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Patients with ANCA-associated vasculitis have low serum thiol levels, and these low levels are correlated with higher disease activity scores

ANCA ilişkili vaskülit hastaları düşük serum tiyol düzeylerine sahiptir ve düşük tiyol düzeyleri yüksek hastalık aktive skorları ile ilişkilidir

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Abstract

Objective: This study compared thiol/disulfide balance and ischemia-modified albumin (IMA) levels between the patients with anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV) and healthy controls. Additionally, the study investigated the relationship between AAV disease activity scores, organ involvement, and levels of IMA and thiol/disulfide molecules.

Methods: Forty-six AAV patients and 45 healthy volunteers were included in the study. Birmingham vasculitis activity score (BVAS) version 3 was used for AAV disease activity. Disulfide (-SS), native thiol (-SH), and total thiol (-SH+-SS) molecule analyses were performed using an automatic spectrophotometric method, and IMA calculations were performed using the albumin-cobalt binding test. An increase in the -SS/(-SH+-SS) ratio was considered indicative of a shift towards oxidation, while an increase in the -SH/(-SH+-SS) ratio was suggested a shift towards antioxidation.

Results: The -SS/(-SH+-SS) ratio was significantly higher [β: 0.482, odds ratio (OR) confidence interval (CI) 95%: 0.618 (0.432-0.882), p=0.008] and the -SH/(-SH+-SS) ratio was lower [β: -0.242, OR (CI 95%): 1.273 (1.065-1.522), p=0.008] in the AAV group compared to the control group. A significant association was found between -SH and BVAS-3 [β: -8.202, CI 95%: -16.108- (-0.267), p=0.043].

Conclusion: Thiol/disulfide molecules are shifted towards oxidation in AAV patients compared to healthy controls. Furthermore, patients with higher BVAS-3 disease scores exhibit lower serum thiol levels.

Keywords: ANCA-associated vasculitis, thiol, IMA, BVAS-3

Özet

Amaç: Bu çalışma anti-nötrofil sitoplazmik antikor ile ilişkili vaskülit (AAV) hastaları ile sağlıklı kontroller arasında tiyol/disülfit dengesinin ve iskemi modifiye albümin (IMA) düzeylerinin karşılaştırılması amacıyla hazırlanmıştır. Ayrıca bu çalışmada AVV'nin hastalık aktivite skoru ve organ tutulumları ile IMA ve tiyol/disülfit molekülleri arasındaki ilişkiler de araştırılmıştır.

Yöntem: Çalışmaya 46 AVV hastası ile 45 sağlıklı gönüllü dahil edildi. AVV hastalık aktivitesi için Birmingham vasculitis aktivite skoru (BVAS) version-3 kullanıldı. Disülfit (-SS), doğal tiyol (-SH) ve total tiyol (-SH+-SS) moleküllerine ait analizler otomatik spektrofotometrik metod ile, IMA'ya ait hesaplamalar ise albümin-kobalt bağlanma testi ile gerçekleştirildi. -SS/(-SH+-SS) oranında artış oksidasyon lehinde kayma, -SH/(-SH+-SS) oranında artış ise antioksidasyon lehinde kayma kabul edildi.

Bulgular: AVV grubunda kontrol grubuna göre -SS/(-SH+-SS) oranı anlamlı olarak daha yüksek [β: -0,482, risk oranı (OR) güven aralığı (GA) %95: 0,618 (0,432-0,882), p=0,008], -SH/(-SH+-SS) oranı ise daha düşük [β: 0,242, OR (GA %95): 1,273 (1,065-1,522), p=0,008] saptandı. -SH ile BVAS-3 arasında anlamlı bir korelasyon ilişkisi tespit edildi [β: -8,202, GA %95: -16,108- (-0,267), p=0,043].

Sonuç: AVV hastalarında sağlık kontrollere göre tiyol/disülfit molekülleri oksidasyon yönünde kaymıştır ve yüksek BVAS-3 hastalıkları skorları olan hastalar daha düşük serum tiyol düzeylerine sahiptir.

Anahtar Kelimeler: ANCA ilişkili vaskülit, tiyol, IMA, BVAS-3

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Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of necrotizing autoimmune vascular diseases characterized by microvascular damage, primarily affecting small vessels. These include granulomatosis with polyangiitis, microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis.[1] significant advances in the AAV treatment, it remains a potentially life- and organ-threatening disease entity.[2] Optimal assessment of AAV disease activity is critical in planning treatment because accurate assessment influences the physician's choice of treatment and subsequent drug therapy management.[3] The Birmingham vasculitis activity score version-3 (BVAS-3) is considered a reliable measurement score that best reflects localized or global inflammation by evaluating the disease activity of AAV. [4] However, the significant disadvantages of this index are that the measurement of BVAS-3 is complex, can cause bias among researchers, and requires considerable time for calculation. Therefore, developing objective laboratory markers that can successfully reflect inflammation and damage in AAV which are inexpensive and easy to apply is a critical necessity.

Under physiological conditions, any of the metalic elements such as nickel, cobalt, or copper can bind to the terminal of human albumin's first amino acids. Hypoxic and ischemic events can disrupt this metal-binding capacity to albumin. This albumin, whose structure has been disrupted by the effect of hypoxia, has been defined as ischemia-modified albumin (IMA). Events resulting in ischemic reperfusion injury cause an increase in plasma IMA levels. [5,6] It has been reported that IMA may be an essential biomarker that can predict the oxidative status in processes with increased oxidative stress.[7-10] In acute coronary events, plasma IMA level can be a biomarker that accurately predicts ischemic myocardial damage.[7,11] Serum IMA levels also increase in autoimmune diseases associated with increased oxidative status, such as psoriasis, ankylosing spondylitis (AS), rheumatoid arthritis (RA), Behçet's disease, systemic sclerosis, familial Mediterranean fever (FMF), Henoch-Schönlein purpura and gout.[12-19]

Antioxidant defense systems include many enzymatic and non-enzymatic molecules, including thiols. Reactive oxygen species (ROS) formed from different reactions enable the transfer of excess electrons in the human body to thiols, thus converting thiol molecules into oxidized form. Reversible disulfide bonds formed from these reactions are converted back to thiol forms when necessary, maintaining the oxidative balance. This antioxidant protection system, called dynamic thiol/disulfide homeostasis (Dtdh), plays a role in many vital processes, such as critical enzymatic systems, apoptosis, detoxification events, intracellular signaling networks, and transcription reactions.[20-22] The structures responsible for Dtdh mainly comprise cysteine residues in the structure of albumin and other proteins.[23] Plasma thiol molecules bind ROS in an environment with increased ROS and convert it into an oxidized form. Thus, significant decreases occur in thiol molecule levels in oxidative environments.[24] Serum thiol levels are known to be decreased in rheumatological diseases such as psoriatic arthritis, RA, gout, AS, Kawasaki disease, FMF, systemic lupus erythematosus, and primary Sjögren syndrome.[25-31]

To our knowledge there is no study comparing IMA levels and Dtdh between AAV patients and healthy controls. The inadequacy of serum markers that can be used in the follow-up of disease activity in AAV patients can be pretty challenging in the management of AAV. In addition, the inadequacy of biomarkers in AAV can cause a delay in the selection of the appropriate medical treatment type. This study used a new idea to compare serum IMA and Dtdh between AAV patients and healthy controls. In addition, this study investigated how serum thiol molecules and IMA levels change according to the disease activity score, systemic organ involvement, and drugs used in AAV patients.

Materials and Methods

Study Design

This study was conducted as a case-control study between 01/06/2023 and 01/05/2024. The patient group included 46 AAV patients who were followed up and treated in the Rheumatology Department of Ankara Bilkent City Hospital. The control group included 45 healthy volunteers not previously diagnosed with rheumatological disease. The control group was selected from those with similar demographic characteristics, age, gender, and comorbidities to the patient group. Individuals with active or chronic infectious disease, malignancy, pregnancy, and non-AAV rheumatic diseases were excluded. The International Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides criteria[32] was used for the classification of AAV, and the BVAS-3 (a score created by a combination of symptoms and findings involving a total of nine organ systems; score range=0 to 63)[33] was used for the follow-up of disease activity. Current data on systemic organ involvement and medical treatments in AAV patients were obtained from the hospital's online registration system or patient files. For comorbid diseases, the presence of chronic obstructive pulmonary disease, coronary artery disease, hypertension, and diabetes mellitus were taken into account. An informed voluntary consent form was obtained from all individuals participating in the study.

Analysis of Serum IMA Levels and Thiol-disulfide Molecules and Preparation of Venous Blood Samples

Venous blood samples taken from the participants in the groups for the study were centrifuged in 10 mL vacuum tubes at 1300 x g for 10 minutes. Then, the obtained sera were taken into Eppendorf tubes and stored at -80 °C until the analysis time. Analysis measurements of Dtdh parameters were carried out with the automatic spectrophotometric method previously described by Erel and Neselioglu.[34] According to this method, the calculations were briefly as follows: First, disulfide bonds were reduced with the help of sodium borohydride to form free plasma functional thiol groups. After the molecules belonging to the thiol groups reacted with 5.5'-dithiobis-2 nitrobenzoic acid, the natural thiol (-SH) and reduced thiol groups were calculated. Disulfide (-SS) levels were determined by taking the difference between total thiol (-SS+-SH) and -SH. After the determination of -SS and -SH, the percentages of native thiol/total thiol [-SH/(-SH+-SS)] and disulfide/total thiol [-SS/(-SH+-SS)] were determined. The increase in the percentage of -SS/(-SH+-SS) was considered a shift in the direction of oxidation, while the increase in the rate of -SH/(-SH+-SS) was evaluated as a shift in the direction of antioxidation.

For the calculations of IMA levels, venous blood samples were kept at room conditions for approximately 30 minutes and centrifuged at 3500 rpm for 5 minutes. Then, the samples separated into Eppendorf tubes were stored at -80 °C until the calculation day. The calculation of IMA levels was performed with an albumin-cobalt binding test (Sigma/Aldrich Chemie GmbH Riedstrasse-2, Steinheim/Germany). In this test method, serum samples obtained from the study groups were mixed with 50 mL of 0.1% cobalt (II) chloride ($CoCl_2$, $6H_2O$) solution and kept in a room for approximately 10 minutes. After this process, 50 mL of 1.5 mg/mL dithiothreitol was added to the solution to ensure cobalt binding to albumin. After a waiting period of two minutes, 1.0 mL of 0.9% sodium chloride solution was added to the solution to determine the binding capacity. Finally, absorbance measurements of the samples were carried out using a spectrophotometer with a wave sensitivity of 470 nm. The unit of IMA levels was shown as absorbance unit.[35]

Indirect immunofluorescence assay (IIA) was used to determine cytoplasmic (C)-ANCA and perinuclear (P)-ANCA. Antigen-specific tests were used for PR3-ANCA and MPO-ANCA tests. IIA was used as the initial screening test for ANCA tests.[36] Antigen-specific tests Phadia ELiA (Thermo-Fisher Scientific-Phadia-Freiburg-Germany) were used for PR3-ANCA and MPO-ANCA tests and their levels were determined using the Phadia250 analyzer. Patients with positive IIA and specific antigen tests were considered MPO-ANCA or PR3-ANCA positive.

The following methods were used in for the calculation of other biochemical analyses: C-reactive protein (CRP) nephelometric method (IMMAGE-Immunochemistry Systems-Ireland); erythrocyte sedimentation rate Westergren method (Berkhum-SDM100-Türkiye); uric acid uricase method (Siemens-Healthineers-Germany); serum creatinine modified-Jaffe method.

Ethical approval for this study was obtained from Ankara Bilkent City Hospital No. 2 Ethics Committee dated 10/05/2023 and numbered E2-23-3791.

Statistical Analysis

Version 22. 0 Statistical Packages for the Social Sciences package program was used to evaluate the statistical analyses of the study. Shapiro-Wilk or Kolmogorov-Smirnov tests, as well as histograms or probability graphs, were used to determine the normality distribution in continuous variables. The results of descriptive statistical analyses were shown in the format of mean-standard deviation (mean ± standard deviation) for variables showing normal distribution and median-interquartile range [interquartile range, (25-75%)] for variables not showing normal distribution. Statistical evaluations in pairwise comparisons were performed with Independent Samples t-test for variables with normal distribution and Mann-Whitney U test for variables not normally distributed. Correlation analyses of continuous variables were performed using Spearman correlation analysis. Bonferroni correction was first performed for multiple comparisons. Then, a One-Way ANOVA post-hoc Tukey test was performed for quantitative variables with normal distribution. Independent Samples-Kruskal-Wallis test was performed for quantitative variables that did not have a normal distribution. Fisher or chi-square analyses were used in comparative tests of categorical variables. The predictive effects of independent variables on categorical dependent variables were analyzed using multinomial or binary logistic regression tests. The predictive effects of independent variables on continuous dependent variables were evaluated using linear regression tests. P values <0.05 were considered statistically significant.

Results

Table 1 shows the results of comparing demographic characteristics and biochemical data between AAV and control groups. The median age of the AAV group was 49.2 (38.8-59.1), and the mean age of the control group was 47.2

(36.9-57.5) (p>0.05). In the AAV group, -SH (p<0.0001), -SH+-SS (p<0.0001) levels, and -SH/(-SH+-SS) ratio (p=0.003) were significantly lower, and -SS/(-SH+-SS) ratio (p=0.004) and IMA levels (p=0.039) were significantly higher compared to controls.

In binary logistic regression analysis, -SS/(-SH+-SS) [β: 0.482, odds ratio (OR) confidence interval (CI) 95%: 0.618 (0.432-0.882), p=0.008], CRP [β: 0.305, OR (CI 95%): 0.737 (0.600-0.905), p=0.004] and creatinine [β: 6.616, OR (CI 95%): 0.01 (0.00-0.056), p=0.001] levels were significantly higher, SH/(-SH+-SS) [β: -0.242, OR (CI 95%): 1.273 (1.065-1.522), p=0.008] and albumin [β: -0.272, OR (CI 95%): 1.313 (1.094-1.577), p=0.004] levels were significantly lower in the AAV group compared to controls. IMA levels were similar between the groups [β: -2.527, OR (CI 95%): 0.08 (0.006-1.044), p=0.054].

Table 2 shows the relationship between some clinical conditions or the type of medical treatment used in AAV patients and thiol parameters and IMA levels. Thiol parameters or IMA levels were similar in the presence of any systemic organ involvement in AAV or the type of medical treatment used (p>0.05).

Table 3 shows the correlation relationship between some continuous variables and thiol parameters and IMA levels in the AAV group. A significant correlation was found between -SH and creatinine (r: -0.347, p=0.018), serum albumin (r: 0.651, p<0.0001), and BVAS-3 score (r: -0.391, p=0.007). Also, a significant correlation was found between -SH+-SS and creatinine (r: -0.352 p=0.016), serum albumin (r: 0.596 p<0.0001) and BVAS-3 score (r: -0.361 p=0.014).

In linear regression analysis, a significant association was found between -SH and BVAS-3 [β: -8.202, CI 95%: -16,108- (-0.267), p=0.043] and serum albumin [β: 6.421, CI 95%: (3.537-9.306), p<0.001], but no significant association was found between -SH and creatinine [β: -7.269, CI 95%: (-18.753-4.215), p=0.209]. A significant association was found between -SH+-SS and serum albumin levels [β: 6.552, CI 95%: (3.263-9.306), p<0.001], but no significant association was found between -SH+-SS and BVAS-3 [β: -8.099, CI 95%: (-16.862-0.664), p=0.064] and creatinine [β: -8.088, CI 95%: (-20.648-4.472), p=0.201].

Table 1. Comparison of demographic characteristics and biochemical data between AAV and control groups

	ANCA	Control	p-value
n (total)	46	45	-
Age, median (IQR) (years)	49.2 (38.8-59.1)	47.2 (36.9-57.5)	0.246
Gender female/male, n	23/23	25/20	0.679
Body mass index, mean ± SD	25.4 (18.5-30.1)	26.2 (19.3-31.9)	0.407
Presence of comorbid disease			
Hypertension, n	6	5	0.777
COPD, n	4	2	0.414
Diabetes mellitus, n	5	5	0.971
CAD, n	5	2	0.250
Smoking, n (%)	6	3	0.308
Disease duration, median (IQR) [year]	1 (0.5-2)	-	-
Dialysis, n (%)	9	-	-
Creatinine, median (IQR) [mg/dL]	1.1 (0.74-1.97)	0.7 (0.6-0.7	**<0.0001**
eGFR, median (IQR) [mL/min/1.73 m^2]	63 (26.5-101.25)	105 (98.5-117)	**<0.0001**
Albumin, median (IQR) [g/dL]	41.5 (37.5-45)	45 (43-47)	**<0.0001**
CRP, median (IQR) [mg/L]	6 (1.3-16.6)	1 (0-3.25)	**<0.0001**
ESR, median (IQR) [mm/h]	22.5 (6-41.75)	15 (6-19)	**0.019**
IMA, median (IQR) [ABSU]	0.79 (0.68-0.9)	0.72 (0.61-0.81	**0.039**
-SH, median (IQR) [μmol/L]	331.2 (289.4-394.4)	410.6 (382.2-435.3)	**<0.0001**
-SH+-SS, median (IQR) [μmol/L]	368 (328.2-437.6)	447.3 (416.3-472)	**<0.0001**
-SS, median (IQR) [μmol/L]	18.6 (13.7-23.4)	17.9 (16.4-19.5)	0.987
-SH/(-SH+-SS), median (IQR) [%]	90.2 (87.5-92.3)	91.8 (91-92.7)	**0.003**
-SS /(-SH+-SS), median (IQR) [%]	4.87 (3.81-6.2)	4.09 (3.63-4.49)	**0.004**
PR3 ANCA ELISA, median (IQR) [RU/mL] (n=23)	127 (45-200)	-	-
MPO ANCA ELISA, median (IQR) [RU/mL] (n=23)	128 (54.5-168.7)	-	-
BVAS-3 (version-3), median (IQR)	4 (2-7.25)	-	-

AAV: ANCA-associated vasculitis, ANCA: Anti-neutrophil cytoplasmic antibody, BVAS: Birmingham vasculitis activity score, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRP: C-reactive protein, eGFR: Estimated Glomerular filtration rate, ESR: Erythrocyte sedimentation rate, IMA: Ischemia modified albumin, IQR: Interquartile range, MPO: Myeloperoxidase, PR3: Proteinase 3, SD: Standard deviation, -SH: Native thiol, -SS: Disulphide, -SH+-SS: Total thiol

Table 2. Relationship between some clinical conditions or medical treatment type and thiol parameters and IMA levels in AAV patients

		n	IMA	p-value	-SS/(-SH+-SS), median (IQR) [%]	p-value	-SH/(-SH+-SS), median (IQR) [%]	p-value
ANCA type	PR3	23	0.81 (0.68-0.9)	0.878	4.9 (2.7-6.2)	0.621	90 (87.6-94.6)	0.621
	MPO	23	0.77 (0.69-0.91)		4.7 (3.9-6.4)		90.4 (87.9-95.1)	
Dialysis	Available	9	0.76 (0.70-0.88)	0.765	5.0 (3.2-7.9)	0.605	89.8 (84-0-93.5)	0.605
	No	37	0.81 (0.9-0.65)		4.7 (3.8-6.1)		90.4 (87.7-92.3)	
Nasal involvement	Available	11	0.88 (0.68-0.9)	0.410	5.9 (3.8-7.1)	0.226	88 (85.6-92.3)	0.221
	No	35	0.77 (0.69-0.087)		4.6 (3.8-6)		90.6 (87.8-92.3)	
Pulmonary involvement	Available	27	0.77 (0.68-0.9)	0.947	4.7 (3.8-6.1)	0.435	90.4 (87.8-92.3)	0.428
	No	19	0.81 (0.69-0.88)		5 (3.9-6.7)		89.8 (86.4-92.1)	
Auditory system involvement	Available	8	0.73 (0.58-0.9)	0.618	6.0 (4.2-8.3)	0.102	87.9 (83.3-91.6)	0.109
	No	38	0.81 (0.70-0.89)		4.6 (3.5-6.1)		90.6 (87.7-92.9)	
Eye involvement	Available	7	0.72 (0.63-0.87)	0.306	2.7 (2.0-6.7)	0.154	94.5 (86.4-95.8)	0.154
	No	39	0.82 (0.69-9)		4.9 (3.9-6.2)		90.0 (87.6-92.1)	
Skin involvement	Available	5	0.9 (0.68-1.07)	0.186	5.0 (2.9-6.1)	0.918	89.9 (87.6-94.1)	0.918
	No	41	0.77 (0.68-0.88)		4.7 (3.8-6.2)		90.4 (87.5-92.3)	
Neurological involvement	Available	4	0.90 (0.57-1.0)	0.315	5.5 (4.1-6.9)	0.417	88.8 (86.1-91.6)	0.439
	No	42	0.77 (0.68-0.88)		4.7 (3.7-6.2)		90.5 (87.5-92.5)	
Cardiac involvement	Available	6	0.8 (0.65-1.01)	0.667	4.8 (3.3-6.7)	0.962	90.2 (86.4-93.3)	0.987
	No	40	0.79 (0.68-0.89)		4.8 (3.8-6.1)		90.3 (87.6-92.3)	
Renal involvement	Available	30	0.79 (0.67-0.9)	0.926	4.9 (3.8-6.5)	0.454	90 (86.9-92.3)	0.460
	No	16	0.79 (0.68-89)		4.6 (3.8-5.9)		90.7 (88.2-92.3)	
GIS involvement	Available	2	0.79 (0.47-)	0.893	5.3 (3.9-)	0.773	89.3 (86.4-)	0.812
	No	44	0.79 (0.69-0.89)		4.8 (3.8-6.1)		90.2 (87.6-92.3)	
Hemoptysis	Available	6	0.75 (0.67-0.8)	0.397	5.6 (3.6-6.8)	0.555	88.6 (86.3-92.6)	0.555
	No	40	0.82 (0.68-9)		4.7 (3.8-6.1)		90.5 (87.6-92.3)	
Pulmonary tomography findings	Normal	19	0.85 (0.75-0.92)	0.063	5.0 (3.9-6.4)	0.953	89.9 (87.1-92.1)	0.950
	Nodule	14	0.77 (0.59-0.87)		4.5 (3.5-6.3)		90.8 (87.2-92.9)	
	Cavity	8	0.80 (0.70-0.92)		5.0 (3.8-6.0)		89.9 (87.8-92.2)	
	Hemorrhage	5	0.72 (0.49-0.74)		4.3 (2.1-7.8)		91.3 (84.2-95.6)	
Corticosteroids	Available	36	0.72 (0.67-0.79)	0.804	4.4 (4.1-4.8)	.585	90.1 (88.1-92.6)	0.520
	No	10	0.73 (0.69-0.85)		4.2 (3.8-4.6)		91.4 (89.7-92.8)	
Methotrexate	Available	16	0.75 (0.66-0.87)	0.703	4.5 (4.2-4.6)	0.506	90.7 (90.1-91.5)	0.774
	No	30	0.73(0.63-0.84)		4.3 (3.6-4.4)		91.0 (90.4-91.9)	
Rituximab	Available	10	0.78 (0.67-0.85)	0.632	4.1 (3.6-4.6)	0.472	90.2 (88.2-92.6)	0.685
	No	36	0.74 (0.63-0.81)		4.4 (4.5-5.3)		91.1 (89.4-93.1)	
Cyclophosphamide	Available	8	0.72 (0.67-0.79)	0.798	4.0 (3.6-4.5)	0.421	90.2 (89.0-91.8)	0.495
	No	38	0.74 (0.68-0.83)		4.3 (4.1-4.8)		91.6 (90.4-92.9)	
Azathioprine	Available	7	0.70 (0.67-0.84)	0.781	4.6 (3.9-5.1)	0.503	90.4 (87.5-91.8)	0.234
	No	39	0.72 (0.70-0.87)		4.4 (3.7-4.8)		92.6 (91.6-92.2)	
Mycophenolate mofetil	Available	5	0.72 (0.57-0.85)	0.767	4.1 (3.6-4.7)	0.622	92.0 (90.2-96)	0.408
	No	41	0.74 (0.71-0.83)		4.2 (3.8-5.0)		91.4 (89.4-91.9)	

AAV: ANCA-associated vasculitis, ANCA: Anti-neutrophil cytoplasmic antibody, GIS: Gastrointestinal system, IMA: Ischemia modified albumin, IQR: Interquartile range, MPO: Myeloperoxidase, PR3: Proteinase 3, -SH: Native thiol, -SS: Disulphide, -SH+-SS: Total thiol

Discussion

In this study, we compared IMA levels and Dtdh between AAV patients and healthy people and evaluated the correlation relationship between these molecules and the disease activity score used in AAV. In our study, it was found that -SH/(-SH+-SS) median values were significantly lower and -SS/(-SH+-SS) median values were higher in the AAV group compared to healthy controls. We also found that there was a significant negative correlation between BVAS-3, one of the indicators of AAV disease activity, and serum -SH levels in linear regression analysis. These data showed that Dtdh shifted in the oxidative direction in AAV patients compared to healthy people and in those with high AAV disease activity. Serum IMA levels were similar between

Table 3. Correlation analyses between some continuous variables and thiol parameters and IMA levels in the AAV group

r (p)	Age	Creatinine	BVAS-3	Serum albumin	CRP	ESR
-SS	-0.189 (0.208)	-0.196 (0.192)	-0.055 (0.715)	0.180 (0.242)	0.162 (0.281)	-0.021 (0.889)
-SH	-0.249 (0.095)	**-0.347 (0.018)**	**-0.391 (0.007)**	**0.651 (<0.0001)**	-0.079 (0.604)	-0.250 (0.094)
-SH+-SS	-0.286 (0.076)	**-0.352 (0.016)**	**-0.361 (0.014)**	**0.596 (<0.0001)**	-0.016 (0.917)	-0.201 (0.180)
-SS/(-SH+-SS)	-0.052 (0.732)	-0.055 (0.714)	0.221 (0.141)	-0.223 (0.145)	0.275 (0.069)	0.195 (0.193)
-SH/(-SH+-SS)	0.051 (0.738)	0.055 (0.714)	-0.219 (0.143)	0.244 (0.144)	-0.278 (0.065)	-0.196 (0.191)
IMA	-0.095 (0.529)	-0.136 (0.369)	0.012 (0.935)	-0.139 (0.369)	0.236 (0.114)	0.046 (0.760)

AAV: ANCA-associated vasculitis, BVAS: Birmingham vasculitis activity score, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, IMA: Ischemia modified albumin, -SH: Native thiol, -SS: Disulphide, -SH+-SS: Total thiol, r: Correlation coefficient

the AAV and control groups. It was determined that thiol/disulfide and IMA levels did not change in the presence of any organ involvement or the types of medical treatment used in AAV.

In the present study, the median values of -SH/(-SH+-SS) were found to be lower and the median values of -SS/(-SH+-SS) were found to be higher in the AAV group compared to the controls. In addition, a negative significant correlation was found between BVAS-3, one of the AAV disease activity indicators, and -SH in our study. Dtdh has not been evaluated before between AAV patients and healthy controls, and our study is the first to demonstrate that Dtdh changes to the oxidative direction in AAV patients and those with high AAV disease activity. The role of oxidative damage in the pathogenesis of some subtypes of small vessel vasculitis has been evaluated in some clinical studies.[37,38] Possible mechanisms of this pathogenesis include activation of inflammatory cells by polymorphic nuclear leukocytes, monocytes, and macrophages due to immune complex accumulation in small vessels, increased oxidative stress triggering lipid peroxidation, and ultimately increased ROS production.[39] Under physiological conditions, the amount of ROS increases during oxygen metabolism, and the antioxidant system removes these ROS from the body. Increased ROS causes tissue damage due to disruption of the oxidant-antioxidant system balance.[39,40] It has been previously shown that myeloperoxidase (MPO) antibodies in AAV trigger oxidative bursts in neutrophils and hypochlorous acid (HOCl) production associated with increased oxidative stress and ultimately play a critical role in the development of endothelial damage and vasculitis.[41,42] Increased ROS and decreased antioxidant levels caused by oxidative stress have been shown to contribute to processes such as inflammation and fibroblast proliferation[43] that play a role in fibrosis in tumoral or systemic sclerosis[44-47] and in AAV-associated pulmonary fibrosis.[48] Interestingly, thiol compounds reduce tissue damage by suppressing leukocyte margination[49,50] and reverse the effects of MPO and HOCl through chloramine scavenging.[51,52] In our study, serum thiol levels were significantly lower in patients with AAV compared to healthy controls and those with high AAV disease activity. Although further studies are needed in this area, our study results suggest that thiols may constitute a physiological defense mechanism against vascular inflammation and tissue damage that play a role in AAV pathogenesis and AAV-related complications.

In our study, serum IMA levels were similar between the AAV and control groups. In addition, no significant correlation was observed between BVAS-3 score and serum IMA levels. Studies have shown that serum IMA levels may be a helpful biomarker reflecting the oxidative status. IMA may be essential in treating inflammatory and vascular endothelial dysfunction diseases.[13,53-55] Serum IMA levels have not been previously compared between AAV patients and healthy controls. However, in a prospective study, serum IMA levels were similar in active and remission AAV patients. In addition, this study found no significant correlation between serum IMA levels and BVAS-3 scores.[56] IMA levels were not affected by AAV disease activity in our study because of the relatively small number of subjects in the study group or the possible effects of drugs used in medical treatment on serum IMA levels. However, considering the close relationship of IMA with diseases that progress with inflammatory processes, numerous and more advanced studies are needed to understand its possible roles in AAV pathophysiology better.

In our study, although a significant relationship was found between AAV disease activity score BVAS-3 and -SH, it was found that thiol parameters and IMA levels did not change in any organ involvement of AAV or the types of medical treatment used. Except for one study in which thiol levels were measured with the old method and their relationship with the presence of AAV renal crescent was evaluated,[57] neither Dtdh and IMA levels nor the types of medical treatment used have not been previously evaluated in AAV patients. In this study in which thiol levels were measured with the old method, serum thiol levels were lower in those with active crescent than those without. The authors of this study suggested that high thiol levels may be protective against renal damage caused by vasculitis.[57] In this study,

in which we evaluated serum thiol levels according to AAV systemic organ involvement with the new method, we did not find a significant relationship between organ damage and thiol levels, but further studies are needed to understand better the possible roles of thiol and IMA molecules in the pathophysiology of AAV organ damage.

Study Limitations

The limitations of this study include the possible effects of medical treatments used in the treatment of AVV on IMA levels and Dtdh, and the fact that it was a cross-sectional study.

Conclusion

Dtdh was shifted in favor of oxidation in AAV patients compared to controls, and patients with higher AAV disease scores had lower serum thiol levels. Thiol molecules may be a potential candidate for understanding AAV disease pathophysiology and predicting disease activity.

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Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from Ankara Bilkent City Hospital No. 2 Ethics Committee dated 10/05/2023 and numbered E2-23-3791.

Informed Consent: Informed consent forms were obtained from all participants of the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., Ö.E., Concept: A.K., E.F.O., S.C.G., Y.M., Ş.E., Design: A.K., E.F.O., S.C.G., Ş.E., Data Collection and Processing: A.K., S.A., E.F.O., S.C.G., Y.M., Ö.E., Analysis or Interpretation: A.K., S.A., E.F.O., Ö.E., Literature Search: A.K., S.A., Writing: A.K.

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