Background
Fetuin-A is a hepatic secretory protein that binds to insulin receptors and inhibits insulin resistance (IR) kinase activity as well as IR autophosphorylation in vivo and in vitro.

Aim
This study aimed to investigate fetuin-A levels in patients with type 2 diabetes mellitus (T2DM) and their relation to microvascular complications.

Patients and methods
This descriptive study was conducted on 160 patients. Group 1 included (n=40) diabetic patients without microvascular complications, group 2 (n=40) included diabetic patients with nephropathy, group 3 (n=40) included diabetic patients with retinopathy, and group 4 represented (n=40) healthy control. Serum fetuin-A and insulin were measured by enzyme-linked immunosorbent assay. Glucose was measured, and homeostasis model assessment for IR (HOMA-IR) was estimated.

Results
Fetuin-A levels were significantly higher in all T2DM groups compared with controls. There was a significant positive correlation between fetuin-A, insulin, and HOMA-IR in all studied groups. There was a significant positive correlation between fetuin-A and some of metabolic syndrome criteria in all diabetic patients. There were high significant increases in the mean levels of fetuin-A, insulin, and HOMA-IR in the diabetic patients with nephropathy group than other groups. There was a nonsignificant increase in fetuin-A levels in diabetic patients with retinopathy than the diabetics without microvascular complications.

Conclusion
Fetuin-A may be used as a marker for microvascular complications in T2DM, especially the diabetic nephropathy. Antifetuin drugs may be invented to delay diabetic microvascular complications.

Keywords:
diabetes mellitus, diabetic nephropathy and diabetic retinopathy, fetuin-A, insulin

Introduction
Diabetes mellitus (DM) has routinely been described as a metabolic disorder characterized by hyperglycemia that develops because of defects in insulin secretion, insulin action, or both. Type 2 diabetes mellitus (T2DM) encompasses individuals who have insulin resistance (IR) and usually relative (rather than absolute) insulin deficiency [1]. T2DM has emerged as a major worldwide public health problem; according to Diabetes Atlas (7th ed.), the global prevalence of diabetes is estimated at 415 million (8.8%), which is predicted to increase to 642 million in the next 25 years [2].

Diverse biomarkers were studied for identifying of patients with T2DM at microvascular and macrovascular risk. Most of these markers are inflammatory, metabolic, or procoagulant molecules, indicating an unfavorable metabolic and vascular repute in patients with T2DM. However, different biomarkers display huge variations in hazard prediction depending on metabolic repute and disease severity of the study groups [3]. Posted records suggest that most novel biomarkers do not enhance hazard prediction while brought to fashions based totally on traditional hazard scores [4]. Yet, associations of novel biomarkers such as fetuin-A with metabolic markers or complications do assist to understand their position within the pathophysiology of the vascular disease [5].

Fetuin-A [also referred to as α-2 Heremans Schmid glycoprotein (AHSG)] is a multifunctional glycoprotein which is exclusively secreted from the hepatocytes in human [6]. For a long time, fetuin-A
Epidemiological research confirmed that serum inflammation [8], which led to IR [9]. It also became pronounced that fetuin-A could inhibit insulin receptor substrate-1 and stimulated a lower-grade IR. Prognostically, fetuin-A could inhibit insulin and phosphorus in serum [7]. It also became regarded as playing a key role in vascular calcification by solubilizing calcium [10]. In mice with knockout of fetuin-A, feeding of a mineral/vitamin D-rich food regimen led to arterial or soft-tissue calcification or both [7]. In human, so far, the available facts have been inconsistent. Lower fetuin-A ranges are associated with mortality and cardiovascular disorder occasions in cohorts with end-stage renal disease [12], whereas a population-based study linked excessive plasma fetuin-A to an increased hazard of myocardial infarction and ischemic stroke [13].

This study aimed to investigate fetuin-A levels in patients with T2DM and their relation to microvascular complications [diabetic retinopathy (DR) and diabetic nephropathy].

### Patients and methods

This descriptive study was conducted in Medical Biochemistry Department, Faculty of Medicine, Cairo University, in the period between September 2015 and May 2016. This study was approved by the Ethical Committee of Kasr Al-Ainy Medical Hospital and was conducted in accordance with the principle of Helsinki Declaration II. A written informed consent was obtained from each participant. A total of 60 patients with T2DM were selected from the outpatients and the inpatients section of the Internal Medicine Department at Beni-Suef University Hospital. They were diagnosed as diabetic patients according to the American Diabetes Association criteria [14]. Moreover, 40 (age and sex matched) apparently healthy individuals were selected from workers in Medical Biochemistry Department, Beni-Suef University. Group 1 included 40 patients with T2DM without complications (with a mean age of 59.8 years). Group 2 included 40 patients with T2DM with nephropathy (with a mean age of 57.0 years). Group 3 included 40 patients with T2DM with retinopathy (with a mean age of 60.0 years). Group 4 included 40 healthy age-matched and sex-matched control volunteers.

### Exclusion criteria

Patients having serum creatinine above 2.5 mg/dl, liver disorders, chronic inflammatory disease, infectious disease, a history of cardiovascular disease, malignant neoplasm, type 1 diabetes, other renal disease by urinary sediments and medical records, taking calcium or vitamin D supplementation, and taking insulin-sensitizing treatment such as pioglitazone were excluded. All participants were subjected to the following: full medical history and full clinical examination; anthropometric measurement including waist and hip circumference in cm, weight in kg, and height in cm; BMI calculated as weight in kilograms divided by square of height in meters [15]; fundus examination to assess DR; estimation of biochemical variables (fasting and postprandial glucose, total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), urea, creatinine clearance, and 24-h urinary protein) after an overnight fasting of at least 10 h; and estimation of serum levels of fetuin-A and insulin using enzyme-linked immunosorbent assays. Homeostasis model assessment for IR (HOMA-IR) values were calculated based on fasting value of plasma glucose and insulin according HOMA model formula: HOMA-IR = fasting insulin (mIU/l)×fasting glucose (mg/dl)/405. IR was arbitrarily considered altered when it wasmore than <2 [16].

Estimation of serum fetuin-A was done by using enzyme immunoassay kit procured from BioVendor Laboratory Medicine Inc. (Brno, Czech Republic). Estimation of insulin was done by enzyme-linked immunosorbent assay kit procured from Monobind Inc. (Lake Forest, California, USA). To estimate the fasting blood glucose and postprandial blood glucose, plasma glucose was measured by the glucose oxidase method using a commercially available kit supplied by Diamond, Egypt. Estimation of total cholesterol was estimated by Quantitative-Enzymatic-Colorimetric determination of total cholesterol though a serum kit, which was provided by StainBio Company (Texas, USA) [17]. Estimation of triglycerides was done by Quantitative-Enzymatic-Colorimetric determination of triglycerides through a serum kit GPO-POD provided by Diamond Company (Cairo, Egypt) [18]. Estimation of HDL-c was estimated by Quantitative-Enzymatic-Colorimetric determination of HDL-c through a serum kit, which was provided by StainBio Company [19]. Estimation of LDL-c value was calculated using Friedewald equation [20]: LDL=total cholesterol-HDL-TG/5. Estimation of creatinine was done by kinetic method according to modified Jaffé method kit, which was provided by StainBio Company [21]. Estimation of urea was done by an enzymatic micromethod according to modified...
urease method kit, which was provided by StainBio Company [22]. To estimate urinary protein, 24-h urine sample was collected by instructing participants to begin collection immediately after completion of first voiding in morning and to collect all urine into same container for 24 h, including final void at completion of 24-h period [23]. Urinary total protein was analyzed using Thermo microprotein kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Urinary excretion of protein is normally less than 0.15 g/24 h (150 mg/24 h). The proteinuria of at least 300 mg/dl in 24-h urine sample was considered as significant proteinuria [24].

Statistical analysis
Analysis of data was performed using SPSS 21 for Windows SPSS (IBM Corp., Released 2013, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY, USA). Description of variables was presented as follows: description of qualitative variables was in the form of numbers (n) and percentages (%). Comparison between quantitative variables was carried out by student t-test of two independent samples. Results were expressed in the form of P values. Comparison between nonparametric quantitative variables was carried out by Mann–Whitney U-test. Kruskal–Wallis test was used when comparing between more than two groups of independent variables. Results were expressed in the form of P values. Comparison between qualitative variables was carried out by χ²-test. Fisher’s exact test was used instead of χ²-test when one expected cell or more were 5 or less. Binary correlation was carried out by Spearman’s correlation test. Results were expressed in the form of correlation coefficient (R) and P values. The following points are the accepted guidelines for interpreting the correlation coefficient: 0 indicates no linear relationship and +1 indicates a perfect positive linear relationship: as one variable increases in its values, the other variable also increases in its values by an exact linear rule.

Results
Table 1 showed the demographic, laboratory, and measured data of the studied groups. A highly significant increase was seen in the mean level of systolic blood pressure (SBP), diastolic blood

| Parameters               | Group 1 diabetic without complications | Group 2 diabetic nephropathy group | Group 3 diabetic retinopathy group | Group 4 control group |
|--------------------------|----------------------------------------|------------------------------------|------------------------------------|-----------------------|
| Age                      | 59.8±6.3                               | 57.0±9.6                           | 60.0±8.3                           | 57.9±7.3              |
| Sex [n (%)]              |                                        |                                    |                                    |                       |
| Male                     | 5 (25)                                 | 5 (25)                             | 6 (30)                             | 8 (40)                |
| Female                   | 15 (75)                                | 15 (75)                            | 14 (70)                            | 12 (60)               |
| Disease duration (years) | 12.1±2.8                               | 14.2±3.5(c)                        | 11.8±4.2                           |                       |
| SBP (mmHg)               | 140.5±11.6(a)*                         | 142.3±16.7(a)*                     | 145.5±11.2(a)*                     | 119.8±8.0             |
| DBP (mmHg)               | 91.5±6.5(a)*                           | 89.5±12.6(a)*                      | 94.8±8.7(a)*                       | 78.3±3.7              |
| Waist circumference (cm) | 87.2±10.3(a)*                          | 94.6±5.0(a)(b)(c)                  | 89.4±9.4(a)*                       | 72.6±6.2              |
| Hip circumference (cm)   | 96.8±7.7(a)*                           | 103.3±4.7(a) (b) (C)               | 98.0±4.0(a)*                       | 85.3±6.9              |
| WH ratio                 | 0.9±0.1(a)*                            | 0.9±0.0(a)*                        | 0.9±0.0(a)*                        | 0.8±0.0               |
| BMI (kg/m²)              | 32.9±5.4(a)*                           | 34.0±4.5(a)*                       | 32.5±4.8(a)*                       | 25.5±2.2              |
| TAG (mg/dl)              | 146.0±11.1                             | 208.9±49.1(a)(b) (c)*              | 170.4±36.3(a)(b)                   | 137.4±9.4             |
| Cholesterol (mg/dl)      | 173.6±24.3                             | 220.0±27.7(a)(b)(c)*               | 184.7±32.0(a)                      | 163.9±10.9            |
| HDL-c (mg/dl)            | 61.7±7.1                               | 50.7±11.3(a) (b)                   | 50.9±14.5(a)(b)*                   | 62.4±5.6              |
| LDL-c (mg/dl)            | 105.2±58.2                             | 156.6±35.9(a)(b)(c)*               | 126.1±40.2(a)(b)                   | 95.4±13.7             |
| Urinary protein (mg/24 h)| 135.2±13.8                             | 690.8±275.1(b)(c)*                 | 131.1±21.1                         |                       |
| Creatinine (mg/dl)       | 0.8±0.2                                | 1.9±0.3(a)(b)(c)*                  | 0.7±0.3                            | 0.7±0.2               |
| Urea (mg/dl)             | 35.4±4.3                               | 87.2±23.2(a)(b)(c)*                | 35.8±4.8                           | 34.5±5.7              |
| FBG (mg/dl)              | 249.3±58.2(a)(c)*                      | 184.0±23.4(a)(b)*                  | 165.5±28.0(a)*                     | 87.4±11.3             |
| PPBG (mg/dl)             | 318.9±73.9(a)*                         | 379.9±81.9(a)(b)                   | 348.6±82.5(a)*                     | 111.0±11.7            |
| Insulin (μU/ml)          | 15.1±3.8(a)                            | 23.0±5.8(a)(b)(c)*                 | 17.6±7.0(a)                        | 7.6±2.2               |
| HOMA-IR                  | 9.2±2.7(a)(c)*                         | 10.5±3.4(a)(c)*                    | 7.4±3.7(a)                         | 1.6±0.6               |
| Fetuin-A (mg/dl)         | 254.8±45.6(a)*                         | 323.8±50.8(a)(b)(c)*               | 259.7±57.4(a)*                     | 159.5±33.6            |

Letter (a) denotes a significant difference as compared with group 4, letter (b) denotes a significant difference as compared with group 1, letter (c) denotes a significant difference as compared with group 3. Results are expressed as means±SD. FBG, fasting blood sugar; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; LDL-c, low-density lipoprotein-cholesterol; PPBG, postprandial blood sugar; TAG, triacyl glycerides. *High significance (P<0.01).
pressure (DBP), waist–hip ratio (W/H) ratio, and BMI in diabetic patients without complications group (group 1), diabetic nephropathy group (group 2), and DR group (group 3) compared with the control group (group 4). A highly significant increase was seen in the mean level of waist circumference and hip circumference in diabetic nephropathy group (group 2) compared with both diabetic without complications group (group 1) and DR group (group 3). There was a significant increase in the mean level of the disease duration in diabetic nephropathy group (group 2) compared with the DR group (group 3). No significant differences were detected in the mean levels of age and sex between the studied groups.

A significant increase was seen in the mean level of triacylglycerol (TAG), cholesterol, and LDL-c in DR group (group 3) versus both control group (group 4) and diabetic without complications group (group 1). A highly significant increase was seen in the mean levels of TAG, cholesterol, LDL-c, 24-h urinary protein, creatinine, and urea in diabetic nephropathy group (group 2) versus other groups.

A highly significant decrease was seen in the mean level of HDL-c in diabetic nephropathy group (group 2) versus both diabetic without complications group (group 1) and control group (group 4). A highly significant decrease was seen in the mean level of HDL-c in DR group (group 3) versus both diabetic without complications group (group 1) and control group (group 4). No significant differences were detected in laboratory data between the diabetic without complications group (group 1) versus the control group (group 4). A significant increase was seen in the mean levels of fasting blood glucose (FBG) and HOMA-IR (Fig. 1) in diabetic without complications group (group 1) versus the DR group (group 3). A highly significant increase was seen in the mean levels of FBG, postprandial blood glucose (PPBG), insulin (Fig. 2), and fetuin-A (Fig. 3) in the diabetic nephropathy group (group 2) versus the diabetic without complications group (group 1). A highly significant increase was seen in the mean levels of insulin, HOMA-IR, and fetuin-A in diabetic nephropathy group (group 2) versus the DR group (group 3). No significant differences were

Figure 1

The mean levels of serum homeostasis model assessment for insulin resistance (HOMA-IR) in the studied groups. DM, diabetes mellitus.

Figure 2

The mean levels of serum insulin in the studied groups. DM, diabetes mellitus.
detected in the mean levels of PPBG, insulin, and fetuin-A between diabetic without complications group (group 1) and DR group (group 3). No significant differences were detected in the mean levels of FBG and PPBG between diabetic nephropathy group (group 2) and DR group (group 3). No significant differences were detected in the mean level of HOMA-IR between the diabetic without complications group (group 1) and diabetic nephropathy group (group 2).

Table 2 showed the correlations of serum level of fetuin-A with data of the studied groups. In the diabetic without complications group (group 1), there was a significant positive correlation between fetuin-A and BMI ($r=0.59$, $P=0.006$), SBP ($r=0.77$, $P<0.001$), DBP ($r=0.55$, $P=0.012$), waist circumference ($r=0.72$, $P<0.001$), W/H ratio ($r=0.78$, $P<0.001$), TAG ($r=0.47$, $P=0.038$), cholesterol ($r=0.66$, $P=0.004$), LDL-c ($r=0.68$, $P=0.001$), insulin ($r=0.61$, $P<0.001$, Fig. 4), HOMA-IR ($r=0.76$, $P<0.001$, Fig. 5), and the duration of the disease ($r=0.7$, $P<0.001$). There was a significant negative correlation between fetuin-A and HDL-c ($r=-0.45$, $P=0.046$).

In the diabetic nephropathy group (group 2), there was a significant positive correlation between fetuin-A and SBP ($r=0.47$, $P=0.037$), DBP ($r=0.54$, $P=0.015$), waist circumference ($r=0.65$, $P=0.002$), hip circumference ($r=0.63$, $P=0.003$), TAG ($r=0.60$, $P=0.005$), LDL-c ($r=0.47$, $P=0.038$), 24-h urinary protein ($r=0.51$, $P=0.023$, Fig. 6), FBG ($r=0.60$, $P=0.006$), insulin ($r=0.69$, $P=0.001$), and HOMA-IR ($r=0.78$, $P<0.001$).

In the DR group (group 3), there was a significant positive correlation between fetuin-A and BMI ($r=0.77$, $P<0.001$), SBP ($r=0.52$, $P=0.019$), DBP ($r=0.58$, $P=0.007$), waist circumference ($r=0.45$, $P=0.046$), W/H ratio ($r=0.62$, $P=0.003$), TAG ($r=0.53$, $P=0.016$), cholesterol ($r=0.61$, $P=0.004$), LDL-c ($r=0.62$, $P=0.004$), FBG ($r=0.51$, $P=0.023$), insulin ($r=0.84$, $P<0.001$), HOMA-IR ($r=0.85$, $P<0.001$), and the duration of the disease ($r=0.7$, $P=0.001$).

**Figure 3**

The mean levels of serum fetuin-A in the studied groups. DM, diabetes mellitus.

**Figure 4**

Correlation between fetuin-A (mg/dL) and insulin in diabetic without complications group (group 1) ($r=0.61$, $P<0.001$).

**Figure 5**

Correlation between fetuin-A (mg/dL) and homeostasis model assessment for insulin resistance (HOMA-IR) in diabetic without complications group (group 1) ($r=0.76$, $P<0.001$).
Table 2: Correlation between fetuin-A (mg/dl) and the data of the studied groups (N=40)

| Parameters                  | Group 1 diabetic without complications | Group 2 diabetic nephropathy group | Group 3 diabetic retinopathy group | Group 4 control group |
|-----------------------------|----------------------------------------|------------------------------------|-----------------------------------|-----------------------|
| Age (years)                 | -0.42                                  | -0.38                              | -0.34                             | -0.09                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| BMI (kg/m²)                 | 0.065                                  | 0.100                              | 0.137                             | 0.695                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| SBP (mmHg)                  | 0.59**                                 | 0.35                               | 0.77**                            | 0.79**                |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| DBP (mmHg)                  | <0.001                                 | 0.037                              | 0.019                             | 0.470                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Waist circumference (cm)    | 0.72**                                 | 0.65**                             | 0.45*                             | 0.68**                |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Hip circumference (cm)      | 0.63**                                 | 0.29                               | 0.56*                             |                      |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Waist–hip ratio             | 0.78**                                 | 0.13                               | 0.62**                            | 0.27                  |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| TAG (mg/dl)                 | 0.47**                                 | 0.60**                             | 0.53*                             | 0.52*                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Cholesterol (mg/dl)         | 0.66**                                 | 0.34                               | 0.61**                            | 0.23                  |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| HDL-c (mg/dl)               | 0.001                                  | 0.138                              | 0.004                             | 0.333                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| LDL-c (mg/dl)               | -0.45*                                 | -0.31                              | -0.41                             | -0.04                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Urinary protein (mg/24 h)   | 0.68**                                 | 0.47*                              | 0.62**                            | 0.20                  |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Creatinine (mg/dl)          | 0.001                                  | 0.005                              | 0.016                             | 0.019                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Urea (mg/dl)                | 0.20                                   | 0.51*                              | 0.18                              |                      |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| FBG (mg/dl)                 | -0.31                                  | 0.02                               | 0.07                              | 0.00                  |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| PPBG (mg/dl)                | 0.191                                  | 0.929                              | 0.760                             | 0.984                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Insulin (\(\mu\)IU/ml)     | -0.10                                  | 0.02                               | 0.10                              | -0.25                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| HOMA-IR                     | 0.662                                  | 0.920                              | 0.684                             | 0.282                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Insulin (\(\mu\)IU/ml)     | 0.066                                  | 0.006                              | 0.023                             | 0.090                 |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| Duration of the disease (years) | 0.69**                              | 0.69**                             | 0.84**                            | 0.72**                |
|                            | \(r\)                                  | \(P\)                              | \(r\)                             | \(P\)                 |
| DBP, diastolic blood pressure; FBG, fasting blood sugar; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; LDL-c, low-density lipoprotein cholesterol; PPBG, postprandial blood sugar; SBP, systolic blood pressure; TAG, triacyl glycerides; W/H ratio, waist–hip ratio. *Correspondence to Correlation is significant, \(P<0.05\) level. **Correlation is significant, \(P<0.01\) level.
In the control group (group 4), there was a significant positive correlation between fetuin-A and BMI ($r=0.79$, $P<0.001$), waist circumference ($r=0.68$, $P=0.001$), hip circumference ($r=0.56$, $P=0.010$), TAG ($r=0.52$, $P=0.019$), insulin ($r=0.72$, $P<0.001$), and HOMA-IR ($r=0.72$, $P<0.001$).

**Discussion**

The gene encoding fetuin-A is located on chromosome 3q27, the chromosomal location that was formerly mapped as a T2DM and MetS susceptibility locus [25]. It was well known that IR is the underlying mechanism of T2DM [26].

In this study, we evaluated the associations of parameters of microvascular disease in patients with T2DM, DR, and diabetic nephropathy as the degree of protein excretion and renal function (serum urea and serum creatinine) with fetuin-A. This study included four main groups: T2DM without complications (group 1), diabetic nephropathy (group 2), DR patients (group 3), and normal healthy control (group 4). Of the 160 included participants, 98 were females and 62 were males, and their ages ranged between 47 and 76 years. There were no significant differences detected in the mean levels of age and sex between the studied groups.

We observed a highly significant increase in the mean levels of fetuin-A in all diabetic groups (1, 2, 3) than the control group (4), and there was a significant positive correlation between fetuin-A, insulin, and HOMA-IR in all studied groups.

Our results coincided with Guo *et al.* [27] who showed that higher circulating fetuin-A levels were associated with increased risk of T2DM.

Sujana *et al.* [28] agreed with us because they concluded that higher fetuin-A levels are associated with incident T2DM in both male and female individuals. They added that increase in fetuin-A levels was independent of subclinical inflammation, adiponectin, and liver fat content.

Pinnaduwage *et al.* [29] agreed with us as they concluded that circulating hepatic markers, particularly fetuin-A, track with changes in insulin sensitivity and β-cell function, supporting a pathophysiologic basis in their prediction of diabetic risk.

Roshanzamir *et al.* [30] concurred with our results as they showed a significant relationship between the fetuin-A levels with T2DM risk.

In accordance with our results, Yin and colleagues suggested that the plasma fetuin-A levels may be associated with macroangiopathies in patients with new-onset T2DM. Therefore, detecting early plasma fetuin-A levels in patients with new-onset T2DM provides an opportunity to intervene in the formation of carotid artery disease in diabetic patients and deliver timely treatment for the prevention of diabetic vascular disease [31].

Ou *et al.* [32] agreed with us because they suggested that fetuin-A may further aggravate increased arterial stiffness in diabetes.

We concur with Iyidir *et al.* who indicated that serum fetuin-A concentrations are increased in women with gestational DM and decrease after delivery. Therefore, fetuin-A might have a role in the development of IR and the metabolic changes in gestational DM [33].

In accordance with these results, a previous study by Ix *et al.* [11] found that serum fetuin-A was related to incident diabetes, independent of other markers of IR.

In accordance with our results, Song *et al.* showed that serum fetuin-A concentrations have been drastically higher in T2DM patients than subjects with normal glucose tolerance and impaired glucose tolerance, but they disagreed with us regarding the correlation between fetuin-A and IR, as they showed that higher fetuin-A concentrations had been independently related to IR in not only nondiabetic participants but also T2DM patients [34].

Stefan *et al.* [35] agreed with us, as they showed in a 7-year follow-up study significant associations among
fetuin-A and extended risk for DM, specifically in people with increased plasma glucose levels within the nondiabetic variety.

We agreed with Takata et al. [36] who have shown that high concentrations of glucose induced transactivation of the AHSG gene, which encodes fetuin-A protein, expression in cultured human hepatoma cells, and such finding approved the relation between high levels of fetuin-A and the development of DM.

This correlated also with Ishibashi et al. [37] who found that serum fetuin-A concentrations were significantly associated with IR. They confirmed that the serum fetuin-A level is independently associated with IR in Japanese patients.

Kröger et al. [38] disagreed with us as they concluded although there was mechanistical evidence for an effect of fetuin-A on insulin sensitivity and secretion, their study did not support a strong, relevant relationship between circulating fetuin-A and diabetes risk in the general population.

Our results did not coincide with Eleftheriadou et al. [39] who showed that plasma fetuin-A levels were lower in patients with T2DM.

In contrast to our study, Eraso et al. [40] showed that circulating fetuin-A was lower in T2DM with vascular complications than diabetes controls. Similar disagreement was shown by Roos et al. [41] who found that lower fetuin-A levels seem to be associated with prevalent macrovascular disease in T2DM.

Mori et al. [42] disagreed with us as they evaluated serum fetuin-A in T2DM and nondiabetic individuals and did no longer discover any distinction between groups in terms of fetuin-A and any significant correlation between IR and fetuin-A.

Our results did not coincide with Yilmaz et al. [43] who observed significantly lower fetuin-A levels in the diabetic group owing to urinary loss of fetuin-A; moreover, Fethiye and colleagues disagreed with us as they found that lower fetuin-A levels in the T2DM group compared with the control group [44].

In this work, there was a significant increase in the mean levels of TAG, cholesterol, and LDL-c in diabetic patients with retinopathy group and diabetic patients with nephropathy group than the control group, and there was a significant decrease in the mean levels of HDL-c in the diabetic patients with retinopathy group and the diabetic with nephropathy group than the diabetic group and the control group. There was a significant positive correlation between fetuin-A, TAG, cholesterol, and LDL-c in the diabetic and the diabetic with retinopathy groups. In the diabetic with nephropathy group, there was a significant positive correlation between fetuin-A, TAG, and LDL-c. There was a significant negative correlation between fetuin-A and HDL-c in the diabetic group. We also found there was a significant positive correlation between fetuin-A and some clinical and metabolic parameters such as blood pressure, BMI, waist circumference, and W/H ratio in all studied groups.

Zhou et al. agreed with us because they showed that higher serum fetuin-A levels in obese T2DM patients compared with nonobese patients and obese normal glucose tolerance patients, which supports the hypothesis that fetuin-A may be a bridge connecting obesity and obesity-related T2DM [45].

The study by Reinehr et al. [46] coincided with ours, as it concluded that the increase of fetuin-A levels in obese adolescents with T2DM supports the hypothesis that fetuin-A is involved in the pathogenesis of T2DM, because this hepatokine leads to IR.

Stepień and colleagues showed similar results who aimed to estimate the association between anthropometric obesity parameters, and HOMA-IR in obese nondiabetic insulin-sensitive and insulin-resistant patients and explained those by inhibition of the insulin receptor by fetuin-A may lead to increased lipolysis and efflux of free fatty acids from adipose tissue. This may, in turn, lead to increased production of apolipoprotein B-containing lipoproteins (very-low density lipoprotein) [47]. Furthermore, hypertriglyceridemia may lead to a decrease in the cholesterol content of HDL, which may enhance HDL clearance from the circulation, thereby potentially leading to the atherogenic lipid profile observed in this study [48].

In accordance with our results, Ayako and colleagues found a significant association between serum fetuin-A and atherogenic lipid profile in individuals without any coronary artery disease. They explained that another factor may promote the elevation in fetuin-A and LDL-c levels; for example, transcriptional factors that regulate cholesterol homeostasis could be involved in the regulation of hepatic synthesis of fetuin-A [37].

We agreed with Xu et al. [49] who found that serum fetuin-A is correlated with MetS in middle-aged and
In contrast to these results, Yilmaz et al. [10] agreed with us as they reported an association between human fetuin-A and the MetS nondiabetic outpatients with coronary artery disease.

In contrast to our study, Ayako and colleagues, Eraso and colleagues, Roos and colleagues, and Fethiye and colleagues found that fetuin-A levels did not correlate with metabolic parameters in their patients with T2DM with prevalent late complications. They explained their results by exclusion of patients with chronic diseases as CAD from their study which could explain the higher prevalence of MetS in the previous studies [40,41,44].

In our study, there was a high significant increase seen in the mean levels of insulin, HOMA-IR, and fetuin-A in the diabetic with nephropathy group versus other groups. There was a significant positive correlation between fetuin-A and 24-h urinary protein in the diabetic with nephropathy group.

As previously known, there was a direct correlation between adiponectin levels and overt proteinuria [8]. Fetuin-A suppresses mRNA encoding adiponectin in cultured human adipocytes, and treatment of wild-type mice with fetuin-A lowered serum adiponectin levels [50]. Lower adiponectin levels reduce AMP-activated protein kinase in podocytes to promote foot process effacement and albuminuria [51]. All these previous reports could explain the relation between fetuin-A and albuminuria.

In accordance with our results, Inoue et al. [52] showed that urinary excretion of fetuin-A significantly increased during the progression of albuminuria, and they concluded that urinary fetuin-A was demonstrated as a risk factor for both microalbuminuria and reduction of glomerular filtration rate in diabetic nephropathy.

Other studies were in accordance with our results [40,41,44]. Fetuin-A levels were not different according to the presence of proteinuria [44]. In contrast to our results, Eraso et al. [40] showed that fetuin-A serum levels were not associated with 24-h urinary albumin excretion in patients with early diabetic nephropathy.

This study showed that there was a nonsignificant increase in the mean levels of fetuin-A in patients with DR compared with diabetic patients without microvascular complications.

In contrast to our results, Yilmaz and colleagues suggested an association between fetuin-A levels and DR stage. In diabetic patients, the risk of retinopathy development increases with higher fetuin-A values. Fetuin-A may play an important role in the pathophysiology and progression of DR [54].

We disagreed with Zhao et al. [55] as they suggested the occurrence and severity of DR is correlated with serum and vitreous fetuin-A concentrations.

**Conclusion**

The study proved that there were high levels of fetuin-A in patients with T2DM which were associated with IR and metabolic parameters. The results suggested that higher levels of fetuin-A were associated with the diabetic nephropathy. Fetuin-A may be used as a marker for microvascular complications in T2DM, especially the diabetic nephropathy group. Antifetuin agents may be used to delay diabetic microvascular complications.

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**Conflicts of interest**

There are no conflicts of interest.

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