
































## ORIGINAL ARTICLE

# Moderation analysis exploring associations between age and mucocutaneous activity in Behçet's syndrome: A multicenter study from Turkey

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## ABSTRACT

The aim of the present study was to examine the effects of age on mucocutaneous activity by using moderation analysis in Behçet's syndrome (BS). In this cross-sectional study, 887 BS patients (female : male, 481:406; mean age, 38.4 ± 10.9 years) followed in 13 tertiary centers in Turkey were included. Mucocutaneous activity was evaluated by using the Mucocutaneous Index (MI) according to sex and disease course. Moderation analysis was performed to test the effect of age on mucocutaneous activity. A moderator variable is a third variable and affects the relationship between independent and outcome variables. Age was chosen as a potential moderator variable (interaction effect), MI score as the outcome variable and sex as an independent variable in the analysis. The moderation analysis tested the effects of age in three steps: whole BS patient group, patients without systemic involvement and those with systemic involvement. The moderation model was only significant in BS patients with systemic involvement ( $P = 0.0351$ ), and a significant relationship was observed between female sex and MI score ( $P = 0.0156$ ). In addition, the interaction plot showed that female patients had increased MI scores compared with male patients, especially in the 28-year-old age group ( $P = 0.0067$ ). Moreover, major organ involvement was newly diagnosed in the majority of these young female BS patients. Our results suggest that the relationship between sex and mucocutaneous activity was moderated by age in the systemic involvement group. Also, increased mucocutaneous activity may be associated with new major organ involvement in young female BS patients with systemic involvement.

**Key words:** Behçet's syndrome, moderation analysis, mucocutaneous activity, Mucocutaneous Index, sex.

## INTRODUCTION

Currently, the assessment of patients' needs and impacts of symptoms on patients' lives is essential in value-driven and

patient-centric health care. In this regard, patient-reported outcome measures (PROM) that reflect the patient's perspective within the evidence-based health-care culture are valuable tools in chronic disease management.<sup>1</sup>

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Behçet's syndrome (BS) is a chronic systemic inflammatory disorder with oral and genital ulcers and cutaneous, ocular, arthritic, vascular, central nervous system and gastrointestinal involvement. Mucocutaneous manifestations with musculoskeletal involvement comprise a relatively milder clinical spectrum of the disease, whereas the others are in the severe disease spectrum.<sup>2,3</sup>

Because there are no specific serological and/or biochemical activity markers in BS,<sup>2</sup> PROM are valuable tools for improving decision-making processes in the clinical practice of BS management.<sup>2,4,5</sup> They can have a disease- or organ-specific pattern that comprises the clinical characteristics of BS. The Total Activity Index,<sup>6</sup> Behçet's Disease Current Activity Form (BDCAF)<sup>7</sup> and Behçet's Syndrome Activity Scale<sup>8</sup> are validated disease-specific PROM in BS. Oral Ulcer Severity Score,<sup>9</sup> Genital Ulcer Severity Score,<sup>10</sup> Composite Index (CI)<sup>5</sup> for oral ulcers and Mucocutaneous Index (MI) for mucocutaneous manifestations are validated organ-specific PROM.<sup>2,11</sup>

Mucocutaneous manifestations are the most commonly observed clinical features of BS, especially in women.<sup>12-14</sup> Oral ulcer activity is shown to be a predictive factor for the new onset of major organ involvement in young male patients with BS.<sup>15</sup> However, mucocutaneous activity may decrease with increasing age, except oral ulcers which may persist.<sup>3,16</sup> As a result, there are close relationships between disease course, sex and age in BS.<sup>17</sup> In this context, the study question was designed to explore whether age could have a moderating effect on disease activity in BS. A moderator variable is defined as a third variable affecting the relationship between independent and outcome variables. It is not a part of the causal structure in a model.<sup>18-20</sup> In the present study, we aim to examine the effects of age on mucocutaneous activity according to the disease course by using moderation analysis in BS.

## METHODS

In this cross-sectional study, 887 patients with BS (female : male, 481:406; mean age, 38.4 ± 10.9 years) diagnosed by international study group criteria<sup>21</sup> and followed in 13 tertiary centers from Turkey were included. Data were collected from September 2017 to July 2018.

Clinical manifestations of BS patients are presented in Table 1. The mean disease duration of the group was 9.1 ± 7.7 years. BS patients were categorized as those without systemic involvement (mucocutaneous manifestations and musculoskeletal) and with systemic involvement (ocular, vascular, central nervous system and gastrointestinal). Disease severity scores (4.5 ± 1.9)<sup>22</sup> were assessed in the whole group. Treatment protocols were also analyzed in the two main groups. Colchicine, sulfasalazine, non-steroidal anti-inflammatory drugs and antibiotics formed the non-immunosuppressive medication group (non-IS; *n* = 549, 61.9%), whereas azathioprine, corticosteroids, anti-tumor necrosis factor agents and interferons (immunomodulators) were included in the immunosuppressive/immunomodulator group (IS; *n* = 289, 32.6%). Patients (*n* = 49, 5.5%) who were not using any medication were not included in the analysis.

**Table 1.** Study group profile

	<i>n</i>	%
Oral ulcer	887	100
Genital ulcer	722	82.9
Cutaneous involvement	682	76.8
Musculoskeletal involvement	385	43.4
Vascular involvement	106	11.9
Neurological involvement	34	3.9
Gastrointestinal involvement	13	1.5
Pathergy test positivity	481	54.5
Patients without systemic involvement	631	71.1
Male	264	41.8
Female	367	58.2
Total	631	100
Patients with systemic involvement	256	28.9
Male	142	55.5
Female	114	44.5
Total	256	100
Treatment protocols		
Non-IS	549	61.9
IS	289	32.5
No medication	49	5.5
Total	887	100
	Mean	SD
Age (years)	38.35	10.89
Disease duration (years)	9.11	7.68
PROM in the study group		
Mucocutaneous Index score ( <i>n</i> = 887)	5.6	5.4
Composite Index	4.1	3.5
Genital ulcer Index	0.9	2.5
Erythema nodosum Index	0.6	1.9
Behçet disease current activity form ( <i>n</i> = 510)	4.3	3.1

Two PROM were used in the study. The validated MI and its subgroup activity indices regarding oral ulcer (CI), genital ulcer (GI) and erythema nodosum (Erythema Nodosum Index [EI]) were assessed during the previous month. Presence of active mucocutaneous symptom (1 point), pain evaluated by 100-mm visual analog scale (5 points) and functional limitations (4 points) were evaluated for each involvement. Scores of each subgroup ranged between 0 (inactive) and 10 (very active). The total MI score was composed of these subgroups (0–30 points) (Fig. 1).<sup>2</sup> As the second PROM, the BDCAF was used for global disease activity during the last 4 weeks. Transformed BDCAF score was used to evaluate global activity.<sup>23,24</sup>

Two methods were chosen to assess the role of age on mucocutaneous activity, which was the primary question of the present study. As the first step, age was categorized into two groups as younger versus older age groups according to a previous study.<sup>15</sup> The cut-off value for the young age group was determined as 25 years (*n* = 219, 24.75%; 25 ± 3.54 years; median, 25), whereas the other patients (*n* = 668, 74.25%) were in the older age group (42.9 ± 8.6 years; median, 41). In the second step, moderation

**COMPOSITE INDEX FOR ORAL ULCER ACTIVITY (Total score: 0-10)**

**1. Oral ulcer activity:** (0-1 points) 1) The number of oral ulcers during the last month: 0= 0 point, ≥1= 1 point

**2. Pain status:** (0-5 points)

Please place a vertical mark on the scale below to describe how bad you felt pain due to oral ulcer during the last month.

0 (no pain) |-----| 100  
(severe pain )

**3. Functional status:** Please describe effects of oral ulcers on your oral functions in the last month? (0-4 points)

How often.....	None of the time (0)	Little of the time (1)	Some of the time (2)	Most of the time (3)	All of the time (4)
Did you feel unpleasant <i>taste</i> in your mouth due to oral ulcers?					
Did you have difficulty in <i>speaking</i> due to oral ulcers?					
Did you have difficulty in <i>eating/chewing/swallowing</i> due to oral ulcers?					

**GENITAL ULCER ACTIVITY (Total score: 0-10)**

**1. Genital ulcer activity:** (0-1 points) 1) The number of genital ulcers during the last month: 0= 0 point, ≥1= 1 point

**2. Pain status:** (0-5 points)

Please place a vertical mark on the scale below to describe how bad you felt pain due to genital ulcer during the last month.

0 (no pain) |-----| 100  
(severe pain)

**3. Functional status:** Please describe effects of genital ulcers on your functions in the last month? (0-4 points)

How often.....	None of the time (0)	Little of the time (1)	Some of the time (2)	Most of the time (3)	All of the time (4)
Did you feel limitation of your <i>simple movements</i> such as sitting due to genital ulcers?					
Did you have difficulty in <i>your sexual life</i> due to genital ulcers?					
Did you have difficulty in doing your <i>personal care and needs</i> (bathroom, toilet, cleaning) due to genital ulcers?					

**ERYTHEMA NODOSUM ACTIVITY (Total score: 0-10)**

**1. Erythema nodosum activity:** The number of erythema nodosum during the last month: 0= 0 point, ≥1= 1 point

**2. Pain status:** (0-5 points)

Please place a vertical mark on the scale below to describe how bad you felt pain due to tender nodules on your legs during the last month.

0 (no pain) |-----| 100  
(severe pain)

**3. Functional status:** Please describe effects of aching tender nodules on your legs in the last month? (0-4 points)

How often.....	None of the time (0)	Little of the time (1)	Some of the time (2)	Most of the time (3)	All of the time (4)
Did you have difficulty in doing <i>daily activities</i> ?					
Did you have difficulty in doing <i>physical activity</i> (sports, walking, going up and down stairs)?					

**Figure 1.** Mucocutaneous Index for patients with Behçet's syndrome.

analysis was performed to test the potential effect of age on the analysis. In this analysis, age was determined as a potential moderator variable, MI score as the outcome (dependent) variable and sex as an independent variable.

Because age is associated with the disease duration, the possible effect of the disease duration on mucocutaneous activity was also evaluated by a mediator analysis.

The inclusion criteria for the study were age of 18 years or more and being under medical control for BS. The presence of other chronic conditions leading to mucocutaneous manifestations was an exclusion criterion. The study was performed according to the principles of the Declaration of Helsinki and was approved by the ethics committee of Marmara University Medical School (14 July 2017, no. 09.2017.497). Informed consent was obtained from all patients.

### Statistical analysis

Analyses were carried out by using SPSS version 26 statistical software (SPSS, Chicago, IL, USA). Non-parametric analysis regarding the Mann–Whitney *U*-test and Spearman's rank correlation coefficient were used due to the non-normal distribution of data. The  $\chi^2$ -test was used for categorical variables.  $P \leq 0.05$  was accepted as statistically significant. Cronbach alpha values for internal reliability of functional limitation section were found to be very high in the group (CI, 0.908; GI, 0.863; EI, 0.972).

### Moderation analysis

The analysis allows testing of the influence of a third variable on the relationship between the dependent and independent variables.<sup>19,20</sup> The PROCESS macro in the SPSS software was used for the analysis.<sup>18</sup> In this study, we used a dichotomous independent variable (sex) and continuous dependent variable (MI score), which was modified by a continuous moderator variable (age). Sex was coded as dichotomous (1, male; 2, female). Age was used as a continuous variable to find the cut-off value by using this analysis.

### Mediation analysis

Both direct and indirect effects of variables on a dependent variable are evaluated by mediation analysis.<sup>18–20</sup> The PROCESS macro in the SPSS software was used for the analysis.<sup>18,20</sup> Sex (1, male; 2, female) as independent variable, MI score as dependent variable (continuous data) and disease duration (continuous data) as a possible mediator were used in the analysis.

## RESULTS

In this cross-sectional study, 887 BS patients (female : male, 481:406; mean age,  $38.4 \pm 10.9$  years) followed in 13 tertiary centers from Turkey were included. The disease duration was  $9.11 \pm 7.68$  years. The majority of the BS patients had no systemic involvement (mucocutaneous manifestations and musculoskeletal) ( $n = 631$ , 71.1%), whereas ocular, vascular, neurological and gastrointestinal involvement were present in the systemic involvement group ( $n = 256$ , 28.9%) (Table 1).

Active manifestations were present in 620 patients (69.9%) during the last month (oral ulcers, 95.6% [ $n = 593$ ]; genital ulcers, 19.6% [ $n = 120$ ]; cutaneous, 25.2% [ $n = 156$ ]; musculoskeletal, 46.8% [ $n = 289$ ]; vascular, 0.3% [ $n = 2$ ]).

Mucocutaneous activity ( $n = 620$ ) was more frequent in patients without the systemic involvement ( $n = 471$ , 74.6%), compared with those with the systemic involvement ( $n = 149$ , 24%) ( $P < 0.001$ ). In the active group, non-IS medication use was significantly higher in women ( $n = 243$ , 68.6%) compared with men ( $n = 131$ , 55.3%) ( $P = 0.001$ ). In addition, active patients were younger ( $37.2 \pm 10.5$  years) than inactive ones ( $41.2 \pm 11.2$  years) ( $P < 0.001$ ).

### MI score in active patients

The scores of MI and its subgroups regarding CI, GI and EI in the whole group are shown in Table 1. In this group, significant associations were observed regarding oral ulcer pattern (number,  $3.03 \pm 2.9$ ; healing time,  $7.1 \pm 4.1$  days) and CI score ( $r = 0.4$  and  $P < 0.001$  for both) as well as genital ulcer pattern (number,  $1.3 \pm 1.7$ ; healing time,  $6.6 \pm 6.3$  days) and GI score ( $r: 0.6$   $P < 0.001$ ;  $r: 0.7$   $P < 0.001$ ). Similarly, the number of erythema nodosum lesions ( $2.1 \pm 2.8$ ) was associated with EI score ( $r: 0.8$   $P < 0.001$ ). Pain levels in subgroups significantly correlated with the scores of subgroups ( $r = 0.9$ ,  $P < 0.001$ ).

In active patients, MI score correlated with CI score ( $r = 0.7$ ,  $P < 0.001$ ), GI score ( $r = 0.5$ ,  $P < 0.001$ ) and EI score ( $r = 0.4$ ,  $P < 0.001$ ). Moreover, MI score was lower in patients with systemic involvement ( $6.9 \pm 3.6$ ) than those without ( $8.4 \pm 4.9$ ) among active patients ( $P = 0.005$ ).

In the study group, 61.9% ( $n = 549$ ; 470 patients without systemic involvement vs 79 with) were using non-IS medications, whereas 32.6% ( $n = 289$ ; 112 patients without systemic involvement vs 177 with) were using IS medications. A subset of 49 patients was not using their medications regularly (Table 1). MI score was significantly higher in the IS group without systemic involvement (IS vs non-IS,  $7.4 \pm 6.3$  vs  $5.5 \pm 4.9$ ;  $P = 0.003$ ), whereas it was lower in the IS group with systemic involvement (IS vs non-IS,  $3.6 \pm 4.6$  vs  $5.1 \pm 3.9$ ;  $P = 0.001$ ).

### MI score according to sex, disease course and age group

In BS patients, MI, CI and GI scores were significantly higher in women than men ( $P < 0.05$ ), except for EI ( $P = 0.165$ ). In addition, MI score and subgroup scores were found to be high in both younger patients and in those without systemic involvement compared with others ( $P < 0.05$ ) (Table 2).

In young patients ( $n = 219$ ), women ( $n = 21$ ) had poor MI ( $7.5 \pm 4.2$ ) and CI scores ( $5.3 \pm 3.5$ ) compared with men ( $n = 44$ ) with systemic involvement ( $3.5 \pm 4.3$  and  $2.5 \pm 3.3$ ,  $P = 0.001$  and  $P = 0.002$ , respectively) (Table 3). Because the majority of young female patients ( $n = 17$ ) were newly diagnosed to have major organ involvement, IS use was lower in women (42.9%) than men (93.2%) ( $P < 0.001$ ). However, among patients without systemic involvement, MI and subgroup scores were similar in both sexes ( $P > 0.05$ ) (Table 3).

**Table 2.** Mucocutaneous Index scores and its subgroups according to disease-related factors

	Mucocutaneous Index			Composite Index			Genital Index			Erythema Nodosum Index			BDCAF-transformed score		
	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>
Male ( <i>n</i> = 406)	4.7	4.9	<b>0.000</b>	3.6	3.4	<b>0.000</b>	0.6	1.9	<b>0.003</b>	0.5	1.8	0.165	3.8	3.3	<b>0.000</b>
Female ( <i>n</i> = 81)	6.4	5.7		4.6	3.5		1.1	2.8		0.7	2.04		4.8	2.9	
Young age ( <i>n</i> = 219)	6.7	5.3	<b>0.000</b>	4.6	3.3	<b>0.033</b>	1.3	2.8	<b>0.000</b>	0.8	2.1	<b>0.03</b>	3.8	2.9	<b>0.012</b>
Old age ( <i>n</i> = 668)	5.3	5.4		3.9	3.5		0.8	2.3		0.6	1.9		4.6	3.2	
Patients without systemic involvement ( <i>n</i> = 631)	6.3	5.7	<b>0.000</b>	4.4	3.4	<b>0.000</b>	1.1	2.7	<b>0.000</b>	0.7	2.1	<b>0.001</b>	4.6	3.04	<b>0.003</b>
Patients with systemic involvement ( <i>n</i> = 256)	4.1	4.4		3.3	3.5		0.4	1.7		0.3	1.4		3.7	3.3	

Bold value indicates  $P < 0.005$  statistically significant.

**Table 3.** Mucocutaneous Index scores and its subgroups according to sex, disease course and age groups

	Mucocutaneous Index			Composite Index			Genital Index			Erythema Nodosum Index			BDCAF-transformed score		
	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>
Young patients ( <i>n</i> = 219)															
Patients without systemic involvement ( <i>n</i> = 154)															
Male ( <i>n</i> = 72)	6.9	5.1	0.243	4.8	3.03	0.447	1.4	2.8	0.320	0.8	2.2	0.519	4.5	3.2	0.811
Female ( <i>n</i> = 82)	8.03	5.7		5.3	3.1		1.8	3.1		0.9	2.2		4.3	2.7	
Patients with systemic involvement ( <i>n</i> = 65)															
Male ( <i>n</i> = 44)	3.5	4.3	<b>0.001</b>	2.5	3.3	<b>0.002</b>	0.5	2.1	0.554	0.5	1.8	0.345	1.6	1.9	<b>0.000</b>
Female ( <i>n</i> = 21)	7.5	4.2		5.3	3.5		0.9	2.8		1.01	2.3		5.8	2.4	
Old patients ( <i>n</i> = 668)															
Patients without systemic involvement ( <i>n</i> = 477)															
Male ( <i>n</i> = 192)	4.8	5.1	<b>0.000</b>	3.6	3.4	<b>0.001</b>	0.5	1.9	<b>0.018</b>	0.6	1.8	0.224	3.7	3.3	<b>0.000</b>
Female ( <i>n</i> = 285)	6.7	5.9		4.6	3.5		1.2	2.9		0.8	2.2		5.2	2.8	
Patients with systemic involvement ( <i>n</i> = 191)															
Male ( <i>n</i> = 98)	3.7	4.4	0.534	3.2	3.4	0.598	0.3	1.2	0.318	0.3	1.3	0.285	4.5	3.5	0.199
Female ( <i>n</i> = 93)	3.9	4.3		3.5	3.5		0.4	1.6		0.1	0.9		3.6	3.2	

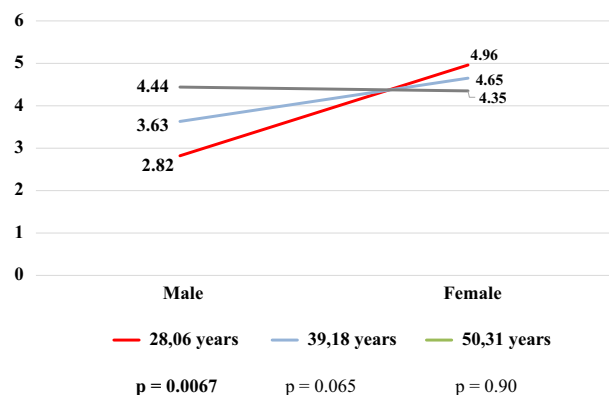
Bold value indicates  $P < 0.005$  statistically significant.

Female patients in the older age group without systemic involvement had significantly poorer MI, CI and GI scores ( $P < 0.05$ ), except for EI ( $P = 0.224$ ). No significant difference was observed in MI and subgroup scores according to sex in the systemic involvement group ( $P < 0.05$ ) (Table 3).

### BDCAF

The transformed BDCAF score was found to be  $4.3 \pm 3.1$  in the whole group (Table 1). It weakly correlated with MI score ( $r = 0.3$ ,  $P < 0.001$ ). It was also significantly higher in women and in groups of older age and those without systemic involvement ( $P < 0.05$ ) (Table 2). Increase in BDCAF score was observed in the non-IS treatment protocol ( $4.5 \pm 3.02$ ) compared with IS ( $3.5 \pm 3.3$ ) ( $P = 0.039$ ).

In women, the BDCAF score was higher in the younger age group with systemic involvement (female vs male,  $5.8 \pm 2.4$  vs  $1.6 \pm 1.9$ ) and in the older age group without systemic



**Figure 2.** Moderational effect of age on the relationship between female sex and mucocutaneous activity in Behçet's syndrome patients with systemic involvement.

**Table 4.** Results of moderation analysis in patients with systemic involvement and conditional effect of sex on Mucocutaneous Index score at different age values in Behçet's disease

	Coeff	SE	t	P	LLCI	ULCI
Constant	0.7816	1.3569	0.576	0.5651	-1.8908	3.454
Age	0.0727	0.0331	2.196	<b>0.0290</b>	0.0075	0.138
Sex	4.9646	2.0388	2.4351	<b>0.0156</b>	0.9491	8.9801
Interaction	-0.1005	0.0501	-2.0041	<b>0.0461</b>	-0.1992	-0.0017

Conditional effect of sex on MI at values of the moderator (s)

Age	Effect	SE	t	P	LLCI	ULCI
28.0612	2.1454	0.7852	2.7323	<b>0.0067</b>	0.599	3.6919
39.1897	1.0274	0.5554	1.8497	0.0655	-0.0666	2.1213
50.3183	-0.0906	0.7092	-0.1149	0.9087	-1.6451	1.4638

Bold value indicates  $P < 0.005$  statistically significant. Coeff, coefficient; LLCI, lower level confidence interval; MI, Mucocutaneous Index; SE, standard error; ULCI, upper level confidence interval.

involvement (female vs male,  $5.2 \pm 2.8$  vs  $3.7 \pm 3.3$ ) compared with others ( $P < 0.001$  for both) (Table 3).

### Moderation analysis

In a preliminary analysis, close associations were observed among sex, disease course and age groups. Therefore, moderation analysis was carried out to evaluate the effect of age on the relationships between sex and MI score according to the disease course. Sex was the predictive variable, and the MI score was an outcome variable in the analysis. Age as a continuous data was used as a possible mediator variable in the analysis. The moderation analysis tested the effects of age in three steps: whole BS patient group, patients without systemic involvement and those with systemic involvement.

The moderation model was only significant in patients with systemic involvement ( $P = 0.0351$ ). In this group, significant relations between female sex and MI score ( $P = 0.0156$ ), as well as age and MI score ( $P = 0.0290$ ), were found (Fig. 2). The interaction effect (sex and age) was statistically significant in the model ( $P = 0.0461$ ) (Table 4).

The moderation analysis also determined three different cut-off values for age (28-year group, 39-year group and 50-year group) in the dataset (Table 4). The interaction plot showed that the younger female patients had increased MI scores compared with male patients, especially in the 28-year age group ( $P = 0.0067$ ). A similar trend was not seen in the other age groups ( $P = 0.065$  in 39-year age group;  $P = 0.90$  in 50-year age group) (Fig. 3). A significant model was not found in

subgroups of the index and BDCAF score in the study ( $P > 0.05$ ).

In the study group, disease duration correlated with age ( $r = 0.57$ ,  $P = 0.000$  in the whole group). It was longer in female ( $9.81 \pm 7.99$  years) than male patients ( $8.29 \pm 7.22$  years) ( $P = 0.004$ ).

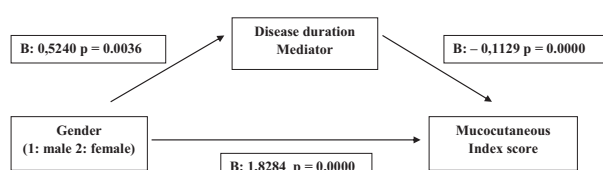
According to simple mediation analysis in the whole group, female sex was associated with elevated MI score by direct path ( $B = 1.8284$ ,  $P = 0.000$ ). Disease duration as the mediator was inversely related with MI score ( $B = -0.1129$ ,  $P = 0.000$ ) by indirect path. Moreover, elevated disease duration was associated with female sex ( $B = 0.5240$ ,  $P = 0.0036$ ) (Fig. 3). According to a bootstrap analysis with 5000 replications, the model was found to be significant by both direct path (1.1182 and 2.5386) and indirect path (-0.3193 and -0.0526).

Although age was also correlated with disease duration in patients with and without systemic involvement ( $r = 0.57$ ,  $P = 0.000$ ;  $r = 0.58$ ,  $P = 0.000$ , respectively), the same mediation model was not seen in these groups.

### DISCUSSION

In chronic disease management, objective criteria such as mortality, morbidity, number of unplanned visits and length of stay in hospital are assessed in order to monitor progress towards achieving symptom control.<sup>25</sup> In addition, subjective criteria such as PROM are used to understand patient experience with chronic diseases.<sup>26</sup> Therefore, user-friendly, patient-driven activity indices are valuable tools to measure clinical progress from the BS patient's perspective. Yet, "what to measure" and "how to measure" are critical points owing to the diversity of organ involvement in BS.<sup>4,7-11,27-31</sup>

Mucocutaneous manifestations are common clinical conditions and affect the patients' lives poorly.<sup>9,10,32,33</sup> In the present study, validated MI as an organ-specific activity index was used to evaluate mucocutaneous activity regarding oral ulcer, genital ulcer and erythema nodosum.<sup>2</sup> Because pain and functional limitation were the main domains in each subgroup score correlating with symptom patterns, clinical



**Figure 3.** Mediator role of the disease duration on the mucocutaneous activity in Behçet's syndrome.

manifestations were easily monitored by MI. This structure is in accordance with the OMERACT recommendations which endorse duration, number and pain as basic variables for oral and genital ulcers and erythema nodosum in clinical trials.<sup>27,34</sup> From the BS patient's perspective, pain is shown to be an indicator of mucocutaneous activity.<sup>35</sup>

In our study, the MI score was found to be high in BS patients without systemic involvement, of female sex and in the younger age group. Patients with active disease were mainly treated with non-IS medications. These results are in accordance with previous studies.<sup>9,10,12,36</sup> These patients are not aggressively treated, as their manifestations do not have mortality risk and do not lead to permanent damage compared with those with major organ involvement.<sup>3,10,37-39</sup>

The effect of age on mucocutaneous manifestations was shown with two different analyses in our study. MI score was found to be high in younger female BS patients with systemic involvement. Because the majority of these patients were newly diagnosed and shown to have major organ involvement, IS medications had not been commenced yet. BDCAF score was also observed to be higher in this group. More aggressive treatments could be considered for mucocutaneous activity that could not be eliminated by non-IS medications in a limited period.<sup>2,11-14</sup> These results were in accordance with the previous studies reporting that BS patients with systemic involvement were preceded by mucocutaneous manifestations, especially in younger patients.<sup>40,41</sup>

Second, we addressed the moderational effect of age on mucocutaneous manifestations in BS. Age was found to be a moderator variable for the mucocutaneous activity in BS patients with systemic involvement, especially in 28-year-old female patients. In other words, mucocutaneous manifestations were more dependent on this level of age in female BS patients with systemic involvement. A moderator variable explains when an independent variable and outcome variable are associated with each other. Moreover, this effect may be stronger or weaker at different values of the moderator variable.<sup>17,19,20,42</sup> The stronger effect was seen in 28-year-old women in the severe group. This result highlighted the need to focus on which female patients with BS were at risk of the development of new major organ involvement.

A mediation analysis tests how an independent variable affects a dependent variable with mediator variable.<sup>18,19,20</sup> In the present study, female sex as the dependent variable and decrease in disease duration as a mediator were associated with poor MI score as the independent variable in the whole group. In our previous study, both oral ulcer activity and CI score for oral ulcer activity were found to be high in the early period of the disease.<sup>12</sup> BS generally starts during the second and/or third decades of life.<sup>43</sup> Disease activity, especially mucocutaneous activity, is commonly seen in young patients and early period of the disease.<sup>12,15,38,44,45</sup> Our results gave information about the need for careful follow-up procedure in young active female patients. Because follow-up procedure and treatment strategies are organized according to the individual needs of patients, age, sex and disease severity are critical points for the disease management.<sup>29,37,39,46</sup>

The CI score-related results were in accordance with our previous studies.<sup>5,12</sup> GI and EI scores were also higher in younger age groups and in those without systemic involvement. In addition, GI and EI scores were related to the number and healing time of oral ulcers, and with the number of erythema nodosum lesions, respectively. Genital ulcer, as a second common manifestation, is a discriminatory finding in BS<sup>3</sup> and has a negative effect on the quality of daily life of the patients.<sup>10</sup>

The BDCAF score was found to be higher in female patients without systemic involvement in an older age group. These patients are not aggressively treated because they do not have a mortality risk.<sup>3,10,37,38</sup> In the present study, it was weakly correlated with MI score as an organ-specific activity index. Because the BDCAF is a global activity index and evaluates organ involvement together,<sup>23,24</sup> this result could be predicted by means of the different measurement methods.

The present study has some strengths. First, it included a very large sample size. Second, the role of age on mucocutaneous activity was evaluated by implementing two different methods. Moderation analysis explored the moderational role of age, as well as giving a critical cut-off point of age for the increased mucocutaneous activity. This analysis provided detailed information to help the development of individual follow-up and treatment strategies in active young female patients with BS. Third, the relationship between clinical presentation for new onset of major organ involvement and age was clarified in young women. Finally, the disease duration was inversely associated with the poor mucocutaneous activity according to the mediation analysis.

In this context, clinicians could improve these patients' prognoses through performing visits at appropriate time intervals and establishing more effective treatment protocols in active young female BS patients.

The main limitation of the study was its cross-sectional design. Therefore, longitudinal studies are necessary to confirm these results in diverse BS patient populations.

In conclusion, the relationship between sex and mucocutaneous activity was moderated by age in a severe disease course. In addition, increased mucocutaneous activity could be a clue for new major organ involvement in young female BS patients in this group. These findings may lead the clinicians to perform regular visits at appropriate time intervals and to use more effective treatment protocols in active young female BS patients to improve the prognosis.

**CONFLICT OF INTEREST:** None declared.

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