Increased Circulating Levels of EDA in Newly Diagnosed Type 2 Diabetic Patients

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Abstract

BACKGROUND

Etodysplasin A (EDA), a newly discovered hepatokine, has recently been considered to be closely related to glycolipid metabolism disorders, but the pathophysiological effects of EDA are still poorly understood. This study was the first time to determine the level of serum EDA in newly diagnosed type 2 diabetes mellitus (T2DM) patients, and to explore the relationships between serum EDA levels and various metabolic indexes.

METHODS

A total of 184 subjects were enrolled in the study, including 92 subjects with newly diagnosed T2DM and 92 subjects with age- and sex-matched normal glucose tolerance (NGT). Serum EDA levels were determined using enzyme-linked immunosorbent assay (ELISA). And oral glucose tolerance test, glycosylated hemoglobin c (HbA1c), blood lipid and insulin were also measured. Insulin resistance (IR) and islet β-cell function were assessed by homeostasis model assessment (HOMA).

RESULTS

Serum EDA levels were significantly increased in the T2DM group than in the NGT group (359.91 ± 117.99 vs. 265.82 ± 86.51 pg/mL, \(P<0.001\)). Serum EDA levels were positively correlated with body mass index (BMI), waist-to-hip ratio (WHR), fasting plasma glucose (FPG), HbA1c, 2-hour postprandial plasma glucose (2hPG), fasting plasma insulin (FIns), fasting C peptide (FCP), triglyceride (TG), HOMA-IR, and negatively correlated with high-density lipoprotein cholesterol (HDL-c) and HOMA-β \(P<0.05\). Multiple stepwise regression analysis demonstrated that 2hPG and FIns were independent influencing factors of serum EDA level \(P<0.05\). Logistic regression analysis showed that serum EDA level was significantly independent correlated with T2DM \(P<0.05\).

CONCLUSIONS

Serum EDA level are significantly higher in T2DM patients, indicating that circulating EDA may be involved in the pathogenesis of T2DM.

Background

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia caused by a variety of reasons, of which 90% are type 2 diabetes mellitus (T2DM), and the main pathophysiological mechanisms are insulin resistance and relatively insufficient insulin secretion[1, 2]. Liver is the central organ of glucose and lipid metabolism, which plays an important role in the development of T2DM[3]. Hepatic insulin resistance was considered to be the main driving factor of insulin resistance[4]. Previous studies have shown that the most obvious pathophysiological characteristics of hepatic insulin resistance were gluconeogenesis and glycolysis dysfunction, and liver lipid accumulation[5]. However, in
addition to the role of glucose and lipid metabolism, the latest research also showed that the liver is an important endocrine organ, which can secrete thousands of proteins, of which about 25% can be released into the blood circulation [6]. Further studies have found that a variety of protein factors secreted by the liver form a regulatory network and affect the energy metabolism of the liver and other organs through inter tissue communication [7], thus affecting the occurrence and development of metabolic related diseases, such as obesity, insulin resistance, diabetes, fatty liver, etc[8-11]. A quantitative protein expression profile based on isobaric tagging for relative and absolute quantification (iTRAQ) showed that there were 69 differentially expressed proteins in the plasma of T2DM patients compared with non-diabetic individuals, including a variety of proteins secreted by the liver and related to insulin resistance in diabetic patients, including a 2-macroglobulin, selenoprotein P, retinol binding protein 4 (RBP4)[12].

Ectodysplasin A (EDA), a newly discovered hepatokine, was considered to be closely related to chronic diseases such as fatty liver, obesity and insulin resistance[13, 14]. It is mainly secreted by hepatocytes in vivo, and is significantly higher than that in white adipose tissue, brown adipose tissue, skeletal muscle cells and other tissues[14]. The gene was initially considered as a member of tumor necrosis factor (TNF) related cytokines family, belonging to type II transmembrane protein [15], which can be secreted into extracellular domain after cleavage of endoprotease furan [46]. Previous studies have shown that EDA plays an important role in the development and maintenance of skin derived structures such as teeth, hair and sweat glands [48], and EDA gene mutations can lead to X-linked hypohidrotic ectodermal dysplasia [16] and selective nonsyndromic tooth dysplasia[17]. With the deepening of research, in 2017, awazawa et al. claimed to have found a new function of this gene expression-regulated systemic glucose metabolism, and led to impaired insulin sensitivity of skeletal muscle, which was considered as a hepatokine. In that study, the results showed that the liver and serum levels of EDA were significantly increased in HFD mice and db/db mice. what’s more, the liver EDA mRNA levels increased with the severity of steatosis. Furthermore, it was found that overexpression of EDA exacerbated the impaired glucose tolerance in mice, while knockdown of EDA significantly improved insulin sensitivity in db/db mice[14].

Although knockdown EDA has strong anti-diabetic properties, the exact understanding of its biological activity and mode of action remains to be further studied. In order to explore the clinical significance of EDA in human, we measured the serum concentration of EDA in healthy control subjects and newly diagnosed type 2 diabetes mellitus patients, and analyzed the relationship between EDA concentration and anthropometric and metabolic parameters.

**Research Design And Methods**

**Study Population**

A total of 184 adults were recruited: ninety-two subjects with normal glucose tolerance and ninety-two patients with T2DM matched with gender and age. None of the healthy controls took drugs known to affect glucose tolerance and lipid metabolism. All patients with T2DM were newly diagnosed and did not
take oral hypoglycemic and hypolipidemic drugs. The diagnosis of T2DM was based on the diagnostic criteria of the American Diabetes Association in 2011[18]. Patients with impaired fasting glucose and/or impaired glucose tolerance, type 1 diabetes mellitus, gestational diabetes mellitus, active hepatitis/cirrhosis, hemodialysis chronic renal failure, congestive heart failure, or other known major diseases were excluded from the study. Each subject was asked about smoking and alcohol consumption. Approval for the study was obtained from the Clinical Research Ethics Committee, Affiliated Hospital of Jiangsu University. Informed consent was obtained from each of the participants.

**Anthropometric and Biochemical Measurements**

General anthropometric parameters such as height, weight, waist circumference, hip circumference and blood pressure were collected and recorded by professional doctors, and BMI [weight (kg)/the square of height(m)] and WHR (the ratio of waist circumference to hip circumference) were calculated. The plasma glucose at each time point of OGTT were measured by glucose oxidase-based assay, and the level of insulin and c-peptide were detected by chemiluminescence method. High-performance liquid chromatography (HPLC) (Arkray Inc., Kyoto, Japan) method was used to detect the Glycosylated hemoglobin (HbA1c). The high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), and triglyceride (TG) parameters of blood lipid profile were measured by appropriate enzymatic assays (Beckman Coulter Inc., Brea, CA, USA). The hepatic insulin resistance, was estimated by the homeostasis model assessment (HOMA): HOMA-IR= FIns×FPG/22.5 [19]. The β-cell function, was estimated by the HOMA of β-cell function: HOMA-β= 20×FINS/(FPG-3.5).

**Estimation of serum EDA levels**

The fasting blood samples of each subject were collected and centrifuged immediately at 1 000 × g at 4 °C for 20 min, and then the serum samples were separated and labeled, and stored in -80 °C refrigerator. Serum EDA levels were determined using a commercially available human enzyme-linked immunosorbent assay (ELISA) (Wuhan Eiaab Science Co., China; Catalog No. E1976h). The sensitivity of the kit was less than 20pg/ml, the intra assay CV was ≤ 7.8%, and the inter assay CV was ≤ 8.9%. The operation process was carried out according to the instructions of the kit. The absorbance value at 450nm was detected by enzyme labeled instrument (ThermoFisher, Multiskan GO), and the standard curve was drawn. Further, the concentration of the sample was calculated by using the standard curve. The detection range of ELISA was 78-5000 pg/mL.

**Statistical analysis**

All statistical analyses were performed using SPSS version 20.0. Continuous variables were expressed as mean ± SD and median (quartile) or categorical variables as cases (percentage). ALT, AST, GGT, HOMA-IR and HOMA-β values are converted logarithmically due to their nonnormal distribution. The differences between the two groups were compared by independent student t test. The categorical variables were tested by χ2 test. One-way ANOVA test were used for multiple comparisons. Pearson correlation analysis was used to evaluate the associations between serum EDA and various variables. After adjusting for the
effects of gender, age and BMI, the correlation was analyzed by partial correlation. The independent influencing factors of EDA were analyzed by linear stepwise regression. Binary logistic regression analysis was used to examine the significant trend of the increase in the tertiles, and the lowest tertile was used as a reference category to estimate the odds ratio of diabetes in each tertile. A double tailed test value of $P<0.05$ was considered statistically significant.

Results

Characteristics of Study Subjects

Table 1 summarizes the clinical baseline characteristics of the study subgroups. There was no significant difference in gender, age, smoking history, alcohol consumption, and TC between T2DM group and NGT group. In addition, compared with the NGT group, BMI, WHR, SBP, DBP, HbA1c, FPG, 2hPG, Flns, FCP, TG, LDL-c, ALT, AST, GGT, and HOMA-IR in T2DM group were significantly ascended ($P<0.05$), while HDL-c and HOMA-β were significantly reduced ($P<0.05$).

Serum EDA levels

Most importantly, the serum level of EDA in the T2DM group was significantly increased than that in the NGT group (265.82 ± 86.51 vs. 359.91 ± 117.99), and the difference was statistically significant ($P<0.001$) (Table 1). However, no difference in serum EDA levels was observed between men and women in T2DM (360.80 ± 128.35 vs. 358.85 ± 105.87, $P=0.938$) or NGT (261.62 ± 87.85 vs. 271.53 ± 85.46, $P=0.590$) groups (Figure 1).

Clinical features of the participants according to the tertiles of asprosin

Compared with the lower serum EDA tertile group, prevalence of diabetes, BMI, WHR, HbA1c, FPG, 2hPG, Flns, FCP, TG, and HOMA-IR in the middle or upper serum EDA tertile group were significantly increased ($P<0.05$), while HDL-c were significantly decreased ($P<0.05$). Other metabolic parameters, such as sex, age, smoking history, alcohol consumption, SBP, DBP, TC, LDL-c, ALT, AST, GGT, and HOMA-β did not reach statistical significance (Table 2).

Correlation of EDA With metabolic parameters

Serum EDA concentrations were positively correlated with BMI, WHR, HbA1c, FPG, 2hPG, Flns, FCP, TG, and HOMA-IR, but inversely correlated with HOMA-β and HDL-c. In addition, after adjusting for gender, age and BMI, the HbA1c, FPG, 2hPG, Flns, TG, HDL-c, HOMA-IR and HOMA-β were still significantly correlated with serum EDA levels (Table 3). Furthermore, stepwise multiple linear regression models revealed that 2hPG and Flns were independently related to the serum EDA levels (Table 4).

Logistic regression analyses for T2DM
Binary logistic regression analyses (Table 5, model 1) demonstrated that EDA was significantly associated with T2DM ($P<0.05$ in unadjusted model). Moreover, the middle tertile [OR (95%CI) :1.954 (1.357~2.812), $P<0.05$] and upper Tertile [OR (95%CI) :1.954 (1.357~2.812), $P<0.001$] were significantly correlated with the development of T2DM even after adjustment for age, sex, BMI, WHR, SBP, DBP, and liver function (Table 5, model 2).

**Discussion**

To our knowledge, this is the first time to investigate the relationship between circulating EDA levels and T2DM. Our results showed that serum EDA levels were significantly higher in newly diagnosed and untreated T2DM patients compared with healthy controls. Serum EDA concentration was positively correlated with BMI, WHR, HbA1c, FPG, 2hPG, fins, FCP, TG, HOMA-IR, but negatively correlated with HOMA-$\beta$ and HDL-C. What's more, the prevalence of type 2 diabetes was more obvious in the upper serum EDA tertile group.

The mechanisms underlying increased EDA levels in newly diagnosed T2DM patients remain unclear. Some studies suggested that the adult liver was very similar to the pancreas in the steps of initiating fate. Both the liver and pancreas come from the endoderm of the embryonic intestinal tract[20]. It has been suggested that there is trans-differentiation between pancreatic cells and hepatocytes[21, 22]. In addition, the liver can secrete a variety of cytokines, which further affect the energy metabolism of liver, pancreas, skeletal muscle and other organs through tissue communication [23]. Recently, Awazawa et al. [14]reported that hepatic expression of EDA was upregulated in animal models of diabetes and obese. Further studies showed that the overexpression of EDA in mice could aggravate the impaired glucose tolerance induced by high-fat diets fed (HDF). While EDA knockdown could improve insulin sensitivity in diabetic mice. Further mechanism study found that the expression of EDA in mouse liver was regulated by peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) and retinoid X receptor (RXR)-$\alpha$, which further promoted c-Jun N-terminal kinase (JNK) activation and inhibited the serine phosphorylation of IRS1 in skeletal muscle, resulting in impaired insulin sensitivity of skeletal muscle in obesity. In addition, clinical studies have shown that the expression of EDA in liver was negatively correlated with glucose infusion rate (GIR). Finally, in another group of morbidly obese patients who received bariatric surgery intervention, the expression of EDA in liver was significantly decreased one year after surgery, while weight loss and insulin sensitivity were improved. In our study, serum EDA concentrations were positively correlated with FPG, 2hPG, FIns, and HOMA-IR, but negatively correlated with HOMA-$\beta$. Most importantly, 2hPG and FIns were independently interfering factor of EDA. Therefore, we speculate that the increase of serum EDA in T2DM patients may be a positive feedback effect, which may represent the ability of liver to adapt to insulin resistance or elevated blood glucose concentration by increasing the secretion of EDA.

Previous studies have shown that hyperlipidemia was an important factor leading to insulin resistance [24, 25]. Diabetic patients often have mixed dyslipidemia, which is mainly characterized by higher TG and lower HDL-C [24-26]. In this study, compared with the normal control group, the T2DM group had higher TG and lower HDL levels. Furthermore, our results showed that the level of circulating EDA was positively
correlated with TG and negatively correlated with HDL-C. However, our results are not completely consistent with a recent study by Yang et al.\[13\], which found that serum EDA levels in nonalcoholic fatty liver disease (NAFLD) patients were only correlated with HDL-c, but not with TG. This divergence may be due to the differences of sample size, drugs and diseases. In that study, 176 subjects were recruited, including 88 normal subjects and 88 patients with NAFLD. The subjects in our study were newly diagnosed diabetic patients. Although both of them are metabolic diseases and interact with each other, their pathogenesis and pathophysiology were different. In addition, none of the patients in our study received hypoglycemic and/or lipid-lowering drugs. We don't know whether patients with NAFLD in that study received medications or not. However, mounting evidence from recent fundamental studies suggested that EDA plays an important role in lipid metabolism. Clinical study demonstrated that the expression of liver EDA was positively correlated with liver fat content and visceral fat area, and was positively correlated with histologically determined inflammation and steatosis score of nonalcoholic steatohepatitis (NASH) in 33 obese male patients\[14\]. In animal studies, mice deficient EDA significantly reduced the increase of liver lipid droplets by HFD, decreased the content of hepatic TG, and reduced the levels of ALT and AST in HFD model mouse. What's more, in HepG2 cells, free fatty acids (FFA) intervention significantly increased the expression of EDA protein in cells and cell culture supernatant. Further mechanism studies showed that EDA gene knockout could increase the expression of carnitine palmitoyltransferase 1A (CPT1A), the key enzyme of fatty acid oxidation, decrease the expression of sterol regulatory-element-binding protein-1c (SREBP-1c), acetyl coA carboxylase (ACC) and fatty acid synthase (FAS), thus reduced the accumulation of TG induced by FFA\[13\]. Previous studies have shown that excessive fat accumulation can induce insulin resistance and destroy the function of islet cells\[27\], while insulin resistance can promote hyperlipidemia\[4, 28\], which is a vicious circle. The latest research found that hepatokines plays a very important role in it\[7\]. Combined with the above results, high glucose and high fat can induce the secretion of EDA, and the increase of EDA further aggravates the disorder of glycolipid metabolism and insulin resistance. Therefore, we speculate that EDA, a new hepatokine, may be also involved in this vicious circle.

Obesity, especially visceral obesity, not only affects body shape, but also is considered to be a key risk factor for insulin resistance, diabetes, fatty liver and even cardiovascular diseases \[29, 30\]. In our study, we have shown that BMI and WHR are positively correlated with circulating EDA levels, which is consistent with Yang et al.\[13\] in NFALD. The limitations of our research are also worth comment. First, this study was limited by a cross-sectional design and could not infer a causal relationship between elevated serum EDA levels and the development of T2DM. Second, all the study participants were recruited from a province, and the population was underrepresented. Third, our analysis was based on a single measurement in the blood, which may not reflect the EDA over time. Circulating EDA levels should be measured at different stages to further clarify its role in the pathogenesis of type 2 diabetes.

**Conclusions**

Taken together, our results indicate for the first time that serum EDA levels were significantly increased in newly diagnosed T2DM patients. Moreover, circulating EDA concentrations were closely correlated with
glycolipid metabolism, insulin resistance. Further studies are required to clarify the potential pathophysiological role of EDA in T2DM.

**Declarations**

**Authors’ contributions**

GY designed the study, analyzed data and drafted the manuscript. XD and YL participated in the design of the study and coordination of the whole work, and had the equal contributions. LZ, LY, and HL interprets the data, revises the manuscript, and recruited participants and collected data. XW, KC, PZ, ZZ, and YQ participated in acquisition of data and analyzed the data. All authors read and approved the final manuscript for publication.

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All the authors of this manuscript have made substantial contributions to this work.

**Consent for publication**

All authors read and approved the final manuscript for publication.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Ethics approval and consent to participate**

The study was approved by the Biomedical Research Ethics Committee of Affiliated Hospital of Jiangsu University, Zhenjiang, China, and performed in accordance with the Declaration of Helsinki. All subjects in this study have fully informed consent.

**Competing interests**

The authors declare that they have no competing interests.

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Abbreviations

T2DM, Type 2 diabetes mellitus; BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin c; FPG, fasting plasma glucose; 2hPG, 2-hour postprandial plasma glucose; FIns, fasting plasma insulin; FCP, fasting C peptide; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, g-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance index; HOMA-β, homeostasis model assessment-β; OR, Odds ratio.

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**Table**
| Parameters                  | NGT (n = 92)            | T2DM (n = 92)            | P value  |
|-----------------------------|-------------------------|-------------------------|----------|
| Age [years]                 | 47.016 ± 11.61          | 48.30 ± 11.07           | 0.440    |
| Sex [female %]              | 53 (57.6%)              | 50 (54.3%)              | 0.656    |
| BMI [kg/m²]                 | 23.89 ± 3.66            | 25.89 ± 3.43            | <0.001   |
| WHR                         | 0.88 ± 0.06             | 0.93 ± 0.05             | <0.001   |
| SBP (mmHg)                  | 120.03 ± 15.50          | 129.90 ± 14.31          | <0.001   |
| DBP (mmHg)                  | 72.91 ± 13.31           | 82.50 ± 10.66           | <0.001   |
| Smoking status, n (%)       |                         |                         |          |
| Never                       | 66 (71.7%)              | 62 (67.4%)              | 0.522    |
| Former smoker               | 5 (5.4%)                | 7 (7.6%)                | 0.550    |
| Current smoker              | 21 (22.8%)              | 23 (25.0%)              | 0.730    |
| Alcohol use, n (%)          |                         |                         |          |
| Never                       | 77 (83.7%)              | 71 (77.2%)              | 0.265    |
| Occasional drinker          | 6 (6.5%)                | 10 (10.9%)              | 0.295    |
| Regular drinker             | 9 (9.8%)                | 11 (12.0%)              | 0.636    |
| HbA1c (%)                   | 5.71 ± 0.29             | 9.78 ± 1.88             | <0.001   |
| FPG (mmol/L)                | 4.87 ± 0.55             | 11.09 ± 2.81            | <0.001   |
| 2hPG (mmol/L)               | 6.23 ± 0.92             | 20.22 ± 4.30            | <0.001   |
| FIns (µIU/ml)               | 5.69 ± 4.11             | 8.21 ± 4.62             | <0.001   |
| FCP (ng/ml)                 | 2.29 ± 0.88             | 3.09 ± 0.93             | <0.001   |
| TG (mmol/L)                 | 1.56 ± 0.85             | 2.54 ± 1.44             | <0.001   |
| TC (mmol/L)                 | 4.97 ± 0.85             | 5.12 ± 1.06             | 0.280    |
| LDL-c (mmol/L)              | 2.81 ± 0.75             | 3.08 ± 0.87             | 0.026    |
| HDL-c (mmol/L)              | 1.44 ± 0.41             | 1.07 ± 0.29             | <0.001   |
| ALT (U/L)                   | 16.15 (10.63-31.38)     | 33.3 (18.13-50.05)      | <0.001   |
| AST (U/L)                   | 17.15 (14.25-20.88)     | 20.3 (15.28-31.23)      | 0.003    |
| GGT (U/L)                   | 20.8 (15.30-75)         | 37 (25-60.5)            | <0.001   |
| HOMA-IR                     | 1.02 (0.65-1.48)        | 3.47 (2.37-4.85)        | <0.001   |
| HOMA-β                      | 70.10 (45.46-123.11)    | 18.45 (11.12-34.39)     | <0.001   |
| Ectodysplasin A (pg/mL)     | 265.82 ± 86.51          | 359.91 ± 117.99         | <0.001   |

Data are presented as means ± SD, medians (inter-quantile range (IQR)), and number (percentages).

*Log-transformed variable, values given are medians (inter-quantile range (IQR)).

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin c; FPG, fasting plasma glucose; 2hPG, 2-hour postprandial plasma glucose; FIns, fasting plasma insulin; FCP, fasting C peptide; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, g-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance index; HOMA-β, homeostasis model assessment-β.
| Parameters                  | Lower Tertile (n=62) | Middle Tertile (n=62) | Upper Tertile (n=60) | P value   |
|---------------------------|----------------------|-----------------------|----------------------|-----------|
| Age (years)               | 48.35 ± 11.71        | 47.89 ± 10.41         | 46.70 ± 11.95        | 0.711     |
| Sex (%)                   | 38 (61.3%)           | 32 (51.6%)            | 33 (55.0%)           | 0.545     |
| BMI (kg/m2)               | 24.42 ± 3.43         | 24.39 ± 3.42          | 25.91 ± 4.01         | 0.033     |
| WHR                       | 0.89 ± 0.06          | 0.90 ± 0.07           | 0.92 ± 0.06          | 0.041     |
| SBP (mmHg)                | 123.95 ± 15.70       | 125.35 ± 17.22        | 125.62 ± 14.12       | 0.820     |
| DBP (mmHg)                | 76.15 ± 13.81        | 78.34 ± 13.04         | 78.67 ± 11.98        | 0.504     |
| Smoking status, n (%)     | 47 (75.8%)           | 42 (67.7%)            | 39 (65.0%)           | 0.401     |
| Never                     | 3 (4.8%)             | 3 (4.8%)              | 6 (10.0%)            | 0.449     |
| Former smoker             | 12 (19.4%)           | 17 (27.4%)            | 15 (25.0%)           | 0.558     |
| Alcohol use, n (%)        | 51 (82.3%)           | 50 (80.6%)            | 47 (78.3%)           | 0.860     |
| Never                     | 4 (6.5%)             | 5 (8.1%)              | 7 (11.7%)            | 0.579     |
| Occasional drinker        | 7 (11.3%)            | 7 (11.3%)             | 6 (10.0%)            | 0.966     |
| HbA1c (%)                 | 7.02 ± 2.31          | 7.68 ± 2.39           | 8.56 ± 2.41          | 0.002     |
| FPG (mmol/L)              | 6.84 ± 3.60          | 8.02 ± 3.77           | 9.11 ± 3.49          | 0.003     |
| 2hPG (mmol/L)             | 10.73 ± 7.40         | 13.13 ± 7.75          | 15.90 ± 7.06         | 0.001     |
| FIIns (µIU/ml)            | 5.19 ± 2.65          | 7.01 ± 4.30           | 8.70 ± 5.59          | <0.001    |
| FCP (ng/ml)               | 2.26 ± 0.80          | 2.79 ± 1.07           | 3.04 ± 0.91          | <0.001    |
| TG (mmol/L)               | 1.72 ± 1.27          | 2.06 ± 1.12           | 2.37 ± 1.36          | 0.019     |
| TC (mmol/L)               | 4.97 ± 1.00          | 5.08 ± 0.81           | 5.09 ± 1.07          | 0.733     |
| LDL-c (mmol/L)            | 2.86 ± 0.91          | 2.99 ± 0.70           | 2.99 ± 0.84          | 0.582     |
| HDL-c (mmol/L)            | 1.36 ± 0.38          | 1.26 ± 0.45           | 1.14 ± 0.34          | 0.008     |
| ALT (U/L)                 | 18.6 (12.9-37.88)    | 22.65 (14.15-38.48)   | 28.65 (15.2-47.8)    | 0.461     |
| AST (U/L)                 | 17.7 (13.95-24.23)   | 17.2 (14.5-23)        | 19.9 (15.95-29.35)   | 0.230     |
| GGT (U/L)                 | 25 (15-41.5)         | 28.5(19-53.25)        | 29 (21.25-50)        | 0.164     |
| HOMA-IR                   | 1.27(0.78-2.17)      | 1.81(1.00-3.62)       | 3.27 (1.80-4.70)     | <0.001    |
| HOMA-β                    | 47.84 (19.72-105.31) | 41.10 (22.77-77.68)   | 33.84 (15.88-53.41)  | 0.154     |
| Ectodysplasin A (pg/mL)   | 200.53 ± 40.28       | 301.73 ± 31.00        | 440.46 ± 85.43       | <0.001    |
| T2DM (%)                  | 18 (29.0%)           | 31 (50.0%)            | 43 (71.7%)           | <0.001    |

* a Significant P ≤0.05 vs group of lower tertile.
* b Significant P ≤0.05 vs group of middle tertile.
Table 3. Partial Correlations Analysis of Variables Associated With Serum Ectodysplasin A Levels in Study Population

| Parameters   | EDA (unadjusted) | EDA (age, BMI, and sex adjusted) |   |   |
|--------------|------------------|----------------------------------|---|---|
|              | $r$              | $P$                              | $r$| $P$|
| Age          | -0.083           | 0.261                            | - | - |
| BMI          | 0.235            | 0.001                            | - | - |
| WHR          | 0.150            | 0.042                            | 0.037| 0.628|
| SBP          | 0.070            | 0.348                            | -0.025| 0.743|
| DBP          | 0.118            | 0.110                            | 0.027| 0.716|
| HbA1c        | 0.321            | <0.001                           | 0.295| <0.001|
| FPG          | 0.325            | <0.001                           | 0.256| 0.001|
| 2hPG         | 0.363            | <0.001                           | 0.336| <0.001|
| FIns         | 0.284            | <0.001                           | 0.192| 0.010|
| FCP          | 0.277            | <0.001                           | 0.184| 0.014|
| TG           | 0.231            | 0.002                            | 0.172| 0.022|
| TC           | 0.111            | 0.134                            | 0.122| 0.104|
| LDL-c        | 0.116            | 0.118                            | 0.103| 0.170|
| HDL-c        | -0.259           | <0.001                           | -0.174| 0.020|
| ALT          | 0.101            | 0.173                            | 0.037| 0.622|
| AST          | 0.102            | 0.169                            | 0.065| 0.386|
| GGT          | 0.128            | 0.082                            | 0.065| 0.388|
| HOMA-IR      | 0.352            | <0.001                           | 0.277| <0.001|
| HOMA-β       | -0.184           | 0.012                            | -0.231| 0.002|

Table 4. Stepwise Multiple Linear Regression Analysis With Ectodysplasin A as the Dependent Variable

| Independent Variable |Regression Coefficient (SE) | $β$ | 95% CI       | $P$  |
|----------------------|-----------------------------|-----|--------------|------|
| (Constant)           | 209.528 (17.97)             | -   | 174.075-244.982| <0.001|
| 2hPG                 | 4.796 (1.01)                | 0.32| 2.806-6.785  | <0.001|
| FIns                 | 5.745 (1.70)                | 0.23| 2.385-9.106  | 0.001|

CI: confidence interval.
The following independent variables were considered for the model: age, BMI, WHR, HbA1c, FPG, 2hPG, FIns, FCP, TC, TG, LDL-c, HDL-c, HOMA-IR, HOMA-β, ALT, AST, GGT. Only the variables that had a $P<0.05$ were considered in the final fitted model.
### Table 5. OR and 95% CI for T2DM by the tertiles of Ectodysplasin A

| Models     | Individuals With and Without T2DM | n   | OR    | 95% CI       | P    |
|------------|-----------------------------------|-----|-------|--------------|------|
|            |                                   |     |       |              |      |
| Model 1    | Lower Tertile                     | 62  | 1     | Ref          | Ref  |
|            | Middle Tertile                    | 62  | 2.783 | 1.166-5.127  | 0.018|
|            | Upper Tertile                     | 60  | 6.183 | 2.821-13.554 | <0.001|
| Model 2    | Lower Tertile                     | 62  | 1     | Ref          | Ref  |
|            | Middle Tertile                    | 62  | 2.783 | 1.118-6.927  | 0.028|
|            | Upper Tertile                     | 60  | 7.216 | 2.758-18.885 | <0.001|

Abbreviation: CI, confidence interval; OR, odds ratio. Adjusted for age, sex, BMI, WHR, SBP, DBP, ALT, and AST.

### Figures

**Figure 1**

Comparison of the serum concentration of EDA between the female and men.