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Plasma Cathepsin V Levels in relation to angiogenesis and obesity in type 2 diabetes mellitus patients with poor control: Implications for oncology

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ABSTRACT

Diabetes and obesity prevalence are rising everywhere in the world. The primary chronic diabetes consequences, which are linked to death and disability, are diabetic vascular problems. One important pathogenic trait of diabetic microvascular problems is angiogenesis.

Aim of study: This study measured the level of cathepsin V and Vascular Endothelial Growth Factor-A (VEGF-A) among a group of patients with type 2 diabetes mellitus who have poor control and its correlation with each other, obesity and hyperglycemia.

Methods: The research study involved 110 patients with type 2 diabetes mellitus and 70 apparently healthy subjects between the ages of 40 and 70. The Roche Cobas Integra 400 Plus was used to calculate the percentage of glycated Hemoglobin (HbA1c) and Fasting Plasma Glucose (FPG) concentration. Cathepsin V and VEGF-A levels in serum were assayed using Enzyme Linked Immunosorbent Assay (ELISA) kit. Weight/height^2 was used as a formula to estimate the Body Mass Index (BMI).

Results: Compared to the controls, patients with type 2 diabetes mellitus exhibited significantly higher levels of VEGF-A and cathepsin V in their blood ($p<0.001$). Significant positive correlation was seen between cathepsin V and VEGF-A in patient's individuals. There was a positive correlation between cathepsin V and VEGF-A levels with the duration of the disease ($p=0.017$). Furthermore, a significant positive correlation was observed between VEGF-A levels and serum glucose levels in patients. It was not observed significant correlation between cathepsin V and VEGF-A with Body Mass Index (BMI) in patient's individuals.

Conclusions: Poor controlled diabetes mellitus patients produce more cathepsin V and VEGF-A than normal level which may contribute in excessive angiogenesis and complication of diabetes mellitus. The increased angiogenesis process in type 2 diabetes mellitus is not associated with obesity. To validate the precise mechanisms of cathepsin V action and its potential as a therapeutic target, more study is required.

Keywords: Cathepsin V, angiogenesis, VEGF-A, carcinomas

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INTRODUCTION

Diabetes mellitus is a chronic disease that is becoming more prevalent worldwide [1]. Type 2 Diabetes Mellitus (T2DM) is the most common type of diabetes, accounting for 90% to 95% of all cases [2]. It is distinguished by a more intricate interplay between genetics and way of life. T2DM appears to have a more complex genetic profile than type 1 Diabetes Mellitus (T1DM). Most people with T2DM have a family history of the condition [3]. Obesity is the primary factor that leads to the development of T2DM [4]. According to the National Diabetes Statistics Report, 87.5% of individuals with diabetes are overweight or obese [5].

A pathophysiological approach proposes that the primary cause of T2DM is the progressive decline in beta-cell insulin synthesis induced by insulin resistance [6]. T2DM is strongly associated with microvascular complications such as nephropathy, retinopathy, and neuropathy, as well as macrovascular complications such as throat cancer, coronary heart disease, stroke, and peripheral artery disease [7].

Diabetes and ageing are characterized by impaired angiogenesis and endothelial dysfunction. Clinical initiatives to stimulate angiogenesis have generally concentrated on growth factor pathways, a novel set of endothelium intracellular molecules required for endothelial metabolism has recently been identified as being crucial for controlling angiogenesis [8].

There are several families of cathepsins, which are protease enzymes. Examples of these enzymes are aspartyl, cysteine, and serine proteases [9]. There are approximately 11 cathepsin classes in humans, and each one serves a different purpose [10]. They may be crucial for physiological processes including digestion, blood clotting, bone resorption, angiogenesis and ion channel activity, much like other enzymes [11-16].

Like other proteolytic enzymes, cathepsins are found in the lysosome, melanosome, and extracellular matrix. They participate in a healthy physiological and health problems condition [17]. The cathepsin family of cysteine proteases includes Cathepsin V, also called cathepsin L2 [8]. It first was discovered in the thymus, and testis. Moreover, it is also found in cornea, thymus, brain, heart and skin [8,11,12]. Cauthepsin V glycosylation may be related to cathepsin V-mediated neurological diseases, myasthenia gravis, and type 1 diabetes mellitus because N-glycosylation controls cathepsin V intracellular trafficking, secretion, and enzymatic activity [13].

Numerous proteases, like cathepsin B, D, and kallikrein 4, have been shown to change in concentration and activities in diabetic individuals' and animal experimental models' urine. Some of these proteases are thought to be indicators for renal problems in diabetes [14].

For adult islet vessel maintenance, blood flow, and precise glucose regulation, VEGF-A and insulin signaling are essential. Type 1 and type 2 diabetes are diseases that start and worsen because of vascular abnormalities. Endocrine vascular maintenance depends on beta cells' VEGF-A signaling to endothelial cells. While persistent VEGF overexpression causes islet hypervascularization, beta cell death, and overt diabetes, VEGF-A loss of function causes islet hypo vascularization and reduced glucose tolerance [15].

The goal of this study is to compare the carcinomas, cathepsin V and VEGF-A levels in the serum of type 2 diabetes mellitus patients to those of controls who appear to be in good health and to look for a relationship between study parameters and obesity.

MATERIALS AND METHODS

In this case-control study, one hundred ten patients with type 2 diabetes mellitus (38 males and 72 females) with age range 40 years -70 years were studied. Seventy apparently healthy subjects (24 males and 46 females) in the same age range (mean 54.90 ± 8.98) who constituted the control group, were taken for comparison.

Blood samples were collected from the patients with type 2 diabetes mellitus at Internal Medicine Unit, Baquba Teaching Hospital, Diyala, Iraq between February 2022 to July 2022. Intravenous blood samples were taken from the patients and controls by inserting disposable 5 ml syringes into their veins after 8 hours to 10 hours of fasting. While the first ml was placed into EDTA tubes for HbA1c estimation, the remaining 4 ml were gradually squeezed into a disposable test tube containing separating gel.

Before freezing the serum, it was separated from the blood to determine the value for fasting plasma glucose, the residual serum will be kept frozen at -20°C until cathepsin V and VEFG-A analysis is performed on it. The estimation of cathepsin V and VEFG-A was made by Enzyme Linked Immunosorbent Assay (ELISA) technique (My BioSource/USA), by the manufacturer's protocol all other tests were assayed by auto analyzer Roche Diagnostics cobas integra 400 plus.

The diagnosis of type 2 diabetes mellitus was made by specialized consultants, according to the ADA protocol [5] considering the clinical history, laboratory tests and clinical features of the patients. In addition, all patients are categorized based on BMI in accordance with WHO guidelines. The average age of type 2 diabetes mellitus among patients was 8.8 years ± 4.1 years. The data for patients and control included information about sex, age, marital status, the questions asked in the study also covered topics such as the length of time the individual had been diagnosed with the disease, the age at which the disease was first detected, any treatments received, family medical history, and the participant's medical and surgical history.

The means of continuous variables were compared between the patient and control groups using a two-tailed independent samples t-test. The link between cathepsin V, VEGF-A, the length of the illness, glucose, HbA1c, and BMI was examined using the Spearman correlation test. Cohen's criterion was applied to assess the strength of the correlations. ROC curve analysis was performed for testing the ability of the study markers to differentiate between patients and control. SPSS version 26.0 (Chicago) and Microsoft excel v.2016 were used for statistical analyses. Less than 0.05 was considered statistically significant.

Exclusion criteria

T1DM patients, presence of other autoimmune disease like Hashimoto's thyroiditis, patients with, renal or liver disease, neurological disorders. subjects with history of acute or chronic infections, any other chronic diseases, under cortisol treatment and pregnant women.

RESULTS

The cancer research groups' characteristics are displayed in table 1 below and figure 1. There was highly significant difference ($p<0.001$) in mean concentration of cathepsin V and VEGF-A for patients with diabetes mellitus type 2 in comparison with apparently healthy control. There was a significant difference in the mean levels of Fasting Plasma Glucose (FPG) and HbA1c between the patient group and the control group ($p<0.001$). The mean BMI concentration for patients (29.41 ± 4.36) was higher than that of the control group (28.13 ± 4.66), but this difference was not statistically significant ($p=0.064$).

Tab. 1. Comparison of the parameters' mean values between the patients and the controls

Variables	Groups	Mean ± SD	p-value
Cathepsin-V ng/ml	Control	0.54 ± 0.19	<0.001
	patients	3.57 ± 0.92	
VEGF-A pg/ml	Control	172.02 ± 24.67	<0.001
	patients	754.83 ± 209.60	
Glucose	Control	101.71 ± 9.26	<0.001
mg/dl	patients	275.58 ± 75.19	
HbA1c%	Control	5.41 ± 0.51	<0.001
	patients	10.47 ± 2.00	
BMI kg/m²	control	28.13 ± 4.66	0.064
	patients	29.41 ± 4.36	

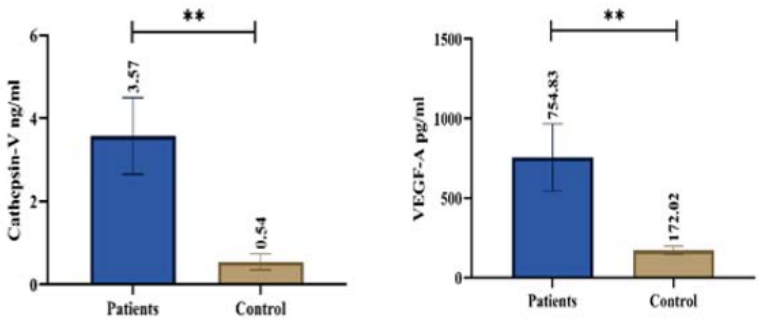

Fig. 1. Mean difference between type 2 Diabetic Mellitus (TDM2) and normal control according to cathepsin V and VEGF-A

Table 2 displays the correlation coefficients (r), sorted by their p values. In patients with T2DM, there was a significant positive correlation between cathepsin V and VEGF-A (r=0.23 and p=0.017). The serum levels of cathepsin V, VEGF-A, and the number of years that a patient had had type 2 diabetes were significantly correlated with one another (p<0.05); additionally, a significant positive correlation was seen between VEGF-A and the patient's fasting plasma glucose level (p=0.03) and no significant correlation with HbA1c and BMI. Non-significant correlation was seen between cathepsin V and glucose, HbA1c and BMI (Figure 2).

Tab. 2. Correlation coefficients between studied parameters and (Glucose, HbA1c, BMI, duration of disease) in diabetes mellitus patients

		VEGF-A	FPG	HbA1c	BMI	Duration of disease
Cathepsin- V	r	0.23	0.01	0.13	-0.04	0.24
ng/ml	p	0.017*	0.91	0.18	0.65	0.013*
VEGFA	r		0.21	0.12	0.06	0.19
pg/ml	p		0.03*	0.22	0.51	0.045*

*Correlation is significant at 0.05and VEGF-A

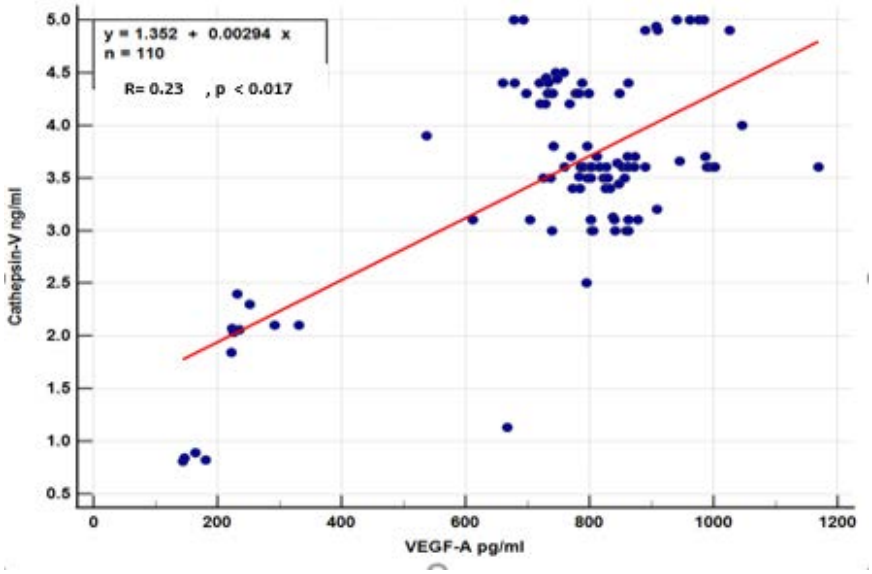

Fig. 2. Scatterplots with the correlation line added for Cathpsin-V and VEGF-A

ROC curve projections for cathepsin V was carried out to assess the area under curve values (AUC ± SE) for discriminating between patients and apparently healthy controls (n=180), AUC=0.997 ± 0.0017, (p<0.0001), projected high sensitivity=96.36% (cut-off cathepsin V value>0.975), projected high specificity=100.0%, and for VEGF-A, AUC=0.975 ± 0.0126, (p < 0.0001), projected high sensitivity=96.36% (cut-off VEGF-A value>215), projected high specificity=97.14 % (Figure 3).

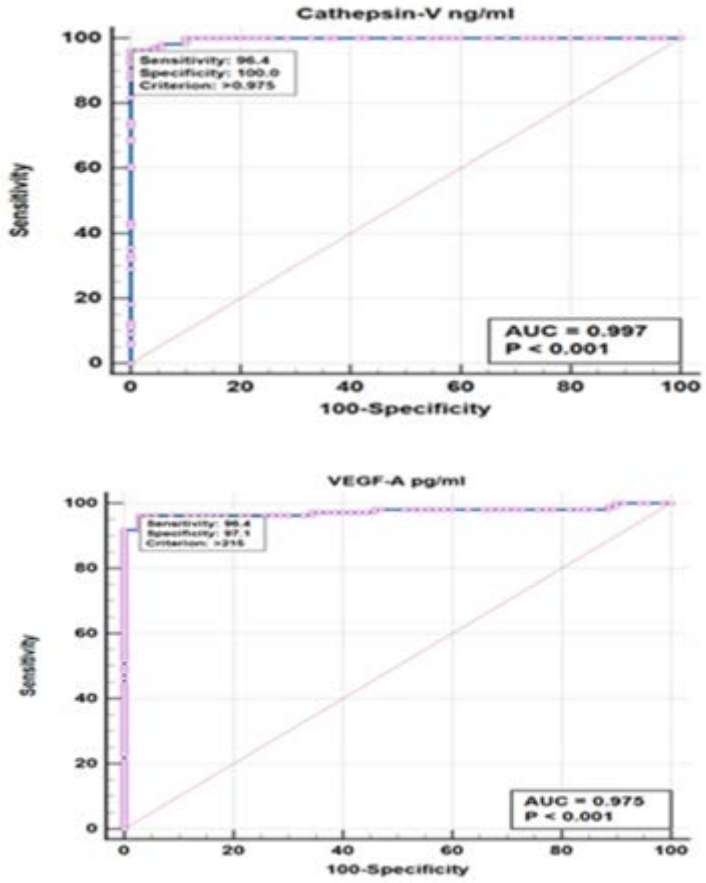

Fig. 3. ROC curve analysis for VEGF-A and Cathepsin-V as they classifying for distinction between patients and control subjects

DISCUSSIONS

The status of blood cancer and VEGF-A levels in type 2 diabetes mellitus patients' group and their relationship with plasma glucose concentration, HbA1c and BMI were evaluated in our investigation.

Cancer can cause serious damage to the human body, but only if acidosis is present and the body pH is below the normal threshold, or Inflammation and hypoxia occur in the human body. It is understandable why dysregulation of their functions is a defining feature of illnesses including cancer, sterility, bone resorption, and neurological disorders [18]. However, to our knowledge, there hasn't been any published research on cathepsin V(Cat) in diabetes mellitus., our study may be the first one which found significant difference in cathepsin V level in group of patients with T2DM comparison with control group.

Another research claimed that the CTSL2 gene in human cortical thymic epithelial cells produces the cysteine protease cathepsin V, which is linked to type 1 diabetes mellitus, autoimmunity, and antigen presentation [19-22]. Podocytes produce and release cathepsin C(CatC), and a hyperglycemic condition raises CatC amounts and activity, according to Polish research [23].

Under various pathological condition such as diabetic retinopathy, rheumatoid arthritis, coronary heart disease and Alzheimer's disease, VEGF-A stimulated VEGFR-2 leading to endothelial cell proliferation, migration and the formation of new blood vessels formation involved in angiogenesis [24].

The current investigation supported the findings of previous study showing type 2 diabetics without complications had VEGF measurements that were significantly greater than those of healthy individuals [25]. In this study of individuals with T2DM, there was a positive correlation between plasma VEGF levels and glycemic markers (FPG and HbA1c). According to our results, an increase in VEGF-A may result in an increase in cathepsin V. This suggests that cathepsin V levels rise as VEGF-A levels rise. The present findings may be parallel with other studies found no significant difference in BMI between patient without diabetic peripheral neuropathy and healthy control [26].

Because there is no connection between VEGF-A, cathepsin V and BMI, it is possible that BMI imbalances associated with diabetes do not affect how angiogenesis is regulated. The significant positive correlation between VEGF-A, cathepsin V, and disease duration as well as the positive correlation between VEGF-A and FBG support the notion that VEGF-A and cathepsin V may play a significant role in raising the risk of complications in people with type 2 diabetes mellitus.

Current research indicates that cathepsins have a crucial function as a regulator of angiogenesis. Therefore, targeting specific cathepsin isoforms is a new and promising approach for treating angiogenic complications may appear in type 2 diabetes patients [27].

Cancer has been shown to contribute to the onset and/or advancement of keratoconus, causing extensive degradation of the corneal matrix and resulting in significant corneal thinning and impairment. The stimulation of hydrogen peroxide generation in the cornea by increased cathepsin activity may also contribute to keratoconus by up-regulating oxidative stress [28]. These results imply that inhibiting cathepsin activity could be an effective way to stop the progression of corneal degeneration [27].

Using Area Under the Curve (AUC), the accuracy of cathepsin V and VEGF-A as biomarkers for angiogenesis was evaluated. The diagnostic value of serum VEGF-A and a new bimolecular marker

cathepsin V for the severity of angiogenesis in type 2 diabetes mellitus was assessed using ROC curve. ROC for blood cathepsin V and VEGF-A levels is significant for distinguishing between cases and controls and has high accuracy for differentiating between people with T2DM and controls. With over 96% projected sensitivity and 97% projected specificity at different cut off values, the AUC values for discriminating between the two research groups demonstrated that blood cathepsin V and VEGF-A levels are sensitive and reliable biomarkers of the severity of angiogenesis. The current study had some limitations. There was no ophthalmologist or neurologist available for the purpose of ascertaining the absence of complications of retinopathy and neuropathy when samples were taken from patients, but reliance was made on the medical history and the patient's question.

CONCLUSION

In conclusion, serum cancer and VEGF-A levels rise as the illness of T2DM advance. Cathepsin V and VEGF-A level in the blood can be used as possible serum risk biomarkers for angiogenesis. The lack of correlation between the studied parameters and BMI suggests that obesity, which is often associated with diabetes, may not be linked to the angiogenesis processes being investigated.

ETHICAL CLEARANCE

The study's goal and procedures were explained to each subject group individually.

They gave their approval in order to take part in the study. The study was consent by Research Committee of Deyaa Health Department-Training and Human Development Centre (no.30887).

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