Research Article

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Evaluation of PADI4 (Human Peptidyl Arginine Deiminase Type 4) levels in patients with type 2 diabetes mellitus with/without complications

Komplikasyon gelişmemiş ve komplikasyon gelişmiş Tip 2 DM (Diabetes Mellitus) hastalarında PADI4 (İnsan Peptidil Arjinin Deiminaz Tip 4) düzeylerinin incelenmesi

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Abstract

Purpose: Human peptidyl arginine deiminase 4 (PADI4) transforms arginine residues on histone tails to citrulline. Recently, researchers have studied PADI4 in inflammation, infection, cancer, and autoimmune disorders. PADI4 also modulates the release of neutrophil extracellular traps (NETs) through a process known as NETosis, which is linked to dysglycemia. PADI4's role in type 2 diabetic mellitus (DM) is not well understood. The goal of this study is to compare PADI4 levels in type 2 DM patients who have not yet experienced diabetic complications, those who have, and a healthy control group.

Materials and methods: This research included three groups of volunteers aged 30-65 years old: a healthy control group, a type 2 diabetic with no diabetic complications, and those with diabetic complications. Serum samples from control and type 2 DM patients were analyzed for complete blood count, biochemistry, and PADI4 (ng/ml).

Results: The control, complicated, and uncomplicated type 2 DM groups did not differ significantly in their serum PADI4 levels (4.94±5.09 ng/ml (med: 2.62, IQR: 2.34-3.87), 5.06±6.58 ng/ml (med: 2.59, IQR: 2.19-2.99), and 4.51±4.76 (med: 2.74, IQR: 2.1- 3.71). Vitamin B12 levels were significantly higher in the complicated type 2 DM group than in the control group (647.83±461.37 vs. 357.32±136.15; p=0.03). The correlation analysis found no significant relationship between nephropathy, retinopathy, or neuropathy and serum PADI4 in the complicated type 2 DM group. There was a statistically significant, negative, and slight correlation with metformin use in the uncomplicated DM group (r=-0.325, p=0.05).

Conclusion: Finally, there was no significant difference in serum PADI4 levels between the type 2 DM groups with complications, those without complications, and the healthy control group. PADI4 is seen as a viable target for illness treatment and surveillance. Prospective studies involving larger patient populations on this subject are needed.

Keywords: PADI4 protein, diabetes mellitus, NETosis, complication.

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Öz

Amaç: İnsan peptidil arjinin deiminaz 4 (PADI4), histon kuyruklarındaki arginin kalıntılarını sitrüline dönüştürür. Son yıllarda araştırmacılar, PADI4'ün inflamasyon, enfeksiyon, kanser ve otoimmün bozukluklardaki rolünü araştırmaktadır. PADI4 ayrıca, disglisemi ile bağlantılı olan NETosis olarak bilinen bir süreç aracılığıyla nötrofil ekstraselüler tuzaklarının (NET) salınımını da düzenler. PADI4'ün tip 2 diyabet mellitustaki (DM) rolü tam olarak anlaşılamamıştır. Bu çalışmanın amacı, henüz diyabetik komplikasyonları olan ve olmayan tip 2 DM hastalarındaki ve sağlıklı kontrol bireylerindeki PADI4 seviyelerini karşılaştırmaktır.

Gereç ve yöntem: Bu çalışma 30-65 yaş aralığındaki üç grup gönüllüden oluşmuştur: sağlıklı bir kontrol grubu, diyabetik komplikasyonları olan ve olmayan tip 2 DM hastaları. Kontrol ve tip 2 DM hastalarından alınan serum örneklerinde tam kan sayımı, biyokimya ve PADI4 (ng/ml) tetkikleri çalışılmıştır.

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Bulgular: Kontrol, komplike ve komplike olmayan tip 2 DM gruplarında serum PADI4 seviyelerinde anlamlı bir farklılık bulunmamıştır (4,94±5,09 ng/ml (orta: 2,62, IQR: 2,34-3,87), 5,06±6,58 ng/ml (orta: 2,59, IQR: 2,19-2,99) ve 4,51±4,76 (orta: 2,74, IQR: 2,1-3,71). B12 vitamini seviyeleri komplike tip 2 DM grubunda kontrol grubuna göre anlamlı derecede daha yüksek olarak bulunmuştur (647,83±461,37 vs. 357,32±136,15; *p*=0,03). Komplike tip 2 DM grubunda nefropati, retinopati veya nöropati ile serum PADI4 arasında anlamlı bir korelasyon bulunamamıştır. Komplike olmayan DM grubunda metformin kullanımıyla istatistiksel olarak anlamlı, negatif ve hafif bir korelasyon saptanmıştır (r=-0,325, *p*=0,05).
Sonuç: Sonuç olarak, komplikasyon gelişmiş veya gelişmemiş tip 2 DM hastaları ve sağlıklı kontrol grubu arasında serum PADI4 seviyelerinde istatistiksel olarak anlamlı bir fark saptanmamıştır. PADI4 birçok hastalık tedavisi ve gözetimi için uygulanabilir bir hedef olarak görülmektedir. Bu konuda daha fazla hasta popülasyonlarını içeren prospektif çalışmalara ihtiyaç vardır.

Anahtar kelimeler: PADI4 protein, diabetes mellitus, NETosis, komplikasyon.

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Introduction

Diabetes mellitus (DM) is a significant worldwide health issue, impacting one in ten individuals aged 20 to 79 and leading to several consequences. Its incidence and prevalence are rising, with an estimated 784 million cases by 2045 [1, 2]. Insulin resistance and inadequate insulin production are hallmarks of type 2 DM [1]. This is caused by oxidative stress, mitochondrial malfunction, and β-cell dedifferentiation [3].

Type 2 DM is a condition marked by systemic inflammation [1, 3]. Neutrophils, the innate immune system's initial line of defense, play an important role in both inflammation and host defense. When activated, neutrophils release neutrophil extracellular traps (NETs) made up of nucleic acids, granular proteins, and histones that collect and destroy foreign pathogens [4]. In addition to their role in host defense, NETs' pathophysiological significance in the etiology of systemic illnesses such as DM is gaining attention. In vitro research indicates that high glucose levels promote the creation of NETs, which are extracellular strands of DNA [4].

The citrullination of histones by human peptidyl arginine deiminase 4 (PADI4) is pivotal to the creation of neutrophil extracellular traps (NETs) in vivo, and this process is known as NETosis. Peptidyl arginine deiminase (PADI) enzymes convert peptidyl arginine to peptidylcitrulline, which has significant implications for protein structure, function, and relationships [5]. PADI4 is a PADI isoform that can be found in neutrophils and monocytes. Numerous studies are being undertaken to investigate the role of PADI4 in inflammation, infection, cancer, and autoimmune disorders [6-8].

Limited research exists regarding the significance of PADI4 in DM and related patients. Type 2 DM can lead to both microvascular consequences like retinopathy, neuropathy, and nephropathy, as well as macrovascular complications including peripheral artery, coronary artery, and cerebrovascular diseases. The purpose of this study is to investigate PADI4 levels in patients with type 2 DM who have not yet developed diabetic complications, those who have, and a healthy control group.

Materials and methods

This research included three groups of volunteers aged 30-65 years: a healthy control group, a type 2 diabetic with no diabetic complications, and those with diabetic complications. Type 2 DM was diagnosed using the American Diabetes Association (ADA) criteria [9]. Complications of type 2 diabetes mellitus are generally classified into two major groups: microvascular complications, which include retinopathy, nephropathy, and neuropathy, and macrovascular complications.

Participants in the control and type 2 DM groups had their fasting venous blood samples taken early in the morning. After the blood samples were centrifuged, the serum was kept

at -20^{0}C until the serum PADI4 levels (ng/ml) were determined. On the day that the samples were taken, measurements were made of the complete blood count, biochemistry, and CRP (0-0.5 µg/dl). A 5 cc sample of venous blood was collected using a serum separator tube, left at room temperature for 15 minutes, and centrifuged at 3500 rpm for 10 minutes to facilitate the measurement of PADI4 levels in human serum.

Commercial kits from BT Lab (Bioassay Technology Laboratory, Shanghai, China) were used to measure human PADI4 levels. All kits and samples were allowed to come to room temperature prior to analysis. After preparing the study kits' reagents and standards, the standards were dispensed into the wells of the microplate. In accordance with the protocol provided in the product insert, color development was carried out based on the test concentrations. Once the color change was evident, absorbance at 450 nanometers (nm) was measured using the BioTek Elx800 microplate reader (BioTek Instruments Inc., USA). The Gen5 data analysis software was utilized to determine concentrations based on the serum absorbance measurements. The PADI4 levels were measured in ng/ml units.

Prior to the study, the Non-Interventional Clinical Research Ethics Committee of Pamukkale University Medical Faculty granted approval (06.08.2024/number 14), and it was performed in compliance with the Helsinki Declaration's legal and regulatory standards.

It was determined that 80% power could be obtained at a 95% confidence level if at least 111 people (at least 37 for each group) were included in the study. This is because there is no comparable study to the one we will conduct, and the power analysis was carried out in accordance with expectations and information obtained from the literature. It was assumed that the effect size of the difference between the groups to be examined would be at a medium level (f=0.3).

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 25.0 (Armonk, NY, USA). Categorical data were expressed as frequencies and percentages, whereas continuous variables were summarized using mean±standard deviation or median with interquartile range (25th–75th percentiles), depending on data distribution. The Shapiro–Wilk test was applied to evaluate the normality of continuous variables.

For data fulfilling the assumptions of parametric testing, comparisons were made using one-way ANOVA (with Tukey post hoc analysis) and the independent samples t-test. In cases where parametric assumptions were not met, the Mann–Whitney U test and Kruskal–Wallis test were employed; post hoc comparisons following Kruskal–Wallis were performed using Bonferroni-adjusted Mann–Whitney U tests.

Spearman's rank-order correlation was employed to evaluate associations between continuous variables. For categorical data, the chi-square test or Fisher's exact test was applied, depending on the suitability for expected frequencies. Statistical significance was determined at a threshold of $p<0.05$.

Results

This study included three groups: a control group and type 2 DM patients with and without diabetic complications aged 30-65. In addition to 37 healthy control group participants, the study included 41 type 2 DM patients with diabetic complications and 37 type 2 DM patients without diabetic complications. There was no significant difference between DM patient groups in terms of gender, BMI, or smoking (Table 1). The type 2 DM group without complications had a greater rate of hypertension ($p=0.007$); however, these individuals' hypertension was present prior to the type 2 DM diagnostic date. The type 2 DM patient group with complications had a higher risk of hyperlipidemia than the diabetic group without complications ($p=0.025$). Compared to the group with uncomplicated DM, the complicated DM group had a considerably longer duration of DM ($p=0.0001$) (Table 1). Twenty-three (80.5%) of the patients in the complicated diabetic patient group had diabetic neuropathy, four (9.8%) had diabetic retinopathy, and fifteen (36.6%) had diabetic nephropathy. In certain individuals, several microvascular complications were noted (Table 2).

Table 1. Duration of DM and other accompanying diseases in complicated and uncomplicated type 2 DM patients

		Complicated DM	Uncomplicated DM	*p*
Body mass index (BMI)	A.O±S.D	30.63±5.17	33.59±7.46	0.07 (z=-1.809)
	Med (IQR)	30 (27-33.2)	32.65 (27.95-38.25)	
Duration of DM	A.O±S.D	13.5±7.44	3.76±4.68	0.0001* (z=-5.853)
	Med (IQR)	14 (6-20)	3 (0-4)	
Hyperlipidemia	No	9 (22%)	17 (45.9%)	0.025* (cs=5.039)
	Yes	32 (78%)	20 (54.1%)	
Smoking	No	36 (87.8%)	35 (94.6%)	0.436 γ
	Yes	5 (12.2%)	2 (5.4%)	
Hypertension	No	14 (34.1%)	24 (64.9%)	0.007* (cs=7.346)
	Yes	27 (65.9%)	13 (35.1%)	
Coronary artery disease	No	31 (75.6%)	33 (89.2%)	0.119 (cs=2.435)
	Yes	10 (24.4%)	4 (10.8%)	
Myocardial infarction	No	40 (97.6%)	36 (97.3%)	1 γ
	Yes	1 (2.4%)	1 (2.7%)	
Peripheral artery disease	No	39 (95.1%)	37 (100%)	0.495 γ
	Yes	2 (4.9%)	0 (0%)	
Cerebrovascular disease	No	39 (95.1%)	37 (100%)	0.495 γ
	Yes	2 (4.9%)	0 (0%)	
Trombosis	No	37 (90.2%)	36 (97.3%)	0.362 γ
	Yes	4 (9.8%)	1 (2.7%)	

*p<0.05 statistically significant, S.D: Standard Deviation, Med (IQR): Median (25^{th}-75^{th} percentiles), z: Mann Whitney U test
cs: Chi-square test, γ: Fisher exact test

Table 2. Distribution of microvascular complications in the type 2 DM patient group with complications

		N (%)
Nephropathy	No	26 (63.4%)
	Yes	15 (36.6%)
Retinopathy	No	37 (90.2%)
	Yes	4 (9.8%)
Neuropathy	No	8 (19.5%)
	Yes	33 (80.5%)

Table 3 provides a summary of the patients' antidiabetic medication types, antiaggregant, anticoagulant, and antihypertensive use rates. Therefore, compared to the uncomplicated group, the complicated DM group's rates of use of DPP4 inhibitors, SGLT inhibitors, insulin, ACE inhibitors, and antiaggregants were statistically substantially higher (Table 3).

Table 3. Types of antidiabetic drugs. antiaggregant, antihypertensive, anticoagulant used by patients

		Complicated DM	Uncomplicated DM	*p*
Metformin	No	6 (14.6%)	10 (27%)	0.176 (cs=1.832)
	Yes	35 (85.4%)	27 (73%)	
Dipeptidyl peptidase 4 (DPP-4) inhibitors	No	16 (39%)	30 (81.1%)	0.0001* (cs=14.218)
	Yes	25 (61%)	7 (18.9%)	
SGLT-2 inhibitors	No	12 (29.3%)	26 (70.3%)	0.0001* (cs=13.087)
	Yes	29 (70.7%)	11 (29.7%)	
Glukagon like peptid 1 (GLP-1) agonist	No	38 (92.7%)	33 (89.2%)	0.702 γ
	Yes	3 (7.3%)	4 (10.8%)	
Sulfonylurea	No	35 (85.4%)	36 (97.3%)	0.111 γ
	Yes	6 (14.6%)	1 (2.7%)	
Glinid	No	41 (100%)	37 (100%)	-
Insulin	No	24 (58.5%)	32 (86.5%)	0.006* (cs=7.503)
	Yes	17 (41.5%)	5 (13.5%)	
Pioglitazone	No	38 (92.7%)	36 (97.3%)	0.617 γ
	Yes	3 (7.3%)	1 (2.7%)	
Angiotensin converting enzyme (ACE) inhibitor	No	30 (73.2%)	35 (94.6%)	0.011* (cs=6.427)
	Yes	11 (26.8%)	2 (5.4%)	
Angiotensin receptor blocker (ARB)	No	29 (70.7%)	32 (86.5%)	0.092 (cs=2.832)
	Yes	12 (29.3%)	5 (13.5%)	
Beta bloker	No	31 (75.6%)	33 (89.2%)	0.119 (cs=2.435)
	Yes	10 (24.4%)	4 (10.8%)	
Antiplatelet	No	6 (14.6%)	13 (35.1%)	0.035* (cs=4.436)
	Yes	35 (85.4%)	24 (64.9%)	
Anticoagulant	No	39 (95.1%)	37 (100%)	0.495 γ
	Yes	2 (4.9%)	0 (0%)	
Diuretic	No	35 (85.4%)	36 (97.3%)	0.111 γ
	Yes	6 (14.6%)	1 (2.7%)	

*p<0.05 statistically significant, cs: Chi-square test, γ: Fisher exact test

Both the complicated and uncomplicated DM groups had higher fasting blood glucose (FBG) levels than the control group. Additionally, the group with complicated DM had higher FBG levels than the group with uncomplicated DM (p=0.0001). The group with complicated DM had higher creatinine levels than the control group (p=0.027). GFR values were considerably lower in the group with complicated DM than in the group with uncomplicated DM and the control group (p=0.0001).

Microalbuminuria was found in 25 (61%) out of 41 individuals in the complicated group, whereas it was not found in the uncomplicated DM group. The levels of spot urine microalbumin varied statistically significantly among the diabetic groups. Microalbumin levels were

substantially greater in the complicated type 2 DM group than in the uncomplicated DM group (p=0.002). Spot urine creatinine levels did not significantly differ across the groups (p=0.339). There was a statistically significant difference between the groups in terms of microalbumin/creatinine ratio. This ratio was higher in the complicated DM group than in the uncomplicated DM group (p=0.0001) (Table 4).

Table 4. Biochemical characteristics of complicated and uncomplicated type 2 DM patients

		Control (1)	Complicated DM (2)	Uncomplicated DM (3)	*p*
HbA1c	A.O±S.S	-	7.47±1.56	6.31±0.76	0.0001*
	Med (IQR)	-	7.1 (6.25-8.5)	6.1 (6-6.5)	(z=-3.546)
Fasting blood sugar	A.O±S.S	90.97±6.69	135.66±32.04	114.13±35.06	0.0001* (kw=54.54)
	Med (IQR)	92 (88- 6)	128 (116.5-151.5)	109 (94-124)	(1-2. 1-3. 2-3)
Creatine	A.O±S.S	0.74±0.15	0.86±0.22	0.77±0.15	0.027* (kw=7.2)
	Med (IQR)	0.76 (0.65-0.85)	0.81 (0.74-0.93)	0.76 (0.63-0.86)	(1-2)
GFR (glomerular filtration rate)	A.O±S.S	105.89±15.81	87.1±17.52	98.38±15.28	0.0001* (kw=20.871)
	Med (IQR)	109 (95-118.5)	90 (76-101.5)	100 (84.5-112)	(1-2. 2-3)
Spot urine microalbumin	A.O±S.S	-	42.48±61.36	9.97±6.92	0.002*
	Med (IQR)	-	18.2 (6.55-49.45)	8.4 (4.4-16.4)	(z=-3.096)
Spot urine creatine	A.O±S.S	-	97.71±73.67	102.89±57.02	0.339
	Med (IQR)	-	76.45 (53.88-126.6)	88.6 (67.3-135.7)	(z=-0.956)
Microalbumin_to creatinine_ratio	A.O±S.S	-	70.03±232.86	11.41±11.85	0.0001*
	Med (IQR)	-	17.38 (7.87-50.05)	7.49 (4.97-12.44)	(z=-3.547)
ALT (alanine aminotransferase)	A.O±S.S	17.95±12.32	22.39±10.86	24.19±15.56	0.016* (kw=8.323)
	Med (IQR)	13 (10-23)	21 (15.5-28)	20 (15-23.5)	(1-2. 1-3)
AST (aspartate aminotransferase)	A.O±S.S	17.57±5.62	19.12±6.88	18.16±7.6	0.39
	Med (IQR)	17 (14-21)	19 (14-22.5)	17 (13-20.5)	(kw=1.882)
Albumin	A.O±S.S	4.54±0.48	4.61±0.3	4.59±0.34	0.759
	Med (IQR)	4.62 (4.42-4.78)	4.65 (4.49-4.76)	4.58 (4.35-4.85)	(kw=0.553)
Total cholesterol	A.O±S.S	164.2±47.02	173.59±36.02	204.05±42.9	0.002* (F=6.527)
	Med (IQR)	149 (130.5-205.5)	179 (148-191.5)	209 (169.5-236)	(2-3)
Triglyceride	A.O±S.S	99±48.39	161.78±80.91	160.89±73.63	0.129
	Med (IQR)	85 (63-142)	139 (107.5-181.5)	151 (102.5-202)	(kw=4.089)
HDL (high density lipoprotein)	A.O±S.S	60.2±21.64	53.2±16.35	53.16±12.73	0.832
	Med (IQR)	50 (45-80.5)	51 (42.5-61.5)	53 (45.5-58.5)	(kw=0.367)
LDL (low density lipoprotein	A.O±S.S	84.2±28.91	87.98±29.44	118.65±38.37	0.0001* (F=8.736)
	Med (IQR)	83 (61.5-107.5)	89 (65.5-108)	115 (93.5-149.5)	(2-3)
CRP (C-reactive protein)	A.O±S.S	2.37±1.02	2.52±2.42	3.73±3.56	0.253
	Med (IQR)	2.13 (1.73-3.21)	1.85 (0.88-3.16)	2.23 (1.2-5.76)	(kw=2.748)

*p<0.05 statistically significant, S.D: Standard Deviation, Med (IQR): Median (25^{th}-75^{th} percentiles), t: Independent samples t test
z: Mann Whitney U test, F: One Way analysis of variance, KW: Kruskal Wallis Variance Analysis, cs: Chi-square test

ALT levels were considerably higher in the complicated and uncomplicated DM groups compared to the control group, but they remained within the normal reference range. The uncomplicated DM group had considerably higher total cholesterol and LDL levels than the complicated DM group. This could be attributed to the hyperlipidemia-lowering medications utilized in the complicated group (Table 4).

There was no statistically significant difference between the three groups in terms of hemogram parameters such as hemoglobin, hematocrit, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, platelet count, MCV (mean corpuscular volume), MPV (mean platelet volume), Pct (plateletcrit), and neutrophil/lymphocyte ratio. Only the RDW (red cell distribution width) value was found to be greater in the complicated group than in the control group (p=0.028). The complicated DM group had significantly higher vitamin B12 levels than the control group (647.83±461.37 vs. 357.32±136.15; p=0.03) (Table 5).

Table 5. Control group, hemogram values, anemia parameters and coagulation values of complicated and uncomplicated type 2 DM patients

		Control (1)	Complicated DM (2)	Uncomplicated DM (3)	*p*
Hemoglobin	A.O±S.D	14.34±1.41	13.89±1.9	13.9±1.82	0.444 (F=0.817)
	Med (IQR)	14 (13.3-15.5)	14.1 (12.75-15.2)	14 (12.3-15.2)	
Hematocrit	A.O±S.D	42.48±3.78	42.05±4.94	41.99±4.51	0.878 (F=0.13)
	Med (IQR)	42.1 (39.95-45.05)	42.7 (39.15-45.5)	42.5 (37.5-45.25)	
Mcv (mean corpuscular volume)	A.O±S.D	86.21±5.9	85.05±6.9	85.17±5.32	0.554 (kw=1.18)
	Med (IQR)	87.1 (83.4-89.6)	87.2 (82.05-89)	86 (82.85-88.4)	
RDW (Red blood cell distribution width)	A.O±S.D	13.79±1.24	14.55±1.82	14.11±1.27	0.028* (kw=7.156) (1-2)
	Med (IQR)	13.5 (12.9- 4.15)	14.2 (13.45-15.4)	13.8 (13.4-14.7)	
WBC (White blood cell)	A.O±S.D	7419.19±1430.27	8066.59±1565.04	8035.14±1827.18	0.15 (F=1.93)
	Med (IQR)	7410 (6770-8540)	8180 (7100-8740)	7960 (6585-9320)	
Neu# (absolute neutrophil count)	A.O±S.D	4368.65±1152.4	4851.95±1221.49	4821.89±1623.67	0.205 (kw=3.165)
	Med (IQR)	4500 (3355-4995)	4890 (4100-5470)	4820 (3840-5570)	
Lymph# (absolute lymphocyte count)	A.O±S.D	2391.89±683.75	2476.1±720.57	2538.92±672.99	0.659 (F=0.419)
	Med (IQR)	2410 (1785-2880)	2470 (1905-2785)	2610 (2035-2975)	
Platelet	A.O±S.D	258216.22±50357.68	256097.56±66094.93	273621.62±67182.32	0.604 (kw=1.007)
	Med (IQR)	256000 (226000-285500)	252000 (207000-302000)	261000 (224000-314500)	
Neu/lymph (neutrophil/ lymphocyte ratio)	A.O±S.D	2.01±0.88	2.11±0.8	2.13±1.69	0.535 (kw=1.25)
	Med (IQR)	1.75 (1.47-2.42)	1.9 (1.58-2.49)	1.75 (1.52-2.22)	
MPV (mean platelet volume)	A.O±S.D	9.78±0.97	9.6±1.06	9.87±1.01	0.499 (F=0.699)
	Med (IQR)	9.7 (9.1-10.7)	9.6 (8.95-10.15)	10 (8.85-10.5)	

Table 5. Control group, hemogram values, anemia parameters and coagulation values of complicated and uncomplicated type 2 DM patients (continued)

		Control (1)	Complicated DM (2)	Uncomplicated DM (3)	*p*
Pct (plateletcrit)	A.O±S.D	0.25±0.04	0.25±0.06	0.27±0.07	0.372
	Med (IQR)	0.26 (0.23-0.27)	0.25 (0.2-0.29)	0.25 (0.21-0.32)	(kw=1.975)
Ferritin	A.O±S.D	56.97±33.26	66.31±75.43	88.98±105.97	0.711
	Med (IQR)	57.5 (26.9-76)	40.75 (16.68-93.45)	52.4 (16.35-123.25)	(kw=0.683)
Iron	A.O±S.D	88.38±34.22	67.82±25.74	88.44±44.72	0.18
	Med (IQR)	83 (60-120)	63 (52.5-93.5)	84 (54-104)	(F=1.799)
TIBC (total iron binding capacity)	A.O±S.D	344.69±81.77	387.14±81.88	337.94±26.39	0.355
	Med (IQR)	375 (305.5-384.5)	392 (308.5-459.75)	334 (319.5-356.4)	(kw=2.072)
Transferrin saturation	A.O±S.D	27.15±13.33	26.53±38.24	26.78±15.35	0.235
	Med (IQR)	24 (15-40.5)	18 (10.75-26.75)	25 (15-31)	(kw=2.9)
Vitamin B12	A.O±S.D	357.32±136.15	647.83±461.37	498.31±215.79	0.03*
	Med (IQR)	339 (275-397)	433 (320.5-930.5)	439 (308-677)	(kw=7.03) (1-2)
Folic acid	A.O±S.D	10.09±4.61	9.22±5.23	11.28±4.43	0.579
	Med (IQR)	9.15 (6.15-13.2)	8.18 (4.63-12.33)	9.64 (7.9-0)	(kw=1.093)
INR (international normalized ratio)	A.O±S.D	1±0.07	0.99±0.08	0.99±0.08	0.878
	Med (IQR)	0.99 (0.95-1.06)	0.99 (0.94-1.05)	0.97 (0.96-1.03)	(F=0.13)
PT (prothrombin time)	A.O±S.D	11.84±0.81	11.96±0.96	11.91±1.01	0.608
	Med (IQR)	11.6 (11.33-12.38)	12.05 (11.43-12.6)	11.8 (11.2-12.3)	(kw=0.997)
APTT (activated partial thromboplastin time)	A.O±S.D	27.88±3.76	25.55±3.04	25.98±2.74	0.121
	Med (IQR)	26.95 (24.73-31.5)	25.8 (23.08-27.43)	25.8 (24.2-27.1)	(kw=4.217)

*p<0.05 statistically significant, S.D: Standard Deviation, Med (IQR): Median (25^{th}-75^{th} percentiles)
t: Independent samples t test, z: Mann Whitney U test, F: One Way analysis of variance, KW: Kruskal Wallis Variance Analysis

There was no significant difference in serum PADI4 levels between the control, complicated, and uncomplicated type 2 DM groups (4.94±5.09 ng/ ml (med: 2.62, IQR: 2.34-3.87), 5.06±6.58 ng/ ml (med: 2.59, IQR: 2.19-2.99), and 4.51±4.76 (med: 2.74, IQR: 2.1-3.71).

The correlation study revealed no significant link between nephropathy, retinopathy, and neuropathy and serum PADI4 in the complicated type 2 DM group (Table 6). In the uncomplicated DM group, there was a statistically significant, negative, and mild connection with metformin use (r=-0.325, p=0.05). Similarly, there was a statistically significant negative and modest connection with antiplatelet use (r=-0.387, p=0.018).

Pioglitazone use was associated with a mild, positive, and statistically significant connection in the complicated DM group (r=0.332, p=0.034). Beta-blocker use was associated with a mild, negative, and statistically significant connection (r=-0.384, p=0.013) (Table 7).

In the complicated DM group, a positive, significant and moderate correlation was found with MPV (r=0.422, p=0.006). In the control group, a statistically significant, positive and weak correlation was found with hemoglobin (r=0.342, p=0.039); and a significant, negative and moderate correlation was found with RDW (r=-0.417, p=0.01) (Table 8).

Table 6. Association between PADI4 and microvascular complications in complicated type 2 DM patients

		PADI 4 Complicated DM
Nephropathy	**r**	-0.169
	p	0.291
Retinopathy	**r**	0.035
	p	0.829
Neuropathy	**r**	0.14
	p	0.381

*p<0.05 statistically significant correlation, r: Spearman correlation coefficient

Table 7. Relationship between PADI4 and drugs used in complicated and uncomplicated type 2 DM patients

		PADI 4	
		Complicated DM	**Uncomplicated DM**
Metformin	**r**	0.222	-0.325*
	p	0.164	0.05
Dipeptidyl peptidase 4 (DPP-4) inhibitors	**r**	0.057	0.036
	p	0.723	0.835
SGLT-2 inhibitors	**r**	0.039	-0.163
	p	0.811	0.334
Glukagon like peptid 1 (GLP-1) **agonist**	**r**	-0.028	0.033
	p	0.863	0.848
Sulfonylurea	**r**	-0.114	0.125
	p	0.479	0.461
Insulin	**r**	0.002	0.037
	p	0.99	0.828
Pioglitazone	**r**	0.332*	-0.273
	p	0.034	0.102
Angiotensin converting enzyme (ACE) inhibitor	**r**	0.023	-0.179
	p	0.885	0.289
Angiotensin receptor blocker (ARB)	**r**	-0.091	-0.311
	p	0.573	0.061
Beta_bloker	**r**	-0.384*	-0.057
	p	0.013	0.737
Antiplatelet	**r**	0.082	-0.387*
	p	0.612	0.018
Anticoagulant	**r**	-0.182	-
	p	0.255	-
Diuretic	**r**	0.082	-0.14
	p	0.612	0.407

*p<0.05 statistically significant correlation, r: Spearman correlation coefficient

Table 8. Evaluation of the relationship between PADI4 and hemogram values in the control group. Complicated and uncomplicated type 2 DM patients

		PADI 4		
		Complicated DM	**Uncomplicated DM**	**Control**
Hgb (Hemoglobin)	**r**	-0.006	-0.278	0.342*
	p	0.971	0.095	0.039
Hct (Hematocrit)	**r**	-0.015	-0.302	0.238
	p	0.925	0.069	0.156
MCV (mean corpuscular volume)	**r**	0.024	-0.195	0.028
	p	0.881	0.247	0.87
RDW (Red blood cell distribution width)	**r**	0.046	0.229	-0.417*
	p	0.776	0.173	0.01
WBC (White blood cell)	**r**	-0.302	0.185	-0.185
	p	0.055	0.274	0.274
Neu# ((absolute neutrophil count))	**r**	-0.262	0.238	-0.098
	p	0.098	0.156	0.565
Lymph# (absolute lymphocyte count)	**r**	-0.108	-0.01	-0.228
	p	0.5	0.953	0.174
Platelet	**r**	-0.043	0.111	-0.303
	p	0.79	0.515	0.069
Neu/Lymph (neutrophil/lymphocyte ratio)	**r**	-0.179	0.12	0.101
	p	0.264	0.48	0.551
MPV (mean platelet volume)	**r**	0.422*	0.032	0.09
	p	0.006	0.851	0.598
PCT (plateletcrit)	**r**	0.156	0.171	-0.218
	p	0.329	0.312	0.195

*$p<0.05$ statistically significant correlation; r: Spearman correlation coefficient

Discussion

PADI4 levels in type 2 DM patients with and without complications were to be investigated in this study. Serum PADI4 levels did not differ statistically significantly between the control group, the complicated, type 2 DM group, and the uncomplicates type 2 DM group (4.94±5.09 ng/ml (med: 2.62, IQR: 2.34–3.87); 5.06±6.58 ng/ml (med: 2.59, IQR: 2.19–2.99); and 4.51±4.76 (med: 2.74, IQR: 2.1–3.71).

According to certain theories in the literature, hyperglycemia triggers NETosis. Accordingly, glucose increases reactive oxygen species (ROS) and has an impact on mitochondria and NADPH oxidase. Given that glucose is a primary metabolic substrate and neutrophils' primary energy source, this is to be expected. Furthermore, NETosis has been shown to be activated by fungi, liposaccharides, cholesterol crystals, interleukins, and both gram-positive and gram-negative bacteria [10, 11]. Research indicates that the products of NET release in patients with T2DM contribute to the pathogenesis of various diabetes-related problems, including cardiovascular disease, diabetic renal disease, delayed wound healing, and diabetic retinopathy. However, there may be mechanisms associated with NETosis that are not well known, as it has been observed that NETosis can also occur in diabetic patients with tightly regulated glucose levels [12]. In our study,

there was no statistical difference in serum PADI4 levels between the control, complicated, and uncomplicated type 2 DM groups, and no significant relationship was found between nephropathy, retinopathy, and neuropathy and serum PADI4 in the complicated type 2 DM group. This could be due to the fact that the patients in this study did not have an acute infection or hyperglycemia at the time the serum samples were collected, and the samples were collected while a chronic process was ongoing.

In a 2022 study by Aukrust et al. [10], no significant difference in circulating NET levels was identified when long-term type 1 DM patients were compared to a control group. Furthermore, PADI4 and PADI4 gene expression did not alter depending on the existence of type 1 DM. Chronic hyperglycemia was assumed to be one reason for this, and because the majority of research in the literature investigating NET markers in type 1 DM was conducted at the time of diagnosis or during the first few years, it was suggested that NET indicators may take a different course in long-term type 1 DM. Furthermore, it has been observed that decreased neutrophil function in type 1 DM may produce a reduction in NETosis. Also, whereas PADI4 activation is regarded as a crucial event during NETosis, circulating PADI4 protein levels are not considered a typical NET marker, and this has been advised to be considered when analyzing the data [10]. There could be similar reasons for the lack of statistically significant variations in serum PADI4 levels among type 2 DM individuals in our investigation.

Smyth DJ et al. [13] genotyped 27 disease-related polymorphisms from thirteen gene regions in type 1 DM patients, including PADI4. They found no significant link between the PADI4 gene polymorphism and type 1 DM [13]. Although the pathology of type 1 and type 2 DM differs, our study also found no significant connection with the PADI4 protein, which is the result of this gene polymorphism. This could be due to their shared history of chronic hyperglycemia.

Citrullination and NET formation have been shown to be suppressed by PADI4 inhibitors. The majority of PADI4 research is still in the experimental or preclinical development stage, despite the fact that selective and efficient molecules with enhanced pharmacokinetic properties have been provided [14]. It is still unclear how exactly PADI4 functions in many disease processes. Whether or not type 2 DM patients experienced complications, our study found no rise in PADI4 levels, but no decrease either. Thus, we might conclude that type 2 DM may still be a topic for PADI4 inhibitor research.

PADI4 is also being studied for its involvement in acute infections and sepsis [15]. A study published in 2024 showed that GSK484 treatment, a PADI4 inhibitor, effectively suppressed H3Cit expression in septic mice and improved sepsis-induced endothelial dysfunction [16]. Another reason why no statistically significant difference was seen in serum PADI4 levels in type 2 DM patients in our study may be that there was no acute infection or wound infection in the type 2 DM patient group included in the study. Accordingly, there was no leukocytosis or increased neutrophils in the type 2 DM patients. This may not have caused an increase in NETosis or serum PADI4 levels.

The medications and their various combinations may also be the cause of the lack of a distinguishable difference in blood PADI4 levels between type 2 DM patients and the control group. For instance, the use of metformin and antiplatelet medications in the uncomplicated type 2 DM group may be one factor contributing to the lack of a difference in PADI4 levels. Because there was a negative and statistically significant association between the usage of metformin and also antiplatelet medications and PADI4 in the uncomplicated DM group.

The fact that each patient with type 2 DM has unique dietary preferences, behavioral traits, and levels of physical activity could also be a contributing factor in the lack of a discernible variation in serum PADI4 levels. We are still unclear on how these factors affect serum PADI4 levels.

Vitamin B12 is essential for both humans and animals, acting as a coenzyme in many biological processes such as mitochondrial and cytosolic pathways, as well as DNA and protein synthesis. It is commonly used as a dietary supplement and to treat hematological and neurological disorders, although the mechanisms behind its biological activities

are little known [17, 18]. A 2024 study found that high vitamin B12 levels increased the risk of cancer and were linked to higher all-cause mortality in adults [19]. Another study found that vitamin B12 inhibited peptidylarginine deiminase and improved rheumatoid arthritis in mice [20]. In our study, vitamin B12 levels differed significantly between the complicated type 2 DM and control groups. Vitamin B12's inhibitory action on PADI4 may have contributed to the low serum PADI4 levels in the complicated type 2 DM group. Furthermore, a high proportion of the type 2 DM participants in the research were using metformin. The evidence in literature suggests that long-term usage of metformin may result in vitamin B12 insufficiency [21-24]. The lack of a rise in PADI4 levels in our study may be due to the high vitamin B12 levels in type 2 DM mellitus patients who experienced complications even after using metformin. The usage of metformin was also found to be statistically significant, negative, and weak in the group with uncomplicated type 2 DM.

This study carries some limitations. The study's participant count may have been increased. Furthermore, the study may have included other indicators of NETosis outside serum PADI4 levels. We used a variety of patients in our investigation. Future research could examine changes in serum PADI4 levels in the same person at the time of type 2 DM diagnosis, in the early stages of the disease, and when complications arise.

In conclusion, the type 2 DM groups with complications, those without complications, and the healthy control group did not differ statistically significantly in their serum PADI4 levels. PADI4 is seen as a promising target for surveillance and disease therapy. Further studies can be planned on this subject.

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References

1. Strati M, Moustaki M, Psaltopoulou T, Vryonidou A, Paschou SA. Early onset type 2 diabetes mellitus: an update. *Endocrine*. 2024;85(3):965-978. doi:10.1007/s12020-024-03772-w
2. International Diabetes Federation. IDF Diabetes Atlas 10th Edition, 2021. https://diabetesatlas.org/. Accessed 29 July, 2025
3. Młynarska E, Czarnik W, Dzieża N, et al. Type 2 Diabetes Mellitus: New Pathogenetic Mechanisms, Treatment and the Most Important Complications. *Int J Mol Sci*. 2025;26(3):1094. doi:10.3390/ijms26031094
4. Cai Y, Yang Q, Tang X, et al. Baicalin mitigates hyperglycemia-linked intestinal epithelial barrier impairment in part by inhibiting the formation of neutrophil extracellular traps. *Front Immunol*. 2025;16:1551256. doi:10.3389/fimmu.2025.1551256
5. Karimian M, Mohammadzadeh FZ. Association of PADI4 Gene Polymorphisms With Susceptibility to Rheumatoid Arthritis: Evidence From 24 Case-Control Studies. *Int J Immunogenet*. 2025;52(1):1-23. doi:10.1111/iji.12701
6. Albiero M, Migliozzi L, Boscaro C, et al. Padi4-Dependent NETosis Enables Diet-Induced Gut Hyperpermeability, Translating Dysbiosis Into Systemic Inflammation and Dysmetabolism. *Diabetes*. 2025;74(5):705-719. doi:10.2337/db24-0481
7. Rycyk Bojarzynska A, Kasztelan Szczerbinska B, Cichoz Lach H, Surdacka A, Rolinski J. Neutrophil PAD4 Expression and Its Pivotal Role in Assessment of Alcohol-Related Liver Disease. *Int J Mol Sci*. 2024;25(14):7597. doi:10.3390/ijms25147597
8. Zheng B, Cai P, Chen Y, Wu Y, Liu H. PADI4 and IL-33 gene polymorphisms with susceptibility to systemic lupus erythematosus and juvenile idiopathic arthritis, a systematic review and meta-analysis. *Medicine (Baltimore)*. 2023;102(18):e33700. doi:10.1097/MD.0000000000033700
9. American Diabetes Association Professional Practice Committee. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2024. *Diabetes Care*. 2024;47(Suppl 1):S20-S42. doi:10.2337/dc24-S002
10. Aukrust SG, Holte KB, Opstad TB, Seljeflot I, Berg TJ, Helseth R. NETosis in Long-Term Type 1 Diabetes Mellitus and Its Link to Coronary Artery Disease. *Front Immunol*. 2022;12:799539. doi:10.3389/fimmu.2021.799539
11. Rodríguez Espinosa O, Rojas Espinosa O, Moreno Altamirano MM, López Villegas EO, Sánchez García FJ. Metabolic requirements for neutrophil extracellular traps formation. *Immunology*. 2015;145(2):213-224. doi:10.1111/imm.12437

12. Njeim R, Azar WS, Fares AH, Azar ST, Kfoury Kassouf H, Eid AA. NETosis contributes to the pathogenesis of diabetes and its complications. *J Mol Endocrinol*. 2020;65(4):R65-R76. doi:10.1530/JME-20-0128
13. Smyth DJ, Howson JM, Payne F, et al. Analysis of polymorphisms in 16 genes in type 1 diabetes that have been associated with other immune-mediated diseases. *BMC Med Genet*. 2006;7:20. doi:10.1186/1471-2350-7-20
14. Aiken SG, Grimes T, Munro S, Zarganes Tzitzikas T, La Thangue NB, Brennan PE. A patent review of peptidylarginine deiminase 4 (PAD4) inhibitors (2014-present). *Expert Opin Ther Pat*. 2025;35(6):611-621. doi:10.1080/13543776.2025.2484366
15. Costa NA, Gut AL, Azevedo PS, et al. Peptidylarginine deiminase 4 concentration, but not PADI4 polymorphisms, is associated with ICU mortality in septic shock patients. *J Cell Mol Med*. 2018;22(10):4732-4737. doi:10.1111/jcmm.13717
16. Su X, Li L, Dai J, Xiao B, Jin Z, Liu B. GSK484, a PAD4 inhibitor, improves endothelial dysfunction in mice with sepsis-induced lung injury by inhibiting H3Cit expression. *Nan Fang Yi Ke Da Xue Xue Bao*. 2024;44(12):2396-2403. doi:10.12122/j.issn.1673-4254.2024.12.16
17. Ünlü A, Ünver Koluman B, Hacıoğlu S, Akgün Çağlıyan G, Güler N. Predictive value of serum vitamin B12 elevation in acute leukemia. Pam Tıp Derg 2023;16(2):230-236. doi:10.31362/patd.1210492
18. Pardo Cabello AJ, Manzano Gamero V, Puche Cañas E. Vitamin B12: For more than just the treatment of megaloblastic anemia? *Rev Clin Esp (Barc)*. 2023;223(2):114-119. doi:10.1016/j.rceng.2022.11.004
19. Amado Garzon SB, Molina Pimienta L, Vejarano Pombo A, Vélez Bonilla M, Moreno Chaparro J, Buitrago Lopez A. Elevated Vitamin B12, Risk of Cancer, and Mortality: A Systematic Review. *Cancer Invest*. 2024;42(6):515-526. doi:10.1080/07357907.2024.2366907
20. Yang CW, Hsu HY, Lee YZ, Lee SJ. Vitamin B12 inhibits peptidylarginine deiminases and ameliorates rheumatoid arthritis in CAIA mice. *Biochem Biophys Res Commun*. 2024;704:149668. doi:10.1016/j.bbrc.2024.149668
21. Ramzan NUH, Shahjahan K, Dhillon RA, et al. Vitamin B12 Deficiency in Patients Taking Metformin: Pathogenesis and Recommendations. *Cureus*. 2024;16(9):e68550. doi:10.7759/cureus.68550
22. Shivaprasad C, Gautham K, Ramdas B, Gopaldatta KS, Nishchitha K. Metformin Usage Index and assessment of vitamin B12 deficiency among metformin and non-metformin users with type 2 diabetes mellitus. *Acta Diabetol*. 2020;57(9):1073-1080. doi:10.1007/s00592-020-01526-4
23. Hurley Kim K, Vu CH, Dao NM, et al. Effect of Metformin Use on Vitamin B12 Deficiency Over Time (EMBER): A Real-World Evidence Database Study *Endocr Pract*. 2023;29(11):862-867. doi:10.1016/j.eprac.2023.06.013
24. Al Fawaeir S, Al Odat I. Influence of metformin intake on serum vitamin B12 levels in patients with type 2 diabetes mellitus. *PLoS One*. 2022;17(12):e0279740. doi:10.1371/journal.pone.0279740