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CONTENTS

RESEARCH ARTICLES

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Research Article

The Relationship Between Disease Activity, Comorbidity Burden, and Functional Status with Self-Esteem in Patients with Rheumatoid Arthritis

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Abstract

Objectives: Rheumatoid arthritis (RA) is a chronic inflammatory disease with systemic involvement that gives rise not only to physical but also to psychosocial consequences. The purpose of this study was to evaluate the relationship between self-esteem and disease activity, functional status, and comorbidities in patients with RA.

Methods: This cross-sectional study enrolled patients with a confirmed diagnosis of rheumatoid arthritis. Disease activity was measured using the DAS28 score, functional status was assessed with the Health Assessment Questionnaire (HAQ), and self-esteem was evaluated using the Rosenberg Self-Esteem Scale.

Results: A statistically significant association was found between increased disease activity, higher comorbidity burden, and lower self-esteem scores.

Conclusion: The psychosocial dimension must be taken into consideration when evaluating patients with RA.

Keywords: Rheumatoid arthritis, self-esteem, disease activity, comorbidity, functional status

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Rheumatoid arthritis (RA) is a chronic, autoimmune, and inflammatory disease that constitutes a complex clinical entity affecting multiple systems, with the synovial joints bearing the primary burden.[1] The disease follows a progressive course and may result in joint destruction, loss of function, and a marked deterioration in quality of life. Genetic susceptibility, environmental factors, and immunological mechanisms all play a role in the pathophysiology of RA.[1,2]

RA is not solely a physical disease; it also encompasses psychological and social dimensions. Chronic pain, restricted mobility, and the requirement for long-term treatment can lead to depression, anxiety, and diminished self-esteem in affected individuals.[3] Self-esteem is an important psychological parameter that reflects a person's self-assessments and their perception of self-worth.[4]

The literature indicates that self-esteem in patients with RA is associated with disease activity, functional status, and comorbidities. For this reason, psychosocial factors—in addition to purely clinical parameters—must be considered when evaluating patients with RA.[5,6]

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Methods

This retrospective, single-center study was conducted at the Faculty of Medicine. The study protocol was reviewed by the Health Sciences Ethics Committee of Manisa Celal Bayar University Faculty of Medicine (decision date/no: October 21, 2020; 20.478.486/584). The study was conducted in accordance with the principles of the Declaration of Helsinki.

This study was designed as a cross-sectional investigation. Patients who had been diagnosed with rheumatoid arthritis were enrolled. All participants were evaluated with respect to demographic characteristics, disease duration, comorbid conditions, and current medications.

Disease activity was assessed using the Disease Activity Score 28 (DAS28). The DAS28 is a composite index that incorporates the number of tender and swollen joints, the erythrocyte sedimentation rate or C-reactive protein level, and the patient's global assessment.[7]

Functional status was measured with the Health Assessment Questionnaire (HAQ), a validated and reliable instrument for assessing patients' ability to perform activities of daily living.[8]

Self-esteem was evaluated using the Rosenberg Self-Esteem Scale, which measures both positive and negative attitudes an individual holds toward the self.

Statistical analyses were performed using appropriate parametric and non-parametric tests. A p-value of less than 0.05 was considered statistically significant.

Results

The majority of the patients included in the study were female. The mean age was in the middle-aged range. A considerable proportion of patients had at least one comorbid condition (Table 1).

An inverse relationship was observed between DAS28 scores and self-esteem scores. Patients with high disease activity demonstrated significantly lower self-esteem ($r=-0.42$, $p<0.001$).

A negative correlation was identified between HAQ scores and self-esteem ($r=-0.46$, $p<0.001$). Patients with greater functional limitation had lower self-esteem scores.

Self-esteem was found to decrease as the number of comorbidities increased ($p=0.002$). This association was particularly pronounced in patients with cardiovascular disease and diabetes mellitus.

In multivariable regression analysis, DAS28 ($\beta=-0.31$, $p=0.004$) and HAQ ($\beta=-0.35$, $p=0.002$) remained independent determinants of self-esteem (Table 2).

Table 1. Demographic characteristics

Variable	n	%
Female	60	75
Male	20	25
Mean age (years)	52	—

Table 2. Clinical parameters

Parameter	Mean	SD
DAS28	4.5	1.2
HAQ	1.3	0.5
Self-esteem score	18	4

DAS 28: Disease activity score 28; HAQ: Health assessment questionnaire; SD: Standart deviation.

Discussion

This study demonstrated that self-esteem in patients with RA is associated with disease activity and comorbidities, findings that are consistent with the existing literature.

Chronic pain and functional loss in patients with RA generate considerable psychological stress. This burden can erode an individual's confidence and lead to a decline in self-esteem.[9]

The presence of comorbid conditions adversely affects patients' overall health status and amplifies the psychological load they carry.[9] Accordingly, patients with RA should be evaluated through a holistic approach that addresses both physical and psychological dimensions.[10]

Psychosocial support and appropriate treatment strategies may help improve self-esteem and, in turn, enhance adherence to therapy.

Conclusion

Self-esteem in patients with rheumatoid arthritis is closely related to disease activity and comorbidities. Psychosocial factors must be incorporated into clinical assessments to ensure comprehensive patient care.[10]

Disclosures

Ethics Committee Approval: This retrospective, single-center study was conducted at Manisa Celal Bayar University Faculty of Medicine. The study protocol was approved by the Health Sciences Ethics Committee (approval date: October 21, 2020; approval no: 20.478.486/584).

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Research Article

Assessment of Serum Uric Acid Levels and Their Relationship with Serum Lipid Parameters in Prediabetic Patients

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Abstract

Objectives: Serum uric acid has been implicated in the pathogenesis of metabolic syndrome; however, its association with cardiometabolic risk in prediabetes remains unclear. This study aimed to evaluate the relationship between uric acid levels and cardiometabolic risk factors in prediabetic individuals compared with normoglycemic subjects.

Methods: A total of 329 patients (155 normoglycemic, 174 prediabetic; aged 31–59 years) were included. Prediabetes was defined according to American Diabetes Association criteria (impaired fasting glucose [IFG] or impaired glucose tolerance. [IGT]). Serum uric acid (UA), lipid parameters (total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]), and atherogenic index of plasma (AIP; log[TG/HDL-C]) were assessed. Patients were categorized according to uric acid levels (<5.4, 5.4–6.4, and >6.4 mg/dL).

Results: Prediabetic patients had significantly higher uric acid levels than normoglycemic individuals ($p<0.001$). Uric acid levels differed significantly across groups and were positively correlated with age, TC, LDL-C, and AIP, and negatively correlated with HDL-C in prediabetic subjects ($p<0.001$).

Conclusion: Elevated uric acid levels are associated with adverse lipid profiles and increased cardiometabolic risk in prediabetes and may serve as a predictor of diabetes development.

Keywords: Glucose tolerance, prediabetic, uric acid

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Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia resulting from decreased insulin secretion, impaired insulin action, or both.[1] Early recognition of diabetes and the implementation of appropriate preventive measures, particularly during the prediabetic stage, are of great importance for slowing disease progression and preventing complications.

Prediabetes, defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), is an important risk factor for the development of type 2 diabetes and cardiovascular diseases.[1,2] A significant proportion of individuals with prediabetes develop overt diabetes in subsequent years, and these individuals show a predisposition to cardiovascular complications similar to patients with diabetes.[3]

Uric acid is the final product of purine metabolism, and elevated serum levels have been associated with various diseases. Recent studies have suggested that hyperuricemia may be related to metabolic syndrome, hypertension, diabetes, and dyslipidemia.[4–6] However, the relationship between serum uric acid levels and prediabetes, particular-

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ly their association with cardiovascular risk factors in prediabetic individuals, has not yet been fully clarified.[7]

In addition to classical lipid parameters used in cardiovascular risk assessment, the atherogenic index of plasma (AIP), calculated from the triglycerides (TG) to high-density lipoprotein cholesterol (HDL-C) ratio, has emerged as an important biomarker in recent years.[8]

The aim of this study was to evaluate the relationship between serum uric acid levels and cardiovascular risk factors, such as serum lipid levels and AIP in prediabetic individuals, compared with individuals with normal glucose tolerance.

Methods

This study was based on the retrospective evaluation of medical records of 329 patients (181 women and 148 men), aged between 31 and 59 years, who were admitted to the Internal Medicine outpatient clinics of Istanbul Training and Research Hospital between December 1, 2012, and December 1, 2014, had an indication for an oral glucose tolerance test (OGTT) (Table 5), and underwent a 2-hour OGTT with 75 g glucose.

Inclusion Criteria

1. Patients admitted to the Internal Medicine outpatient clinics of Istanbul Training and Research Hospital
2. Patients with an indication for OGTT who underwent a 2-hour OGTT with 75 g glucose
3. Patients with fasting plasma glucose ≥ 100 mg/dL and <126 mg/dL and/or 2-hour plasma glucose during OGTT ≥ 140 mg/dL and <200 mg/dL

Exclusion Criteria

1. Fasting plasma glucose ≥ 126 mg/dL and/or 2-hour plasma glucose ≥ 200 mg/dL
2. Known diagnosis of diabetes mellitus
3. Pregnant women
4. Serum creatinine > 1.3 mg/dL

Patient Classification

Patient groups were classified as follows:

- Individuals with normal fasting and 2-hour plasma glucose levels were classified as normoglycemic.
- Patients with fasting plasma glucose between 100–125 mg/dL were classified as having impaired fasting glucose (IFG).
- Patients with 2-hour plasma glucose between 140–199 mg/dL were classified as having impaired glucose tolerance (IGT).

Patients with HbA1c levels above 6.4% and serum creatinine levels > 1.3 mg/dL were excluded from the study.

Serum uric acid, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, and plasma TG levels of all patients were recorded. These parameters were obtained from measurements performed in the hospital's biochemistry laboratory.

After comparing uric acid levels among the three groups (normoglycemic, IFG, and IGT), normoglycemic and prediabetic subjects were further divided into three subgroups according to serum uric acid levels:

- Uric acid <5.4 mg/dL
- 5.4 mg/dL ≤ Uric acid ≤ 6.4 mg/dL
- Uric acid > 6.4 mg/dL

The lipid profiles of these three groups were compared separately in normoglycemic and prediabetic subjects.

To assess cardiovascular risk, plasma lipid profiles and the atherogenic index of plasma (AIP) were evaluated. AIP was calculated using the following formula:

AIP=log (plasma TG / HDL-C)

Statistical Analysis

Statistical analyses were performed using SPSS version 15.0 for Windows (Armonk, New York: IBM Corp.). Descriptive statistics were presented as numbers and percentages for categorical variables, and as mean, standard deviation, minimum, and maximum values for numerical variables. Comparisons among more than two groups were performed using one-way ANOVA when normal distribution was satisfied, and the Kruskal–Wallis test when it was not. Subgroup analyses were performed using the Tukey test for parametric tests and the Mann–Whitney U test with Bonferroni correction for nonparametric tests. Comparisons between two independent groups were performed using Student's t-test when normal distribution was satisfied, and the Mann–Whitney U test otherwise. Categorical variables were compared using chi-square analysis. When assumptions were not satisfied, Monte Carlo simulation was applied. Relationships between numerical variables were evaluated using Spearman correlation analysis, since parametric assumptions were not met. Factors determining numerical variables were evaluated using linear regression analysis with the backward elimination method. The statistical significance level was accepted as $p<0.05$. This retrospective study was approved by the Istanbul Training and Research Hospital Clinical Research Ethics Committee (decision number: 595, approval date: January 23, 2015). The study was conducted in accordance with the Declaration of Helsinki.

Results

A total of 329 patients were included in the study: 155 normoglycemic patients (72 men and 83 women) with a mean age of 41.2±6.4 years, 97 prediabetic patients with IFG (39

men and 58 women) with a mean age of 43.1±6.1 years, and 77 prediabetic patients with IGT (37 men and 40 women) with a mean age of 45.6±6.1 years (Fig. 1).

The difference in mean age among the groups was statistically significant (p<0.001). Pairwise comparisons also demonstrated statistically significant differences between all groups (normoglycemic vs. IFG, p=0.009; normoglycemic vs. IGT, p<0.001; IFG vs. IGT, p=0.004). The highest mean age was observed in the IGT group, followed by the IFG group and the normoglycemic group.

There was no statistically significant difference in sex distribution among the groups (p=0.516).

Significant differences were observed in mean uric acid levels and their distribution across groups (HDL-C, p=0.027; all other comparisons, p<0.001). Mean uric acid levels were significantly higher in prediabetic patients with IFG and IGT compared with normoglycemic individuals. However, no statistically significant difference was observed between the two prediabetic groups (Table 1).

There was a statistically significant difference in uric acid categories among the groups (p<0.001). In the normoglycemic group, only one patient had a uric acid level above 6.4 mg/dL, while 70.3% of patients had uric acid levels below 5.4 mg/dL.

In the normoglycemic group, uric acid levels showed a statistically significant positive correlation with age, TC, and LDL-C (p=0.001, p<0.001, and p=0.001, respectively). However, no statistically significant association was observed between uric acid levels and TG, HDL-C, or AIP (p=0.609, p=0.331, and p=0.645, respectively).

In the prediabetic group, uric acid levels were positively correlated with age, TC, TG, LDL-C, and AIP, and negatively correlated with HDL-C, all of which were statistically significant (p<0.001 for all comparisons) (Fig. 2, 3).

Among normoglycemic patients, those with uric acid levels between 5.4–6.4 mg/dL had significantly higher mean values of age, TC, TG, LDL-C, and AIP compared with patients whose uric acid levels were below 5.4 mg/dL (p=0.004, p<0.001, p=0.019, p<0.001, p=0.001, and p=0.009, respectively). However, no statistically significant difference was observed in mean HDL-C levels (p=0.170).

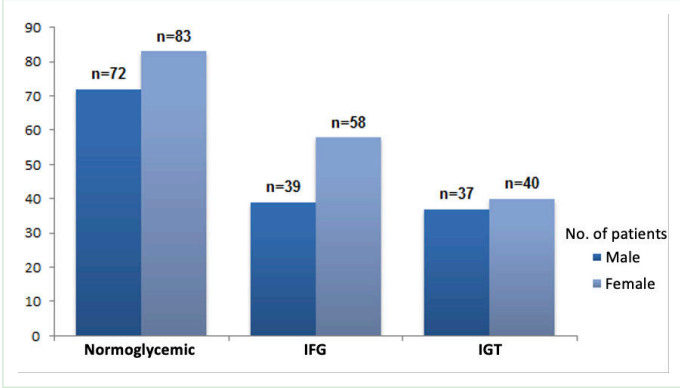

Figure 1. Number of patients in the study groups.
IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance.

In prediabetic patients, significant differences were observed among uric acid groups in terms of age, TC, TG, LDL-C, HDL-C, and AIP levels (p<0.001 for all comparisons). These differences were statistically significant between all groups except for HDL-C levels between the groups with uric acid levels below 5.4 mg/dL and those with levels between 5.4–6.4 mg/dL (Tables 2 and 3).

In the model constructed to evaluate the factors determining serum uric acid levels (sex, age, TC, TG, LDL-C, HDL-C, and AIP), age, TC, HDL-C, and AIP were identified as the most significant predictors in the normoglycemic group, whereas age, LDL-C, AIP, and sex were identified as the most significant predictors in the prediabetic group (Table 4).

When prediabetic male and female patients were grouped separately according to uric acid levels, statistically signif-

Table 1. Mean values and distributions of gender, age, and uric acid levels in Normoglycemic, IFG and IGT groups

	Normoglycemic	IFG	IGT	p
Age (years)	41.2±6.4 (31-59)	43.1±6.1 (31-59)	45.6±6.1 (32-59)	**<0.001**
Gender				
Male	72 (46.5)	39 (40.2)	37 (48.1)	0.516
Female	83 (53.5)	58 (59.8)	40 (51.9)	
Uric Acid(mg/dL)	4.8±0.9 (2.6-6.6)	5.6±1.1 (2.6-7.1)	5.9±1.0 (3.6-8)	**<0.001**
<5.4	109 (70.3)	40 (41.2)	23 (29.9)	**<0.001**
5.4-6.4	45 (29.0)	25 (25.8)	27 (35.1)	
>6.4	1 (0.6)	32 (33.0)	27 (35.1)	

IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance.

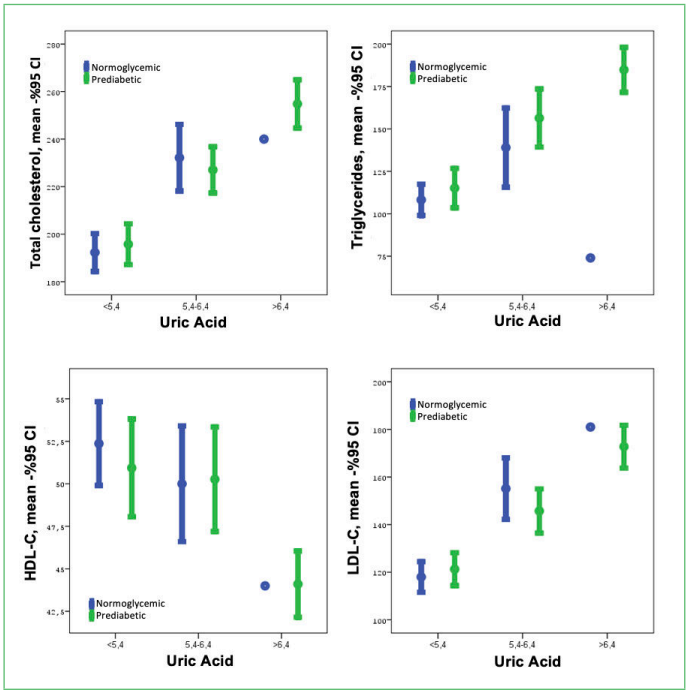

Figure 2. Mean values and confidence intervals of total cholesterol, triglycerides, HDL-C, and LDL-C according to uric acid categories in normoglycemic and prediabetic groups.

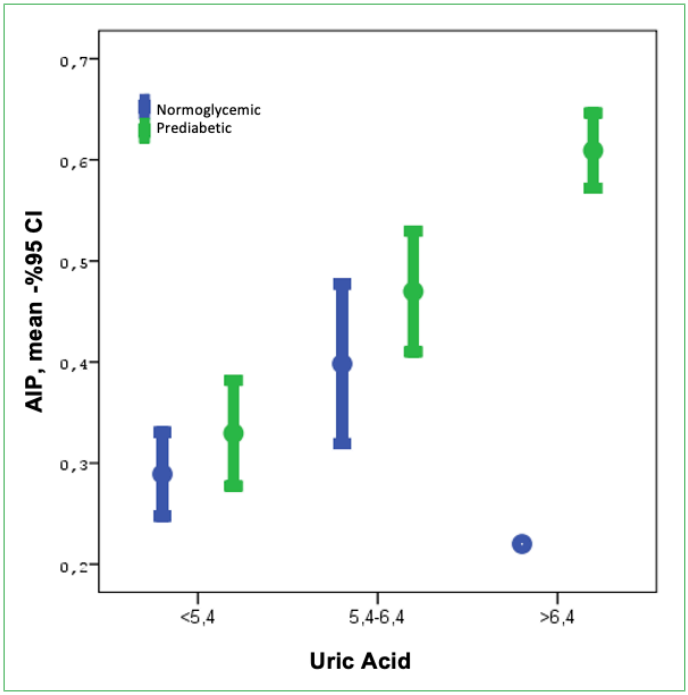

Figure 3. Mean AIP values and confidence intervals according to uric acid categories in normoglycemic and prediabetic groups.

icant differences were observed across almost all parameters.

Among prediabetic male patients, significant differences were found between uric acid groups for all parameters (age, p=0.008; HDL-C, p=0.015; all other parameters, p<0.001).

Similarly, among prediabetic female patients, significant differences were observed among the three uric acid groups for all parameters (HDL-C, p=0.022; all other parameters, p<0.001) (Table 5).

Discussion

Prediabetes, defined as impaired fasting glucose and/or impaired glucose tolerance.[1], is an important risk factor for the development of overt diabetes and cardiovascular disease (CVD).[1,9] Prediabetic individuals are at an increased risk of cardiovascular disease and mortality compared with normoglycemic individuals; in most cases, prediabetic patients remain clinically asymptomatic.[10]

This retrospective study was conducted to evaluate serum uric acid levels and their relationship with serum lipid parameters in individuals with prediabetes and those with normal glucose tolerance. In our study population, the proportion of prediabetic patients was 52.8%. This rate differs from the 28.7% prevalence reported in the TURDEP-II study conducted by Satman et al.[11] This discrepancy may be explained by the fact that our study population consisted only of patients who had an indication for an OGTT, particularly individuals with a family history of diabetes or suspected metabolic syndrome.

The pathophysiological relationship between elevated uric acid levels and prediabetes has not yet been fully clarified. Hyperuricemia is most commonly caused by reduced renal excretion of uric acid, and it has been shown to correlate with the severity of insulin resistance.[12] In addition, insulin resistance is associated with reduced nitric oxide levels, and increased uric acid levels have been shown to reduce nitric oxide availability.[13] Elevated insulin levels may impair renal tubular function, thereby decreasing uric acid excretion.[14] Diets rich in fructose are also known to increase uric acid production. Another study demonstrated that fructose-induced hyperuricemia may impair glucose tolerance and contribute to the development of metabolic syndrome.[15]

One of the studies investigating the association between uric acid levels and the risk of prediabetes and diabetes was conducted in China. In this study, individuals with higher baseline uric acid levels had a significantly increased risk of developing diabetes during a 9-year follow-up period.[16] Similarly, a study conducted by Dehghan et al.[17] in 2008 demonstrated that the future risk of diabetes was correlated with serum uric acid levels. In our study, serum uric acid levels were also significantly higher in the prediabetic group compared with the normoglycemic group. Consistent with previous studies, our findings suggest that serum

Table 2. Distribution of mean age, lipid parameters, and AIP values according to uric acid categories in normoglycemic and prediabetic groups

	Normoglycemic				Prediabetic			
	Uric acid				Uric Acid			
	<5.4	5.4-6.4	>6.4*	p	<5.4	5.4-6.4	>6.4	p
Age(years)	40.0±5.4	44.0±7.4	56.0	**0.004**	41.1±5.0	43.0±4.9	48.6±6.0	**<0.001**
TC (mg/dL)	192.3±42.1	232.2±46.7	240.0	**<0.001**	195.8±34.1	227.1±3.8	254.8±38.9	**<0.001**
Triglycerides (mg/dL)	108.2±48.3	139.0±77.6	74.0	**0.019**	115.1±46.1	156.4±6.5	184.8±50.7	**<0.001**
HDL-C (mg/dL)	52.4±13.0	50.0±11.3	44.0	0.170	50.9±11.4	50.3±11.0	44.1±7.5	**<0.001**
LDL-C (mg/dL)	118.0±33.9	155.1±43.0	181.0	**<0.001**	121.3±27.3	145.7±33.3	172.8±34.6	**<0.001**
AIP	0.29±0.22	0.40±0.26	0.22	**0.009**	0.33±0.21	0.47±0.21	0.61±0.14	**<0.001**

*Excluded from statistical analysis due to inclusion of only one patient. TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AIP: Atherogenic index of plasma.

Table 3. Spearman correlation coefficients (ρ) between uric acid levels and age, lipid parameters, and AIP in normoglycemic and prediabetic patients

	Uric acid			
	Normoglycemic		Prediabetic	
	Rho	p	rho	p
Age (years)	0.260	**0.001**	0.540	**<0.001**
TC (mg/dL)	0.376	**<0.001**	0.639	**<0.001**
TG (mg/dL)	0.041	0.609	0.590	**<0.001**
HDL-C (mg/dL)	-0.079	0.331	-0.244	**<0.001**
LDL-C (mg/dL)	0.425	**<0.001**	0.635	**<0.001**
AIP	0.037	0.645	0.581	**<0.001**

TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AIP: Atherogenic index of plasma.

Table 4. Results of multivariate linear regression analysis (backward method) for determining uric acid levels in normoglycemic and prediabetic patients

	B	Beta	p
Normoglycemic			
Intercept	0.469		
Age	0.037	0.262	**<0.001**
TC	0.010	0.513	**<0.001**
HDL-C	-0.027	-0.372	**0.001**
AIP	-0.831	-0.220	**0.028**
Prediabetic			
Intercept	-0.336		
Age	0.033	0.198	**<0.001**
LDL-C	0.010	0.352	**<0.001**
AIP	1.300	0.280	**<0.001**
Sex	-0.366	-0.176	**0.001**

Normoglycemic R: 0.337 Prediabetic R:0.617 Model: sex, age, TC, TG, LDL-C, HDL-C, AIP, TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AIP: Atherogenic index of plasma.

uric acid levels are associated with prediabetes and may serve as a potential predictor for its development.

Chen et al.[18] reported a negative correlation between HDL-C and uric acid levels. The probable mechanism underlying this relationship is the association between insulin resistance and decreased HDL-C levels. In our study, when patient groups were compared according to uric acid levels, the group with the highest uric acid levels had the lowest HDL-C values, while the group with the lowest uric acid levels had the highest HDL-C values. However, in individuals with normal glucose tolerance, no significant differences were observed between groups in terms of HDL-C levels.

In a study conducted by Agamah et al.[19], individuals with elevated uric acid levels were found to have higher total cholesterol levels compared with individuals with lower uric acid levels. Another study by Akbas et al.[20] conducted in diabetic patients demonstrated that total cholesterol levels were significantly higher in patients with uric acid levels above 6 mg/dL compared with those with lower levels. Similarly, in our study, prediabetic patients with uric acid levels above 6.4 mg/dL had higher total cholesterol levels compared with those with uric acid levels between 5.4–6.4 mg/dL and those with levels below 5.4 mg/dL. A similar pattern was also observed among normoglycemic individuals, where higher uric acid levels were associated with higher total cholesterol levels.

One explanation for the relationship between hyperuricemia and elevated plasma triglyceride levels is that the synthesis

Table 5. Mean age, lipid parameters, and AIP values according to uric acid categories in male and female prediabetic patients

		Uric acid		p
Male	<5.4 (n=15)	5.4-6.4 (n=18)	>6.4 (n=43)	
Age (years)	42.6±4.8	44.4±5.7	47.9±6.1	**0.008**
TC (mg/dL)	198.1±39.6	231.4±36.0	259.8±34.2	**<0.001**
TG (mg/dL)	118.2±37.4	143.7±46.7	189.7±51.5	**<0.001**
HDL-C (mg/dL)	52.2±11.5	51.9±15.1	44.5±6.8	**0.015**
LDL-C (mg/dL)	122.0±30.4	151.0±26.2	176.7±31.5	**<0.001**
AIP	0.34±0.16	0.43±0.25	0.62±0.15	**<0.001**
Female	<5.4 (n=48)	5.4-6.4 (n=34)	>6.4 (n=16)	
Age (years)	40.7±5.0	42.2±4.3	50,5±5,5	**<0.001**
TC (mg/dL)	195.0±32.7	224.7±34.5	241.4±48.2	**<0.001**
TG (mg/dL)	114.2±48.8	163.1±67.8	171.9±47.7	**<0.001**
HDL-C (mg/dL)	50.5±11.5	49.4±8.3	43.1±9.1	**0.022**
LDL-C (mg/dL)	121.0±26.7	142.9±36.6	162.2±40.9	**<0.001**
AIP	0.3±0.2	0.5±0.2	0.6±0.1	**<0.001**

TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AIP: Atherogenic index of plasma.

of fatty acids and triglycerides requires large amounts of NADPH, which also promotes hepatic purine synthesis and consequently uric acid production. Nakagawa et al.[21] suggested that uric acid may be associated either with excess triglyceride production or decreased triglyceride clearance. Another study reported that reduced activity of lipoprotein lipase (LPL), which is responsible for the lipolysis of both triglycerides and LDL-C, may lead to increased serum lipid levels in patients with hyperuricemia.[22]

In a study conducted by Shelmadine et al.[23], allopurinol was administered to 12 patients with end-stage renal disease to reduce uric acid levels, and its effects on metabolic syndrome parameters were evaluated. The study demonstrated an average decrease of 3.53 mg/dL in uric acid levels and 14.00 mg/dL in LDL-C levels. Similarly, in our study, higher TG and LDL-C levels were observed in parallel with increasing uric acid levels in prediabetic individuals.

In the study conducted by Soğut et al.[24], a TG/HDL-C ratio greater than 3.69 was found to be significantly associated with the risk of atherosclerosis, with 82.5% sensitivity and 58.1% specificity. In another study conducted by Lippi et al.[25] in 2010, hyperuricemic individuals had higher AIP values compared with individuals with normal uric acid levels. Similarly, in our study, patients with higher uric acid levels also had significantly higher AIP values compared with those with lower uric acid levels.

Another noteworthy finding in our study was that only 18.3% of normoglycemic individuals with uric acid levels below 5.4 mg/dL were classified in the low-risk group. This may be explained by the fact that many of these patients had a family history of diabetes or a higher body mass index, despite having normal uric acid levels and glucose tolerance.

Considering the high sensitivity of AIP in predicting ischemic heart disease risk, our findings suggest that uric acid may represent an important risk factor for ischemic cardiovascular disease.

Study Limitations

The primary limitation of our study is that the available data were obtained solely from laboratory records. It was not possible to determine whether patients were receiving medications that could influence laboratory parameters such as uric acid, glucose, HbA1c, or lipid levels. In addition, the absence of important clinical data such as family history, body mass index, smoking and alcohol history, and blood pressure measurements limited the scope of our analysis.

Conclusion

In this study evaluating laboratory data from 329 individuals with prediabetes and normal glucose tolerance, our findings were generally consistent with previous studies reported in the literature.

First, the observation that serum uric acid levels were higher in prediabetic individuals compared with normoglycemic individuals supports the hypothesis that uric acid may serve as a strong positive predictor for diabetes risk.

When prediabetic individuals were grouped according to uric acid levels, uric acid was found to be positively associated with TC, LDL-C, TG, and AIP levels, and negatively associated with HDL-C levels.

The strong relationship observed between uric acid and cardiovascular risk factors suggests that uric acid may represent an important risk factor that should be monitored and controlled in the early stages among prediabetic individuals.

Nevertheless, larger, prospective, well-designed studies are needed to better understand the interaction between serum uric acid levels and cardiovascular risk in prediabetic patients.

Disclosures

Ethics Committee Approval: This retrospective study was approved by the İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 595, approval date: 23 January 2015).

Informed Consent: Informed consent was waived due to the retrospective design of the study.

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Research Article

Comparative Prognostic Value of the Durie–Salmon, ISS, and mSMART Staging Systems in Multiple Myeloma

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Abstract

Objectives: Multiple myeloma (MM) is a plasma cell malignancy characterized by marked interpatient variability in clinical course and prognosis. Accurate staging systems are essential for risk stratification and survival prediction. We aimed to compare the prognostic performance of the Durie–Salmon (DS), International Staging System (ISS), and Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) systems in predicting overall survival.

Methods: This retrospective study included 110 patients diagnosed with MM at a tertiary hematology center. Patients were staged according to the DS, ISS, and mSMART systems. Overall survival (OS) was evaluated using the Kaplan–Meier method, and prognostic factors were evaluated using univariate and multivariate Cox regression analyses.

Results: Advanced disease stages were significantly associated with poorer overall survival across all staging systems (Durie–Salmon, p<0.001; ISS, p=0.007; mSMART, p=0.001). Among the evaluated models, only DS stage IIIB remained an independent predictor of mortality in multivariate analysis (HR=11.55, 95% CI=3.29–40.56, p < 0.001). ISS stage and mSMART risk categories did not retain independent prognostic significance after adjustment.

Conclusion: The DS staging system showed the strongest association with overall survival among the evaluated models. Although biologically based risk stratification has advanced considerably, traditional clinical staging systems still provide valuable and practical guidance in routine clinical practice.

Keywords: Durie–Salmon; ISS, Multiple myeloma, mSMART, prognosis

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Multiple myeloma (MM) is a hematological cancer driven by the clonal expansion of plasma cells that produce monoclonal immunoglobulins. It constitutes a notable proportion of hematological malignancies and shows wide variability in clinical presentation and prognosis.[1] The International Myeloma Working Group (IMWG) has defined diagnostic criteria for MM, including clonal plasma cell infiltration in the bone marrow, the presence of monoclonal proteins, and evidence of end-organ damage.[2] Because of this variability, assessing prognosis at the time of diagnosis is particularly important. In everyday clinical practice, staging systems are commonly used to guide treatment choices, estimate risk, and inform patients about their disease. Among these, the International Staging System (ISS) is widely adopted, mainly due to its simplicity and reliance on routinely available laboratory parameters.[3] Nevertheless, other clinical and biological factors affecting survival have also been reported.[4] Moreover, variations in

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treatment response and patient-specific factors contribute to discrepancies in survival outcomes among multiple myeloma patients.

As knowledge of disease biology has evolved, more refined prognostic models have been developed. For example, the Revised International Staging System (R-ISS) includes biological markers in addition to traditional clinical variables to more accurately determine risk stratification.[5] Furthermore, the serum free light-chain ratio has been identified as a useful prognostic indicator in multiple myeloma patients; in parallel, risk-adapted approaches such as the Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) classification have been developed to integrate cytogenetic risk factors into clinical decision-making.[6] These developments aim to improve risk stratification and provide a more comprehensive understanding of the biological heterogeneity of the disease, including the effects of cytogenetic abnormalities and molecular subtypes.[7,8] Furthermore, response criteria and minimal residual disease (MRD) assessment have gained increasing importance in evaluating treatment response and long-term outcomes in multiple myeloma.[9]

Despite these advances, outcomes in multiple myeloma remain influenced by both biological factors and the initial disease burden. In many real-world settings, access to comprehensive cytogenetic and molecular analyses remains limited, which maintains the practical importance of conventional clinical staging systems. The Durie–Salmon (DS) system, one of the first clinically based staging systems, continues to be used in various centers due to its ability to reflect tumor burden and organ involvement. Owing to this feature, it maintains its importance as a valuable and practical tool in routine clinical practice, especially in settings where access to advanced molecular diagnostic methods is limited.

However, the comparative performance of these staging systems in real-world clinical settings remains to be fully clarified. Moreover, evidence from real-world patient cohorts remains relatively limited. In particular, direct comparisons between commonly used staging systems in routine clinical practice are still scarce.

In this context, we aimed to evaluate and compare the prognostic value of the DS, ISS, and mSMART staging systems in predicting overall survival in patients with multiple myeloma.

Methods

Study Population

This retrospective study included a total of 110 patients aged between 18 and 90 years who were diagnosed with multiple myeloma (MM) and followed at a tertiary hematology center. All patients had sufficient clinical and follow-up data available for evaluation. Patients with incomplete medical records or inadequate follow-up were excluded from the study. The diagnosis of MM was established according to standard international criteria.

Data Collection

Demographic characteristics, baseline laboratory parameters, and radiologic findings related to bone involvement were obtained from institutional medical records. Available cytogenetic data were reviewed when present. Patients were staged according to the Durie–Salmon (DS), International Staging System (ISS), and Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) systems at the time of diagnosis. Information regarding treatment responses and survival outcomes was also extracted from patient records. Treatment responses were assessed based on routine clinical evaluations documented in the patient files.

Ethical Approval

The study protocol was reviewed by the local Health Science University Haseki Education and Research Hospital Clinical Reserach Ethics Committee. According to the committee's decision (Approval No.: 298, Date: 02.12.2015), formal ethics committee approval was deemed unnecessary due to the retrospective nature of the study.

Statistical Analysis

Overall survival (OS) was defined as the time from diagnosis to death or the date of the last follow-up. Patients who were alive at the last follow-up were censored. Survival analyses were performed using the Kaplan–Meier method, and differences between groups were assessed with the log-rank test. Prognostic factors associated with overall survival were evaluated using univariate and multivariate Cox proportional hazards regression models. Variables that showed statistical significance in univariate analysis were included in the multivariate model. Statistical analyses were performed using SPSS software (version 22; IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The basic demographic and clinical characteristics of the study population are presented in Table 1. The patient group exhibited a heterogeneous structure in terms of age distribution, clinical characteristics, and disease stages at diagnosis. Staging of patients according to the Durie–Salmon, International Staging System (ISS), and Mayo Strat-

Table 1. Baseline demographic and clinical characteristics of the study population

Characteristic	Value
Age (years)	67.3±11.1 (range: 37–86)
Sex, n (%)	
Male	63 (57.3)
Female	47 (42.7)
Myeloma type, n (%)	
IgG (κ/λ combined)	62 (56.3)
IgA (κ/λ combined)	23 (20.9)
Light chain only	18 (16.4)
IgD	4 (3.6)
Solitary plasmacytoma	3 (2.7)
Hypercalcemia, n (%)	13 (11.8)
Renal impairment, n (%)	25 (22.7)
Anemia, n (%)	56 (50.9)
Lytic bone lesions, n (%)	58 (53.2)

Data are presented as mean ± standard deviation or number (%). κ/λ indicates combined kappa and lambda light chain subtypes.

ification of Myeloma and Risk-Adapted Therapy (mSMART) systems revealed the presence of different prognostic groups within the study population.

In overall survival analyses, significant differences were found between disease stages according to all three staging systems. Survival analyses based on the Durie–Salmon, ISS, and mSMART classifications showed significant differences between stages (Table 2; Figures 1–3). Kaplan–Meier curves demonstrated decreasing survival probabilities with advancing disease stage across all three models (Durie–Salmon, p<0.001; ISS, p=0.007; mSMART, p=0.001). Median overall survival was shorter in advanced-stage groups compared to early-stage patients.

Multivariate Cox proportional hazards regression analysis showed that only Durie–Salmon stage IIIB was independently linked to a higher risk of death (HR=11.55, 95% CI=3.29–40.56, p<0.001) (Table 3). After controlling for other variables in the model, ISS stage and mSMART risk category lost their independent prognostic significance. These results indicate that Durie–Salmon stage IIIB, which signifies advanced tumor burden and end-organ involvement, is the prognostic marker most closely correlated with overall survival in this cohort.

In addition to survival differences between staging categories, the distribution of patients across stages revealed a predominance of advanced disease at diagnosis. A substantial proportion of patients were classified as advanced stage according to the Durie–Salmon and ISS systems, while a smaller subset was categorized as high risk by mSMART. Advanced-stage patients more frequently showed clinical features indicative of higher disease bur-

Table 2. Overall survival outcomes according to staging systems

Staging system	Category	Median OS (months)	95% CI	p
Durie–Salmon	IA	52	29.8–74.2	<0.001
	IIA	47	9.1–84.9	
	IIB	17	6.6–27.4	
	IIIA	19	11.3–26.7	
	IIIB	6	2.3–9.7	
ISS	I	52	35.4–68.6	0.007
	II	33	1.9–64.1	
	III	12	5.3–18.7	
mSMART	Standard risk	47	31.0–62.9	0.001
	High risk	7	0.0–15.6	

Overall survival (OS) was estimated using the Kaplan–Meier method. p values were calculated using the log-rank test. Median OS is presented in months, OS: Overall survival; ISS: International Staging System; mSMART: Mayo Stratification of Myeloma and Risk-Adapted Therapy.

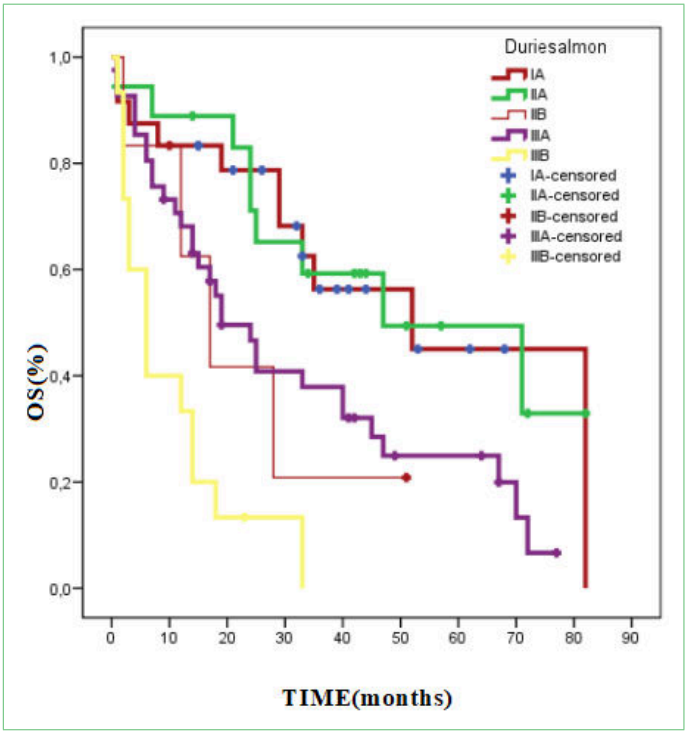

Figure 1. Durie–Salmon survival curves.
Kaplan–Meier overall survival curves according to Durie–Salmon staging system. Overall survival differed significantly among Durie–Salmon stages, with progressively shorter survival observed in advanced stages (log-rank test, p<0.001).

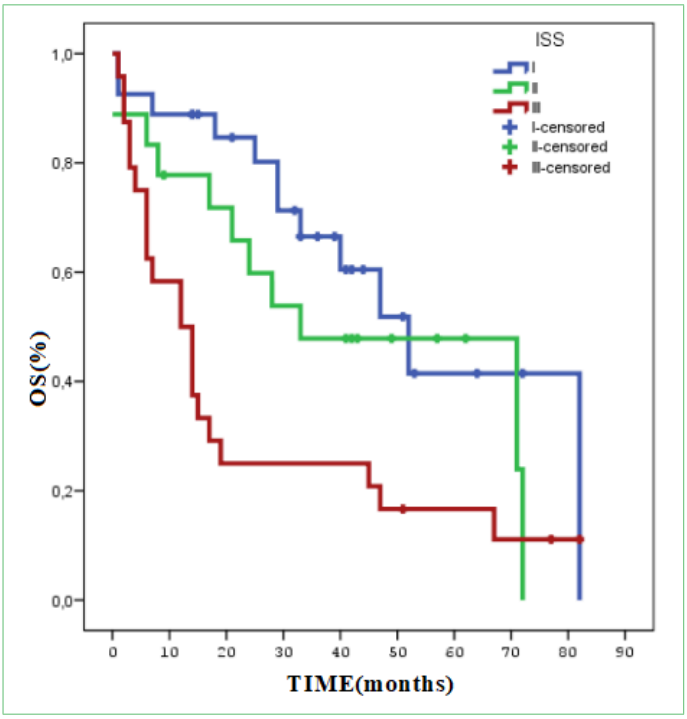

Figure 2. ISS survival curves.
Kaplan–Meier overall survival curves stratified by International Staging System (ISS). Patients with higher ISS stage demonstrated significantly inferior overall survival compared with lower stages (log-rank test, p=0.007).

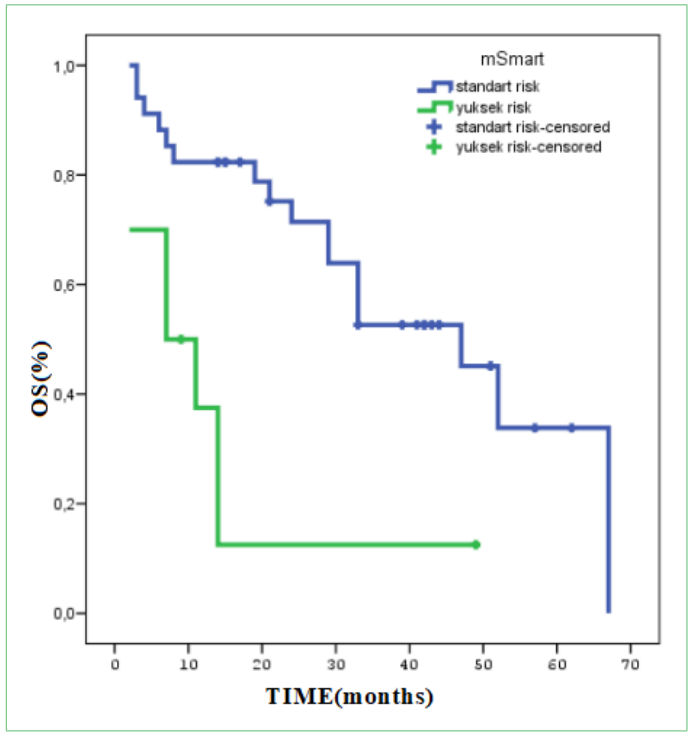

Figure 3. mSMART survival curves.
Kaplan–Meier overall survival curves according to mSMART risk stratification. High-risk patients exhibited significantly reduced overall survival compared with standard-risk patients (log-rank test, p=0.001).

den, including renal impairment, anemia, and extensive skeletal involvement (Table 1). These findings further support the observed differences in survival outcomes across staging groups.

When the staging systems were compared, greater agreement was observed in early-stage disease, whereas variability increased among advanced-stage categories. Notably, some patients categorized as advanced stage by the Durie–Salmon system were classified as intermediate or high risk according to ISS and mSMART. Despite these differences, each system was able to distinguish patients with poorer outcomes. Overlapping classifications indicate that disease severity may be captured differently across staging approaches used in routine clinical practice.

Table 3. Multivariate Cox regression analysis of prognostic factors associated with overall survival

Variable	Hazard Ratio (HR)	95% CI	p
Durie–Salmon stage			
IIIA	0.57	0.11–3.11	*0.520*
IIIB	11.55	3.29–40.56	*<0.001*
ISS stage			
II	0.98	0.24–3.94	*0.975*
III	3.14	0.55–17.83	*0.196*
mSMART risk category			
High risk	1.62	0.22–11.85	*0.635*

Multivariate Cox proportional hazards regression analysis included Durie–Salmon stage, ISS stage, and mSMART risk category. Durie–Salmon stage IA, ISS stage I, and standard-risk mSMART category were used as reference groups. Hazard ratios (HRs) and 95% confidence intervals (CIs) are shown. ISS: International Staging System; mSMART: Mayo Stratification of Myeloma and Risk-Adapted Therapy.

Discussion

In this retrospective cohort, the Durie–Salmon (DS) staging system was most strongly associated with overall survival compared with ISS and mSMART. This finding suggests that, even in the era of modern MM management, clinical burden and organ involvement—as reflected by the DS system—remain highly relevant for predicting patient outcomes at diagnosis.

DS stage IIIB appears to identify a subgroup of patients with more advanced disease, where renal impairment and extensive organ involvement play an important role in determining outcomes. In our cohort, Durie–Salmon stage IIIB was the strongest predictor of mortality (HR=11.55, 95% CI=3.29–40.56, p<0.001). Renal dysfunction is a frequent

complication of multiple myeloma and is linked to worse outcomes.[10] In this setting, staging systems that capture tumor burden and end-organ damage may provide clinically relevant prognostic information.[11]

The ISS is still commonly used in clinical practice, largely because it is simple and easy to apply across different settings. In our cohort, however, it did not appear to fully capture the complexity of disease burden and organ involvement. This may partly explain why its prognostic effect was no longer evident after adjustment for DS stage in the multivariate model. Similar patterns have been described in previous studies, particularly in real-world cohorts, where the performance of ISS has been less consistent.[3,4]

Multiple myeloma shows marked biological diversity, with molecular and cytogenetic features influencing both the disease course and response to treatment.[12] In particular, high-risk cytogenetic abnormalities are known to be associated with poorer clinical outcomes.[13] It is well established that multiple myeloma is often preceded by precursor conditions such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma, which follow a stepwise progression pattern.[14,15] The incorporation of cytogenetic abnormalities into prognostic models, as seen in the Revised International Staging System (R-ISS), represents an important step toward more refined risk stratification. However, the applicability of such models may be limited by the availability of comprehensive cytogenetic testing in routine clinical practice.

Advanced molecular diagnostics are not always available in many centers, especially those with limited resources. In these situations, clinically based staging systems like DS may still be very useful because they are easy to use and understand. Our findings affirm the enduring significance of these conventional models, particularly in instances where biological data are insufficient or inaccessible.

Recent advancements in treatment, encompassing autologous stem cell transplantation and the introduction of innovative therapeutic agents like proteasome inhibitors and immunomodulatory drugs, have significantly enhanced survival outcomes in patients with multiple myeloma.[16–18] Despite these therapeutic advances, baseline disease characteristics remain critical determinants of prognosis, underscoring the importance of accurate staging at diagnosis.

Frailty, age, and comorbidities also play an important role in determining outcomes, particularly in elderly patients.[19] These patient-related factors may influence both treatment selection and survival independently of disease stage. Therefore, integrating clinical staging with patient-specific factors may provide a more comprehensive approach to prognostic assessment.

Another significant finding in our study is the inconsistency noted among various staging systems, especially in advanced-stage disease. There is a general consensus in early-stage classifications; however, discrepancies become more evident in high-risk groups. This indicates that various staging methodologies may represent distinct facets of the disease's biology and clinical impact.

From a clinical perspective, these findings offer important practical implications. In routine clinical practice, where rapid decision-making is necessary and access to advanced diagnostic methods may be limited, simple and easily applicable staging systems like DS continue to provide valuable guidance.[20] Clinicians may benefit from using traditional staging systems in conjunction with modern prognostic tools to obtain a balanced and clinically relevant risk assessment. Newer approaches, such as the second revision of the International Staging System (R2-ISS), have been developed to make prognostic classification in multiple myeloma more precise.[21,22] In addition, some genomic profiles, defined as "double-hit" multiple myeloma, have been shown to be associated with a poor prognosis and a more aggressive disease course.[23] Minimal residual disease (MRD) status also stands out as a strong predictor of long-term outcomes in multiple myeloma.[24] Furthermore, patient-specific factors such as frailty and geriatric assessment have been reported to have a significant impact on survival outcomes, particularly in elderly patients.[25] When all these findings are considered together, it appears that while the importance of biology-based prognostic models is increasing, classical clinical staging systems—particularly the Durie–Salmon system—retain their status as a reliable source of prognostic information in routine clinical practice. The use of these systems is especially important in real-life situations where comprehensive molecular data are not always available.

Study Limitations

Several factors should be considered when interpreting these findings. The study was conducted retrospectively, which introduces an inherent risk of selection bias. The patient population was relatively limited and drawn from a single center, which may influence how broadly the results can be generalized. In addition, complete cytogenetic and molecular data were not consistently available across all cases, restricting a more detailed assessment of biologically based risk models. Variations in treatment practices among patients may also have affected survival outcomes and were not fully captured in this analysis.

Conclusion

Our results show that the Durie–Salmon (DS) staging sys-

tem remains closely related to overall survival in patients with multiple myeloma. Compared with ISS and mSMART, DS seemed to better identify patients with more advanced disease and less favorable outcomes.

While biologically driven risk models are becoming more common in clinical practice, traditional staging systems still have a clear role in everyday decision-making, particularly in settings with limited access to advanced molecular testing where clinicians rely on routinely available clinical data.

Expanding the evidence base with larger and more diverse patient cohorts may help clarify how clinical staging can be more effectively combined with emerging biology-based approaches in multiple myeloma.

Disclosures

Ethics Committee Approval: The study protocol was reviewed by the local Health Science University Haseki Education and Research Hospital Clinical Reserach Ethics Committee. According to the committee's decision (Approval No.: 298, Date: 02.12.2015), formal ethics committee approval was deemed unnecessary due to the retrospective nature of the study.

Informed Consent: Informed consent was waived due to the retrospective nature of the study.

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Research Article

Ischemia-Modified Albumin as a Predictor of Anemia in Hemodialysis Patients: An Exploratory Cross-Sectional Study

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Abstract

Objectives: Ischemia-modified albumin (IMA) is a biomarker of oxidative stress. Its relationship with anemia in hemodialysis (HD) patients remains unclear. This study aimed to evaluate the association between IMA and hemoglobin (Hb) in HD patients and to determine whether IMA can predict anemia.

Methods: This cross-sectional study enrolled 85 maintenance HD patients. IMA was measured using the albumin cobalt-binding assay. Anemia was defined as Hb<11 g/dL according to the KDIGO guidelines.

Results: IMA correlated inversely with Hb (r=–0.341; p=0.001). Multivariate linear regression identified Hb (β=–0.033; p=0.002) and ultrafiltration volume (β<0.001; p=0.001) as independent predictors of IMA (adjusted R^2=0.43). ROC analysis yielded an optimal IMA cutoff of ≥0.635 absorbance units (ABSU) (AUC=0.700; 95% CI, 0.582–0.817; p=0.002; sensitivity, 61.3%; specificity, 63.0%). In logistic regression, IMA≥0.635 ABSU (OR, 7.21; p=0.037) and EPO resistance (OR, 14.09; p=0.002) were independent predictors of anemia.

Conclusion: IMA levels were significantly elevated in anemic HD patients. An IMA cutoff of ≥0.635 ABSU showed moderate predictive capacity for anemia, suggesting its potential as a supplementary biomarker. Larger prospective studies are needed for validation.

Keywords: Anemia, chronic kidney disease, erythropoietin resistance, hemodialysis, ischemia-modified albumin, oxidative stress

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The worldwide burden of chronic kidney disease (CKD) continues to increase, with population-based surveys estimating that 10%–16% of adults are affected globally.[1,2] When kidney function deteriorates to end-stage kidney disease (ESKD), patients become dependent on renal replacement therapy. Among the available modalities, hemodialysis (HD) is the predominant treatment approach in numerous healthcare systems.[3]

Anemia represents one of the most prevalent and clinically consequential complications encountered in the CKD pop-

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ulation, affecting over 90% of individuals with advanced renal impairment and approximately 70% of those undergoing HD.[4] Deficient erythropoietin (EPO) synthesis by the failing kidneys constitutes the principal mechanism, although additional contributors—namely, iron depletion, chronic systemic inflammation, secondary hyperparathyroidism, and the accumulation of uremic solutes—also play important roles.[5] Notably, all of these pathways converge on enhanced oxidative stress, which has gained increasing recognition as a key driver in the pathobiology of uremic anemia. From a clinical standpoint, anemia in CKD impairs cardiac function, diminishes exercise tolerance and cognitive ability, reduces overall well-being, and independently predicts adverse outcomes, including higher rates of hospitalization and death.[6–8]

Ischemia-modified albumin (IMA) arises when the metal-binding capacity at the amino-terminal end of human serum albumin is compromised. Cobalt, nickel, and copper normally bind at this site; however, exposure to oxidative radicals, ischemic insult, hypoxic conditions, an acidotic milieu, or direct free-radical attack alters the N-terminal structure and reduces its affinity for these transition metals.[9,10] Following an ischemic episode, circulating IMA concentrations increase rapidly—within minutes—and remain elevated for roughly 6–12 hours before normalizing over 24 hours, positioning IMA as a responsive early indicator of tissue-level ischemia.[11] IMA was first shown by Sinha et al.[11] to detect myocardial ischemia before frank necrosis in the acute coronary setting and has subsequently been found to rise in diverse noncardiac disorders in which tissue oxygen delivery is compromised, such as cerebrovascular events, diabetes mellitus, and peripheral arterial disease.[12,13] Specifically within the ESKD population, Sharma et al.[14] published the earliest investigation of IMA in dialysis patients and reported that higher IMA values predicted mortality while correlating with unfavorable left ventricular structural changes. Turedi et al.[15] later confirmed that IMA concentrations measured both before and after a dialysis session exceeded those of age-matched healthy individuals. Collectively, these observations positioned IMA as a promising oxidative stress indicator warranting further study in the HD context.

A growing body of data indicates that the tissue-level oxygen deficit caused by low Hb concentrations may drive IMA elevation through enhanced oxidative modification at the albumin N-terminus.[16–18] Nevertheless, IMA has been examined in relation to anemia in the HD population by only a handful of investigators using relatively small cohorts, and a clinically useful threshold for identifying anemic patients has yet to be defined.

The present study was therefore designed to investigate whether circulating IMA concentrations correlate with Hb values in maintenance HD patients, to delineate the variables that independently determine IMA levels, and to assess the capacity of IMA to function as a predictive marker of anemia in this clinical setting.

Methods

Study Design and Population

A cross-sectional investigation was carried out in the Hemodialysis Unit affiliated with the Division of Nephrology at a tertiary university hospital in Ankara, Türkiye, from September through December 2019. The primary objective was to examine the interplay between circulating IMA concentrations, Hb values, and other routine biochemical measures in CKD patients maintained on HD.

Adults (≥18 years) with a diagnosis of CKD who were actively receiving HD, possessed a complete set of routine laboratory values dated within the previous 60 days, and consented to participation were deemed eligible. The following conditions led to exclusion: age below 18 years, unavailability of recent laboratory data, unwillingness to consent, a personal history of any malignant neoplasm, an active systemic inflammatory condition (e.g., systemic lupus erythematosus), or a confirmed episode of infection or acute cardiovascular compromise during the 3 months preceding enrollment. Eighty-five patients satisfied these criteria and were included in the analysis.

Ethical clearance was granted by the institutional clinical research ethics committee (Decision No. 2012-KAEK-15/2042, dated January 8, 2020). Every participant received a thorough verbal and written explanation of the study procedures, after which each individual provided signed informed consent.

Data Collection

Blood for IMA determination was drawn through routine venipuncture performed just before the dialysis session; no additional needle insertion was required. Five milliliters of whole blood were collected into a plain (red-top) vacutainer from each individual. Specimens were left undisturbed at ambient temperature for 20–30 minutes to allow clotting and were then centrifuged at 2000×g for 10 minutes. The resulting serum aliquots were transferred to cryovials and maintained at −80 °C pending batch analysis. All preanalytical processing was conducted in partnership with the university's allied health sciences teaching laboratory.

The following analytes were abstracted retrospectively from each patient's most recent routine workup, obtained

within 60 days of the IMA sampling date: hemoglobin (Hb), hematocrit (Htc), total leukocyte count (WBC), platelet count (PLT), blood urea nitrogen (BUN), serum creatinine, calcium, phosphorus, uric acid, albumin, C-reactive protein (CRP), serum iron (Fe), total iron-binding capacity (TIBC), transferrin saturation (TSAT), ferritin, vitamin D, parathyroid hormone (PTH), glycated hemoglobin (HbA1c), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides.

Baseline demographic and clinical information was documented for each participant: age, sex, body mass index (BMI), tobacco use, comorbid conditions (diabetes mellitus, hypertension, coronary artery disease, peripheral artery disease, and cerebrovascular disease), type of dialysis access, presence or absence of residual kidney function, adequacy indices, EPO prescription and cumulative dose, parenteral iron supplementation history, and antihypertensive regimen.

IMA Measurement

Circulating IMA was quantified using the albumin cobalt-binding (ACB) test according to the protocol of Bar-Or et al.[9] In brief, each serum specimen was mixed with a cobalt chloride ($CoCl_2$) solution, after which dithiothreitol (DTT) and physiological saline (0.9% NaCl) were introduced. The optical density of the final reaction mixture was measured at 470 nm using a spectrophotometer. Values are reported in absorbance units (ABSU); the accepted reference interval for healthy individuals is 0–0.5 ABSU.

Definitions

A hemoglobin concentration below 11 g/dL was used to classify anemia, in accordance with the lower maintenance target endorsed by the Kidney Disease: Improving Global Outcomes (KDIGO) practice guidelines.[19] The erythropoietin resistance index (ERI) was derived by dividing the weight-adjusted weekly EPO dose (U/kg/week) by the concurrent hemoglobin level (g/dL).[20] For exploratory subgroup evaluation, EPO-treated patients were categorized into three ERI strata: <5, 5–15, and >15 U/kg/week per g/dL. Dialysis adequacy was gauged using Kt/V and the urea reduction ratio (URR).

Statistical Analysis

All computations were performed using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The normality of the distribution of continuous measures was assessed through graphical inspection (histograms and Q-Q plots), supplemented by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Because all continuous variables departed from normality, they are reported as the mean±standard deviation, alongside the median and range (minimum–maximum). Categorical data are presented as absolute counts and proportions. Between-group differences in continuous outcomes were tested using the Mann-Whitney U test; categorical comparisons were performed using the chi-square test. The strength and direction of bivariate associations between IMA and laboratory indices were estimated using Spearman rank-order correlation coefficients.

Candidate predictors reaching $p<0.10$ in univariate testing were carried forward into a multivariable linear regression model; age, sex, cumulative EPO exposure, and coronary artery disease status were forced into the model as clinically plausible confounders. A receiver operating characteristic (ROC) curve was then constructed to evaluate whether IMA could discriminate between anemic and nonanemic patients, with calculation of the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Guided by the ROC-derived threshold, patients were recategorized and entered into a multivariable logistic regression analysis, adjusted for age and sex, to obtain odds ratios (ORs) for potential independent determinants of anemia. Two-sided p values below 0.05 were deemed statistically significant.

Results

Patient Characteristics

Eighty-five individuals constituted the final analytic sample: 50 men (58.8%) and 35 women (41.2%), with a mean age of 57.01±18.04 years and a mean BMI of 24.95±5.32 kg/m^2. Hypertension was the leading comorbidity (49.4%), followed by coronary artery disease (28.2%) and diabetes mellitus (18.8%). Thirty-two patients (37.6%) had a documented history of vascular disease, encompassing coronary artery disease (n=24), peripheral artery disease (n=11), and cerebrovascular disease (n=4). Vascular access consisted of an arteriovenous fistula (AVF) in 63 cases (74.1%) and a central venous catheter (CVC) in 22 cases (25.9%). Residual kidney function was detectable in 26 patients (30.6%), and 54 (63.5%) were receiving EPO therapy. The mean Hb level was 11.06±1.62 g/dL; 31 participants (36.5%) fulfilled the anemia criterion (Hb<11 g/dL). The cohort's mean IMA value was 0.61±0.15 ABSU (Table 1).

Correlations Between IMA and Laboratory Parameters

IMA exhibited a statistically significant inverse association with Hb ($r=-0.341$; $p=0.001$; Fig. 1) and total leukocyte count ($r=-0.235$; $p=0.030$). A direct relationship was noted between IMA and ferritin ($r=0.232$; $p=0.039$), whereas an inverse association emerged with CRP ($r=-0.279$; $p=0.011$).

Table 1. Baseline demographic, clinical, and laboratory characteristics of the study cohort (n = 85)

Characteristic	Value
Male sex, n (%)	50 (58.8)
Age (years), mean±SD	57.01±18.04
BMI (kg/m^2), mean±SD	24.95±5.32
Diabetes mellitus, n (%)	16 (18.8)
Hypertension, n (%)	42 (49.4)
Coronary artery disease, n (%)	24 (28.2)
Peripheral artery disease, n (%)	11 (12.9)
Cerebrovascular disease, n (%)	4 (4.7)
HD access: AVF, n (%)	63 (74.1)
Residual renal function, n (%)	26 (30.6)
EPO use, n (%)	54 (63.5)
Hemoglobin (g/dL), mean±SD	11.06±1.62
Hematocrit (%), mean±SD	33.99±5.03
IMA (ABSU), mean±SD	0.61±0.15
Serum albumin (g/dL), mean±SD	3.78±0.36
Ferritin (ng/mL), mean±SD	429.65±273.71
CRP (mg/dL), mean±SD	10.39±12.15
Mean UF volume (mL), mean±SD	2787±1059

AVF: Arteriovenous fistula; BMI: Body mass index; CRP: C-reactive protein; EPO: Erythropoietin; HD: Hemodialysis; IMA: Ischemia-modified albumin; UF: Ultrafiltration.

The strongest bivariate association observed was between IMA and mean ultrafiltration (UF) volume (r=0.658; p<0.001). No meaningful correlations were identified between IMA and age, BMI, albumin, calcium, phosphorus, creatinine, uric acid, iron, TIBC, TSAT, PTH, HbA1c, lipid fractions, Kt/V, URR, cumulative EPO dose, or ERI (all p>0.05; Table 2).

IMA Levels According to Clinical Subgroups

AVF users demonstrated significantly higher IMA concentrations than CVC users (p=0.015). Conversely, patients who developed intradialytic hypertension had lower IMA values (p=0.022). Among EPO-treated individuals stratified by ERI, those in the highest category (ERI>15 U/kg/week per g/dL) exhibited the greatest IMA concentrations relative to the other two strata (p=0.038). Anemic participants (Hb<11 g/dL) had markedly elevated IMA levels compared with their nonanemic counterparts (p=0.002). Sex, tobacco use, diabetes, hypertension, coronary artery disease, peripheral artery disease, cerebrovascular disease, residual kidney function, and medication categories showed no significant influence on IMA (all p>0.05).

Table 2. Spearman correlation analysis between IMA and selected laboratory and clinical parameters

Parameter	r	p
Hemoglobin (g/dL)	−0.341	0.001*
WBC (K/µL)	−0.235	0.030*
CRP (mg/dL)	−0.279	0.011*
Ferritin (ng/mL)	0.232	0.039*
Mean UF volume (mL)	0.658	<0.001*
Hematocrit (%)	−0.119	0.276
BUN (mg/dL)	−0.011	0.917
Creatinine (mg/dL)	0.053	0.629
Serum albumin (g/dL)	0.098	0.373
Iron (µg/dL)	0.079	0.485
TIBC (µg/dL)	0.005	0.967
TSAT (%)	0.062	0.583
PTH (pg/mL)	0.063	0.567
Kt/V	0.172	0.120
Cumulative EPO dose (IU)	0.163	0.136

* Statistically significant (p<0.05). BUN: Blood urea nitrogen; CRP: C-reactive protein; EPO: Erythropoietin; IMA: Ischemia-modified albumin; PTH: Parathyroid hormone; TIBC: Total iron-binding capacity; TSAT: Transferrin saturation; UF: Ultrafiltration; WBC: White blood cell.

Independent Predictors of IMA

After multivariable adjustment for age, sex, cumulative EPO dose, and coronary artery disease, Hb (β=−0.033; p=0.002) and mean UF volume (β<0.001; p=0.001) emerged as the sole independent determinants of IMA (adjusted R^2=0.43; model p<0.001; Table 3). The negative β coefficient for Hb indicates that declining hemoglobin levels are accompanied by rising IMA levels, a pattern congruent with the notion that anemia-driven hypoxia accelerates the oxidative modification of albumin.

IMA as a Predictor of Anemia: ROC Analysis

The ROC curve yielded an AUC of 0.700 (95% CI, 0.582–0.817; p=0.002), confirming that IMA possesses a statistically meaningful ability to distinguish anemic from nonanemic HD patients. At the optimal threshold of ≥0.635 ABSU, sensitivity reached 61.3%, specificity 63.0%, PPV 48.7%, and NPV 73.9% (Fig. 2).

Multivariate Logistic Regression for Anemia

Patients were then dichotomized at the ROC-derived IMA threshold, and predictors of anemia were reassessed. In the age- and sex-adjusted logistic model, two variables attained independent significance: IMA≥0.635 ABSU (OR, 7.21; 95% CI, 1.12–46.19; p=0.037) and EPO resistance (OR,

Table 3. Multivariate linear regression analysis for independent predictors of IMA levels (adjusted R^2=0.43; model p<0.001)

Variable	β coefficient	p
Hemoglobin (g/dL)	−0.033	0.002*
Mean UF volume (mL)	<0.001	0.001*
WBC (K/μL)	<−0.001	0.449
CRP (mg/dL)	−0.002	0.141
Ferritin (ng/mL)	<0.001	0.873
HD access type (AVF vs. CVC)	0.066	0.084
Vitamin D use	−0.035	0.291
Intradialytic hypertension	0.005	0.920

Adjusted for age, sex, cumulative EPO dose, and coronary artery disease status. * Statistically significant. AVF: Arteriovenous fistula; CRP: C-reactive protein; CVC: Central venous catheter; HD: Hemodialysis; UF: Ultrafiltration; WBC: White blood cell.

14.09; 95% CI, 2.69–73.95; p=0.002) (Table 4). Mean UF volume did not retain significance in this model (p=0.280).

Table 4. Multivariate logistic regression analysis for independent predictors of anemia (Hb < 11 g/dL)

Variable	OR (95% CI)	p
IMA ≥0.635 (ref: <0.635)	7.21 (1.12–46.19)	0.037*
EPO resistance (ref: ERI < 5)	14.09 (2.69–73.95)	0.002*
Mean UF volume	0.98 (0.94–1.02)	0.280

Adjusted for age and sex. * Statistically significant. CI: Confidence interval; EPO: Erythropoietin; ERI: Erythropoietin resistance index; IMA: Ischemia-modified albumin; OR: Odds ratio; UF: Ultrafiltration.

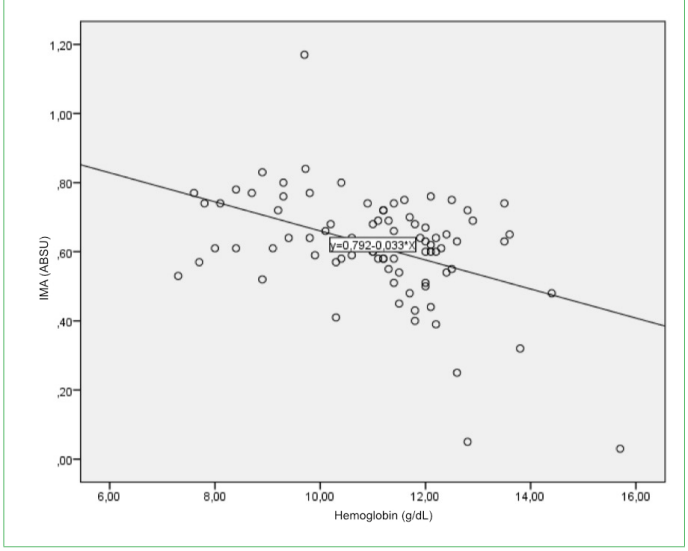

Figure 1. Scatter plot depicting the correlation between hemoglobin (Hb) and ischemia-modified albumin (IMA) levels in hemodialysis patients (r = -0.341; P = 0.001). The regression line equation: y = 0.792 - 0.033x.

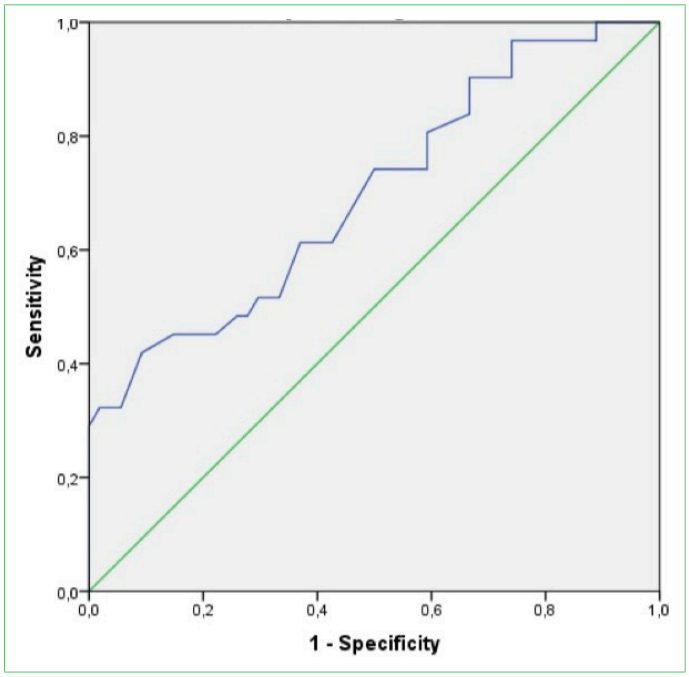

Figure 2. Receiver operating characteristic (ROC) curve for ischemia-modified albumin (IMA) as a predictor of anemia (hemoglobin < 11 g/dL) in hemodialysis patients. The area under the curve (AUC) was 0.700 (95% CI, 0.582-0.817; P = 0.002). The optimal cut-off value of ≥0.635 ABSU yielded a sensitivity of 61.3% and specificity of 63.0%.

Discussion

The present analysis of 85 maintenance HD patients revealed a robust inverse relationship between circulating IMA and Hb. After controlling for relevant confounders, Hb remained a significant independent determinant of IMA. ROC-based evaluation further established that an IMA threshold of ≥0.635 ABSU discriminated between anemic and nonanemic patients with moderate accuracy, conferring a 7.2-fold increase in anemia risk in multivariate logistic analysis.

These observations align with the work of Cichota et al.[17], who documented higher IMA concentrations and a negative IMA–Hb correlation (r=−0.344; p=0.040) in CKD patients with anemia than in healthy volunteers. In a peritoneal dialysis cohort, Su et al.[21] likewise identified an inverse IMA–Hb association (r=−0.71; p=0.024). Importantly, neither of these studies detected meaningful associations between IMA and serum calcium, phosphorus, creatinine, or uric acid—a pattern mirrored in our data. Taken together, these concordant findings reinforce the concept that diminished Hb impairs tissue oxygen delivery, thereby accelerating oxidative alteration at the albumin N-terminus and augmenting IMA generation.

One particularly noteworthy result was the strong direct association between IMA and mean UF volume (r=0.658;

p<0.001). Despite reaching statistical significance as an independent predictor in linear regression, the negligible magnitude of the UF coefficient (β<0.001) suggests limited clinical impact. Nonetheless, high intradialytic fluid removal may trigger transient intravascular volume contraction and relative tissue hypoperfusion, potentially explaining the observed link. To the best of our knowledge, no prior report has explored this specific IMA–UF relationship, and the finding merits confirmation in dedicated studies.

The direct correlation observed between IMA and ferritin (r=0.232; p=0.039) echoes the report of Awadallah et al.[16] in beta-thalassemia major. Elevated ferritin may signal excess body iron stores, which, in turn, fuel Fenton chemistry, generating hydroxyl radicals capable of oxidatively modifying albumin. Moreover, ESKD is characterized by a well-documented triad of compromised antioxidant defenses, heightened reactive oxygen species production, and chronic tissue hypoxia. Together, these derangements offer a coherent mechanistic framework for the elevated IMA concentrations observed in our cohort. Chronic anemia, sustained hypoxia, and iron-driven oxidative damage thus likely act in concert to modify the albumin N-terminus and promote IMA formation in patients with advanced kidney disease.

An unanticipated observation was the negative association between IMA and CRP (r=−0.279; p=0.011). This finding runs counter to the positive IMA–CRP relationship documented by Duarte et al.[22] in hypercholesterolemic subjects. However, Su et al.[21] similarly detected no meaningful correlation between IMA and high-sensitivity CRP in peritoneal dialysis patients. The discordance may stem from the complex bidirectional interaction between inflammation and oxidative stress in the uremic milieu, where inflammation-associated hypoalbuminemia could confound IMA interpretation. A further consideration is that our facility measured standard rather than high-sensitivity CRP, potentially underestimating subclinical inflammatory activity and contributing to the divergent result.

IMA concentrations were paradoxically higher in patients with AVF access than in those with CVC access (p=0.015), contrasting with data from Dukkipati et al.[23] and Banerjee et al.[24], who observed a greater inflammatory burden in catheter-dependent patients. The pathophysiological basis of this unexpected finding warrants further clarification.

A novel aspect of our work is the observation that patients with the highest degree of EPO resistance (ERI>15 U/kg/week per g/dL) displayed the greatest IMA levels (p=0.038). No previous investigation has directly stratified IMA across ERI categories. The finding is, however, pathophysiologically plausible: EPO hyporesponsiveness in HD patients is closely intertwined with chronic inflammation and oxidative stress, both of which favor IMA generation. Macdougall et al.[25] demonstrated that poor EPO responsiveness was accompanied by higher CRP and interleukin-6 concentrations, implying that inflammatory mediators simultaneously blunt erythroid proliferation and foster oxidative albumin modification. ERI may therefore function as a clinical surrogate for the underlying pro-oxidant environment that concurrently undermines erythropoiesis and elevates IMA.

In line with earlier observations by Sinha et al.[11] and Christenson et al.[26], neither age nor sex influenced IMA concentrations in our cohort. The transient pharmacokinetics of IMA—a rapid postischemic rise followed by normalization within a day—likely render it insensitive to fixed demographic traits.

The IMA threshold of ≥0.635 ABSU derived from our ROC analysis—although yielding only moderate sensitivity (61.3%) and specificity (63.0%)—constitutes the first such cutoff proposed specifically for CKD-associated anemia. Previous ROC-based IMA thresholds have targeted myocardial ischemia and hematologic malignancy.[11,18,27] Notably, the 0.64 ABSU cutoff reported by Erkut et al.[18] for acute leukemia (AUC=0.846; sensitivity, 65.9%; specificity, 91.7%) is strikingly similar to our value, despite being derived from a fundamentally different clinical context. No analogous threshold existed for anemia in the dialysis literature before the present study.

Several methodological constraints deserve mention. First, the cross-sectional framework prevents any causal attribution in the IMA–anemia relationship. Second, the single-center design and limited sample size (n=85) restrict external applicability and account for the broad confidence intervals in our logistic model. Third, without a healthy reference group, absolute IMA levels could not be benchmarked against those of the nonuremic population. Fourth, IMA was measured only before the dialysis session; postdialysis values, which can differ appreciably,[15] were not captured. Fifth, high-sensitivity CRP was unavailable; reliance on the standard assay may have masked low-grade inflammation.

Despite these limitations, the study furnishes preliminary evidence supporting a role for IMA as an ancillary biomarker in evaluating oxidative stress related to anemia in the HD setting.

Conclusion

IMA concentrations are substantially higher in HD patients with anemia and display an inverse relationship with Hb, most likely reflecting hypoxia-driven oxidative modification of the albumin molecule. A threshold of ≥0.635 ABSU afforded moderate discrimination of anemia in our cohort,

pointing to a potential supplementary role for IMA in clinical assessment. Relatively few studies have addressed this association, and none have previously proposed a specific diagnostic threshold. Prospective investigations enrolling larger, multisite cohorts are warranted to confirm the proposed cutoff and to delineate the added prognostic contribution of IMA in HD risk stratification.

Disclosure

Ethics Committee Approval: This study was approved by the institutional clinical research ethics committee (Decision No. 2012-KAEK-15/2042, dated January 8, 2020).

Informed Consent: Written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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Research Article

Investigation of the Clinical and Demographic Characteristics of Our Patients With Breast Cancer Diagnosed Before the Age of 40

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Abstract

Objectives: Breast cancer diagnosed before the age of 40 accounts for 7% of all cases and is associated with aggressive disease. This study aimed to investigate the clinical, demographic, and pathological characteristics of young patients with breast cancer in Türkiye.

Methods: A retrospective analysis was conducted on 73 patients diagnosed with breast cancer before the age of 40 at Necmettin Erbakan University between 2011 and 2020. Demographic data, clinical presentation, pathology, treatment, and recurrence/metastasis were analyzed.

Results: All patients were female, with a mean age of 30.9±4.1 years. The most common symptom was a palpable breast mass (61.6%). Invasive ductal carcinoma was predominant (87.7%). The molecular subtypes were as follows: luminal, 65.8%; HER2-positive, 20.5%; and triple-negative, 13.7%. Metastasis at diagnosis occurred in 21.9% of patients, and recurrence during follow-up occurred in 23.3%. In the luminal subgroup, recurrence was more common than metastasis (82.4% vs. 43.8%, $p<0.05$). In the HER2-positive subgroup, metastasis was more common than recurrence (56.3% vs. 5.9%, $p<0.05$). Among the 13 patients tested for BRCA mutations, 3 (23%) had pathogenic variants.

Conclusion: Young Turkish patients with breast cancer showed high rates of aggressive molecular subtypes. The different recurrence and metastasis patterns between the luminal and HER2-positive subtypes suggest the need for subtype-specific follow-up strategies.

Keywords: Breast cancer, clinical characteristics, Türkiye, under 40, young

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Breast cancer is the most frequently diagnosed cancer among women worldwide, with an estimated 2.3 million new cases in 2020.[1] Although breast cancer predominantly affects older women, approximately 7% of newly diagnosed cases occur in women under 40 years of age.[2] In Türkiye, breast cancer accounts for 23.9% of all female cancers.[3]

Young-onset breast cancer presents unique clinical challenges. These patients are not included in routine screening programs, leading to delayed diagnosis and a more advanced stage at presentation.[4] A young age at diagnosis is associated with more aggressive tumor biology, a higher grade, greater lymph node involvement, and a poorer prognosis.[5,6]

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Table 1. Demographic and clinical characteristics (N=73)

Characteristic	n (%)
Mean age (years, ±SD)	30.9±4.1
Married	66 (90.4)
Oral contraceptive use	10 (13.7)
IUD use	10 (13.7)
Nulliparous	11 (15.1)
Presenting symptom: breast mass	45 (61.6)
Tumor location: upper outer quadrant	42 (57.5)
Clinical T4	16 (21.9)
Axillary lymph node positive	43 (58.9)

SD: Standard deviation; IUD: Intrauterine device.

Table 2. Pathological and molecular characteristics

Characteristic	n (%)
Invasive ductal carcinoma	64 (87.7)
Grade 3	15 (20.5)
ER positive	46 (63.0)
PR positive	40 (54.8)
HER2 3+	26 (35.6)
Luminal	48 (65.8)
HER2-positive	15 (20.5)
Triple-negative	10 (13.7)

ER positive: Estrogen receptor-positive breast cancer; PR positive; Progesterone receptors-positive breast cancer; HER2: Human epidermal growth factor receptor 2.

Table 3. Metastasis and recurrence by molecular subtype

Subtype	Metastasis (n)	Recurrence (n)
Luminal	7 (43.8)	14 (82.4)
HER2-positive	9 (56.3)	1 (5.9)
Triple-negative	0 (0)	2 (11.8)
Total	**16 (100)**	**17 (100)**

HER2: Human epidermal growth factor receptor 2.

The molecular landscape of breast cancer in young women differs from that in older patients, with higher proportions of triple-negative and HER2-positive subtypes.[7,8] Genetic predisposition also plays a prominent role, with BRCA mutations accounting for a substantial proportion of early-onset cases.[9]

Data from Turkish populations remain limited. This study aimed to investigate the clinical, demographic, and pathological characteristics of patients with breast cancer diagnosed before the age of 40 at a tertiary care center in Türkiye.

Methods

Study design and population

This retrospective, single-center study was conducted at Necmettin Erbakan University Meram Faculty of Medicine, Department of Medical Oncology. The study protocol was approved by the Institutional Ethics Committee (approval date: 2022; approval no.: 2022/3665) and was conducted in accordance with the Declaration of Helsinki.

Patients were included if they: 1) were diagnosed with breast cancer between January 2011 and December 2020; 2) were under 40 years of age at diagnosis; 3) received at least part of their treatment at our institution; and 4) had complete medical records available.

Data Collection

Data were extracted from electronic medical records. The collected variables included demographic data, clinical presentation, imaging findings, pathological features (ER, PR, HER2, Ki-67, and grade), treatment modalities, and clinical outcomes (metastasis at diagnosis and recurrence during follow-up).

ER and PR positivity was defined as ≥1% nuclear staining. HER2 positivity was defined as 3+ staining by immunohistochemistry or 2+ staining with FISH amplification. Molecular subtypes were classified as luminal (ER/PR-positive and HER2-negative), HER2-positive, and triple-negative.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0. Categorical variables were compared using the chi-square test. Continuous variables were expressed as the mean±standard deviation. A p-value of <0.05 was considered statistically significant.

Results

Demographic characteristics

A total of 73 patients were included. All patients were female, with a mean age of 30.9±4.1 years (range: 20–39 years). At diagnosis, 90.4% (n=66) were married. Oral contraceptive use was reported in 13.7% (n=10), and intrauterine device (IUD) use was reported in 13.7% (n=10). The nulliparity rate was 15.1% (n=11) (Table 1).

Clinical presentation

The most common presenting symptom was a palpable breast mass (61.6%; n=45). Tumors were most frequently localized in the left breast (56.1%; n=41) and the upper outer quadrant (57.5%; n=42). The clinical T-stage distribution

was as follows: T1, 38.4% (n=28); T2, 38.4% (n=28); and T4, 21.9% (n=16). Axillary lymph node positivity was present in 58.9% (n=43) (Table 1).

Pathological and molecular characteristics

Invasive ductal carcinoma was the most common histological type (87.7%; n=64). The tumor grade was grade 2 in 31.5% (n=23) and grade 3 in 20.5% (n=15). ER positivity was observed in 63.0% (n=46), and PR positivity was observed in 54.8% (n=40). HER2 3+ expression was observed in 35.6% (n=26). The molecular subtype distribution was as follows: luminal, 65.8% (n=48); HER2-positive, 20.5% (n=15); and triple-negative, 13.7% (n=10) (Table 2).

Metastasis, recurrence, and molecular subtypes

Metastatic disease at diagnosis was present in 21.9% (n=16). Recurrence during follow-up developed in 23.3% (n=17). In the luminal subgroup, recurrence was significantly more frequent than metastasis (82.4% vs. 43.8%, $p<0.05$). In the HER2-positive subgroup, metastasis was significantly more frequent than recurrence (56.3% vs. 5.9%, $p<0.05$) (Table 3).

BRCA mutations

BRCA1/2 mutation analysis was performed in 13 patients (17.8%). Pathogenic variants were detected in 3 patients (23%).

Discussion

In this study, we described the clinical, demographic, and pathological characteristics of 73 patients diagnosed with breast cancer before the age of 40 in Türkiye. The mean age of 30.9 years is consistent with the literature.[2,8]

The finding that a palpable breast mass was the most common presenting symptom (61.6%) reflects the absence of routine screening in this age group.[4,10] The 87.7% rate of invasive ductal carcinoma is consistent with previous reports.[11,12]

Our triple-negative rate (13.7%) was lower than those reported in Western series (20%–30%).[7,8], which may be due to the sample size, genetic differences, or environmental factors. HER2 positivity (20.5%) was consistent with the literature.[7]

A striking finding was the difference in recurrence and metastasis patterns between the luminal and HER2-positive subgroups. The higher recurrence rate in luminal patients suggests the need for closer long-term follow-up. The higher rate of metastasis at diagnosis in HER2-positive patients indicates a more aggressive initial course, although current anti-HER2 therapies appear to be effective.[13,14]

The prevalence of BRCA mutations (23% among those tested) emphasizes the importance of genetic counseling in young patients.[9,15]

Study limitations

The limitations of this study include its single-center, retrospective design, small sample size, and high rates of missing data for tumor grade (45.2%) and Ki-67 (61.6%). BRCA testing was performed in a limited number of patients.

Conclusion

Young Turkish patients with breast cancer showed high rates of aggressive molecular subtypes. The different recurrence and metastasis patterns between the luminal and HER2-positive subgroups suggest the need for subtype-specific surveillance strategies. Larger multicenter studies are needed.

Disclosure

Ethical Committee Approval: This retrospective, single-center study was conducted at Necmettin Erbakan University Meram Faculty of Medicine, Department of Medical Oncology. The study protocol was approved by the Institutional Ethics Committee (approval date: 2022; approval no: 2022/3665) and conducted in accordance with the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all study participants before enrollment.

Conflict of Interest: The authors declare no conflict of interest.

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Research Article

Detection of Antibiotic Resistance by Phenotypic and Genotypic Methods in Helicobacter Pylori and its Association with Genotypes

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Abstract

Objectives: This study aimed to determine the prevalence of Helicobacter pylori infection in patients with dyspeptic symp-toms, to evaluate antibiotic resistance rates using both phenotypic and genotypic methods, and to investigate the association between resistance and the presence of cagA and vacA virulence genotypes.

Methods: A total of 103 consecutive patients (64 female, 39 male; mean age 47.7 +/- 15.9 years) who underwent upper gastrointestinal endoscopy for dyspepsia were included in this cross-sectional study. Biopsy samples were obtai-ned from both the antrum and corpus of each patient. *H. pylori* culture was performed on selective media under microaerophilic conditions. Antibiotic susceptibility testing for amoxicillin, clarithromycin, tetracycline, and le-vofloxacin was conducted using the E-test method. DNA extracted from biopsy samples was amplified by polymerase chain reaction (PCR) using specific primers targeting the glmM, cagA, and vacA genes. Clarith-romycin resistance was confirmed by PCR-restriction fragment length polymorphism (PCR-RFLP) targeting the 23S rRNA gene. Levofloxacin resistance was confirmed by DNA sequence analysis of the gyrA gene. Histopat-hological examination was performed using Giemsa staining.

Results: *H. pylori* positivity was detected in 76 of 103 patients (73.8%) by glmM PCR, whereas culture positivity was observed in only 58 patients (56.3%), indicating significantly higher sensitivity of PCR ($p < 0.001$). Among the 58 culture-positive isolates, E-test resistance rates were as follows: amoxicillin 1.7% (1/58), clarithromycin 10.3% (6/58), tetracycline 0% (0/58), and levofloxacin 6.9% (4/58). No statistically significant association was found between antibiotic resistance and patient gender or endoscopic findings ($p > 0.05$). Among the 76 PCR-positive samples, the cagA gene was positive in 46 (60.5%) and the vacA gene was positive in 73 (96.0%). The ca-gA+/vacA+ (Type I) genotype was present in 46 patients (60.5%). No significant association was detected between antibiotic resistance and the presence of cagA or vacA genotypes ($p > 0.05$). Molecular analysis revea-led that among clarithromycin-resistant isolates, the A2143G mutation was present in 71.4% (5/7) and the A2142G mutation in 28.6% (2/7). All four levofloxacin-resistant isolates harbored gyrA point mutations: Asp-91 in three isolates (75%) and Asn-87 in one isolate (25%).

Conclusion: The clarithromycin resistance rate (10.3%) in the Kahramanmaraş region remains within the range reported in European countries and below the 15% threshold. However, levofloxacin resistance (6.9%) is emerging and requires careful monitoring. Amoxicillin and tetracycline resistance remain low. No association was found between cagA or vacA genotypes and antibiotic resistance. PCR-based methods demonstrated superior sensiti-vity compared to culture for *H. pylori* detection. Periodic regional surveillance of antibiotic resistance patterns is essential to guide appropriate empiric treatment and preserve the efficacy of *H. pylori* eradication regimens.

Keywords: *Helicobacter pylori*, antibiotic resistance, clarithromycin, levofloxacin, cagA, vacA

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More than half of the global population carries Helicobacter pylori in their stomachs. This Gram-negative, spiral-shaped, microaerophilic organism colonizes the gastric lining and serves as a primary cause of several upper gastrointestinal disorders, including persistent gastritis, peptic ulcers, gastric cancer, and MALT lymphoma.[1] Prevalence rates vary considerably between nations: developing countries report figures between 60% and 85%, whereas developed nations have seen declines to 10–30% owing to better sanitation and effective treatment protocols.[2] Turkish studies indicate an average infection rate of approximately 85%.[3]

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Standard first-line therapy for H. pylori combines a proton pump inhibitor with two antibiotics—amoxicillin and clarithromycin. Nevertheless, treatment fails in 15–40% of cases, largely because of emerging primary and acquired resistance, especially toward clarithromycin and levofloxacin.[4,5] According to the Maastricht III consensus, clarithromycin-based regimens should be administered cautiously in areas where resistance rates are elevated.[6]

Geographic differences in resistance patterns are substantial. European studies report clarithromycin resistance between 2% and 15%, while Turkish data show figures exceeding 27%.[7,8] Worldwide, resistance to amoxicillin and tetracycline remains uncommon (<1% and <0.5–9%, respectively).[7] Fluoroquinolone resistance, particularly to levofloxacin, is an increasing global concern.[9]

Among the bacterial factors influencing disease severity, cagA (cytotoxin-associated gene A) and vacA (vacuolating cytotoxin A) are the most extensively studied. Strains carrying cagA (designated Type I) have been linked to more serious clinical manifestations, including peptic ulcers and gastric malignancy.[10,11] Whether these virulence determinants correlate with antibiotic susceptibility, however, remains poorly understood.

The present investigation sought to assess H. pylori infection frequency, establish phenotypic and genotypic resistance profiles for amoxicillin, clarithromycin, tetracycline, and levofloxacin, and explore potential links between resistance and cagA/vacA genotypes among dyspeptic patients from the Kahramanmaraş province of Türkiye.

Methods

Study Population

This cross-sectional investigation enrolled 103 consecutive dyspeptic patients (64 women, 39 men; mean age 47.7±15.9 years) who underwent upper gastrointestinal endoscopy at Kahramanmaraş Sütçü İmam University Faculty of Medicine's Gastroenterology Clinic between January and June 2013. Individuals were excluded if they had previously received H. pylori eradication, used antibiotics or PPIs within 12 weeks before enrollment, were pregnant or lactating, had a history of gastroduodenal surgery, or reported allergies to the study antibiotics.

This study was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with decision number 2013/01-7 on January 29, 2013, and all participants provided written informed consent.

Endoscopy and Biopsy Collection

Upper gastrointestinal endoscopy was performed using a fiberoptic endoscope. Five biopsy samples were obtained from each patient: two from the antrum and two from the corpus (one each for culture and PCR) and one for histopathological examination. Biopsy samples for culture were placed in Brain Heart Infusion Broth containing 3% glycerol and transported to the microbiology laboratory within 2 hours.

Culture and Identification

After mechanical homogenization, biopsy materials were plated onto modified Columbia Agar enriched with 7% horse blood, Helicobacter selective supplement (Dent, Oxoid, Hampshire, England), and Vitox supplement (Oxoid). Plates were kept under microaerophilic conditions (5% O2, 10% CO2, 85% N2) at 37°C for 4–7 days. Colonies exhibiting suspicious growth were confirmed as H. pylori through Gram staining and positive oxidase, catalase, and urease reactions.[12]

Antibiotic Susceptibility Testing (E-test)

Susceptibility testing employed the E-test method (bioMérieux, France). A bacterial suspension matching McFarland 3 turbidity was spread onto Mueller-Hinton Agar supplemented with 7% horse blood. E-test strips for amoxicillin, clarithromycin, tetracycline, and levofloxacin were placed on the inoculated plates. Following microaerophilic incubation at 37°C for 3–5 days, MIC values were determined at the point where the inhibition ellipse intersected the strip. Resistance interpretation followed the British Society for Antimicrobial Chemotherapy (BSAC) 2013 criteria.[13]

DNA Extraction and PCR

The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from biopsy specimens according to the manufacturer's instructions. H. pylori presence was confirmed by amplifying the glmM gene (294 bp).[14] Detection of cagA (349 bp) and vacA (259/286 bp) was per-

formed using specific primer sets. PCR products underwent electrophoresis on 2% agarose gels and were visualized with ethidium bromide staining.

Molecular Detection of Clarithromycin Resistance (PCR-RFLP)

A 135 bp segment of the 23S rRNA gene was amplified. The resulting products were digested with *MboII* (to detect the A2142G mutation) and *Eco3II* (to detect the A2143G mutation) restriction enzymes (Fermentas, USA). Digestion patterns were resolved on 3.5% agarose gels.[15]

Molecular Detection of Levofloxacin Resistance (DNA Sequencing)

Amplification of a 428 bp fragment of the *gyrA* gene was carried out. Purified PCR products were sequenced using the Sanger dideoxy chain-termination method on an automated sequencer. Obtained sequences were aligned with the reference *H. pylori* 26695 strain.[16]

Histopathological Examination

Biopsy samples fixed in 10% formalin were paraffin-embedded, sectioned, and stained with Giemsa for microscopic evaluation of *H. pylori* and gastritis assessment.

Statistical Analysis

SPSS version 21.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Continuous variables are presented as mean ± standard deviation, while categorical variables are shown as numbers and percentages. Group comparisons employed Student's t-test for continuous data and the chi-square test for categorical data. Statistical significance was defined as $p<0.05$.

Artificial Intelligence Statement

During the preparation of this work, the authors used DeepSeek (a Large Language Model) for language editing, grammar correction, and formatting assistance. All AI-generated suggestions were reviewed, fact-checked, and approved by the corresponding author. No AI was used for data collection, statistical analysis, interpretation of results, or conclusion generation. The authors take full responsibility for the final content of the manuscript.

Results

Patient Demographics and H. pylori Positivity

The study group comprised 103 individuals, of whom 64 (62.1%) were female and 39 (37.9%) were male, with an average age of 47.7±15.9 years. *H. pylori* was detected in 56.3% (58/103) of patients by culture and in 73.8% (76/103) by *glmM* PCR, demonstrating that PCR is significantly more sensitive ($p<0.001$).

Endoscopic and Histopathological Findings

Among the 103 patients, 40 (38.9%) presented with gastric or duodenal ulcers or mucosal lesions (Group A), whereas 63 (61.1%) showed normal mucosa (Group B). *H. pylori* positivity rates were 75.0% (30/40) in Group A and 73.1% (46/63) in Group B, with no statistically significant difference ($p>0.05$). Histopathological examination identified *H. pylori* in 75 patients (72.8%).

Antibiotic Resistance Rates (Phenotypic)

Table 1 summarizes E-test results for the 58 culture-positive isolates. Resistance rates were as follows: amoxicillin 1.7% (1/58), clarithromycin 10.3% (6/58), tetracycline 0% (0/58), and levofloxacin 6.9% (4/58). No significant associations emerged between resistance and either patient sex or endoscopic group ($p>0.05$ for all comparisons).

Genotype Distribution

Among the 76 PCR-positive samples, *cagA* was positive in 46 (60.5%), and *vacA* was positive in 73 (96.0%). The combined *cagA*+/*vacA*+ (Type I) genotype occurred in 46 patients (60.5%). *cagA* positivity was 70.0% (21/30) in Group A versus 54.3% (25/46) in Group B ($p>0.05$) (Table 2).

Association Between Genotypes and Antibiotic Resistance

No statistically significant relationship was observed between antibiotic resistance and the presence of either *cagA* or *vacA* ($p>0.05$ for all comparisons) (Table 3).

Table 1. Antibiotic resistance rates determined by E-test (n=58)

Antibiotic	Susceptible n (%)	Resistant n (%)	Resistance rate (%)
Amoxicillin	57 (98.3)	1 (1.7)	1.7
Clarithromycin	52 (89.7)	6 (10.3)	10.3
Tetracycline	58 (100)	0 (0)	0
Levofloxacin	54 (93.1)	4 (6.9)	6.9

Table 2. Distribution of *cagA* and *vacA* genotypes according to endoscopic findings

Endoscopic Group	*cagA* Positive n (%)	*vacA* Positive n (%)
Group A (n=30)	21 (70.0)	29 (96.6)
Group B (n=46)	25 (54.3)	44 (95.6)
p	>0.05	>0.05

Group A: Patients with gastroduodenal ulcer/mucosal lesions; Group B: Patients with normal mucosa.

Table 3. Antibiotic resistance according to *cagA* and *vacA* status

Virulence Factor	Amoxicillin R	Clarithromycin R	Levofloxacin R
cagA Positive (n=46)	1 (2.2%)	4 (8.7%)	3 (6.5%)
cagA Negative (n=30)	0 (0%)	2 (6.7%)	1 (3.3%)
vacA Positive (n=73)	1 (1.4%)	6 (8.2%)	4 (5.5%)
vacA Negative (n=3)	0 (0%)	0 (0%)	0 (0%)

R: resistant; no statistically significant differences were observed (p>0.05 for all)

Table 4. Molecular characterization of antibiotic resistance mutations

Antibiotic	Mutation	n	Frequency (%)
Clarithromycin (n=7)	A2143G	5	71.4
	A2142G	2	28.6
Levofloxacin (n=4)	*gyrA* Asp-91	3	75.0
	gyrA Asn-87	1	25.0

Molecular Resistance Mechanisms

Of the seven clarithromycin-resistant isolates (six phenotypic plus one molecular), PCR-RFLP identified the A2143G mutation in five (71.4%) and the A2142G mutation in two (28.6%).[15] All four levofloxacin-resistant isolates carried *gyrA* point mutations: Asp-91 in three isolates (75%) and Asn-87 in one isolate (25%)[16] (Table 4).

Discussion

The present work offers a detailed examination of *H. pylori* infection frequency, antibiotic resistance patterns, and virulence genotype distribution within the Kahramanmaraş region of Turkey. The 73.8% positivity rate detected by PCR aligns with the elevated prevalence typical of developing countries and matches earlier Turkish investigations reporting figures between 60% and 85%.[17] PCR proved markedly more sensitive than culture (73.8% versus 56.3%, p<0.001), underscoring the advantage of molecular techniques for *H. pylori* detection, especially when bacterial viability may be compromised during specimen transport.[18]

The 10.3% clarithromycin resistance rate observed here falls within the 2–15% range documented across Europe and remains below the 15% threshold. This figure is substantially lower than the 27–56% reported in some recent Turkish studies.[19] Such discrepancies likely stem from regional differences in antibiotic utilization and highlight the necessity for local resistance monitoring. The predominance of the A2143G mutation (71.4%) among clarithromycin-resistant isolates concurs with earlier findings that this alteration is the most frequent cause of high-level macrolide resistance.[15]

The 6.9% levofloxacin resistance rate is a matter of concern, given that fluoroquinolone resistance has been rising worldwide due to extensive use of these agents across various infections.[9] Although this rate is lower than the 16.8–23% reported in certain European countries[20] and the 21.5% from Korea,[21] any detectable levofloxacin resistance carries clinical importance, as fluoroquinolones are increasingly employed in second-line and salvage therapies. The identification of *gyrA* mutations (Asp-91 and Asn-87) in all resistant isolates confirms that these point mutations constitute the primary mechanism underlying fluoroquinolone resistance in *H. Pylori*.[16]

Amoxicillin (1.7%) and tetracycline (0%) resistance remained infrequent, consistent with global data.[7] These agents continue to hold value as components of *H. pylori* eradication regimens, particularly within quadruple therapy protocols.

The 60.5% *cagA* positivity rate observed falls within the 50–70% range typical of Western populations.[10,11] While *cagA*-positive strains are generally associated with more aggressive clinical courses, the lack of a statistically significant relationship between *cagA* status and endoscopic findings (70.0% in Group A versus 54.3% in Group B, p>0.05) suggests that host factors and other bacterial determinants also contribute significantly to disease outcomes.[22]

Importantly, no association emerged between *cagA* or *vacA* genotypes and antibiotic resistance. This finding indicates that a strain's virulence potential does not predict its antimicrobial susceptibility profile, nor does resistance development appear to be influenced by the presence of these virulence genes.

Study Limitations

Several limitations warrant acknowledgment. First, the sample size was relatively modest, and the single-center design may limit generalizability. Second, metronidazole resistance—which is highly prevalent in many regions—was not assessed. Third, only the signal region of *vacA* was examined; the mid-region, which also modulates toxin activity, was not analyzed.

Conclusion

In the Kahramanmaraş region, the clarithromycin resistance rate (10.3%) remains within acceptable limits, whereas levofloxacin resistance (6.9%) is an emerging concern that warrants vigilant monitoring. Amoxicillin and tetracycline resistance stay low. No relationship exists between *cagA* or *vacA* genotypes and antibiotic resistance. PCR-based methods surpass culture in sensitivity for *H. pylori* detection. Regular regional surveillance of antibiotic resistance profiles is crucial to inform appropriate empiric therapy and preserve the effectiveness of *H. pylori* eradication regimens.

Disclosures

Ethical Committee Approval: This study was approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with decision number 2013/01-7 on January 29, 2013.

Informed Consent: All participants provided written informed consent.

Conflict of Interest: The authors declare no conflict of interest.

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