Research Square

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# Circulating levels of asprosin and its association with insulin resistance and renal function in type2 diabetes mellitus and diabetic nephropathy patients

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**Research Article**

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## Abstract

# Introduction

: Adipokines have an important role in development and progression of type 2 diabetes mellitus (T2DM) and its complications such as nephropathy. Asprosin is a recently discovered adipokine that involve in glucose metabolism and inflammation process. The present study sought to evaluate asprosin levels in patients with T2DM and T2DM + nephropathy (NP) compared to controls and its relation with markers of insulin resistance, inflammation and renal function.

# Methods

Serum levels of asprosin, adiponectin, IL-6 and TNF-α were measured in 55 control, 54 T2DM and 55 T2DM + NP patients using ELISA kits.

# Results

Asprosin was found to be higher in T2DM (6.73 ± 1.67) and T2DM + NP (7.11 ± 1.54) compared to controls (4.81 ± 1.09) ($p < 0.001$), while adiponectin indicated a lower concentration in both patient groups compared with controls. Moreover, IL-6 and TNF-α indicated higher levels in both patients group compared with controls. Asprosin indicated a positive correlation with HbA1c, FBG, TC, LDL-C, IL-6 and TNF-α in T2DM group. In the patients with T2DM + NP asprosin positively correlated with BMI, HbA1c, insulin, HOMA-IR, Cr, UAE, IL-6 and TNF-α and inversely correlated with eGFR.

# Conclusion

Higher concentration of asprosin in T2DM and T2DM + NP and its relation with glucose and lipid metabolism, and markers of renal function and inflammation suggested a possible role for this adipokine in the pathogenesis of T2DM and nephropathy.

## Introduction

In the recent decades, type 2 diabetes mellitus (T2DM) has been becoming one of major risks to human health which its prevalence is anticipated to rise to 10.4% in 2040 in adults [1]. T2DM characterized by chronic hyperglycemia, and insulin resistance, and with the persistent of high levels of blood glucose and non-esterified fatty acids which result in more damage in function and apoptosis in pancreatic β-cell, and consequently by higher discharge of inflammatory factors [2], it will be developed [3]. Progress in T2DM contributes to several disorders including cardiovascular diseases (CVD), retinopathy, nephropathy, and neuropathy. Diabetic nephropathy (DN) is considered as a main reason for end-stage renal failure and its association with the occurrence of atherosclerosis and CVD in these patients.[4]. One of the important

and preliminary indexes to evaluate the incidence and progression of DN is microalbuminuria [5]. Pathophysiology of DN showed that it results in arterial narrowing, and medial hypertrophy thickens the vessel wall [6]. In the meantime, the secretion of inflammatory cytokines such as TNF-α, IL-6 plays an important role in the formation of inflammation in the arteries, which exacerbates this process [7].

Obesity as one of the major and independent risk factors for diabetes and CVD, is related with hyperinsulinemia and insulin resistance[8]. On the other hand, adiposity induces a low grade inflammation in the body which is associated with more penetration of macrophages, especially M1 macrophages, into adipose tissue. In addition, adipose tissue has endocrine function to secrete bioactive components called adipokines [9,10]. The influence of these macrophages along with changing in the secretion of adipokeins from adipose tissue, can affect various aspects of metabolism and inflammation [11]. Several adipokeins such as resistin, adiponectin, and leptin have found to be related with regulating blood glucose levels, inflammation, and insulin sensitivity [12]. The adipokeins have been changed their concentrations in T2DM [13,14] and in the other hand excessive amount of adipocyte cells disrupt the adipokeins normal functions [15]. Besides that, the renal physiology can be affected by changes in concentration of adipokeines including leptin and adiponectin, oxidative stress, and inflammation in obesity and insulin resistance [16]. It was also confirmed that IL-18 and Hs-CRP were meaningfully increased in the severe nephropathy [17]. Secretion of this adipokeines and inflammatory factors negatively are related with endothelial function.

Asprosin is produced by white adipose tissue (WAT) which has been recognized in 2016 by Romere et al. They have found that asprosin is a 140-amino-acid protein that encoded by FBN1 gene and it is product of C-terminal cleavage which generated by profibrillin [18]. They also revealed the mediatory role of asprosin in glucose and insulin levels in blood. Mechanistically, this hormone increases during starvation and controls the release of glucose from liver cells via G protein-cAMP-PKA way to prevent hypoglycemia [18]. Moreover, in excremental studies have seen that in the insulin resistance circumstances and type 2 diabetes, the level of asprosin in blood is extremely increased [19]. In contrast, the insulin resistance will be ameliorating while the asprosin concentration is decreased by specific antibody of asprosin [18]. Moreover, the association of asprosin with inflammation (JNK phosphorylation TLR4-dependent pathway) [20] and ER stress (ER stress/inflammation-dependent pathways) [21] has been confirmed by several studies.

In order to orexigenic hormonal and glucogenic function of asprosin as well as its relation to inflammation, attenuating asprosin can be helpful in treating type 2 diabetes, obesity, and metabolic syndrome with hyperinsulinemia [22].

On the other hand, insulin resistance and increased levels of lipids in the blood, followed by deposition in blood vessels, especially small blood vessels such as renal vessels, as well as inflammation, can play a major role in the development of diabetic nephropathy. However, no previous study has been conducted to determine whether the protein changes in patients with diabetic nephropathy, and whether the protein has the potential to be a factor in the disease or a biomarker for the disease. To the best of authors

knowledge this case- control study was conducted to assess asprosin concentration in the serum of patients with T2DM, diabetic nephropathy and healthy control groups and its correlation with metabolic indicators.

## Methods

# Study participants

This case control study included 110 type 2 diabetic patients which were diagnosed according to the criteria of American Diabetes Association and 56 healthy volunteers as control group. Based on urinary albumin excretion (UAE) levels, 54 participants of diabetic patients who had UAE > 20 µg/min were considered as diabetic nephropathy group. All individuals were recruited from outpatients referred to Shohadaie Tajrish hospital and Institute of Endocrinology and Metabolism, Tehran, Iran from Jan 2019 to Jan 2020. Subjects with a history or evidence of cancer, autoimmune disease, type 1 diabetes or infectious diseases as well as patients who received GLP-1 receptor agonists and thiazolidinediones were excluded. Written informed consent form was signed by all participants and the study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (code: IR.SBMU.RETECH.REC.1398.432).

# Anthropometric data and biochemical measurements

Height and weight were measured for calculating body mass index (BMI) and a standard sphygmomanometer was utilized to determine systolic blood pressure (SBP) and diastolic blood pressure (DPB). Five milliliter (mL) of was taken after an overnight fasting from all the participants. Moreover, fasting plasma sugar (FBS), lipids profiles including: total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) as well as Cr, urinary albumin excretion (UAE), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by applying commercial available kits (ParsAzmoon, Iran). The traditional 4-variable Modification of Diet in Renal Disease (MDRD) equation was used to calculate Estimated glomerular filtration rate (eGFR). Insulin was measured by ELISA kit (Monobind, USA) and HOMA-IR was calculated by a standard formula: FBS (mg/dL)/insulin (uU/ml) × 405.

# Measuring serum adipokines and cytokines

ELISA kit was utilized to measure adiponectin levels (Adipogen, South Korea) with intra- and inter-assay coefficient of variations (CV) of 4.6% and 4.4%, respectively. The levels of asprosin were also determined using ELISA kit (AvisceraBioscience, USA) with intra- and inter-assay CV <8%. Moreover, the levels of tumor necrosis factor-α (TNF-α) and interleukine-6 (IL-6) were determined by applying ELISA kits (R & D Systems, USA) with minimum detectable doses of 1.6 and 0.7 pg/mL, respectively.

# Statistical analysis

All the statistical analyses were performed using SPSS software version 16. Chi-square test utilized for comparing categorical data and showed as frequency and percentage. Continuous data were presented as mean and standard deviation (SD) and tested using student t-test. Correlation analysis was performed using Spearman correlation test. In addition, multinomial logistic regression was conducted to estimated odd ratio of diseases status according to serum levels of asprosin. P value less than 0.05 considered as significance threshold.

## Results

# Basic characteristics of the study population

The groups were matched in terms of age, sex and BMI and there were no significant differences between the groups. Blood pressures including SBP and DBP indicated a higher levels in both patient groups compared to controls. As expected, patients with T2DM and T2DM+NP indicated elevated levels of glucose metabolism parameters including FBS, insulin, HOMA-IR and HbA1c. TG levels indicated higher levels in T2DM+NP compared with control and T2DM groups, while TC, LDL-C and HDL-C had no considerable difference between the groups. Markers of liver functions including AST and ALT were different between the groups, AST demonstrated higher levels in the both patient's groups compared to the controls, while ALT were higher in T2DM+NP compared with controls. Cr and eGFR were considerably elevated in the both patient's groups compared to the controls, in addition these variables indicated higher concentration in T2DM+NP in comparison to the controls. Moreover, UAE was higher in T2DM+NP compared with T2DM and control groups (Table 1).

# Serum levels of cytokines and adipokines

IL-6 indicated a higher concentration in both T2DM (8.28 ± 3.5) and T2DM+NP (9.87 ± 2.98) compared with controls (5.49 ± 1.76), moreover, T2DM+NP group indicated elevated levels of IL-6 compared with T2DM group (Fig 1a). TNF-α were found to be higher in both groups of T2DM (27.11 ± 6.58) and T2DM+NP (29.15 ± 7.77) compared to controls (21.91 ± 7.51) (Fig 1b). However, adiponectin decreased in both T2DM (9.53 ± 2.88) and T2DM+NP (8.56 ± 2.69) groups compared to controls (11.79 ± 3.47) (Fig 1 c). Furthermore, levels of asprosin were found to be higher in both T2DM (6.73 ± 1.67) and T2DM+NP (7.11 ± 1.54) groups compared to controls (4.81 ± 1.09) (Fig 1 d).

The possible influence of covariates (e. g. age, sex and BMI) on serum levels of Asprosin was adjusted using ANCOA and the results showed that serum levels of Asprosin remained higher in T2DM (6.71 ± 1.34) and T2DM+NP (7.09 ± 1.34) compared to controls (4.84 ± 1.36) ($p<0.001$). In addition, multinomial logistic regression was performed to estimate the odd ratio of diseases status according to one unit

change in Asprosin serum levels. The results indicated that asprosin had a significant association with disease statues in both crude and adjusted (for age, sex and BMI) models (Table 2).

In addition, the ability of Asprosin for differentiation of diseases status was tested using ROC curve analysis. Asprosin indicated a relatively good ability to differentiate T2DM (AUC [CI]: 0.828 [0.751, 0.904], p<0.001, cutoff: 5.46, sensitivity: 72% and specificity: 71%) and T2DM+NP (AUC [CI]: 0.890 [0.831, 0.949], p<0.001, cutoff: 5.89, sensitivity: 80% and specificity: 82%) from controls (Fig 2).

# Association of asprosin with anthropometric and biochemical variables

Correlation analysis and multiple stepwise linear regression were performed in control, T2DM and T2DM+NP groups separately and detailed results are shown in Table 3. In the control group, asprosin indicated a positive correlation with BMI, insulin and HOMA-IR, and multiple stepwise linear regression indicated the association of asprosin with insulin. In patients with T2DM, asprosin was positively correlated with HbA1c, FBG, TC, LDL-C, IL-6 and TNF-α and multiple stepwise linear regression indicated the association of asprosin with HbA1c, LDL-C and IL-6. Moreover, in patients with T2DM+NP asprosin indicated a positive correlation with BMI, HbA1c, insulin, HOMA-IR, Cr, UAE, IL-6 and TNF-α and inversely correlated with eGFR. In addition, asprosin indicated independent association with BMI, UAE, eGFR and IL-6 (Table 3).

## Discussion

The results of this case-control showed that the asprosin levels in T2DM+NP and T2DM diabetes groups were higher than the control group and it remained significant after adjusting for age, BMI, and sex. Diabetic nephropathy patients had a higher asprosin concentration than patients with T2DM groups, but this difference didn’t reach statistical significance.

The regulatory role of adipose tissue as an endocrine organ in metabolism and energy homeostasis has been confirmed [9]. The insulin function can be affected by the secreted components of adipose tissue [12]. Excess adiposity can cause insulin resistance which is known as a major reason for T2DM, therefore, obesity is associated with several metabolic disorders including T2DM and metabolic syndrome [23,24]. Asprosin, a newly discovered adipokine, is secreted by white adipose tissue (WAT) and has an important role in discharge of glucose from hepatic cells to maintain serum glucose level in normal condition. In addition, the level of asprosin is increased in pathological conditions such as insulin resistance, and T2DM, whereas in animal study showed that reduction of asprosin concentration through treatment with its specific antibody leads to ameliorating insulin resistance [18]. Nevertheless, the definite association of asprosin in T2DM has not been well established yet due to diversity in race and sample types.

The results of this study showed that asprosin concentration is positively correlated with T2DM and T2DM+NP, which was in line with Romere C et al. study [18]. They reported that the levels of asprosin in the serum of newly diagnosed T2DM patients were elevated compared to controls. Zhang et al. also showed that the levels of asprosin were increased considerably in insulin resistance and T2DM [19]. Overall, it may be proposed that the higher asprosin concentration in serum is a risk factor related with the development of T2DM. In line with this concept the results of the present study indicated that asprosin is positively associated with markers of glucose metabolism and insulin resistance.

In spite of the unclear mechanism for increased level of asprosin in T2DM, the glycogenic role of this adipokine suggested to explain this phenomenon. Glucose acts as a suppressor of asprosin in a negative-feedback axis. According to previous results, the asprosin concentration is extremely increased in the insulin resistance subjects and insulin sensitivity has been improved by lowering the asprosin [18]. In addition, it was shown that the abnormal secretion of asprosin by WAT, leads to higher levels of asprosin in T2DM [19]. The asprosin increases the glucose production in liver cells, and then due to hyperinsulinemia, consequently the insulin resistance will exacerbate [18]. Glucose dysregulation in individuals with insulin resistance is narrowly associated with the pathogenesis of T2DM [25,26], further studies are warranted to explore the exact mechanism.

The current study also showed a positive association between asprosin and BMI. It was proposed that excessive adipose tissue can disrupt the normal function and secretion of adipokeins and metabolic dysfunction [27,15]. Therefore, it can be concluded that obesity is a pivotal factor for elevated serum levels of asprosin.

Furthermore, the results showed serum asprosin associated with lipid metabolism. In the T2DM patient group the level of asprosin was positively and independently related with LDL-C and TC. The target organ for asprosin is hepatic cells, therefore, it is assumed that asprosin may be related with dyslipidemia [19]. Animal study showed that using antibody against asprosin reduced lipid profile include TG, TC and LDL-C which could be a result from the impact of asprosin on insulin sensitivity. These results needed more study to dissect possible underlying mechanisms.

Strikingly, the results of the present study showed association of asprosin with the markers of kidney function (eGFR, UAE and Cr) in patients with T2DM+NP. Previous study has shown that asprosin associated with markers of kidney function in diabetic patients, while the present study showed that the relation of asprosin with markers of kidney function were detected only in T2DM+NP. In diabetic patients, several factors including hyperglycemia, oxidative stress, and renin-angiotensin system are the initiators of the inflammatory procedure in the kidneys and micro inflammation is proposed that as a major mechanism for development and progression of diabetic nephropathy [28]. Furthermore, asprosin indicated a positive correlation with inflammatory cytokines. The effect of asprosin on inflammation has been confirmed. Asprosin upregulates JNK phosphorylation TLR4-dependent pathway which causes inflammation in the body [20]. On the other hand, it was reported that several adipokeins such as resistin

and adiponectin have the regulatory effect on the insulin action and are associated with inflammation [28].

Collectively, we showed that asprosin is inversely associated with inflammatory markers, insulin resistance obesity and renal function indicators in T2DM+NP, while in T2DM patients asprosin was associated with glucose and cholesterol metabolism markers and inflammation. These results suggested a possible role for asprosin in the pathogenesis of T2DM and T2DM+NP through their pathological mechanism such as inflammation, insulin resistance and obesity. Regarding the cross sectional design of the study we were limited to conclude a casual relation between asprosin and mentioned factors and future studies are needed in this regard.

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# Tables

Table 1. Anthropometric and biochemical characteristics of the study population.

| les | Control (n=55) | T2DM (n=54) | T2DM-NP (n=55) | p |
|---|---|---|---|---|
| g/m2) | 25.72 ± 3.45 | 26.72 ± 4.21 | 26.47 ± 4.27 | 0.401 |
| ear) | 58.71 ± 7.93 | 61.83 ± 7.78 | 61.84 ± 9.36 | 0.082 |
| ale) | 36 (65.5%) | 32 (59.3) | 35 (63.6%) | |
| ımHg) | 126.13 ± 15.3 | 137.56 ± 19.82 | 145.71 ± 18.73 | < 0.001 |
| ımHg) | 78.00 ± 12.46 | 86.30 ± 15.2 | 92.76 ± 13.56 | < 0.001 |
| (%) | 4.52 ± 0.92 | 8.19 ± 1.43 | 8.17 ± 1.26 | < 0.001 |
| ıg/d) | 93.55 ± 11.5 | 163.02 ± 22.75 | 167.64 ± 21.45 | < 0.001 |
| (µU/mL) | 4.34 ± 2.46 | 10.29 ± 5.09 | 11.66 ± 5.63 | < 0.001 |
| ·IR | 1.02 ± 0.62 | 4.27 ± 2.47 | 4.86 ± 2.5 | < 0.001 |
| g/dL) | 126.76 ± 49.56 | 150.56 ± 44.57 | 175.73 ± 60.33 | < 0.001 |
| /dL) | 174.82 ± 39.8 | 180.44 ± 45.99 | 193.07 ± 47.53 | 0.092 |
| (mg/dL) | 106.51 ± 32.33 | 107.13 ± 36.38 | 119.33 ± 35.92 | 0.099 |
| (mg/dL) | 47.24 ± 6.86 | 44.44 ± 7.37 | 44.60 ± 5.6 | 0.050 |
| /dL) | 1.03 ± 0.18 | 1.28 ± 0.18 | 2.57 ± 0.79 | < 0.001 |
| /L) | 19.60 ± 5.66 | 22.66 ± 5.72 | 24.05 ± 6.96 | 0.001 |
| /L) | 19.43 ± 7.79 | 22.1 ± 7.67 | 25.01 ± 8.18 | 0.001 |
| ıg/min) | 10.35 ± 4.48 | 11.07 ± 5.16 | 254.31 ± 147.3 | < 0.001 |
| mL/min/1.73 m2) | 82.02 ± 25.00 | 65.20 ± 15.37 | 32.41 ± 13.31 | < 0.001 |

Table 2. The odd ratio of diseases status according to one unit change in serum asprosin.

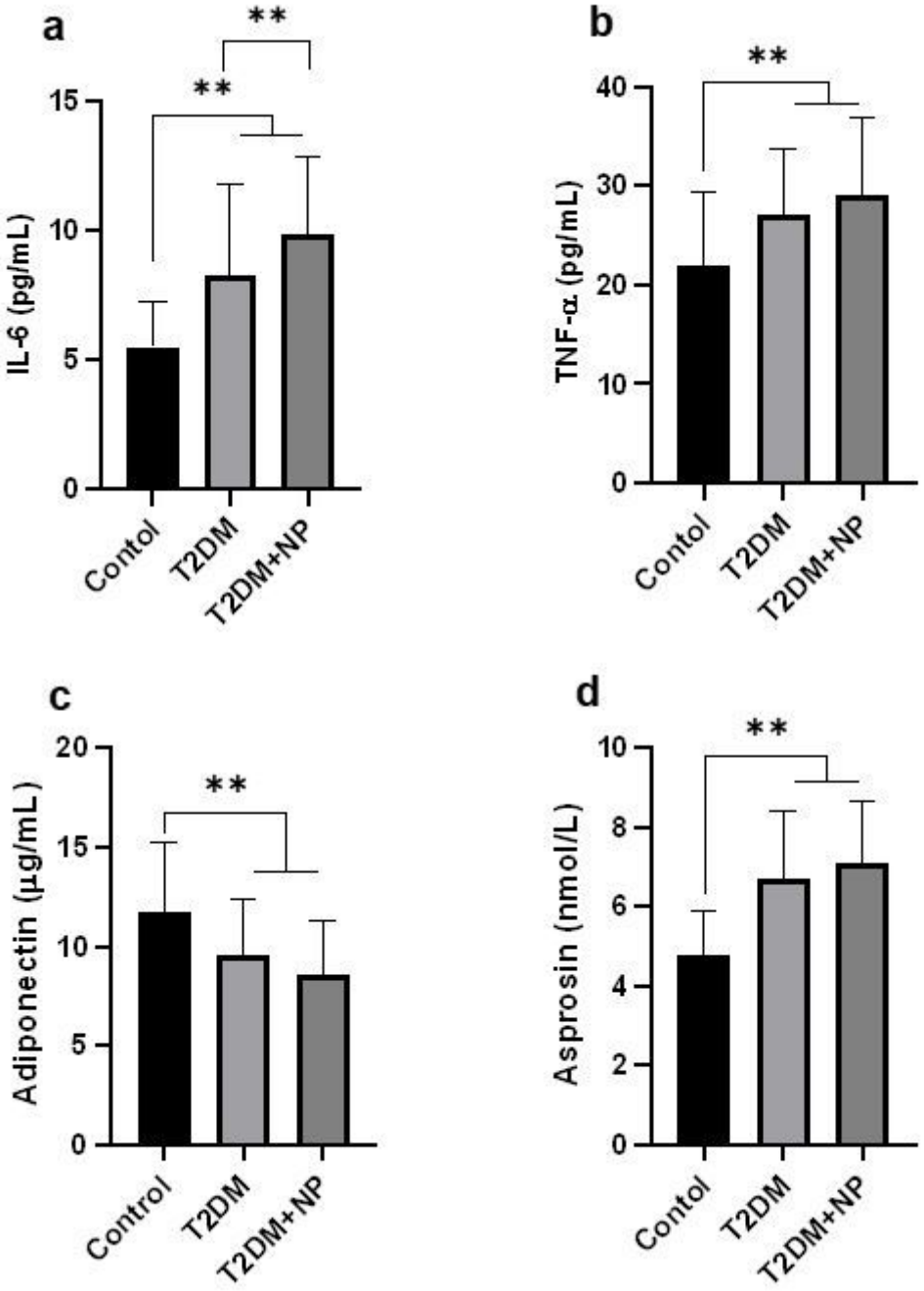
| Group | Model | B | Odd ratio | 95% Confidence Interval for Odd ratio | | |
|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | P value |
| T2DM | Crude | 1.053 | 2.865 | 1.962 | 4.185 | <0.001 |
| | Adjusted | 1.431 | 4.183 | 2.507 | 6.980 | <0.001 |
| T2DM-NP | Crude | 1.203 | 3.331 | 2.250 | 4.929 | <0.001 |
| | Adjusted | 1.611 | 5.009 | 2.961 | 8.473 | <0.001 |

Table 3. Spearman correlation and multiple linear regression of asprosin with anthropometric and biochemical measurement.

| | Control | | T2DM | | T2DM+NP | |
|---|---|---|---|---|---|---|
| Variables | r | B (95%CI) | r | B (95%CI) | r | B (95%CI) |
| BMI | 0.273* | | 0.259 | | 0.314* | 0.095 (0.016, 0.174)* |
| Age | 0.154 | | -0.076 | | -0.043 | |
| SBP | 0.206 | | 0.062 | | 0.104 | |
| DBP | 0.050 | | 0.050 | | 0.175 | |
| HbA1c | 0.105 | | 0.422** | 0.014 (0.003, 0.024)* | 0.314* | |
| FBG | 0.161 | | 0.317* | | 0.262 | |
| Insulin | 0.292* | 0.202 (0.094, 0.311)** | 0.171 | | 0.357** | |
| HOMA-IR | 0.309* | | 0.241 | | 0.409** | |
| TG | 0.070 | | 0.144 | | 0.121 | |
| TC | -0.177 | | 0.345* | | -0.060 | |
| LDL-C | -0.143 | | 0.374** | 0.346 (0.064, 0.624)* | -0.090 | |
| HDL-C | 0.006 | | 0.078 | | 0.046 | |
| Cr | 0.060 | | 0.153 | | 0.453** | |
| UAE | -0.037 | | -0.050 | | 0.363** | 0.003 (0.000, 0.005)* |
| eGFR | -0.114 | | -0.194 | | -0.446** | -0.042 (-0.068, -0.016)** |
| AST | 0.073 | | 0.068 | | 0.132 | |
| ALT | 0.043 | | 0.035 | | 0.049 | |
| IL-6 | -0.113 | | 0.329* | 0.014 (0.046, 0.274)** | 0.392** | 0.135 (0.020, 0.249)* |
| TNF-α | 0.131 | | 0.311* | | 0.334* | |
| Adiponectin | -0.051 | | -0.159 | | -0.239 | |

*p<0.05, **p<0.01

# Figures




## Figure 1

Serum levels of cytokines and adipokines. a) Serum levels of IL-6 increased in both T2Dm and T2DM+NP compared to controls as well as T2DM+NP compared to T2DM group. b) TNF-α indicated higher concentration in both patient groups in comparison to controls. c) Adiponectin serum levels were found to be lower in the both patient groups compared to controls. d) Asprosin serum levels increased in T2DM and T2DM+NP groups compared with controls.

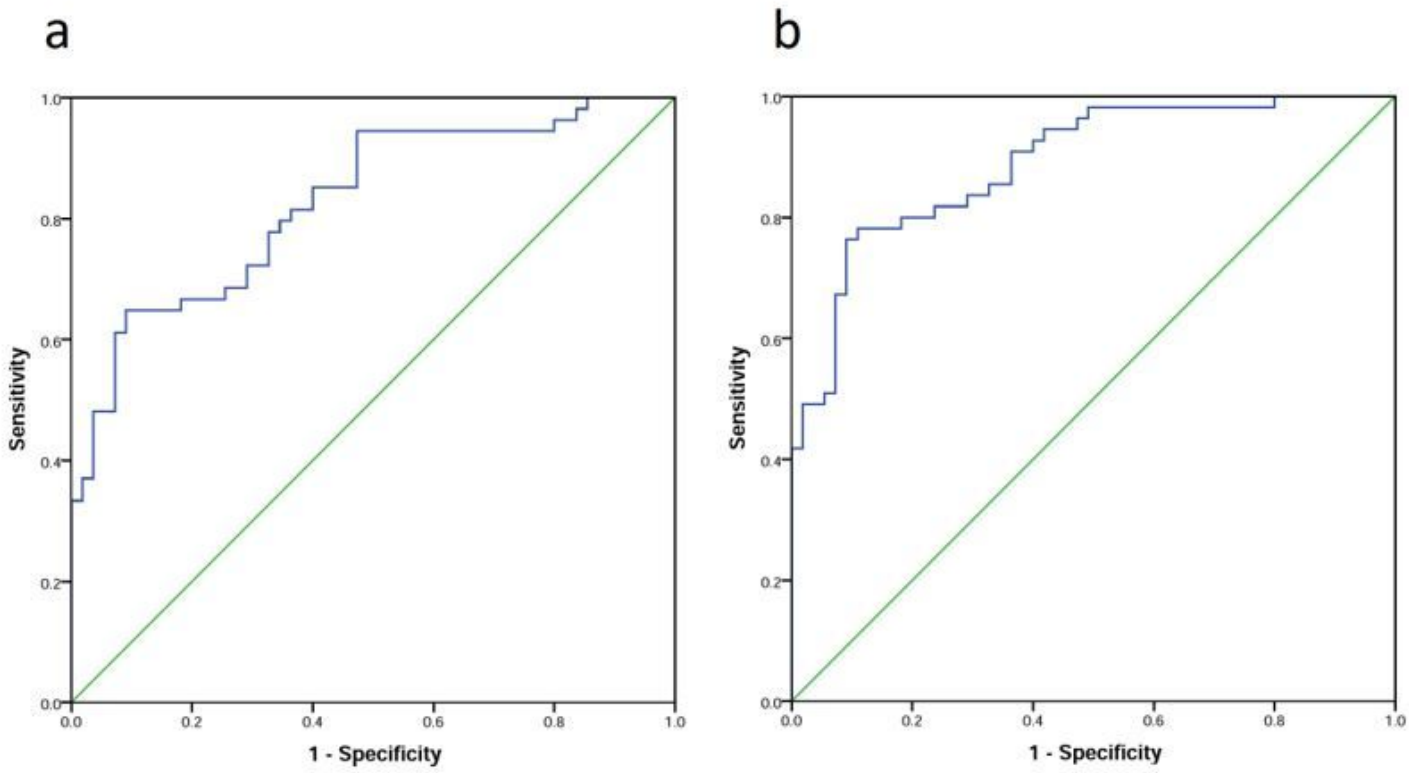



**Figure 2**

ROC curve analysis of diagnostic ability of asprosin to differentiate diseases status from control. a) T2DM from control and b) T2DM+NP from control.

# Supplementary Files

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