of LH (miu/ml) in lean, obese and control group was 11, 9.5 and 2 respectively.

The maximum levels of LH (miu/ml) in lean, obese and control group was 20, 20 and 6.2 respectively.

The mean levels of LH (miu/ml) in lean, obese and control group was 16 (SD 2.6, SEM ± 0.46, 95% CI 15-17), 12 (SD 2.6, SEM ± 0.46, 95% CI 11-13) and 3.8 (SD 1, SEM ± 0.18, 95% CI 3.4-4.2) respectively.

The **Table 3** depicts the ratios of LH:FSH in lean, obese and control group respectively

Mean Values	Lean	Obese	Control
LH	16	12	3.8
FSH	4.1	4.2	3.3
Ratios	3.9	2.85	1.15

The LH: FSH ratio were high in PCOS (both lean and obese), compared to controls.

2 b. The Fasting Blood Sugar (FBS) (mg/dl) in Lean, Obese and the control group were compared. (Figure 5)

The minimum levels of FBS (mg/dl) in lean, obese and control group was 78 in each group respectively.

The maximum levels of FBS (mg/dl) in lean, obese and control group was 83, 86 and 83 respectively.

The mean levels of FBS (mg/dl) in lean, obese and control group was 83 (SD 3.1, SEM \pm 0.55, 95% CI 82-84), 86 (SD 7.1, SEM \pm 1.3, 95% CI 84-89) and 83 (SD 2.9, SEM \pm 0.52, 95% CI 82-84) respectively.

The differences were statistically significant ($p = 0.0047$)

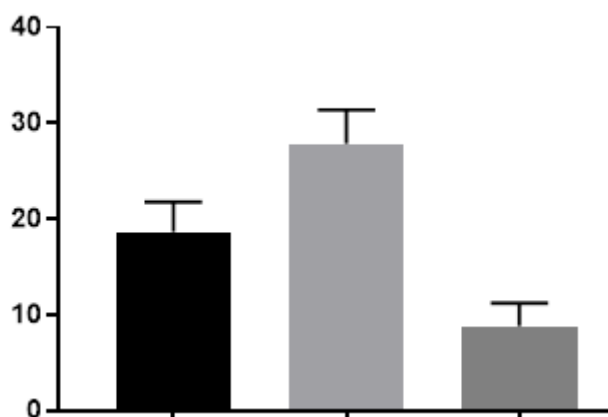
2 c. The Insulin (miu/ml)in Lean, Obese and the control group were compared. (Figure 6)

The minimum levels of Insulin (miu/ml) in lean, obese and control group was 16, 20 and 5.6 respectively.

The maximum levels of Insulin (miu/ml) in lean, obese and control group was 29, 32 and 13 respectively.

The mean levels of Insulin (miu/ml) in lean, obese and control group was 19 (SD 3.1, SEM \pm 0.55, 95% CI 18-20), 28 (SD 3.5, SEM \pm 0.63, 95% CI 27-29) and 8.8 (SD 2.4, SEM \pm 0.43, 95% CI 7.9-9.7) respectively.

The differences were statistically significant ($p < 0.0001$)



2 d. The HOMA- IRin Lean, Obese and the control group were compared. (Figure 7)

The minimum levels of HOMA -IR in lean, obese and control group was 3, 4 and 1.2 respectively.

The maximum levels of HOMA-IR in lean, obese and control group was 6.5, 8.4 and 2.7 respectively.

The mean levels of HOMA-IR in lean, obese and control group was 3.8 (SD 0.73, SEM \pm 0.13, 95% CI 3.6-4.1), 6 (SD 0.99, SEM \pm 0.18, 95% CI 5.6-6.3) and 1.8 (SD 0.53, SEM \pm 0.093, 95% CI 1.6-2) respectively.

The differences were statistically significant ($p < 0.0001$)

2 e. The Testosterone (ng/ml)in Lean, Obese and the control group were compared. (Figure 8)

The minimum levels of testosterone (ng/ml) in lean, obese and control group was 0.24, 0.29 and 0.2 respectively.

The maximum levels of testosterone (ng/ml) in lean, obese and control group was 0.62, 0.69 and 0.44 respectively.

The mean levels of Testosterone (ng/ml) in lean, obese and control group was 0.45 (SD 0.1, SEM \pm 0.018, 95% CI 0.41-0.48), 0.47 (SD 0.14, SEM \pm 0.025, 95% CI 0.42-0.52) and 0.29 (SD 0.057, SEM \pm 0.01, 95% CI 0.27-0.31) respectively.

The differences were statistically significant ($p < 0.0001$)

2 f. The Bisphenol (ng/ml) in Lean, Obese and the control group were compared. (Figure 9,10)

The minimum levels of Bisphenol (ng/ml)in lean, obese and control group was 0.47, 0.48 and 0.33 respectively.

The maximum levels of HOMA-IR in lean, obese and control group was 1.7, 1.6 and 1 respectively.

The mean levels of Bisphenol (ng/ml)in lean, obese and control group was 0.96 (SD 0.4, SEM \pm 0.07, 95% CI 0.82-1.1), 0.99 (SD 0.43, SEM \pm 0.076, 95% CI 0.84-1.1) and 0.69 (SD 0.22, SEM \pm 0.039, 95% CI 0.61-0.77) respectively.

The differences were statistically significant ($p = 0.0015$)

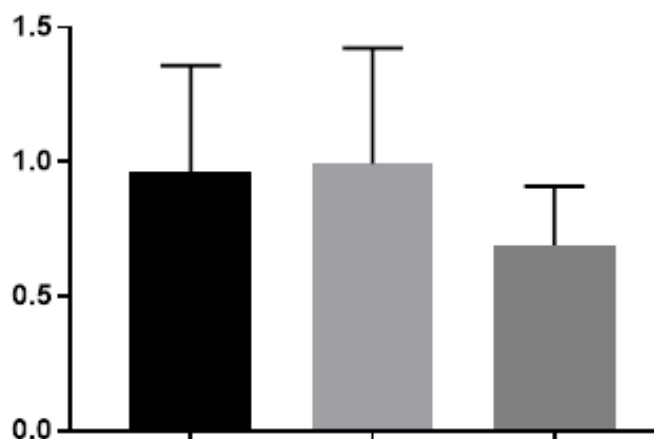


Table 4 Baseline characteristics and results in the Lean, Obese and Control

Mean ± SD	Lean	Obese	Control
Age (year)	24 ± 3.7	26 ± 3.7	28 ± 2.4
BMI (kg/m ²)	21 ± 1	27 ± 1.7	23 ± 1.7
FSH (miu/ml)	4.1 ± 0.85	4.2 ± 1.1	3.3 ± 1.4
LH (miu/ml)	16 ± 2.6	12 ± 2.6	3.8 ± 1
LH:FSH Ratios	3.9	2.85	1.15
FBS (mg/dl)	83 ± 3.1	86 ± 7.1	83 ± 2.9
Insulin (miu/ml)	19 ± 3.1	28 ± 3.5	8.8 ± 2.4
HOMA-IR	3.8 ± 0.73	6 ± 0.99	1.8 ± 0.53
Testosterone (ng/ml)	0.45 ± 0.1	0.47 ± 0.14	0.29 ± 0.057
Bisphenol (ng/ml)	0.96 ± 0.4	0.99 ± 0.43	0.69 ± 0.22
17-Hydroxyprogesterone (17 OHP) (ng/ml)	1.1 ± 0.39	1.2 ± 0.39	0.63 ± 0.22

Discussion

Our study was a cross sectional study of 64 PCOS and 32 normal women, age and BMI matched, in a university hospital setting. Anthropometric, hormonal, metabolic parameters and BPA blood levels were determined.

This data show that mean age is comparable between all three groups.

The data demonstrate that serum BPA levels are higher in women with PCOS in comparison to their normal ovulating non hyperandrogenemic peers.

This is independent of the degree of obesity. Though, the pathogenesis of the syndrome has not yet been fully demystified, the role of environmental factors is certainly being implicated in PCOS development^[14,15].

BPA is a potent SHBG ligand and, accordingly, therefore, in increased concentrations, displaces

androgens from SHBG binding sites and leads to increased circulating free androgen concentrations^{[16],[17]}.

It has been well documented that BPA significantly inhibited the activity of two different testosterone hydroxylases (2-and 6-hydroxylase), leading to decreased testosterone catabolism and indirectly to increased testosterone concentrations^[18].

The mechanisms appear to be linked with increased mRNA expression of key enzymes involved in steroid production pathway, including 17 α hydroxylase, cholesterol side chain cleavage enzyme, and steroidogenic acute regulatory protein^[19].

Our study showed elevated testosterone levels in both obese and lean PCOS groups compared to control group, which is in line with above discussed previous studies^[20].

Interestingly, PCOS ovarian hyperandrogenism is partly attributed to activation of this steroidogenic pathway^[21]

The connection of androgens with BPA is further supported by the positive correlation of BPA with androgens, a finding in agreement with previous reports in a small number of anovulatory women^[22]

It has been documented that the increased frequency of hirsutism in obese compared with lean women with PCOS is associated with increased bio-availability of androgens to peripheral tissues and enhanced activity of 5 alpha-reductase in obese subjects^[23].

The LH: FSH ratios were quite striking in our work with lean (3.9) and obese (2.85) groups reporting higher values compared to the control group (1.15) similar to earlier published data^[24].

Obese PCOS women had significantly higher levels of plasma insulin, and lower insulin sensitivity as compared to lean PCOS patients. However, lean PCOS women were more hyperinsulinemic and insulin resistant than women in control group. Obesity is the important factor determinating the insulin sensitivity, hyperinsulinemia and hyperandrogenemia

This hypothesis is further corroborated by recent animal data showing that environmentally relevant doses of BPA have been linked to disturbance of pancreatic physiology and glucose metabolism, thus enhancing the risk for the development of insulin resistance in intact animals^{[25],[26]}. The impact of BPA in insulin action and PCOS needs to be further studied.

Data on neonatal exposure to BPA and the subsequent development of PCOS in animals are suggestive of a possible interaction of with the hormonal and metabolic abnormalities observed in women with PCOS^{[27],[28]}.

The current study is not designed to detect causality in the pathophysiological mechanism linking BPA with PCOS.

Conclusion

In conclusion, our study demonstrates that in women with PCOS, BPA levels are higher in the lean and the obese as compared to the controls with the differences being statistically significant. BPA may have a potential role in PCOS pathophysiology. Mechanistic insights could be further explored for a precise understanding of the underlying association between the BPA and PCOS, through multi-centric trials and retrospective collaborative evaluation. The role of environmental factors and epigenetics is further needed to be corroborated.

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