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Meta-Analysis

# Circulating prolactin levels and Behcet's disease: A meta-analysis

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**Abstract:** This study aimed to summarize the current evidence on the relationship between circulating prolactin levels and Behcet's disease (BD). We performed a meta-analysis comparing the serum/plasma prolactin levels in patients with BD with those of controls and performed a subgroup analysis based on ethnicity, disease activity, and sex. Ten articles with a total of 320 patients with BD and 259 controls were included. The prolactin levels were not significantly higher in the BD group than in the control group (SMD=0.208, 95% CI=-0.012–0.428, $p$=0.064). Stratification by ethnicity indicated no elevation in prolactin level in Turkish patients with BD (SMD=0.127, 95% CI=-0.111–0.366, $p$=0.295). Stratification by disease activity revealed no elevation in prolactin level in both the active and inactive BD groups compared with the control group (SMD=0.373, 95% CI=-0.095–0.841, $p$=0.119; SMD=0.055, 95% CI=-0.243–0.354, $p$=0.717). Stratification by sex revealed no elevation in prolactin level in both the female and male BD groups (SMD=0.031, 95% CI=-0.398–0.460, $p$=0.888; SMD=0.279, 95% CI=-1.411–1.969, $p$=0.746). The prolactin levels were not significantly elevated in patients with BD, regardless of the adjustments for age/sex, sample size, or data type evaluated. This meta-analysis of current evidence suggests that circulating prolactin levels may not be higher in patients with BD than in controls.

***Key words:*** Prolactin level; Behcet's disease.

## Introduction

Behcet's disease (BD) is a chronic inflammatory disease characterized by recurrent oral and genital ulcers, skin lesions, and uveitis (1). BD also affects all types and sizes of the blood vessels, joints, central nervous system, lungs, and gastrointestinal system (2). Although its etiology is not fully understood, neutrophil hyperfunction, vasculitis, and autoimmune and inflammatory responses are its major pathological features.

Prolactin is a polypeptide that not only plays an important role in lactogenesis, but also functions as a cytokine with immunomodulatory properties (3). Prolactin is produced in the pituitary gland and in extrapituitary sites, including immune cells (4), and its receptor belongs to the family of type I cytokine receptors that are distributed throughout the immune system (5). Prolactin may influence both humoral and cell-mediated immune reactions and play an important role in autoimmune and inflammatory disease development. Prolactin exerts its actions via specific receptors expressed on many cells, including T and B lymphocytes (5), and enhances Th1 and Th17 responses and VEGF production (6). Furthermore, prolactin has an immune stimulatory effect and may promote autoimmunity by encouraging the development of antigen-presenting cells expressing MHC class II and co-stimulatory molecules (7). Because prolactin stimulates both cellular and humoral immunity by modulating IFN-γ secretion and dendritic cell maturation (8), it may be a critical immune stimulator, detrimental in autoimmune and inflammatory diseases (9,10). Taken together, prolactin may have a possible role in BD pathogenesis.

However, studies comparing the circulating prolactin levels between patients with BD and healthy controls have shown mixed results (11-20). Such disparities may be a result of small sample sizes, low statistical power, and/or clinical heterogeneity. To overcome the limitations of individual studies and resolve inconsistencies, we performed a meta-analysis (21). The present study aimed to determine the serum/plasma prolactin levels in patients with BD compared with those in healthy controls.

## Materials and Methods

### Eligible study identification and data extraction

We searched the MEDLINE, EMBASE, and Cochrane databases (up to February 2017) for studies that compared the prolactin levels between patients with BD and controls. The key words and subject terms used in the search were "prolactin" and "Behcet's disease." All references cited were also reviewed to identify additional studies not available in the above-mentioned electronic databases. Studies were considered eligible if they met the following criteria: (1) case-controlled studies and (2) available data on prolactin levels in cases and controls. No language or race restriction was applied. The following articles were excluded: (1) articles with overlapping or insufficient data or (2) reviews or case reports. Methods and results were extracted from the original studies by two independent reviewers. Any discrepancies between the reviewers were resolved by a consensus, and the meta-analysis was conducted in accordance with the PRISMA guidelines (22). The following information was extracted from each study: primary author, publication year, country, ethnicity, age and/or sex matching, number of participants, and mean

and standard deviation (SD) of the prolactin levels. When the data were presented as medians, interquartile ranges, or ranges, we computed the means and SDs using previously described formulae (23). Finally, we conducted a sensitivity test on the imputed values. We scored the quality of each study included in the meta-analysis based on the Newcastle–Ottawa Scale (24). Scores ranging from 6–9, with 9 being the highest score possible indicated high methodological quality.

### Statistical association evaluation

We performed a meta-analysis to examine the relationship between the prolactin levels and BD. For data continuity, the results were presented as standardized mean differences (SMDs) and 95% confidence intervals (CIs). SMDs were calculated by dividing the mean difference between the two groups by the pooled SD and were used when the data based on different scales that measured the same phenomenon were integrated. This measure allowed a comparison of case and control arms with a standardized measure. The magnitudes of the SMDs were as follows: 0.2–0.5, small effect; 0.5–0.8, medium effect; ≥0.8, large effect (25). We also assessed the within- and between-study heterogeneities using the Cochran's Q-statistic (26). This heterogeneity test was used to assess the null hypothesis that all studies were evaluating the same effect. A significant Q-statistic ($p$<0.10) indicated heterogeneity across the studies; the random effects model was used for the meta-analysis (27). When heterogeneity was not indicated, the fixed effects model was used, which assumed that all studies estimated the same underlying effect; we considered only the within-study variation (26). We quantified the heterogeneity effect using $I^2=100\%\times(Q-df)/Q$(28), where $I^2$ indicated the inconsistency degree between studies, and determined whether the percentage total variation across the studies was due to heterogeneity rather than chance. $I^2$ ranged between 0% and 100%; $I^2$ values of 25%, 50%, and 75% were referred to as low, moderate, and high estimates, respectively (28). Statistical analyses were performed using the Comprehensive Meta-Analysis program (Biostat Inc., Englewood, NJ).

### Heterogeneity, sensitivity, and publication bias evaluation

To examine possible heterogeneities in the meta-analysis, a subgroup analysis was performed using the following variables: ethnicity, age and sex adjustments, sample size, and data type. A sensitivity test was performed to assess the influence of each study on the pooled odds ratio (OR) by omitting each study individually. Although funnel plots are often used to detect a publication bias, they require diverse study types of varying sample sizes, and their interpretation involves a subjective judgment. Therefore, we evaluated the publication bias using the Egger's linear regression test (29), which measures funnel plot asymmetry based on a natural logarithm scale of ORs.

## Results

### Studies included in the meta-analysis

We identified 25 studies via electronic and manual searches. Eleven of these studies were selected for full-text review based on their titles and abstracts, and one was excluded because it was a review article (30). Thus, 10 reports met the inclusion criteria (11-20) (Fig. 1). In addition, one study contained data on four different groups (18) and three on two different groups (16,17,20); we analyzed these studies independently. Therefore, a total of 16 separate studies were considered in the meta-analysis, which included a total of 320 patients and 259 controls (Table 1). The quality assessment score of each study ranged between 4 and 7. The selected characteristics of these studies are summarized in Table 1.

### Meta-analysis comparing the circulating prolactin levels between the patients with BD and controls

The prolactin levels were not significantly higher in the BD group than in the control group (SMD=0.208, 95% CI=-0.012–0.428, p=0.064) (Table 2, Fig. 2). Stratification by ethnicity indicated no elevated prolactin level in Turkish patients with BD (SMD=0.127, 95% CI=-0.111–0.366, p=0.295) (Table 2). A single Brazilian study showed no association between prolactin level and BD; however, one Caucasian and one Arab study showed a significantly higher prolactin level in the BD group (SMD=0.825, 95% CI=0.202–1.448, p=0.009; SMD=0.581, 95% CI=0.018–1.144, p=0.043, respectively). The subgroup analysis based on disease activity indicated that both the active and inactive BD groups had no higher prolactin levels than the control group (SMD=0.373, 95% CI=-0.095–0.841, p=0.119; SMD=0.055, 95% CI=-0.243–0.354, p=0.717) (Table 2). Stratification by sex revealed no elevation in prolactin level in both the female and male BD groups (SMD=0.031, 95% CI=-0.398–0.460, p=0.888; SMD=0.279, 95% CI=-1.411–1.969, p=0.746) (Table 2). The subgroup analysis by sample size showed no association between the adiponectin levels and BD group with large sample sizes (N≥50) or small sample sizes (N<50) (Table 2). Stratification by data type revealed that the BD group had no higher prolactin level based on the original and calculated data (Table 2). Stratification by the adjustment for age and/or sex revealed a

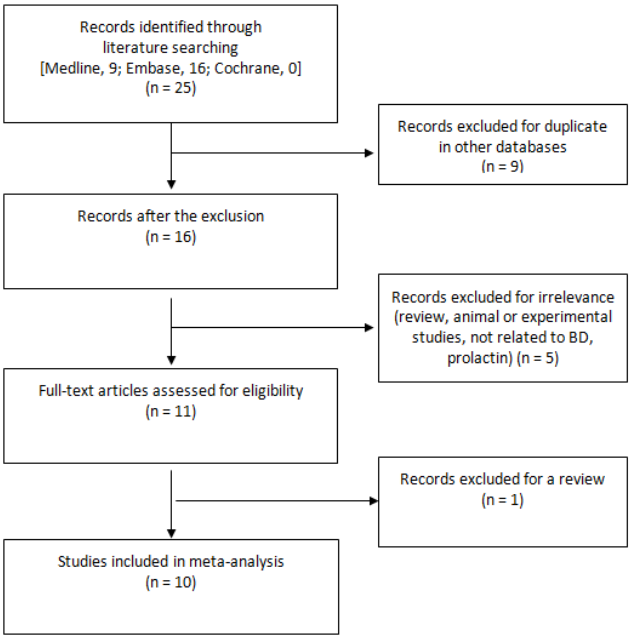


**Figure 1.** Flow diagram of the study selection process.

**Table 1.** Characteristics of the individual studies included in the meta-analysis

| Authors | Country | Ethnicity | Number | | Data type | Matching | Results | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cases | Controls | | | SMD | Magnitude* | *p*-value |
| Mont'Alverne, 2015(11) | Brazil | Brazilian | 10 | 22 | Calculated | Age, sex | 0.186 | No effect | 0.627 |
| Sahin, 2015(12) | Turkey | Turkish | 35 | 35 | Calculated | Age | -0.182 | No effect | 0.449 |
| Avci, 2013(13) | Turkey | Turkish | 43 | 20 | Calculated | Age, sex | 0.135 | No effect | 0.619 |
| Karalus-1, 2012(18) | Turkey | Turkish | 12 | 22 | Calculated | Age, sex | -0.350 | Small | 0.333 |
| Karalus-2, 2012(18) | Turkey | Turkish | 15 | 18 | Calculated | Age, sex | 1.123 | Large | 0.003 |
| Karalus-3, 2012(18) | Turkey | Turkish | 9 | 22 | Calculated | Age, sex | 0.325 | Small | 0.415 |
| Karalus-4, 2012(18) | Turkey | Turkish | 7 | 18 | Calculated | Age, sex | -0.602 | Medium | 0.184 |
| Cil, 2010(14) | Turkey | Turkish | 20 | 31 | Original | Age | -0.280 | Small | 0.332 |
| Proenca, 2007(15) | Portugal | Caucasian | 22 | 21 | Calculated | NA | 0.825 | Large | 0.009 |
| Atasoy-1, 2006(16) | Turkey | Turkish | 18 | 20 | Original | Age, sex | 0.703 | Medium | 0.036 |
| Atasoy-2, 2006(16) | Turkey | Turkish | 14 | 20 | Original | Age, sex | 0.677 | Medium | 0.059 |
| Houman, 2001(19) | Tunisia | Arab | 28 | 23 | Original | NA | 0.581 | Medium | 0.043 |
| Apaydin-1, 2000(17) | Turkey | Turkish | 17 | 17 | Calculated | NA | 0.038 | No effect | 0.912 |
| Apaydin-2, 2000(17) | Turkey | Turkish | 20 | 17 | Calculated | NA | -0.041 | No effect | 0.900 |
| Keser-1, 1999(20) | Turkey | Turkish | 20 | 30 | Original | NA | 0.370 | Small | 0.203 |
| Keser-2, 1999(20) | Turkey | Turkish | 30 | 30 | Original | NA | -0.110 | No effect | 0.671 |

SMD: Standardized mean difference. *Magnitude of Cohen's d effect size: 0.2–0.5, small effect; 0.5–0.8, medium effect; ≥0.8, large effect, NA: Not available.

**Table 2.** Meta-analysis of the association between the circulating prolactin levels and BD.

| Groups | Population | No. of Studies | Test of association | | | Test of heterogeneity | | |
|---|---|---|---|---|---|---|---|---|
| | | | SMD* | 95% CI | *p*-value | Model | *p*-value | $I^2$ |
| All | Overall | 16 | 0.208 | -0.012–0.428 | 0.064 | R | 0.016 | 48.4 |
| Ethnicity | Turkish | 13 | 0.127 | -0.111–0.366 | 0.295 | R | 0.034 | 46.3 |
| Disease activity | Active | 5 | 0.373 | -0.095–0.841 | 0.119 | R | 0.042 | 59.6 |
| | Inactive | 5 | 0.055 | -0.243–0.354 | 0.717 | F | 0.194 | 34.0 |
| Sex | Female | 3 | 0.031 | -0.398–0.460 | 0.888 | F | 0.403 | 0 |
| | Male | 2 | 0.279 | -1.411–1.969 | 0.746 | R | 0.003 | 88.3 |
| Age- and/or sex-matched | Yes | 10 | 0.166 | -0.149–0.481 | 0.301 | R | 0.015 | 56.2 |
| | NA | 6 | 0.269 | 0.029–0.509 | 0.028 | F | 0.164 | 36.3 |
| | NA[a] | 6 | 0.274 | *0.029–0.577 | 0.076 | R | 0.164 | 36.3 |
| Sample size | N<50 | 11 | 0.251 | -0.057–0.559 | 0.111 | R | 0.012 | 55.8 |
| | N≥50 | 5 | 0.128 | -0.116–0.371 | 0.305 | F | 0.197 | 33.6 |
| Data type | Original | 6 | 0.294 | -0.047–0.634 | 0.091 | R | 0.074 | 50.2 |
| | Calculated | 10 | 0.149 | -0.150–0.449 | 0.328 | R | 0.031 | 51.1 |

SMD: Standardized mean difference. *Magnitude of Cohen's d effect size: 0.2–0.5, small effect; 0.5–0.8, medium effect; ≥0.8, large effect. F: Fixed effects model, R: Random effects model, NA: not available, [a]Random effects model.

significantly higher prolactin level in the BD group only by non-adjustment (Table 2). However, the meta-analysis using the random effects model showed no significantly elevated prolactin level in the BD group by non-adjustment (Table 2).

### Heterogeneity, sensitivity, and publication bias

A between-study heterogeneity was identified in the meta-analysis of prolactin levels in the patients with BD (Table 2). The heterogeneity decreased based on ethnicity, female sex, and large sample size (Table 2). Some studies significantly affected the pooled SMDs, indicating that the results of this meta-analysis are not robust (Fig. 2). A publication bias can lead to a disproportionate number of positive studies, which poses a problem for meta-analyses. However, the funnel plot showed a symmetry, and the Egger's regression test showed no evidence of a publication bias (Egger's regression test p=0.469) (Fig. 3).

## Discussion

In this meta-analysis, we reviewed combined data on circulating prolactin levels in patients with BD compared with those in healthy controls. This meta-analysis of 10 articles included data from 320 patients with BD and

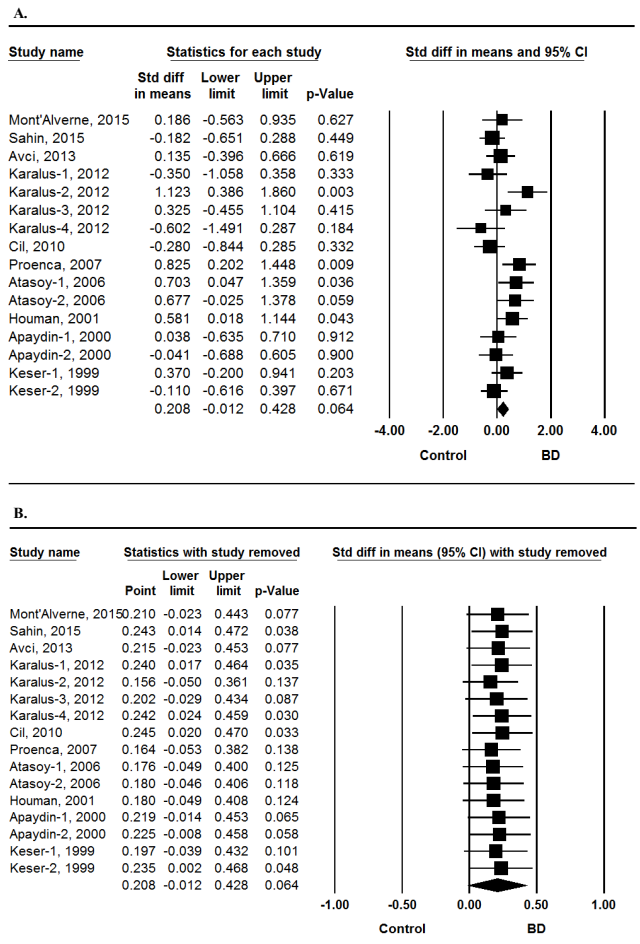


**Figure 2.** Meta-analysis (A) and sensitivity analysis (B) of the relationship between the prolactin levels and BD.

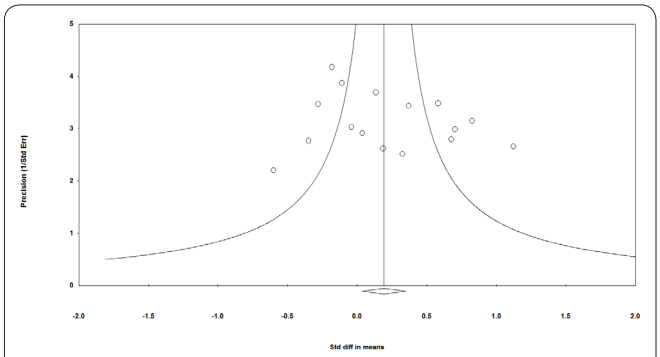


**Figure 3.** Funnel plot of the studies that examined the association between the prolactin levels and BD (Egger's regression $p$=0.469).

259 controls and showed that prolactin levels were not significantly higher in the BD group than in the control group, regardless of ethnicity, disease activity, sex, age and/or sex adjustments, sample size, or data type evaluated. It suggests that circulating prolactin levels may not play an important role in BD pathogenesis.

In this meta-analysis, the total heterogeneity was moderate ($I^2$=48.4%). The heterogeneity decreased in some subgroup analyses; however, it did not resolve. Other unknown factors affecting heterogeneity may contribute to the difference in the relationship between the prolactin level and BD. Our ethnicity-specific meta-analysis indicated no elevated prolactin level in Turkish patients with BD; however, a single Caucasian and Arab study showed a significantly higher prolactin level in the BD group. Although there was one Caucasian and Arab study conducted on prolactin levels in BD, their finding indicates the presence of possible racial prolactin level differences. Given the small number of studies, further studies are warranted in various ethnic groups.

Prolactin maintains immune competence and is an important factor in the immune response (3). Specifically, prolactin promotes T and B lymphocyte and NK cell proliferation and dendritic cell maturation, possibly leading to immune tolerance breakdown (31). Prolactin has an immune stimulatory effect and may promote autoimmunity by encouraging the development of antigen-presenting cells expressing MHC class II and co-stimulatory molecules (7). Prolactin may play an important role in autoimmune and inflammatory disease development by influencing both humoral and cell-mediated immunity, and several studies suggest a possible role of prolactin in BD pathogenesis. However, our meta-analysis showed that higher prolactin levels may not be associated with BD; such a finding does not coincide with those from functional studies in this regard, considering that BD is a complex disease, and multiple genes, different genetic backgrounds, and environmental factors contribute to its development. Moreover, we cannot rule out that the results of our meta-analysis can be due to a Type II error (false negative) or heterogeneity.

This meta-analysis has a few limitations. First, most of the studies had small sample sizes. Thus, this meta-analysis may be underpowered. Second, the studies included were heterogeneous in demographic characteristics and clinical features. Several factors can increase prolactin production in patients with BD, including pregnancy, stress, hypothyroidism, diet, and drug use (32). Exclusion of subjects taking medicine or nutritional factors interfering with the oestrogen levels is required. Heterogeneity, confounding factors, and limited clinical information available from the study participants may have affected our results. These limited data did not allow for further analyses. Third, it is needed to determine the cause or consequence of the prolactin levels as well as measuring prolaction during intervention studies on this disease. Fourth, precision on the prolactin laboratory testing among all the studies is important for a meta-epidemiological study. However, there was no a centralized laboratory which could be required to avoid false-positive prolactin values. Nevertheless, this meta-analysis also has its strengths. To the best of our knowledge, our meta-analysis is the first to provide combined data on prolactin levels in patients with BD. Previous studies only included 7–43 participants, whereas we presented a pooled analysis of 320 patients. Similarly, we believe that our findings on the relationship between the prolactin levels and BD were more accurate than those of previous studies because of the increased statistical power and resolution achieved by pooling the independent analyses results.

In conclusion, circulating prolactin levels may not be higher in patients with BD than in controls. Thus, our meta-analysis does not support the notion that prolactin may play an important role in BD pathogenesis. A better characterization of the quality criteria of the basal studies is the critical point for the negative results. Given the limited number of available studies, small sample sizes, study quality, and significant heterogeneities, larger, well-designed randomized studies are needed.

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**Conflict of interest statement**
The authors have no financial and non-financial conflicts of interest to declare.

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