Gremlin-1 and Wagner Classification: Potential Biomarker for Amputation in Diabetic Foot Patients

STUDY PROTOCOL

This study is a cross-sectional, single-center study conducted between 20xx and 20xx. A total of 85 patients aged 18 years and older, diagnosed with DM, and who either had diabetic foot ulcers or were at high risk for DF, were recruited from the Endocrinology outpatient clinic or assessed by the diabetic foot council of XXX University Faculty of Medicine Hospital. All patients agreed to participate in the study and to share their data in accordance with the principles of the Helsinki Declaration (2001). Approval from the XXX University Clinical Research Ethics Committee was obtained on December XX, XXXX (approval number XXX).

Patients with autoimmune diseases, malignant conditions, a history of prior amputation, those on hemodialysis, and pregnant women were excluded from the study.

Data were collected on patient age, smoking status, type of diabetes, duration of diabetes, treatments received, and diabetes-related complications.

Hematocrit, C-reactive protein (CRP), white blood cell (WBC) count, platelets, HbA1c, triglycerides, cholesterol levels, glomerular filtration rate (GFR), and albuminuria data were extracted using electronic medical records.

Diabetic peripheral neuropathy was evaluated based on the patient's medical history, clinical assessment, and diagnostic tests. The Semmes-Weinstein monofilament test was used to assess diabetic peripheral neuropathy.

Retinopathy was defined based on ophthalmological evaluations as the presence of non-proliferative or proliferative diabetic retinopathy.

Cardiovascular disease (CVD) was considered positive if the patient had a history of bypass surgery, stent placement, positive angiography, or myocardial infarction. Patients with normal angiography and exercise test results or without a history of coronary artery disease were classified as CVD negative.

Albuminuria was defined as an albumin-to-creatinine ratio >30 mg/g during a spot urine test.

DF severity was classified using the Wagner diabetic foot classification system, based on clinical and imaging assessments after identifying the ulcer site and presence of infection. Patients with DF ulcers in stages 1 and 2 (limited to tissue) were evaluated together with patients in stages 4 and 5 (with necrosis).

The Ankle Brachial Index (ABI) of the patients was measured using both the ankle and both arms after a 30-minute rest period.

Symptomatic peripheral artery disease (PAD) was considered present if the ABI was <0.9, if imaging was consistent with PAD, or if the patient had a history of stent placement or revascularization. Contrast-enhanced extremity imaging techniques included computed tomography angiography, magnetic resonance angiography, or Doppler ultrasonography. ABI values between 0.9-1.3 were considered normal, ABI <0.9 was classified as PAD, and values >1.3 (calcified) were excluded.

In addition to routine tests, a 5 mL blood sample was drawn from the antecubital veins of all patients for the measurement of serum Gremlin-1 levels. The blood samples were sent to the Department of Medical Biochemistry at XXX University Faculty of Medicine. Blood collected in serum tubes was centrifuged at 4000 rpm for 5 minutes, and the serum was separated. The serum samples were stored at -80°C in 1.5 mL Eppendorf tubes until the day of analysis. On the day of analysis, the serum samples were brought to room temperature, and Gremlin-1 protein levels were analyzed using the ELISA method (Novus) following the procedure. The Gremlin-1 protein levels were evaluated in ng/mL.

20.12.2019

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STATISTICAL ANALYSIS PLAN

In the statistical analysis of the data, categorical variables were examined using frequency tables, and descriptive statistics for continuous variables were calculated. Pearson's chi-square test was used for categorical variables, and the levels of the variables were analyzed using cross-tabulations. The Shapiro-Wilk normality test was used to determine whether continuous variables were normally distributed. Since the assumption of normal distribution was not met, the Mann-Whitney U test was used to compare two independent groups. For the comparison of non-normally distributed variables among more than two groups, the Kruskal-Wallis test was used. Correlations between continuous variables were examined using the Spearman correlation coefficient. A significance level of 0.05 was applied to all group comparisons. IBM SPSS Version 25.0 statistical software package was used for the statistical analysis.

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INFORMED CONSENT FORM
PLEASE READ CAREFULLY!!! You have been invited to participate in this study. Before accepting to take part in this study, you must understand the purpose of the study and make your decision freely after this information. Please read this information prepared specifically for you carefully and ask for clear answers to your questions.
What is the name of this study? Investigation of the Relationship between Gremlin-1 Levels and Wagner Classification in Diabetic Foot Patients and Testing Its Usability as a Biomarker for Amputation
What is the purpose of this study? In patients with diabetic foot wounds, gremlin-1 levels will be studied in the blood to predict the degree of the wound, to determine the course of amputation (the surgical removal of an organ that is deemed impossible to heal), and to determine the treatment method for this. If it is significant, it will be in the interest of patients to determine the severity of the disease with a blood test and to apply early treatment methods.
How will you be treated? Your routine examinations and tests will not be affected. For this study, we will only need to take a 5 ml blood sample from the vein in your arm for testing. The blood collection procedure will be performed by a doctor or healthcare personnel in accordance with the blood collection procedure.
What is the probability of being randomly assigned to different treatment groups?

Our study is not testing any treatment method. There will be no changes to your current treatment.

How much time will it take?

Aside from your routine examination, 5-10 minutes will be enough time for us to take a blood sample.

What is the estimated number of volunteers expected to participate in the study?

90

What will happen to the biological materials that will be taken from you, and where will the analyses be done? (explanation of where the biological materials will be sent if the analyses are done abroad),

5 ml blood samples taken from the participants will be examined in the Biochemistry Laboratory of the Ege University Faculty of Medicine and then destroyed.

What is expected of you? What are your responsibilities?

It will be sufficient to provide a 5 ml blood sample.

What will you benefit from participating in the study?

A method that is likely to be a guide in the follow-up and treatment of your disease will be tested. The results are expected to provide innovation/contribution to the future follow-up and treatment of you and other patients.

What are the situations that would require termination of participation in the study?

You may withdraw from the study at any time if you withdraw your written consent.

Could participating in the study cause you any harm?

No

What happens if you do not want to participate?

There will be no change for you. Your follow-up and treatment will continue as is.

What alternative methods can be applied to you?

There is no alternative method for the study.

Will I be paid anything for participating in this study?

No

Will I pay any fees for participating in this study?

All kinds of tests, physical examinations, and other research expenses will not be paid by you or any official or private institution or organization that you are under the guarantee of.

Confidentiality of information: All your personal and medical information will remain confidential and will only be used for scientific purposes. Even if the research results are published, your identity will remain confidential.

Contact information of the person responsible for this study

1- **Name, surname: Prof. Dr. xxxxxxx**
2- **Reachable phone number: xxxxxxX**
3- **Position: XXX University Department of Internal Medicine, Department of Endocrinology**

Consent to Participate in the Study

I have read and listened to the information provided above, which indicates that the subject should be given before the study begins. I have asked the researcher all questions that come to mind and have fully understood all the explanations given to me in writing and verbally. I have been given sufficient

time to decide whether I wish to participate in the study. Under these conditions, I authorize the researcher to review, transfer and process my medical information and I accept the invitation to participate in this study with great willingness, without any coercion or pressure. I understand that my participation in the study is voluntary and that I may withdraw from the study with or without giving any reason. I understand that by signing this form I will not lose any rights granted to me by local law.

I understand that I will be given a signed and dated copy of the informed consent.

Please select one of the following options:

Biological samples taken within the scope of the research titled 'Investigation of the Relationship Between Gremlin-1 Levels and Wagner Classification in Diabetic Foot Patients and Testing Its Usability as a Biomarker for Amputation'

-I only allow it to be used in the above-mentioned research.

-I allow it to be used in future research limited to the relevant disease group.

-I do not allow it to be used under any circumstances.

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VOLUNTEER'S		SIGN
NAME-SURNAME		
ADRESS		
TELEPHONE		
DATE		

For those under guardianship or trusteeship, the parent or guardian		SIGN
NAME-SURNAME		
ADRESS		
TELEPHONE		
DATE		

A competent researcher who is a part of the research team and provides information about the research.		SIGN
NAME-SURNAME		
ADRESS		
TELEPHONE		
DATE		