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Serum Bilirubin Concentrations and Insulin Resistance in Advanced Maternal Age without Diabetes Mellitus

Short Title: Serum Bilirubin and IR in Pregnant Women without DM

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Abstract: Aim: We estimated the correlation between serum bilirubin concentrations and insulin resistance in advanced maternal age without diabetes mellitus. **Method:** The cross-sectional study involved consecutive 50 women of advanced maternal age without diabetes mellitus, and insulin resistance (IR) was diagnosed according to the homeostatic model assessment insulin resistant (HOMA-IR) formula and the cut-off point was set to more than 2.5. Serum bilirubin and HOMA-IR were compared between the IR (+) and IR (-) groups. **Results:** We found that serum bilirubin levels in the IR subjects were significantly decreased compared to those without IR ($p = 0.0252$). Serum bilirubin concentrations were negatively correlated with the weight and the fasting insulin concentrations in all subjects ($r=-0.282$, $p=0.047$, $r=-0.424$, $p=0.002$). Additionally, serum bilirubin had a negatively correlation with HOMA-IR ($r = -0.410$, $p = 0.003$) in all subjects. The multiple linear regression analysis found that serum total bilirubin levels were independently correlated with HOMA-IR (beta = -0.392, $p= 0.015$) in these subjects. **Conclusions**: The study demonstrated that serum bilirubin concentrations had negatively correlation with insulin resistance in advanced pregnant women without diabetes mellitus, and decreased serum bilirubin may forebode increased gestational diabetes mellitus risk in our study population.

Keywords: serum bilirubin; pregnant women; insulin resistance; advanced maternal age

Introduction

Advanced maternal age is considered as over 35 years of age at the time of delivery in our nation [1]. aged has been considered as a main risk factor for adverse pregnancy outcomes, especially for gestational diabetes mellitus [2–4]. Pregnancy is characterized by a series of metabolic changes, especially in late gestation, and normal pregnancy is characterized by insulin-mediated glucose, and insulin secretion increases by more than 200% to maintain the mother's euglycemia [5–7]. During a normal pregnancy, pro- and anti-inflammatory cytokines have a fine balance for normal development [8]. However, chronic enhanced insulin resistance may disturb this balance, which is involved with the development of diabetes mellitus risk in pregnant women [8,9].

Bilirubin, as an end-product of heme catabolism, is an important indicator to assess liver function. Interestingly, recently literatures have reported that serum bilirubin had strong anti-inflammatory and anti-oxidative features [10]. The linear evidences have demonstrated that lower serum bilirubin was demonstrated to be associated with rheumatic disease, cardiovascular disease and diabetes mellitus [11–13]. From the above view, lower serum bilirubin may increase the risk of these diseases by the oxidative stress and inflammation mechanisms [13–15]. Serum bilirubin as an anti-inflammatory molecule has been suggested to be associated with insulin resistance in diabetes

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mellitus patients [16]. However, the correlation between serum bilirubin and insulin resistance has not been understood in subjects without diabetes mellitus, especially in advanced maternal age. Thus, we estimated the correlation between serum bilirubin concentrations and insulin resistance in these pregnant women without diabetes mellitus.

Patients and materials

Study population

The cross-sectional study included consecutive 50 women of advanced maternal age without diabetes mellitus who undergone pregnant health examinations at the Suzhou Hospital of Anhui Medical University. All women were diagnosed as intrauterine pregnancy by menstrual history, pregnancy tests and ultrasonic examinations in outpatient clinics. Pregnant women (≥35 years) were included in this study. Exclusion criteria were defined: diabetes mellitus, hepatic and renal disorders, active infection, malignant tumor and mental disorders. Our study was approved study was approved by the Ethics Committee of Zhongda Hospital,School of Medicine, Southeast University.

Anthropometric and biochemical data

We collected the maternal baseline clinical and laboratory characteristics, such as age, weight, height, gestational weeks and blood pressure. Moreover, laboratory indexes included serum bilirubin, fasting blood-glucose (FBG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST),. In the present study, we used IR to assess the homeostatic model assessment (HOMA), and HOMA-IR index was calculated by formula (http://www.dtu.ox.ac.uk/) [17,18]. Insulin resistance was considered to be present if the HOMA-IR value was > 2.5 according to the findings of the study [19].

Statistical analysis

Data were analyzed statistically by using SPSS 19.0 software. Continuous variable were exhibited as mean ± standard deviation and median (interquartile range). We checked the homoscedasticity of continuous variables with Kolmogorov-Smirnov test. We used the Student's t-test, Mann–Whitney U tests and Chi-square tests where appropriate for the differences comparison between the two groups. The correlations of serum bilirubin with other continuous variables were estimated by Spearman analysis. The diagnostic efficiency of serum bilirubin in evaluating insulin resistance was analyzed by using a receiver operating characteristic (ROC) curve. Multiple linear regression analysis was adopted to determine these correlations in all subjects. p values < 0.05 had significant statistical difference.

Results

1 Clinical features in all participants

Table 1 showed baseline clinical characteristics non-diabetic pregnant women. The median age of pregnant women in this study was 36 years. The median level of serum bilirubin was 8.0 μmol/L. The insulin resistance was defined if the HOMA-IR index was greater than 2.5 [19]. Thus, according to this standard, we divided these subjects into IR(+) groups and IR(-) groups in Table 2. We found that serum bilirubin levels in the IR groups were significantly decreased compared with individuals without IR (p = 0.0252) (Figure 1).

2 The correlation between serum bilirubin and selected indexes

The correlations of serum bilirubin with other clinical parameters were evaluated in all subjects, as shown in Table 3. We found serum bilirubin had negatively correlations with the weight and fasting insulin concentrations in all subjects (r =-0.282, p = 0.047, r = -0.424, p = 0.002). Furthermore, serum bilirubin levels were found to be correlated with HOMA-IR (r =-0.410, p =0.003) in subjects without diabetes mellitus.

3 Serum bilirubin and HOMA-IR in multivariate linear regression analysis

In order to exclude the effects of other factors on the correlation of serum bilirubin and HOMA-IR in the study population, a multivariate linear regression analysis was used in this study. The

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multivariate linear regression analysis results suggested that serum bilirubin levels still were correlated with HOMA-IR (beta = -0.392, p = 0.015) in all subjects, when age, body mass index, gestational weeks and FBG were considered as independent variables

4 The ROC curve analysis for predicting IR subjects in whole pregnant women

We used the ROC curve analysis estimate the performance of serum bilirubin in IR in the study population. Our results showed that the cut-off points and AUC for serum bilirubin in predicting IR pregnant women were 5.7 umol/L and 0.70, respectively. Insulin-resistant pregnant women could be recognized with well sensitivity 94.9% and specificity 36.4% in Figure 2.

Disscusion

Our study was first to analyze the correlation of serum bilirubin with insulin resistance in pregnancy of advanced maternal age without diabetes mellitus. We found that IR(+) subjects had lower serum bilirubin levels. The present study main found that serum bilirubin maintained negatively correlation with insulin resistance in pregnant women of advanced maternal age without diabetes mellitus in multiple linear regression analysis, thus, this study suggests that serum bilirubin levels may evaluate gestational diabetes mellitus risk in the study individuals.

Recently, several studies showed that higher serum bilirubin levels can prevent metabolic syndrome, cardiovascular disease and diabetes mellitus [20–22]. It have been reported that the inverse relationship of serum bilirubin with HOMA-IR in patients abdominal obesity [20], insulin resistance [23] and diabetes mellitus [13,24]. After adjusting for conventional risk factors, serum bilirubin was negatively correlated with HOMA-IR in these pregnant women without diabetes, suggesting that decreased serum bilirubin concentrations may forebode increased gestational diabetes mellitus risk during pregnancy. The mechanism for the correlation may be unclear in the present study. There are several possible mechanisms. First, serum bilirubin is a potent endogenous antioxidant that protects cells from oxidative stress [13,14], recent studies revealed that serum bilirubin significantly reduced hyperglycemia and increased insulin sensitivity [23], this protective effect of serum bilirubin may be its antioxidant actions in patients with insulin resistance. Second, serum bilirubin has protective effect on inflammation [13]. We all know that C-reactive protein levels (CRP) is a sensitive inflammation marker, and serum bilirubin was found to be inversely proportional to CRP in several studies. Middle-aged women have a reduction in insulin sensitivity and in individuals with impaired glucose tolerance, pancreatic β cell function falls and obesity [25], and aging is related to a low-grade inflammation known as “inflamm-aging” [26]. Thus, pregnant women of advanced maternal age are prone to low inflammation during pregnancy [8]. We found serum bilirubin levels were lower in IR subjects without diabetes mellitus, the finding supports the possibility that lower serum bilirubin could be implicated in increased low-grade systemic inflammation in these pregnant women. Finally, heme products, including biliverdin and its key enzymes, have been also considered to be the possible factors for preventing diabetes mellitus [13], heme oxygenase and biliverdin reductase are given with potent antidiabetic and insulin sensitizing effects [27]. Hence, the oxidative stress and inflammation may explain the interesting phenomenon in our study.

The study has several limitations. First, the study was a small sample in the cross-sectional design. Second, there were no assessments the relation between serum bilirubin and classical inflammatory parameters in clinical laboratory. Third, the study did not analyze the relation between serum bilirubin concentrations and gestational diabetes. In conclusion, the study demonstrated that serum bilirubin concentrations had negatively correlation with insulin resistance in pregnant women of advanced maternal age without diabetes mellitus, and decreased serum bilirubin concentrations may forebode increased gestational diabetes mellitus risk in our study population in our study population.

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