

## To evaluate the role of interleukin-6 in alopecia areata

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### Abstract

**Objective:** To investigate the level of interleukin-6 in alopecia areata patients.

**Method:** The exploratory study was conducted from September to December 2021 at the Sindh Institute of Skin Disease, Karachi, and comprised alopecia areata patients regardless of age and gender in group A, while healthy controls matched for age and gender formed group B. Alopecia areata classification and severity were done using the Severity of Alopecia Tool. Serum interleukin-6 was measured using enzyme-linked immune sorbent assay. Data was analysed using R statistical software v4.2.1.

**Results:** Of the 100 subjects, 50(50%) with mean age  $15.52 \pm 10.14$  years were cases in group A; 26(52%) females with mean age  $16.78 \pm 10.77$  years, and 24(48%) males with mean age  $16.44 \pm 10.3$  years. The remaining 50(50%) were controls in group B. Interleukin-6 concentration was significantly higher in group A ( $p < 0.05$ ). The concentration was not significantly different between the genders ( $p > 0.05$ ). The concentration was the highest in patients aged 11-20 years, followed by 21-30 years, 31-40 years and 1-10 years.

**Conclusion:** The concentration of circulatory pro-inflammatory interleukin-6 was significantly higher in alopecia areata patients than in the healthy controls.

**Keywords:** Alopecia areata, Autoimmune, Cytokines, Interleukin-6, Skin disease. (JPMA 74: 930; 2024)

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### Introduction

Alopecia areata (AA) is a common cause of hair loss in humans. This autoimmune condition causes patchy hair loss on the scalp and other body areas. Although AA can start at any age, it is observed that 50-60% people experience the onset of disease before turning 20 years.<sup>1</sup> According to the global burden of disease (GBD) database 1990-2019, the age-standardised disability adjusted life years (DALYs) rate of AA was 7.51 (4.73-11.14) per 100,000 persons globally.<sup>2</sup>

AA often manifests as an abrupt loss of hair in clearly defined areas. The affected region typically consists of a round-shaped patch of alopecia that may be widespread or localised. Previous reports stated that although scalp hair involvement is the most typical complication of this condition, other body hair, such as eyelashes, eyebrows, beard, underarm and pubic hair, may also be affected.<sup>3</sup> Based on the locality of hair loss, there are two sub-categories of AA; alopecia totalis (AT) and alopecia universalis (AU). Patients in the former group completely lose their terminal scalp hair, while those in the latter group completely lose both their terminal scalp and body hair.<sup>4</sup>

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The aetiology of AA is not well understood, and there is still much to explore about the disease. A family history of AA indicates a hereditary risk in about 20% AA patients.<sup>5</sup> The major histocompatibility complex and cytokine genes are among those with which associations are reported, indicating that the genetic propensity is multifactorial in character. Viral infections, trauma and physical and mental stress are the triggers of AA.<sup>6</sup>

Histologically, a 'swarm of bees' is a distinctive hallmark of AA lesions which indicate T lymphocytes surrounded the hair bulb.<sup>7</sup> The molecular processes driving this cell accumulation is not identified yet. According to some theories, the hair follicle from autoreactive T cells' immune surveillance and the loss of immunity privilege are major factors in AA pathogenesis. Additionally, it has been hypothesised that autoantigens found in hair follicles, like melanin-associated protein, may be recognised by cytotoxic T lymphocytes.<sup>8</sup> The connection between AA and autoimmune conditions raises the possibility that AA is an autoimmune disorder. Most of the data points to varied T-cell-mediated immunity as a factor in the pathophysiology of AA. The immune process of AA is derived primarily through different cytokines and chemokines.<sup>9</sup>

Two inflammatory cytokines, interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- $\alpha$ ), are significant contributors to a variety of inflammatory diseases, including psoriasis and rheumatoid arthritis (RA).<sup>10</sup> The IL-6 and other cytokines are produced by T helper 2 (Th2) cells which initiate the activation process and induce conversion

of B cells into subsequent antibody-producing plasma cells.<sup>11</sup> Recent research on the key role of these two inflammatory cytokines in AA has opened new avenues for AA treatment.<sup>10,12,13</sup>

Pakistan is the fifth most populous country in the world and lacks adequate healthcare facilities due to poor socio-economic conditions. Prevalence of infectious and non-infectious diseases is higher. There are only a few reports on clinico-epidemiological aspects of AA from some selected areas in the country.<sup>14-16</sup> The current study was planned to evaluate the concentration of circulatory pro-inflammatory cytokine IL-6 in AA patients, and to compare it with healthy controls.

### Subjects and Methods

The exploratory study was conducted from September to December 2021 at the Sindh Institute of Skin Disease, Karachi, and comprised AA patients regardless of age and gender in group A, while healthy controls matched for age and gender formed group B. The study was approved by the hospital's ethical committee. With a life time risk of 2.1% for AA<sup>17</sup> and considering the 99.9% confidence interval, the sample size was calculated to be 90, by using the formula  $n = z^2 * p * (1-p) / e^2$ , where  $z = 3.29$  for a confidence level ( $\alpha$ ) of 99.9%,  $p$  = proportion (expressed as a decimal), and  $e$  = margin of error.<sup>18</sup> For this study, haphazard sampling technique was adopted i.e., all the AA patients who were readily available were included in the study. AA classification and severity were done using the Severity of Alopecia Tool (SALT), as per the Alopecia Areata Foundation (AAF) clinical assessment guidelines.<sup>19</sup> Those with SALT value  $\geq 25\%$  were designated as severe AA, while those with SALT value  $< 25\%$  were considered moderate AA patients.

Those excluded were patients who had received systemic immunosuppressive therapy or local treatment with glucocorticoids or dithranol prior in the preceding 3 months. Also excluded were those with a history of any chronic disease or smoking. Children who showed indicators of other infections were also left out. Clinical assessment through SALT score and trichoscopy was used to confirm the inclusion requirements. The controls were unrelated individuals, and were free of AA and any other immunological condition which could interfere with IL6 level.

After taking written informed consent from all the participants, 10mL blood was drawn from the peripheral veins each subject. Histopaque-1077 media was used in gradient centrifugation to rapidly recover the viable lymphocytes and other mononuclear cells.

By using the enzyme-linked immunosorbent assay (ELISA)

method, the concentrations of serum cytokine IL-6 (Invitrogen, United States) were determined quantitatively. The serum, separated from the freshly collected blood samples, were used in the quantification through ELISA testing as per the manufacturer's recommendations. Before ELISA testing, all the components were given time to reach room temperature. In the 96-well plate, 100uL each of the standards and samples were added in their corresponding wells. The assay wells were then filled with 50uL of human IL-6 Biotin Conjugate solution, except the blank. For 2 hours, the plate was left at room temperature with the cover on. Following incubation, the solution was aspirated, and using the 1X Wash buffer, the wells were cleaned 4 times. After the addition of 100uL of 1X Streptavidin-horseradish peroxidase (HRP) solution to each well except the blank, the plate was incubated for 30 minutes at room temperature in the dark. Following the incubation, the solution was aspirated, and 1X Wash buffer was used to wash the wells 4 times. After adding 100uL of stabilised chromogen into each well, the plate was incubated for 30 minutes at room temperature in the dark. Then 100uL of Stop solution was mixed into each well. Using a spectrophotometer, the absorbance in each well was determined at 450nm. The equation generated from the standard curve was used to calculate the serum cytokine levels in the samples. Age groups and gender were compared with respect to serum cytokine levels.

Data was analysed using R statistical software v4.2.1. To ascertain how cytokine levels were distributed, one-way analysis of variance (ANOVA) test was employed. Median and mean values of the studied variables in the groups were computed using MS Excel. ANOVA with the Tukey post-hoc comparative test was used for testing the significance. The correlation between quantitative measures was investigated using the Spearman correlation test.  $P < 0.05$  was considered significant.

### Results

Of the 100 subjects, 50(50%) with mean age  $16.62 \pm 10.45$  years were cases in group A; 26 (52%) females with mean age  $16.78 \pm 10.77$  years and 24(48%) males with mean age  $16.44 \pm 10.30$  years. The remaining 50(50%) were controls in group B. In group A, 4(8%) patients had SALT score 80% with the involvement of whole scalp and eyebrows, 40(80%) had SALT score 20% with only 1 patch, and 6(12%) had SALT score 40%. On dermatoscopic analysis, tapering hairs, broken hairs, black and yellow dots, and clustered short vellus hairs were observed (Figure 1).

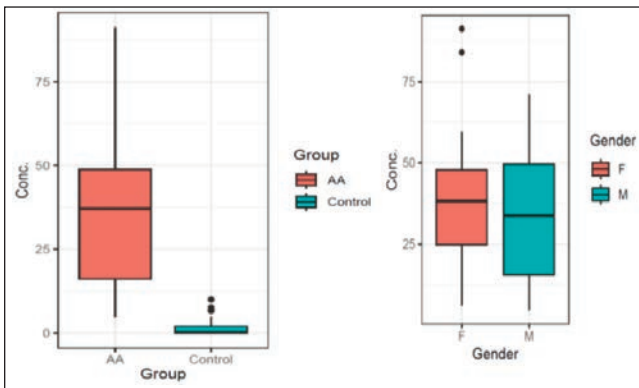
The mean IL-6 concentration in group B was  $1.679 \pm 2.705$  pg/mL, and it was  $35.939 \pm 21.970$  pg/mL in group A ( $p < 0.001$ ). The median IL-6 concentration in group B was

0.166 pg/mL (IQR 0.0079-1.8477), and it was 37.032 pg/mL (IQR 15.5743-49.0232) in group A (Figure 2).

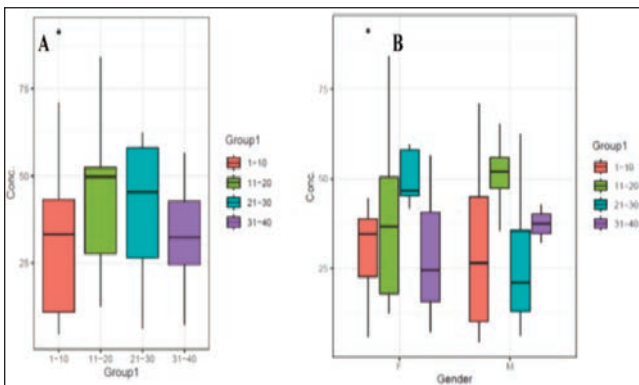
Average IL-6 concentration in group A males was



**Figure-1:** Dermatoscopic findings of exclamation mark, black dot, broken hairs and vellus hairs on the scalp of an alopecia areata patient.



**Figure-2:** Boxplot showing the concentrations of interleukin-6 (IL-6) (pg/mL) in the healthy controls (A) and alopecia areata (AA) patients (B). The mid lines within the boxes represent the median values. The IL-6 concentration was significantly higher in AA than in controls ( $p < 0.001$ ).



**Figure-3:** Boxplots showing the concentrations of interleukin-6 (IL-6) (pg/mL) in different age groups (A) and genders (B).

33.991±21.337 pg/mL and in females it was 37.70±22.632 pg/mL ( $p > 0.05$ ). Mean IL-6 concentration in those aged 1-10 years in group A was 31.911±22.358 pg/mL, it was 44.16±3.882 pg/mL in those aged 11-20 years, 40.25 pg/mL in those aged 21-30±3.270 years and 32.68±2.739 pg/mL in those aged 31-40 years (Figure 3A-B).

In group A females aged 1-10 years, mean IL-6 concentration was 33.64±22.67 pg/mL, and in males it was 30.42±21.73 pg/mL. In female cases aged 11-20 years, mean IL-6 concentration was 39.67±27.21 pg/mL, and it was 51.20±12.27 pg/mL in males. In female cases aged 21-30 years, mean IL-6 concentration was 50.27±8.01 pg/mL, and it was 27.74±24.56 pg/mL in males. In females aged 31-40 years, mean IL-6 concentration was 29.48±24.95 pg/mL, and it was 37.48±7.44 pg/mL in males.

### Discussion

AA is an autoimmune disease for which detailed pathophysiology is unknown. It appears to be linked to a complicated interplay between immune system performance and genetic susceptibility.<sup>5</sup> The current study revealed a strong association between AA and pro-inflammatory marker IL-6.

In the development of various autoimmune illnesses and chronic inflammatory conditions, a key role is played by the pro-inflammatory cytokine IL-6. In the current study, AA patients had significantly higher levels of IL-6 than the healthy controls ( $p < 0.001$ ). Previously, it has been demonstrated that balding dermal papilla cells express more IL-6 transcripts than the dermal papilla cells that were non-balding, and IL-6 could impede the lengthening of the hair shaft as per the varying doses.<sup>20</sup> Regardless of the dominant response, IL-6 levels are frequently high in autoimmune disorders, and this is also true for multiple sclerosis (Th1 dominant), systemic lupus erythematosus (Th2 dominant), and psoriasis (where Th17 is critical in its pathogenesis).<sup>4</sup> The balding cells might be prone to more immune-infiltration, resulting in the release of higher concentration of IL-6. The higher level of IL-6 in AA is also correlated with the notion that the recombinant humanised antihuman monoclonal antibody tocilizumab (TCZ) has been approved by the Food and Drug Administration (FDA) to treat various immunological conditions, such as cytokine release syndrome, giant cell arteritis, juvenile idiopathic arthritis, and refractory rheumatoid arthritis.<sup>21</sup> In a study, 7 months of TCZ administration resulted in substantial hair regrowth in AA cases.<sup>22</sup> TCZ selectively inhibits IL-6 receptor, which is known for having a wide range of applications.

The current study has limitations as it had a small sample size from a single centre.

## Conclusion

The concentration of circulatory pro-inflammatory interleukin-6 was significantly higher in AA patients than in healthy controls.

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**Conflict of Interest:** None.

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### Author Contribution:

SWM: Data collection, writing and editing, responsible for integrity of research.

MSP: Reviewing, finalize analysis, final approval.