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## ORIGINAL ARTICLE

### Serum Level of Anti-Tubulin-Alpha-1c Antibody in Behçet Disease and Its Relation to Disease Activity and Severity

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#### ABSTRACT

Behçet's Disease (BD) is a chronic, multisystem inflammatory condition with unpredictable activity and diverse clinical manifestations. Reliable biomarkers for monitoring disease severity and activity are still needed. This study aimed to evaluate serum anti-tubulin-alpha-1c antibody levels and their association with clinical features, disease activity and severity of BD. **Methods:** This case-control study included 84 participants: 42 BD patients from the Rheumatology and Rehabilitation Department, Zagazig University Hospitals, and 42 healthy controls. Serum anti-tubulin-alpha-1c antibody levels were measured using ELISA. **Results:** Serum anti-tubulin-alpha-1c levels were significantly higher in patients with arthritis ( $P=0.01$ ), anterior uveitis ( $P=0.03$ ), panuveitis ( $P=0.02$ ), optic atrophy ( $P<0.001$ ), skin lesions ( $P=0.01$ ), vascular involvement ( $P=0.043$ ), stroke ( $P=0.048$ ), and psychosis ( $P=0.04$ ). A positive significant correlation was observed between antibody levels and both the Behçet's Disease Damage Index ( $r=0.652$ ,  $P<0.001$ ) and the Birmingham Vasculitis Activity Score ( $r=0.361$ ,  $P=0.02$ ). Antibody levels were significantly higher in active compared to inactive disease ( $P=0.04$ ). ROC analysis showed good diagnostic accuracy of anti-tubulin-alpha-1c in distinguishing active from inactive disease ( $AUC=0.731$ ), detecting vascular lesions ( $AUC=0.694$ ), and identifying high disease severity ( $AUC=0.824$ ). **Conclusion:** Serum anti-tubulin-alpha-1c antibody levels are significantly associated with disease activity, severity, and multiple clinical features of BD. These findings suggest that it may serve as a valuable biomarker for monitoring disease activity and identifying patients at higher risk for severe or multisystem involvement.

**Keywords** Behçet's disease, anti-tubulin-alpha-1c, disease activity.

#### INTRODUCTION

Behçet's disease (BD) is a chronic multisystemic inflammatory disease, and usually manifests as recurring oral and vaginal ulcers, ocular lesions, and a wide range of systemic symptoms, including arthritis, neurological, vascular, and gastrointestinal involvement [1].

The vast majority of Behçet's disease clinical manifestations are caused by small,

medium, or large blood vessel vasculitis of the venous and arterial systems. One of the main characteristics of BD is heterogeneity, which makes diagnosis and treatment difficult [2].

Although the precise cause of BD is unknown, the generally accepted theory holds that a complex interplay between environmental factors and genetic susceptibility results in immunological

dysfunction, which is thought to be an inflammatory disorder that lies between autoimmune and autoinflammatory conditions[3].

Microtubules are vascular cells' fundamental cytoskeletal structural elements, made up of  $\alpha$ - $\beta$ -tubulin heterodimers. There are six different families in the tubulin superfamily, and each has a variety of isotypes. Alpha, beta, gamma, delta, epsilon, and zeta tubulins [2].

Alpha/beta-tubulin heterodimer is the structural element of microtubules. They undergo a range of posttranslational modifications, exist in several forms of isotypes, and share 40% of their amino acid sequence. Alpha-tubulin and beta-tubulin have almost the same structures. One member of the alpha-tubulin family is tubulin- $\alpha$ 1-C, sometimes referred to as tubulin alpha-6. Human tissues contain three distinct isoforms of tubulin- $\alpha$ 1-C: isoforms a, b, and c [4].

After lung transplantation, in patients who suffer from persistent allograft rejection, alpha-tubulin has been identified as an autoantigen. and its autoantibodies are a contributing factor to bronchiolitis obliterans syndrome (BOS). Patients with SLE had higher levels of serum anti-tubulin- $\alpha$ 1C autoantibodies, which was linked to increased disease activity, vasculitis symptoms, and primarily cutaneous lesions [4]. In order to identify a biomarker for future disease activity and severity assessments, this study sought to determine the serum level of anti-tubulin-alpha-1c antibody in Behçet's illness and its relationship to disease activity and severity.

### METHODS

This case-control study was carried out in Rheumatology and Rehabilitation and Clinical Pathology Departments of Zagazig University Hospitals between December 2023 and December 2024. Eighty-four people were divided into two equal groups

at this time: Group (1) included 42 Behçet's Disease patients selected from Rheumatology and Rehabilitation Department Zagazig University Hospitals. Group (2) had 42 apparently healthy subjects who served as a control group. All participants' informed consent was acquired in compliance with the local ethical committee's guidelines (IRB# 11126/9/2023). This study complies with the Declaration of Helsinki's ethical standards for research involving human subjects. Inclusion criteria: Patients with Behçet's Disease were diagnosed according to the International Criteria for Behçet's Disease (ICBD) [5]. The study excluded patients with primary vasculitis or other autoimmune disorders.

A comprehensive medical history was obtained for each patient, including details on their name, age, sex, address, marital status, and occupation, and any unusual behaviors they might have had, like smoking. Every BD patient received a comprehensive clinical evaluation.

The patients were assessed using the Behçet's Disease Current Activity Form score (BDCAF) [6]: a score of 4 or above out of 12 denotes high disease activity, whereas a score of less than 4 denotes low disease activity [2]. The BDCAF score is a number between 0 and 12. Headache, vaginal and oral ulcers, skin lesions, joint involvement, gastrointestinal, ophthalmic, neurological, and major vascular involvement are among the investigated signs.

The Birmingham Vasculitis Activity Score (BVAS) (version 3) is used to quantify vascular activity at the time of diagnosis. The weighted score is based on the nine different organ systems: the cardiovascular, abdominal, renal, neurological, cutaneous, ophthalmic, ENT, chest, and general. The score, which goes from 0 to 63 [7], evaluates

clinical characteristics linked to active vasculitis during the preceding four weeks. Score for assessment of damage by Behçet disease Damage Index (BDI) [8]. It comprises 73 items within 11 organ-specific categories, with a total possible score of 100 points.

Laboratory investigation included Measurement of anti Tubulin alpha-1C antibody (anti-TUBA1C) serum level by ELISA in which Kit was provided from Wuhan Fine Biotech Company (China) with Catalogue No. 201-12-8732C as directed by the manufacturer's instruction in which absorbance was read on sunrise (Tecan Austria GmbH, 5082 Groding, Austria).

### Statistical analysis

IBM SPSS version 23.0 for Windows was used to code, enter, and analyze the gathered data (SPSS Inc., Chicago, IL, USA). The number (n) and percentage (%) of observations within each category were used to summarize the qualitative data. The mean, median (particularly for skewed data because of its resistance to extreme values), standard deviation (as a measure of dispersion), Quantitative data were described using range (the difference between the highest and lowest values) and interquartile range (IQR). Fisher's exact test was employed when expected frequencies in more than 20% of cells fell below 5, and the Chi-square ( $\chi^2$ ) test was utilized to assess the relationship between the qualitative variables. When comparing quantitative variables between two independent groups, the non-parametric Mann-Whitney U test (also known as the Wilcoxon rank-sum test) was used, whereas the independent t-test was used for data that was regularly distributed. Two quantitative variables were correlated using Spearman's rank correlation for ordinal or non-normally distributed data and Pearson's correlation for parametric data. Additionally, the receiver operating characteristic (ROC) curve was used to

assess the sensitivity and specificity of quantitative diagnostic markers in splitting cases into two groups.  $P < 0.05$  was regarded as a significant p-value.

### RESULTS

The 84 participants in this case-control study were divided into two groups: **Group I** (Behçet's disease group) included 42 patients who were diagnosed according to the International Criteria for Behçet's Disease (ICBD). Their ages ranged between 19 and 58 years with a mean of  $35.5 \pm 10.05$ . They were 28 males (66.7%) and 14 females (33.3%). **Group II** (control group) comprised forty-two healthy volunteers. Their ages ranged from 19 to 56 years with a mean of  $33 \pm 9.54$ , and. They were 29 males (69%) and 13 females (31%). Regarding demographic data, **Table 1** revealed no discernible differences between the groups under study ( $P > 0.05$ ).

Disease duration ranged between 1 to 20 years with a mean of  $7.24 \pm 4.89$ . Regarding clinical data, the most common manifestation was oral ulcers which were detected in 38 patients (90.5%), followed by genital ulcers and skin lesions among 33 (78.6%) of the patients. Articular manifestations were detected in form of arthralgia among 20 (47.6%) of the patients and arthritis among 19 (45.2%) of the patients. Total neurological lesions were found in 33 patients (78.6%), the most common was headache among 31 (73.8%) of the patients. Total eye lesions were detected in 37 patients (88%), the most common were retinal vasculitis among 17 (40.4%) of the patients and panuveitis among 17 patients (40.4%), while total vascular lesions were found in 14 patients (33.3%), the most common was deep vein thrombosis (DVT) among 9 (21.4%) of the patients.

Regarding serum anti-tubulin-alpha-1c antibody levels, the Behçet's disease group (The median value=1194) had a greater level than the control group (The

median value=506) ( $P<0.001$ ), indicating a statistically significant difference between the two groups (table 2).

Additionally, there was a noteworthy positive significant association between serum anti-tubulin-alpha-1c and each of the following: disease duration ( $r=0.333$ ,  $P=0.03$ ) (table 3), patients with arthritis ( $P=0.01$ ), anterior uveitis ( $P=0.03$ ), panuveitis ( $P=0.02$ ), optic atrophy ( $P<0.001$ ), skin lesions ( $P=0.01$ ), vascular lesions ( $P=0.043$ ), stroke ( $P=0.048$ ) and psychosis ( $P=0.04$ ) (table 3,4).

A significant positive correlation between serum anti-tubulin-alpha-1c with BDCAF score ( $r=0.417$ ,  $P=0.006$ ), BVAS score ( $r=0.361$ ,  $P=0.02$ ) and BDI score ( $r=0.652$ ,  $P<0.001$ ) was found (figure 1).

On conducting ROC analysis (Receiver operation Curve) to determine the optimal

cutoff value to discriminate active Behçet's disease from inactive disease, the analysis showed that serum anti-tubulin-alpha-1c had the highest sensitivity (74.29%) and specificity (71.43%) at 1195.4 ng/ml with the curve's area under it was (0.731), and to discriminate patients with vascular lesions from patients with no vascular lesions, the analysis showed that serum anti-tubulin-alpha-1c had the highest sensitivity (71.43%) and specificity (78.57%) at 1490.26 ng/ml with area under the curve of (0.694). Finally, in order to separate patients with severe illnesses from those with less severe illnesses, the analysis showed that serum anti-tubulin-alpha-1c had the highest sensitivity (79.41%) and specificity (87.5%) at 994.805 ng/ml where the area under the curve of (0.824) (figure 2).

**Table (1):** Demographic data among the studied groups

Variables		Behçet's disease group (n=42)	Control group (n=42)	P Value
Age (years)	Mean±SD	35.5 ± 10.05	33 ± 9.54	0.25
	Range	(19 – 58)	(19 – 56)	
Sex (n. %)	Male	28 (66.7%)	29 (69%)	0.82
	Female	14 (33.3%)	13 (31%)	
Smoking status (n. %)	Non-smokers	32 (76.2%)	33 (78.6%)	0.79
	Smokers	10 (23.8%)	9 (21.4%)	
Occupation (n. %)	None	19 (45.2%)	24 (57.1%)	0.28
	Worker	23 (54.8%)	18 (42.9%)	
BMI (kg/m <sup>2</sup> )	Mean ± SD	26.4 ± 3.48	27.1 ± 3.9	0.38
	Range	(21 – 35.3)	(20.4–33.9)	

\*Student T-test, Chi-square test, Non-significant:  $P>0.05$ , Significant:  $P\leq 0.05$

**Table (2):** Serum anti-tubulin-alpha-1c antibody level among the studied groups

Variables		Behçet's disease group (n=42)	Control group (n=42)	P Value
Anti-tubulin-alpha-1c (ng/ml)	Median (IQR)	1194 (1005)	506 (218)	<0.001**
	Range	(349 – 4594)	(209–676)	

\*Mann-Whitney U test, Non-significant:  $P>0.05$ , Significant:  $P\leq 0.05$

\* IQR=Interquartile range

**Table (3):** Correlation of Serum anti-tubulin-alpha-1c antibody with demographic data among the Behçet's disease group

Variable	Anti-tubulin-alpha-1c	
	R	P
Age	0.161	0.31
BMI	-0.136	0.39
Disease duration	0.333	<b>0.03*</b>

\*Pearson correlation, Spearman rank correlation test, Non-significant:  $P > 0.05$ , Significant:  $P \leq 0.05$ , \*BMI=Body mass index

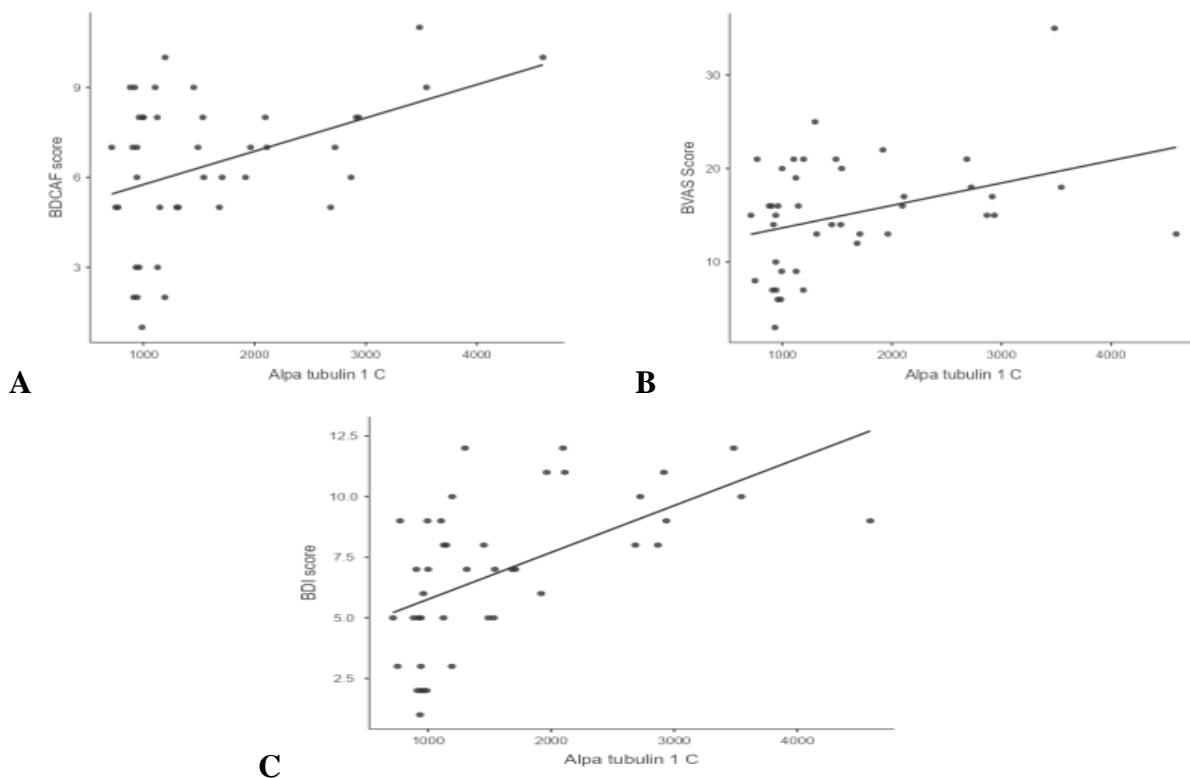
**Table (4):** Association between clinical data and serum anti-tubulin-alpha-1c antibody level among the Behçet's disease group

Variables		Anti-tubulin-alpha-1c Median (IQR)	P Value
Oral ulcers	Absent	1309 (765)	0.76
	Present	1194 (1006)	
Genital ulcers	Absent	1300 (765)	0.98
	Present	1193 (1003)	
Skin lesions	Absent	961 (73.4)	<b>0.01*</b>
	Present	1452 (1110.8)	
Pathergy test	Absent	1128 (753)	0.59
	Present	1314 (1125)	
Arthralgia	Absent	1149 (918)	0.89
	Present	1307 (1028)	
Arthritis	Absent	1125 (497)	<b>0.01*</b>
	Present	1919 (1743)	
Anterior uveitis	Absent	1000 (220)	<b>0.03*</b>
	Present	1490 (1426)	
Posterior uveitis	Absent	1136 (600)	0.26
	Present	1247 (1569)	
Panuveitis	Absent	1115 (600)	<b>0.02*</b>
	Present	1580 (1859)	
Optic atrophy	Absent	1115 (707)	<b>&lt;0.001**</b>
	Present	2416 (1610)	
Retinal vasculitis	Absent	1147 (742)	0.22
	Present	1314 (1684)	
Visual loss	Absent	1128 (1000)	0.24
	Present	1314 (961)	

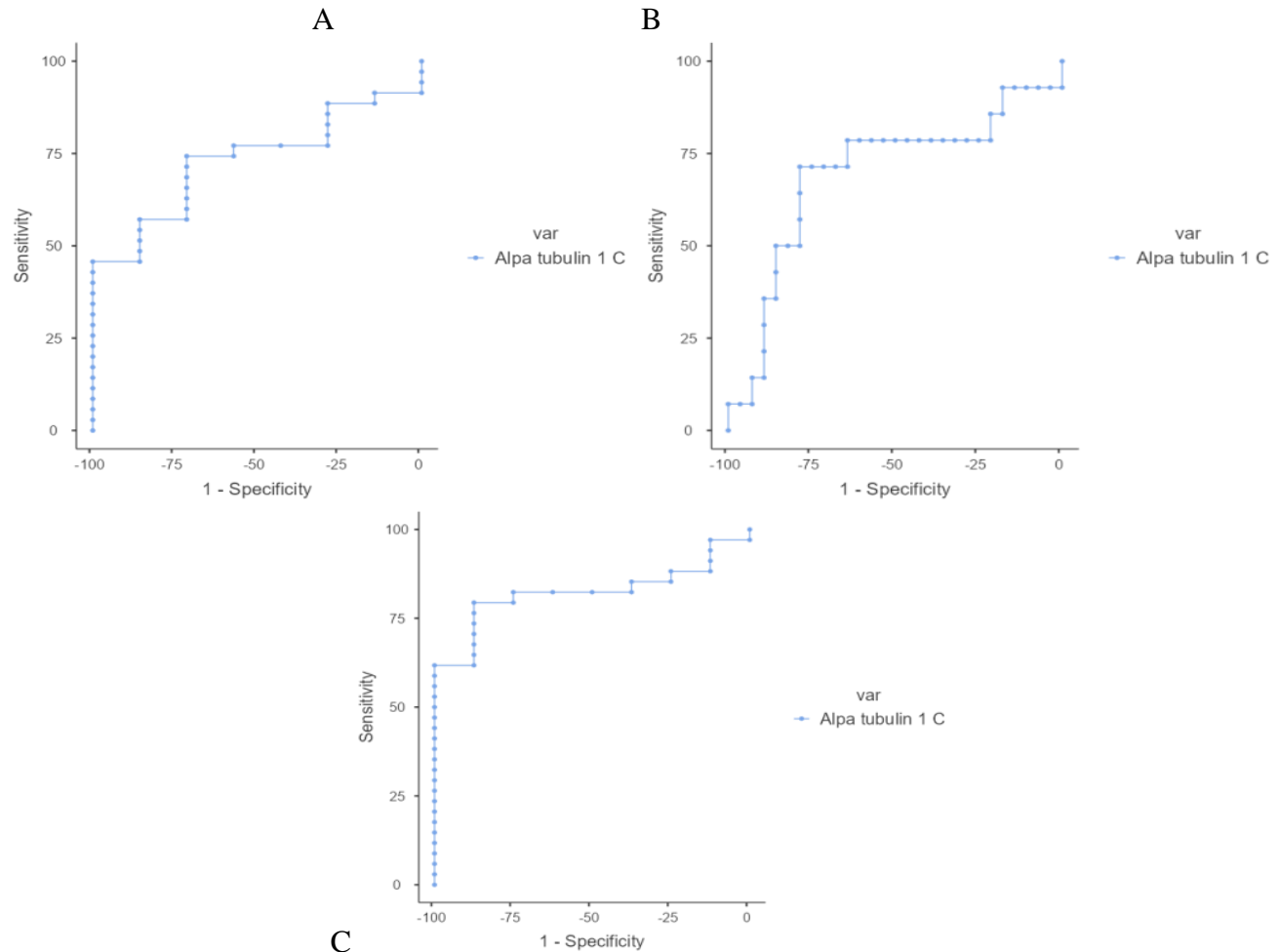
Variables		Anti-tubulin-alpha-1c Median (IQR)	P Value
Total vascular lesions	Absent	1115 (519)	<b>0.043*</b>
	Present	1823 (1434)	
Stroke	Absent	1193 (975)	<b>0.048*</b>
	Present	4594 (0)	
Headache	Absent	988 (425)	0.09
	Present	1314 (1419)	
Hemiplegia	Absent	1193 (975)	0.52
	Present	1964 (0)	
Neuropathy	Absent	1128 (666)	0.3
	Present	1490 (1053)	
Psychosis	Absent	1147 (671)	<b>0.04*</b>
	Present	2916 (2033)	

\*Mann-Whitney U test, Non-significant:  $P > 0.05$ , Significant:  $P \leq 0.05$

\***IQR**=Interquartile range, **DVT**=Deep venous thrombosis



**Figure (1):** Scatter plots showing the correlation between anti-tubulin-alpha-1c and different scores; **A)** BDCAF score , **B)** BVAS score, **C)** BDI score among the Behçet's disease group.



**Figure (2):** ROC curve analysis of serum anti-tubulin-alpha-1c antibody; **A)** differentiating active disease from inactive disease. **B)** differentiating patients with vascular lesions from patients with no vascular lesions. **C)** differentiating patients with high disease severity from patients with low disease severity.

### DISCUSSION

Behçet's disease (BD) is a chronic inflammatory vascular disease of unclear etiology, affecting multiple organs and systems. Skin lesions, uveitis, and recurring oral and vaginal ulcers are among the most prevalent clinical signs of BD [9].

While the exact cause of BD is still unknown, several researchers have proposed that genetic vulnerability rather than environmental variables plays a larger role in the etiopathogenesis of BD. Genome-wide Association Studies (GWAS) have found several other

important genomic variants of BD in addition to HLA-B51, which is currently thought to be the main genetic susceptibility factor of BD [10].

Numerous antigens found in peripheral blood's circulating immune complexes (CICs) may be linked to underlying illnesses. CICs of Behçet's disease yielded a total of 17 new putative autoantigens. Further research revealed that the autoantibody against tubulin- $\alpha$ -1c, one of these autoantigens, may be a sensitive and specific diagnostic biomarker for BD. Anti-tubulin- $\alpha$ -1c antibodies were linked to

disease activity and could be involved in the disease's development [11].

Therefore, Assessing the blood level of anti-tubulin-alpha-1c antibody in Behçet's sickness and its relationship to clinical symptoms, disease activity, and severity is the aim of our research.

In the present study; regarding the frequency of clinical signs and symptoms in Behçet's illness patients, the most frequent clinical manifestation was oral ulcers which occurred in (90.5%) of patients and eye lesions in (88%) of the patients. Genital sores occurred in (78.6%) of patients, skin lesions among (78.6%) of the patients, neurological involvement among (78.6%) of the patients, while joint involvement was evident in patients in the form of arthralgia (47.6%) and arthritis (45.2%), and vascular involvement was evident in (33.3%) of the patients.

**Wann et al.**, reported that mouth ulcers were the most common clinical manifestation in all BD patients (100%), with genital ulcers coming in second in 80% of cases, eye lesions in 60% of the patients, and joint manifestations like arthralgia in 60% of the cases they studied [11].

According to a study on the clinical expression of Behçet's illness in Upper Egypt, mouth ulcers accounted for 100% of all clinical manifestations, with genital ulcers coming in second at 88%. the skin lesions appeared in (80 %) and ocular lesions appeared in (68 %) of studied patients. Meanwhile, the vascular lesions appeared in (26 %) and neurological manifestation occurred in (10%) of patients [13].

Patients with Behçet's disease had a higher serum anti-tubulin-alpha-1c antibody level than those in the control group ( $P<0.001$ ), in this respect, our analysis shows a statistically significant difference between the two groups.

These results are consistent with those of Amin et al., who looked into anti-tubulin-alpha-1c antibody as a potential marker for Behçet syndrome and discovered that BD patients had far greater levels of the antibody than did healthy controls [2].

In another study, Cheng et al. discovered anti-tubulin- $\alpha$ -1c, an autoantibody associated with inflammation that shows promise as a diagnostic tool for Behçet's disease, using circulating immune complexome analysis. Additionally, they verified that BD patients had considerably greater levels of tubulin- $\alpha$ -1c compared to both healthy controls and patients with other autoimmune disorders [11].

Our research revealed a strong positive relationship between duration of the disease and serum anti-tubulin-alpha-1c ( $r=0.333$ ,  $P=0.03$ ), that may be due to long standing disease lead to more immune complex deposition within the vascular endothelium can worsen tissue damage and trigger an inflammatory reaction.

This study found a statistically significant difference in serum anti-tubulin-alpha-1c antibody levels based on clinical data. Arthritis patients had greater serum levels of anti-tubulin-alpha-1c antibodies ( $P=0.01$ ), anterior uveitis ( $P=0.03$ ), panuveitis ( $P=0.02$ ), optic atrophy ( $P<0.001$ ), skin lesions ( $P=0.01$ ), vascular lesions ( $P=0.043$ ), stroke ( $P=0.048$ ), and psychosis ( $P=0.04$ ).

This is in line with Amin et al.'s findings that anti-tubulin-alpha-1c was substantially associated with venous thrombosis, panuveitis, and posterior uveitis (p values of 0.023, 0.034, and 0.009, respectively), with instances exhibiting different clinical manifestations [2].

In line with our findings, Cheng et al. showed that anti-tubulin- $\alpha$ -1c autoantibodies were linked to erythema nodosum and issues with deep vein thrombosis in BD [11]. Anti-tubulin- $\alpha$ -1c

autoantibodies may target endothelial cells that express tubulin- $\alpha$ -1c. This may eventually result in local vascular inflammation and endothelial cell death in BD, according to the study's findings.

According to a different study by Zhao et al., The only vasculitis symptoms associated with an elevation in anti-tubulin- $\alpha$ -1C in SLE patients were minor vessel damages, such as cutaneous lesions and mouth ulcers [4].

Serum anti-tubulin-alpha-1c antibody levels and disease activity among the Behçet's disease group were found to differ statistically significantly in the current study. Patients with active disease had higher serum anti-tubulin-alpha-1c levels than patients with inactive disease ( $P=0.04$ ), and there was a significant positive correlation between serum anti-tubulin-alpha-1c and the BDCAF score ( $r=0.417$ ,  $P=0.006$ ).

This aligns with Cheng et al.'s findings, which showed that anti-tubulin- $\alpha$ -1c autoantibodies were significantly positively correlated with Behçet's disease activity [11], and Amin et al., who reported that the anti-tubulin- $\alpha$ -1c autoantibody was positively correlated with disease activity in Behçet's disease patients with ( $p=0.002$ ) [2].

Serum anti-tubulin-alpha-1c had a substantial positive connection with both the BDI score ( $r=0.652$ ,  $P<0.001$ ) and the BVAS score ( $r=0.361$ ,  $P=0.02$ ) in the current study.

This is consistent with Amin et al.'s findings that anti-tubulin- $\alpha$ -1c had a positive connection with venous thrombosis and a substantial direct link with the BVAS score. According to this study, tubulin- $\alpha$ -1c antibody liability is involved in the vascular etiology of BD [2].

Anti-tubulin- $\alpha$ -1c's suggestive value on BD's total vasculitis activity was also assessed by Cheng et al., who discovered a

strong positive correlation between anti-tubulin- $\alpha$ -1c autoantibodies and BVAS ( $r=0.419$ ,  $p<0.05$ ) as well as the occurrence of complications such DVT and Erythema nodosum. This suggests that anti-tubulin- $\alpha$ -1c may have a role in the pathophysiology of vascular injury[11].

In this study on conducting ROC analysis to determine the optimal cutoff value to discriminate active Behçet's disease from inactive disease, serum anti-tubulin-alpha-1c had the highest sensitivity (74.29%) and specificity (71.43%) at 1195.4 ng/ml with the curve's area under it was (0.731).

On conducting ROC analysis to determine the optimal cutoff value to separate individuals with severe illnesses from those with less severe illnesses, the analysis showed that serum anti-tubulin-alpha-1c had the highest sensitivity (71.43%) and specificity (78.57%) at 1490.26ng/ml with area under the curve of (0.694).

Also on conducting ROC analysis to determine the optimal cutoff value to discriminate patients with vascular lesions from other BD patients, the analysis showed that serum anti-tubulin-alpha-1c had the highest sensitivity (79.41%) and specificity (87.5%) at 994.805 ng/ml with the curve's area under it (0.824).

Despite these findings, there are still several limitations to our study, including no follow-up and a small sample size. Therefore, long-term follow-up is necessary to ascertain how the tubulin- $\alpha$ -1c antibody contributes to the pathogenesis of BD.

### Conclusion

In conclusion our findings indicate a substantial correlation between tubulin- $\alpha$ -1c antibody and disease activity, severity, and several clinical manifestations in BD. It represents a promising sensitive biomarker for monitoring Behçet's Disease activity and identifying patients at risk of severe and multisystem involvement.

**Conflict of Interest:** None

**Financial Disclosures:** None

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## Citation

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