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Assessment of serum bisphenol a levels in autoimmune thyroiditis: a case-control study

Otoimmün tiroidit'te serum bisfenol a düzeylerinin değerlendirilmesi: bir vaka-kontrol çalışması

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ABSTRACT

Aim: The precise mechanism underlying autoimmune thyroiditis is still unclear. The female preponderance suggests the role of estrogens in autoimmunity, and there is strong evidence about this role. Bisphenol A (BPA), a widely used endocrine disruptor, may have estrogenic effects and affect autoimmunity through estrogenic and non-estrogenic mechanisms. We aimed to determine the relationship between serum BPA levels and thyroid autoimmunity.

Materials and Methods: We performed a cross-sectional case-control study involving 143 autoimmune thyroiditis patients and 95 age-matched healthy controls. Serum BPA levels were measured using ELISA method.

Results: BPA levels were not significantly different between autoimmune thyroiditis and control groups. The percentage of antibody-positive patients, thyroid function, thyroid antibody levels, or BMI were not different between BPA quartiles. Also, there was no correlation between BPA levels and thyroid autoantibody levels.

Conclusion: Serum BPA levels were not significantly different in autoimmune thyroiditis. Also, we could not show any relationship between BPA, thyroid function tests, and thyroid antibody levels.

Keywords: Autoimmune thyroiditis, Bisphenol A, Endocrine disruptors

ÖZ

Amaç: *Otoimmün tiroiditin altında yatan kesin mekanizma hala belirsizdir. Kadınların baskın olması, östrojenlerin otoimmünitedeki rolünü düşündürmektedir ve bu rol hakkında güçlü kanıtlar bulunmaktadır. Yaygın olarak kullanılan bir endokrin bozucu olan Bisfenol A (BPA), östrojenik etkilere sahip olabilir ve östrojenik ve östrojenik olmayan mekanizmalar aracılığıyla otoimmüniteyi etkileyebilir. Serum BPA düzeyleri ile tiroid otoimmünitesi arasındaki ilişkiyi belirlemeyi amaçladık.*

Gereç ve Yöntem: *143 otoimmün tiroidit hastası ve 95 yaş eşleştirilmiş sağlıklı kontrol içeren kesitsel bir olgu-kontrol çalışması gerçekleştirdik. Serum BPA düzeyleri ELISA yöntemi kullanılarak ölçüldü.*

Bulgular: *BPA düzeyleri otoimmün tiroidit ve kontrol grupları arasında anlamlı ölçüde farklı değildi. Antikor pozitif hastaların yüzdesi, tiroid fonksiyonu, tiroid antikor düzeyleri veya BKİ açısından BPA dörtlükleri arasında fark saptanmadı. Ayrıca, BPA düzeyleri ile tiroid oto antikor düzeyleri arasında bir korelasyon yoktu.*

Sonuç: *Serum BPA düzeyleri otoimmün tiroiditte önemli ölçüde farklı değildi. Ayrıca, BPA, tiroid fonksiyon testleri ve tiroid antikor düzeyleri arasında herhangi bir ilişki gösteremedik.*

Anahtar Sözcükler: *Otoimmün tiroidit, bisfenol A, endokrin bozucular.*

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INTRODUCTION

Autoimmune thyroid disease is the most prevalent cause of hypothyroidism in iodine-sufficient areas. It is characterized by the presence of thyroid autoantibodies, mainly anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg). The precise mechanism underlying autoimmune thyroid disease is unclear. Both genetic and environmental factors are implicated in pathogenesis. Autoimmune thyroid disorders demonstrate a female preponderance, suggesting the role of estrogens in autoimmunity. Immune cells express estrogen receptors. There is compelling evidence of the relationship between estrogen and autoimmunity via diverse and complex modulatory and stimulating roles (1). This cascade of complex interaction of hormones and environmental factors in genetically susceptible individuals leads to autoimmune disorders, including autoimmune thyroiditis (2).

Bisphenol A (BPA) is widely used in plastics (3). Its widespread use results in high levels of exposure in humans and animals (4). It can bind to estrogen receptors and exert estrogenic or anti-estrogenic effects (5). Enhancement of the immune-stimulating effects of the estrogens. T cell aggregation, effects on prolactin levels and cytochrome P450, and promoting reactive oxygen species formation are the other components of BPA induced autoimmunity(6).

Given the higher prevalence of autoimmune thyroid disease in women, the potential role of BPA's estrogenic effects in its pathogenesis is a compelling area of study. Our aim was to assess serum BPA levels in autoimmune thyroiditis and control subjects, with the potential to reveal intriguing implications for this relationship.

MATERIALS and METHODS

Ethics statement

The study was rigorously approved by the local ethics committee of Ege University Faculty of Medicine with approval number 13-11/13 at 04/12/2013. All the participants provided oral and written informed consent, and we ensured that all study steps were in strict alignment with the Declaration of Helsinki Principles revised in 2008, instilling confidence in the study's integrity.

Subjects and study design

This is a cross-sectional study including 143 patients with autoimmune thyroiditis and 95 age-matched thyroid autoantibody-negative healthy controls. Autoimmune thyroiditis was diagnosed in patients with anti-TPO and/or anti-Tg antibody positivity and sonographic findings of thyroiditis (i.e., hypo-echogenicity and heterogeneity of the parenchyma). The patients under levothyroxine replacement treatment were included as long as their thyroid hormones were in the euthyroid range. Subjects negative for anti-thyroid antibodies and normal sonographic appearance of the thyroid were recruited as controls. Subjects with overt hypo/hyperthyroidism, ALT/AST levels over three times the upper limit of normal, estimated glomerular filtration rate under 60 ml/min/1.73 m^2, pregnancy, oral contraceptive use, acute systemic illness, and active malignancy were excluded. A detailed medical history and thorough physical examination were performed on all participants. Height (centimeters) and weight (kilograms) were measured. We calculated body mass index (BMI) by dividing weight (kg) by square meter of height (m^2). Circulating levels of TSH, Free Triiodothyronine (FT3), Free thyroxine (FT4), anti-TPO, and anti-Tg were measured using immunochemical methods (Beckman Coulter DXI 800, USA)

The venous blood of both the patient and control groups was drawn following an overnight fasting. The serum was separated and stored at -80°C. Using a competitive ELISA method, Serum BPA levels were determined using a commercial kit (IBL Co., Ltd., Gunma, Japan). Spectrophotometric measurement was performed at a wavelength of 450 nm using the Thermo Scientific Multiskan GO model ELISA reader (Finland). The absorbance curves were used to determine BPA concentrations. The results were expressed in ng/mL.

Statistical analysis

IBM SPSS version 25.0 (Chicago, IL, USA) was used for statistical analysis. Descriptive statistics of continuous variables were reported as median (interquartile range-IQR) and mean ± standard deviation (SD), frequencies and percentages were provided for categorical variables. For comparison of two continuous variables Mann-Whitney *U* or Student's *t-test* was used while for more than two variables, one-way ANOVA or Kruskall-Wallis test was employed. Depending on distribution, Spearman's rank or Pearson's correlation coefficient was used for correlations. We performed logistic regression analysis to investigate the relationship between autoimmunity status and BPA. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 238 age and BMI matched subjects were included in the study (26 Male/ 212 Female). One hundred forty-three (60.1%) of the patients were antibody positive while 95 (39.9%) were controls. The groups were not different in terms of thyroid hormone levels. BPA levels were not significantly different between autoimmune thyroiditis and control groups (*p*=0.998) (Table-1).

When the groups were compared by their levothyroxine replacement status, there was no significant difference in terms of BPA and thyroid function tests. Patients were grouped by their BMI (<25, 25-30, and >30 kg/m2), and there was no significant difference in BPA levels (data not shown).

Only a minority of the patients were male (10.1%). There were no significant differences in BPA levels between male and female groups (p=0.086). Likewise, thyroid function tests and thyroid antibody levels were similar according to gender.

No significant differences were detected in terms of thyroid function tests, thyroid autoantibody levels, age, or BMI between BPA quartiles (Table-2). Also, the ratio of antibody-positive subjects was not different between BPA quartiles (*p*=0,87).

There was no correlation between the levels of BPA and anti-Tg, anti-TPO, FT3, FT4, TSH, age, and BMI in autoimmune thyroiditis and control groups (Table-3).

Logistic regression analysis with a full model including age, BMI, BPA, TSH, FT4, and FT3 as independent variables and autoimmunity presence as dependent variable, the final model was significant for only TSH with an OR of 1.2 (%95 CI 1.01-1.42 *p*=0.04).

Table-1. Comparison of the demographic and laboratory characteristics of the subjects.

Variables	**Autoimmune thyroiditis n=143**	**Controls n=95**	***P***
Sex, F/M	14/129	12/83	0.529
Age, years	42.57 ± 11.86	44.53 ± 13.26	0.666
BMI, kg/m^2	27.61 ± 4.97	27.23 ± 5	0.512
TSH(mU/L)	2.00 (2.4)	1.48 (1.82)	0.073
fT4(ng/dL)	0.86 (0.23)	0.87 (0.19)	0.943
fT3(ng/L)	2.92 (0.48)	2.93 (0.54)	0.183
Anti-T(IU/mL)	4.7 (75.9)	0.20 (0.95)	<0.001*
Anti-M(IU/mL)	255.3 (617.9)	0.70 (1.1)	<0.001*
BPA(ng/mL)	1.13 (62.74)	0.99 (65.81)	0.998

Table-2. Comparison of groups according to BPA Quartiles.

	p	Q1	Q2	Q3	Q4
BPA	<0.001	0.37(0.09)	0.84 (0.19)	1.85 (18.43)	85.6 (20.69)
Age	0.964	43.3 ± 10.91	42.67 ±12.18	44.69 ±12.88	42.78 ±14.07
BMI	0.767	27.63 ±5.84	27.88 ±4.72	26.96± 4.75	27.27± 4.58
TSH	0.511	1.87 (2.26)	1.78 (2.5)	1.46 (1.74)	1.91 (2.97)
FT4	0.63	0.82 (0.21)	0.87 (0.23)	0.88 (0.23)	0.87 (0.18)
FT3	0.91	2.97 (0.44)	2.92 (0.49)	2.97 (0.59)	2.9 (0.5)
Anti M	0.63	17.3 (273.7)	19.4 (265.5)	12.4 (297.2)	40.4 (642.8)
Anti T	0.99	0.5 (4.8)	1.4 (26.8)	1 (18.75)	1.4 (26.5)

Table-3. Evaluation of the relation between BPA and the other demographic and laboratory variables.

	Control		Autoimmune thyroiditis	
	r	***p***	**r**	***p***
BMI	-0.099	0.354	0.071	0.438
TSH	-0.092	0.373	0.071	0.402
fT4	0.149	0.150	-0.050	0.550
fT3	-0.139	0.183	-0.016	0.847
Anti-T	-0.187	0.071	0.071	0.404
Anti-M	0.128	0.290	0.103	0.223

DISCUSSION

The potential effects of BPA on autoimmunity, either through estrogenic or direct effects, have drawn attention to possible pathogenetic effects on autoimmune thyroiditis. However, the current literature has revealed inconsistent results. We couldn't demonstrate any significant difference between autoimmune thyroiditis and control groups as regards serum BPA levels. Also, we failed to show any association between serum BPA levels and antibody titters or antibody positivity. There was no difference in BPA, thyroid function tests and thyroid antibodies in terms of gender.

Chailurkit et al were the first to evaluate BPA in autoimmune thyroiditis. They found that increasing BPA quartiles were accompanied by an increasing proportion of antibody-positive patients. They also found that the BPA quartile, age, and gender were independent determinants of anti-TPO antibody presence (7). Nevertheless, Choi et al. could not reveal any association between thyroid antibodies and urinary bisphenol A (8). Recently, in line with the aforementioned results, Yuan et al. failed to demonstrate any association between urinary BPA and thyroid autoantibodies in women of childbearing age (9).

Conflicting results may arise for various reasons, such as the study population, the detection method for BPA, and the stability and half-life of BPA. Different detection methods may yield different results. Also, the stability of phenolic compounds varies according to the type of sample, such as urine or blood, and storage method (4). ELISA method is less sensitive than liquid chromatography-mass spectrophotometry (LC-MS) technique. (3, 10) Because of their relatively short half-life, serum BPA levels may be less reliable compared to urinary BPA for reflecting daily BPA exposure. Somehow, all of our study participants had detectable BPA serum levels which indicates the widespread exposure to BPA in the community.

The urinary BPA levels have been found to be 42 times higher than the serum levels. Also, the timing of the urine or blood sampling is important in relation to meals and the BPA content of the meal. Albeit, serum BPA may be more accurate in reflecting tissue concentrations, so it should not be entirely overlooked (11). However, there is still controversy surrounding the clinically significant levels of BPA. Some studies suggest that levels in the nanomolar range are enough to exert its effects (12), (13)

Apart from thyroid autoimmunity, BPA affects the thyroid in other aspects as well. Kitamura et al stated that BPA acts as a thyroid hormone agonist (14) on the contrary, others showed antagonistic effects on thyroid hormone receptors (TR) (15, 16) Some in vitro studies suggest that BPA causes thyroid disruption at the translational level by upregulating or down-regulating the genes involved in thyroid hormone synthesis or their transcription factors (17).

A recent meta-analysis showed a positive correlation between FT4 levels and BPA levels in females (18). On the other hand, some other studies failed to indicate any association between BPA and thyroid function (19, 20). In our study, we could not define any differences in thyroid hormone levels between autoimmune thyroiditis and control groups. Also, thyroid hormones were not significantly different among BPA quartiles. There was no correlation between BPA levels and TSH, FT4, and FT3 levels.

A recent study examined the relationship between serum and urinary BPA and analogs and thyroid volume and function. They failed to show any relationship between thyroid volume and function and serum and urine levels of

bisphenols except bisphenol C (BPC) (21). Choi et al. demonstrated that in patients with thyroid autoantibody positivity, the risk of thyroid nodules increased with increasing urinary BPA levels (8) . In our study, we did not have access to thyroid volume data of all patients, but the nodule prevalence was not different between BPA quartiles (data not shown).

Epidemiological studies to evaluate the effects of BPA on thyroid function are limited for several reasons. BPA exposure begins in utero and continues for a lifetime. BPA exposure during the early stages of life may have a transgenerational effect, predisposing the subsequent generations to the risk of BPA-related disease. Since autoimmunity is a lifelong process, it is hard to build a cause-effect relationship between BPA and thyroid autoimmunity. Another obstacle to EDC studies is their lipophilic nature, leading them to accumulate on tissues, which eventually results in leakage from deposits and longstanding toxicity.

Main Points

Although we could not define any relationship between serum BPA levels and autoimmunity, it is hard to draw firm conclusions with EDC studies for several reasons. One of which is the multiple endocrine disruptor exposure in a time. Secondly, they exert their effects for an extended period of time due to their lipophilic nature and possible epigenetic effects. Hence epidemiological and prospective studies are hard to perform.

CONCLUSION

In conclusion, our study did not show any difference in BPA serum levels between autoimmune thyroiditis and control groups. Moreover, we did not determine any correlation between BPA and thyroid autoantibodies and thyroid function tests. Future studies are needed to determine the effects of endocrine disruptors on the thyroid, including novel biomarkers to detect tissue effects and studies including various endocrine disruptors to ascertain possible synergistic effects on autoimmunity or thyroid disruption.

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Conflict of interest: The authors declare that they have no conflict of interest.

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