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Serum angiostatin levels in diabetic patients with heart failure taking oral antidiabetic therapy or basal insulin and its clinical significance

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Abstract

Diabetes mellitus is a metabolic disorder with an increasing incidence all over the world leading to high sequelae and high mortality rates behind other microvascular and macrovascular complications. The deterioration in angiogenesis in particular is known to cause deterioration of vascular complications of diabetes. After the discovery of a natural angiogenesis inhibitor, angiostatin in the etiopathogenesis of retinopathy and nephropathy, which are frequent complications of diabetes, several successful clinical trials have been made. However, the most lethal complication of diabetes, diabetic heart failure, lacks any trial about effectiveness of angiostatin. In this study, the levels and the clinical significance of angiostatin were investigated in oral antidiabetic or Insulin treated diabetic patients with heart failure. The patient group consisted of 31 patients with a diagnosis of diabetes mellitus and heart failure and the control group included 30 patients with heart failure without diabetes mellitus. Serum levels of angiostatin were studied. A total of 61 subjects were enrolled in the study. The patient group consisted of 31 patients; between the age of 56-88 (73.06 ± 8.7) years, of which 16 (51.6%) were female and 15 (48.4%) were male. The control group included 30 patients; 15 (50%) women and 15 (50%) of were male, between the ages of 57 to 85 (74.23 ± 8.27). In the case group, the average angiostatin levels were 133.25 ± 78.46 and in the control group it was found to be 121.7 ± 71.81 . The average angiostatin levels were similar in diabetic and non-diabetic heart failure groups ($p=0.55$). The average serum angiostatin levels showed a significant negative correlation with the level of fasting blood glucose. In our study, diabetic patients with heart failure, when compared with non-diabetic patients with heart failure, showed no significant difference in the levels of angiostatin. Levels of angiostatin are not affected by the level of HbA1c. Fasting blood glucose level has a negative correlation with the level of angiostatin. In order to be used in determining the prognosis in diabetic patients with heart failure, further studies are needed on angiostatin levels.

Keywords: Diabetic heart failure, angiogenesis, angiostatin, HbA1c

Introduction

The majority of patients with diabetes mellitus (DM) develop various complications over time. These complications have significant effects on mortality and sequelae cessation [1]. 70-80% of deaths in DM patients are due to cardiovascular diseases, the mortality rate due to cardiovascular disease is 2-4 times higher in patients with DM compared to those without DM [2]. As a result of clinical studies, DM is the most common cause of cardiovascular disease. It may be accepted as equivalent to coronary artery disease (CAD) in patients with a genetic predisposition [3,4].

Diabetic heart failure is a consequence of macrovascular complications of DM. Also, disrupted angiogenesis formation plays a crucial role in the development of DM related complications

[4]. Studies have accelerated with the discovery of angiostatin, an inhibitor of angiogenesis. Angiostatin is a proteolytic part of plasminogen [5-7]. The anti-angiogenic effect of angiostatin is evident and acts by inhibiting proliferation, in particular, vascular endothelium, and triggering programmed cell death [8-10]. Successful clinical studies have been conducted on the effect of angiostatin in patients with diabetic retinopathy and nephropathy. However, no research has been done on the efficacy of angiostatin in diabetic heart failure, the most significant complication of DM [11].

This study aimed to determine the effects of the use of angiostatin blood level in diagnosis, treatment, and follow-up in patients with diabetic heart failure receiving oral antidiabetic or basal insulin therapy.

Material and Methods

We included the study heart failure patients with or without DM applied to the GATA School of medicine outpatient settings or clinics between May 2012 and March 2013. We enrolled a total

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of 61 heart failure patients meet inclusion criteria Thirty-one of them have DM, and 30 of them were not. We recorded age, gender, height, body weight measurements, echocardiography reports performed last year, and results of hemogram, routine biochemistry, sedimentation rate, C-reactive protein, pro-BNP, and receiving treatments to the follow-up cards. We determined angiotensin levels from the serums of by using commercially available ELISA kits and using the Synergy HT plate reader (Bio-Tek Instruments Inc, Winooski, VT, USA) according to the manufacturer's procedure (Eastbiofarm, Hangzhou, China, catalog no. CKE90461).

The local ethics committee approved the study, and we obtained informed consent from all attendants.

Statistical Analysis

Descriptive statistics are shown as number (%) for discrete data and as mean $\pm$ standard deviation for continuous data. In intergroup comparisons, if the assumptions are met in paired comparisons where the difference is investigated, "t test for independent samples" is used. The nonparametric version of the Mann Whitney U Test was used in cases where the number of measurements in the comparison groups was low or the assumptions were not met. One Way Anova was required when the number of groups compared was more than 2, but the non-parametric Kruskal Wallis Test was preferred because the assumptions of this test were not met. Pearson Product-Moment Correlation Coefficient was used to investigate the relationship between two variables. The significance of the relationships between categorical variables was investigated by Chi-Square Test. The values of the study data which were $p < 0.05$ were considered statistically significant.

Results

The demographic characteristics of the case and control groups are shown in Table-1 and the distribution by gender is shown in Table-2. A total of 31 patients (16 women (51.6%) and 15 women (48.4%) between the ages of 56-88 (73.06 ± 8.7) were included in the study. A total of 30 patients, 15 (50%) female and 15 (50%) male, aged 57-85 (74.23 ± 8.27) years were included in the control group. The mean length of the patient group was 163.06 ± 7.16 cm, body weight was 75.11 ± 13.41 kg and body mass index was 28.24 ± 4.41 kg / m². In the control group, mean length was 163.57 ± 7.28 cm, body weight was 72.23 ± 11.09 kg, and body mass index was 26.78 ± 3.58 kg / m².

Table 1. Demographic characteristics of case and control groups

Variable	Group	Median	Standard Deviation	p
Age	Patient	73.06	8.7	0.59
	Control	74.23	8.27	
Height	Patient	163.06	7.16	0.79
	Control	163.57	7.28	
Weight	Patient	75.11	13.41	0.36
	Control	72.23	11.09	
Body Mass Index	Patient	28.24	4.41	0.16
	Control	26.78	3.58	

Table 2. Distribution of case and control groups by gender

Gender	Patient	Control
Women	Patient	15(50%)
Men	Control	15(50%)
p=0.9		

Diabetic retinopathy in 16 patients (51.6%), 38 diabetic nephropathy in 15 patients (48.4%), history of coronary artery disease in 12 patients (38.7%), retinopathy in 16 patients (53.3%) and retinopathy in 16 patients (53.3%). (53.3) had nephropathy and 11 (36.7%) had coronary artery disease.

According to the NYHA (New York Heart Association) staging of the case and control groups, the stages are given in Table-3. There were no patients with stage-1 heart failure, 7 (22.6%) had stage-2 heart failure, 12 (38.7%) had stage-3 heart failure and 12 (38.7%) had stage-4 heart failure. In the control group, 1 (3.3%) stage 1 heart failure, 9 (30%) stage 2 heart failure, 11 (36.7%) stage 3 heart failure and 12 (38.7%) stage -4 had heart failure clinic.

Table 3. Distribution of case and control groups according to heart failure stages

Heart Failure Stage	Group	Number	Ratio (%)
Stage-1	Patient	0	0
	Control	1	3.3
Stage-2	Patient	7	22.6
	Control	9	30
Stage-3	Patient	12	38.7
	Control	11	36.7
Stage-4	Patient	12	38.7
	Control	9	30

The results of the angiotensin levels of the participants are shown in Table-4. The mean angiotensin level of the patient group was 133.25 ± 78.46 , while the mean angiotensin level of the control group was 121.7 ± 71.81 . The difference was not statistically significant ($p=0.55$).

Table 4. Angiotensin levels of case and control groups

Variable	Group	Median	Standard Deviation	p
Angiotensin	Patient	133.25	78.46	0.55
	Control	121.7	71.81	

Angiotensin levels were correlated with white blood cell, hemoglobin, hematocrit, platelet, urea, creatine, high density lipoprotein, low density lipoprotein, sedimentation, crp, pro-BNP. Since the case group consisted of DM patients and there was no diagnosis of DM in the control group, the correlation analysis between fasting blood sugar and HbA1c levels and angiotensin was evaluated separately in the case and control groups. There was a negative correlation between mean angiotensin level and fasting blood glucose level in laboratory data ($p=0.01$), but not with other laboratory data.

Retinopathy was present in 16 (51.6%) of the patient group and 16 (53.3%) of the control group. The mean angiostatin level was 127.74 ± 84.83 in patients with retinopathy and the mean angiostatin level in patients without retinopathy was 139.13 ± 73.56 . In the control group, the mean angiostatin level was 132.61 ± 72.23 in patients with retinopathy and the mean angiostatin level in patients without retinopathy was 109.23 ± 71.90 . The difference between retinopathy complication and angiostatin level in patient and control groups was not statistically significant ($p = 0.57$).

Nephropathy was present in 15 (48.4%) of the patient group and 16 (53.3%) of the control group. The mean angiostatin level was 131.44 ± 86.46 in patients with nephropathy and the mean angiostatin level in patients without nephropathy was 134.95 ± 72.99 . In the control group, the mean angiostatin level was 132.61 ± 72.23 in patients with nephropathy and the mean angiostatin level in patients without nephropathy was 109.23 ± 71.90 . There was a significant difference in the correlation between angiostatin levels and nephropathy complications in the patient and control groups, but this difference was not statistically significant ($p = 0.62$).

Coronary artery disease was present in 12 (38.7%) of the patient group and 11 (36.7%) of the control group. The mean angiostatin level was 138.07 ± 88.50 in patients with coronary artery disease and 130.21 ± 73.81 in patients without coronary artery disease. In the control group, the mean angiostatin level was 144.34 ± 84.12 in patients with coronary artery disease and the mean angiostatin level in patients without coronary artery disease was 108.59 ± 62.31 . Correlation between coronary artery disease and angiostatin levels was found to be different in the patient and control groups, but this difference was not statistically significant ($p = 0.64$).

17 patients (54.8%) were using insulin in the patient group. The mean angiostatin level was 134.65 ± 72.09 in the insulin group, 131.76 ± 87.29 in the non-insulin group and 121.70 ± 71.81 in the control group. Angiostatin level was found to be different between the insulin users and non-users, but this difference was not statistically significant ($p = 0.78$).

In the patient group, 3 (9.7%) people were using meglitinide. The mean angiostatin level was 116.73 ± 50.44 in the patients receiving meglitinide, 135.02 ± 81.35 in the patients not using meglitinide, and 121.70 ± 71.81 in the control group. There was a significant difference in the correlation between angiostatin levels between participants receiving meglitinide and those who did not, but this difference was not statistically significant ($p = 0.87$).

Nine patients (29%) were using sulfonylurea DM. The mean angiostatin level was 150.3 ± 31.8 in the sulfonylurea group DM, 135.2 ± 33.06 in the sulfonylurea group and 121.70 ± 71.81 in the control group. There was a significant difference in the correlation between the levels of angiostatin between participants using glylazide and those who did not, but this difference was not statistically significant ($p = 0.19$).

Seven patients (22.6%) were taking metformin in the patient group. The mean angiostatin level was 110.60 ± 66.03 in the patients receiving metformin, 139.86 ± 81.79 in the patients not using metformin and 121.70 ± 71.81 in the control group. There was a significant difference in the correlation between the levels of angiostatin between the participants using metformin and those

who did not, but this difference was not statistically significant ($p = 0.71$).

Discussion

In our study, we tried to show angiostatin level and clinical significance in patients with diabetic heart failure receiving oral antidiabetic or insulin therapy. In our study, age, BMI, gender, HbA1c level, Pro-BNP level, inflammation marker sedimentation and angiostatin levels were not found to be correlated with the average. In addition, no significant difference was found between angiostatin levels of diabetic retinopathy, nephropathy, and CAD.

Xi et al, 311 patients underwent coronary angiography in their study pro-BNP was observed to stimulate angiogenesis [11]. They observed that increased levels of proBNP increased collateral formation and more regular collateral formation in patients with high pro-BNP levels. Depending on the study, angiostatin with anti-angiogenic effect was expected to have a negative relationship between pro-BNP, but no significant correlation was found between angiostatin and Pro-BNP. Pro-BNP is significantly elevated in patients with heart failure. In our study, it was evaluated that no significant relationship was observed due to the diagnosis of heart failure in all case and control groups and due to high levels of pro-BNP due to heart failure.

Patel et al., 102 heart failure and 110 healthy participants evaluated the level of angiogenesis stimulating angiogenesis in the study, angiogenin level of heart failure (according to the NYHC class) have determined that the level of angiogenin increases [12]. In our study, no significant relationship was found between EF and mean angiostatin level. This situation was attributed to the lack of a healthy control group in our study.

Papathanassiou and colleagues in their study of 28 patients with DM, sulfonylureas have been found to have no effect on the endothelium [12]. In our study, we found that the average of angiostatin level in the sulfonylurea group was higher than the other treatment group. This was not clinically significant. These results are consistent with the literature and can be considered to be effective on endothelial function in DM patients receiving treatment other than sulfonylurea.

Studies have shown that metformin has an antiangiogenic effect by suppressing VEGF and proangiogenic factors in women with polycystic ovary syndrome. In the study of Soraya et al., Metformin has been shown to have antiangiogenic effect by reducing VEGF-A mRNA secretion [13]. Similar results were obtained in our study. Since the high level of angiostatin causes damage to coronary collateral vasculature, the lower mean level of angiostatin levels of 7 participants receiving metformin may be due to the protective effect of impaired angiogenesis. The difference was not statistically significant, which may be related to insufficient number of patients.

In the ACCORD and VA-DT studies, which were published in 2007 and 2008, it was shown that strict metabolic control increases the risk of cardiovascular disease in elderly and DM groups with a duration of more than 10 years and the increase in risk is associated with a decrease in blood glucose levels [14]. The negative correlation between glucose and angiostatin, which is one

of the results of our study, was evaluated in accordance with this literature.

Limitations of our study; compared to other studies in the literature, the number of participants is less, the lack of a healthy control group, and age, inflammation and renal failure, such as conditions that may affect angiogenesis status was not excluded during the choice of case and control groups.

Conclusion

In conclusion, angiostatin level was not different when diabetic heart failure patients were compared with non-diabetic heart failure patients. In addition, angiostatin level was not affected by HbA1c level. There is a negative relationship between fasting blood glucose level and angiostatin level. We think that angiostatin blood level is not effective in the diagnosis, treatment and clinical follow-up of patients with diabetic heart failure. However, more comprehensive studies are needed in patients with diabetic heart failure to better address this issue.

Competing interests

The authors declare that they have no competing interest.

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Ethical approval

Ethical approval was obtained from GATA Ethics Committee

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