ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 29(3): 276-287, 2024
DOI: 10.5505/ejm.2024.78614

# A Novel Indicator for Erectile Dysfunction: S100A4

**Murat Demir[1*], Zübeyir Huyut[2], Mehmet Tahir Huyut[3], Kasım Ertaş[1], Rahmi Aslan[1], Recep Eryılmaz[1], Kadir Körpe[4], Muhammed Kotan[1], Kerem Taken[1]**

*[1]Department of Urology, Faculty of Medicine, Van Yuzuncu Yil University, 65080-Van, Turkey*
*[2]Department of Biochemistry, Faculty of Medicine, Van Yuzuncu Yil University, 65080-Van, Turkey*
*[3]Department of Bioistatistics, Faculty of Medicine, Erzincan Binali Yıldırım University, 24100-Erzincan, Turkey*
*[4]Department of Urology, Van Training and Research Hospital, 65050-Van, Turkey*

**ABSTRACT**

This study aimed whether S100A4 would be useful in predicting Erectile Dysfunction (ED) and ED severity.
This prospective study included 88 male volunteers aged 18-80 years. The control group consisted of 44 healthy patients and the diabetic group consisted of 44 patients with type-2 diabetes (T2DM). Age, body mass index, smoking status, hypertension status, International Index of Erectile Function 1-5 scores of all the volunteers were evaluated, and also glucose, HbA1c, cholesterol, testosterone, prolactin and S100A4 levels were measured in the serum samples. The relationships between S100A4 and erectile functions were investigated with appropriate statistical analyzes.
The mean age of the T2DM group was 51.98 ±10.91 years, while the control group's mean age was 53.77 ±12.46 years and there was no significant difference between them ($p=0.31$). Glucose, HbA1c, mean ED severity and S100A4 levels in the T2DM group were higher than in the control group, while testosterone level was lower than in the control ($p\leq0.05$). In addition, it was found that S100A4 levels increased due to the increase in ED severity in both groups ($p<0.001$). In addition to ED severity, smoking and hypertension were seen as the factors that most affected S100A4.
The results indicated that S100A4 may be a useful biomarker in determining ED and ED severity.

**Keywords**: Atherosclerosis, Diabetes Mellitus, Erectile Dysfunction, S100 Calcium-Binding Protein A4

## Introduction

Erectile dysfunction (ED) is the inability to initiate or maintain a successful erection for vaginal intercourse. It is a disease with strong negative psychosocial effects, especially affecting men over the age of 40. Although ED is multifactorial, the main etiological causes can be listed as smoking, obesity, a sedentary lifestyle, diabetes mellitus (DM), and/or hypercholesterolemia (1). These etiological causes are the main cause of endothelial dysfunction. Endothelial dysfunction progresses to endothelial damage and atherosclerosis. Therefore, ED is often associated with systemic atherosclerosis, which causes inadequate endothelial nitric oxide secretion, insufficient vascular dilatation and blood supply to the corpora cavernosa (2). In addition, ED can develop with peripheral nerve damage, prolonged pudendal nerve conduction and autonomic nervous system dysfunction in Diabetes Mellitus disease besides vascular pathogens (3).

The literature states that inflammation causes endothelial dysfunction and is involved in all stages of progression toward atherosclerosis. Endothelial cells are normally protected against the adhesion of inflammatory cells and their mediators. However, in the face of stimuli such as smoking, hypertension (HT), hyperglycemia, and hypercholesterolemia, the expression of adhesion molecules such as P-selectin and vascular cell adhesion molecule-1 in the endothelium is impaired, causing circulating monocytes and leukocytes to adhere to the endothelium (4). In addition, the migration of smooth muscle cells into the intima is important in the development of atherosclerosis. Vascular intima, which starts with the arrival of inflammatory cells and their mediators and smooth muscle migration, turns into a non-contractile synthetic structure. Cell differentiation occurs with the deterioration of the structure of contractile proteins as well as the increase in extracellular matrix components. In addition, fibrotic plaques occur in the vessel with foamy cells formed by macrophages. All these events begin with the migration of smooth muscle cells (5). Recently, the S100A4, which has been found to increase in smooth muscle cells with atherosclerosis, has attracted attention (6). In

***Corresponding Author:** Murat Demir, Department of Urology, Faculty of Medicine, Van Yuzuncu Yıl University, 65080-Van, Turkey
E-mail: urologmurat72@gmail.com, Phone: +90 432 225 17 01-05, Fax: +90 432 236 1054
**ORCID ID: Murat Demir:** 0000-0001-5029-8800, **Zübeyir Huyut:** 0000-0002-7623-1492, **Mehmet Tahir Huyut:** 0000-0002-2564-991X, **Kasım Ertaş:** 0000-0003-4300-1399, **Rahmi Aslan:** 0000-0002-4563-0386, **Recep Eryılmaz:** 0000-0002-4506-8784, **Kadir Körpe:** 0000-0001-7159-1272, **Muhammed Kotan:** 0009-0009-3504-3725, **Kerem Taken:** 0000-0002-4370-4222
**Received:** 25.01.2024, **Accepted:** 29.05.2024

addition, it has been reported that S100A4 release is increased in nerve cell damage (7).

S100 proteins, which belong to the calcium-binding protein family, are involved in many mechanisms in the body, such as cell apoptosis, cell proliferation, energy metabolism and inflammation. Consisting of 25 members, the S100 proteins have intracellular, extracellular, and both intracellular and extracellular acting members. Intracellular S100 proteins interact with a variety of proteins, including enzymes, receptors, and transcription factors. These intracellular proteins are involved in proliferation, differentiation, apoptosis, $Ca^{2+}$ hemostasis, energy metabolism, inflammation and cell migration. On the other hand, extracellular S100s regulate the activation of surface receptors, G protein-coupled receptors, heparan sulfate proteoglycans or N glycans with autocrine and paracrine effects. Extracellular S100s have been shown to activate cell proliferation, differentiation, cell survival, cell migration, and inflammation in normal and pathological conditions (8).

The S100A4 has been found in the intima of arteries and other vessels as well as in the endothelium of capillaries. Apart from this, it is also found in many places such as kidney tubules, salivary glands, the stomach and the spleen. The S100A4 can be secreted by a wide variety of inflammatory and immune cells, the main sources of which are leukocytes, fibroblasts and macrophages. Moreover, binding of the S100A4 to target proteins leads to pro-inflammatory processes such as chemotaxis, cell migration, smooth muscle cell remodeling and angiogenesis. Although the relationship of the S100A4 with malignancy formation and prognosis has been investigated, studies have recently been published stating that it increases atherosclerosis and high levels of S100A4 are correlated with metabolic risk. It has also been reported that elevated S100A4 levels are associated with insulin resistance and cause diabetic retinopathy (8, 9 10, 11) The other studies have shown that S100A4 levels increase in nerve cell damage (3, 7,12 )

In this study, we investigated the relationship of S100A4 with ED and whether it could be an indicator in determining ED and ED severity.

## Material and Method

**Ethical approval and Volunteers:** This prospective study was started after obtaining approval from the Van Yuzuncu Yil University Interventional Ethics Committee (date: 12.01.2022, no:06). The study was performed according to the criteria of the Declaration of Helsinki and consent forms were obtained from all patients. Between 01.02.2022 and 28.02.2022, a total of 88 volunteers aged 18-80, 44 with T2DM and 44 without DM, were included in the study. All T2DM patients were selected from the patients who applied to the internal medicine outpatient clinic and were examined by the internal medicine doctor. The control group was selected from healthy individuals.

**Inclusion and Exclusion Criteria for Type 2 DM:** The study had two groups; the subject group was composed of Type 2 diabetes mellitus (T2DM) patients, and the control group was composed of non-DM patients. The inclusion criteria for the subject group for this study were to be between the ages of 18-80 and have a diagnosis of T2DM. The exclusion criteria were patients with psychiatric or with an additional systemic disease (cardiac, cerebral, thyroid diseases, inflammatory diseases, cancer), major pelvic surgery, penile or testicular abnormality or operation, prolactin or testosterone abnormality, patients that were outside the age range of 18-80 years or were using drugs such as steroids or anti-inflammatory. At the beginning of the study, patients' age and body mass index (BMI) values, fasting glucose levels, glycosylated hemoglobin, prolactin and testosterone were recorded for all patients. All blood samples were taken from patients after fasting for at least 12 hours (no food, drink, or smoke), between 08:00 and 10:00 in the morning.

**Inclusion and exclusion criteria for the control group:** The criteria for inclusion in the control group were to be between the ages of 18-80 and not have DM diagnoses. The exclusion criteria for the control group were patients that were diagnosed with DM, psychiatric or additional systemic disease (cardiac, cerebral, thyroid diseases, inflammatory diseases, cancer), undergoing major pelvic surgery, having a penile or testicular abnormality or operation, having prolactin or testosterone abnormality, being outside the age range of 18-80 years, using drugs such as steroids or anti-inflammatory.

**Erectile function evaluation:** The erectile function status of the patients was evaluated with the International Index of Erectile Function-5 (IIEF-5). This form consists of 6 questions, each of which consists of 6 options. The score varies between 0 and 30 according to the answers given by the individuals. Patients with an ED score of 26-30 are considered healthy, while 22-25 is mild, 17-21 is mild-moderate, 11-16 is moderate, and 0-10 is severe (13). According to these scores, it was accepted as level 0, level 1, level 2, level 3 and level 4, respectively.

**Obtaining Serum Samples:** Whole blood samples taken from both the T2DM and control groups were placed in dry biochemistry tubes with yellow caps and

were centrifuged at 3500 *xg* for 10 min. Routine biochemistry parameters, prolactin, and testosterone measurements were examined in the serum samples remaining in the upper part of the tube. Serum prolactin and testosterone measurements were performed in the Abbott Architect I6200 SR device using the chemiluminescent microparticle immunological method, with the appropriate calibrator, control and kit. The remaining serum samples were stored at -80$^{o}$ C until other parameters were measured. In addition, HbA1c measurements were taken from the blood that was drawn into hemogram tubes.

**Measurement of S100A4 in Serum Samples:** Measurement of S100A4 in serum samples was measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method using commercially purchased ELISA kits (ELISA, YL biont, Shanghai YL Biotech Co., Ltd) by following the kit insert.

**Statistical Analysis:** Statistical analysis was performed using the SPSS package program (Version 21). Descriptive statistics for the groups were presented as the mean and standard deviation. To assess the normal distribution of data within the groups, the Shapiro-Wilk test was employed. Given the non-normal distribution of the groups, the Mann-Whitney U test was utilized to assess significant differences between groups for the same parameter. Furthermore, the relationship between S100A4 and erectile dysfunction (ED) levels was examined through one-way ANOVA analysis. Then, multiple comparisons of ED levels were made with the post-hoc test. In addition, the relationship structure between S100A4 and ED levels was analyzed by Pearson biserial correlation analysis. In addition, multiple ordinal-logit regression analysis was used to determine the predictors affecting the ED level. The complementary-logit model was used for the connection function of the regression model established between the predictors and ED levels. The significance of the parameters related to the predictions of the predictors was checked by chi-square analysis. The validity of the regression model was examined with the -2 Log-Likelihood test and the coefficient of determination ($R^2$) was calculated for the performance of the model. Results with a p-value of 0.05 or less were considered significant.

## Results

**Demographic Data:** While the mean age of the 44 T2DM patients participating in our study was 51.98 ±10.91, the mean age of the 44 patients in the control group was 53.77 ±12.46 years (p=0.312) and there was no significant difference between them. This result showed that the age distribution of the volunteers was similar in both the T2DM and healthy control groups. In addition, statistical data in terms of the presence of hypertension, smoking and dyslipidemia in both healthy control and T2DM groups are seen in detail in Table 1. Furthermore, information on drug use and sugar regulation in the T2DM group is shown in detail in Table 1.

**Biochemical Results:** Table 2 represents the results of the HbA1c analysis of the whole blood samples and the biochemical parameters that were measured in the serum samples. According to the results obtained, the prolactin values of the T2DM group were close to the control group's values and there was no significant difference between them (p=0.970). However, the testosterone levels of the T2DM group were significantly lower than the control group's (p=0.011). When the serum glucose levels were analyzed, the serum glucose levels of the T2DM group were higher than those of the control group (p=0.001). In addition, the mean HbA1c of the T2DM group was 7.71 ±0.11 while it was 4.66 ±0.08 in the control group (p=0.001). The other results were shown in Table 2 and Figure 1.

When the situation was examined in terms of ED rate or mean, the ED means of the T2DM group were considerably higher than that of the control group (p=0.010).

In this study, we investigated how the S100A4 levels changed in the control and patient groups according to their ED levels (Table 4). S100A4 levels corresponding to ED levels in the control group showed that ED levels also increased in response to increasing S100A4 levels (Table 3 and Figure 2). However, the increase in the S100A4 levels corresponding to the 1st and 2nd ED levels in the control group were not significant according to each other. In addition, the increase in the S100A4 levels corresponding to the 4th ED level was significant according to the other ED levels. Additional detailed results were showed in Table 3.

In this study, we determined the relationship structure between ED levels and S100A4 levels in the control group using a Pearson Point-Biserial Correlation analysis (Figure 3). According to the Pearson Point-Biserial Correlation curve results, the S100A4 levels increased as the ED levels increased in the control group. However, the highest increase was observed in the S100A4 values corresponding with the 4th ED level. In addition, the degree of correlation between the ED level and S100A4 levels in the control group was 80% (r=0.8, p=0.001).

**Table 1:** Demographic Data of Healthy and Diabetes Volunteers

| | | Healthy Control Group (n=44) Mean ± SD | Diabetes Group (n=44) Mean ± SD |
|---|---|---|---|
| Age (years) | | 53.77±12.46 | 51.98±10.91 |
| BMI ( kg/m² ) | | 23.14 ±4.82 | 26.31 ±4.03 |
| Dyslipidemia | + | 8 (18.2%) | 13 (29.6%) |
| | - | 36 (81.8%) | 31 (70.4%) |
| Hypertension | + | - | 24 (54.5%) |
| | - | - | 20 (45.5%) |
| Smoking status | Smoking | 17 (38.6%) | 20 (45.4%) |
| | No smoking | 27 (61.4%) | 24 (54.6%) |

BMI: Body Mass Index, SD: Standard Deviation

**Table 2**: Mean and Standard Error of Mean Values of Biochemical Parameters

| | Control Mean ± SD | Diabetes Mean ± SD | *p value |
|---|---|---|---|
| Prolactin (ng/ml) | 8.86±0.55 | 8.82±0.57 | 0.970 |
| Testosterone (nmol/L) | 18.66±0.86 | 15.88±0.85* | 0.011 |
| Glucose (mg/dL) | 85.09±1.17 | 175.32±12.24* | 0.001 |
| HbA1c | 4.661±0.08 | 7.71±0.11* | 0.001 |
| S100A4 | 1.20±0.02 | 1.32±0.02* | 0.001 |
| ED score | 17.68±6.30 | 26.31±4.03* | 0.001 |

*p: Significant compared to the control group (p<0.05), SD: Standard deviation

**Table 3:** Mean and Standard Deviation Values of S100A4 Levels Corresponding To Different ED Levels In The Control And Diabetes Groups

| | ED levels | | | | | |
|---|---|---|---|---|---|---|
| | 0th level | 1st level | 2nd level | 3rd level | 4th level | p value |
| Groups | Mean ±SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | |
| Control | 1.130±0.03d | 1.189± 0.015c | 1.227±0.024c | 1.272 ±0.038b | 1.72 ± 0.020a | 0.001 |
| Diabetes | 1.199±0.48d | 1.241± 0.027d | 1.311±0.023c | 1.390± 0.055b | 1.533 ±0.038a | 0.001 |

[a,b,c,d]p: Values with different letters in the same line are significantly different from each other. (p<0.001) SD: Standard deviation

Furthermore, we conducted an investigation into the diverse correlations between levels of erectile dysfunction (ED) and S100A4 levels within the control group using multiple correspondence analysis (MCA) as outlined in Table 4. Analyzing the relationship structure between variables in scenarios with a substantial number of categories can be challenging when utilizing cross tables. MCA facilitates the quantification of nominal (categorical) data by assigning numerical values to cases (objects) and categories. This approach divides variables into homogeneous subgroups based on categories. Through MCA analysis, the relationship structure between categorical and continuous variables can be easily interpreted on a diagram, utilizing a dimension reduction method to represent them in a two-dimensional space. This diagram illustrates a strong correlation between objects within the same category, situated closely to each other, while indicating a weaker correlation between objects in different categories, positioned farther apart (14,15,16).

Analyzing the two-dimensional ED levels and S100A4 levels graph (Figure 4), the initial zero ED level was associated with low S100A4 levels (1.05-1.17), while the 1st, 2nd, and 3rd ED levels were

**Table 4:** Model Performance Results of the MCA Diagram to Summarize The Multi-Correlation Structure Between The ED And S100a4 Levels of The Control Group

| Dimension | Cronbach's Alpha | Variance Accounted For | | |
|---|---|---|---|---|
| | | Total (Eigenvalue) | Inertia | % of Variance |
| 1 | 1.000 | 2.000 | 1.000 | 100.000 |
| 2 | 0.955 | 1.913 | 0.956 | 95.650 |

1: Vertical dimension, 2: Horizontal dimension

**Table 5:** Model performance results of the MCA Diagram to Summarize the Multi-correlation Structure Between the ED and S100A4 levels of the DM Group

| Dimension | Cronbach's Alpha | Variance Accounted For | | |
|---|---|---|---|---|
| | | Total (Eigenvalue) | Inertia | % of Variance |
| 1 | 0.962 | 1.926 | 0.963 | 96.297 |
| 2 | 0.877 | 1.781 | 0.890 | 89.041 |

1: Vertical dimension, 2: Horizontal dimension

**Table 6:** Mean and Standard Deviation Values of S100A4 Levels Corresponding to Different S100A4 Scores In The Control and Diabetes Groups

| | ED Levels | | | | | |
|---|---|---|---|---|---|---|
| | 0th level | 1sd level | 2nd level | 3rd level | 4th level | p value |
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | |
| Total S100A4 Levels | 1.15 ± 0.05[c] | 1.21 ± 0.03[c] | 1.28 ± 0.05[b] | 1.34 ± 0.08[b] | 1.56 ± 0.08[a] | 0.001 |

[a,b,c,d]p: Values with different letters on the same line are significant relative to each other. ($p<0.001$)
SD: Standard deviation

associated with low to moderate S100A4 levels (1.18-1.28). However, the 4th ED level was found to be associated with the highest S100A4 levels (1.69-1.72) (Figure 4). The total variation rate of the MCA diagram (Figure 4), which explains the relationship structure between the ED and S100A4 levels of the control group, was found to be 97.8%. This result showed that the diagram explained a high percentage of the relationship structure between the variables (Figure 4)

In this study, we determined how the S100A4 levels changed with different ED levels in the T2DM group. Our results showed that as ED levels increased in the T2DM group, their corresponding S100A4 levels also increased (Table 3 and Figure 2). However, although the increase in S100A4 levels corresponding to the zero and 1st ED levels was not significant, the increase in S100A4 levels corresponding to the 2nd, 3rd, and 4th ED levels was significant ($p<0.001$).

In this study, we determined the relationship between ED and S100A4 levels in the T2DM group using a Pearson Point-Biserial Correlation analysis (Figure 3). According to the Pearson Point-Biserial Correlation curve results, S100A4 levels increased as the ED level increased in the T2DM group ($p<0.001$). However, the sharpest increases were observed in the S100A4 levels that corresponded to the 2nd, 3rd and 4th ED levels ($p<0.001$). In addition, the degree of correlation between ED and S100A4 levels in the T2DM group was 91% ($r=0.91$, $p=0.001$).

In addition, the multi-correlation structure between different ED levels and S100A4 levels in the T2DM group was examined using multiple correspondence analysis (MCA) (Figure 4). When the two-dimensional ED and S100A4 levels graph is examined (Figure 4), baseline zero and 1st ED levels were associated with low S100A4 levels (1.16-1.22), while level 2 ED was associated with a low (1.26-1.35) S100A4 level. A level 3 ED level was found to be associated with a moderate (1.35-1.40) S100A4 level. However, a level 4 ED level was found to be associated with the highest S100A4 levels (1.43-1.58) (Figure 4). The total variation rate of the MCA diagram (Figure 4), which explains the relationship structure between the ED levels and the S100A4 levels in the T2DM

**Table 7:** Model Performance Results of The MCA Diagram To Summarize The Multi-Correlation Structure Between The ED and S100A4 Levels of the DM and Control Groups

| | | Variance Accounted For | | |
|---|---|---|---|---|
| Dimension | Cronbach's Alpha | Total (Eigenvalue) | Inertia | % of Variance |
| 1 | 0.963 | 1.930 | 0.965 | 96.476 |
| 2 | 0.901 | 1.820 | 0.910 | 90.987 |

1: Vertical dimension, 2: Horizontal dimension

**Table 8:** The Effect of Different Factors on S100A4 Levels In The Control and Diabetes Groups

| | Use of the drug in the diabetes group | | | | |
|---|---|---|---|---|---|
| | OAD | | Insulin | | *p |
| | Mean | SEM | Mean | SEM | |
| S100A4 | 1.308 | 0.017 | 1.412 | 0.048 | 0.030 |
| | Sugar regulation in the diabetes group | | | | |
| | 1.00 | | 2.00 | | *p |
| | Mean | SEM | Mean | SEM | |
| S100A4 | 1.300 | 0.015 | 1.492 | 0.043 | 0.001 |
| | Dyslipidemia in all the volunteers | | | | |
| | non-dyslipidemia | | Dyslipidemia | | *p |
| | Mean | SEM | Mean | SEM | |
| S100A4 | 1.229 | 0.011 | 1.358 | 0.036 | 0.002 |
| | Smoking history in all the volunteers | | | | |
| | No smoking | | Smoking | | *p |
| | Mean | SEM | Mean | SEM | |
| S100A4 | 1.219 | 0.011 | 1.317 | 0.025 | 0.001 |
| | Hypertension status in the diabetes group | | | | |
| | non-hypertension | | hypertension | | *p |
| | Mean | SEM | Mean | SEM | |
| S100A4 | 1.240 | 0.012 | 1.390 | 0.096 | 0.001 |

OAD: Oral Antidiabetic, 1.00: Sugar Regulated, 2.00: Sugar non Regulated, SEM: Standard Error of Mean

group, was found to be 92.67%. This result showed that the diagram explained a high percentage of the relationship structure between the variables (Table 5).

We determined how the S100A4 levels changed with different ED levels of all the patients in both the control and T2DM groups. Our results showed that S100A4 levels increased as the ED levels of all patient groups increased ($p<0.001$). Although the increases in the S100A4 levels corresponding to the zero and 1st ED levels of all samples and the ED levels corresponding to the 2nd and 3rd ED levels were not significant in and of themselves, there was a significant difference in the 4th ED level ($p<0.001$, Table 6 and Figure 2).

In this study, we determined the relationship between ED levels and S100A4 levels of all the control and T2DM groups' patients using a Pearson Point Biserial Correlation analysis (Figure 3). According to the Pearson Point Biserial Correlation curve results, the S100A4 levels increased as the ED level increased ($p<0.001$). However, the sharpest increase was observed in S100A4 levels corresponding to the 4th ED level ($p<0.001$). In addition, the degree of correlation between ED levels and S100A4 levels of the patients of both groups participating in the study was 84.4% ($r=0.844$, $p=0.001$).

When we look at the MCA analysis shown in figure 4, we found that the lowest S100A4 levels of the patients of both groups were highly correlated with the initial zero and 1st ED levels, and moderate S100A4 levels were highly correlated with the 2nd and 3rd ED levels. Additionally, the highest S100A4 levels were found to be highly correlated with the 4th ED level. As can be seen in Figure 4, the S100A4 levels associated with the 4th ED level were found

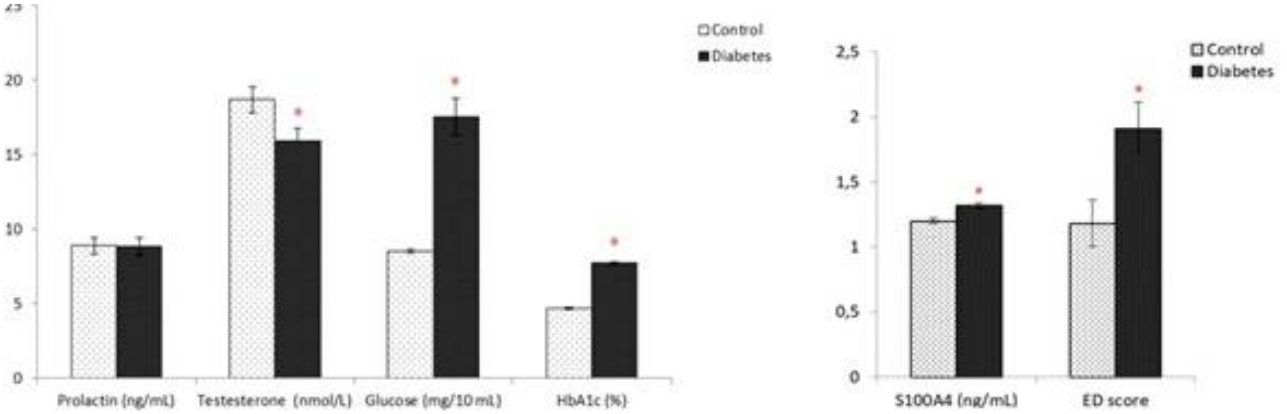


**Fig. 1.** Comparison of the results of the parameters and ED scores between the control and T2DM groups. *p: significant compared to the control group ($p<0.05$)

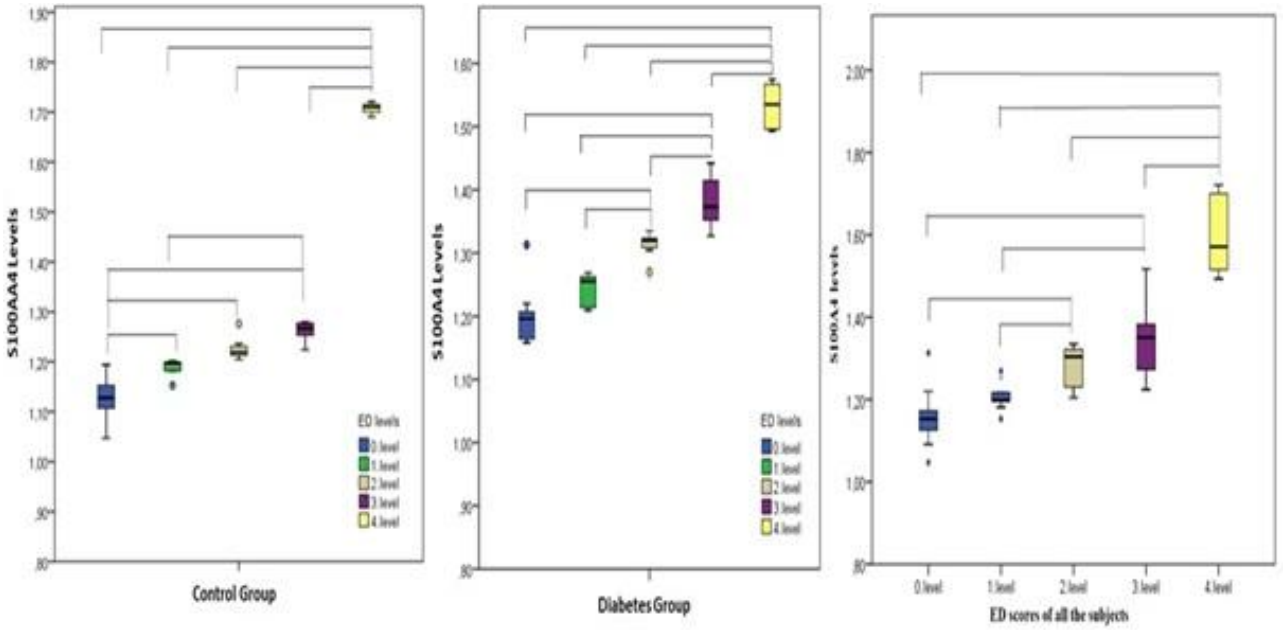


**Fig. 2**. Comparison of S100A4 levels corresponding to different ED scores of the control group, T2DM group and all the patients in both groups with Box Plot graph

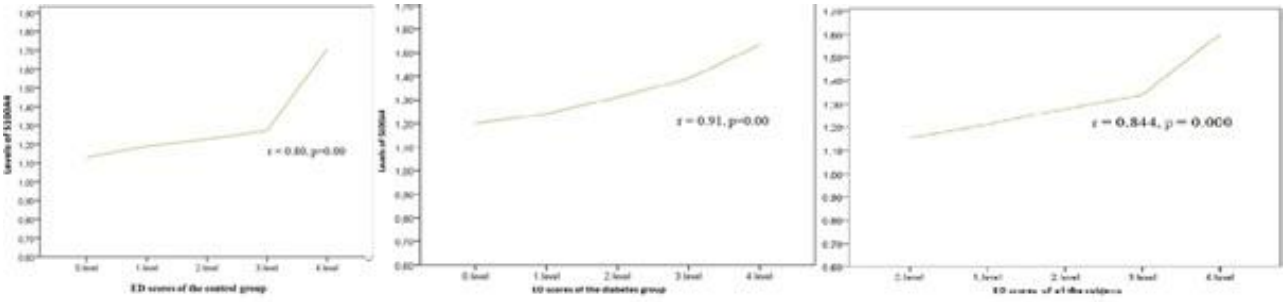


**Fig. 3.** Comparison of S100A4 levels corresponding to different ED scores of the control group, T2DM group and all the patients in both groups with the person correlation curve

to be significantly different from other S100A4 levels. The variance explanation rate was taken into account as the model performance criterion of the MCA diagram. The total variance rate of the two dimensions was found to be 93.73% on average (Table 7). This result shows that the diagram explains a high percentage of the relationship structure between the variables.

In addition, it was investigated how S100A4 levels changed with factors such as drug use, smoking and hypertension. In the diabetes group, as well as sugar regulation and hypertension, insulin use was found to increase S100A4 levels according to OAD. In addition, it was determined that the presence of dyslipidemia and smoking increased S100A4 levels in healthy and diabetic groups. Other detailed information can be seen in Table 8.

It was examined with the -2 Log-Likelihood method that the multivariate ordinal-logit regression model used to determine the predictors affecting ED levels gave sufficient estimates and it was found that the model was sufficient ($p=0.000$). In addition, the coefficient of determination (R2) for the performance of the obtained regression model was calculated and found to be 68.1%. Accordingly, it was observed that the predictors who worked with the obtained regression model contributed 68.1% to the change in ED levels.

## Discussion

Our thorough literature review indicates that this study represents the pioneering effort to explore the connection between S100A4 and ED. In our study, we showed that as the S100A4 level increased, the severity of ED in both the T2DM and the control group also increased.

T2DM is one of the most common comorbidities of ED. The rate of ED in men with T2DM ranges from 35% to 90%. In addition, the annual incidence rate of ED in men with T2DM is twice that of the normal population. There is a multifactorial relationship between T2DM and ED. However, the most alleged cause is vascular causes and peripheral and autonomic neuropathy. Endothelial damage that is caused by hyperglycemia progresses toward atherosclerosis. Since penile vessels are thin, they are affected earlier by atherosclerosis. Therefore, ED is considered an early marker of atherosclerosis (17). ED may also develop in patients with DM due to autonomic nervous system dysfunction and peripheral nerve damage. As expected, in our study, there was a significant difference between fasting blood glucose values and HbA1c levels between the T2DM and control groups. In a study conducted by Maalmi et al., the mean ED levels of the T2DM patients were higher than the mean ED levels of the control group (18). In our study, we also found the mean of ED levels of the T2DM group to be higher than the mean of ED levels of the control group. This result was consistent with other studies in the literature as well (19,20)

The reason why we included T2DM patients in our study was to obtain a patient group with a higher probability of developing atherosclerosis and peripheral neuropathy. We found that the T2DM group had higher mean S100A4 and ED levels compared to the control group, which met our expectations in this study. In a study by Cheung et al., they reported that the testosterone level of patients with T2DM decreased (21). Likewise, Dianatti and Grossmann (2020) stated in a study that testosterone levels may decrease in T2DM patients (22). In our study, we also found that serum testosterone levels decreased in the T2DM group compared to the control group. The results of our study also revealed similar results, by following the literature. However, since we excluded patients with testosterone levels outside the reference range and the decreasing testosterone levels in the T2DM group were also within the reference range, we think that the change in ED levels was not due to decreased testosterone levels.

One of the mechanisms of erectile dysfunction in diabetic men is neuropathy. This mechanism acts on both the autonomic and peripheral nerves. It has been shown in the literature that this damage in patients with diabetes is associated with early somatic and autonomic nerve dysfunction resulting from delayed stimulation of the pudendal nerve (23). In addition, it has been stated that another synthesis site of S100A4s besides vessels is neurons. Studies have reported that its level is increased in central and peripheral nerve injuries (12, 24,25 ). In a study in which neurons were cultured, it was reported that S100A4 level did not increase in healthy neurons, but S100A4 level increased in damaged nerves (26). In addition, it has been shown that S110A4 level increases in cases of brain trauma and epilepsy (27). In another study, it was reported that S100A4 level increased due to peripheral nerve neuropathy (28). Our study showed that increased ED scores were associated with increased S100A4 levels. In diabetic patients, ED is particularly associated with vascular and peripheral neuropathy. Although increased S100A4 levels in cases of smoking, dyslipidemia and hypertension suggest that this is more related to vascular causes, increased S100A4 scores may be associated with both vascular and neuropathic causes.

Today, it is known that vascular causes are involved in 80% of the etiology of ED. If the vessels feeding the penile corpus cavernosum

expand with appropriate stimuli but cannot convey enough blood to the penis, an erection cannot occur. A relationship was found between the severity of ED and the occluded vessels in cardiovascular diseases. The incidence of both ED and coronary artery disease is increased in smokers, patients with T2DM, HT, and hypercholesterolemia. The reason for this is that endothelial dysfunction lies in the pathogenesis of both diseases. In the case of endothelial dysfunction, the Nitric Oxide-Cyclic Guanosine-3-5-Monophosphate (NO-cGMP) system in both arterial structures and cavernosal structures is adversely affected and adequate dilatation cannot be achieved (29,30). This mechanism illustrates the commonalities of vascular ED and arterial diseases. ED is an expression of endothelial dysfunction, because the penis, which has a smaller artery structure, may be more sensitive to endothelial dysfunction than the vessels of the brain, heart and other organs. Another important reason is that penile arteries are end arteries and cannot compensate for arterial damage with collateral arteries like other organs can. Therefore, ED has been considered a predictor of cardiovascular diseases (30,31).

The relationship of the S100A4, which is known to be associated with cancer diseases, has recently attracted attention (8, 32) Studies have found that occludin and cadherin, which are intercellular tight junction proteins, decrease in parallel with high S100A4 expression and cause endothelial damage. After the damage to the endothelium, inflammation begins in the tissue. With the onset of inflammation, the S100A4 adheres to the vascular intima and causes smooth muscle cell migration to the intima. Macrophages that settle on the intima cause foamy cells to form, and fibrotic tissue and plaques form in the artery (33). Sakic et al. reported that inflammation and atherosclerosis were reduced, while lipid metabolism was not affected after administration of S100A4 protein-specific neutralizer mouse monoclonal IgG1 isotype (clone 6B12) in mice with atherosclerosis induced by a high cholesterol diet for 6 weeks (6). This showed that S100A4 increased inflammation and atherosclerosis, while the monoclonal IgG1 isotype could eliminate these effects. In a study, it was shown that the S100A4 is not found in cardiac myocytes, but that there is a strong S100A4 expression in cardiac myocytes after myocardial infarction (MI). This indicates that the S100A4 may be associated with increased inflammation, fibrosis and angiogenesis in the damaged area (34).

In a research investigation, it was noted that the existence of plexiform lesions in the pulmonary arteries of a mouse exhibiting heightened levels of S100A4/Mts1 could play a significant role in the development of this protein and contribute to the pathogenesis of clinical exogenic arteriopathy and pulmonary hypertension (35). Moreover, S100A4 was identified in the endothelial cells of external lesions in mice and the smooth muscle cells of the neointima in human pulmonary arteries. The expression of this protein was found to be particularly intense in obstructive lesions (35). Furthermore, an increase in S100A4 levels was not observed in the initial stages of pulmonary hypertension in lung biopsies conducted on children with the condition. It was reported that the excessive secretion of S100A4 in the later stages of the disease may indicate impaired arteriopathy (36).

Our study found that S100A4 levels increased significantly in both the T2DM group and the control group, in line with the increase in ED severity found in the above studies (34,35). Our study also revealed a strong positive correlation between ED levels and S100A4 levels. In addition, smoking and hypertension were found to be positively associated with ED severity and increased S100A4.

**Limitations:** The limitations of our study are the lack of a clear marker to show ED and the fact that this procedure could not be performed because the patients did not voluntarily accept the procedure due to the side effects of the phenyl doppler ultrasonography procedure, which is used to show penile vascular damage.

Our study also showed a strong positive correlation between ED levels and S100A4 levels. In addition, smoking and hypertension were found to be positively associated with ED severity and increased S100A4. The data indicates that the S100A4 may be used in the diagnosis and/or scoring of ED. However, to verify this result, more detailed experimental studies are needed to compare penile Doppler Ultrasonography diagnosed vascular injury and ED with S100A4 levels. In addition, studies to be carried out with antibody treatment can be a guide for possible ED treatment.

**Acknowledgment:** This study was supported by the Department of Biochemistry, Medical Faculty, Van Yuzuncu Yil University.

**Conflicts of interest:** The authors have no conflicts

**Ethics approval:** The study was approved by the Ethical Committee of the Van Yuzuncu Yil University Medical Research Centre, Van, Turkey (date: 12.01.2022, no:06)

## References


1. McCabe, M. P., Sharlip, I. D., Lewis, R., Atalla, E., Balon, R., Fisher, A. D. & Segraves, R. T. Risk factors for sexual dysfunction among women and men: a consensus statement from the Fourth International Consultation on Sexual Medicine 2015. The journal of sexual medicine 2016; 13: 153-167.
2. Demir, S., & Barlas, İ. Ş. An independent indicator of erectile dysfunction is C-reactive protein/albumin ratio. Andrologia 2021; 53: e14073.
3. Kozlova, E. N., & Lukanidin, E. Metastasis-associated mts1 (S100A4) protein is selectively expressed in white matter astrocytes and is up-regulated after peripheral nerve or dorsal root injury. Glia 1999; 27: 249-258.
4. Packard, R. R., & Libby, P. (). Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clinical chemistry 2008; 54: 24-38.
5. Ervinna, N., Mita, T., Yasunari, E., Azuma, K., Tanaka, R., Fujimura, S., Sukmawati, D., Nomiyama, T., Kanazawa, A., Kawamori, R., Fujitani, Y., & Watada, H. Anagliptin, a DPP-4 inhibitor, suppresses proliferation of vascular smooth muscles and monocyte inflammatory reaction and attenuates atherosclerosis in male apo E-deficient mice. Endocrinology 2013; 154: 1260-1270.
6. Sakic, A., Chaabane, C., Ambartsumian, N., Klingelhöfer, J., Lemeille, S., Kwak, B. R., Grigorian, M., & Bochaton-Piallat, M. L. Neutralization of S100A4 induces stabilization of atherosclerotic plaques: role of smooth muscle cells. Cardiovascular research 2022; 118: 141-155.
7. Serrano, A., Apolloni, S., Rossi, S., Lattante, S., Sabatelli, M., Peric, M., Andjus, P., Michetti, F., Carrì, M. T., Cozzolino, M., & D'Ambrosi, N. The S100A4 Transcriptional Inhibitor Niclosamide Reduces Pro-Inflammatory and Migratory Phenotypes of Microglia: Implications for Amyotrophic Lateral Sclerosis. Cells, 2019; 8: 1261.
8. Donato, R., Cannon, B. R., Sorci, G., Riuzzi, F., Hsu, K., Weber, D. J., & Geczy, C. L. (). Functions of S100 proteins. Current molecular medicine 2013; 13: 24-57.
9. Gonzalez, L. L., Garrie, K., & Turner, M. D. Role of S100 proteins in health and disease. Biochimica et biophysica acta. Molecular cell research 2020; 1867: 118677.
10. Barraclough R. Calcium-binding protein S100A4 in health and disease. Biochimica et biophysica acta 1998; 1448: 190-199.
11. Anguita-Ruiz, A., Mendez-Gutierrez, A., Ruperez, A. I., Leis, R., Bueno, G., Gil-Campos, M., Tofe, I., Gomez-Llorente, C., Moreno, L. A., Gil, Á., & Aguilera, C. M. The protein S100A4 as a novel marker of insulin resistance in prepubertal and pubertal children with obesity. Metabolism: clinical and experimental 2020; 105: 154187.
12. Abu El-Asrar, A. M., Nawaz, M. I., De Hertogh, G., Alam, K., Siddiquei, M. M., Van den Eynde, K., Mousa, A., Mohammad, G., Geboes, K., & Opdenakker, G. S100A4 is upregulated in proliferative diabetic retinopathy and correlates with markers of angiogenesis and fibrogenesis. Molecular vision 2014; 20: 1209-1224.
13. Lei, L., & Tang, L. Schwann cells genetically modified to express S100A4 increases GAP43 expression in spiral ganglion neurons in vitro. Bioengineered, 2017; 8: 404-410.
14. 13Mahmood, M. A., Rehman, K. U., Khan, M. 56+A., & Sultan, T. Translation, cross-cultural adaptation, and psychometric validation of the 5-item International Index of Erectile Function (IIEF-5) into Urdu. The journal of sexual medicine 2012; 9: 1883-1886.
15. Huyut MT, Soyguder S. The Multi-Relationship Structure between Some Symptoms and Features Seen during the New Coronavirus 19 Infection and the Levels of Anxiety and Depression post-Covid. East J Med 2022; 27: 1-10.
16. Huyut MT, Keskin S. Determination of Factors Affecting of Mathematics Success: Environmental factors with Multiple Correspondence Analysis. Turk J Appl Sci Technol 2017; 1: 48-59.
17. Huyut MT, Keskin S. The Success of Restricted Ordination Methods in Data Analysis with Variables at Different Scale Levels. Erzincan University J Sci Technol 2021; 14: 215-231.
18. Malavige, L. S., & Levy, J. C. Erectile dysfunction in diabetes mellitus. The journal of sexual medicine 2009; 6: 1232-1247.
19. Maalmi, H., Herder, C., Bönhof, G. J., Strassburger, K., Zaharia, O. P., Rathmann, W., Burkart, V., Szendroedi, J., Roden, M., Ziegler, D., & GDS Group. Differences in the prevalence of erectile dysfunction between novel subgroups of recent-onset diabetes. Diabetologia 2022; 65: 552-562.
20. Fan, J., Peng, T., Hui, J., Ding, W., He, B., Zhang, H., & Wei, A. Erectile Dysfunction in Type-2 Diabetes Mellitus Patients: Predictors

of Early Detection and Treatment. Urologia internationalis 2021; 105: 986-992.

21. Weldesenbet, A. B., Kebede, S. A., & Tusa, B. S. Prevalence of erectile dysfunction and its associated factors among patients with diabetes in Ethiopia: a systematic review and meta-analysis. The Journal of international medical research 2021; 49: 300060521993318.
22. Cheung, K. K., Luk, A. O., So, W. Y., Ma, R. C., Kong, A. P., Chow, F. C., & Chan, J. C. Testosterone level in men with type 2 diabetes mellitus and related metabolic effects: A review of current evidence. Journal of diabetes investigation 2015; 6: 112-123.
23. Gianatti, E. J., & Grossmann, M. Testosterone deficiency in men with Type 2 diabetes: pathophysiology and treatment. Diabetic medicine: a journal of the British Diabetic Association 2020; 37: 174-186.
24. Thorve, V. S., Kshirsagar, A. D., Vyawahare, N. S., Joshi, V. S., Ingale, K. G., & Mohite, R. J. Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. Journal of diabetes and its complications, 2011; 25: 129-136.
25. Dmytriyeva, O., Pankratova, S., Owczarek, S., Sonn, K., Soroka, V., Ridley, C. M., Marsolais, A., Lopez-Hoyos, M., Ambartsumian, N., Lukanidin, E., Bock, E., Berezin, V., & Kiryushko, D. The metastasis-promoting S100A4 protein confers neuroprotection in brain injury. Nature communications 20123; 1197.
26. Sandelin, M., Zabihi, S., Liu, L., Wicher, G., & Kozlova, E. N. Metastasis-associated S100A4 (Mts1) protein is expressed in subpopulations of sensory and autonomic neurons and in Schwann cells of the adult rat. The Journal of comparative neurology 2004; 473: 233-243.
27. Fang, Z., Forslund, N., Takenaga, K., Lukanidin, E., & Kozlova, E. N. Sensory neurite outgrowth on white matter astrocytes is influenced by intracellular and extracellular S100A4 protein. Journal of neuroscience research 2006; 83: 619-626.
28. Hahn, C. G., Wang, H. Y., Cho, D. S., Talbot, K., Gur, R. E., Berrettini, W. H., Bakshi, K., Kamins, J., Borgmann-Winter, K. E., Siegel, S. J., Gallop, R. J., & Arnold, S. E. (). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nature medicine 2006; 12: 824-828.
29. Wack, G., Metzner, K., Kuth, M. S., Wang, E., Bresnick, A., Brandes, R. P., Schröder, K., Wittig, I., Schmidtko, A., & Kallenborn-Gerhardt, W. Nox4-dependent upregulation of S100A4 after peripheral nerve injury modulates neuropathic pain processing. Free radical biology & medicine 2021; 168: 155-167.
30. Stuckey, B. G., Walsh, J. P., Ching, H. L., Stuckey, A. W., Palmer, N. R., Thompson, P. L., & Watts, G. F. Erectile dysfunction predicts generalised cardiovascular disease: evidence from a case-control study. Atherosclerosis 2007; 194: 458-464.
31. Chiurlia, E., D'Amico, R., Ratti, C., Granata, A. R., Romagnoli, R., & Modena, M. G. (). Subclinical coronary artery atherosclerosis in patients with erectile dysfunction. Journal of the American College of Cardiology 2005; 46: 1503-1506.
32. Thompson, J. F., Durham, L. K., Lira, M. E., Shear, C., & Milos, P. M. CETP polymorphisms associated with HDL cholesterol may differ from those associated with cardiovascular disease. Atherosclerosis, 2005; 181: 45-53.
33. Li, Z., Li, Y., Liu, S., & Qin, Z. Extracellular S100A4 as a key player in fibrotic diseases. Journal of cellular and molecular medicine 2020; 24: 5973-5983.
34. Herwig, N., Belter, B., & Pietzsch, J. Extracellular S100A4 affects endothelial cell integrity and stimulates transmigration of A375 melanoma cells. Biochemical and biophysical research communications 2016; 477: 963-969.
35. Schneider, M., Kostin, S., Strøm, C. C., Aplin, M., Lyngbaek, S., Theilade, J., Grigorian, M., Andersen, C. B., Lukanidin, E., Lerche Hansen, J., & Sheikh, S. P. S100A4 is upregulated in injured myocardium and promotes growth and survival of cardiac myocytes. Cardiovascular research, 2007; 75: 40-50.
36. 35)Dempsie, Y., Nilsen, M., White, K., Mair, K. M., Loughlin, L., Ambartsumian, N., Rabinovitch, M., & Maclean, M. R. Development of pulmonary arterial hypertension in mice over-expressing S100A4/Mts1 is specific to females. Respiratory research, 2011; 12: 159.
37. Greenway, S., van Suylen, R. J., Du Marchie Sarvaas, G., Kwan, E., Ambartsumian, N., Lukanidin, E., & Rabinovitch, M. S100A4/Mts1 produces murine pulmonary artery changes resembling plexogenic arteriopathy and is increased in human plexogenic arteriopathy. The American journal of pathology 2004; 164: 253-262.
38. Johannes, C. B., Araujo, A. B., Feldman, H. A., Derby, C. A., Kleinman, K. P., & McKinlay, J. B. Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. The Journal of urology 2000; 163: 460-463.

39. Libby P. Inflammation in Atherosclerosis-No Longer a Theory. Clinical chemistry 2021; 67: 131-142.
40. Sahan, A., Akbal, C., Tavukcu, H. H., Cevik, O., Cetinel, S., Sekerci, C. A., & Tanidir, Y. Melatonin prevents deterioration of erectile function in streptozotocin-induced diabetic rats via sirtuin-1 expression. Andrologia 2020; 52: e13639.