ORIGINAL ARTICLE

Maternal Serum Copper and Some Metabolic Indexes in Late Second Trimester of Gestational Diabetes Mellitus Pregnancy

Mariana P. Genova 1, Irena Ivanova 2, Emilia Naseva 3, Milena Velizarova 1, Bisera Atanasova 1

1 Department of Clinical Laboratory, Faculty of Medicine, Medical University of Sofia, Alexander University Hospital, Sofia, Bulgaria
2 Clinical Laboratory Department, St. Ivan Rilski University Hospital, Medical University of Sofia, Bulgaria
3 Department of Health Economics, Faculty of Public Health "Prof. Tsekomir Vodenicharov, MD, DSc", Medical University of Sofia, Bulgaria

SUMMARY

Background: Copper (Cu) is a physiologically important trace element during pregnancy. The study aim is to assess the altered level of serum Cu and its association with some metabolic indexes in Gestational Diabetes Mellitus (GDM).

Methods: A total of 108 pregnant women (aged 18 - 40, second trimester) are included in the study and divided into two groups (GDM n = 54; pregnant with normal glucose tolerance (NGT), n = 54) after performing a 2-hour 75-g oral glucose tolerance test (OGTT). Maternal blood samples are collected at 26 - 28 gestational week. All biochemical parameters are measured in serum from fasting venous blood. Serum Cu levels are analyzed by flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 300, USA). Body Mass Index (BMI), insulin sensitivity/resistance, triglyceride-glucose (TyG), TyG-BMI (triglyceride glucose-body mass) indexes are calculated by formulas.

Results: The following data were observed: significantly higher levels of serum Cu (p = 0.009), pre-pregnancy BMI (pre-pBMI), BMI at the GDM diagnosis (pBMI), TyG, pregnancy TyG-BMI (pTyG-BMI) p < 0.001, and triglycerides (Tgl) (p = 0.02) in GDM compared to NGT pregnancy. The study presents a positive correlation between serum Cu and pre-pBMI (p < 0.02), pBMI and pTyG-BMI (p < 0.001). Besides, pre-pBMI (mean ≥ 25 kg/m²), pBMI (mean ≥ 30 kg/m²), and pTyG-BMI are associated with 14.5% (OR 1.145, 95% CI: 1.064 - 1.232; p < 0.001), 15.3% (OR 1.153, 95% CI: 1.070 - 1.243; p < 0.001), and 5.9% (OR 1.059, 95% CI: 1.022 - 1.086; p < 0.001) increased risk for GDM development. No association is found between Cu and Tgl levels, fasting plasma glucose (FPG) and TyG. ROC analysis suggests the serum Cu as a possible risk factor for GDM development. The analysis shows that at a cutoff point of ≥ 31.9 µmol/L, serum Cu presents a sensitivity and specificity of 64.8% and 66.7% in the prediction of GDM development (AUC = 0.659, p < 0.012). After adjustment for maternal age, gestational age, and family predisposition, the odds ratios (ORs) (95% CIs) still show association of Cu levels with increased GDM risk (OR 1.099, 95% CI 1.018 - 1.184, p = 0.013).

Conclusions: pTyG-BMI index exhibits a better interaction than TyG index, Tgl, and glucose separately with serum Cu levels where BMI has a mediator’s role.


Correspondence:
Assoc. Prof. Mariana P. Genova, MD, PhD
Department of Clinical Laboratory
Faculty of Medicine, Medical University of Sofia
Alexander University Hospital 1
Sv. G. Sofiyski Str. 1431
Sofia
Bulgaria
Phone: + 359 2 9230927
Email: mariana8sofia@yahoo.com

KEYWORDS
copper, gestational diabetes mellitus, triglyceride-glucose body mass index, triglyceride glucose index

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INTRODUCTION

Gestational diabetes (GDM) is a hyperglycemic state recognized for the first time during pregnancy [1], and its pathophysiology is not fully clarified yet. GDM is one of the most common complications in pregnancy with an increasing percent of prevalence, especially in recent decades (≥ 20% of pregnancies in some parts of the world), both in developed and developing countries, due to increased average age of pregnant women and increased obesity [2]. The prevalence in Europe is 6.1% (1.8 - 31.0%) though the rates among European countries vary widely [3].

The pathophysiology of GDM is not fully understood but it is linked to hormonal imbalances affecting insulin sensitivity (IS) and to pancreatic β-cell dysfunction [4]. Obesity is a modifiable risk factor for GDM, especially visceral obesity in pregnant women [5]. Furthermore, pre-pregnancy overweight or obesity (BMI ≥ 25 kg/m²) is implicated as the most significant risk factor for GDM [6]. By turns, GDM increases the risk, in both mother and offspring, of developing type 2 diabetes mellitus (T2DM), metabolic syndrome, and obesity [7, 8].

The state of obesity is characterized by the adipose tissue inflammation, induces islet β-cell dysfunction, and leads to systemic insulin resistance (IR) and changes in glucose tolerance [9]. Furthermore, IR and β-cell dysfunction in pregnant women are associated with dyslipidemia, an independent predictor for GDM [10]. Close relationship between IR and glucose with lipid metabolism has been established. The TyG index as a product of fasting glucose and Tgl as a non-insulin-based index is proposed as a useful parameter for identifying IR by Simental-Mendía et al. [11]. The efficiency in assessing IR may be improved when TyG is combined with some other obesity indicators such as BMI and such index obtained is namely TyG-BMI. This is a new obesity-related, non-insulin-based parameter developed and used in recent years as a marker for IR [12].

Trace elements are important for humans, but their specific effects on health are not well understood, particularly during pregnancy and especially in pregnancy complicated with GDM. In serum, the majority of Cu is bound to ceruloplasmin (Cp). Total serum or plasma Cu and Cp are both widely used as biomarkers of Cu status. Copper concentration in serum significantly increases by 40% among the pregnant women and this elevation is observed during the three trimesters of pregnancy [13]. The possible mechanism about the role of Cu in gestational diabetes is based on the interaction of sex hormones with Cu metabolism [14].

The aim of the present study is to assess the relation between altered serum Cu levels and pre-pBMI, pBMI, and some metabolic indexes in a complex clinical environment of pathological elevated IR.

MATERIALS AND METHODS

This case-control study was carried out in women in late second trimester of GDM pregnancy (26 - 28 gestational week; gw), during May 2018 - October 2020, after being approved by Medical University Ethics Review Board (Ethics approval number: 45/324/06.03. 2018). The study is conducted in accordance with the Declaration of Helsinki [15].

Informed consent was obtained and signed from each enrolled pregnant woman. The study population included 54 pregnant women with newly diagnosed GDM before treatment and 54 healthy pregnant women based on OGTT results.

Exclusion criteria were smoking, multiparity, pregnancy with complications (severe heart, liver, and kidney diseases) or mental disorders, chronic hypertension, stillbirth, preeclampsia, eclampsia, fetoplacental abnormalities, presence of pre-gestational diabetes, presence of GADA-antibodies, previous pregnancy with GDM, patients with long-term glucocorticoid therapy and other drugs affecting glucose levels. The selection of all participants was carried out from a cohort of pregnant women without anamnestic data about polycystic ovary syndrome (PCOS).

Information about maternal socio-demographic characteristics as maternal age, smoking habit, drinking habit, gestational age, height and weight before pregnancy and during pregnancy, parity, and gestational age at enrollment was collected using a structured questionnaire. All identifying patient information was anonymized to protect patient identity.

Diagnostic Criteria for GDM

The diagnosis of GDM is made based on 2-hours 75-g OGTT results. According to the criteria of International Association of Diabetes and Pregnancy Study Groups, if any of OGTT results meet or exceed the following criteria: FPG ≥ 5.1 mmol/L; 1-hour post-glucose load ≥ 10.0 mmol/L; and 2-hour post-glucose load ≥ 8.5 mmol/L, the pregnant women were diagnosed with GDM (American Diabetes Association 2021) [16].

Design of the study

The study participants were instructed to fast from 10 p.m. the night before. Blood samples were collected from the antecubital vein. About 7 mL of fasting venous blood was drawn from each woman enrolled by an experienced laboratory assistant. After that, every participant was given a 300 mL water solution of 75-g of glucose and instructed to drink it within 5 minutes for OGTT. Venous blood samples were collected at 60 and 120 minutes.

Fasting venous blood samples were centrifuged for 10 minutes at 3,000 rpm to obtain blood serum. The released serum samples for Cu were stored at refrigerated temperature for up to 2 days until the same analysis. Part of the aliquoted serum in plastic microcentrifuge tubes was frozen at -80°C for analysis of leptin. Serum
leptin levels were determined using a Human Leptin ELISA kit (DiaSource Immuno Assays). Leptin ELISA (enzyme-linked immunosorbent assay) is a solid phase enzyme-linked immunosorbent assay, based on the sandwich principle and is used according to manufacturer’s protocol. The range of the assay is between 0.7 ng/mL and 100 ng/mL. The working range for leptin was assessed from the precision profile and defined as the concentration range with coefficient of variation < 9.6%. FPG, 1-hour-, and 2-hour-blood glucose levels post-load were analyzed by amperometric method (Analyzer Biosen C-line, Germany).

Analysis of lipids: Total Cholesterol (TC) was measured with CHOD-PAP method; Triglycerides (TG) measured with GPO-PAP method; Low Density Lipoprotein Cholesterol (LDL-C) measured with homogenous enzymatic colorimetric assay and High-Density Lipoprotein Cholesterol (HDL-C) measured with homogenous enzymatic colorimetric assay using commercially available kits (Roche Diagnostics, Germany) using the automatic analyzer Cobas 6000 (Roche Cobas 6000 Chemistry Analyzer Roche Diagnostics, Germany). C-reactive protein (CRP) and ceruloplasmin (Cp) were measured with immunoturbidimetric assay, using commercially available kits (Roche Diagnostics, Germany) using the automatic biochemical analyzer Cobas Integra 400 Plus, (Roche Diagnostics, Germany). Fast- ing serum insulin (FSI) concentration was measured with an electrochemiluminescence immunoassay (ECLIA) using a commercially available kit (Roche Diagnostics, Germany) on an automatic analyzer Elecsys 2010 (Roche Diagnostics, Germany). Serum Cu levels were analyzed by flame atomic absorption spectrophotometry (Perkin Elmer Analyst 300, USA) with CV% day-to-day variation being 3.5% and bias < 1%. Analytical quality was also guaranteed by regular participation for serum Cu in systems of EQAS: National System of Quality Assurance and INSTAND-Germany. Pre-pBMI is a self-reported weight, expressed in kilograms (kg) and the height, expressed in squared meters (m²) during the interview are used to calculate maternal BMI (kg/m²) and is classified as underweight (pre-pBMI ≤ 18.5), normal (18.5 ≤ pre-pBMI ≤ 24.9), overweight (pre-pBMI 25 - 29.9) and obese (pre-pBMI ≥ 30.0) (https://www.who.int/europe/news-room/factsheets/item/a-healthylifestyle-who-recommendations).

The detailed procedure for defining pregnancy TyG-BMI was as follows: TyG-BMI = BMI × TyG index, where TyG index = ln [FPG (mg/dL) / TG (mg/dL)/2] and BMI = weight/height² [17]. TyG index is calculated by formula: ln [TG (mg/dL) × FPG (mg/dL)/2] [11]. The insulin resistance score was assessed as homeostatic model assessment (HOMA index) using the HOMA2 calculator. This calculator is available at http://www.dtu.ox.ac.uk/homacalculator/index.php (updated January 8, 2013). QUICKI index was calculated as 1/[\log_{10} (FPG [mg/dL]) + \log_{10} (FI [µU/mL])] [18]. The ratios leptin/BMI and insulin/BMI were calculated.

Statistical methods
All data were analyzed using IBM Statistical Package for Social Science (SPSS) software Windows version 23. Kolmogorov-Smirnov test (sample size > 50) is used for normality testing the continuous data in this study. Comparisons between groups are performed using Mann-Whitney or independent samples “t” test. Binary logistic regression is used to assess the significant factors for binary output (gestational diabetes or healthy pregnancy). Results are expressed as frequencies or percentages for qualitative variables and mean (± SD) in normally distributed quantitative variables and median and interquartile range (IQR; both 25th and 75th percentile) in not normally distributed ones. Furthermore, the area under the curve (AUC) was calculated by receiver operating characteristic (ROC) curves. The optimal cutoff value of each risk factor was calculated at the maximum Youden Index. Second, univariate and multivariate binary logistic regression analyses are performed on the Cu levels and GDM to estimate an independent association between the mentioned trace element and risk of GDM development at late second trimester and the results are presented as odds ratio (OR) with 95% confidence interval (95% CI).

RESULTS
Baseline characteristics of the study population are shown in Table 1. The results are presented as medians and IQRs for the cases and controls. The pre-pBMI, pBMI, TyG index, pTyG-BMI, levels of Cu and Tgl are higher in women with GDM than NGT pregnant (p < 0.05) at this period of pregnancy. The mean differences in Tgl levels are ~0.6 mmol/L between two pregnant groups and the difference is to an approximately 18 to 20% increase in Tgl levels in women with GDM (the average Tgl level in women with GDM is 2.3 mmol/L; in NGT it is 1.7 mmol/L). Similar significant difference is observed for FPG, HOMA-IR, QUICKI index, CRP, leptin, leptin/ BMI ratio, FSI.

The recommendation about weight gain in pregnancy and rates of weight gain varied between different pre-pBMI groups and are presented in Table 2. To better understand the relationship between Cu and some metabolic indexes and parameters in GDM pregnancy, the associations between serum Cu and Tgl, TyG, pTyG-BMI, pBMI, pre-pBMI, HOMA-IR, QUICKI index, FPG, and FSI are examined. The association between serum Cu levels and some metabolic indexes of interest are presented at Table 3. Univariate analysis of the data in the GDM group revealed a positive association between Cu levels and pre-pBMI (r = 0.419, p = 0.02; Figure 1), Cu and pBMI (r = 0.390, p < 0.001; Figure 2), Cu and pTyG-BMI (r = 0.433, p < 0.001; Figure 3). As shown in Table 3, weak positive/negative correlations are observed for Cu levels and HOMA-IR.
Table 1. The baseline characteristics of pregnant women in the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GDM pregnant women (n = 54)</th>
<th>NGT pregnant women (n = 54)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD, years)</td>
<td>32 ± 5.8</td>
<td>30.4 ± 5.1</td>
<td>0.078</td>
</tr>
<tr>
<td>Maternal age over 40 years n (%)</td>
<td>7 (12.9)</td>
<td>4 (7.4)</td>
<td>0.38</td>
</tr>
<tr>
<td>Pre-pBMI (mean ± SD, kg/m²)</td>
<td>27.6 ± 7.2</td>
<td>23.3 ± 4.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>pBMI (mean ± SD, kg/m²)</td>
<td>30.4 ± 7.0</td>
<td>26.1 ± 4.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gestational age at blood drawing (mean ± SD, weeks)</td>
<td>26.5 ± 3.2</td>
<td>25.9 ± 2.8</td>
<td>0.522</td>
</tr>
<tr>
<td>Family history of diabetes n (%)</td>
<td>15 (27.7)</td>
<td>6 (11.1)</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Smoking habit n (%)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Drinking habit n (%)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>FPG (mmol/L) median (IQR)</td>
<td>5.3 (5.1 - 5.4)</td>
<td>4.4 (4.1 - 4.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OGTT-1h (mean ± SD, mmol/L)</td>
<td>8.3 ± 2.3</td>
<td>6.7 ± 1.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OGTT-2h (mean ± SD, mmol/L)</td>
<td>6.0 (4.7 - 7.6)</td>
<td>5.0 (4.4 - 6.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>FSI (µU/mL) median (IQR)</td>
<td>10.3 (5.4 - 16.1)</td>
<td>7.3 (5.0 - 10.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMA-IR median (IQR)</td>
<td>2.0 (1.3 - 3.4)</td>
<td>0.9 (0.6 - 1.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>QUICKI index median (IQR)</td>
<td>0.3 (0.3 - 0.4)</td>
<td>0.4 (0.3 - 0.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Copper (mean ± SD, µmol/L)</td>
<td>31.9 ± 6.9</td>
<td>28 ± 5.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Ceruloplasmin (ng/L) median (IQR)</td>
<td>0.56 (0.5 - 0.6)</td>
<td>0.6 (0.5 - 0.6)</td>
<td>0.623</td>
</tr>
<tr>
<td>CRP (mg/L) median (IQR)</td>
<td>5.4 (2.6 - 7.6)</td>
<td>3.3 (1.4 - 5.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Leptin (ng/mL) median (IQR)</td>
<td>55.6 (29 - 109)</td>
<td>25.4 (14.7 - 42.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC (mmol/L) median (IQR)</td>
<td>6.1 (5.1 - 7.0)</td>
<td>5.7 (5.0 - 6.4)</td>
<td>0.126</td>
</tr>
<tr>
<td>Tgl (mmol/L) median (IQR)</td>
<td>2.3 (1.8 - 2.9)</td>
<td>1.7 (1.2 - 2.5)</td>
<td>0.024</td>
</tr>
<tr>
<td>LDL-C (mmol/L) median (IQR)</td>
<td>3.58 (2.9 - 4.48)</td>
<td>3.15 (2.89 - 3.83)</td>
<td>0.061</td>
</tr>
<tr>
<td>HDL-C (mean ± SD, mmol/L)</td>
<td>1.72 ± 0.43</td>
<td>1.73 ± 0.44</td>
<td>0.967</td>
</tr>
<tr>
<td>TyG index median (IQR)</td>
<td>5.0 (4.8 - 5.1)</td>
<td>4.7 (4.5 - 4.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>pTyG-BMI (mean ± SD)</td>
<td>149.9 ± 35.3</td>
<td>122.1 ± 19.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leptin/BMI median (IQR)</td>
<td>2.23 (1.01 - 3.90)</td>
<td>0.64 (0.01 - 1.36)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin/BMI median (IQR)</td>
<td>0.36 (0.21 - 0.50)</td>
<td>0.27 (0.18 - 0.39)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

BMI - body mass index, CRP - C-reactive protein, FPG - fasting plasma glucose, FSI - fasting serum insulin, GDM - gestational diabetes mellitus, HDL-C - high-density lipoprotein cholesterol, HOMA-IR - homeostasis model assessment of insulin resistance, LDL-C - low-density lipoprotein cholesterol, OGTT-1h - 1-h post-glucose load, OGTT-2h - 2-h post-glucose load, pTyG-BMI - pregnancy triglyceride-glucose-body mass index, Tgl - triglycerides, TyG index - triglyceride-glucose index, TC - total cholesterol, QUICKI - quantitative insulin sensitivity check index, p < 0.05.
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Table 2. Recommended weight gain ranges in pregnancy according to pre-pregnancy BMI. Source: The US Institute of Medicine [35].

<table>
<thead>
<tr>
<th>Pre-Pregnancy BMI (kg/m²)</th>
<th>Recommended Weight Gain (kg)</th>
<th>Rates of Weight Gain Second and Third Trimester, Average (kg/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td>12.5 - 18</td>
<td>0.51</td>
</tr>
<tr>
<td>18.5 - 24.9</td>
<td>11.5 - 16</td>
<td>0.42</td>
</tr>
<tr>
<td>25.0 - 29.9</td>
<td>7.0 - 11.5</td>
<td>0.28</td>
</tr>
<tr>
<td>≥ 30</td>
<td>5.0 - 9.0</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Table 3. Correlation coefficients between serum Cu levels and metabolic indexes in GDM pregnant women.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Copper (µmol/L)</th>
<th>Spearman’s rho correlation coefficient</th>
<th>p-value</th>
<th>Copper (µmol/L)</th>
<th>Spearman’s rho correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate analysis, GDM pregnant</td>
<td></td>
<td></td>
<td>Univariate analysis, NGT pregnant</td>
<td></td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>0.115</td>
<td>0.408</td>
<td>0.047</td>
<td>0.253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pBMI</td>
<td>0.419</td>
<td>≤ 0.021 ***</td>
<td>0.217</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pBMI</td>
<td>0.390</td>
<td>≤ 0.001 **</td>
<td>0.196</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSI (µU/mL)</td>
<td>0.286</td>
<td>≤ 0.036 *</td>
<td>-0.042</td>
<td>0.730</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (OGTT1h) µU/mL</td>
<td>0.347</td>
<td>&lt; 0.01 **</td>
<td>0.051</td>
<td>0.677</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTyG-BMI</td>
<td>0.433</td>
<td>&lt; 0.001 ***</td>
<td>0.107</td>
<td>0.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TyG</td>
<td>0.119</td>
<td>0.390</td>
<td>-0.145</td>
<td>0.235</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tgl (mmol/L)</td>
<td>0.130</td>
<td>0.348</td>
<td>-0.111</td>
<td>0.363</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>-0.041</td>
<td>0.771</td>
<td>-0.148</td>
<td>0.226</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.291</td>
<td>&lt; 0.033 *</td>
<td>-0.149</td>
<td>0.222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>-0.292</td>
<td>≤ 0.032 *</td>
<td>0.098</td>
<td>0.425</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.225</td>
<td>0.102</td>
<td>0.362</td>
<td>0.002 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>-0.166</td>
<td>0.264</td>
<td>0.202</td>
<td>0.160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin/BMI</td>
<td>-0.227</td>
<td>0.126</td>
<td>0.112</td>
<td>0.359</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin/BMI</td>
<td>0.136</td>
<td>0.327</td>
<td>-0.104</td>
<td>0.394</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


* - Correlation is significant at the 0.05 level
** - Correlation is significant at the 0.01 level. The significant correlations are underlined.

The incidence of GDM is increased with increasing level of pre-pBMI, pBMI, and pTyG-BMI. Every 1-SD increase in every one of these parameters: pre-pBMI (mean value ≥ 25 kg/m²), pBMI (mean value ≥ 30 kg/m²), and pTyG-BMI is associated with 14.5%, 15.3%, and 5.9% increase in the risk of developing of GDM (OR 1.145; 95% CI: 1.064 - 1.232; p < 0.001); (OR 1.153; 95% CI: 1.070 - 1.243; p < 0.001); (OR 1.059; 95% CI: 1.022 - 1.086; p < 0.001), respectively. The univariate logistic regression demonstrated that Cu
Figure 1. Spearman’s correlation between serum copper levels and BMI before pregnancy. GD, gestational diabetes.

Figure 2. Spearman’s correlation between serum copper levels and BMI during pregnancy (pBMI). GD, gestational diabetes.
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Figure 3. Spearman’s correlation between serum copper levels and TyG-BMI during pregnancy (pTyG-BMI). GD, gestational diabetes.

Figure 4. ROC analysis of serum copper levels as a risk factor for GDM development in the mid pregnancy. AUC 0.659, p < 0.012 (95% CI 1.019 - 1.161).
levels are significantly associated with GDM (OR 1.098 (95% CI 1.019 - 1.181, p = 0.012). Adjusted odds ratios (ORs) (95% CIs) for controlling maternal age, gestational age and family predisposition (at least 1 of the following: mother, father, grandmother, grandfather) showed Cu levels continuing to be associated with increased GDM risk (OR 1.099 (95% CI 1.018 - 1.184, p = 0.013).

A ROC analysis is performed to determine the scope of serum Cu levels to be used as a possible risk factor for GDM evolution. Analysis of the result indicated that at a cutoff point of ≥ 30.9 μmol/L, serum Cu measurements showed a sensitivity and specificity of 64.8% and 66.7%, respectively, in order to predict GDM development (AUC = 0.659, p < 0.012) (Figure 4).

**DISCUSSION**

The presented study demonstrates significant differences in serum Cu levels among pregnant women with GDM and NGT. The results are in agreement with the published meta-analysis by Lian et al. [14], enrolling 14 relevant articles. They strongly support that the serum Cu concentrations in GDM women are remarkably increased in comparison to the pregnant women without GDM. Currently, related research on the levels of serum Cu concentration in GDM are limited with inconsistent results. A couple of studies indicate that no statistically significant differences are found in serum Cu concentrations between healthy pregnant and women with GDM [19,20]. Other studies found significantly higher values of serum Cu in GDM individuals in the second trimester [21,22].

Normally, maternal Cu concentration increases during pregnancy, from early pregnancy, and reportedly plateaus by the 2nd trimester and is doubled at full term [23]. Probably, the hormonal changes during pregnancy could be part of the explanation for higher circulating Cu concentrations observed in pregnant women in comparison to non-pregnant females. Our previous pilot study showed a highly significant statistical increase of serum Cu in the GDM group and in NGT pregnant patients in comparison to healthy non-pregnant controls [24]. The pointed differences may be a result of several factors such as geographic and cultural proximity, indicating that local dietary and environmental factors influence Cu concentrations [25].

Healthy pregnancy is characterized with mildly increased lipidemia occurring in early pregnancy with an increase of serum concentration of Tgl after 10 gw and a more pronounced elevation during the second and third trimesters [26]. Data have been proposed for hypertriglyceridemia as one of the most prevalent conditions in GDM. Most studies show that circulating lipid levels are different between GDM and normal pregnancy. This finding is consistent in the 1st, 2nd, and 3rd trimesters of pregnancy [27]. The pointed higher Tgl level findings in the present study are similar to most of those in the meta-analyses by Hu et al. [28] and Rahnamaei et al. [29]. The role of Cu in lipid metabolism is recently emerged [30]. Understanding the complex relationship between Cu and lipids during GDM is an important knowledge for the role of Cu in pathophysiological processes of GDM and its influence on the fetus. The results of this study present no significant association between Cu and Tgl levels in both groups. Tgl levels could be influenced by several variables such as BMI. So far, limited studies examine the relationship between serum Cu concentration, pre-pBMI, and pBMI in late second trimester of pregnancy. The present results about association between pre-pBMI and Cu concentrations are similar to other studies in European populations of pregnant women during the first and the second trimesters [31]. No data are found about the link between Cu and pBMI in the late second trimester of GDM.

Some authors demonstrate a positive association between Cu levels in serum or adipose tissue and BMI with a presumption that Cu status is strongly related to BMI. According to Yang et al., Cu levels positively correlate with leptin, insulin, and leptin/BMI ratio in non-pregnant, healthy individuals, suggesting that Cu and/or cuproproteins may be functionally linked to fat accumulation [30]. The reported investigation of leptin/BMI ratio does not point to an association with serum Cu in both pregnant groups.

Increasing interest is focused in TyG index [11] as a marker for IR. Nevertheless, the presented study does not investigate the relationships between OGTT-derived IR indexes and TyG. However, studies on TyG potential and relevance in pregnancy are still relatively scarce and most of them analyze the connection between TyG level and GDM risk [32]. TyG index is significantly different for GDM and NGT groups in the present study and the results are in accordance with other published works [33,34]. To our surprise, TyG index is not associated with serum Cu in contrast to other indexes of IR. According to our latest information, this is the first study to examine the connection between pregnancy TyG index and the levels of Cu during the second trimester of pregnancy.

TyG-BMI combines Tgl, FPG, and obesity status and has better diagnostic value in the differentiation of IR [17]. The presented study finds that TyG-BMI is a remarkably better surrogate marker than the traditional TyG to assess a connection between Cu levels, Tgl, and serum glucose in mid GDM pregnancy. We hypothesize that the serum Cu is not directly connected with Tgl, glucose, and derivative TyG index. Also, we suggest that Cu needs a mediator at this GDM stage with pathological IR manifestation. In this context, we assume the potential mediation effect of BMI between serum Cu, Tgl and glucose levels. The TyG-BMI index is better than TyG index. Tgl and glucose separately in their tie-up to Cu levels.

It is important to underline the significant role of BMI in interactions and influences, specific for GDM.
ferences in BMI values before and during pregnancy highlight the importance of obesity-related pathogenetic factors contributing to increasing IR, carbohydrate, and metabolic changes in women with GDM. Therefore, the effect of weight changes over the follow-up of pointed above connections is important [35]. Furthermore, we suggest that a number of other factors participate in these mechanisms: copper-proteins, pro-inflammatory factors, and some specific changes in the second trimester of GDM pregnancy. Finally, associations between BMI, Tgl, and glucose and Cu levels in GDM at late second trimester might be causal elements in the pathway for the development of GDM. However, up to now, the observations on the relationship between Cu concentration, GDM, and new, non-insulin-based index TyG-BMI are extremely limited. According to our information from PubMed sources about such research, this is the first study to examine the complicated link between TyG-BMI index and serum Cu levels in the second trimester of pregnancy.

Copper exhibits positive correlations with GDM [22] and is directly involved in developing this pathology independent of other established risk factors [36]. Zhu et al. found that Cu and other trace elements are significantly associated with the risk of GDM in early pregnancy [37]. Previous retrospective studies show a positive correlation between high plasma Cu levels and the risk of GDM; however, until now, studies on the relationship between Cu concentration and GDM at second trimester of pregnancy are limited [14,22]. Based on certain studies during different trimesters of pregnancy, serum Cu level could be considered as a possible risk factor for GDM developing. The presented results of the study are in concordance with the above-mentioned information and studies by Lian et al. [14] and Li et al. [22]. The advantages of the present study show a positive and significant correlation between new indexes pTyG-BMI, pre-pBMI, and pBMI and serum Cu levels in the late second trimester of GDM pregnancy. Furthermore, here the reported results suggest a role of Cu as a possible risk factor for GDM development at this phase of GDM pregnancy.

One limitation of the current investigation is the relatively small sample size in both studied groups. Second, due to the nature of the case-control study, the specific mechanisms and the causal associations could not be explored. Therefore, a larger sample size and well-designed cohort studies are warranted for further exploration and verification. Despite the aforementioned limitations, this study had several strengths. First, we explored the association between Cu levels, pre-pBMI, pBMI, and pTyG-BMI index and their role in determining the risk of GDM. To our knowledge, it is the first study to explore these interactions at late second trimester of GDM pregnancy. Second, we established that the Cu levels are a possible risk factor for GDM development at this time of the pregnancy.

CONCLUSION

The results of the study demonstrate that the pTyG-BMI index has a better potential than TyG index, Tgl, and glucose separately in the connection to altered Cu levels with a possible mediator role of BMI. The incidence of GDM increases with increasing level of pre-pBMI, pBMI, and pTyG-BMI indexes. The altered Cu levels might be a risk factor for GDM development with pathological elevation of IR at the late second trimester. An eventual role of Cu levels in GDM, especially in this specific phase of the pregnancy, is indicated. The current clinical study is a step ahead in understanding the complicated pathophysiology of GDM. It is focused on the cross-talks between copper as a dual trace element (on the one hand essential for life, especially in pregnancy and, on the other hand, in high concentration with toxic effects) and metabolic imbalance in GDM. It is an example of modern knowledge of trace elements in precision medicine: genetic predisposition, individual life style, and influence of certain environmental factors. In any case, such integrated scientific and clinical approach is a step ahead in better care for women with GDM not only during pregnancy but even further after this specific physiological period and even better care for the offspring. Undoubtedly, this is the most important outcome of the current research.

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Authors declare no conflict of interest.

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