Is Ischemia-Modified Albumin a Biomarker in Wagner Classification in Diabetic Foot Ulcers?

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Abstract

Background: We aimed to determine the relationship of ischemia-modified albumin (IMA) with diabetic foot ulcers and its predictive value in the Wagner classification. Methods: Our cross-sectional study was conducted in 120 diabetic foot patients and 60 healthy individuals with similar body mass index and age. Patients with a diabetic foot were classified according to the Wagner classification. Biochemical parameters, C-reactive protein (CRP) and IMA levels were measured in all patients and healthy volunteers. Screening performance characteristics of CRP and IMA were calculated according to Wagner classes and the presence of osteomyelitis. Results: CRP and IMA levels in the patient group were significantly higher than the control group. The highest IMA levels were detected in Wagner grade 5. CRP had higher sensitivity and specificity than IMA in the discrimination of other grades, except for grade 4-5 separation. For Wagner grade 4-5 distinction, IMA had 84.6% sensitivity and 94.7% specificity. Conclusion: IMA may play a role in the pathogenesis of diabetic foot ulcers and had a higher predictive value in discrimination of the Wagner grade 4 and 5. In the management of diabetic foot patients, it may be recommended that IMA is evaluated by clinicians.

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Conclusion: IMA may play a role in pathogenesis diabetic foot ulcers and had a higher predictive value in discrimination of the Wagner grade 4 and 5. In the management of diabetic foot patients, it may be recommended that IMA is evaluated by clinicians.

Keywords: Diabetic foot, Ischemia modified albumin, Wagner classification, CRP

What is already known about this topic?

- Radiological findings and infection markers such as C-reactive protein are used in the diagnosis of diabetic foot, which is one of the most important complications of diabetes. - As a result of diabetic foot ulcers, amputation of the lower extremity is performed at various levels. - Vascular occlusion and ischemic necrosis are important criteria in determining the amputation level. - Wagner Classification is used in the classification of diabetic foot ulcers and amputation is frequently performed in the advanced stages of this classification. - One of the important markers in determining ischemic damage is IMA. - There are increased levels of IMA in diabetic foot patients.**What does this article add?**- IMA is increased in diabetic foot patients, so it may play a role in your diabetic foot pathogenesis. - IMA has high sensitivity and specificity in the distinction between phases in Wagner classification. - Determination of IMA levels together with radiological findings will provide information about the wounds in the decision of diabetic foot amputations.

Introduction

Diabetes mellitus, one of the most common chronic metabolic diseases worldwide, is characterized by high blood sugar and leads to many serious complications.[1] The complications caused by long-term high blood glucose include nephropathy, retinopathy and neurovascular complications.[2] One of the most serious conditions of diabetes-related neurovascular complications is foot ulcers. It was estimated that approximately 15% of people with type 2 diabetes would be affected by foot ulcers.[3] However, in 2015, the International Diabetes Federation reported that this rate was approximately 34%.[4] Diabetic foot ulcers lead to amputation at various levels, depending on severity, in approximately 20% of patients. Furthermore, diabetic foot ulcers increase the risk of death. Patients with diabetic foot ulcers have a 2.5-fold higher risk of death at 5 years than those without diabetic foot ulcers.[5] Unfortunately, despite significant improvements in diabetic foot care and treatment, the rate of amputation due to diabetic foot ulcers is increasing.

One of the classifications of diabetic foot ulcers was developed by Wagner in the 1970s, in which ulcers were graded between 0-5. In this classification, only the depth of the ulcer, osteomyelitis and gangrene are evaluated, but the presence of ischemia is not considered.[6, 7], New classification systems have been developed after this classification; however, the Wagner classification is simple and very effective in predicting low extremity amputation.[8, 9] Ischemia in tissues due to the decreased blood flow in diabetic foot ulcers is not considered in the Wagner classification, making it difficult to determine the level of amputation. Therefore, a biomarker to help determine the level of ischemia in these patients may be more effective in determining the degree of amputation. Classical blood parameters such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), procalcitonin and white blood cells (WBC) are used to determine the severity of infection in diabetic foot ulcers. However, there is no blood biomarker to determine the level of ischemia in diabetic foot ulcer.[10]

Ischemia-modified albumin (IMA) is a structure formed by the change of the N-terminal end as a result of ischemic damage of circulating albumin; due to this modification, the transport of elements such as cobalt and nickel is impaired.[11] In recent years, many studies have been reported on whether IMA may be a novel predictor in ischemic injury-related diseases such as stroke, acute mesenteric ischemia, acute pulmonary embolism and coronary syndrome.[12] In previous studies, IMA levels were examined for diabetes and diabetes-related complications such as diabetic nephropathy and diabetic ketoacidosis.[13] It has also been reported that IMA may be an indicator of diabetes-related complications.[14] In the literature review we conducted, we found only one study that examined IMA levels in diabetic foot patients, but there is no study examining the relationship of IMA levels with the Wagner classification in these patients.[15]

In this study, we aimed to compare IMA levels in diabetic foot patients and a healthy control group. In addition, we aimed to determine the relationship of IMA levels with diabetic foot severity by grouping diabetic foot patients according to the Wagner classification.

Materials and Methods

Study Design and Population

This study was designed as a cross-sectional study. Ethical approval was obtained from the local ethical

committee of Van Yuzuncu Yil University and was conducted in accordance with the Declaration of Helsinki. Our study was conducted with patients who applied to the Orthopaedics and Traumatology outpatient clinic of the Research Hospital of Van Yuzuncu Yıl University Medicine Faculty. Informed consent was obtained from all patients who participated in the study. The patient group consisted of adult (age >18 years) diabetic patients diagnosed with a diabetic foot. Patients who had received antimicrobial treatment in the last six months, had renal failure, cardiovascular diseases, neuropathy, retinopathy, septic shock, unstable hemodynamic, malignancy, musculoskeletal injury, a history of thyroid or liver disease and those who had suffered a stroke were excluded from the study. In addition, patients with a history of amputation or comorbid diseases were also excluded from the study.

The patients were evaluated according to the Wagner classification.[16, 17] The diabetic foot ulcers in patients were graded from 1 to 5, according to the presence of infection and/or gangrene using the following criteria:

Grade 1: The presence of superficial ulcers limited to the epidermis**Grade 2:** Infection extending to the dermis, muscle and tendons but without evidence of osteomyelitis**Grade 3:** Presence of deep soft tissue infection and osteomyelitis**Grade 4:** Gangrene localized to the distal foot and osteomyelitis**Grade 5:** Extensive gangrene and osteomyelitis

All diabetic foot patients were evaluated in terms of osteomyelitis. To evaluate osteomyelitis, we used the probe-to-bone test and, if necessary, magnetic resonance imaging (MRI).[18] In our study, the patient group was divided into subgroups according to the Wagner classification and evaluated using all the parameters we detected. We also classified our patient group according to the presence of osteomyelitis. The "osteomyelitis absent" group consisted of grade 1 and 2 ulcers, and the "osteomyelitis present" group consisted of ulcers of grade 3, 4 and 5.

The healthy control group was composed of healthy volunteers who were in the gender and age range similar to the patient group, had no chronic disease, and had not applied to the hospital for any reason in the past 6 months. Consequently, our study was conducted with 120 diabetic foot patients and 60 healthy volunteers.

Sample Collection and Measurements

Blood samples were obtained from patients with diabetic foot ulcers before treatment was initiated. A total of 5 mL blood samples were taken from the volunteers: 3 mL blood samples were placed in tubes containing anticoagulants to obtain whole blood, and 2 mL blood samples were placed into dry tubes. Serum samples were obtained by centrifuging the dry tube blood samples at 3500 g for 10 minutes. Whole blood and serum samples were kept at -80°C until the study day. The body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the body height in meters (kg/m^2).

In the whole blood samples, the amount of glycated haemoglobin (HbA1c) was determined on an analyzer (Arkray Adams HA-8160 from Japan) working with a high pressure liquid chromatography method. The serum levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, total cholesterol and glucose were measured by autoanalysis using photometric methods (Architect C 16200 Abbott Laboratories Inc., Abbott Park, IL, USA). The levels of CRP were detected by a BNR II SYSTEM SN 442176 instrument.

The serum IMA levels in both groups were measured according to the method described by Bar-Or et al.[19] A 200 µL serum sample was placed in a glass tube, 50 µL cobalt chloride added and the mixture then incubated for 10 min at 24°C before 50 µL dithiothreitol was added to the mixture. After 2 minutes, 1 mL of sodium chloride solution was added. The IMA concentrations were calculated by measuring the absorbance of the coloured complex at 470 nm wavelength. The serum IMA levels were expressed as absorubans (ABSU).

Statistical Analysis

Statistical analyses were performed using Microsoft Excel (Microsoft, Redmond, WA, US), Analyse-it (Analyse-it Software Ltd., Leeds, UK) and MedCalc version 12.7.2.0 (MedCalc Software, Ostend, Belgium). The normal distribution of the data was determined by the Shapiro-Wilks test. An independent sample t

-test was used to compare two independent groups, and a one-way ANOVA test was used for comparison of more than two groups. G-power 3.1 software was used to determine the sample size. We assigned the effect size as 0.8, alpha error as 5%, and power as 95%. Since we divided our patient group into subgroups, the allocation ratio was determined as 6.[20] The required sample size was estimated to be 140 in total (20 for group 1 and 120 for group 2).

Results

First, we divided the study group into the diabetic foot group and the healthy control group and examined the groups in terms of age, gender and BMI values. The age of the individuals ranged between 20-65 in the patient group and between 20-66 in the healthy control group, and there was no statistically significant difference between the two groups. The BMI levels in the patient group were significantly higher, statistically, than those of the healthy control group. The level of HbA1c, glucose and CRP in the patient group was also significantly higher than those of the healthy control group. The levels of LDL, triglyceride and total cholesterol were higher in the patient group than the healthy control group, but were not statistically significant, while HDL levels were statistically, significantly lower. When we investigated the IMA levels, we found significantly higher IMA levels in the patient group than in the healthy control group (Table 1).

Table 1: Demographic, clinical and laboratory data of patients in the diabetic foot and healthy control groups.

		Patients with Diabetic Foot (N=120)	
Gender	**Gender**		
Female count (%)	Female count (%)	45 (37.5)	3
Male count (%)	Male count (%)	75 (62.5)	3
Duration of dibates (years)	**Duration of dibates (years)**	14.5±8.12	
Age (years)	**Age (years)**	43.1±11.7	4
BMI (kg/m^2)	**BMI (kg/m^2)**	32.1±3.82	2
HbA1c(%)	**HbA1c(%)**	7.25±2.31	4
LDL (mg/dL)	**LDL (mg/dL)**	105.8±40.8	9
HDL (mg/mL)	**HDL (mg/mL)**	35.7±13.7	6
Colesterol (mg/dL)	**Colesterol (mg/dL)**	178.4±57.2	1
Triglyceride (mg/dL)	**Triglyceride (mg/dL)**	165.5±89.5	1
Glucose (mg/dL)	**Glucose (mg/dL)**	142.4±52.7	8
CRP (mg/L)	**CRP (mg/L)**	28.7±17.3	3
IMA (ABSU)	**IMA (ABSU)**	2,61±0,26	1
Wagner Clasification count (%)	**Wagner Clasification count (%)**		
Grade 1	Grade 1	28 (23.3)	
Grade 2	Grade 2	37 (31.7)	
Grade 3	Grade 3	22 (18.3)	
Grade 4	Grade 4	19 (15.8)	
Grade 5	Grade 5	13 (10.9)	
Osteomyelitis Count (%)			
Absent	65 (54.2)		
Present	55 (45.8)		

Values are given as mean (range) or n (%

We summarized demographic, clinical and laboratory data according to the Wagner classidication in Table 2. The age levels of the grade 3 and 4 groups were significantly higher than the other groups (p=0.006). There was no significant difference between sub-groups in terms of BMI (p=0.665) and duration of diabtetes (p=0.521). When we investigated the lipid profile of patients, the level of LDL in grade 5 patients was

significantly higher than those of the grade 1 and 2 patients (p=0.001 and 0.003, respectively). The level of LDL in grade 5 patients was higher than those of the grade 3 and 4 patients, but not statistically significant (p=0.386 and 0.072, respectively). There was no significant diffrence between sub-groups in terms of HDL, triglyceride and total cholesterol levels. In addition, there was no significant difference between sub-groups in terms of HbA1c and glucose levels. When we investigated the CRP and IMA levels, we found the highest CRP and IMA levels in grade 5 patients, and these differences were statistically significant. The lowest CRP and IMA levels were found in grade 1 patients. There was no significant difference between grade 1 and 2 patients in terms of CRP and IMA levels. In addition, the level of IMA in grade 2 patients was significantly lower than those of the grade 4 and 5 patients, and higher than those of the grade 1 and 2 patients, but not statistically significant.

Table 2: Demographic, clinical and laboratory data according to the Wagner classification in the patient group.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Gender					
Female (%)	9 (32.1)	9 (24.3)	10 (47.6)	8 (42.1)	7 (53.8)
Male (%)	19 (67.9)	28 (75.7)	11 (52.4)	11 (57.9)	6 (46.2)
Duration of dibates (years)	10.7±5.74^{a}	11.8±6.75^{a}	11.5±5.68^{a}	12.9±7.11^{a}	13.1±7.22^{a}
Age (years)	47.2±14.1^{b}	49.5±13.4^{b}	59.3±13.6^{a}	60.1±10.9^{a}	53.1±13.7^{b}
BMI (kg/m^2)	32.7±2.22^{a}	32.1±3.46^{a}	32.7±5.72^{a}	31.8±3.91^{a}	32.1±3.82^{a}
HbA1c (%)	6.25±1.75^{a}	7.68±2.61^{a}	7.40±2.52^{a}	7.41±2.19^{a}	7.25±2.31^{a}
LDL (mg/dL)	76.1±52.3^{b}	94.4±28.5^{b}	119.1±38.5^{ab}	105.4±14.4^{ab}	147.6±60.6^{a}
HDL (mg/mL)	35.7±10.3^{a}	38.1±13.6^{a}	34.2±10.5^{a}	36.2±10.2^{a}	29.5±22.9^{a}
Cholesterol (mg/dL)	167.1±73.3^{a}	161.9±53.4^{a}	180.9±65.2^{a}	191.5±33.4^{a}	253.1±32.9^{a}
Triglyceride (mg/dL)	171.1±53.1^{a}	166.3±45.1^{a}	151.5±42.3^{a}	170.7±88.4^{a}	171.4±52.7^{a}
Glucose (mg/dL)	130.5±45.1^{a}	148.4±62.1^{a}	118.9±31.1^{a}	160.9±65.2^{a}	157.9±32.4^{a}
CRP (mg/L)	16.7±8.47^{d}	17.9±5.98^{d}	26.11±8.89^{c}	50.9±8.75^{a}	56.7±8.81^{a}
IMA (ABSU)	2.51±0.12^{c}	2.48±0.13^{c}	2.51±0.14^{c}	2.74±0.15^{b}	3.12±0.19^{a}

Different letters in the same row indicate significant difference

When we investigated demographic and clinical finding in the osteomyelitis groups, there was no significant difference between the osteomyelitis absent and osteomyelitis present groups in terms of gender, age, BMI, HbA1c, HDL, cholesterol, triglyceride and glucose levels. The levels of LDL, CRP and IMA in the osteomyelitis present group were significantly higher than those of the osteomyelitis absent group (p<0.001) (Table 3)

Table 3: Demographic, clinical and laboratory data according to the absence or presence of osteomylitis in patient groups.

	Osteomyelitis absent (n=65)	**Osteomyelitis present (n=55)**	**P value**
Gender			
Female	19 (29.2%)	24 (45.3%)	0.085
Male	46 (70.8%)	29 (54.7%)	
Age (years)	48.6±13.5	59.2±11.6	
BMI (kg/m^2)	32.4±2.99	31.4±4.71	0.168
HbA1c (%)	7.15±2.41	7.41±2.18	0.61
LDL (mg/dL)	91.5±33.9	121.5±42.3	0.001
HDL (mg/mL)	38.2±13.1	33.1±14.1	0.112

	Osteomyelitis absent (n=65)	Osteomyelitis present (n=55)	P value
Cholesterol (mg/dL)	164.1±55.4	193.4±56.1	0.066
Triglyceride (mg/dL)	165.7±73.5	165.2±64.2	0.914
Glucose (mg/dL)	139.9±55.9	145.5±48.6	0.571
CRP (mg/L)	17.5±7.16	42.3±10.1	<0.001
IMA (ABSU)	2.49±0.12	2.74±0.31	<0.001

We also performed ROC analysis (Figure 1). We compared CRP and IMA ROC curves for each Wagner classification. In addition, we estimated the cut-off value, AUC, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CPR and IMA for each one Wagner classification (Table 4). When we investigated the ROC curves for Wagner grade 1 and 2, we found relative lower AUC, sensitivity and specificity in terms of CRP and IMA. For Wagner grade 1 and 3, the AUC, sensitivity and specificity values of CPR were higher than those of IMA. When ROC curves of Wagner grade 1-4 and 1-5 classifications were examined, it can be said that both CRP and IMA had relatively high AUC, sensitivity and specificity values, but CPR values were higher than IMA. Similar to the Wagner grade 1-3 comparison ROC curve, relatively low AUC, sensitivity and specificity values were obtained in the Wagner grade 2-3 ROC curve, in terms of CPR and IMA. We found relatively high AUC, sensitivity and specificity values for both CRP and IMA in Wagner grade 2-4 and 2-5 comparison ROC curves. In addition, for Wagner grade 3-4 and 3-5 comparison ROC curves, we found relatively high AUC, sensitivity and specificity values for both CRP and IMA. Unlike other Wagner grade ROC comparisons, the AUC, sensitivity and specificity values of IMA for Wagner grade 4-5 were higher than those of CRP. When the patient group was classified according to the presence of osteomyelitis and the ROC curves were examined, it was observed that the AUC, sensitivity and specificity values of CRP were higher than those of IMA. We summarise all AUC, sensitivity, specificity, PPV and NPV data in Table 4.

Table 4: Screening performance characteristics of CRP and IMA in predicting Wagner classification.

G1-G2	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
CRP	14.5	0.54(0.48-0.61)	62.1(44.8-77.5)	42.9(24.5-62.8)	59(42.6-74.2)	46.2(26.6-66.6)
IMA	2.48	0.58(0.43-0.64)	62.2(44.8-77.5)	60.7(40.6-78.5)	67.6(49.5-82.6)	54.8(36-72.7)
G1-G3						
CRP	18.8	0.76(0.62-0.87)	80.9(58.1-94.6)	64.3(44.1-81.4)	63(42.4-80.6)	81.8(59.7-94.8)
IMA	2.56	0.53(0.39-0.97)	76.2(52.8-91.8)	39.3(21.5-59.4)	48.5(30.8-66.5)	68.7(41.3-89)
G1-G4						
CRP	31.2	0.98(0.92-1)	94.7(74-99.9)	100(87.7-100)	100(81.5-100)	96.6(82.2-99.9)
IMA	2.66	0.89(0.77-0.96)	68.4(43.4-87.4)	100(87.7-100)	100(75.3-100)	82.4(65.5-93.2)
G1-G5						
CRP	31.2	1.00(0.91-1.00)	100(75.3-100)	100(87.7-100)	100(75.3-100)	100(87.7-100)
IMA	2.66	0.93(0.81-0.99)	84.6(54.6-98.1)	100(87.7-100)	100(71.5-100)	93.3(77.9-99.2)
G2-G3						
CRP	16.6	0.75(0.62-0.86)	90.5(69.6-98.8)	51.3(34.4-98.1)	51.4(34.4-68.1)	89.1(71.2-98.3)
IMA	2.39	0.56(0.42-0.69)	80.9(58.1-94.6)	40.5(24.8-57.9)	43.6(27.8-60.4)	78.9(54.4-93.6)
G2-G4						
CRP	28.9	0.99(0.93-1.00)	94.7(74-99.9)	100(90.5-100)	100(81.5-100)	97.4(86.2-99.9)
IMA	2.68	0.90(0.78-0.96)	68.4(43.4-87.4)	94.6(81.8-99.3)	86.7(59.5-98.3)	85.4(70.8-94.4)
G2-G5						
CRP	28.9	1.00(0.93-1.00)	100(75.3-100)	100(90.5-100)	100(75.3-100)	100(90.5-100)

G1-G2	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
IMA	2.91	0.95(0.84-0.99)	84.6(54.6-98.1)	100(90.5-100)	100(71.5-100)	94.9(82.7-99.4)
G3-G4						
CRP	37.4	0.96(0.85-0.99)	89.5(66.9-98.7)	100(83.9-100)	100(80.5-100)	91.3(72.1-98.9)
IMA	2.55	0.88(0.75-0.96)	94.7(74-99.9)	71.4(47.8-88.7)	75(53.3-90.2)	93.7(69.8-99.8)
G3-G5						
CRP	37.4	1.00(0.89-1.00)	100(75.3-100)	100(83.9-100)	100(75.3-100)	100(83.9-100)
IMA	2.64	0.94(0.79-0.99)	84.6(54.6-98.1)	95.2(76.2-99.9)	91.7(61.5-99.8)	90.9(70.8-98.9)
G4-G5						
CRP	58.7	0.69(0.50-0.83)	53.8(25.1-80.8)	89.4(66.9-98.7)	77.8(40.0-97.2)	73.9(51.6-89.8)
IMA	2.94	0.86(0.69-0.96)	84.6(54.6-98.1)	94.7(74.1-99.9)	91.7(61.5-99.8)	90.0(68.3-98.8)
Osteomyelitis						
CRP	31.2	0.89(0.82-0.94)	71.7(57.7-83.2)	100(94.5-100)	100(97.7-100)	81.2(71.0-89.1)
IMA	2.68	0.75(0.66-0.82)	47.2(33.3-61.4)	96.9(89.3-99.6)	92.6(75.7-99.1)	69.2(58.7-78.5)

G: Grade, AUC: Area under the ROC curve, PPV: Positive predictive value, NPV: Negative predictive value

Figure 1: The comparision ROC curves

Discussion

Diabetic foot ulcers are one of the most serious complications of diabetes mellitus that can result in amputation. Diabetic foot ulcers usually progress with infection, and early diagnosis and effective treatment is very important in preventing amputation.[4]For this reason, previous studies have emphasized biomarkers that show both the severity of infection and the amputation rate, and which can be used in early diagnosis.[21] Among these biomarkers, CRP, WBC, procalcitonin and ESR levels related to infection were especially emphasized[22, 23], and HbA1c levels were examined with regard to the amputation rate.[24] These parameters can provide an evaluation of diabetic foot ulcers in terms of infection and diabetes, while IMA can allow these patients to be evaluated from a different angle. IMA is a molecule formed by the modification of albumin as a result of ischemic damage. The popularity of IMA has been increasing recently and its association with ischemic injury-related diseases has been demonstrated.

In this present study, we aimed to determine the predictive value of IMA in diabetic foot ulcer patients and compare them to CRP results, as well as reviewing the relationship of IMA levels with diabetic foot ulcers according to the Wagner classification. We found that the levels of IMA in the diabetic foot patient group was significantly higher than those of the healthy control group. In previous studies, IMA levels have been considered in patients with diabetes and in patients with diabetes complications. Piwowar et al. reported that IMA levels in patients with type 2 diabetes were higher than the healthy control group.[13] In addition, IMA levels in diabetic nephropathy[25] and diabetic retinopathy[26] have been reported to be higher than the corresponding control group. Gunduz et al. reported that IMA levels of lower extremity ischemia patients and a healthy control group were compared and IMA levels of the patient group were significantly higher.[27] Muhtaroğlu et al. examined IMA levels in diabetic foot patients and reported that they were higher than the healthy control group.[28] The results from our study support the results of Muhtaroğlu et al. When we investigated the CRP result, the CRP level in the diabetic foot patient group was significantly higher than the healthy control groupSimilar results were reported in previous studies.[21-23]. These results show that IMA also plays an important role in the pathogenesis of diabetic foot patients.

In our study, we investigated the IMA levels in subgroups created according to the Wagner classification, and this is the first study that reports IMA levels in diabetic foot patients in terms of the Wagner classification. We found the highest IMA levels in Wagner grade 5. There was no significant difference between Wagner grade 1, 2 and 3 in terms of IMA levels. The level of IMA in Wagner grade 4 was significantly higher than those of Wagner grade 1, 2 and 3. We are unable to discuss these results in detail since IMA levels

in diabetic foot patients, which were previously classified according to the Wagner classification, were not examined. The highest CRP levels were determined in grade 5 in subgroups created according to the Wagner classification, but there was no statistically significant difference between grades 4 and 5. Also, there was no statistically significant difference between grade 1 and 2 in terms of CRP. Grade 3 CRP levels were found to be significantly higher than grade 1 and 2, and significantly lower than grade 4 and 5. Raheem et al. divided diabetic foot patients into subgroups according to the Wagner classification and examined their CRP levels. They reported that there was no statistically significant difference between grade 1 and 2 and that the highest CRP levels were detected in grade 5.[29] Hadavand et al. compared only the CRP levels of class III and IV and found that the CRP levels of class IV were statistically significantly higher than the class III.[22] Jeandrot et al. created subgroups using a different method of diabetic foot classification and examined their CRP values. While determining the highest CRP value in grade 4 in their studies, they reported that there was no statistically significant difference between the grade 1 and healthy control groups.[23] According to our results, both IMA levels and CRP levels are closely related to the Wagner classification, which evaluates according to the severity of infection, osteomyelitis, and necrosis. In our study, we classified the diabetic foot patients according to the presence of osteomyelitis and examined the IMA and CRP levels. We found that IMA and CRP levels were significantly higher in diabetic foot patients with osteomyelitis than in patients without osteomyelitis. These results support that IMA is related to the severity of infection in diabetic foot patients.

In our study, ROC analysis was performed to show the predictive value of IMA and CRP in subgroups created according to the Wagner classification. When the ROC curves are examined, it can be seen that the predictive value of CRP is higher than IMA in the distinction between grades other than grade 4-5. In distinguishing between Wagner grades 4 and 5, IMA AUC, sensitivity and specificity values were higher than those of CRP. According to our knowledge, there is no study examining the predictive value of IMA in the Wagner classification: this assessment was made for the first time in our study. Studies investigating the predictive value of CRP in distinguishing the classification, severity and presence of osteomyelitis have been conducted. Hadavand et al. reported that CRP has high sensitivity and specificity, especially in determining the presence of osteomyelitis in diabetic foot patients.[22] However, it should not be forgotten that CRP is an acute phase reactant and naturally increases in many infection-related diseases. In other words, CRP levels can also increase in a different complication, not associated with the diabetic foot. Jeandrot et al. examined the predictive value of CRP and procalcitonin in the separation of non-infected (grade 1) and infected (grade 2) patients, reporting that there was no significant difference between CRP and procalcitonin in terms of predictive value and that the combination of CRP and procalcitonin gave much better results.[9] IMA was more specific and sensitive than CRP in the distinction of grade 4 and grade 5 in patients with diabetic foot ulcers . This may be due to the development of endothelium-induced ischemia in tissues. Therefore, in these patients, besides blood, glucose level regulation, control of HbA1c levels and detection of infectious agents, ischemic conditions may also be considered.

There are some limitations in our study. One of these limitations is that the duration of diabetes in patients is unknown, so we could not clarify whether the duration of diabetes has an effect on IMA levels. Another limitation is that the sample size of our control group is relatively low. In advanced studies, the effects of diabetes duration on IMA levels can be examined by creating larger sample sizes.

In conclusion, our data showed that IMA may play a role in the pathogenesis of diabetic foot ulcers. In addition, it has been determined that IMA levels have high sensitivity and specificity in distinguishing Wagner grade 4 and 5 diabetic foot ulcers, especially when the infection is severe. Therefore, it may be clinically useful to examine IMA levels in the classification, progression and management of diabetic foot ulcers.

Conflicts of interest

The authors have no conflicts of interest associated with the publication of this article.

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