Evaluation of CD4 and CD8 in Al-Diwaniyah alopecia Patients by ELIZA

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Abstract: Background: According to recent beliefs, certain immunological variations discovered in patients may be related to alopecia, particularly the alopecia areata kind.

Objective: The current study's objective is to detect, evaluate, and estimate certain hematological parameters in alopecia areata (AA).

Material and methods: A retrospective analysis was done between July 2022 and February 2023. A total of 120 alopecia patients from AL-Qadisiyah hospitals, private clinics, and AL-Karamah private hospitals were examined. Blood was drawn from the patients, spun apart to separate the serum, and then processed using immunological methods.

Results: Out of 120 samples, the results showed that 80 (66.66%) had alopecia areata, whereas 40 (33.22%) had the other forms (alopecia universalis and alopecia totalis). From the findings, immunological alterations in alopecia areata patients were discovered. The results of CD4 showed that the patients with alopecia areata was the highest (n=80) 4.06 ± 0.93 compared with control (n=60) 3.18 ± 0.65, results of CD8 showed that the patients with alopecia areata was the highest (n=80) 323.7± 127.3 compared with control group(n=60) 159.8±62.1 .

Conclusion: By revealing the existence of particular immunological components in patients who acquire alopecia, the study laid the groundwork for a wider and more in-depth investigation of immunological abnormalities in people with alopecia areata or totalis.

Keywords: Alopecia areata, Alopecia universalis, Alopecia totalis, Immunological variables.
Introduction:
Alopecia is the medical term for hair loss on the body or head. The head is typically at least[1]. Alopecia is one of the most often encountered dermatological conditions. Despite the fact that (non-cicatricial) alopecia is a prevalent cause of hair loss, a clear diagnosis is not always easy to make and there are few effective medical therapies [2]. An autoimmune illness is suggested by the circular, finely defined regions of alopecia areata (AA) and the non-scarring, pounding T-cell response to the hair follicle autoantigen [3]. characterized by sporadic body and head hair loss without swelling [4]. The epidermis is most affected. Everyone is impacted by AA [5]. Form and amount of hair loss are AA categories. It can affect the hair on the scalp, torso, cheeks, goatee, eyebrows, and other body parts [6, 7]. AA sisiapho, widespread, reticularis, patchy, and ophiasis are examples of clinical variations. Total hair loss is caused by A. totalis (AT), whereas scalp hair loss is caused by A. universalis.[8,9]. Some of the recognized etiopathogenesis of AA include genetics, infections, melanocyte abnormalities, immunological variables, keratinocyte degeneration, neurological reasons, and emotional stress. It is believed that all stimuli could be contributors[10]. The incidence of psychological comorbidities and this illness are strongly correlated[11]. The initial sign of autoimmunity in AA was a clump of inflammatory cells that looked like a swarm of bees moving in the direction of the hair follicles[12]. According to Strazzulla et al. (2018), cytotoxic T lymphocytes in the bulb region may be able to recognize the auto-antigen present on hair follicles, such as the protein related with melanin[13,14,15]. The hair follicle is a structure with special immunity. This Immune Privilege (IP) covers the bulge area that protects the matrix and hair stem cells[16]. Similar to NK cell receptors being downregulated, a number of factors[17] affect the hair bulb's capacity to withstand immune system responses to melanocyte peptides and/or self-keratinocytes.

Many inflammatory dermatological conditions, including psoriasis, have been diagnosed using hematological traits as biomarkers[18]. The CD4, CD8 are proven to be active throughout the AA etiopathogenesis, Patients with AA who have CD8 cell expression in lesional skin biopsies in connection to the disease's intensity, activity, duration, and trichoscopic findings[19]. in the patients' blood serum [20, 21]. Alopecia areata is an autoimmune disorder of the hair follicle that has a genetic origin. In alopecia areata, the lymphocytic infiltration around the hair follicle is what causes hair loss[22].

Aims of the study: Examining serum levels will help the study's researchers identify whether these biomarkers and baldness are related (CD4,CD8). This will make it easier to understand how alopecia and immunological and hematological factors are related.

Materials and Methods

Patients characterization
A total of 120 patients' serum was donated by the dermatology departments at Al-Diwaniyah Teaching Hospital, Al-Karama Private Hospital, and private clinics.

Using a clean needle, draw 5 ml of blood from a vein and place it in the transport tube from patients of all ages (1-55) with alopecia who have been admitted to AL-Diwaniyah Teaching Hospital, AL-Karamah Private Hospital, and private clinics. Next, separate the serum by centrifuging and freezing it until use under aseptic conditions during the study period from 1/7/2022 to 1/2/2023.

Ethical Considerations
The Medical Ethics Commission at the Iraqi Ministry of Health gave its approval to this study.
Measurement of serum CD4, CD8 levels by ELIZA

Each patient, as well as the several groups of healthy controls, had five milliliters of venous blood taken from them. Around 30 minutes were given for a part of the sample to coagulate in a gel tube. The serum was drawn out. It was kept until the research at 20°C. To measure the blood amounts of CD4, CD8, ELISA was used. (Mybiosource, USA). The remaining sample (whole blood) was gathered and put in an EDTA tube for hematological examination using an auto-hematology apparatus (Mindary, China). An ELISA reader operating at 450 nm assessed the optical density of each well, and the results for the concentrations of CD4, CD8 were extrapolated from the standard curve.

Statistical analysis

The results are expressed by Pearson’s chi-square test and Fisher’s exact test were used to compare the risk factors. A p-value < 0.05 was considered statistically significant.

Results

Patients characterization

120 A total number of patients with alopecia, 80 of whom were infected with alopecia areata (35 of males, 45 of females) and 40 were infected with alopecia totalis and universalis (11 of males, 29 of females) The results in table (1) show that alopecia areata represents 80 sample 2.19a±26.51 , alopecia universalis and totalis represents 40 out of 120 sample 1.70a±27.45 compare with 60 sample of control 1.71a±26.27 . As shown in table (1), (2).

<table>
<thead>
<tr>
<th>Table (1): The age means among the studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Age mean ± SE (years)</td>
</tr>
</tbody>
</table>

According to the Duncan test, a difference between letters that were equivalent indicated that the difference was not significant (P > 0.05), while a difference between letters that were different indicated that the difference was significant. This was determined by comparing the letters’ similarities and differences. (P ≤ 0.05).

<table>
<thead>
<tr>
<th>Table (2): the distribution of studied groups according to their gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Gender number percentage(%)</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>
Figure(1): The number of alopecia areata, universalis and totalis of males and females

The patients with alopecia areata had the highest CD4 findings ($n=80$), according to table (3) and curve. 4.06 ± 0.93 against control ($n = 60$) 3.18 ± 0.65

Table (3): T-estvariables' descriptive statistics for the groups under study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients group ($n = 80$)</th>
<th>Control group ($n = 60$)</th>
<th>P by t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.93±4.06</td>
<td>0.65±3.18</td>
<td></td>
</tr>
</tbody>
</table>

Duncan test: similar letters referred to a non-significant difference ($P > 0.05$), different letters referred to a significant difference ($P \leq 0.05$)

The results through the table (3), Immune biomarkers (CD4) in blood were not significantly different ($p > 0.05$) across disease groups compared to healthy people.

Table (4): T-estvariables' descriptive statistics for the groups under study

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Control group ($n = 60$)</th>
<th>P by t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>127.3±323.7</td>
<td>62.1±159.8</td>
<td></td>
</tr>
</tbody>
</table>
The results of CD8, which are shown in table 4, show that the number of patients with alopecia areata (n=80) 323.7 127.3 was considerably higher than the number of patients in the control group (n=60) 159.8 62.1. The reality that there were significantly more individuals with alopecia areata served as evidence of this. Patients who have been diagnosed with AA and have lesional skin tissues that exhibit CD8 cell expression [23]. This expression is discovered to be related to trichoscopic findings as well as the severity, activity, and duration of the illness. CD8+ T cells are thought to act as effector cells in the pathogenesis of inflammatory arthritis illness. Interferon (IFN), the substance that aids in the breakdown of HF-IP, is produced in large quantities by these lethal T cells. Without a doubt, research has shown that alopecia areata is an inflammatory condition linked to the growth of CD4+ and CD8+ T cells near hair follicles [24]. Several science investigations have backed this claim [24].

Discussion

The causes of baldness are unclear [25]. This theory, based on a recent undirected data study [26]. Inflammation of hair cells is the most widely known cause of this illness [26]. (HFs). An increase in class I and II ma, a systemic illness that can affect hair, nails, and skin, and a lymphocytic invasion around and inside hair bulbs during active disease all contribute. Another factor is immunoglobulin and complement buildup around HFs, especially at open lesions. This hypothesis's largest flaw is that it wasn't until recently that AA's HFs were the target of an aberrant immune response. This issue is new. Study suggests that CD4 and CD8 may be vital to its growth, even though it may change in many ways.

In the active stage of AA, circulating CD4, CD8 T, and NK cells decline and the CD4/CD8 T cell ratio rises [30]. This result persisted even though these cells increased at the same pace as the disease. We found that immune pathways cause AA. T-lymphocytes are mostly CD4+ T helper and CD8+ T apoptosis cells [31]. CD4 T cells induce and divide into B cells, CD8 T cells, mast cells, and macrophages, which are needed to handle the immune reaction. Immunomodulation achieves this. CD4+ T cells can induce hair loss by costimulating CD8+ T cells [32,33]. The rise in Lee et al.'s CD4:CD8 T cell ratio is consistent with our study's finding that patients' peripheral blood had fewer CD4 and CD8 T cells than healthy people's. We found CD4 and CD8 T cells. (1996). The 1988 study by Lutz and colleagues supported this. The study found that, while patients killed fewer CD8 T cells than the control group, they killed a similar number of CD4 T cells. Zöller et al. (2004) found that the peripheral blood of patients with active AA had a higher percentage of T regulatory cells (T reg), which suppress the proliferative activity of CD4+ and CD8+ T cells, compared to healthy controls or patients with stable or regressive AA. This may explain the decline in CD4+ and CD8+ T cells in our study. Kubo et al. (2017) found that recent patients had more peripheral blood Treg cells. T regulatory cells (Treg cells) can block CD4+, CD8+, and NK cell growth and early disease start [34]. When the illness is more severe and lasts longer, the disease develops faster due to fewer peripheral blood modulating T cells. Because the illness has lasted a long time. Patients with long-term alopecia totalis and universalis had higher peripheral blood CD4+ and CD8+ T cell ratios [35,36]. Alopecia unilocularis was tied to lower peripheral blood CD4+ and CD8+ T-cells. These types of baldness were linked to higher CD4+ and CD8+ T-cell counts using this information. These results illuminated the relationship between a patient's disease length and the community's peripheral blood CD4+ and CD8+ T cell proportion [37]. How can an excess of CD4+ and CD8+ T-cells around hair follicles' immune defenses kill them when the proportion of these cells in AA patients' peripheral blood decreases [38]? According to Hamed et al., more CD4+ and CD8+ T cells are near hair fibers, explaining this. (2019). AA patients have 40% more T regulatory cells in their peripheral blood than controls, but active AA
skin has 90% fewer T regulatory cells than normal skin. Why? Active AA epidermis has more T regulating cells than normal skin. [39,40].

References:


16. Aldzhabali, M. A. M., & Kuts, L. V. (2022). Serum levels of IL-2 and IL-17A are related to clinical type and severity of alopecia areata


