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RESEARCH

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Study of serum bisphenol-A and the mRNA of galactosidase beta 1 and tumor necrosis factor alpha in Egyptian patients with type 2 diabetes mellitus

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Abstract

Background The pathogenesis of type 2 diabetes mellitus (T2DM) originates from multiple tissue defects. Environmental pollutants such like endocrine-disrupting chemicals (EDCs) have lately gotten special attention in triggering the metabolic abnormalities in relation to insulin resistance and T2DM. Bisphenol-A (BPA), which is one of the EDCs and simulates natural endogenous estrogens, has been implicated in the pathogenesis of several diseases, and, over the last decade, it has been revealed to have probable diabetogenic and obesogenic actions.

Aim To study the environmental exposure of BPA by measuring its serum level together with the relative expression level of galactosidase beta 1 (GLB1), tumor necrosis alpha (TNF-α) mRNAs and malondialdehyde (MDA) as an oxidative stress marker determine the association of these markers with the glycemic control of Egyptian patients having T2DM.

Patients and methods The present study was conducted on 90 participants categorized into group 1: 60 patients with T2DM and group 2: 30 subjects with normal glucose tolerance. Serum BPA levels were measured by enzyme-linked immunosorbent assay (ELISA) kit, and biochemical determinations were done by standard protocols. Because of the pro-oxidizing role of BPA, we measured serum level of malondialdehyde (MDA) as an oxidative stress marker. Peripheral blood mononuclear cells (PBMCs) were used for detecting the gene expression changes in GLB1 mRNA, a major marker of cellular senescence, and the mRNA of the proinflammatory cytokine, TNF-α.

Results Serum levels of BPA in T2DM patients were significantly higher compared to their control group, and they were significantly and positively correlated to poor glycemic control and insulin resistance. T2DM patients showed significantly high serum MDA, mRNA levels of GLB1, and TNF-α. There was positive correlation between serum BPA levels and oxidative stress marker, senescence indicator, and inflammatory marker; however, there was no statistical significance for this correlation.

Conclusion Our study demonstrates that there is a link between increased serum BPA levels, poor glycemic control, and insulin resistance in Egyptian patients with T2DM.

Keywords T2DM, Insulin resistance, BPA, MDA, GLB1, TNF-α, Cellular senescence

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Background

Worldwide, the number of diabetic people has increased to fourfold during the previous three decades, and diabetes mellitus is estimated to be the ninth main cause of death [1]. Egypt was marked by the International Diabetes Federation as the 9th leading country in the world with several patients with type 2 diabetes mellitus (T2DM) [2]. In addition to the known risk factors for T2DM, a certain group of environmental pollutants identified as endocrine disruptor chemicals (EDCs) seem to play a pathophysiological role in the origin of metabolic diseases including T2DM [3]. EDCs are exogenous substances that can disrupt any component of hormone activity and have detrimental metabolic effects [4]. A common EDC and the primary component of polycarbonate plastics are bisphenol-A (BPA) [5]. It is utilized in recycled paper, the production of epoxy resins, and the lining of food cans [5]. Chlorinated BPA derivatives are created when BPA from these goods combines with chlorinated tap water [6]. Oral consumption is the main way that people are exposed to BPA, and research on human pharmacokinetics shows that after a single exposure, it is conjugated in the liver and eliminated through the urine and bile with a half-life of around 5.3 h [7]. Many recent studies also have highlighted that BPA could be a supplemental risk factor to be considered in the development of insulin resistance and T2DM [8].

Increasing evidences suggest that the reactive oxygen species (ROS) induction by BPA is the most important causing factor of its toxicity. Moreover, as a result of BPA exposure, oxidative stress is stimulated, and this starts the aging process [9]. Malondialdehyde (MDA), a marker of lipid peroxidation, has been commonly believed as a possible biomarker for oxidative stress, and numerous findings show its rise in BPA-exposed organisms [10].

It was assumed that BPA could enhance the risk of cells being senescent because of its pro-oxidizing function [11]. Cellular senescence, an unavoidable cell cycle stop, is a known impediment to the spread of cancer [12]. Senescent cells may influence the function of pancreatic beta cells, accelerate tissue damage through the senescence-associated secretory phenotype (SASP), and enhance and propagate a number of tissue abnormalities [12]. The identification of senescent cells is dependent on high level of lysosomal β-galactosidase activity which is a protein in humans, which is encoded by the galactosidase beta 1 (GLB1) gene.

When senescent cells are induced by genotoxic stress, they secret a compound number of factors that are linked to inflammation, and this is identified as the SASP phenomena [13]. Although senescent cells stop dividing, they are still metabolically active and employ a complicated pro-inflammatory response secreting cytokines, chemokines, interleukins, and proteinases that are mutually recognized as SASP [14]. The SASP is intermediated by the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) that mainly stimulates the expression of inflammatory cytokines like tumor necrosis factor alpha (TNF-α) [15]. As an adipokine, TNF-α promotes insulin resistance and is highly connected to obesity-induced T2DM [15]. BPA can induce inflammation and insulin resistance by activating toll-like receptors (TLRs) resulting in activation of c-Jun N-terminal kinases (JNK) and NF-κB signaling pathways with successive release of some pro-inflammatory cytokines as TNF-α [16]. As a result of that, studying the relationship between BPA and TNF-α mRNA together with the senescent marker GLB1 mRNA at their transcriptional level could have a clue on the glycemic control and insulin resistance of T2DM patients.

Patients and methods

To achieve the goal of the study, we enrolled 60 T2DM patients (group 1) and 30 nondiabetic subjects of matched age and sex (group 2). Patients of group 1 were chosen from the outpatient clinic of Alexandria Main University Hospital. We excluded patients with type 1 diabetes mellitus, liver failure, end-stage renal disease, malignancy, and patients on steroids and hormonal therapies. Also, pregnant females and patients who are unwilling to participate, and patients from whom we cannot take consent reports, have been excluded from this work.

Following a full explanation of the procedures they might experience, patients and volunteers signed written informed permission forms. The Ethics Review Board of the Faculty of Medicine at Alexandria University as well as the ethics committee approved the study. 0,201,511 was the approval number. For a case–control study, the sample size was calculated using the Epi Info software, accounting for power of 80% and confidence level of 95%.

All patients and healthy volunteers underwent clinical evaluations with specific emphasis on anthropometric measures in the form of height and weight to calculate body mass index (BMI) according to the following formula: BMI = weight (kg)/height (m^2) kg/m^2; additionally, waist circumference and vital signs were assessed. Ten milliliters of venous blood was collected from them in the morning following an overnight fast. Then, each blood sample was divided into two aliquots: one in an EDTA tube for dividing peripheral blood mononuclear cells (PMCs) and the other in a plain tube for dividing serum samples. In the latter, after allowing blood to coagulate, samples were centrifuged at $1200 \times g$ for 10 min, and they were then stored at − 80 °C until use. Blood was processed for PBMC isolation utilizing Histopaque-1077

(Sigma-Aldrich) matching to the standard protocol by transferring the blood on density gradient solution and centrifugation at 1500–1800 rpm for 30 min. The buffy coat layer that contains the PBMCs was suctioned and washed three times with phosphate-buffered saline (PBS; pH 7.2–7.4).

Routine laboratory tests for all patients and healthy subjects include liver test profile, renal test profile, and fasting lipid profile. Fasting blood glucose, glycated hemoglobin (HbA_{1c}), fasting insulin assessed using the enzyme-linked immunosorbent assay (ELISA) technique, and insulin resistance determined using the homeostasis model assessment of insulin resistance (HOMA-IR) formula were all included in the glycemic control blood tests: HOMA-IR is calculated as (fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5). The recommended healthy range of HOMA-IR is < 1 which denotes optimal tissue sensitivity to insulin. More than 1.9 denotes early insulin resistance, while 3 indicates insulin resistance [17].

Serum malondialdehyde (MDA) was measured by enzymatic colorimetric method provided by biodiagnostic (catalog no. MD 25 29, website: www.bio-diagnostic.com) [18].

Serum BPA was measured by ELISA technique; the kit was provided by Chongqing Biospes Co., Ltd. (catalog no. BZEK1424, website: www.biospes.com) [19]. This kit was dependant on the basic sandwich enzyme-linked immunosorbent assay technique. The purified anti-BPA antibody was pre-coated on the well plates, and the anti-BPA-horseradish peroxidase (HRP) enzyme conjugate was utilized as capturing antibody. The standards, test samples, and HRP-conjugated detection antibody were included to the wells subsequently, mixed and incubated, and then, unbound conjugates were removed with wash buffer. A solution of tetramethyl benzidine (TMB) substrates (A&B) were utilized to assess the HRP enzymatic reaction. TMB reaction with HRP produces a blue color product that changes into yellow after adding up acidic stop solution. The density of yellow color is proportional to the BPA amount of sample captured in plate, and it was measured spectrophotometrically at 450 nm, and then, the concentration of BPA can be calculated.

Total RNA isolation from serum samples was done utilizing Qiagen® miRNeasy Mini Kit (cat. no. 217004). The concentration and purity of RNA were assessed via NanoDrop, and then, complementary deoxyribonucleic acids (cDNA) were synthesized utilizing High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA, cat. no. Archive) [19, 20]. In each experiment, 10 μg of RNA extract, 2 μl of reverse transcriptase buffer, 0.8 μl of deoxynucleotide triphosphate (dNTP), 1 μl of reverse transcriptase, 1 μl of RNase inhibitor, and 2 μl of RT random primers were used. Finally, 20 μl of nuclease-free water was added to make the entire volume. The thermal cycle was set up to last 10 min at 25 °C, 120 min at 37 °C, and 5 min at 85 °C, after which the temperature was reduced to 4 °C and the run was terminated. For use in real-time quantitative polymerase chain reaction (RT-qPCR) investigations, cDNA was stored at − 20 °C after reverse transcription. Thermo Scientific Maxima SYBR Green qPCR Master Mix (2 ×) (Thermo Scientific, cat. no. K0251) was used for RT-qPCR that was made on Applied Biosystems StepOne Real-Time utilizing specific primers for GLB1 mRNA with a forward primer 5′-ACGGTGGACTTT GGAACAG-3′ and a reverse primer 5′-TGATTGTGG AGTGAGGTTGG-3′, TNF-α mRNA with a forward primer 5′-GGACGAACATCCAACCTTCCC-3′ and a reverse primer 5′-GTGGTCTTGTTGCTTAAAGTT CTAAG-3′, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control with a forward primer 5′-GAAGGTGAAGGTCGGAGTCAAC-3′ and a reverse primer 5′-CAGAGTTAAAAGCAGCCCTGGT-3′. A total of 12.5 μl of the Maxima SYBR Green qPCR Master Mix (2 ×), 1 μl of the forward primer (50 pmol), 1 μl of the reverse primer (50 pmol), 0.1 μl of the ROX Solution, 7.4 μl of nuclease-free water, and 3 μl of cDNA were used in each reaction. Samples were examined in duplicates. In each test, a no template control was created. The following is how RT-qPCR was programmed: a 10-min, 95 °C first cycle, followed by a 3-step cycle: forty cycles denaturation at 95 °C for 15 s; annealing at 65 °C for 30 s for the GLB1, TNF-α mRNAs, and GAPDH gene; and finally, extension at 72 °C for 30 s. Melting curve analysis was done to evaluate the PCR products' specificity and identity. For GLB1 and TNF-α mRNAs, the relative quantification method ($RQ = 2^{-\Delta\Delta CT}$) was used to calculate the fold change between a sample and a normal control, and the outcomes were assessed using the StepOne™ software.

Statistical analysis [21]

Using version 20.0 of the IBM SPSS software package, the data have been analyzed and added to the computer (Armonk, NY: IBM Corp) [22].

Results

The mean age of the control group was 35.63 ± 7.63 with 15 males (50%) and 15 females (50%). The mean age of T2DM patients was 58.53 ± 6.56 years. Twenty-seven (45%) subjects were females, and thirty-three (55%) subjects were males. There was statistical significance among both groups regarding the age (Table 1).

Regarding anthropometric measurements, the mean of BMI and waist circumference were significantly less

Table 1 Demographic data and laboratory results of the studied groups

	Control ($n=30$)	T2DM ($n=60$)	p
Sex			
Male	15 (50%)	33 (55%)	0.654
Female	15 (50%)	27 (45%)	
Age (years)	53.63 ± 7.63	58.53 ± 6.56	< 0.001*
BMI (kg/m^2)	24.07 ± 2.39	29.77 ± 3.98	< 0.001*
Waist circumference (cm)	82.57 ± 8.41	93.97 ± 9.04	< 0.001*
Systolic blood pressure (mmHg)	110.7 ± 10.15	114.7 ± 12.41	0.130
Diastolic blood pressure (mmHg)	79.67 ± 9.28	81.17 ± 8.65	0.451
Mean arterial blood pressure	90.0 ± 7.17	92.33 ± 8.08	0.184
AST (U/L)	25.87 ± 6.54	29.68 ± 16.22	0.298
ALT (U/L)	28.77 ± 12.91	31.0 ± 6.70	0.140
Albumin (g/dl)	3.83 ± 0.85	3.50 ± 0.86	0.088
Urea (mg/dl)	15.73 ± 4.63	24.02 ± 19.01	0.111
Serum creatinine (mg/d)	1.10 ± 0.17	1.60 ± 1.79	0.264
e-GFR (ml/min/1.7)	72.53 ± 16.05	69.69 ± 32.61	0.581
UACR (mcg/mg creat.)	16.46 ± 4.16	16.35 ± 3.36	0.885
Total cholesterol (mg/dl)	171.8 ± 17.27	172.4 ± 16.91	0.890
HDL (mg/dl)	70.67 ± 7.48	69.25 ± 7.02	0.380
TG (mg/dl)	140.8 ± 10.15	143.6 ± 7.87	0.179
LDL (mg/dl)	72.97 ± 21.32	74.43 ± 17.93	0.734
Fasting glucose level (mg/dl)	102.6 ± 14.28	113.7 ± 22.34	0.005*
HbA_{1c} (%)	4.97 ± 0.66	7.09 ± 1.21	< 0.001*
Fasting insulin level (mU/L)	6.85 ± 3.23	12.90 ± 11.18	0.044*
Insulin resistance (HOMA-IR)	1.74 ± 0.88	3.86 ± 3.49	0.042*

AST aspartate aminotransferase. *ALT* alanine aminotransferase. *e-GFR* estimated glomerular filtration rate. *UACR* urinary albumin creatinine ratio. *HDL* high-density lipoproteins. *TG* triglycerides. *LDL* low-density lipoproteins. Quantitative data was expressed using mean ± SD. *SD* standard deviation. *p* *p*-value for comparing between the studied groups. *Statistically significant at $p \leq 0.05$

in the control group compared to the T2DM group ($p < 0.001$, $p < 0.001$, respectively) (Table 1).

It was revealed that the mean of fasting glucose level, HbA_{1c}, fasting insulin, and insulin resistance (HOMA-IR) in the T2DM patients also was significantly less in the control group compared to the T2DM group ($p = 0.005$, $p < 0.001$, $p = 0.044$, $p = 0.042$, respectively) (Table 1).

Multivariate logistic regression analysis adjusted for age was performed for serum BPA, TNF-α mRNA, GLB1 mRNA, and serum MDA to identify the association between these markers and diabetes. This association remained statistically significant even after adjusting for age ($p = 0.047$, $p = 0.044$, $p = 0.049$, $p = 0.043$, respectively) (Table 2).

Serum BPA level was significantly lower in the control group compared to the T2DM group, mean (48.59 ± 21.88 ng/ml and 81.28 ± 49.77 ng/ml, respectively) ($p = 0.004$). In males, the mean serum BPA level in the control group was less than that of the T2DM group (49.59 ± 24.22 ng/ml and 79.24 ± 52.13, respectively ($p = 0.109$). In females, the mean serum BPA level in the control group was significantly less than that of the T2DM group (47.60 ± 20.07 ng/ml and 83.78 ± 47.59 ng/ml, respectively) ($p = 0.014$) (Table 3) (Fig. 1).

Table 2 Multivariate logistic regression analysis for the different markers adjusted for age to discriminate between T2DM patients and control

	[a]Multivariate	
	p	AOR (LL–UL 95% *CI*)
Serum BPA	**0.047***	**1.045 (1.001–1.092)**
TNF-α mRNA	**0.044***	**4.428 (1.040–18.854)**
GLB1 mRNA	**0.049***	**1.892 (1.002–3.575)**
Serum MDA	**0.043***	**1.667 (1.016–2.734)**

AOR adjusted odd's ratio

CI confidence interval, *LL* lower limit, *UL* upper limit

[a] Adjusted with age (years)

* Statistically significant at $p \leq 0.05$

Serum MDA level was significantly decreased in the control group compared with patients in the T2DM group, mean (6.27 ± 3.24 nmol/ml and 20.97 ± 14.87 nmol/ml, respectively) ($p < 0.001$) (Fig. 2).

Table 3 Comparison between the two studied groups according to serum BPA in each gender

Serum BPA	Control	T2DM	*p*
In male	**(*n* = 15)**	**(*n* = 33)**	
Min.–max	20.0–112.1	22.80–187.0	0.109
Mean ± SD	49.59 ± 24.22	79.24 ± 52.13	
Median	45.0	55.10	
In female	**(*n* = 15)**	**(*n* = 27)**	
Min.–max	22.0–93.0	25.80–182.6	0.014*
Mean ± SD	47.60 ± 20.07	83.78 ± 47.59	
Median	45.0	68.80	

p *p*-value for comparing between the studied groups

* Statistically significant at $p \leq 0.05$

The mean level of GLB1 mRNA in the control subjects (0.021 ± 0.012) was significantly less than in patients with T2DM (0.12 ± 0.34) ($p = 0.042$) (Fig. 3).

TNF-α mRNA level was significantly decreased in the control group compared with those in the T2DM group, mean (0.364 ± 0.77 and 0.38 ± 0.69, respectively) ($p = 0.002$) (Fig. 4).

In the control group, serum BPA level was significantly and positively correlated with TNF-α mRNA. On the other hand, serum BPA was positively correlated with BMI, waist circumference, fasting glucose concentration, HbA_{1c}, fasting insulin level, and insulin resistance (HOMA-IR); however, there was no statistical significance for this correlation. Also, there was negative correlation between serum BPA, serum MDA, and GLB1 mRNA without statistical significance for this correlation (Table 4).

In T2DM group, circulating serum BPA level was significantly and positively correlated with fasting glucose level, HbA_{1c}, fasting insulin level, and insulin resistance (HOMA-IR). There was positive association between serum BPA and BMI, serum MDA, and the relative expression levels of TNF-α and GLB1 mRNAs; however, this link lacked statistical significance. Additionally, there was a negative association between serum BPA and waist circumference without statistical significance for this correlation (Table 4).

Discussion

In the current study, serum BPA was significantly greater in T2DM group relative to the control group, and there was positive significant correlation between serum BPA, fasting glucose concentration, HbA_{1c}, fasting insulin level, and insulin resistance (HOMA-IR). In agreement with our work, Soundararajan et al. (2019) [19] declared positive significant correlation between serum BPA, fasting glucose levels, HbA_{1c}, and insulin resistance. Also, it was revealed in a study by Ahmadkhaniha et al. (2014) [23] that urinary BPA were observed to be linked to diabetes independent of established diabetes risk factors. In their study, there was a positive correlation between HbA_{1c} and urinary BPA levels.

Additionally, Mourad et al.'s study from 2021 [24] in Egypt sought to determine the incidence of insulin resistance and the likelihood that nondiabetic BPA-exposed plastic sector workers would go on to develop T2DM.

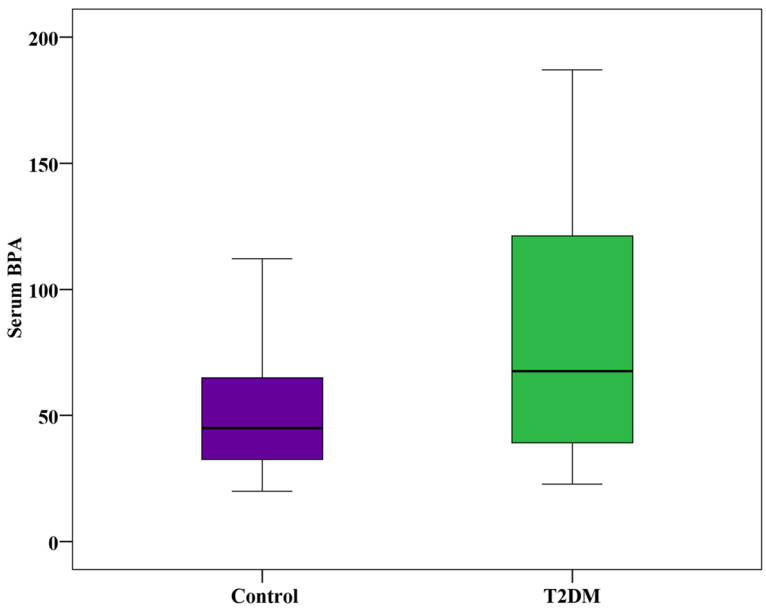

Fig. 1 Box plot showing the comparison between the two studied groups according to serum BPA (μg/ml) ($p = 0.004$)

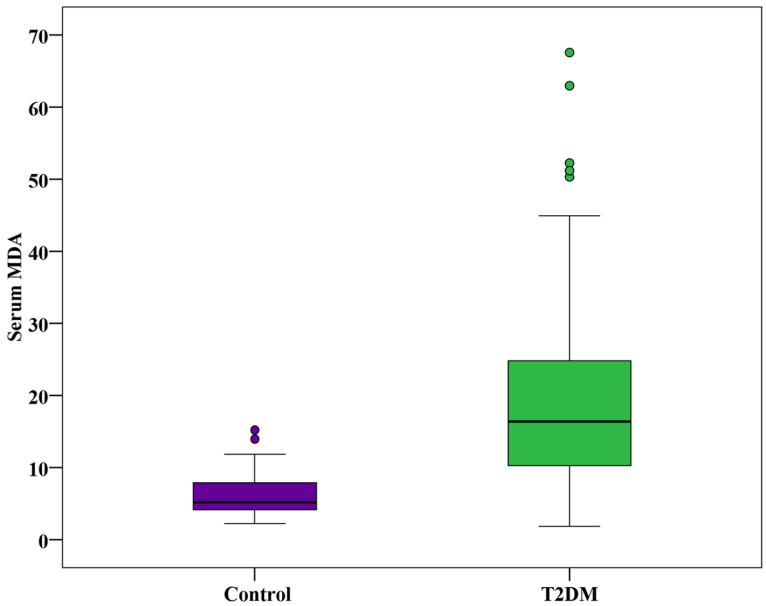

Fig. 2 Box plot showing the comparison between the two studied groups according to serum MDA (nmol/ml) ($p < 0.001$)

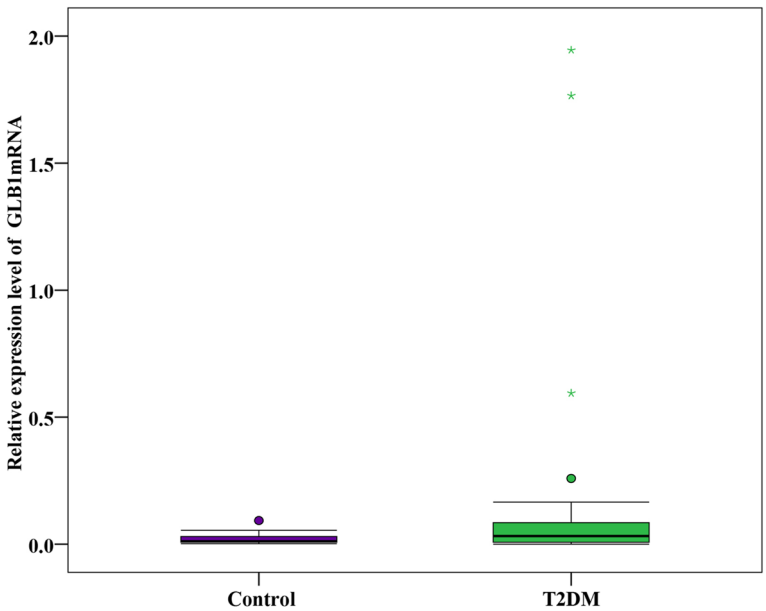

Fig. 3 Box plot showing the comparison between the two studied groups according to the relative expression of GLB1 mRNA ($p = 0.042$)

According to their research, BPA-exposed workers had significantly greater serum BPA levels, fasting insulin levels, and insulin resistance (HOMA-IR) than their control group. Additionally, there was a statistically positive link between BPA and HOMA-IR.

In contrast to our study, Piecha et al. (2016) [25] reported that there is no connection between BPA and T2DM, hypertension, and dyslipidemia. Carlsson et al. (2018) [26] also revealed an inverted relation between BPA and insulin resistance in healthy normal-weight children. They mentioned that other cross-sectional research studies showing positive relationship between BPA and obesity might be confounded by lack of data on caloric intake and energy expenditure.

Regarding if there is gender difference in serum level of BPA, our results revealed that serum BPA levels in female patients with T2DM were significantly higher compared to their respective control group; however, serum BPA

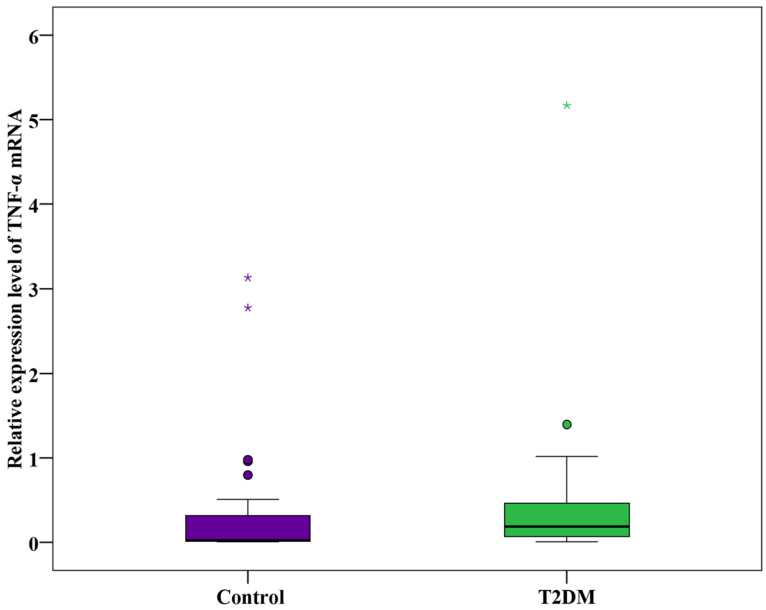

Fig. 4 Box plot showing the comparison between the two studied groups according to the relative expression of TNF-α mRNA ($p = 0.002$)

Table 4 Correlation between serum BPA with different parameters

Serum BPA vs	Control ($n = 30$)		T2DM ($n = 60$)	
	r	*p*	*r*	*p*
BMI	0.009	0.964	0.083	0.528
Waist circumference	0.057	0.763	−0.012	0.928
Fasting glucose concentration	0.250	0.182	0.273	0.035*
HbA_{1c}	0.065	0.733	0.303	0.018*
Fasting insulin level	0.136	0.475	0.347	0.007*
Insulin resistance (HOMA-IR)	0.245	0.192	0.363	0.004*
Serum MDA	−0.242	0.198	0.197	0.131
TNF-α mRNA	0.376	0.040*	0.172	0.189
GLB1 mRNA	−0.159	0.400	0.200	0.126

r Pearson coefficient. *Statistically significant at $p \leq 0.05$

levels in T2DM male patients were elevated but without statistical significance relative to their corresponding control subjects ($p = 0.109$). In agreement with our study, Soundararajan et al. (2019) [19] reported significant high serum BPA in T2DM females than in females of the control group.

In contrast to our study, Takeuchi et al. (2004) [27] studied the serum concentrations and the metabolism of BPA in rats by an high-performance liquid chromatography system. Their study revealed that serum BPA concentrations in rats were significantly elevated in males more than in females. They reported that the gender difference in serum BPA concentrations may be explained by the difference in clearance based on the UDP-glucuronosyltransferase activities that was elevated in females more than in males. Additionally, Caporossi et al. (2015) [28] revealed that the BPA serum concentrations were higher in male subjects, possibly due to the difference in androgen-related enzyme activity levels, compared with the healthy female subjects, to equal levels of exposure.

Different studies have found elevated oxidative stress levels in poor glycemic than in good glycemic control groups [29]. In our study, we assessed the serum level of MDA as an oxidative stress marker, and it was significantly higher in T2DM group compared to the control group; moreover, there was positive correlation between serum MDA and serum BPA levels. However, there was no statistical significance for this correlation.

This was in agreement with Gunawardena et al. (2019) [30] who reported that MDA levels were significantly higher in T2DM patients than in controls ($p < 0.001^{*}$). They explained that increased nonenzymatic and autooxidative glycosylation is one possible mechanism for free radical-induced lipid peroxidation in DM.

In a study by Moghaddam et al. (2015) [31], they attempted to investigate the effects of BPA on adult male mice's hyperglycemia, lipid abnormalities, and oxidative stress. Their research showed that, in comparison with the control group, the tested groups' blood glucose, lipid profiles, and MDA levels rose as BPA doses increased. Comparing exposed animals to the control group, BPA injection also elevated MDA levels and lowered levels of other antioxidant measures in the pancreas.

In another animal study by Amraoui et al. (2018) [32] to evaluate the role of selenium, vitamin E, and natural antioxidants against BPA-induced oxidative stress, there was significant increase in MDA level after the BPA administration to rats as compared to control ones. While rats treated concurrently with BPA, selenium and vitamin E demonstrated a significant decline in MDA level in relation to BPA-treated group.

In contrast to our study, Erden et al. (2014) [18] revealed that BPA levels were significantly higher in the chronic obstructive pulmonary disease (COPD) patients than control subjects; however, there was no difference between groups for MDA levels. This may be explained by the high MDA levels only during the acute attack and in the untreated cases, and most of their patients were on inhaled corticosteroid, and they were stable in terms of COPD.

To analyze the impact of cellular senescence in T2DM, we assessed the relative expression of GLB1 and TNF-α mRNAs at the transcriptional level, and they were significantly elevated in T2DM patients compared to control subjects and positively correlated to serum levels of BPA; however, there was no statistical significance for this correlation.

Similarly, a study by Sathishkumar et al. (2018) [20] showed significant elevation of senescence markers including GLB1mRNA and proinflammatory markers including TNF-α mRNA at their transcriptional level, and they were elevated in patients with T2DM significantly relative to control subjects.

In a study by Alzamil et al. (2020) [33], they tried to assess the association between TNF-α, obesity, glycemic control, and insulin resistance. Their work revealed that there is correlation between serum level of TNF-α, insulin resistance level, and HbA_{1c} in diabetic patients. They reported that these findings indicate that TNF-α has an important role T2DM pathogenesis through mechanisms linked to insulin peripheral action.

In contrast to our study, Spazzafumo et al. (2017) [34] assessed the plasma β-galactosidase activity by fluorometric assay, and their work revealed that it was significantly reduced in T2DM patients compared to the control group. They reported that this could be explained by different phenomena like the fact that diabetic condition could impair lysosomal disfunction, and that chronic hyperglycemia/diabetic condition could provoke several differences in enzymes implicated in hexoses metabolism, involving galactosidases. Also, high release of proteins originated from lysosomes, and vascular epithelium into the urine may develop from the inflammation induced by hyperglycemia in the kidney vasculature. Moreover, hyperglycemia could affect the cellular secretory abilities.

In agreement with our study, Jain et al. (2020) [35] revealed that TNF-α serum levels were higher in T2DM patients compared to their control subjects; however, there was no relation between both circulatory BPA and TNF-α levels in either group. This could be explained by the accumulation of BPA in adipose tissue, although its blood levels in a cross-sectional study may not indicate its true action level in the tissue. BPA accumulates in adipose tissue because of its lipophilic nature, which may have an impact on adipogenesis and the release of adipocytokines, which are known to have an impact on insulin resistance, beta-cell dysfunction, and diabetes. As a result, altered adipocytokine production from adipose tissue may be one of the mechanisms behind BPA's ability to cause diabetes.

In a similar study by Soundararajan et al. (2019) [19], they studied the link between serum BPA, glycemic control, cellular senescence, and insulin resistance in T2DM patients. Their work revealed significant and positive correlation between serum BPA, GLB1, and TNF-α mRNAs.

Additionally, Soundararajan et al. (2019) [36] treated zebrafish embryos with BPA in the lack and existence of metabolic stress (hyperglycemia). They noticed that the elevation in glucose levels under the effect of hyperglycemia seen in zebrafish embryos was significantly boosted in the presence of BPA. In combination of the metabolic stress and BPA exposure, the transcriptional levels of senescence markers including GLB1 were raised. Their work concluded that BPA potentiated the effect of the metabolic stress which induces increased senescence resulting in aggravation of T2DM.

This study is important due to its novelty to study the relationship between BPA, insulin resistance, and cellular senescence in Egyptian patients with T2DM. Also, with better knowledge of environmental toxicants like BPA, it could be possible to look forward to national and global guidelines and rules to control the use and human exposure to these toxicants.

Conclusion

In Egyptian patients with T2DM, the current investigation was able to identify a link between serum BPA, glycemic control, and insulin resistance. On the other hand, it is important to address some of the study's shortcomings. The study participants were initially limited to Alexandria city. Second, the study's sample size may not have sufficient statistical power to investigate the true link because it is relatively small. Thus, extensive population-based prospective studies with a broad ethnic population are necessary.

Abbreviations

BMI Body mass index

BPA	Bisphenol A
cDNA	Complementary deoxyribonucleic acid
COPD	Chronic obstructive pulmonary disease
dNTP	Deoxynucleotide triphosphate
EDCs	Endocrine disruptor chemicals
ELISA	Enzyme-linked immunosorbent assay
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLB1	Galactosidase beta 1
HBA_{1C}	Glycated hemoglobin
HOMA-IR	Homeostasis model assessment of insulin resistance
HRP	Horseradish peroxidase
JNK	C-Jun N-terminal kinases
MDA	Malondialdehyde
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
ROS	Reactive oxygen species
RT-qPCR	Real-time quantitative polymerase chain reaction
SASP	Senescence-associated secretory phenotype
T2DM	Type 2 diabetes mellitus
TLRs	Toll-like receptors
TMP	Tetramethyl benzidine
TNF-α	Tumor necrosis factor alpha

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Authors' contributions
Yasmin Ibrahim Zanet, Enayat Mohamed Hashem, and Magdy Helmy Megalla provided the study concept and design. Yasmin Ibrahim Zanet and Mohamed Naguib Dessouky performed the experiments and collection of data. Yasmin Ibrahim Zanet and Enayat Mohamed Hashem analyzed and interpreted the data. Yasmin Ibrahim Zanet and Iman Samy Dessouky drafted the manuscript. All authors critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate
Written informed consent of patients and volunteers was obtained. The Ethics Review Board of the Faculty of Medicine at Alexandria University as well as the ethics committee approved the study. 0201511 was the approval number.

Consent for publication
Available consent for publication.

Competing interests
The authors declare that they have no competing interests.

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