



The Relationship Between Heavy Metal Exposure, Trace Element Level, and Monocyte to HDL Cholesterol Ratio with Gestational Diabetes Mellitus

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Abstract

The objective of this study is to assess the levels of heavy metals (cadmium, lead, antimony, mercury, and arsenic), which are also called endocrine-disrupting chemicals, and trace elements (chromium-III, chromium-VI, zinc, copper, and selenium) vs. monocyte to HDL ratio among pregnant women with gestational diabetes mellitus (GDM). A total of 112 pregnant women (60 with GDM and 52 healthy women) were included in this case-control study. Analysis of heavy metals and trace elements were performed in inductively coupled plasma mass spectrometer. Heavy metals (cadmium, lead, antimony, mercury, and arsenic), trace elements (chromium-III, chromium-VI, zinc, copper, and selenium), and metabolic parameters were assessed in both groups. It was determined that the levels of cadmium, lead, antimony, and copper were higher ($p < 0.05$) and levels of chromium-III, zinc, and selenium were lower ($p < 0.05$) among the GDM group compared to the control group, whereas there was a statistically insignificant difference between the two groups, regarding the levels of copper, mercury, and arsenic ($p > 0.05$). Moreover, the monocyte to HDL ratio was higher in the GDM group ($p < 0.05$), and the insulin resistance was significantly higher as well ($p < 0.05$). The results of our study demonstrated that environmental factors could be effective in the etiology of GDM. Toxic heavy metals, through inducing Cu, OS, and chronic inflammation, and other trace elements, either directly by impacting insulin secretion or through weakening the body's antioxidant defense system, could play a role in the occurrence of GDM.

Keywords Gestational diabetes mellitus · Chromium-III · Cadmium · Antimony · Monocyte to HDL ratio

Introduction

Gestational diabetes mellitus (GDM) is one of the most prevalent complications of pregnancy, which is diagnosed for the first time during pregnancy and progresses with different degrees of glucose intolerance [1]. It is a public health issue with

an increasing prevalence, in line with the increasing prevalence of obesity and diabetes mellitus across the world. Even though its prevalence is higher in Far East Asian countries, the International Diabetes Federation estimates this rate as 14% worldwide [2, 3]. GDM prevalence in studies conducted in Turkey is between 4.8 and 6.2% [4, 5]. GDM, with its short-

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and long-term complications, threatens maternal and fetal health. Risk factors of GDM include advanced maternal age, obesity, race, medical history of GDM in previous pregnancies, and history of type 2 diabetes mellitus (T2DM) in parents; however, information related to the impact of environmental factors on GDM is limited [6].

Heavy metals are used in industrial products. Heavy metal exposure occurs through food intake, drinking water, and inhaling polluted air [7]. It has now been determined that environmental chemical exposure could lead to an increase in the incidence of GDM [8, 9]. The fact that it can show toxic effects even in small amounts indicates the significance of heavy metal exposure. Some heavy metals such as cadmium (Cd), arsenic (As), mercury (Hg), antimony (Sb), and lead (Pb) have been termed as endocrine disruptors. It has been revealed that Cd damages β cells through increasing oxidative stress in the pancreas [10]. It has been determined in animal trials that Pb can induce hyperglycemia and glucose intolerance through hepatic gluconeogenesis [11]. Although it is not clearly understood how As affects the formation of GDM, experimental studies have shown that As can affect β cell functions [12]. Moreover, the negative effects of Sb exposure on glucose metabolism during pregnancy have been demonstrated [13]. It has been determined that Hg may play a role in the development of diabetes mellitus through inflammation and oxidative stress [14].

Trace elements, which consist of elements such as copper (Cu), selenium (Se), zinc (Zn), and molybdenum (Mo), are mostly taken with foods. The level of trace elements decreases with oxidative stress, which increases in diabetes mellitus [15]. Se is necessary for the synthesis of selenoproteins, which are known with their antioxidant and cytoprotective properties. Besides, Se has effects such as facilitating the entry of glucose into the cell and regulating the metabolic process, namely, insulin-mimetic [16]. Chromium (Cr) manifests its impact generally through the insulin receptor [17]. It has been determined that the level of Cr in the blood decreases with oxidative stress, which occurs secondary to hyperglycemia [18]. Zn plays an active role in a wide range of different processes, from cytokine production to apoptosis. There are also studies in the literature in which the increase of trace elements compared to the control group is associated with GDM [19].

Oxidative stress (OS) draws the attention as a remarkable component in the pathophysiology of many illnesses [20, 21]. It has been shown that OS increases in GDM, albeit the relationship between GDM and OS has not been explained precisely [21]. Monocyte to high-density lipoprotein cholesterol (HDL-C) ratio (MHR) was considered to be a marker for oxidative stress and inflammation, since monocytes play a vital role in the secretion of circulating pro-inflammatory and oxidant cytokines and due to the fact that the anti-inflammatory and antioxidant properties of HDL-C have been

found out [22–24]. To the best of our knowledge, hitherto MHR has not been studied in GDM.

Although there are studies in the literature related to the relationship of heavy metal exposure and levels of trace elements with GDM, their results are confounding. We aimed in this study to assess the levels of heavy metals (Cd, Pb, Sb, Hg, and As), which are also called endocrine disruptors, and trace elements (Cr-III, Cr-VI, Zn, Cu and Se) vs. MHR among pregnant women with GDM. Besides, we intended to reveal the correlation between these elements and the metabolic parameters of MHR and GDM.

Materials and Method

Our study is a case-control trial and was performed at the Obstetrics and Gynecology Department of Yozgat Bozok University Faculty of Medicine, upon obtaining the approval of the local Ethics Committee (2017-KAEK-189_2018.02.27-09). Written informed consent was obtained from the participants, and the ethical principles of the Declaration of Helsinki were complied with in all phases of the study.

Population of the Study

Our study consists of 2 groups, namely, pregnant women with GDM and healthy (control) pregnant women. Pregestational body mass index (BMI), blood pressure, smoking status, iron, and vitamin supplements were questioned during antenatal follow-ups of pregnant women. Moreover, pregnant women with type I-II diabetes mellitus, hypertension, chronic renal failure, hypo-hyperthyroidism, and heart diseases and those who smoke were excluded from the study.

Diagnosis of GDM

The diagnosis of GDM was made in two phases in our study. Both tests are conducted during fasting. Fifty-gram oral glucose tolerance test (OGTT) was performed to all pregnant women who were between the gestational weeks of 24th and 28th, during routine antenatal follow-ups in our clinic. As a result of this test, 100-g OGTT is applied to pregnant women whose 1st hour serum glucose value (SG) is ≥ 140 mg/dL. There is at least 3 days of interval between the two tests, and pregnant women who will undergo 100-g OGTT are asked to consume at least 150 mg of carbohydrate per day throughout this period. The pregnant women are diagnosed with GDM in the presence of 2 of the following criteria in the 100-g OGTT test [25]: fasting SG > 95 mg/dL, 1st hour SG > 180 mg/dL, 2nd hour SG > 155 mg/dL, and 3rd hour > 140 mg/dL.

Measurement of Biochemical Parameters

Venous blood samples were taken from all participants following 10 to 12 h of fasting, and the samples were centrifuged within 30 to 60 min and kept frozen at -80°C . Furthermore, fasting serum glucose (FSG), total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), and 2nd hour SG were analyzed using assay kits (Abbott®, IL, USA) with an autoanalyzer (Aeroset®, Abbott®, IL, USA). Fasting serum insulin (FSI) was measured by the electrochemiluminescence immunoassay (ECLIA) using commercial kits (Abbott Laboratories) suitable for the autoanalyzer (Architect i2000; Abbott Laboratories, Abbott Park, IL, USA). HbA1c levels were measured using high-performance liquid chromatography (Variant II Turbo, Bio-Rad, Hercules, CA). The cutoff value of SG, HbA1c, TC, TG, and HDL-C are listed below: 109 mg/dL, 6.5%, 200 mg/dL, 200 mg/dL, and 45 mg/dL. The coefficient of variation of SG, FSI, HbA1c, TC, TG, and HDL-C are respectively as follows: 0.7%, 2.0%, 1.6%, 1.1%, 1.1%, and 1.8%. An automated hematology analyzer (BC-6800, Mindray, Shenzhen, China) was used for complete blood count results. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with this formula: $\text{FSG (mg/dL)} \times \text{FSI (mU/mL)} / 405$. IR was considered HOMA-IR > 2.5 .

Measurement of Heavy Metals and Trace Elements

Serum samples were collected for heavy metal and trace element levels measurements and stored at -80°C until being transferred. Subsequently, samples were transferred to Science and Technology Application and Research Center (STARC) Yozgat Bozok University for analysis in a cold chain setting. The samples were pretreated before analysis at this center. In order to perform this phase, 1 mL of each sample was taken and placed in Teflon tubes of microwave digestion unit, which are available in the laboratory (Milestone Ethos, USA). Subsequently, 5 mL of suprapur nitric acid (HNO_3) and 5 mL of ultrapure water were added to these samples, respectively. Samples were dissolved at 180°C for 40 min (Table 1). Afterward, these analyzed samples were transferred into 50-mL polypropylene tubes and completed

with 20 mL of ultrapure water, and the samples were stored at $+4^{\circ}\text{C}$ until analysis.

Analysis of heavy metals and trace elements was performed in inductively coupled plasma mass spectrometer (ICP, Thermo Scientific ICAPQc, USA). An 11-point calibration curve was generated for each element, and internationally validated certified standard samples (CRM, Seronorm, Whole Blood L-2) were used for the validation of these techniques. Plasma power was set to 1500 W and nebulizer pressure to 3.01 bar, and the spray chamber temperature was measured to be 2.9°C . High purity argon gas was used as gas; plasma gas was set to 0.7 L/min, and nebulizer gas was set to 0.9 L/min. The measurement was performed in 0.01 s. Measurements of samples and standard samples were repeated a total of 3 times. The results were obtained as the mean of 3 readings, and it was determined that the relative standard deviation (RSD) did not surpass 5%. Measurement of the blank was performed using 3% nitric acid solution. The r^2 values of the calibration curves were determined to be at least 0.9988.

Statistical Analysis

The statistical analysis was conducted using the software of SPSS 20 (IBM Corp. released 2011. IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.). Data are presented as mean \pm SD, median (interquartile range), and n (%). The normality of the data was tested with the Kolmogorov-Smirnov/Shapiro-Wilks tests. Student t test or the Mann-Whitney U test was performed to compare the continuous variables of the groups, depending on whether they are normally distributed or not. Univariate correlations were analyzed through Spearman's rho test. Categorical data were compared with the chi-square test. The diagnostic performance of the parameters was evaluated by the analysis of the receiver operating characteristic (ROC) curve. The results were considered statistically significant at $p < 0.05$.

Results

Of the 112 pregnant women who participated in our study, 60 were in the GDM, and 52 were in the control group. As presented in Table 2, values of age, pregestational BMI, gravida, and parity were significantly different between the two groups. It was higher among the GDM group, compared to the control group ($p < 0.05$).

FSG, FSI, PPSG, HOMA-IR, HbA1c, triglyceride, HDL-C, and MHR were significantly different between groups. It was determined that FSG, FSI, PPSG, HOMA-IR, HbA1c, triglyceride, and MHR were higher among the GDM group, while HDL-C was higher among the control group (Table 2) ($p < 0.05$). The IR rate was significantly higher in the GDM group (Table 2) ($p < 0.05$).

Table 1 Thawing process steps with microwave oven

	Time (min)	E (W)	T ($^{\circ}\text{C}$)
Conditioning	5	1000	0
Heating	15	1000	180
Waiting	5	1000	
Cooling	20	0	25

Table 2 Comparison of clinical, endocrine, and metabolic parameters in GDM and control groups

	GDM n: 60	Control n: 52	p
Age (years)	32.50 (6.25)	29 (5.00)	< 0.001 ^b
P-BMI (kg/m ²)	26.38 (4.70)	24.61 (1.60)	< 0.001 ^b
Gravidity	3 (2)	2 (2)	< 0.001 ^b
Parity	2 (1)	1 (2)	0.013 ^b
FSG (mg/dL)	87.80 (12.68)	87.80 (6.70)	< 0.001 ^b
FSI (μIU/mL)	12.06 ± 4.79	9.98 ± 3.95	0.014 ^a
PPSG (mg/dL)	113.31 ± 31.10	95.85 ± 13.03	< 0.001 ^a
HbA1C (%)	5.52 (0.91)	5.03 (0.25)	< 0.001 ^b
HOMA-IR	2.62 ± 1.19	1.96 ± 0.81	< 0.001 ^a
Cholesterol (mg/dL)	191.91 ± 22.35	197.47 ± 26.48	0.231 ^a
Triglyceride (mg/dL)	160.50 ± 51.79	136.17 ± 56.18	0.019 ^a
LDL-C (mg/dL)	110.78 ± 19.58	109.06 ± 19.84	0.645 ^a
HDL-C (mg/dL)	48.95 ± 7.24	61.17 ± 10.94	< 0.001 ^a
MHR	10.42 ± 1.81	8.19 ± 1.59	< 0.001 ^a
IR	No	Yes	< 0.001 ^c
	29 (48.3%)	46 (88.5%)	
	Yes	31 (51.7%)	6 (11.5%)

Data are presented as mean ± SD/median (IQR)/n (%). $p < 0.05$ is significant

^a Student *t* test, ^b Mann-Whitney *U* test, ^c chi-square test

GDM gestational diabetes mellitus, P-BMI pregestational body mass index, FSG fasting serum glucose, FSI fasting serum insulin, PPSG post-prandial 2nd hour serum glucose, HOMA-IR homeostatic model assessment insulin resistance index, LDL-C LDL cholesterol, HDL-C HDL cholesterol, MHR monocyte to HDL cholesterol ratio, IR insulin resistance

The levels of trace elements and heavy metals are presented in Table 3. As shown in Table 3, the levels of trace elements including Cr-III, Zn, and Se were lower among GDM group, whereas the level of Cu level was higher among the GDM group ($p < 0.05$). Moreover, the difference between the two groups, regarding the Cr-VI level, was statistically insignificant ($p > 0.05$). Of the heavy metals, Cd, Pb, and Sb levels were higher in the GDM group ($p < 0.05$), whereas the difference between the two groups, regarding the levels of Hg and As, was statistically insignificant ($p > 0.05$).

The correlations between metabolic parameters and trace elements and heavy metals are presented in Table 4. There is a negative correlation between FSG and Cr-III ($r = -0.274$, $p = 0.003$), whereas, there is a positive correlation between Cu ($r = 0.294$, $p = 0.002$), Pb ($r = 0.205$, $p = 0.030$), and Sb ($r = 0.198$, $p = 0.037$) (Table 4, Fig. 1). A negative correlation was determined between PPSG and Cr-III and Zn ($r = -0.294$, $p = 0.002$) ($r = -0.214$, $p = 0.023$), respectively. Of the heavy metals, a positive correlation was found out to be between Pb, Sb, and PPSG ($r = 0.278$, $p = 0.003$) ($r = 0.265$, $p = 0.005$), respectively. A negative correlation was detected

Table 3 Comparison of heavy metals and trace elements between the groups

	GDM n: 60	Control n: 52	p
Cr III (μg/L)	0.19 (0.03)	0.29 (0.08)	< 0.001 ^b
Cr VI (μg/L)	3.62 (0.49)	3.75 (0.42)	0.160 ^b
Se (μg/L)	29.48 ± 9.87	38.21 ± 11.56	< 0.001 ^a
Zn (μg/L)	474.07 (141.70)	545.29 (142.17)	0.003 ^b
Cu (μg/L)	1483.69 (1062.04)	1352.67 (329.69)	< 0.004 ^b
Cd (μg/L)	1.33 (0.42)	1.20 (0.23)	0.005 ^b
Pb (μg/L)	19.76 (15.82)	11.30 (5.24)	< 0.001 ^b
Hg (μg/L)	1.12 (0.52)	1.03 (0.63)	0.278 ^b
Sb (μg/L)	2.51 ± 0.98	2.11 ± 0.71	< 0.001 ^a
As (μg/L)	5.92 (0.93)	6.18 (1.78)	0.172 ^b

Data are presented as mean ± SD/median (IQR)/mean ± SD. $p < 0.05$ is significant

^a Student *t* test, ^b Mann-Whitney *U* test

Cr III chromium trivalent, Cr VI chromium hexavalent, Se selenium, Zn zinc, Cu copper, Cd cadmium, Pb lead, Hg mercury, Sb antimony, As arsenic

Table 4 The correlation of heavy metal and trace elements with oxidative stress marker and glucose metabolism parameters

		FSG	PPSG	HbA ₁ C	HOMA-IR	MHR
Cr III	<i>r</i>	-0.274	-0.294	-0.373	-0.255	-0.419
	<i>p</i>	0.003	0.002	< 0.001	0.007	< 0.001
Se	<i>r</i>	-0.131	-0.165	-0.166	-0.110	-0.337
	<i>p</i>	0.168	0.081	0.080	0.248	< 0.001
Cu	<i>r</i>	0.294	0.129	0.255	0.242	0.229
	<i>p</i>	0.002	0.176	0.007	0.010	0.015
Zn	<i>r</i>	-0.132	-0.214	-0.259	-0.092	-0.058
	<i>p</i>	0.167	0.023	0.006	0.335	0.546
Cd	<i>r</i>	0.111	0.022	-0.067	0.097	0.562
	<i>p</i>	0.243	0.820	0.481	0.308	< 0.001
Pb	<i>r</i>	0.205	0.278	0.125	0.116	0.396
	<i>p</i>	0.030	0.003	0.190	0.223	< 0.001
Hg	<i>r</i>	0.056	0.098	-0.073	0.041	0.159
	<i>p</i>	0.557	0.305	0.445	0.671	0.095
Sb	<i>r</i>	0.198	0.265	0.107	0.224	0.236
	<i>p</i>	0.037	0.005	0.260	0.017	0.012
As	<i>r</i>	-0.023	-0.009	0.019	-0.171	0.001
	<i>p</i>	0.814	0.925	0.844	0.072	0.991

FSG fasting serum glucose, PPSG post-prandial 2nd hour serum glucose, HOMA-IR homeostatic model assessment insulin resistance index, MHR monocyte to HDL cholesterol ratio, Cr III chromium trivalent, Se selenium, Zn zinc, Cu copper, Cd cadmium, Pb lead, Hg mercury, Sb antimony, As arsenic

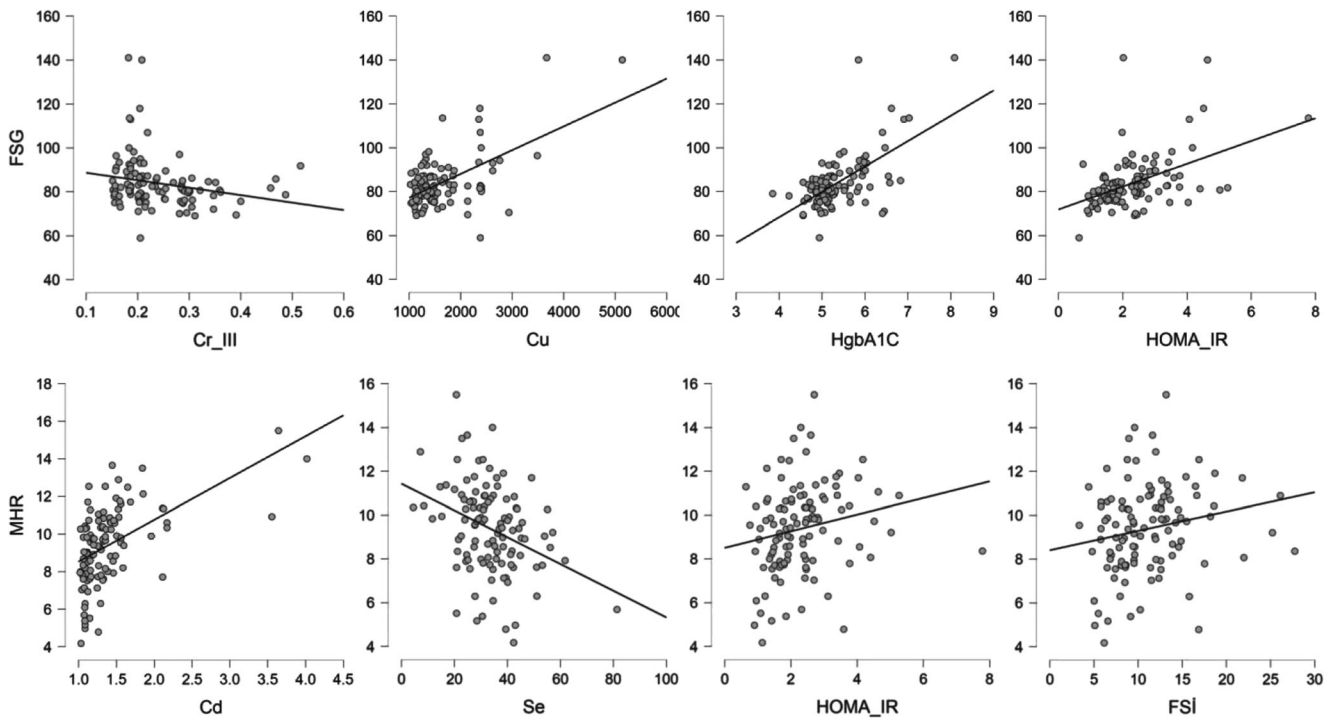


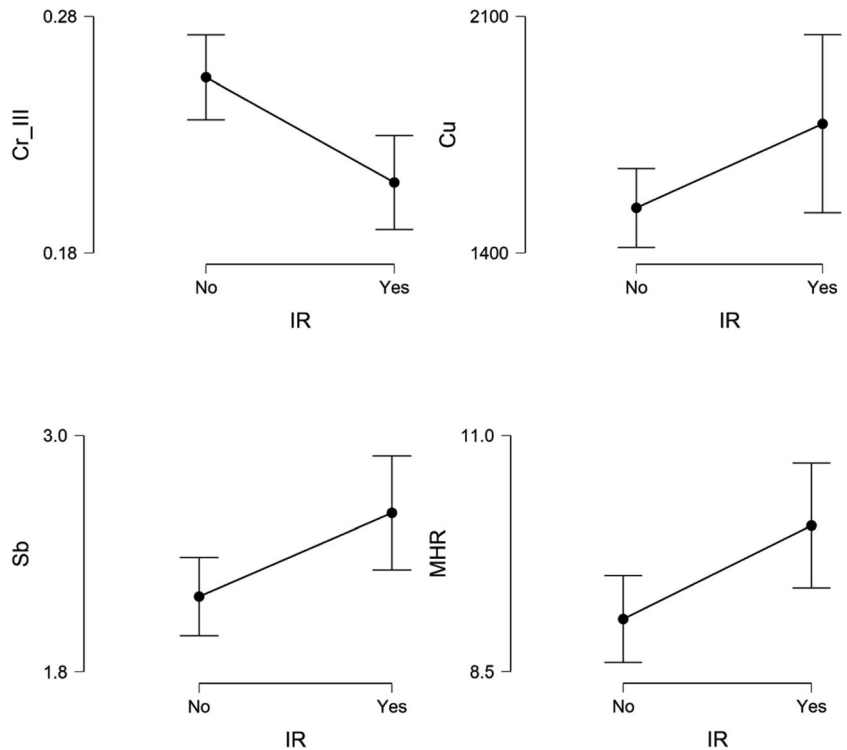
Fig. 1 Scatter plot of relationship between FSG and MHR with some elements and metabolic parameters

between HbA1c and Cr-III and Zn, whereas a positive correlation was detected between HbA1c and Cu, ($r = -0.373$, $p < 0.001$) ($r = -0.259$, $p = 0.006$) ($r = 0.255$, $p = 0.007$), respectively. It has been determined that HOMA-IR was negatively correlated with Cr-III, whereas it was positively

correlated with Cu and Sb ($r = -0.255$, $p = 0.007$) ($r = 0.242$, $p = 0.010$) ($r = 0.224$, $p = 0.017$), respectively.

Furthermore, it was found out that MHR, which is an oxidative stress marker, was negatively correlated with Cr-III and Se ($r = -0.419$, $p < 0.001$) ($r = -0.337$, $p < 0.001$), respectively, whereas it was positively correlated with Cu, Cd,

Fig. 2 Relationship of serum myonectin and irisin levels with IR



Pb, and Sb ($r = 0.229$, $p = 0.015$) ($r = 0.562$, $p < 0.001$) ($r = 0.396$, $p < 0.001$) ($r = 0.236$, $p = 0.012$), respectively (Table 4). In addition to that, a strong relationship was determined between IR and Cr-III, Cu, Sb, and MHR (Fig. 2).

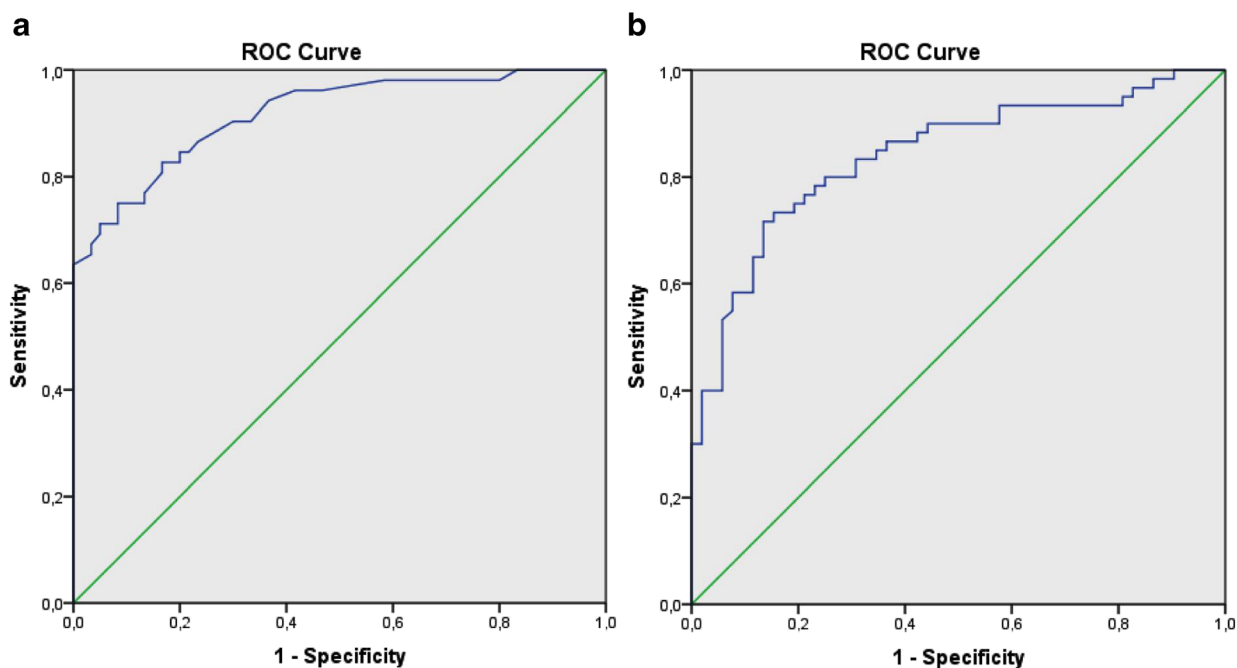
It was found out as a result of the ROC curve analysis of Cr-III and MHR that the cutoff value for Cr-III was 0.213 $\mu\text{g/L}$ with a sensitivity of 82.7% and a specificity of 83.3% (AUC: 0.917) (Fig. 3), while the cutoff value for MHR was 8.97 with a sensitivity of 83.3% sensitivity and a specificity of 69.2% (AUC: 0.840) (Fig. 3).

Discussion

The primary objective of our study is to investigate the correlation of GDM with heavy metal exposure during pregnancy and the levels of trace elements. To the best of our knowledge, our research is the first study in the literature to concurrently assess the heavy metal exposure and trace element levels (especially both forms of chromium) in GDM. It was found out in this study that the serum levels of trace elements such as Cr-III, Se, and Zn were lower among the GDM group, whereas

the serum levels of Cu, which is a trace element, and Cd, Pb, and Sb were higher among the GDM group. Moreover, a correlation was determined between trace elements and heavy metals and glucose metabolism parameters.

Trace elements, which are found in the body as a component of metalloproteins, play an active role in many structural and enzymatic processes. Furthermore, they are a significant part of the antioxidant defense system. The requirement for trace elements increases typically during pregnancy. Cr, which is known for its favorable effects on glucose metabolism, is better absorbed as Cr-III. Cr, which acts through chromodulin that enhances insulin signal [26], also increases insulin receptor count and β cell sensitivity [27]. Król et al. [28] found out that serum glucose level enhanced and Cu to Zn ratio decreased in rats thanks to the Cr-III supplementation. Cr-III supplementation has a favorable impact on individuals with impaired lipid and glucose metabolism rather than healthy individuals [29]. Zhou et al. [30] determined that the serum level of Cr was decreased in T2DM and its complications, such as diabetic nephropathy. The increased levels of Cu are toxic to the cell, albeit it is a trace element. Se is included in the structure of glutathione peroxidase, while Cu



	Cut-off Level	Area Under Curve	Sensitivity (%)	Specificity (%)	P
Cr-III ($\mu\text{g/L}$)	0.213	0.917	82.7	83.3	<0.001
MHR	8.97	0.840	83.3	69.2	<0.001

Fig. 3 ROC curve analysis of Cr-III (a) and MHR (b)

is included in the structure of superoxide dismutase and cytochrome-c oxidase [31]. Cu might lead to oxidative stress directly through the Fenton-like reaction or by reducing the glutathione level [32, 33]. It is well-known that Zn plays a key role in cell division and differentiation. Moreover, it impacts the functions of various hormones, including insulin. The requirement for trace elements increases typically during pregnancy. The potential increase in Cu levels paradoxically decreases Zn absorption [34]. Wilson et al. [35] investigated the correlation between pregnancy complications and serum trace element levels during early pregnancy. They found out as a result of this study that the increased levels of Cu and Zn were associated with pregnancy complications. Another study, which associated Se level with birth weight, also revealed a decreased Se level in preterm births [36]. The levels of Cr-III, Zn, and Se were determined to be lower and Cu levels to be higher in our study, upon comparing women with GDM and healthy pregnant women ($p < 0.05$). On the other hand, the difference between the two groups, regarding Cr-VI, was statistically insignificant. It has been underscored in a meta-analysis, which assessed the correlation between Se and GDM, that the decreased levels of Se are associated with GDM [37]. Another study found out that the increased level of Cu heightens the risk of glucose dysregulation [38]. Mishu et al. [39] identified similar results to ours in patients with GDM.

Cd exposure occurs predominantly through food and smoking cigarettes [40]. It has been well-known for nearly 50 years that Cd has diabetogenic effects [41]. The association of Cd with T2DM and impaired fasting glucose was revealed for the first time in the study of NHANES III [42]. Ting et al. [43] compared 122 newly diagnosed T2DM patients with 429 healthy controls and determined that certain metal levels in plasma, including Cd, were significantly higher among the T2DM group. It has been revealed that Cd induces hyperglycemia and is negatively correlated with the level of insulin [44]. Liu et al. [8] determined in their study, in which they investigated the relationship between Cd level and GDM, that maternal urinary Cd level could be associated with GDM. Soomro et al. [45] found out analogous results in another study. In contrast, Oguri et al. [46] put forward that there was no correlation between Cd exposure and GDM. Cd was determined to be significantly higher among the GDM group in this study. Besides, Cd has the strongest correlation with MHR along with Sb.

Sb exposure, as with the case of other heavy metal exposures, is associated with industrial development. The majority of the population is exposed to chronic low dose Sb. Iyengar et al. [47] has demonstrated that American men aged between 25 to 30 are exposed to a mean of 5 $\mu\text{g}/\text{day}$ Sb. In another study, Sb was detected to be above the range of baseline values for American adults in blood samples of neonates in China [48]. The results of studies, which investigate the correlation between T2DM and Sb, are contradictory. Menke

et al. [49] found out a correlation between T2DM and Sb in their study, whereas in another study, a negative correlation was determined between T2DM and plasma Sb level [50]. Zhang et al. [51] assessed urinary Sb levels in a prospective cohort study in which 2093 pregnant women were included. Ultimately, they highlighted that an increased Sb exposure could be associated with GDM. It has been revealed in another study that the risk of GDM increases even with lower levels of Sb exposure, and it could lead to deterioration of glucose metabolism in pregnant women. Our study also supports these results. In addition to these similar findings, deterioration was also detected in metabolic parameters such as FSG, PPSG, and HOMA-IR, proportional with the Sb level.

The effects of Pb exposure occur in a dose dependent manner. Pb inhibits glutathione reductase and delta-aminolevulinic acid dehydrogenase, which are members of the antioxidant defense system [52]. High dose Pb exposure has an increased risk for GDM, when high dose exposure and low dose exposure are compared [45]. Excluding occupational exposure, the community is subjected to Pb exposure through environmental pollution and smoking [53, 54]. Pb levels in maternal blood samples were compared with obstetric results. Jelliffe-Pawlowski et al. [55] found out that a Pb level of 10 mg/dL in maternal blood increased the risk of preterm delivery and low birth weight. Whereas some studies have found out a correlation between maternal Pb level and GDM [45, 56], other studies have determined that the maternal Pb level is not correlated with the GDM [46, 57]. Maternal Pb levels were significantly higher among the GDM group in our study. Moreover, it has been found out that it correlates positively with FSG and PPSG.

Hg and As are among the toxic heavy metals. It is well-documented that the risk of diabetes increases in the environments, which are remarkably contaminated with As [58]. MIREC study has indicated that the As exposure could be a risk factor for GDM [57]. The same research did not determine a correlation between Hg and GDM [57]. In another study, no correlation was identified between inorganic As and GDM among 230 pregnant women whose urine samples were taken [59]. Ettinger et al. [60] underscored in their study, in which they assessed the As level in maternal hair samples, that As exposure might be associated with the increased risk of GDM. A review, which has examined the metabolic effects of Hg exposure, suggested that the risk of diabetes mellitus and metabolic syndrome may rise with the increased Hg exposure, yet there is not any compelling evidence to explain this cause-effect relationship [61]. No significant difference was determined between the two groups, regarding Hg and As levels, in our study.

OS might occur secondary to hyperglycemia, as it might also lead to GDM. Heavy metals could also cause GDM through increasing OS. For instance, it is well-documented that Cd damages β cells via OS [10]. MHR is a method that

demonstrates oxidative stress and chronic inflammation [62–64]. To the best of our knowledge, MHR has not been previously assessed in patients with GDM. We determined that MHR was significantly higher among the GDM group. In addition to this, MHR is negatively correlated with Se and Cr-III and positively correlated with Cu, Cd, Pb, and Sb. These findings suggest that OS might play a role in the pathophysiology of GDM, regardless of its cause.

Heavy metal exposure and trace elements may play a key role in GDM, albeit the mechanism is not fully understood. Oxidative stress, which is caused by heavy metal exposure, inflammation, inhibition of peroxisome proliferator-activated receptor-gamma, and modification of diabetes-related genes are considered to be the mechanisms that may be effective in the occurrence of GDM [45, 65]. Trace elements can affect this process positively in a direct way through insulin secretion and receptors, and they can also be effective by boosting tissues in their fight against the oxidative stress. Moreover, even though Cu is a trace element, increased levels of it could lead to the occurrence of GDM through increasing oxidative stress [26, 27, 31–33].

Our study has some limitations: Firstly, it has a small sample size. Secondly, demonstrating OS with other OS markers apart from MHR could increase the strength of the study. Another limitation was that the measurement of heavy metal and trace element levels was performed with the material, which was taken from a single sample. Hair, urine, umbilical cord blood, and meconium specimens could be used as well. Furthermore, the robustness of the study was tried to be enhanced by excluding the circumstances that could create a situation of risk for GDM, change the levels of heavy metals, and lead to chronic inflammation, when generating the trial and control groups. In addition to that, various heavy metals and trace elements were assessed in the same participants.

The results of our study demonstrated that environmental factors could be effective in the etiology of GDM. Toxic heavy metals, through inducing Cu, OS, and chronic inflammation, and other trace elements, either directly by impacting insulin secretion or through weakening the body's antioxidant defense system, could play a role in the occurrence of GDM. From this point of view, our results will shed light on further studies; nevertheless, studies with larger sample groups are required to validate our results.

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Analysis or interpretation: T.O., D.A.K., E.S.Y.

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Data Availability The data set has been uploaded to the system.

Compliance with Ethical Standards The study was approved by the Yozgat Bozok University Clinical Research Ethics Committee (2017-KAEK-189_2018.02.27-09), and an informed consent was obtained from all participants.

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

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