



Demodex density increases after adalimumab therapy in ankylosing spondylitis: a pilot study

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Research Article

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Cite this article: Li X, Lam NSK, Yang L, Liang A, Long XX, Guo J, Zhong S (2022). *Demodex* density increases after adalimumab therapy in ankylosing spondylitis: a pilot study. *Parasitology* **149**, 940–943. <https://doi.org/10.1017/S0031182022000348>

Received: 15 October 2021
Revised: 29 January 2022
Accepted: 14 March 2022
First published online: 18 April 2022

Key words:

Adalimumab; ankylosing spondylitis; *Demodex*; tumour necrosis factor- α

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Abstract

Demodex infestation and density changes remain one of the main challenges in some clinical settings. Tumour necrosis factor- α (TNF- α) inhibitors have been recommended as a first-line treatment for ankylosing spondylitis (AS). However, there have been no studies investigating the impact of TNF- α inhibitor adalimumab on changes in the *Demodex* density in patients with AS. The aim of this study was to investigate *Demodex* density changes before and after adalimumab treatment and analyse the relationship between the *Demodex* density and clinical characteristics in AS. It was found that the *Demodex* density was positively correlated with age and C-reactive protein levels and the number of *Demodex* mites could increase after adalimumab treatment in AS.

Introduction

Demodex that reside on humans can be divided into two species *Demodex folliculorum* and *Demodex brevis*. *Demodex folliculorum*, which is approximately 0.3–0.4 mm long, has a longer opisthosoma and usually resides in the hair follicles, and *D. brevis*, which is approximately 0.2–0.3 mm long, has a shorter opisthosoma and usually resides in sebaceous and meibomian glands (Cheng *et al.*, 2015). They are mainly found on the forehead, cheek, nose and nasolabial folds. The immune system of the host seems to tolerate *Demodex* without inflammation as a commensal when the numbers of *Demodex* are low. However, when the number of *Demodex* increases, *Demodex* might cause dermatological or ophthalmological diseases, especially rosacea and blepharitis (Chang and Huang, 2017; Sędzikowska *et al.*, 2018).

Demodex infection can occur in some clinical settings, especially in the status of immunodeficiency and the use of immunosuppressant (Yamaoka *et al.*, 2014; Chovatiya and Colegio, 2016; Arli *et al.*, 2019). The cytokine tumour necrosis factor- α (TNF- α) plays crucial roles in the pathogenic mechanism and development of ankylosing spondylitis (AS) and TNF- α inhibitors have been recommended as a first-line treatment for AS (Ward *et al.*, 2019). However, whether the use of TNF- α inhibitors can cause changes in the *Demodex* density and dermatological diseases in AS remains unknown. The aim of this study was to investigate changes in the *Demodex* density by standardized skin surface biopsy (SSSB) before and after therapy with the TNF- α inhibitor adalimumab in patients with AS.

Materials and methods

Study population

Seventy-three patients with definitive AS were enrolled in this study. The diagnosis of AS was established by the Modified New York Criteria (van der Linden *et al.*, 1984). All the patients were initially diagnosed and did not receive specific treatment before this study. Patients who were pregnant; had a history of heart failure, dermatological diseases, neoplasm, diabetes mellitus, infection or tuberculosis; and those using immunosuppressive drugs were excluded.

Therapy

All the patients with AS were classified into two subtype groups (Group A and Group B) according to whether the patients accepted adalimumab treatment due to their willingness and financial situation. Forty patients in Group A were treated with adalimumab 40 mg once every two weeks subcutaneously and celecoxib 200 mg twice each day orally. Thirty-three patients in Group B were only administered 200 mg celecoxib twice each day orally.

Sample collection

The method of SSSB (Aşkin and Seçkin, 2010) was used to collect *Demodex* samples from patients at the Department of Rheumatology of the Fifth Affiliated Hospital of Sun Yat-Sen University from April 2020 to February 2021. Five standardized skin surface biopsies were

taken from the cheeks, forehead, nose and chin of the patients at the initiation and after three-month adalimumab treatment. For SSSB, a drop of cyanoacrylate adhesive (Aron Alpha Glue, Loctite Corporation, Yantai, China) was placed on a 1 cm² marked area of a microscope slide. The adhesive-bearing surface was pressed over the skin for 1 min, the slide was gently removed from the skin, covered with 2–3 drops of glycerin and then covered with a coverslip. The slides were assessed for parasites by light microscopy at a magnification of $\times 10$. The average value of five sites was considered the *Demodex* count. Demodicosis was defined as the identification of five or more *Demodex* mites in a 1 cm² area (Baima and Sticherling, 2002).

Clinical variables

All demographic data were gathered from the medical records of the patients. Clinical characteristics, including duration, smoking, body mass index (BMI), human leucocyte antigen (HLA)-B27, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), were also collected.

Statistical analysis

The results are described as the mean \pm standard deviation (s.d.). The Kolmogorov–Smirnov test was used to determine whether continuous variables were normally distributed. Normally distributed variables were compared using independent and randomized paired *t*-tests. Non-normally distributed variables were compared using Wilcoxon matched-pairs signed-ranks sum test. Categorical variables were assessed by the χ^2 test. Pearson's rank correlation coefficient was used to examine the relationships between two continuous variables. A *P* value < 0.05 was considered to indicate statistical significance. Statistical analysis was carried out using Statistical Package of Social Science (SPSS) version 24.0.

Results

Seventy-three patients with a definitive diagnosis of AS were enrolled. The *Demodex* test was performed in all AS patients (Fig. 1). At least one *Demodex* mite was detected in 69 (94.52%) out of 73 AS patients. None of the patients had demodicosis before and after treatment. Dermatological symptoms did not occur in patients with AS after adalimumab treatment.

Correlation analysis between the *Demodex* density and clinical characteristics in AS patients

Demodex mites were detected in 96.15% of the male patients (50/52) and 90.48% of the female patients (19/21), but there



Fig. 1. A *Demodex* is observed by light microscopy at a magnification of $\times 40$.

Table 1. Correlation analysis between *Demodex* density and clinical characteristics in AS patients

	<i>Demodex</i> density	
	<i>r</i>	<i>P</i>
Sex	–	0.691 ^a
Age	0.27	0.021 ^a
Smoking	–	1.000 ^b
HLA-B27	–	0.378 ^b
Duration	0.22	0.064
BMI	0.09	0.433
ESR	0.14	0.244
CRP	0.27	0.019 ^a

AS, ankylosing spondylitis; HLA, human leucocyte antigen; BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

^a χ^2 test.

^bFisher's test.

^{*}Statistically significant (*P* < 0.05).

was no significant difference in terms of sex (*P* = 0.691). Three of the sixty-five patients (3/65) with positive HLA-B27 and only one of the eight patients (1/8) with negative HLA-B27 had *Demodex* mites. The incidence of *Demodex* mite detection was 94.12% (16/17) in patients who smoked and 94.64% (53/56) in patients who did not smoke. No significant difference was found in terms of HLA-B27 and smoking (*P* = 0.378, *P* = 1.000, respectively). Table 1 also shows that *Demodex* density was positively correlated with both age and CRP (*P* = 0.021, *P* = 0.019, respectively) but was not correlated with BMI, ESR or duration (*P* = 0.433, *P* = 0.244, *P* = 0.064, respectively).

Comparison of clinical characteristics and the *Demodex* density between Groups A and B before treatment

Among the AS patients, 40 and 33 patients were placed in Groups A and B, respectively. Clinical characteristics and the *Demodex* density are shown in Table 2. The male sex rates were 75.00% (30/40) and 66.67% (22/33) in Groups A and B, respectively. The age and symptom duration were 36.08 ± 10.59 and 9.58 ± 7.22 years in Group A

Table 2. Comparison of clinical characteristics and *Demodex* density between Groups A and B before treatment in AS patients

	Group A (<i>n</i> = 40)	Group B (<i>n</i> = 33)	<i>P</i> value
Male (%)	30 (75.00%)	22 (66.67%)	0.434 ^a
Age (years)	36.08 ± 10.59	35.24 ± 11.87	0.753
Smoking (%)	10 (25.00%)	7 (26.92%)	0.703 ^a
HLA-B27 positive (%)	37 (92.50%)	29 (87.88%)	0.789 ^b
Duration (years)	9.58 ± 7.22	7.28 ± 7.18	0.179
BMI	22.59 ± 3.79	21.79 ± 3.64	0.363
ESR (mm/H)	31.28 ± 30.61	31.52 ± 24.10	0.971
CRP (mg/L)	21.08 ± 28.16	17.34 ± 20.09	0.551
<i>Demodex</i> presence rate (%)	38 (95.00%)	31 (93.94%)	1.000 ^b
<i>Demodex</i> density	0.44 ± 0.26	0.41 ± 0.23	0.694

AS, ankylosing spondylitis; HLA, human leucocyte antigen; BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

^a χ^2 test.

^bFisher's test.

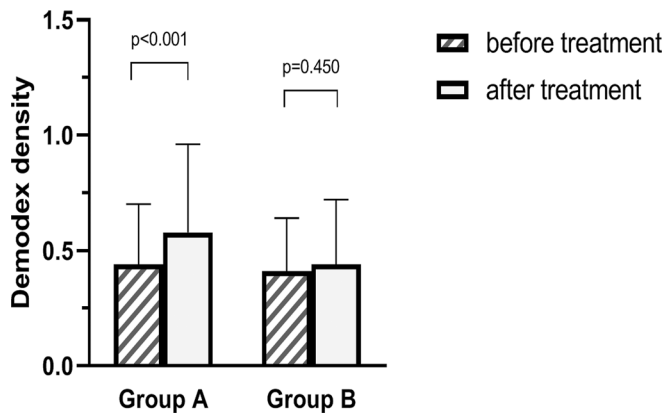


Fig. 2. Increase of the *Demodex* density after adalimumab treatment in Group A but no change of the *Demodex* density in Group B.

and 35.24 ± 11.87 and 7.28 ± 7.18 years in Group B, respectively. There was no significant difference in terms of sex, age or duration between Groups A and B ($P = 0.434$, $P = 0.753$, $P = 0.179$, respectively). Table 2 also shows that there were no significant differences between the two groups regarding the *Demodex* presence rate and *Demodex* density before treatment.

Comparison of the *Demodex* density before and after treatment

The change in the *Demodex* density before and after treatment is shown in Fig. 2. Among the patients in Group A, the *Demodex* densities were 0.44 ± 0.26 and 0.58 ± 0.38 before and after treatment, respectively. The *Demodex* density increased after adalimumab treatment in Group A ($P < 0.001$). The *Demodex* densities before and after treatment were 0.41 ± 0.23 and 0.44 ± 0.28 in Group B, respectively. However, there was no significant difference before and after adalimumab therapy in Group B.

Discussion

In this study, it has been found that the *Demodex* density increased in AS patients after receiving adalimumab therapy. Additionally, *Demodex* density was positively correlated with age and CRP level in AS patients.

Demodex positivity was reported in asymptomatic healthy individuals but at low densities. Using SSSB, a prevalence of approximately 40–50% has been consistently reported in adults aged approximately 50 (Chang and Huang, 2017). Using PCR, a population sample tested positive for *Demodex* in 100% of adults over 18 (Thoemmes et al., 2014). In this study, the presence of *Demodex* was 94.52% (69/73) in patients with AS. Furthermore, there was no significant difference in *Demodex* presence in regard to the sex of AS patients, and a positive correlation was found between the *Demodex* density and age. Similar results were also reported: sex had no impact on *Demodex* infection, but age had a positive correlation with the risk of *Demodex* infection (Sędzikowska et al., 2018; Arli et al., 2019). In addition, it was also found that there was a positive correlation between the *Demodex* density and CRP level. The host immune system seems to tolerate *Demodex* without inflammation as a commensal when they are present at low numbers and it appears that they stimulate inflammation when their number increases (Foley et al., 2021). The elevated CRP level might be attributed to the increase in the *Demodex* density.

Demodicosis can occur in some clinical settings. Diabetes mellitus, metabolic syndrome and immunodeficiency resulting from

active infection with HIV generate an environment that supports *Demodex* infestations (Annam et al., 2010; Keskin et al., 2014; Yamaoka et al., 2014; Arli et al., 2019; Toka et al., 2020). However, there was no correlation between BMI and the *Demodex* density in this study, which was not in accordance with previous studies (Arli et al., 2019; Toka et al., 2020). Because all the samples of such studies were small, so larger samples should be needed to investigate the real relationship. Demodicosis has also been reported in transplant recipients receiving long-term regimens of immunosuppressants (Chovatiya and Colegio, 2016) and in cancer patients receiving targeted treatment with EGFR blockers (Chon and Hassler, 2016). The cytokine TNF- α plays crucial roles in the innate and adaptive immune response, but it also participates in the pathogenic mechanism and development of AS. TNF- α inhibitors have been recommended as the first-line treatment for AS (Ward et al., 2019). This study revealed that the *Demodex* density in patients with AS increased after adalimumab treatment. Immunological studies showed that the *Demodex* mites were able to modulate the inflammatory response. As a survival mechanism, *Demodex* mites may suppress the adaptive immune system by downregulating T-cell levels (Akilov and Mumcuoglu, 2004) or by blocking molecules required for an effective antiparasitic Th2 immune response (Liu et al., 2004). Low *Demodex* mite numbers may also downregulate the host immune TLR signalling pathway to facilitate their survival (Lacey et al., 2018). Moreover, analysis of skin-homing CD4 + T-cell subsets suggested that *Demodex* infestation induced an increase in the regulatory T-cell subpopulation compared to the *Demodex*-negative donors (Gazi et al., 2019). So we speculate that the suppression of TNF- α might create a better environment that promotes the survival of *Demodex* and the increase in the *Demodex* density. However, no clinical symptoms or diseases related to *Demodex* were observed in this study.

There are still limitations to this study. First, though the increase of *Demodex* density is statistically significant, patients with AS receiving adalimumab treatment did not present with clinical manifestation caused by infestation of *Demodex*. Thus, patients need to be observed for a longer time to confirm whether adalimumab therapy can cause dermatological or ophthalmological diseases in AS. Second, though *Demodex* density was positively correlated with both age and CRP level, the correlations were still weak. Moreover, the sample size of this study is relatively small. The change in the *Demodex* density resulting from adalimumab therapy in AS should be confirmed in larger cohorts.

In conclusion, the changes in the *Demodex* density before and after adalimumab therapy were observed in AS patients. These results provide important implications for patients with AS and their treating physicians, and also call for the awareness of long-term complication during the adalimumab therapy for AS patients. Future studies should focus on whether early intervention of *Demodex* infestation could reduce the risk of *Demodex*-related diseases.

Acknowledgements. The authors thank the staff of the Department of Rheumatology, the Fifth Affiliated Hospital of Sun Yat-sen University for providing the necessary facilities.

Author contributions. All the authors contributed to this work. Xuegang Li and Nelson Siu Kei Lam conceived and designed the study. Xuegang Li and Li Yang took samples and conducted data gathering. Anqi Liang and Xin Xin Long performed statistical analyses. Junli Guo and Shuping Zhong wrote the article.

Financial support. This research received no specific grant from any finding agency, commercial or not-for-profit sectors.

Conflict of interest. None.

Ethical standards. Before this study, patients were informed of the clinical requirements and possible risks of all operations and signed informed consent forms were obtained. This study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-Sen University (Approval number: K48-1).

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