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SERUM FRUCTOSAMINE AS A MARKER FOR GLYCEMIC CONTROL IN PATIENTS WITH DIABETIC NEPHROPATHY

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Abstract

Diabetes mellitus (DM) is a metabolic disease of chronic insulin deficiency or resistance. The progression of DM is associated with long-term damage to macro- and micro-vascular body systems and causes serious health complications. Up to 40% of DM patients develop chronic kidney disease (CKD), mainly as a consequence of diabetic nephropathy (DN). Hence, strict glycemic control is recommended to slow down CKD progression. Hyperglycemia causes complications in diabetic patients through the glycation of various proteins. The interaction of glucose with the free NH_2 groups of proteins results in the formation of the early glycation product fructosamine (FA). FA has a short half-life (1 to 2 weeks) and hence it has the potential of an early marker for glycemic control. Although long used in the clinical practice, the FA diagnostic and prognostic significance remains questionable. In this study, we tested the FA potential to serve as a glycemic marker in DM patients with DN. We found that DM patients have significantly higher serum FA levels than non-diabetics. Further, the serum FA level in diabetics correlates positively with the blood glucose concentration. Finally, the mean serum FA level was higher in DM patients with DN, compared to those without DN. These results establish FA as a promising marker for glycemic control in DN patients.

Key words: diabetic nephropathy, fructosamine, glycemic control

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Introduction. Diabetes mellitus (DM) is a chronic metabolic disease, caused by impaired insulin production or cellular uptake. On this basis, DM is divided into type 1 (T1DM) and type 2 (T2DM), respectively. DM is a serious health problem with increasing prevalence. According to the International Diabetes Federation, 537 million people worldwide were living with diabetes in 2021, with an increasing trend. DM patients with uncontrolled hyperglycemia maintain high blood glucose levels, causing damage to various micro- and macro-vascular systems in the body. As a result, such patients develop various DM-associated complications such as cardiovascular disease, diabetic retinopathy, neuropathy and nephropathy. About 30% of patients with T1DM and up to 40% of patients with T2DM develop diabetic kidney disease [1]. Among the long-term DM complications, chronic kidney disease (CKD) is one of the most severe, affecting the patients' quality of life. CKD is characterized by sustained elevated albuminuria > 30 mg/24 h and/or sustained decreased glomerular filtration rate (eGFR) < 60 ml/min, and its progression is associated with deterioration of renal function, leading to End Stage Renal Disease (ESRD) and increased mortality [2].

Diabetes research is focused on improving DM treatment and increasing the life expectancy of people with diabetes. Nowadays, new therapeutic approaches are being developed, including use of stem cells to generate functional β-cells and identification of potential markers for disease control [3]. Undoubtedly, the most effective way to prevent diabetic complications is timely diagnosis of DM and its proper management [4]. Biomarkers allow monitoring of treatment and its efficiency in terms of improving glucose control and reducing the risk of complications. Many studies are focused on biomarkers' accuracy and reliability, as well as on the identification of new biomarkers with enhanced prognostic and diagnostic potential [5].

Keeping blood glucose levels within reference ranges is essential to human health. In particular, biomarkers that provide a reliable and objective assessment of glycaemia are crucial to the monitoring of diabetes. One well-established marker in the clinical practice is the glycated hemoglobin (HbA1c), which reflects the blood glucose levels over a period of 8 to 12 weeks [6]. Laboratory glucose measurements reflect glucose levels at the time of blood sampling. However, neither HbA1c, nor glucose provide reliable information about the real glycemic status of DM patients. In addition, HbA1c is an unreliable marker of glucose control in CKD patients because of the reduced erythrocytes count and turnover in these patients.

Fructosamine (FA) is a ketoamine that is formed in a non-enzymatic reaction (glycation) of glucose with free amino groups of serum proteins, mainly albumin. In DM patients, FA levels increase as a result of the increased blood glucose levels. This positive correlation between blood glucose and serum FA levels makes the latter a good candidate marker for glycemic control. In cases where HbA1c is unreliable, FA could be used as an alternative marker of the diabetic condition.

In contrast to HbA1c, FA reflects the mean blood glucose concentration over a short period, two to three weeks, and thus can be used as a short-term glycemic marker. Also, FA reflects both postprandial and fasting blood glucose levels [7], and therefore appears to be a promising marker for monitoring of glucose fluctuations in DM patients with stable HbA1c. FA has been in the focus of clinical research more than 30 years. However, despite the great number of studies and the FA potential to serve as an alternative glycemic marker [8], its diagnostic and prognostic significance remain unclear. The aim of this study was to investigate the possibility of using FA as a marker for glycemic control in DM patients and particularly in patients with diabetic nephropathy (DN).

Materials and methods. ***Study design.*** A prospective, single-centre, cross-sectional study was performed, including DM patients (with and without DN) and control CKD patients without DM. Serum FA levels were measured and correlated with patients' age, DM duration, eGFR, proteinuria and glycaemia.

Patients. The DM group included 23 patients with T2DM (13 males and 10 females), nine of them diagnosed with DN and the rest with non-diabetic renal disease. Six out of the 23 T2DM patients were on insulin therapy. The CKD control group comprised 21 patients without DM. All patients were admitted to the Clinic of Nephrology at the University Hospital "Tsaritsa Yoanna – ISUL" during the period of October 2016 to May 2017. The patients underwent clinical and anthropometric observations, as well as standard laboratory tests. GFR was calculated using the MDRD (Modification of Diet in Renal Disease) formula based on the serum creatinine concentration. CKD staging was performed according to the recommendations of The Kidney Diseases Improving Global Outcomes (KDIGO) group - 2012. All DM patients were screened for diabetic nephropathy, retinopathy and polyneuropathy. The DN diagnosis was made following the American Diabetes Association criteria – excretion of an abnormal quantity of albumin in the urine (alb $>$ 300 mg/24 h), ophthalmoscopic evidence of diabetic retinopathy and absence of clinical and laboratory evidence of other urologic diseases.

Measurement of serum FA. Serum FA was measured by the FA assay, developed by Johnson et al. [9]. Standard samples were prepared with 1-Deoxy-1-morpholino-D-fructose supplemented with 50 mg/ml bovine serum albumin in the concentration range from 0.25 mM to 8 mM. Ten microliters of either serum or standard samples were pipetted into a polystyrene 96-well plates and mixed with 100 µl 0.1 M carbonate buffer, pH 10.8, containing 0.25 mM Nitro Blue Tetrazolium. After 15 min incubation at room temperature, the absorbance at 550 nm was measured with a plate reader Bio-TEK ELx800.

Statistical analysis. The following analyses were applied: descriptive statistics, including quantitative parameters' mean ± standard deviation (SD), non-parametric Mann–Whitney test to compare differences between two independent samples, Fisher's exact test for statistical associations between two categorical variables, and correlation analysis. Data were processed using Microsoft Ex-

cel®2007 and GraphPad Prism®, ver.5.00 software packages. A significance level of $p < 0.05$ was used for rejecting the null hypothesis.

Results and discussion. Table 1 shows the general and clinical characteristics of the DM patients. The sex distribution of the DM patients was 56.5% males and 43.5% females. The age range was from 39 to 71 years, and according to the WHO age distribution, patients of young age were 13.1%, of middle age – 21.7% and 62.2% were in the elderly group. The mean age of males was 59.08 ± 8.74 years, and for females it was 61.80 ± 12.04 years. The duration of DM was determined by anamnestic information and medical records. The mean duration of DM in men was 96.92 ± 65.08 months and 151.2 ± 136.38 months in women. With fasting glucose levels below 5 mmol/l were 8.7% of the patients, between 5 and 7 mmol/l – 60.87%, and above 7 mmol/l – 30.43%.

T a b l e 1

Characteristics of DM patients

Indicator	Value				
Average age [years]	60.26±10.14				
Average duration of DM [months]	120.52±103.32				
Glomerular filtration rate [ml/min/1.73 m^2]	≥ 90	60–89	30–59	15–29	< 15
Number of patients	3	9	7	4	0
Proteinuria [g/day]	0.15–0.50		0.5–3.5		> 3.5
Number of patients	8		14		1
	Yes		No		
Diabetic nephropathy	7 patients		16 patients		
Diabetic retinopathy	5 patients		18 patients		
Diabetic polyneuropathy	9 patients		14 patients		

The reference serum FA levels were determined based on measurements of FA in sera of 21 non-diabetic CKD patients. The sex distribution in this control group was 12 (57.1%) males and 9 (42.9%) females. The mean age of the control patients was 48.57 ± 10.69 (27 to 63) years. For men it was 49.25 ± 9.22 and for women – 47.67 ± 12.93 years. The mean FA level in the control group was found to be 1.35 ± 0.19 mM. Pathological FA levels in the DM patients were considered those above the cut-off value of 1.9 mM, calculated as:

$$\text{Mean FA}_{\text{control}} + 3\text{SD} = 1.35 + (3 \times 0.19) = 1.9 \text{ mM}.$$

The FA levels in sera of all DM patients were measured and correlated with patients' clinical parameters. The FA levels in sera of DM patients were also compared to those of the control group. This comparative analysis revealed that the FA concentration in the DM patients is significantly higher than that in the control group (1.94 ± 0.53 mM vs. 1.35 ± 0.19 mM, $p = 0.0018$).

The elevated serum FA levels in DM patients support previously published data, indicating that FA might substitute or complement traditional biomarkers for glycemic control like HbA1c [10]. Malmström et al. [10] report a strong positive correlation of serum FA levels with glucose and HbA1c levels among DM patients in the AMORIS cohort, and no clear correlation in subjects with normal glucose tolerance. In other words, in that study serum FA and HbA1c in DM individuals show a tendency to covary over time, which demonstrates the FA potential to be an alternative (to HbA1c) glycemic marker. Similarly, Juraschek et al. [11] have found significantly higher serum FA levels in DM patients, compared to those without diabetes. They also demonstrate a positive correlation of FA with fasting glucose, with the correlation being stronger in individuals with diabetes. In our study, we also observed a positive correlation of patients' serum FA levels with fasting blood glucose levels ($r = 0.3992$, $p = 0.0296$) (Fig. 1). Postprandial glycaemia also tended to correlate positively with FA levels ($r = 0.3399$, $p = 0.0563$).

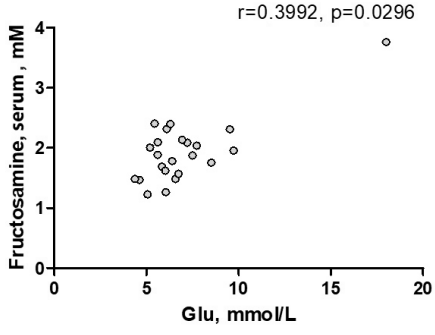

Fig. 1. Correlation of serum FA levels with fasting glycaemia in DM patients

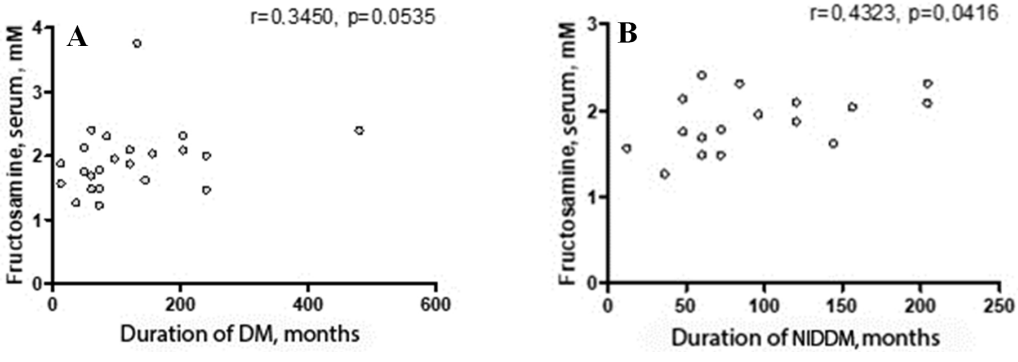

Fig. 2. Correlation of serum FA levels with DM duration in all DM patients (A) and in the cohort of patients with NIDDM (B)

We found no correlations of serum FA levels with patients' age, DM duration and renal function. Although among all DM patients the FA levels did not correlate with disease duration (Fig. 2A), a weak positive correlation was observed in those that were not on insulin therapy, designated as non-insulin dependent diabetes (NIDDM) in Fig. 2B (r=0.4323, p=0.0416).

We compared the serum FA levels in DM patients with DN with the FA levels in DM patients with other (non-diabetic) kidney disease. Our analysis showed a significantly higher serum FA concentration in DM patients with DN compared to those without DN (2.3 ± 0.7 mM vs. 1.8 ± 0.3 mM, $p = 0.0244$) (Fig. 3). Among DM patients with pathologically elevated serum FA levels ($n = 11$), five (45.5%) developed DN, and among DM patients with reference serum FA levels ($n = 12$) only two (16.7%). This distribution showed that despite the different DN frequency in the two groups, the pathologically elevated FA levels in the serum of DM patients did not unambiguously determine the presence of DN ($p = 0.1483$).

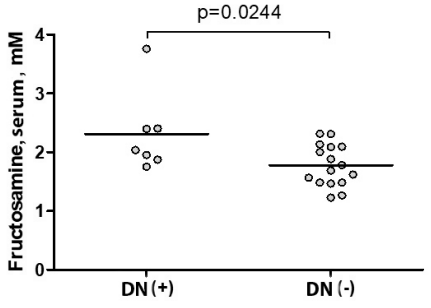

Fig. 3. Comparative analysis of serum FA levels in DM patients with and without DN

Data about the association of FA with CKD in DM patients are controversial. Some studies report that FA correlates positively with blood glucose in DM patients with normal renal function, but not in those with renal impairment [12]. According to other studies, FA reflects the glycemic status of DM patients with CKD more accurately than HbA1c [13]. One limitation for validating FA as a glycemic marker is the insufficient evidence linking FA to long-term complications of diabetes. On the other hand, in CKD patients, HbA1c demonstrates insufficient accuracy in blood glucose monitoring. Any condition affecting erythrocyte survival alters the HbA1c levels. In CKD, the turnover of red blood cells is shortened, resulting in a lower HbA1c levels. Exceptionally, the CKD-associated iron-deficiency anemia results in elevated HbA1c values due to the particular treatment [14]. Therefore, it appears that in DM patients with CKD, HbA1c may be an unreliable marker for glycemic control. The fast metabolism, short half-life and preferential accumulation of FA in blood proteins of DM patients make it a suitable HbA1c substitute.

Vos et al. [15] have studied the accuracy of HbA1c and FA as markers for glycemic control in DM patients with DN. They find a strong positive correlation of HbA1c with glucose concentration in DM patients without DN, but not in those with DN. In contrast, FA has shown a significant correlation with glucose concentration in DN patients. In a study of a small cohort of DM patients with CKD, who were on iron or erythropoietin treatment, Konya et al. [16] observe that during the therapy, FA did not change, while HbA1c underwent a significant decrease. However, at the beginning and at the end of the therapy, HbA1c correlated substantially with the mean glucose concentration. FA has also been studied for association with morbidity and mortality in dialysis patients. Mittman et al. [17] have measured the serum FA in DM hemodialysis patients and found that increased FA levels are associated with enhanced risk of infection. Shafi et al. [18] demonstrate an association of FA with cardiovascular disease (CVD) events, CVD mortality and sepsis among hemodialysis patients.

To date, FA measurement has not been standardized, and there is no consensus on the preferred methods for its measurement and threshold determination. Selvin et al. [19] have analyzed data from the community-based Atherosclerosis Risk in Communities (ARIC) Study. They have searched for FA reference intervals or cutoff values, comparable with clinically accepted values for HbA1c and fasting glucose. Based on the correlation between these biomarkers under hyperglycemic conditions, the authors are optimistic regarding the FA applicability in the clinical practice. In a study of over 1000 DM patients Andrade et al. [20] demonstrate a clear linear relationship between serum FA and the blood glucose levels. They also propose a method for calculation of the mean glucose value from that of FA.

Conclusions. The control over diabetes and CKD involves various approaches aiming to slow down disease progression and minimize complications. Although CKD is often irreversible, proper management can significantly improve the patients' quality of life and delay robust interventions like dialysis and kidney transplantation. Diabetes is one of the main causes of CKD and therefore control over blood glucose levels is crucial. Our study demonstrates that serum FA levels correlate positively with blood glucose in DM patients and are significantly higher than in non-diabetics. Most importantly, FA levels differentiate well between diabetics with, and without DN. This preliminary pilot study demonstrates that it is worthwhile to further explore the FA potential of a glycemic marker in larger cohorts of DM patients with and without DN, and such studies are now in progress.

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