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ORIGINAL ARTICLE

Maternal circulating levels of some metabolic syndrome biomarkers in gestational diabetes mellitus

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Abstract The aims of the present study were to explore serum levels of lipid profile, atherogenic indexes LDL-C/HDL-C, TG/HDL-C, and TC/HDL-C, bilirubin, adiponectin, pseudocholinesterase, activities of gamma-glutamyltransferase (GGT), adenosine deaminase (ADA), and α-amylase, insulin resistance using homeostasis model assessment of insulin resistance (HOMA-IR) formula, and assessment of correlations between them in gestational diabetes mellitus (GDM) compared with normal pregnancy. A cross-sectional study was designed. The patients consisted of 30 women with GDM. The normal group consisted of 30 healthy pregnant women. The Mann–Whitney *U* test and Spearman's correlation analysis were used for statistical analysis. A p value less than 0.05 was considered significant. Serum activities of enzymes GGT ($p = 0.001$) and ADA ($p = 0.02$) were significantly higher in GDM compared with normal pregnancy, while pseudocholinesterase were significantly decreased ($p = 0.02$). However, activity of enzyme α-amylase did not show significant difference between two groups ($p = 0.75$). Serum levels of both HDL-C and adiponectin were significantly decreased in GDM group ($p = 0.001$). The atherogenic indexes and the HOMA-IR index were significantly higher in GDM ($p = 0.001$). Serum activity of ADA showed positive correlation with total cholesterol (TC) ($r = 0.46$, $p = 0.01$) and CRP ($r = 0.66$, $p = 0.001$) in GDM group. Serum levels of total bilirubin correlated negatively with both ADA ($r = -0.38$, $p = 0.04$) and triglyceride ($r = -0.45$, $p = 0.01$) in women with GDM. Serum activity of GGT correlated positively with LDL-C ($r = 0.48$, $p = 0.01$) and TC ($r = 0.52$, $p = 0.003$) in GDM group. Increased atherogenic indexes, ADA, GGT, and decreased pseudocholinesterase might be risk factors for GDM.

Keywords Adenosine deaminase · Amylase · Atherogenic index · Gamma-glutamyltransferase · Insulin resistance · Gestational diabetes mellitus

Introduction

Gestational diabetes mellitus (GDM) is defined as any degrees of impaired glucose tolerance that first appears or is recognized during pregnancy. The prevalence of GDM ranged from 1 to 15 % in all pregnancies depending on the ethnic group studied and diagnostic criteria employed [1, 2]. It has been shown that low serum α-amylase activity is associated with prevalence of metabolic syndrome [3], diabetes [4, 5], and non-alcoholic fatty liver [6] but little data are available in the patients with GDM. There are some conflicting findings of maternal circulating levels of lipids in women with GDM compared with normal pregnancy [7]. It has been reported that increase in gamma-glutamyltransferase (GGT) activity is a risk factor for GDM [8–11]. On the other hand, the enzyme has been proposed as a practical marker to estimate the level of oxidative stress in clinical practice [12–14]. Some studies have reported that pseudocholinesterase could be a marker for metabolic syndrome [15] and atherosclerosis [16]. However, the findings of maternal serum activity of the enzyme in GDM and its association with metabolic

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syndrome biomarkers are conflicting [11, 17, 18]. It has been reported that bilirubin in normal range shows inverse correlation with metabolic syndrome risk factors in type 2 diabetes [19] but little has been found in GDM. There are few reports about serum activity of adenosine deaminase (ADA) in GDM and its association with biomarkers of metabolic syndrome, inflammation, oxidative stress, and insulin resistance [20]. The aims of the present study were (i) to explore circulating levels of metabolic syndrome biomarkers in GDM compared with normal pregnancy, (ii) to use them for explaining some metabolic features of GDM and (iii) to introduce them as probable biomarkers for clinical practice of GDM. Therefore, serum levels of lipid profile including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), atherogenic indexes including LDL-C/HDL-C, TG/HDL-C and TC/HDL-C ratios, bilirubin, adiponectin, pseudocholinesterase, activities of GGT, ADA, and α-amylase, insulin resistance using homeostasis model assessment of insulin resistance (HOMA-IR) formula, and correlations between them were assessed in GDM compared with normal pregnancy.

Materials and methods

This cross-sectional study was conducted at the Obstetrics and Gynecology Hospital of the University after approving by the Institutional Ethical Review Board. Informed consent was obtained from each pregnant woman enrolled in this study. The study population consisted of 30 women with GDM and 30 normal pregnant women who were matched in BMI. All subjects were Iranian with Lor ethnicity. All participants were screened for gestational diabetes at 24–28 weeks of gestation. The diagnosis of gestational diabetes was made according to 75-g oral glucose tolerance test (OGTT) when at least one or more of the following glucose values are elevated: fasting ≥92 mg/dl; 1 h ≥180 mg/dl; and 2 h ≥153 mg/dl [21]. Exclusion criteria were smoking, multiple gestation, diabetes mellitus, chronic hypertension, preeclampsia, inflammatory diseases, hepatitis, tuberculosis, obesity (BMI ≥ 30 kg/m^2), and the patients who were under treatment with insulin.

Commercially available photometric methods were used to measure serum levels of glucose, TC, TG, creatinine, direct and total bilirubin, GGT, aspartate transaminase (AST), alanine transaminase (ALT), α-amylase (Parsazmun, Karaj, Iran), ADA (ZiestChem Diagnostic, Tehran, Iran), and HDL-C (Pishtazteb, Tehran, Iran) using a chemistry analyzer (Hitachi, Germany). C-reactive protein (CRP) was assessed by commercially available method (Enison, Tehran, Iran). The intra-assay and inter-assay coefficients of variations of the methods were <10 % according to the manufacturers. Serum LDL-C levels were estimated by Friedewald's equation [22]. The atherogenic indexes including the LDL-C/HDL-C, the TG/HDL-C, and the TC/HDL-C ratios were also calculated [23, 24].

Commercially available enzyme-linked immunosorbent assay (ELISA) methods were used to measure serum levels of total adiponectin (BioVendor Laboratory Medicine, Inc. Czech Republic), insulin (Monobind Inc., USA), ferritin (Pishtazteb, Tehran, Iran), and pseudocholinesterase (HANGZHOU EASTBIOPHARM CO., LTD., China) using an ELISA reader (STAT FAX 3200, USA). The intra-assay coefficient of variations of all the ELISA assays was <10 % according to the manufactures. The sensitivities of the assays were 26 ng/ml, 0.75 μIU/ml, 1, and 0.25 ng/ml, respectively, according to the manufactures. Insulin resistance was calculated using the HOMA-IR index formula [25].

Statistical calculations were carried out using the Mann–Whitney *U* test and Spearman's correlation analysis. The data that are presented in Table 2 are raw values and not adjusted for age. However, comparison of the values of ADA, GGT, and pseudocholinesterase was also done between GDM and normal pregnant women after adjusting for age. A p value <0.05 was considered statistically significant. Data are presented as mean ± SEM. Statistical computations were carried out using SPSS 19 software.

Results

The anthropometric characteristics of women with GDM and normal pregnancy are presented in Table 1. There were no significant differences in gestational age and BMI between two groups. Women with GDM had a higher mean age than control group. The biochemical characteristics of GDM and control groups are presented in Table 2. Women with GDM had higher levels of fasting plasma glucose (FPG), 1- and 2-h plasma glucose than normal pregnancy. Serum activities of enzymes GGT and ADA were significantly increased in GDM compared with normal pregnancy. Serum levels of enzyme pseudocholinesterase were significantly decreased in GDM. However, circulating activities of enzymes α-amylase, AST, and ALT were not significantly different between two groups. There was no statistically significant difference in serum levels of TC, TG, LDL-C, creatinine, CRP, uric acid, ferritin, and direct and total bilirubin between two groups. Serum levels of both HDL-C and adiponectin were significantly lower in GDM group than in normal pregnant women. Values of the HOMA-IR index and the atherogenic indexes including the LDL-C/HDL-C, the TG/HDL-C, and the TC/HDL-C ratios

Table 1 Demographic characteristic of gestational diabetes mellitus (GDM) and normal pregnant women

Parameter	Normal pregnant	GDM	*p*
Age (years)	28.53 ± 0.94	32.63 ± 0.72	0.001
Gestational age (weeks)	25.03 ± 0.18	25.53 ± 0.19	0.10
Gravidity	1.57 ± 0.13	1.70 ± 0.13	0.40
Parity	0.43 ± 0.12	0.57 ± 0.12	0.36
SBP (mmHg)	104.00 ± 1.11	107 ± 1.55	0.09
DBP (mmHg)	72.00 ± 1.14	75.00 ± 1.63	0.08
Pre-pregnancy BMI (kg/m^2)	24.84 ± 0.28	25.00 ± 0.23	0.61
BMI at OGTT (kg/m^2)	26.97 ± 0.29	27.59 ± 0.23	0.15

Data are presented as mean ± SEM

SBP systolic blood pressure, *DBP* diastolic blood pressure, *BMI* body mass index, *OGTT* oral glucose tolerance test

Table 2 Biochemical characteristic of gestational diabetes mellitus (GDM) and normal pregnant women

Parameter	Normal pregnant	GDM	*p*
Fasting plasma glucose (mg/dl)	76.80 ± 1.03	104.07 ± 3.34	0.001
1-h plasma glucose (mg/dl)	119.10 ± 3.26	201.70 ± 4.90	0.001
2-h plasma glucose (mg/dl)	99.33 ± 2.88	172.10 ± 4.37	0.001
Creatinine (mg/dl)	0.73 ± 0.04	0.74 ± 0.02	0.94
Uric acid (mg/dl)	4.31 ± 0.16	4.00 ± 0.17	0.16
Total bilirubin (mg/dl)	0.21 ± 0.03	0.18 ± 0.02	0.45
Direct bilirubin (mg/dl)	0.12 ± 0.01	0.11 ± 0.00	0.36
TG (mg/dl)	223.97 ± 18.51	278.73 ± 23.17	0.07
TC (mg/dl)	256.13 ± 12.56	234.90 ± 11.51	0.10
HDL-C (mg/dl)	62.07 ± 2.18	36.90 ± 3.25	0.001
LDL-C (mg/dl)	149.27 ± 9.70	142.25 ± 12.66	0.60
TG/HDL-C ratio	3.65 ± 0.31	8.64 ± 0.76	0.001
TC/HDL-C ratio	4.16 ± 0.20	7.66 ± 0.72	0.001
LDL-C/HDL-C ratio	2.43 ± 0.17	4.92 ± 0.67	0.001
CRP (mg/l)	4.63 ± 0.73	7.04 ± 1.10	0.07
Ferritin (ng/ml)	60.68 ± 6.87	61.50 ± 10.21	0.24
Insulin (μIU/ml)	11.89 ± 0.38	14.32 ± 0.65	0.002
HOMA-IR	2.33 ± 0.08	3.57 ± 0.16	0.001
Adiponectin (μg/ml)	5.05 ± 0.29	3.73 ± 0.24	0.001
Alanine transaminase (U/l)	12.70 ± 0.96	12.73 ± 0.96	0.94
Aspartate transaminase (U/l)	18.13 ± 1.37	15.46 ± 0.85	0.17
α-Amylase (U/l)	75.23 ± 5.27	73.07 ± 4.35	0.75
Pseudocholinesterase (ng/ml)	62.68 ± 7.75	40.25 ± 5.21	0.02
Gamma-glutamyltransferase (U/l)	12.83 ± 1.41	60.17 ± 3.91	0.001
Adenosine deaminase (U/l)	17.73 ± 0.79	21.93 ± 1.67	0.02

Data are presented as mean ± SEM

TG triglyceride, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *CRP* c-reactive protein, *HOMA-IR* homeostasis model assessment of insulin resistance

were significantly increased in GDM. Values of GGT, ADA, and pseudocholinesterase were also compared between two groups after adjusting for age. Adjusted values of GGT (33.98 ± 1.58 vs. 39.07 ± 1.23, $p = 0.01$) and ADA (19.77 ± 0.09 vs. 19.93 ± 0.08, $p = 0.03$) were remained significantly lower, and values of pseudocholinesterase (53.33 ± 1.00 vs. 49.27 ± 0.95, $p = 0.01$) were remained significantly higher in normal pregnancy than in GDM.

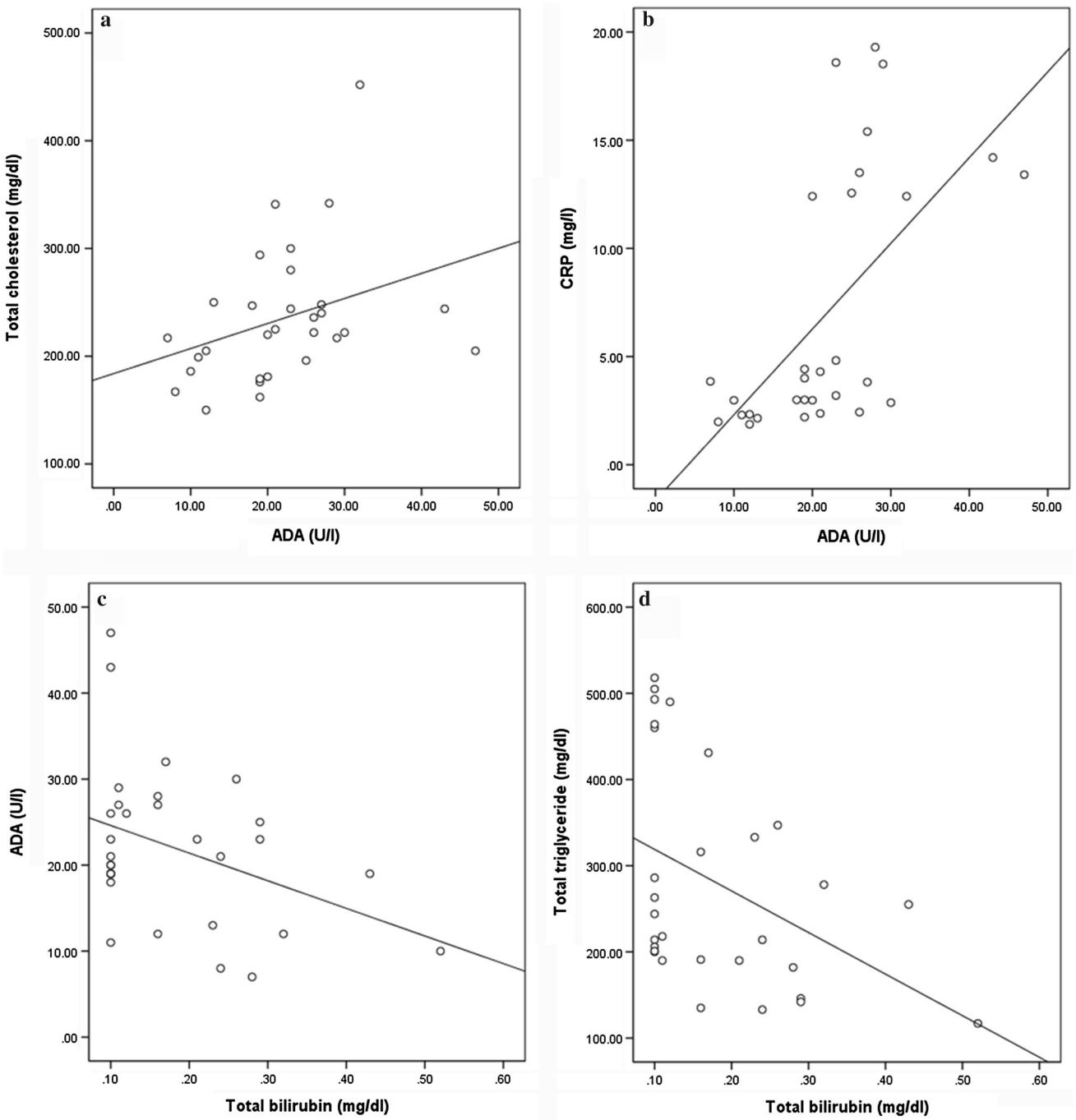

Fig. 1 Correlations between **a** adenosine deaminase (ADA) activity and total cholesterol levels ($r = 0.46$, $p = 0.01$), **b** ADA activity and C-reactive protein (CRP) levels ($r = 0.66$, $p = 0.001$), **c** ADA activity and total bilirubin levels ($r = -0.38$, $p = 0.04$), and **d** total bilirubin and total triglyceride levels ($r = -0.45$, $p = 0.01$) in 30 women with gestational diabetes mellitus

Serum activity of ADA showed positive correlation with TC ($r = 0.46$, $p = 0.01$) (Fig. 1a) and CRP ($r = 0.66$, $p = 0.001$) (Fig. 1b) in GDM group. Serum levels of total bilirubin correlated negatively with both ADA ($r = -0.38$, $p = 0.04$) (Fig. 1c) and TG ($r = -0.45$, $p = 0.01$) (Fig. 1d) in women with GDM. Serum activity of GGT correlated positively with LDL-C ($r = 0.48$, $p = 0.01$) (Fig. 2a), TC ($r = 0.52$, $p = 0.003$) (Fig. 2b), the LDL-C/HDL-C ratio ($r = 0.41$, $p = 0.03$) (data not shown), and the TC/HDL-C ratio ($r = 0.41$, $p = 0.03$) (data not shown)

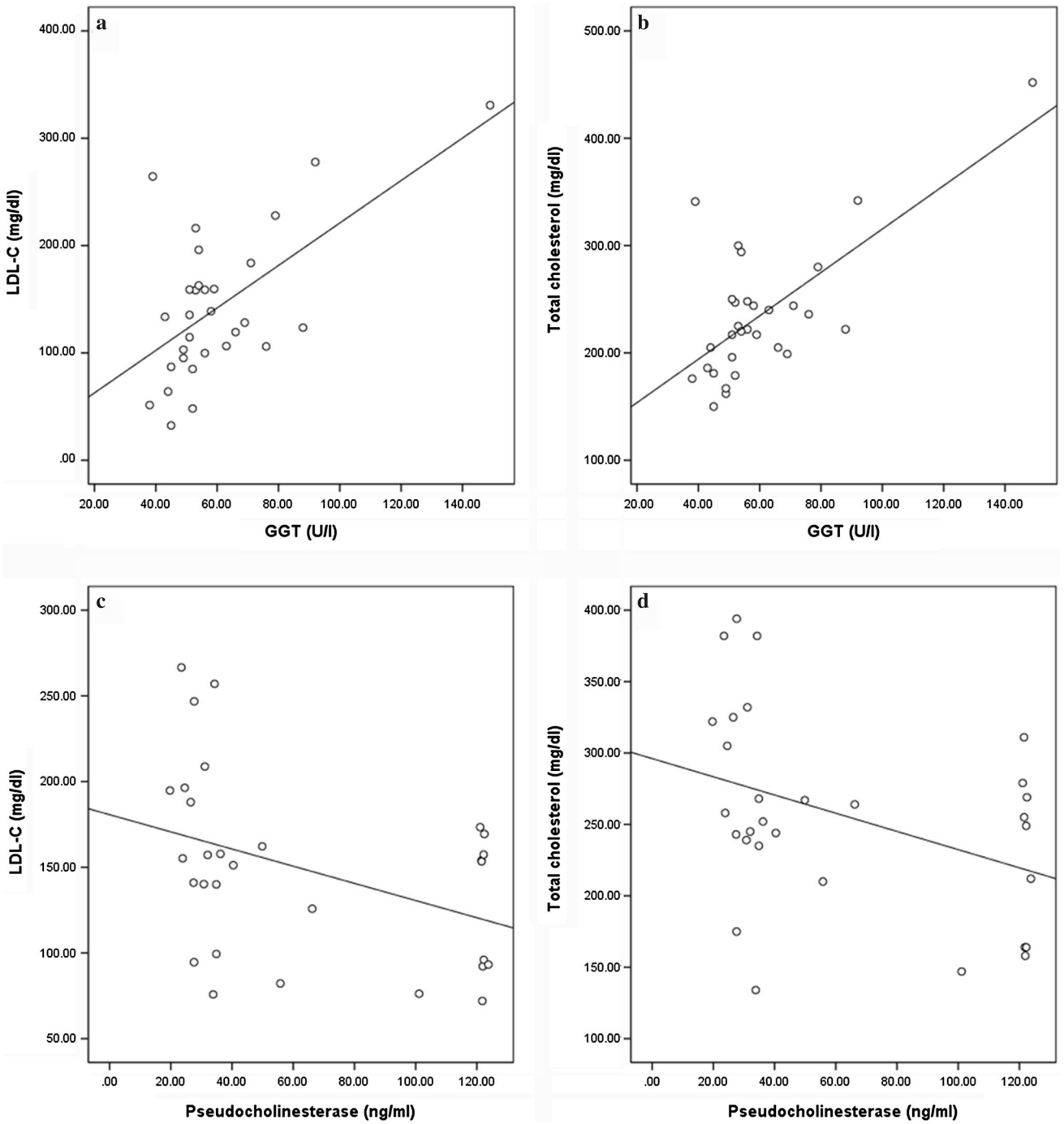

Fig. 2 Correlations between **a** gamma-glutamyltransferase (GGT) activity and low-density lipoprotein cholesterol (LDL-C) levels ($r = 0.48$, $p = 0.01$) and **b** GGT activity and total cholesterol levels ($r = 0.58$, $p = 0.003$) in 30 women with gestational diabetes mellitus. Correlations of serum pseudocholinesterase levels with **c** LDL-C ($r = -0.45$, $p = 0.01$), and **d** total cholesterol ($r = -0.41$, $p = 0.03$) levels in 30 normal pregnant women

in GDM group. In normal control group, pseudocholinesterase showed negative correlation with both LDL-C ($r = -0.45$, $p = 0.01$) (Fig. 2c) and TC ($r = -0.41$, $p = 0.03$) (Fig. 2d). GGT related marginally but not significantly with ADA ($r = 0.36$, $p = 0.055$) in GDM (data not shown). Serum levels of direct bilirubin and TG showed an indirect correlation in GDM group ($r = -0.36$, $p = 0.04$) (data not shown). In GDM, pseudocholinesterase showed slightly negative correlation with HOMA-IR ($r = -0.33$, $p = 0.07$) (data not shown). Serum levels of

total bilirubin correlated negatively with LDL-C ($r = -0.42$, $p = 0.02$) (data not shown), TG ($r = -0.64$, $p = 0.001$) (data not shown), TC ($r = -0.41$, $p = 0.03$) (data not shown), the TG/HDL-C ratio ($r = -0.59$, $p = 0.001$) (data not shown), the TC/HDL-C ratio ($r = -0.51$, $p = 0.004$) (data not shown), and CRP ($r = -0.49$, $p = 0.01$) (data not shown) in normal pregnancy. Serum levels of direct bilirubin showed indirect correlations with TG ($r = -0.47$, $p = 0.01$) (data not shown), TC ($r = -0.57$, $p = 0.001$) (data not shown) the TG/HDL-C ratio ($r = -0.41$, $p = 0.02$) (data not shown), the TC/HDL-C ratio ($r = -0.59$, $p = 0.001$) (data not shown) and the LDL-C/HDL-C ratio ($r = -0.51$, $p = 0.01$) (data not shown) in normal pregnant women. We also examined the correlations in a pooled sample of entire GDM and normal pregnant women ($n = 60$). In the pooled sample, GGT activity correlated positively with FPG ($r = 0.53$, $p = 0.001$) (Fig. 3a), 1-h plasma glucose ($r = 0.70$, $p = 0.001$) (data not shown), 2-h plasma glucose ($r = 0.70$, $p = 0.001$) (data not shown), and CRP ($r = 0.29$, $p = 0.02$) (Fig. 3b), HOMA-IR ($r = 0.51$, $p = 0.001$) (Fig. 3c), the LDL-C/HDL-C ratio (r = 0.42, $p = 0.001$) (data not shown), the TG/HDL-C ratio (r = 0.57, $p = 0.001$) (data not shown), and the TC/HDL-C ratio ($r = 0.53$, $p = 0.001$) (data not shown) and negatively with adiponectin ($r = -0.39$, $p = 0.002$) (Fig. 3d). In the pooled sample, ADA showed a significant positive correlation with GGT ($r = 0.35$, $p = 0.01$) (Fig. 4a), CRP ($r = 0.52$, $p = 0.001$) (Fig. 4b), and FPG ($r = 0.37$, $p = 0.01$) (data not shown) and a negative correlation with adiponectin ($r = -0.26$, $p = 0.04$) (Fig. 4c). In the pooled sample, serum levels of adiponectin showed negative correlation with FPG ($r = -0.38$, $p = 0.003$) (data not shown), 1-h plasma glucose ($r = -0.41$, $p = 0.001$) (data not shown), 2-h plasma glucose ($r = -0.38$, $p = 0.003$) (data not shown), CRP ($r = -0.27$, $p = 0.04$) (data not shown), and HOMA-IR ($r = -0.33$, $p = 0.01$) (Fig. 4d). Levels of pseudocholinesterase correlated negatively with 1-h plasma glucose ($r = -0.31$, $p = 0.02$) (data not shown), 2-h plasma glucose ($r = -0.27$, $p = 0.04$) (data not shown), TC ($r = -0.27$, $p = 0.04$) (Fig. 5a), LDL-C ($r = -0.30$, $p = 0.02$) (Fig. 5b), the TC/HDL-C ratio ($r = -0.28$, $p = 0.03$) (data not shown), and the LDL-C/HDL-C ratio ($r = -0.26$, $p = 0.04$) (data not shown) in the pooled sample. Serum levels of total bilirubin correlated negatively with TG ($r = -0.44$, $p = 0.001$), TC ($r = -0.33$, $p = 0.01$), the TG/HDL-C ratio ($r = -0.37$, $p = 0.004$), and CRP ($r = -0.27$, $p = 0.04$) in the pooled sample (data not shown). Serum levels of direct bilirubin showed negative correlations with TG ($r = -0.26$, $p = 0.03$), TC ($r = -0.33$, $p = 0.02$), and the TG/HDL-C ratio ($r = -0.27$, $p = 0.04$) in the pooled sample (data not shown). The TG/HDL-C ratio showed a significant positive correlation with HOMA-IR index in the pooled sample ($r = 0.49$, $p = 0.001$) (data not shown).

Discussion

The main findings of the current study were (i) increased lipid ratios, ADA and GGT, and decreased pseudocholinesterase and adiponectin in GDM compared with normal pregnancy; and (ii) significant relationships of them with each other, HOMA-IR and bilirubin.

High serum α-amylase activity is related to various conditions such as acute pancreatitis, pancreatic cancer, and kidney dysfunction that has been extensively studied [3–6]. However, recently, low serum α-amylase activity has been shown to be associated with prevalence of metabolic syndrome [3], diabetes [4, 5], and non-alcoholic fatty liver [6]. It has been proposed that systemic ectopic fat deposition in organs including pancreas (i.e., fatty pancreas) might lead to pancreatic dysfunction that results in low serum α-amylase activity [6]. In the current study, no significant difference was observed in α-amylase activity between GDM and normal pregnancy. This observation might be due to the fact that the subjects were nonobese and matched in BMI.

There are some conflicting findings of maternal circulating levels of lipids in GDM. A recent systematic review and meta-analysis study showed that women with GDM have higher levels of TG and lower levels of HDL-C compared with normal pregnancy, while TC and LDL-C do not change significantly [7]. Our findings were nearly in line with this study. Some authors have reported no significant difference in TG between GDM and normal pregnancy [25, 26]. Hyper-HDL cholesterolemia has been also reported in GDM [26]. The atherogenic indexes including the LDL-C/HDL-C, TG/HDL-C,s and TC/HDL-C ratios were higher in GDM compared with normal pregnancy in the current study. Therefore, the GDM group might be at an increased risk of developing cardiovascular disease [27]. The TG/HDL-C ratio showed a significant positive correlation with HOMA-IR index in the current study that confirms Wang et al. study finding [24]. Therefore, the ratio might be used as a simple surrogate marker for assessing insulin resistance in pregnancy, and prediction of women with high risk of GDM.

It has been shown that serum levels of direct bilirubin in normal range are inversely correlated with TC, TG, and LDL-C, and positively with HDL-C in men with type 2 diabetes [19]. A cross-sectional and longitudinal study showed that baseline serum levels of total bilirubin have significantly preventive effects against hypertriglyceridemia in both men and women and against hypo-HDL cholesterolemia in men [28]. In the current study, total and

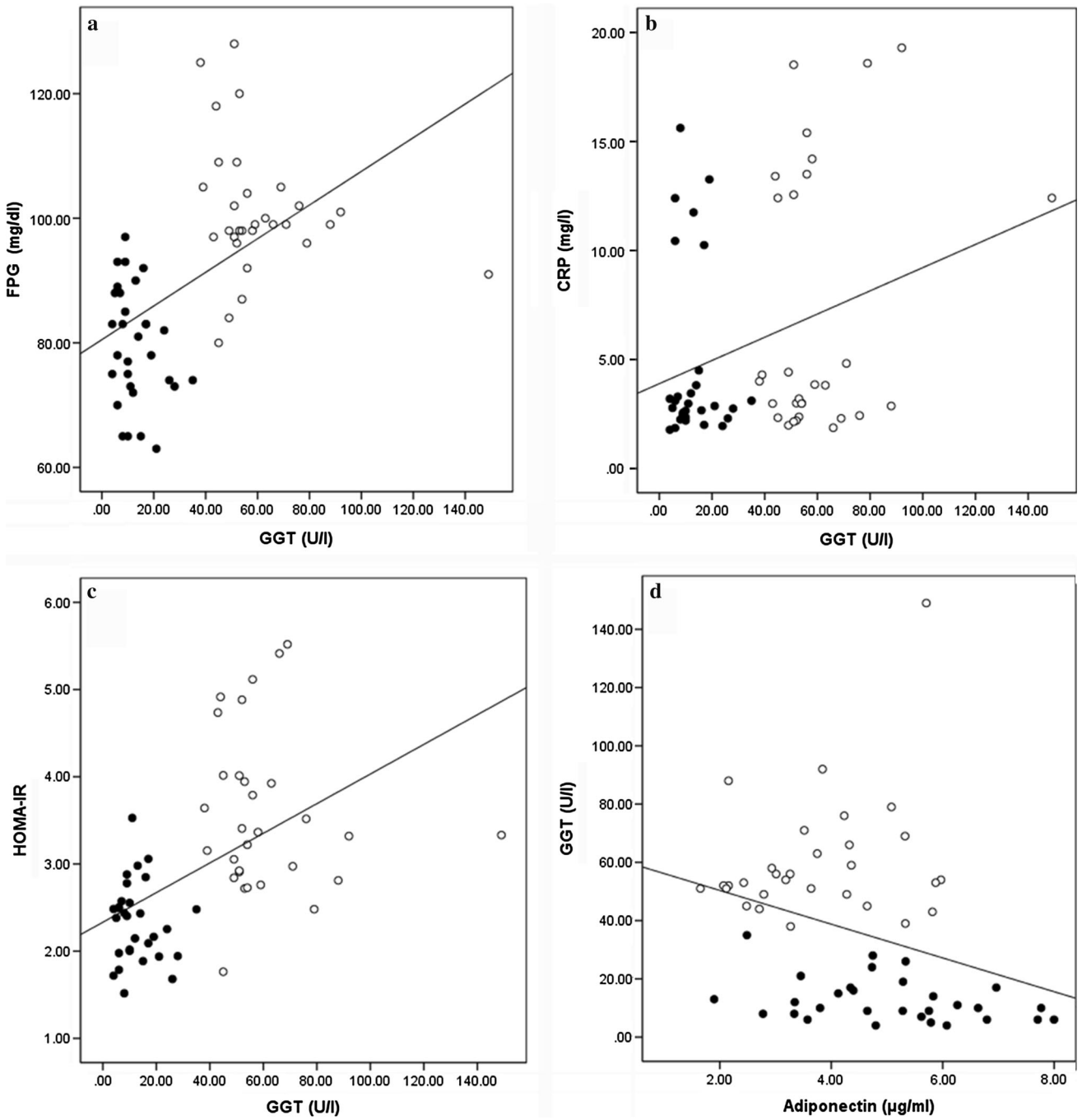

Fig. 3 Correlations of serum gamma-glutamyltransferase (GGT) activity with **a** fasting plasma glucose (FPG) levels ($r = 0.53$, $p = 0.001$), **b** C-reactive protein (CRP) concentrations ($r = 0.29$, $p = 0.02$), **c** Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index ($r = 0.51$, $p = 0.001$), and **d** adiponectin levels ($r = -0.39$, $p = 0.002$) in entire (*filled circle*) 30 normal pregnant and (*circle*) 30 gestational diabetes mellitus women

direct bilirubin showed significant inverse correlations with TC, TG, and lipid ratios. Another study showed that baseline serum levels of total bilirubin are very slightly lower in GDM than normal pregnancy [11] that is confirmed by the current study. Bilirubin has been known as a potent antioxidant, and most of its proposed beneficial effects are resulted from this property. It has been shown that oxidative stress can increase ADA activity in vitro [29]. Therefore, the inverse correlation between total bilirubin and ADA in the normal pregnant group might be resulted from the antioxidant property of bilirubin. It has been suggested that bilirubin can reduce pro-inflammatory

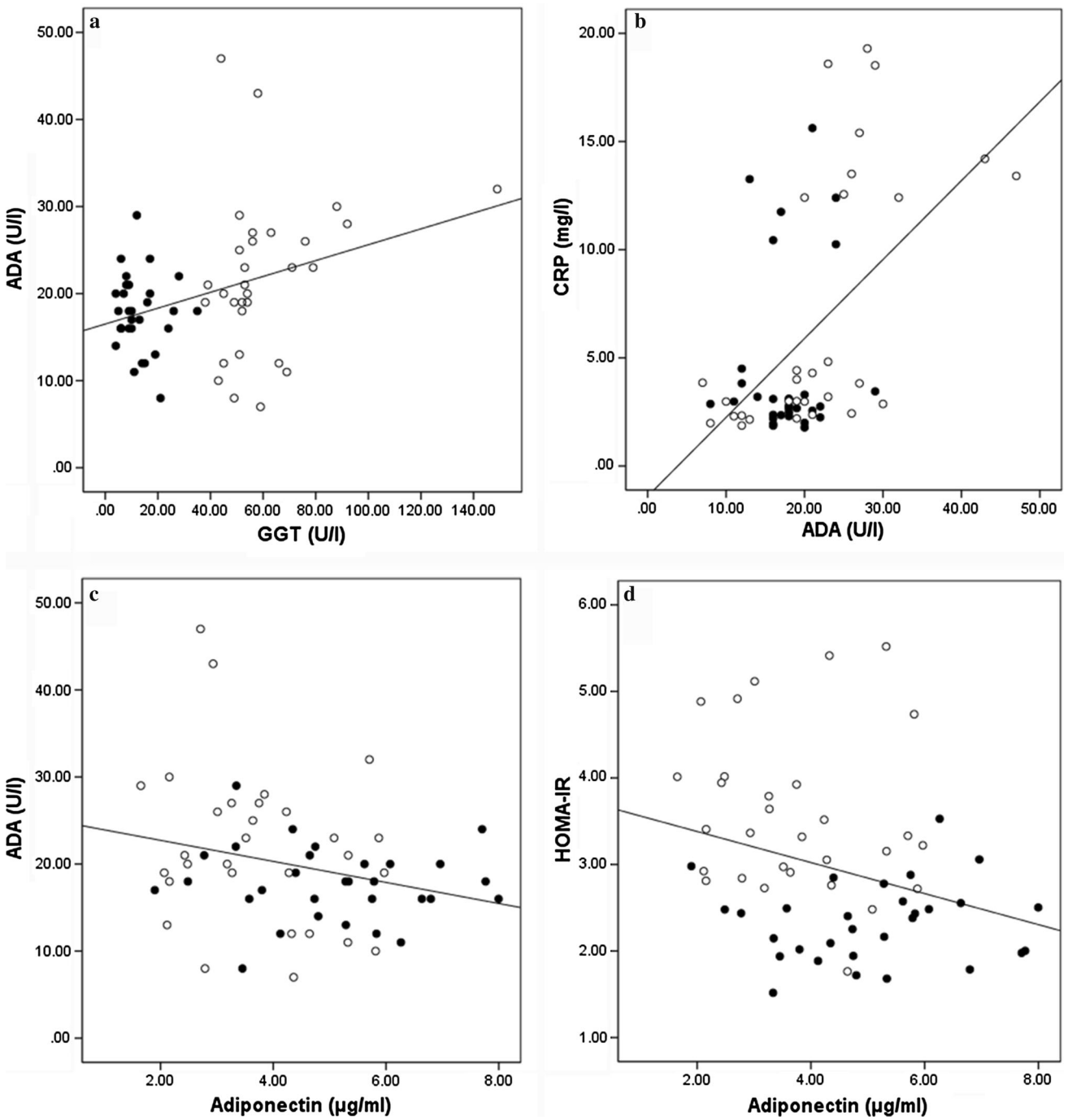

Fig. 4 Correlations between **a** adenosine deaminase (ADA) and gamma-glutamyltransferase (GGT) activities ($r = 0.35$, $p = 0.01$), **b** ADA activity and C-reactive protein (CRP) levels ($r = 0.52$, $p = 0.001$), **c** ADA activity and adiponectin levels ($r = -0.26$, $p = 0.04$), and **d** adiponectin levels and Homeostasis Model assessment of Insulin Resistance (HOMA-IR) index ($r = -0.33$, $p = 0.01$) in serum of entire (*filled circle*) 30 normal pregnant and (*circle*) 30 gestational diabetes mellitus women

status in hyperbilirubinemic subjects [28]. The negative correlation between CRP and total bilirubin that is observed in the current study could be addressed by this property of bilirubin.

It has been shown that increased circulating activity of GGT is associated with cardiovascular disease, mortality, and prevalence of diabetes mellitus in the general population [12], which is confirmed by observation of positive relationship of the enzyme with the atherogenic indexes in the present study. Raised GGT activity has also been proposed as a reliable biomarker of oxidative stress in clinical practice [13].

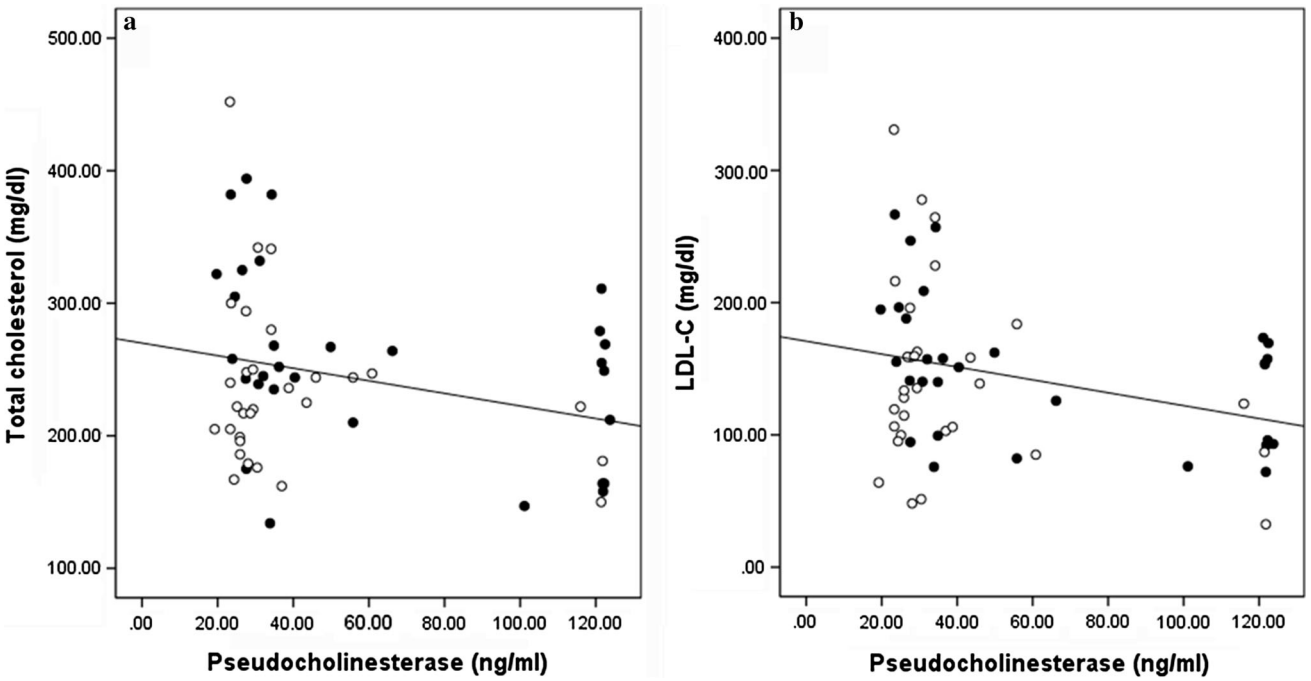

Fig. 5 Correlations of serum pseudocholinesterase levels with **a** total cholesterol levels ($r = -0.27$, $p = 0.04$) and **b** low-density lipoprotein cholesterol (LDL-C) levels ($r = -0.30$, $p = 0.02$) in entire (*filled circle*) 30 normal pregnant and (*circle*) 30 gestational diabetes mellitus women

There are some conflicting findings of maternal serum activity of pseudocholinesterase in GDM compared with normal pregnancy. Decreased [18] and increased [11] activity of the enzyme is reported in GDM, at time of diagnosis and in the second trimester, respectively. In both of these studies, GDM group had a higher BMI than normal group. However, in a BMI-matched study, at 34–40 weeks of gestation, no significant difference in pseudocholinesterase activity has been observed between two groups [17]. In the current study, level of the enzyme decreased significantly in GDM. It has been suggested that pseudocholinesterase activity is decreased in response to oxidative stress [18]. Raised GGT activity could be addressed as an oxidative stress situation in the current study [13]. Therefore, decrease in pseudocholinesterase levels in GDM might be due to oxidative stress situation. In Liu et al. study [11], in the second trimester of pregnancy, pseudocholinesterase activity has been increased in spite of the increase in GGT activity. Our finding was contradicted this study. The negative correlations observed between pseudocholinesterase and TC and LDL-C in Guimarães et al. study [18] were confirmed by the present study. However, Cocelli et al. [17] reported that pseudocholinesterase activity shows direct correlation with LDL-C and inverse correlation with TG. A negative correlation was also observed between pseudocholinesterase and the atherogenic indexes, the TC/HDL-C ratio and the LDL-C/HDL-C ratio, in the current study. Two studies reported that pseudocholinesterase can be a marker for metabolic syndrome [15] and atherosclerosis [16]. It has been proposed that pseudocholinesterase may behave differently in GDM compared with metabolic syndrome and type 2 diabetes mellitus [18]. In the present study, pseudocholinesterase showed negative associations with atherogenic indexes. On the other hand, the enzyme activity was decreased in GDM. Therefore, decrease in pseudocholinesterase levels in GDM compared with normal pregnancy could lead to some metabolic disorders in the patient. Therefore, there are many conflicting results of serum activity of pseudocholinesterase and its correlation with lipid profile in GDM. The only mechanism that could be addressed for decreasing of the enzyme activity in GDM is the negative effect of oxidative stress on it [18]. Therefore, further studies might be needed to explore the role of the enzyme in GDM.

ADA degrades endogenous adenosine through an irreversible deamination reaction. Adenosine can increase insulin sensitivity by stimulating glucose transport into cells [30]. A study showed that there is no significant difference in maternal serum ADA activity between GDM and normal pregnancy [20]. However, the current study showed a significant increase in the enzyme activity in GDM. A study has shown that ADA activity is increased in type 2 diabetes mellitus compared to normal subjects, and

it is correlated positively with FPG [30]. Another study showed that serum activity of ADA does not differ significantly between metabolic syndrome and normal control subjects [15]. In this study, a positive correlation has been observed between ADA and HOMA-IR. In the current study, ADA showed a significant positive correlation with FPG in the pooled sample. In the present study, ADA correlated positively with GGT and negatively with adiponectin and total bilirubin. It has been reported that oxidative stress can increase ADA activity in vitro [29]. It has been shown that adiponectin has antioxidant property [31–33]. Therefore, significantly decrease in maternal serum levels of adiponectin, significantly increase in GGT, and very slightly decrease of total bilirubin could be addressed as an oxidative stress situation in GDM that might lead to increase in ADA activity. It has been reported that during inflammation, the extracellular level of adenosine increases rapidly [15]. Therefore, increased ADA activity in the GDM patients might be also a response to inflammation that could be addressed by the positive correlation between the enzyme and CRP. It could be concluded that oxidative stress and inflammation situations increase ADA activity in GDM resulting in degradation of extracellular adenosine that might lead to insulin resistance.

The major limitations of the current study were small sample size and that the study only focused on the last trimester of pregnancy. However, the presented findings might be a preliminary data for further cohort studies with bigger sample size at different trimesters in different populations for investigation on application of them in clinical practice.

In summary, lipid ratios, ADA, and GGT were increased, and pseudocholinesterase and adiponectin were decreased significantly in GDM compared with normal pregnancy. The lipid ratios might be more important than lipid profile alone as simple surrogate markers for assessing insulin resistance in pregnancy and prediction of women with high risk of GDM. Very little decrease in bilirubin levels showed very important metabolic changes in GDM; this would be considered as the subject of further investigation.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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