# Serum visfatin and its relation to insulin resistance and inflammation in type 2 diabetic patients with and without macroangiopathy

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## ABSTRACT

**الأهداف:** استقصاء مستويات الفيزفاتين وعلاقته بالبدانة ومقاومة الأنسولين وبروفيل الشحميات والالتهاب في النمط الثاني من الداء السكري مع اعتلال الأوعية الكبيرة وبدونه.

**الطريقة:** صُمّم هذا البحث في كلية الطب – جامعة القصيم – المملكة العربية السعودية، وبعد موافقة اللجنة الأخلاقية تم التنفيذ في الفترة ما بين أبريل ٢٠٠٦ ومايو ٢٠٠٧. تضمّن البحث ٨٨ شخصاً قسموا إلى ثلاث مجموعات: ٢٩ مريضاً بالنمط الثاني من الداء السكري بدون اعتلال الأوعية الكبيرة، و٣٣ مريضاً بالسكري بدون مضاعفات، و٢٢ شخصاً من الأصحّاء من غير المصابين بالسكري كمجموعة ضبط. بعد صيام ١٤ ساعة، تم قياس الطول والوزن وضغط الدم والحصول على عينة دم واحدة. تمت معايرة الفيزفاتين والأنسولين والإنترلوكين–٦ والبروتين المتفاعل C بتقنية الإليزا (ELISA) بينما استخدمت الطريقة الإنزيمية اللونية لقياس الجلوكوز ومكونات البروفيل الشحميّ. أما البدانة ومقاومة الأنسولين فقد تمّ تقييمها باستخدام منسب كتلة الجسم وصيغة HOMA–IR، على التوالي.

**النتائج:** على عكس مجموعة الضبط، ترافقت مجموعتا السكّري بالبروفيل الشحميّ المؤهّب للتصلّب العصيدي وبمستويات أعلى من الجلوكوز والأنسولين وHOMA–IR والإنترلوكين–٦ والبروتين المتفاعل C. كما لوحظ وجود ارتفاع ذو دلالة إحصائية في مستويات الفيزفاتين في مجموعة مرضى السكري غير المترافقة بالمضاعفات الوعائية بالمقارنة مع مجموعة الضبط ومجموعة مرضى السكري المصابين باعتلال الأوعية الكبيرة. إلا أنّ الفرق في هذه المستويات بين مرضى السكر المصابين بالمضاعفات الوعائية والأصحّاء كان بدون دلالة إحصائية. لم يكن هناك علاقة بين مستويات الفيزفاتين وقيم كلٍ من منسب كتلة الجسم والجلوكوز والأنسولين ومدى مقاومة الأنسولين وحساسيته. أما العلاقة بين قيم الفيزفاتين وكلٍ من الإنترلوكين–٦ والبروتين المتفاعل C ومدى خطورة البروفيل الشحميّ من حيث إحداث التصلّب العصيديّ فكانت عكسيّةً وذات دلالة إحصائية.

**خاتمة:** تشير النتائج إلى ارتفاع مستويات الفيزفاتين في الداء السكّري بصرف النظر عن قيمة منسب كتلة الجسم ونمط معالجة هذا الداء. ارتبطت هذه المستويات بشكل عكسي بكل من واصمات الالتهاب (IL–6, CRP) ومدى خطورة البروفيل الشحمي كعامل محدث للتصلب العصيديّ

**Objective:** To explore visfatin levels and its relationship to obesity, insulin resistance, lipid profile and inflammation in type 2 diabetes mellitus (T2DM) patients with and without macroangiopathy.

**Methods:** The study was designed in the College of Medicine, Qassim University, Qassim, Kingdom of Saudi Arabia, and implemented between April 2006 and May 2007. It involved 84 subjects divided into 3 groups: 29 T2DM patients without macroangiopathy, 33 T2DM patients with macroangiopathy, and 22 non-diabetic controls. After overnight fast, weight, height, and blood pressure were measured and a single blood sample was obtained. Serum visfatin, insulin, interleukin-6 (IL-6), and high sensitive C-reactive protein (hsCRP) were measured by Enzyme-linked immunosorbent assay (ELISA), whereas glucose and lipid profile were measured using colorimetric enzymatic methods. Obesity was measured using body mass index (BMI) and insulin resistance was measured using Homeostasis Model Assessment-Insulin Resistance (HOMA-IR).

**Results:** Compared to control, both non-macroangiopathic and macroangiopathic diabetic groups were associated significantly with an atherogenic lipid profile, and high levels of glucose, insulin, HOMA-IR, hsCRP and IL-6. Serum visfatin was significantly higher in non-complicated diabetics compared to controls and macroangiopathic diabetics, but difference in visfatin level between macroangiopathic diabetics and controls was insignificant. Visfatin levels were not correlated with BMI, insulin, glucose, or HOMA-IR, but they were negatively and significantly correlated with hsCRP, IL-6, and the atherogenicity of lipid profile.

**Conclusion:** Visfatin levels were increased in T2DM regardless of BMI and type of diabetic treatment. These levels were correlated negatively with inflammatory markers and atherogenicity of lipid profile.


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Type 2 diabetes mellitus (T2DM) profoundly accelerates the development of atherosclerosis and cardiovascular diseases, which are major causes of mortality and morbidity in diabetics.[1,2] Insulin resistance, represented mainly by hyperinsulinemia, hyperglycemia, and dyslipidemia, is a major factor in this regard.[3,4] Inflammation is also known to play a role in atherogenesis and atherosclerotic plaque progression. Many inflammatory cytokines and biomolecules are implicated in these processes and have, therefore, been investigated as potential markers of atherosclerotic plaque progression, and cardiovascular disease risk. Of these, the best characterized and most widely studied is C-reactive protein (CRP). It is the most sensitive marker of inflammation and its association with hyperglycemia and the development of atherosclerotic disease has been observed in experimental and epidemiological studies.[5-8] Epidemiological studies revealed that obesity, especially abdominal, is a risk factor for T2DM and both conditions are associated with insulin resistance, dyslipidemia and atherosclerosis.[9-11] They are also considered as inflammatory conditions as indicated by increased plasma concentrations of CRP, interleukin-6 (IL-6) and other inflammatory markers.[12] Factors in adipose tissue that link obesity to insulin resistance, T2DM and atherosclerosis are not yet clear. During the last decade, it has become increasingly apparent that adipose tissue, besides releasing and storing lipids, is a very active endocrine organ that secretes a wide range of proteins collectively known as "adipocytokines" or "adipokines." Some of these adipokines (for example, leptin, resistant and adiponectin) are known to affects insulin action and sensitivity[13] whereas others (for example, tumor necrosis factor-alpha and IL-6) are related to immune and inflammatory responses.[14,15] So, a study investigating the link between visceral adipokines and insulin resistance and inflammatory markers may help provide clues for understanding the link between obesity, T2DM and atherosclerotic complications. In 1994, Samal et al[16] identified a novel protein expressed in immature lymphocytes and called it pre-B-cell colony-enhancing factor (PBEF). In 2005, Fukuhara et al[17] demonstrated that this protein is also expressed in adipocytes and secreted from adipose tissue and named it visfatin as it is highly expressed in visceral fat. They reported that visfatin has insulin-like metabolic effects on glucose metabolism but has a distinct binding site on insulin receptors. These findings triggered great interest for many researchers to explore the mechanism(s) of regulation of visfatin expression, and the possible relationships between visfatin and obesity, insulin resistance, and T2DM.[18-21] However, the available information on visfatin is still too little and controversial leaving our understanding of this adipokine and its physiological and pathophysiologic roles in humans in its infancy. As yet, there is no published data on the relationship between visfatin and inflammatory markers and atherosclerotic lipid profile, associated with T2DM complicated with macroangiopathy, the aim of this study is to explore the levels of visfatin and its relationship to each of obesity, insulin resistance, lipid profile and inflammation in T2DM with and without macroangiopathy.

**Methods.** ***Study approval, subjects and anthropometric assessments.*** After approval by the ethics committee of the College of Medicine, Qassim University, Saudi Arabia, the study and its purpose were explained to all of the participants and their consent was obtained. The study was implemented between April 2006 and May 2007 and involved 84 subjects divided into 3 groups: 29 T2DM patients (16 males and 13 females) without macroangiopathy (non-complicated group); 33 patients (15 males and 18 females) with T2DM complicated with macroangiopathy in the form of recent (<6 months) myocardial infarction, unstable angina or stroke (macroangiopathic group); and the control group, which involved 22 (10 males and 12 females) gender- and age- matched, apparently healthy non-diabetics having fasting serum glucose less than 110 mg/dl and no family history of T2DM. Diabetes mellitus was diagnosed based on the American Diabetes Association criteria, as reported in 1998.[22] Before inclusion, all participants underwent careful physical examination and detailed laboratory investigations to exclude any condition that may interfere with glucose tolerance and inflammation. Exclusion criteria were smoking, acute or chronic infections, cancer, hepatic or renal disease, or medications that affect inflammation or lipid levels other than those used in T2DM. Standing height and weight were measured with the patients barefoot in light clothing. Body mass index (BMI) was calculated as weight divided by squared height ($kg/m^2$). Individuals with a BMI of 30 or more are considered obese, while those with a BMI of 25 to 29.9 are considered overweight.[23] Blood pressure measurements were made after subjects had remained in the sitting position for 10 minutes. Measurements were made twice; with a 5 minutes rest period and mean value of measurements was used.

***Sampling and laboratory analysis.*** After overnight (14 hours) fasting, blood samples were collected between 08:00 and 09:00 in the morning. Each sample was divided into 2 parts, one part was kept as whole blood for estimation of glycohemoglobin ($HbA_1$) by colorimetry using a kit provided by Stanbio Co. (Texas, USA), and the other part was left to clot and then centrifuged at 2500 g at 4°C for 10 min. Sera to be analyzed for glucose,

lipids and liver, and kidney function tests were stored at 4°C for 2-4 hours till the estimation time. A second aliquot of serum was stored at -80°C for future analysis of other parameters. Fasting serum glucose (FSG), total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) levels were measured by the enzymatic colorimetric method using kits from Crescent Diagnostics (London, UK). Low density lipoprotein cholesterol (LDL-C) level was calculated by Friedwald's formula.[24] Enzyme-linked immunosorbent assay (ELISA) method was used to estimate the levels of each of insulin (Human insulin ELISA kit, Linco Research, St. Charles, Missouri, USA with sensitivity "minimum detectable concentration": 2 µU/ml, Intra-assay <5.96% and Inter-assay <10.3%), visfatin (Human visfatin ELISA kit, AdipoGen Inc., Seoul, Korea with sensitivity: 30 pg/ml, Intra-assay <5.53% and Inter-assay <9.53%), IL-6 (Human IL-6 ELISA kit, R&D System, Inc., Minneapolis, USA with sensitivity <0.7 pg/ml, Intra-assay <4.2% and Inter-assay <6.4%), and the well-known marker of inflammation, high-sensitive C-Reactive protein (hsCRP) (Human hsCRP ELISA kit, Diagnostics Biochem Canada Inc., Ontario, Canada with sensitivity: 0.7 ng/ml, Intra-assay <15.2% and Inter-assay <9.9%). The procedures provided with these kits were applied exactly as mentioned. The insulin resistance index was calculated using the formula of HOMA-IR where HOMA-IR=fasting insulin (µU/ml) X fasting glucose (mg/dl)/405.[25] Low values of HOMA-IR indicate high insulin sensitivity, whereas high values indicate low insulin sensitivity (insulin resistance).

***Statistical analysis.*** GraphPad Prism version 4.00 (GraphPad software Inc.) was used in all statistical processes. Results are presented as means ± SD. The means of variables were compared using ANOVA test and Bonferroni's Multiple Comparison Test as a post-hoc test, whereas Pearson's correlation technique was used to explore the correlations between different parameters. A $p$ value of <0.05 was accepted to indicate statistical significance.

**Results.** The clinical and biochemical characteristics of the studied groups are given in **Table 1**. There was no significant difference in age and gender among the 3 studied groups. Diabetes duration was slightly higher in macroangiopathic T2DM group compared to non-complicated one without any statistical significance. Body mass index was significantly higher in non-complicated and complicated diabetic groups compared to control ($p$<0.01 in both cases). However, BMI did not differ between the 2 diabetic groups (**Figure 1**). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in non-complicated diabetic ($p$<0.01 in both) and complicated ($p$<0.001 in both) diabetic groups compared to control as well as in complicated diabetics compared to non-complicated ones ($p$<0.01 in both). There was no significant difference in the levels of FSG, $HbA_1$, insulin, HOMA-IR, TG, TC, and LDL-C between the 2 diabetic groups. However, all these levels increased significantly in both groups compared to control. However, HDL-C was higher and LDL-C/HDL-C was lower in controls compared to the non-complicated ($p$<0.01 and <0.001), and complicated diabetics ($p$<0.001 in both). Likewise, HDL-C was higher and LDL-C/HDL-C was lower in non-complicated diabetics compared to complicated ones (<0.05 in both). Interlukin-6 and hsCRP measurements showed significant differences among all of the studied groups with the highest level in diabetics with macroangiopathy and the lowest level in the control subjects (**Table 1 & Figure 1**). Visfatin levels were slightly higher in macroangiopathic diabetic patients than in the controls but statistically insignificant. These levels were significantly higher in non-complicated diabetics compared to controls ($p$<0.001) and macroangiopathic diabetics ($p$<0.05) (**Table 1 & Figure 1**). With regard to gender, there were no significant differences between male and female levels of visfatin in control (8.13±3.01 versus 9.58±4.96 ng/ml), non-complicated diabetic (13.75±6.00 versus 14.99±5.63 ng/ml) and macroangiopathic diabetic (10.86±6.74 versus 10.76±6.08 ng/ml) groups. Comparison of visfatin levels among obese and overweight subjects revealed no significant differences in non-complicated diabetics (No. 17 versus 12; visfatin: 12.92±4.97 versus 16.26±6.45 ng/ml) and complicated ones (No. 15 versus 18; visfatin: 9.17±4.21 versus 12.17±7.45 ng/ml). In addition, there was no significant difference in visfatin levels among normal weight and overweight control subjects (No. 10 versus 12; visfatin: 8.41±4.50 versus 9.35±4.00 ng/ml). On the other hand, the levels of visfatin in non-complicated T2DM group did not differ significantly between patients on diet treatment only (14.68±6.73 ng/ml; No. 10) and those on oral hypoglycemic agents (14.11±5.39 ng/ml; No. 19). Similarly, in T2DM patients with macroangiopathy, there was no difference between the 8 patients on diet treatment (7.00±4.47 ng/ml), the 18 patients on oral hypoglycemic agents (12.38±7.13 ng/ml) and the 7 patients on insulin treatment (11.12±4.07ng/ml). Correlations between serum levels of visfatin and other parameters were analyzed, and the significant correlations are shown in **Table 2**. In both genders in all groups, serum visfatin levels were correlated positively with HDL-C and negatively with each of TC, LDL-C and LDL-C/HDL-C ratio. In addition, visfatin levels were correlated negatively with TG in both genders in complicated diabetics and in male non-complicated

**Table 1 -** The clinical characteristics and laboratory results of healthy controls and T2DM patients with and without macroangiopathy.

| Clinical characteristics and laboratory results | Controls (n=22) | Non-complicated T2DM (n=29) | T2DM with macroangiopathy | *P*-value | | |
|---|---|---|---|---|---|---|
| | | | | p1 | p2 | p3 |
| Gender (M/F) | 10/12 | 16/13 | 15/18 | NS | NS | NS |
| Age (years) | 50.50 ± 8.41 | 51.07 ± 6.95 | 53.27 ± 7.78 | NS | NS | NS |
| Duration of diabetes(years) | - - | 8.69 ± 1.95 | 10.06 ± 4.58 | - | - | NS |
| Body mass index (kg/m$^2$) | 25.74±1.23 | 31.49 ± 6.04 | 29.41 ± 2.75 | <0.01 | <0.01 | NS |
| Systolic blood pressure (mmHg) | 128.3 ± 6.36 | 135.9 ± 15.32 | 128.3 ± 6.36 | <0.05 | <0.001 | <0.01 |
| Diastolic blood pressure (mmHg) | 73.55 ± 5.24 | 83.52 ± 13.66 | 94.82 ± 13.50 | <0.01 | <0.001 | <0.01 |
| Fasting serum glucose (mg/dl) | 85.45 ± 7.76 | 178.28 ± 56.80 | 179.15 ± 34.78 | <0.001 | <0.001 | NS |
| $HbA_1$ (%) | 6.11 ± 0.68 | 9.64 ± 1.97 | 10.09 ± 1.49 | <0.001 | <0.001 | NS |
| Insulin (µU/ml) | 11.34±5.38 | 15.26 ± 8.58 | 18.23 ± 11.99 | NS | <0.05 | NS |
| HOMA-IR | 2.45 ± 1.31 | 7.71 ± 5.52 | 8.89 ± 7.69 | <0.05 | <0.001 | NS |
| Diabetic treatment (Diet/Oral/ Insulin) | - | 10/19/0 | 8/18/7 | - | - | - |
| Triglycerides(mg/dl) | 116.95 ± 18.56 | 176.69 ± 71.90 | 169.00 ± 60.76 | <0.01 | <0.01 | NS |
| Total cholesterol (mg/dl) | 177.68 ± 24.36 | 222.55 ± 41.68 | 228.85 ± 42.78 | <0.01 | <0.01 | NS |
| HDL-C (mg/dl) | 42.41 ± 4.96 | 38.10 ± 4.39 | 34.79 ± 4.64 | <0.01 | <0.001 | <0.05 |
| LDL-C (mg/dl) | 111.88 ± 26.09 | 147.41 ± 36.37 | 160.26 ± 37.38 | <0.01 | <0.001 | NS |
| LDL-C/HDL-C | 2.73 ± 0.87 | 4.00 ± 1.32 | 4.80 ± 1.64 | <0.001 | <0.001 | <0.05 |
| Interleukin-6 (pg/ml) | 4.39 ± 2.70 | 7.44 ± 2.96 | 11.26 ± 4.61 | <0.05 | <0.001 | <0.001 |
| High sensitive C-reactive protein (µg/ml) | 2.05 ± 1.09 | 4.31 ± 1.91 | 7.89 ± 4.25 | <0.05 | <0.001 | <0.001 |
| Visfatin (ng/ml) | 8.92 ± 4.16 | 14.30 ± 5.77 | 10.81 ± 6.29 | <0.01 | NS | <0.05 |

Results are expressed as mean ± SD. p1 - for non-complicated T2DM and controls; p2 - for T2DM with macroangiopathy and controls; p3 - for T2DM with macroangiopathy and non-complicated T2DM; NS - not significant (p>0.05); M - males; F - females; T2DM - type 2 diabetes mellitus; $HbA_1$ - glycohemoglobin; HOMA-IR - homeostasis model assessment-insulin resistance; HDL-C - high density lipoprotein-cholesterol; LDL-C - low density lipoprotein-cholesterol

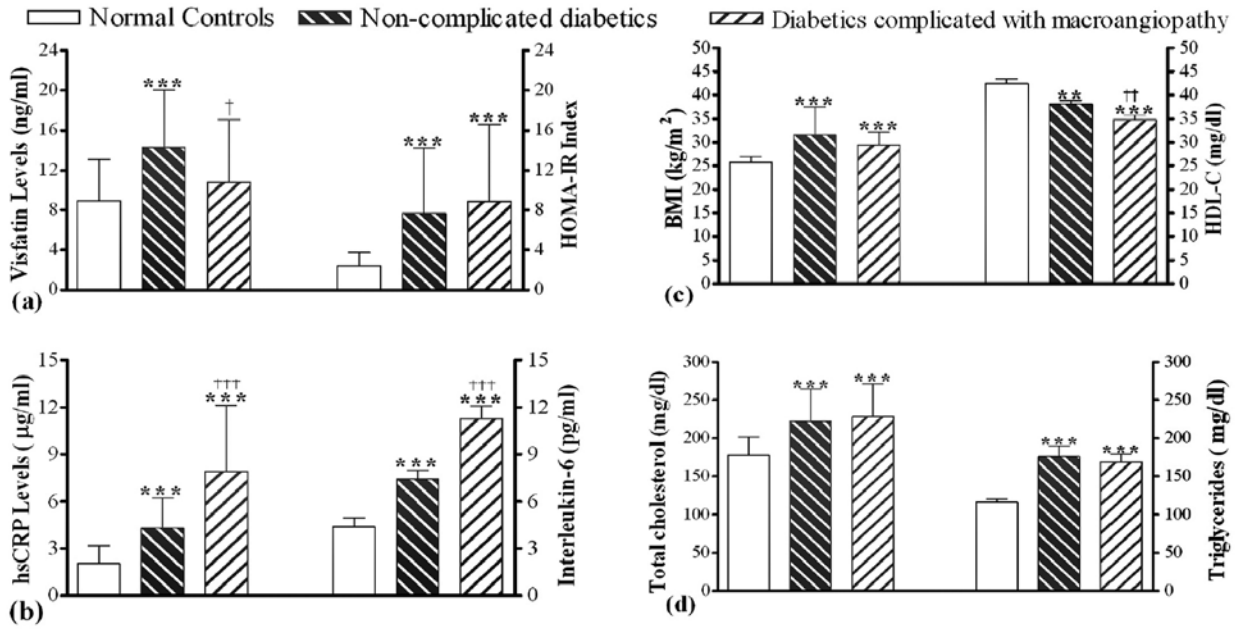


**Figure 1 -** Comparison of the levels of each of **a**) visfatin and homeostasis model assessment-insulin resistance (HOMA-IR), **b**) high sensitive C-reactive protein (hsCRP) and interleukin-6, **c**) Body mass index (BMI), and high density lipoprotein cholesterol (HDL-C), and **d**) total cholesterol (TC) and triglycerides (TG), among the 3 studied groups. Data are represented as mean± SD, *compared to controls; †compared to non-complicated diabetics (only significant differences are marked).

**Table 2 -** Correlations between visfatin and other parameters among males and females of the studied groups.

| Parameters | | Control group | | | Non-complicated T2DM | | | Macroangiopathic T2DM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Males | Females | Both | Males | Females | Both | Males | Females | Both |
| Number | | 10 | 12 | 22 | 16 | 13 | 29 | 15 | 18 | 33 |
| TG | *r* | -0.334 | -0.176 | -0.231 | -0.692 | -0.355 | -0.570 | -0.642 | -0.587 | -0.613 |
| | *P* | NS | NS | NS | <0.01 | NS | 0.001 | 0.01 | <0.05 | <0.001 |
| TC | *r* | -0.635 | -0.708 | -0.694 | -0.865 | -0.722 | -0.804 | -0.641 | -0.651 | -0.605 |
| | *P* | <0.05 | 0.01 | <0.001 | <0.001 | <0.01 | <0.001 | 0.01 | <0.01 | <0.001 |
| HDL-C | *r* | 0.798 | 0.888 | 0.806 | 0.847 | 0.712 | 0.721 | 0.838 | 0.705 | 0.742 |
| | *P* | <0.01 | <0.001 | <0.001 | <0.001 | <0.01 | <0.001 | <0.001 | 0.001 | <0.001 |
| LDL-C | *r* | -0.711 | -0.795 | -0.768 | -0.899 | -0.763 | -0.832 | -0.623 | -0.674 | -0.584 |
| | *P* | <0.05 | <0.01 | <0.001 | <0.001 | <0.01 | <0.001 | <0.05 | <0.01 | <0.001 |
| LDL-C/ HDL-C | *r* | -0.692 | -0.813 | -0.739 | -0.885 | -0.709 | -0.808 | -0.671 | -0.639 | -0.621 |
| | *P* | <0.05 | 0.001 | <0.001 | <0.001 | <0.01 | <0.001 | <0.01 | <0.01 | <0.001 |
| hsCRP | *r* | -0.484 | -0.584 | -0.523 | -0.736 | -0.558 | -0.656 | -0.635 | -0.523 | -0.576 |
| | *P* | NS | <0.05 | <0.05 | 0.001 | <0.05 | <0.001 | <0.05 | <0.05 | <0.001 |
| IL-6 | *r* | -0.645 | -0.576 | -0.400 | -0.670 | -0.645 | -0.659 | -0.658 | -0.626 | -0.638 |
| | *P* | <0.05 | 0.05 | NS | <0.01 | <0.05 | <0.001 | <0.01 | <0.01 | <0.001 |
| T2DM Duration | *r* | - - | - - | - - | -0.060 | -0.070 | -0.024 | -0.380 | -0.379 | -0.376 |
| | *P* | - - | - - | - - | NS | NS | NS | NS | NS | <0.05 |

T2DM - type 2 diabetes, TG - triglyceride, TC - total cholesterol, HDL-C - high density lipoprotein-cholesterol, LDL-C - low density lipoprotein-cholesterol, hsCRP - high sensitive C-reactive protein, IL-6 - interleukin-6, NS=$p$>0.05.

ones. On the other hand, there was negative correlation between visfatin level and the inflammatory markers hsCRP and IL-6 in all groups and in both genders except for hsCRP in male controls and IL-6 in pooled female and male controls. In addition, only in pooled (females and males) macroangiopathic diabetics, visfatin levels were negatively correlated with the duration of T2DM, but this correlation disappeared when gender was considered ($r$ and $p$ values are shown in **Table 2**). However, our study did not show any significant correlation between visfatin and age, BMI, SBP, DBP, FSG, $HbA_1$, insulin or HOMA-IR in both females and males in all the studied groups.

**Discussion.** Our study is designed to investigate the serum levels of visfatin and its relationships to obesity (BMI), insulin resistance and inflammation in T2DM with and without macroangiopathy. The non-complicated diabetics in the present study showed significantly higher serum visfatin levels when compared to healthy controls. This is in line with the findings of Sandeep et al,[21] Dogru et al,[26] and Chen et al[27] who reported elevated levels of visfatin in non-complicated T2DM. The study of Dogru et al[26] was performed on newly diagnosed untreated diabetics whereas ours and those of Sandeep et al[21] and Chen et al[27] involved patients on different hypoglycemic treatments and did not show any significant difference in visfatin levels among diabetic patients on different types of hypoglycemic treatments. This indicates that there was no effect of therapy on visfatin levels. However, the number of patients was too small to consider this conclusion strongly. Our study differed also from those of Sandeep et al,[21] Dogru et al,[26] and Chen et al[27] in that BMI levels were higher in diabetics compared to controls. However, in our study as in those of Dogru et al[26] and Chen et al,[27] there was no correlation between BMI and the levels of visfatin. Moreover, visfatin levels did not vary significantly among the different ranges of BMI. Therefore, from all these results we may suggest that visfatin increases in T2DM regardless of the type of treatment or the presence of obesity. This suggestion is not supported by other studies reporting the absence of any significant change in visfatin levels associated with T2DM,[28,29] the decreased visfatin levels in women with gestational diabetes,[30] and the significant correlation between these levels and BMI.[17,29] Elevated visfatin levels in T2DM patients may suggest the impairment of visfatin signaling in target tissues, dysregulation in biosynthesis or impairment in response to hyperglycemia, hyperinsulinemia, and adipocytokines in the state of diabetes.[27] Furthermore, it has been hypothesized that visfatin may be released from dying adipocytes during

apoptosis rather than some direct metabolic or mechanistic effect.[31] Macroangiopathic diabetic patients investigated in this study showed slightly higher non-significant visfatin levels compared to healthy controls, but showed significantly lower levels of visfatin than those of non-complicated diabetics. In order to clarify the possible cause(s) of this finding we analyzed the correlation of visfatin levels with the abnormalities associated with diabetes complications. We did not find any association of serum visfatin with FSG, fasting serum insulin or insulin resistance as measured by HOMA-IR. This is in accordance with findings of other studies[21,29,32] that showed a lack of association between visfatin and insulin resistance, but not with the results of Chen et al[27] who found that visfatin was positively associated with HOMA-IR and fasting insulin. However, this association disappeared in multiple regression analysis. If visfatin is not influenced by age, gender, glucose homeostasis or BMI, as our study suggests, what if anything, does affect visfatin level? Dyslipidemia and inflammatory state are well known to be associated with obesity, diabetes, and atherosclerotic complications. With the exception of the studies made by Smith et al[33] and Jian et al,[28] all other studies mentioned above[21,27,29,32] failed to find any correlation between visfatin and lipid profile parameters. In agreement with Smith's findings,[33] we found a positive correlation between visfatin and HDL-C. In addition, visfatin levels in our study groups correlated negatively with TC and LDL-C/HDL-C ratio, the main atherosclerotic marker in the lipid profile. These data suggest a link between visfatin and lipoprotein metabolism, as has been shown for other adipokines, supporting the data reported on the association between visfatin gene and glucose and lipid metabolism in a Chinese population.[28] Moreover, and with the exception of female non-complicated diabetics, visfatin was negatively associated with TG in both diabetic groups, which supports the role of visfatin in increasing lipogenesis in adipocytes.[17,34] These correlations would suggest that higher visfatin levels may be associated with a better, less atherogenic, metabolic profile although it is still unknown whether visfatin influences lipid profile parameters or vice versa. This suggestion is supported in our study by the decreased visfatin levels in macroangiopathic diabetics compared to non-complicated ones. In addition, these levels were correlated negatively and significantly with the duration of diabetes in macroangiopathic group, which was slightly longer than in the non-complicated group. In this context, one should consider inflammation, which is known to play important roles in insulin resistance, dyslipidemia and atherosclerosis. In our study, the levels of the inflammatory markers IL-6 and hsCRP were higher in diabetics with the highest levels associated significantly with macroangiopathic ones establishing the increased severity of inflammatory status in these patients. Visfatin has been suggested as an inflammatory cytokine that is produced and released by the adipose tissue derived macrophages.[35] Its levels are increased in acute lung inflammation and sepsis, which is accompanied by an insulin-resistant state.[36,37] However, there are some controversial reports over the correlation of visfatin with inflammatory markers (such as IL-6 & CRP) and the regulation of its gene expression by these markers. Interlukin-6 was reported as a negative regulator of visfatin gene expression in adipocytes.[20] Another study[38] reported that the inflammatory markers, adipose CD68 and plasma TNF-alpha, downregulate visfatin expression in subcutaneous adipose tissue. In contrast, 2 available studies suggested the proinflammatory role of visfatin and correlate it positively with IL-6 & hsCRP.[39,40] However, both studies involved non-diabetic subjects. In addition, these correlations were found only in healthy women in one study[39] and the correlation with CRP was evident only in men in the second one.[40] So, the relationship of visfatin to inflammation needs further more well-designed studies to be undertaken, taking into consideration that visfatin and IL-6 might have an interactive effect on each other. In addition, the designing should consider the different regulation of visfatin gene expression between male and females,[41] between different cell types, as well as acute and chronic inflammation. Another mechanism that might be suggested to cause visfatin drop in complicated T2DM is the impairment of renal function. Actually, although we excluded patients with renal impairment from the study, our exclusion criteria were based on creatinine level. It is believed that low degrees of renal impairment will not be detected unless microalbuminuria is explored. This was one of the limitations of our study suggesting the need for more well-designed studies in this field. Taking altogether, one might suggest that visfatin levels increase in diabetics as a compensation mechanism aiming to decrease the severity of some metabolic disturbances of insulin resistance by partially supplementing the role of insulin once adipocytes or myocytes become insulin-resistant. However, with the increased severity of the disease (insulin resistance, inflammation, renal impairment or other mechanism), this compensation fails allowing more atherogenic lipid profile to be produced and increased severity of atherosclerotic process as well as losing the proposed cardioprotective actions of this adipokine. These protective actions are demonstrated by Hausenloy et al,[42] showing that visfatin reduces myocardial infarct

size when given at time of myocardial reperfusion, and improves cell viability when given at time of reoxygenation. They reported that this protection appears to be mediated through the inhibition of mitochondrial permeability transition pore opening.[42]

Other limitations of this study should also be considered. The marked differences in BMI of our group subjects and the cross-sectional design limited our ability to infer a causal relationship between T2DM and increased visfatin levels. In addition, we estimated insulin resistance using the HOMA-IR model for which some limitations were reported.[25] Further studies designed to evaluate the relationship of visfatin with insulin resistance and its consequences are needed.

In conclusion, our study confirmed the increased levels of visfatin in T2DM regardless of obesity and type of diabetic treatment. Those levels were correlated negatively with inflammatory markers and atherogenicity of lipid profile suggesting that this increase in visfatin might represent a compensation mechanism against metabolic disturbances associated with insulin resistance. Later, with the increased severity of the disease, this compensation fails, possibly as a result of increased inflammation. Further well-designed studies are needed to interpret the controversial data on visfatin and to uncover its relation to insulin resistance, metabolic disturbances, inflammation and T2DM and its complications.

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