ORIGINAL ARTICLE

Bisphenol A Effect on T regulatory and T helper 17 Related Cytokines in Female Mice

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ABSTRACT

Background: *In recent years there has been increasing concern about the potential health effects of exposure to Bisphenol A, especially in pregnant women and their offspring. The potential health effects of BPA exposure have been studied extensively in animal models. There is evidence to suggest that exposure to this compound can have a range of adverse health effects, including reproductive and developmental malformations, metabolic disorders, and immune dysfunction.* ***Objectives:*** *To determine the effect of Bisphenol A consumption on regulatory T cells and Th17 cell-associated cytokines in female mice, as well as whether Bisphenol A exposure could cause abortion in pregnant mice.* ***Methodology*****:** *The study included 60 female mice divided into four groups; each group contained 15 female mice. The first group is the control, non-pregnant female mice, which take water containing 1% ethanol. The second group, female mice who arenŕt pregnant, which given 5 mg/ml/kg of a BPA solution. The third group is a control group of pregnant female mice who take water containing 1% ethanol. The fourth group, pregnant female mice, were given 5 mg/ml/kg of BPA solution. At the end of the trial, blood samples were taken and serum concentrations of Il-10, TGF-beta, and IL-17 were measured using ELISA kits.* ***Results:*** *In both pregnant and non-pregnant female mice, the experimental group exposed to BPA had considerably lower levels of TGF-beta and -IL-10 than the control group. Simultaneously, the experimental group had significantly higher levels of IL-17 than the control group. There was also a substantial change in TGF-beta, IL-10, and IL-17 levels between non-pregnant and pregnant mice.* ***Conclusion:*** *BPA exposure during pregnancy can result in aberrant T reg. and Th 17 cell-related cytokines and abortion in female mice.*

INTRODUCTION

Bisphenol A is an endocrine-disrupting compound produced in large quantities to make polycarbonate plastic food and beverage containers. Researches have revealed that exposure to BPA can cause neurodevelopmental defects, metabolic problems, and reproductive damage[1]. BPA has been connected to autoimmune diseases, with animal studies revealing unbalanced helper T cells and moreover an elevated risk of allergies and asthma, and to impact immune responses via receptors, epigenetic changes, cell signalling pathways, and the gut microbiota[2].

BPA's primary impact on human health is through receptor-mediated mechanisms, specifically acting as ligands for estrogen, androgen, and thyroid hormone nuclear receptors[3]**.** The same for humans and in other primates, BPA metabolized within the liver by sulfation or glucoronidation to be easily eliminated in the urine [4]. After entering the human body, BPA could be detected in various biological samples, including blood, urine, saliva, hair, etc[5]. T cells are types of lymphocytes that develop in the thymus then undergo clonal proliferation and cytokine release. Th1, Th2, T-reg, and Th17 cells are subsets of T cells, but they are not the only ones [6].

Tregs are specialised T cells that express the transcription factor FoxP3, and they are important for moderating the immune responses [7]. Treg cells regulate the immune response by recognizing autoantigens from wounded tissues and modulating the immune response through cytokines, suppressing autoimmunity, and limiting local effector cell functions by preventing T-reg cell activation[8]. TGF-β is essential for the generation, function, and survival of adaptive CD4+ and CD8+ Treg subsets, regulating FoxP3 expression and maintaining Treg homeostasis, dependin on IL-2 .[9,10]

Th17 cells, a subtype of CD4+T lymphocytes, produce IL-17, attracting neutrophils and macrophages to diseased tissues, promoting abscesses and antimicrobial peptide expression, contributing to autoimmunity and host defense [11].

TCR stimulation, TGF-beta, and IL-6 are crucial for naive T cell antagonism towards TH17 lineage, with TGF-beta inducing anti- inflammatory Treg and TGF-β influencing anti-inflammatory Treg or pro-inflammatory TH17 cells [11]. Bettelli et al. established a reciprocal

relationship between the upgrading routes for TH17 effector cells and Treg cells, showing that the equilibrium between TH17 and Treg cell generation depends on cytokine-induced interactions between these transcription factors [12].

During pregnancy, semen influences the maternal immune response, creating a fetus-friendly environment for a healthy gestation. This dampens the mother's inflammatory response, allowing the fetus to grow. The feto-maternal interface plays an important role in maternal tolerance and defense, with Treg cells determining pregnancy fate [6,13].

Our results indicated that small or high dosages of BPA exposure could induce imbalance of Treg/Th17, which was related with down- regulation in TGF-β and IL-10 and up-regulation in IL-17, respectively. Our findings indicate that BPA exposure within the first trimester of pregnancy cause abortion to female mice.

METHODOLOGY

This study used sexually mature sixty albino female mice (250- 300gm) purchased from the animal house at Assiut University's Medical College. Before utilize, mice were examined and acclimated to the laboratory environment for two weeks. The temperature and relative moisture content were kept at 23±2°C and 40±5%, respectively, with a 12 hr: 12 hr light:dark photoperiod. They were kept in stainless steel cages with a standard diet and water. Animal care was in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and local Laboratory Animal Care standards. Sixty female mice were separated into 4 groups (n=15 mice/ group) first group is control female mice who aren't pregnant which received only 1% ethanol , second group is female mice who aren't pregnant were received 5mg/ml/kg bisphenol A (SigmaAldrich Chemical Co., St. Louis, MO, USA) in daily drinking water as daily dose ,third group is control pregnant female mice which received 1% ethanol only and fourth group is pregnant female mice were received 5 mg/ml/kg bisphenol A (Sigma- Aldrich Chemical Co., St. Louis, MO, USA) in daily drinking water as daily dose. Water consumption was recorded daily based on bottle volume reduction, and the body's mass was recorded weekly.

Sample collection

Following an overnight fast, female mice were anesthetized using light ether, blood samples were collected from the abdominal aorta, serum separated, and stored at -40°C.

Serological assays

The concentrations of serum IL-10, TGF-β and IL-17 were determined with ELISA kits according to the manufacturer's protocol (Shanghai Sunred Biological Technology Co., Ltd). The absorbance was read at 450 nm using a spectrophotometer. The average concentrations of serum IL-10, IL-17 were expressed as pg/mL and TGF-β was expressed as ng/ml.

Detection of IL-17 by ELISA

A double antibody sandwich enzyme linked immunosorbent assay (ELISA) was utilized to measure Interleukin 17 (IL-17) level in tested sample according to manufacturer′s protocol (Shanghai Sunred company, China, catalogue No. 201-12-0143) [14].

Detection of IL-10 by ELISA:

A double antibody sandwich enzyme linked immunosorbent assay (ELISA) was used to measure Interleukin 10 (IL-10) level in tested sample according to manufacturer′s protocol (Shanghai Sunred company, China, catalogue No.201-12-0103) [14].

Detection of TGF-β by ELISA

A double antibody sandwich enzyme linked immunosorbent assay (ELISA) was used to measure Transforming Growth Factor β (TGF-β) level in tested sample according to manufacturer′s protocol (Shanghai Sunred company, China, catalogue No. 201-12-5480) [14].

Statistical analysis

The study used Excel for data entry and analysis, IBM SPSS for statistical analysis, and multivariate regression to identify predictors of serum TGF-β, IL-10, and IL-17 levels, with a significance level of 95% and p-value ≤ 0.05.

REAULTS

1. Effect of BPA on pregnant mice:

Serum level of TGF-β and IL-10 was lower among the experimental group treated with BPA compared to the control group and that was statistically significant (3.97±1.06 versus 9.39±1.52, p-value<0.001) and (124.1±14.30 versus 233.58±26.17, p-value <0.001) respectively Whereas, it was higher in IL-17 and it was discovered to be (75.32±7.52 versus 39.49 ± 3.18, p-value <0.001) as demonstrated in table (1).

Table 1: Effect of BPA on serum levels of TGF-β, IL-10 and IL-17 of pregnant mice:

Parameter	Experimental group (Mean ± SD)	Control group (Mean ± SD)	p-value
TGF-β (ng /ml)	3.97 ± 1.06	9.39 ± 1.52	< 0.001
IL-10 (Pg /ml)	124.1 ± 14.30	233.58 ± 26.17	< 0.001
IL-17 (Pg /ml)	75.32 ± 7.52	39.49 ± 3.18	< 0.001

Mann-whitney U test was used. *p-value was significant if ≤ 0.05.

2. Effect of BPA on outcome of pregnancy in pregnant mice

The pregnancy of mice in the experimental group treated with BPA ended by abortion in 10 mice (66.67%) and stillbirth in 5 mice (33.3%), while the pregnancy of all mice in the control group ended by normal delivery of live births as demonstrated in table (2).

Table 2: Effect of BPA on outcome of pregnancy in pregnant mice

Outcome of pregnancy	Experimental group N=15	Control group N=15
Live birth	0 (0%)	15 (100%)
Abortion	10 (66.67%)	0 (0%)
Stillbirth	5 (33.3%)	0 (0%)

3. Comparison of mean level of serum TGF-β, IL-10 and IL-17 according to outcome of pregnancy

Table (3) showed that mean serum level of TGF-β± SD was 9.39±1.52 among mice whose pregnancy was terminated by live birth (control group), 3.4±0.81 among mice whose pregnancy was terminated by abortion (experimental group) and 5.12 ± 0.18 among mice whose pregnancy was terminated by stillbirth.

Mean serum level of IL-10 ± SD was 233.58 ± 26.17 among mice whose pregnancy was terminated by live birth (control group), 116.77±11.38 among mice whose pregnancy was terminated by abortion (experimental group) and 138.76 ±4.66 among mice whose pregnancy was terminated by stillbirth.

Mean serum level of IL-17±SD was 39.45±3.18 among mice whose pregnancy was terminated by live birth (control group), 80.03±2.67 among mice whose pregnancy was terminated by abortion (experimental group) and 65.9±3.95 among mice whose pregnancy was terminated by stillbirth.

Table 3: Comparison of mean level of serum TGF-β, IL-10 and IL-17 according to outcome of pregnancy

	Live birth [a] (Mean ± SD)	Abortion [b] (Mean ± SD)	Stillbirth [c] (Mean ± SD)	p-value
TGF-β (ng /ml)	9.39 ± 1.52	3.4 ± 0.81	5.12 ± 0.18	< 0.001
p-value	[a] vs [b] < 0.001	[b] vs [c] =0.120	[a] vs [c] = 0.028	
IL-10 (Pg /ml)	233.58 ± 26.17	116.77 ± 11.38	138.76 ±4.66	< 0.001
p-value	[a] vs [b] < 0.001	[b] vs [c] =0.120	[a] vs [c] = 0.028	
IL-17 (Pg /ml)	39.45 ± 3.18	80.03 ± 2.67	65.9 ±3.95	< 0.001
p-value	[a] vs [b] < 0.001	[b] vs [c] =0.120	[a] vs [c] = 0.028	

Kruskal-wallis test was used.
*p-value was significant if ≤ 0.05.

4. Effect of BPA on non-pregnant mice:

Serum level of TGF-β and IL-10 was lower among the experimental group treated with BPA in contrast to the control group which was discovered to be statistically significant (3.99±1.21 versus 10.67±1.85, p-value <0.001) and (206.99±30.79 versus 398.27±92.82 respectively, p- value <0.001) . Whereas serum level of IL-17 was higher among the experimental group treated with BPA, when compared to the control group the difference was statistically significant. (154.79 ± 25.06 versus 72.65 ±11.00 respectively, p-value <0.001) as demonstrated in table (4).

Table 4: Effect of BPA on serum TGF-β, IL-10 and IL-17 levels in non- pregnant mice:

Parameter	Experimental group (Mean ± SD)	Control group (Mean ± SD)	p-value
TGF-β (ng /ml)	3.99 ± 1.21	10.67 ± 1.85	< 0.001
IL-10 (Pg /ml)	206.99 ± 30.79	398.27 ±92.82	< 0.001
IL-17 (Pg /ml)	154.79 ± 25.06	72.65 ± 11.00	<0.001

Mann-whitney U test was used.
*p-value was significant if ≤ 0.05.

Table 5 revealed statistically significant variations between mean serum level of IL-10 and IL-17 among pregnant and non-pregnant mice of the experimental group (p-value <0.001 Table 6 revealed statistically significant variations between mean serum level of IL-10 and IL-17 among pregnant and non-pregnant mice of the control group (p-value < 0.001).

Table 5: Comparison of effect of BPA on serum levels of TGF-β, IL-10 and IL-17 between pregnant and non-pregnant experimental group mice:

Parameter	Non-pregnant mice Mean ± SD	Pregnant mice Mean ± SD	p-value
TGF-β (ng /ml)	3.99 ± 1.21	3.97 ± 1.06	0.462
IL-10 (Pg /ml)	206.99 ± 30.79	124.1 ± 124.1	<0.001
IL-17 (Pg /ml)	154.79 ±25.06	75.32 ± 7.52	< 0.001

Mann-whitney U test was used.
*p-value was significant if ≤ 0.05.

Table 6: Comparison of serum levels of TGF-β, IL-10 and IL-17 between pregnant and non pregnant control group mice:

Parameter	Non-pregnant mice Mean ± SD	Pregnant mice Mean ± SD	p-value
TGF-β (ng /ml)	10.67 ± 1.85	9.39 ± 1.52	0.061
IL-10 (Pg /ml)	398.27 ± 92.82	233.58 ± 26.17	<0.001
IL-17 (Pg /ml)	72.65 ± 11.00	39.49 ± 3.18	<0.001

Mann-whitney U test was used.
*p-value was significant if ≤ 0.05.

5. Predictors of serum levels of TGF-β, IL-10 and IL-17 of studied mice

Using multivariate regression, it was found that serum level of TGF-β and IL-10 significantly negative associated with treatment with BPA (p- value < 0.001), while IL-17 significantly positive associated with treatment with BPA (p-value < 0.001) as demonstrated in Table 7.

Table 7: Predictors of serum levels of TGF-β, IL-10 and IL-17 of studied mice:

Parameter	TGF-β		IL-10		IL-17	
	B	p-value	B	p-value	B	p-value
Pregnancy status	-0.64	0.095	-123.79	< 0.001	-56.32	< 0.001
Study group	-6.05	< 0.001	-150.38	< 0.001	58.98	< 0.001

R^2 of TGF-β model = 0.81, R^2 of IL-10 model = 0.76, R^2 of IL-17 model = 0.81

DISCUSSION

T regulatory cells (Tregs) and T helper 17 cells (Th17) are immune cells that play critical functions in immune response regulation. Perinatal BPA has been proven to alter the balance of T regulatory cells (Tregs) and T helper 17 cells (Th17) and to impact the female mice's immune system, leading to greater risk to inflammatory reactions in adult offspring [15].Moreover, BPA can reduce the quantity of Tregs and promote the growth of Th2 cells in maturation, as well as etheir Th1 and Th2 cells in the stages of pregnancy [16].

Consequently, TGF-β, IL-10, and IL-17 are interconnected cytokines which have important roles in immune regulation and disease pathogenesis. In the current study we aimed to investigate the impact of exposure to BPA on regulatory T cells and Th17 cells associated cytokines in female mice and detect if exposure to bisphenol A could cause abortion in pregnant mice.

In the current research, it was noticed that being subjucted to bisphenol A affected the quantities of serum TGF-β in non-pregnant and pregnant female mice . In female mice who aren't pregnant, it was noticed that the experimental group exposed to BPA had significantly lower levels of TGF-β in comparison to the control group (mean ± SD: 3.99 ± 1.21 vs 10.67 ± 1.85, p-value < 0.001). Likewise, in pregnant female mice, the experimental group also had lesser levels of TGF-β in comparison to the control group (mean ± SD: 3.97 ± 1.06 vs 9.39 ± 1.52, p-value <0.001).

In good agreement with Wu et al. who stated that bisphenol A exposure negatively impacts the immunological system, resulting in abnormal responses, dysfunction, and cell damage. It significantly affects macrophage growth, proliferation, and function, decreasing their phagocytic ability, promoting polarization towards M1-type, and inhibiting M2-type polarization [17].

Ruiz et al. found that bisphenol A toxicity disrupts inflammatory responses, promoting tumor growth and inflammatory cell recruitment, thereby promoting a malignant phenotype [18].

Yuruker et al.'s study examines the significance of cord blood Bisphenol A (BPA) levels on cytokine responses, finding that prenatal BPA exposure in mice enhanced Th17 cell activity and IL17 mRNA expression, potentially triggering inflammatory responses [19].

A study found that BPA consumption during pregnancy in mice increased susceptibility to mammary gland cancer, perhaps as a result of decreased TGF-β, a key protein involved in signaling pathways [20].

In this research it was found that toxicity of bisphenol A had a substantial impact on the quantities of serum interleukin-10 (IL-10) in female mice, both pregnant and non-pregnant. In female mice who aren't pregnant, the mean level of IL-10 was substantially less in the experimental group (206.99 ± 30.79) in comparison to the control group (398.27 ± 192.82), with a p-value < 0.001. Also, in pregnant female mice, the mean level of IL-10 was substantially less in the experimental group (124.1 ± 14.30) compared to the control group (233.58 ± 26.17), with a p-value < 0.001.

Alizadeh et al. proved that BPA impacted Th2 and Treg responses and decreased Foxp3 mRNA levels, which are Treg-specific transcription factors [21].

One study investigates the impacts of Bisphenol-A on cytokine production, anti-inflammatory mediators, neurodevelopmental biomarkers, and oxidative stress in placental explant cultures. Results show BPA increases IL-1β, IL-6, and BDNF production, while reducing IL-10 production [22].

Faheem et al.'s study examined the effect of Bisphenol-A (BPA) concentrations on Labeo rohita fish larvae, revealing developmental abnormalities and elevated oxidative damage. The study found that lower BPA concentrations increased catalase activity, while higher concentrations led to lowered activity due to elevated free radical production. This indicates that BPA causes severe oxidative stress by reducing antioxidant levels in larvae [23].

The immune system was impacted by BPA exposure, with lower TNFα and INF-γ levels and increased interleukin-10 expression. A biphasic response of IL-10 was observed, with lower concentrations causing increased expression and higher concentrations decreasing it. IL-10 plays a crucial role in immune response homeostasis. IL-10 secretion in bisphenol A-exposed animals reduces inflammatory response, aids in oral tolerance development, and prevents inflammatory and autoimmune pathologies through immune regulation [24].

IL-10, produced by various cell types, modulates immunological responses in both natural and adaptive immune systems, preventing pro-inflammatory Th1 cytokines and cell development, and promoting MHC-class II and co-stimulatory molecules on antigen-presenting cells [25].

An alternative research proved that BPA triggers Th2 cell polarization in adult mice, leading to higher levels of IL-4, IL-10, and IL-13, consistent with previous research [26,27]

According to our study, BPA exposure had a substantial impact on the quantities of serum IL-17 in both non-pregnant and pregnant female mice. In non-pregnant mice, the experimental group showed substantially greater levels of IL-17 in comparison with the control group (mean ± SD: 154.79 ± 25.06 vs. 72.65 ± 11.00, p<0.001). Similarly, in pregnant mice, the experimental group had substantially greater levels of IL-17 in comparison with the control group (mean ± SD: 75.32±7.52 vs. 39.49±3.18, p<0.001).

A study found a favourable relationship between serum IL-17 concentration and BPA exposure levels, with dose dependent, indicating a major increase in IL-17 levels [28].

Bisphenol A intake during pregnancy increased Th17 cell development and IL-17 serum protein levels in offspring, but VitD3 supplementation ameliorated these effects through vitamin D receptor-dependent regulation [29].

A study found that exposed to bisphenol A during pregnancy and breastfeeding can cause alterations in Th17 cells, including increased Th17 cells, RORt mRNA expression, and cytokine production. These changes are more pronounced in female descendants./+ However, BPA had minimal impact on serum TGF-β, indicating sustained immune disorders due to Th17 cell development [30].

A study on mice found that prenatal exposure to BPA increased IL17 and Th17-Th1 responses in offspring, while decreasing Treg responses [19].

In the current study, the impact of Bisphenol A (BPA) on pregnant mice, pregnancy outcome revealed alarming findings. In the experimental group (N=15), no live births occurred, while the control group (N=15) had a 100% live birth rate. Additionally, the experimental group experienced a 66.67% abortion rate and a 33.3% stillbirth rate, contrasting with zero cases in the control group. These results demonstrate a clear connection between BPA intake and bad effects, including decreased live births and increased abortion and stillbirth rates. Further research is crucial to understanding and addressing the potential dangers of pregnancy exposure to BPA. The mean levels of serum TGF-β, IL-10, and IL-17 were compared according to the result of pregnancy, namely live birth, abortion, and stillbirth. There were significant variances between the groups. The mean level of TGF-β in the live birth group (9.39 ± 1.52 ng/ml) was statically greater compared to the abortion group (3.4 ± 0.81 ng/ml) and the stillbirth group (5.12 ± 0.18 ng/ml) (p < 0.001). Similarly, for IL-

10, the live birth group (233.58 ± 26.17 pg/ml) showed statically greater levels compared to the abortion group (116.77 ± 11.38 pg/ml) and the stillbirth group (138.76 ± 4.66 pg/ml) ($p < 0.001$). Conversely, the IL-17 level was statically greater in the abortion group (80.03 ± 2.67 pg/ml) in comparison with the live birth group (39.45 ± 3.18 pg/ml) and the stillbirth group (65.9 ± 3.95 pg/ml) ($p < 0.001$). These results proved that TGF-β and IL-10 could be related to successful pregnancy outcomes, while raised quantities of IL-17 may be linked to adverse outcomes.

A study found that bisphenol equivalents pose a danger to the reproduction in humans, potentially resulting in female infertility that is idiopathic [31].

Our interesting results, the mean level of TGF-β in non-pregnant mice (3.99±1.21) was identical to pregnant mice (3.97±1.06), there is not a noticeable distinction ($p=0.462$). However, for IL-10, non-pregnant mice (206.99±30.79) had much more levels compared to pregnant mice (124.1 ± 124.1) ($p < 0.001$). Similarly, the IL-17 level was significantly greater in non-pregnant mice (154.79±25.06) compared to pregnant mice (75.32±7.52) ($p<0.001$). These results suggest that BPA exposure may have a more pronounced impact on the levels of IL-10 and IL-17 in non-pregnant mice compared to pregnant mice.

A study comparing immunization-related changes in pregnant and non-pregnant mice revealed differences in immune responses, suggesting pregnancy may impact the immune system's response to BPA exposure [32].

CONCLUSION

This study investigates the effect of BPA exposure on immune function in humans, particularly pregnant women, to understand potential risks and develop interventions. Future studies should consider pregnancy as an issue.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.

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