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Evaluation the role of Interlukin-36 in Atopic dermatitis patients (Eczema)

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ABSTRACT

Background: Atopic dermatitis (atopic eczema) is a widespread inflammatory skin condition characterized by defects in both skin barrier structures and immune response alternations, so that the major cause for structural abnormalities in the epidermidis associated with immune dysregulation in this disease.

Objective: This study aimed to compare serum IL-36α between 3 study groups and the involvement and importance of these interleukins in atopic dermatitis.

Method: ELISA was used to detect the IL-36 level between groups under study. During the current study, blood samples, were collected from only 60 patients with AD in Diyala and Baghdad province, and 30 healthy persons as a control.

Results: there were significant differences between patients age groups, the highest mean of infection was in the patients and under 30 age categories while the lowest infection mean in control group. the statistical analysis shows the mean of IL-36 in non-treated and treated groups increased significantly when compared with control group. In non-exposure groups IL-36 increased significantly when compared with control group. In Hands & Foot, and Back & Chest groups IL-36 increased significantly when compared with control. The statistical analysis shows the mean of IL-36 in acute, and chronic groups increased significantly when compared with control. The mean of IL-36 in generalized, and localized groups increased significantly when compared with control group. In topical steroid, and systemic groups IL-36 increased significantly when compared with control group. p-value was less than 0.05.

Conclusion: The study demonstrates the role of IL-36 in the pathogenesis of atopic dermatitis and a positive correlation between severity of AD symptoms and level of serum IL-36.

Keyword: *Atopic dermatitis, IL-36.*.

1. INTRODUCTION

Atopic dermatitis (AD), also known as atopic eczema, is a chronic inflammatory skin condition that recurs frequently. Its pathophysiology is extremely complex and is influenced by genetic, environmental, and immune system abnormalities as well as a lack of skin barrier function (1). It affects approximately 10%-20% of children and 1%-3% of adults worldwide (2). In a study done by Alwan et al. (3) showed that the prevalence of dermatitis in Diyala province was 45 (18.3%) among other skin diseases.

Grown-up Atopic dermatitis in Iraq has ended up a significant social issue with as numerous as one-third of adult patients with extreme Atopic dermatitis absenting themselves from their occupations or classes due to a flare-up of their condition. Pruritus is one of the foremost important symptoms of Atopic dermatitis in Iraq. Be that as it may, in grown-up AD reddened skin on the confront is the foremost common and problematical side effect, making noteworthy social embarrassment. Neck pigmentation moreover called swell pigmentation messy neck or poikiloderma-like skin changes is additionally a common symptom. These symptoms are safe to different sorts of treatment. In later a long time, expanding numbers of such patients have been recognized in Iraq (4).

Interleukin-36 (IL-36) is a member of the IL-1 superfamily, which has four existing isoforms (5-6), assigned as IL-36α, IL-36β, IL-36γ, and IL-36 receptor antagonist (Ra) (7). IL-36 is increasingly associated with inflammatory diseases, including various inflammatory and infectious skin disorders (8). the IL-36 subfamily, including proinflammatory agonists IL-36α, IL-36β, and IL-36γ, and one antagonist, IL-36Ra, is recognized as a key initiator of inflammation in the skin (9). So, the study aims to compare the serum IL-36α between 3 study groups (non-treated newly diagnosed and treated patients and

healthy people) and its involvement and importance atopic dermatitis (AD).

2. METHODS

Patients and Controls

The study conducted on 60 AD patients (30 treated, 30 untreated with atopic dermatitis (newly diagnosed). Their ages ranged between 5 and 75 years. A secondary control group of 30 healthy adults was recruited from outpatient clinic visitors, colleagues, and workers. The control groups were chosen based on two criteria: first, that they did not suffer from any chronic skin disorders (e.g. dermatitis, eczema, psoriasis, or others); and second, that they did not have any other chronic diseases (e.g. diabetes, malignancy, autoimmune disease, and others).

Collection and preparation of Samples

Approximately 5 ml of venous blood sample was collected from 60 patients and 30 control group in this study, then placed in the gel tubes and allow to clot for 10-20 minutes at room temperature, then centrifuged (at 2000-3000 RPM) for 20 minutes in order to separate serum which was carefully transferred to Eppendorf tubes and preserved at -20°C until used to be analyzed later for detection of IL-36 using ELISA kits. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at-20°C or -80°C to avoid loss of bioactivity and contamination.

Collection of Data

A questionnaire was used to collect data from each AD patient especially for this investigation. Data were obtained through direct patient interviews, including age, gender, specimen, chronic disease, diagnostic cause, past medical history as chemotherapy intake, steroids, immunosuppressive drugs, antibiotics and antifungals, past surgical and medical history.

ELISA procedure to determine Interleukin 36 (IL-36) Level

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Interleukin 36 Alpha (IL-36A). Add Interleukin 36 Alpha (IL-36A) to the wells, which are pre-coated with Interleukin 36 Alpha (IL-36α) monoclonal antibody and then incubated. After that, antiIL-36A antibodies were added that labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Removed unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Interleukin 36 Alpha (IL-36α) are positively correlated.

Assay procedure:

The kit is a sandwich enzyme linked immunoassay for in vitro quantitative measurement of IL-36 in human serum according to manufactures' instruction, as the following:

All reagents and micro-ELISA plate were brought to room temperature (18-25) before use. Then serial dilution for IL-36 were prepared from reference standard. Sample injection: a) Blank well: do not add sample, IL-36 antibody labeled with biotin and streptavidin-HRP; chromogen reagent A & B and stop solution was added, each other step operation was the same. After that, Standard solution well: 50µl standard was added and streptomycin-HRP 50µl (biotin antibodies were united in advance in the standard, so no biotin antibodies were added).

Sample well to be tested: 40µl sample was added and then 10µl IL-36 antibodies, and 50µl streptavidin-HRP were added next. Then solution was covered with seal plate membrane and shaken gently to mix. Solution was incubated at 37℃ for 60 min. Then washing concentration (30x) with distilled water was diluted for later use as a washing solution.

Washing: carefully the seal plate membrane was removed, drain washing solution and shaken off the remainder. Each well was filled with washing solution, stand for 30 sec, then drain. This procedure was repeated for five times then blot the plate. Each well was filled with 50 µl of chromogen reagent A, followed by 50 µl of chromogen reagent B. For color development, the mixture was gently shaken and incubated for 10 min at 37°C away from light. To stop the reaction, 50 µl of stop solution was applied to each well (color changes from blue to yellow immediately at that moment).

The absorbance (OD) of each well was measured one by one under 450 nm wavelength, which should be completed within 10 min after the stop solution was applied. The linear regression equation of the standard curve was determined based on standard concentrations and OD values. The concentration of the related sample was determined based on the OD value of the samples. Statistical software was used as well.

3. RESULTS CALCULATION

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration (which is the concentration of the sample); or calculate the linear regression equation of standard curve according to the concentration of the standard and the OD value. Then substitute with the OD value of the sample to calculate its concentration.

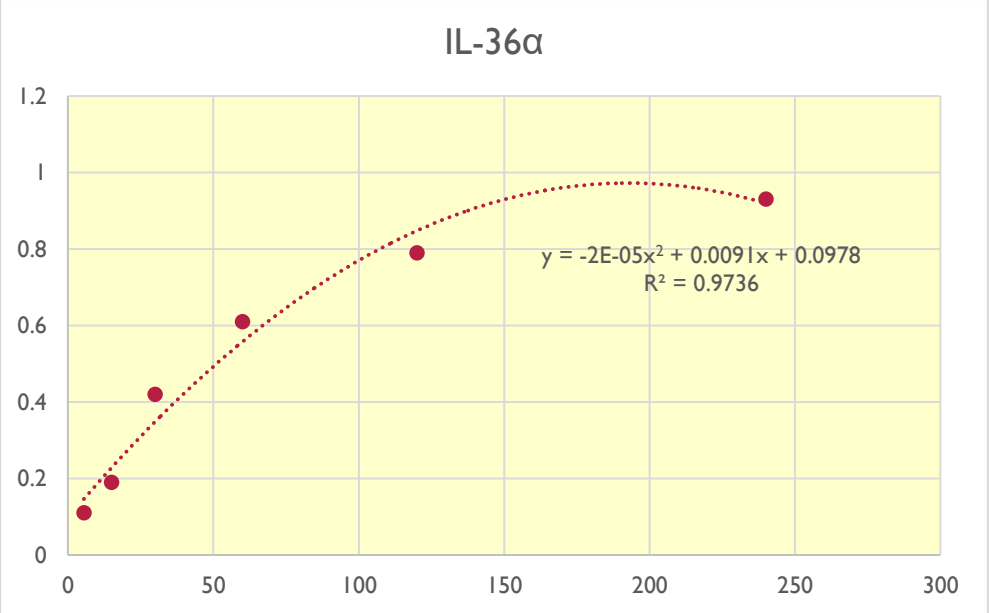

Figure (1): Standard Curve of IL-36

Statistical Analysis

Using the social sciences statistical software package (MiniTab statistical software version17, IBM (Pennsylvania, USA)), statistical analysis was conducted and the results were expressed as mean±standard deviations (Mean±S.D). Using Student's t-test for two independent means and one way ANOVA for more than two independent means, statistical analysis was conducted on the importance of quantitative data differences (P-values of around <0.05) (10).

Ethical Statement

The Medical Microbiology department local committee agreed to the experiments mentioned in this thesis and all volunteers give formal consent and details and advantages were given to the patients based on research and copy of the completed medical analysis. The study was undertaken by the University of Baghdad/College of Medicine under supervision of science unite in college.

4. RESULTS AND DISCUSSION

During the current study, blood samples, were collected from only 60 patients with AD in Diyala and Baghdad province, and 30 healthy persons as a control. There were significant differences between patients age groups, the highest mean of infection was in the patients and under 30 age category while the lowest infection mean in control group.

The mean of IL-36 in control, age less than 30 years, and age more than 30 years groups (9.00 ± 6.59 ng/L, 184.32 ± 229.01 ng/L, and 178.27 ± 331.04 ng/L respectively), the statistical analysis show the mean of IL-36 in age less than 30 years, and age more than 30 years groups increased significantly when compared with control group p-value less than 0.05 table (1)

Table 1: Demonstration of IL-36 Levels According to age

Interleukins	**Control N (30)** **Mean ± SD**	**Age less 30 (23)** **Mean ± SD**	**Age more 30 (37)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	184.32 ± 229.01	178.27 ± 331.04
ANOVA P-Value	Control Vs. age less than 30 (**0.028**) Control Vs. age more than 30 (**0.017**) Age less than30 Vs. age more than30 **0.942** P-value <0.05 significant		

The present results were in agreement with Ramirez et al. (11) who demonstrated that, AD was more prevalent between the

ages group from 2-16. Some studies have shown that AD prevalence is still growing, particularly in countries with low incomes and typically it is first to occurs early in the life and frequently precedes other allergic disorders such as asthma (12).

The mean of IL-36 in control, non- treated, treated groups (9.00 ± 6.59 ng/L, 199.37 ± 345.40 ng/L, and 163.97 ± 242.23 ng/L respectively), the statistical analysis shows the mean of IL-36 in non-treated and treated groups increased significantly when compared with control group p-value less than 0.05 table (2)

Table 2: Demonstration of IL-36 Levels According to Treatment

Interleukins	**Control N (30)** **Mean ± SD**	**Non-Treated (30)** **Mean ± SD**	**Treated N (30)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	199.37 ± 345.40	163.97 ± 242.23
ANOVA P-Value	Control Vs. Non-Treated **0.011** Control Vs. Treated **0.037** Non- Tarted Vs. Treated **0.597** P-value <0.05 significant		

The mean of IL-36 in control, non- exposure, and exposure groups (9.00 ± 6.59 ng/L, 74.16 ± 91.92 ng/L, and 205.68 ± 319.83 ng/L respectively), the statistical analysis shows the mean of IL-36 in non-exposure groups increased significantly when compared with control group p-value less than 0.05 table (3)

Table 3: Demonstration of IL-36 Levels According to Occupation

Interleukins	**Control N (30)** **Mean ± SD**	**Non -Exposure N (11)** **Mean ± SD**	**Exposure N (49)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	74.16 ± 91.92	205.68 ± 319.83
ANOVA P-Value	Control Vs. non-Exposure **0.490** Control Vs. exposure **0.004** Exposure Vs. Non-exposure **0.123** P-value <0.05 significant		

The mean of IL-36 in in control, Handa & Foot, Face & Nack, Back & Chest and groups (9.00 ± 6.59 ng/L, 184.68 ± 307.00 ng/L, 96.29 ± 126.73 ng/L, and 209.50 ± 321.04 ng/L respectively), the statistical analysis shows the mean of IL-36 in Hands & Foot, and Back & Chest groups increased significantly when compared with control p-value less than 0.05 group table (4)

Table 4: Demonstration of IL-36 Levels According to Lesion Site

Interleukins	**Control** **N (30)** **Mean ± SD**	**Hands & Foot** **N (40)** **Mean ± SD**	**Face & Nack** **N (7)** **Mean ± SD**	**Back & Chest** **N (13)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	184.68 ± 307.00	96.29 ± 126.73	209.50 ± 321.04
ANOVA P-Value	Control Vs. Hands & Foot 0.013; Vs. Face & Nack **0.464**; Vs. Back & Chest **0.030** Hands & Foot Vs. Face & Nack **0.433**; Vs. Back & Chest **0.763** Face & Nack Vs. Back & Chest **0.374** P-value <0.05 significant			

Zysk et al (13), Found that the expression level of I-36α was significantly higher in lesional AD skin than in non-lesional AD skin (p = 0.0039) and healthy skin (p = 0.0045). No significant difference in the expression levels of IL36α between the non-lesional AD skin and healthy skin was found (p = 0.3505). Healthy skin was characterized by weak expression of both IL-35 and IL-36α.

The mean of IL-36 in in control, acute, sub-acute, and chronic groups (9.00 ± 6.59 ng/L, 176.54 ± 318.70 ng/L, 68.43 ± 73.32 ng/L, and 259.58 ± 3.9.75 ng/L respectively), the statistical analysis shows the mean of IL-36 in acute, and chronic groups increased significantly when compared with control p-value less than 0.05 group table (5)

Table 5: Demonstration of IL-36 Levels According to Atopic Dermatitis

Interleukins	**Control** **N (30)** **Mean ± SD**	**Acute** **N (35)** **Mean ± SD**	**Sub-acute** **N (10)** **Mean ± SD**	**chronic** **N (15)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	176.54 ± 318.70	68.43 ± 73.32	259.58 ± 3.9.75
ANOVA P-Value	Control Vs. Acute **0.019**; Sub-Acute **0.556**; Chronic **0.004** Acute Vs. Sub-Acute **0.255**; Vs. Chronic **0.290** Sub-Acute Vs. Chronic **0.076** P-value < 0.05 significant			

The mean of IL-36 in in control, generalized, and localized groups (9.00 ± 6.59 ng/L, 196.06 ± 306.06 ng/L, and 176.53 ± 294.84 ng/L respectively), the statistical analysis shows the mean of IL-36 in generalized, and localized groups increased significantly when compared with control p-value less than 0.05 group table (6)

Table 6: Demonstration of IL-36 Levels According to Extent of disease

Interleukins	**Control N (30)** **Mean ± SD**	**Generalized N (15)** **Mean ± SD**	**Localized N (45)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	196.06 ± 306.06	176.53 ± 294.84
ANOVA P-Value	Control Vs. Generalized **0.037** Control Vs. Localized **0.015** Generalized Vs. Localized **0.8.9** P-value <0.05 significant		

The mean of IL-36 in in control, topical steroid, and systemic groups (9.00 ± 6.59 ng/L, 147.24 ± 164.69 ng/L, and 147.24 ± 164.69 ng/L respectively), the statistical analysis shows the mean of IL-36 in topical steroid, and systemic groups increased significantly when compared with control p-value less than 0.05 group table (7)

Table 7: Demonstration of IL-36 Levels According to Treating type

Interleukins	**Control N (30)** **Mean ± SD**	**Topical Steroid N (12)** **Mean ± SD**	**Systemic N (18)** **Mean ± SD**
IL-36 ng /L	9.00 ± 6.59	147.24 ± 164.69	174.44 ± 285.31
ANOVA P-Value	Control Vs. topical steroid **0.047** Control Vs. systemic **0.008** Topical steroid Vs. systemic **0.698** P-value <0.05 significant		

As a result of all the above mentioned conclusions of the present study in addition to the remarks of other researchers in

concern with the mentioned findings, It is necessary to highlight the need of continual skin care in keeping the symptoms of AD patients from worsening by keeping the skin barrier from being compromised or damaged as a result of the pathology of AD, which is a common indicator of filaggrin (a protein found in the skin cells), abnormal growth of gram-positive bacteria and the increase of T helper cell type 2 (Th2) cytokines as Kozo et al. (14) mentioned .

IL-36γ is thought to work in the progression of atopic dermatitis from the acute phase to the chronic phase (15), although the exact mechanism for this remains unclear.

Finally, it is safe to advise AD patients to follow a routine of frequent skin washing while avoiding dryness to help strengthen the barrier's activities, hence suppressing bacterial development issues and achieving the ideal Th1/Th2 ratio balance as proposed by many other researchers including Peng and Novak, (2015). The use of soap and detergents raises the skin pH in AD patients, causing an imbalance between serine proteases and protease inhibitors (16).

5. CONCLUSIONS

AD may arise in people of any age, but with highest incidence rate in age under 30 years specifically. The study showed that a relationship between significant increase in inflammatory marker (IL-36) and AD disease

REFERENCES

1. Sroka-Tomaszewska, J.; Trzeciak, M. Molecular Mechanisms of Atopic Dermatitis Pathogenesis. Int. J. Mol. Sci. 2021, 22, 4130.

[1] Hassan, Z. Y., Hassan, T. Y., & Kanany, A. Y. (2024). The Anti-Inflammatory Effect of Chenopodium Murale in Comparison to Salvia Frigida on Atopic Eczema. Journal of the Faculty of Medicine Baghdad, 66(4), 437-445.

[2] Alwan NK, Shakir SA, Waheeb HH. Epidemiology of Skin Diseases among Displaced People in Diyala Province. JFacMedBagdad. 2018; 60(1):52-6. https://doi.org/10.32007/jfacmedbagdad.60145

[3] Alrawiy, E. A. (2022). Atopic Dermatitis Incidence in Iraqi Population and Psychological Side Effects. Int Jr Infect Dis & Epidemlgy, 3(3), 1-3.

[4] Kumar S, McDonnell PC, Lehr R, Tierney L, Tzimas MN. and Griswold DE. 2000. Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem. 275: 10308–14.

[5] Khazem R. M. and Ibraheem S. R. Detection of Some Immunological Parameters in Psoriatic Iraqi Female Patients. Iraqi Journal of Science, 2020, Vol. 61, No. 10, pp: 2515-2524

[6] Dinarello C, Arend W, Sims J, Smith D, Blumberg H. and O'Neill L. 2010. IL-1 family nomenclature. Nat Immunol. 11: 973.

[7] Foster, A. M., Baliwag, J., Chen, C. S., Guzman, A. M., Stoll, S. W., Gudjonsson, J. E. and Johnston, A. (2014). IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin. J Immunol, 192 (12): 6053-6061.

[8] Sachen, K. L., Greving, C. N. A., & Towne, J. E. (2022). Role of IL-36 cytokines in psoriasis and other inflammatory skin conditions. Cytokine, 156, 155897.

[9] Al-Ghanmi,H.(2016).Haematological indices and iron status among patients with renal failure in Babylon Governorate.(Master Degree in Scince),University of Kerbala, College of Scince.

[10] Ramirez, F D., Shelley Ch, Sinéad M. L, Aric A. P, Charles E. Mc, Sharon A. K, Michael D. Cabana, Mary-Margaret Chren, and Katrina Abuabara. (2019)"Association of atopic dermatitis with sleep quality in children." JAMA pediatrics 173, no. 5: e190025-e190025.

[11] Nutten, S. (2015). Atopic dermatitis: global epidemiology and risk factors. Annals of nutrition and metabolism, 66(Suppl. 1), 8-16.

[12] Zysk, W.; Sitko, K.; Tukaj, S.; Zarycza´ nska, A.; Trzeciak, M. Altered Gene Expression of IL-35 and IL-36α in the Skin of Patients with Atopic Dermatitis. Int. J. Mol. Sci. 2024, 25, 404.

[13] Kozo Nakai, Yasuo Kubota, and Chie Kohchi (2019). The effect of lipopolysaccharide-containing moisturizing Cream on Skin Care in Patients with Mild Atopic Dermatitis. 33(1), 109-114.

[14] Tsoi LC, Rodriguez E, Stölzl D, Wehkamp U, Sun J, Gerdes S, et al. Progression of acute-to-chronic atopic dermatitis is associated with quantitative rather than qualitative changes in cytokine responses. J Allergy Clin Immunol. 2020;145:1406–15.

[15] Hachem, J. P., Wagberg, F., Schmuth, M., Crumrine, D., Lissens, W., Jayakumar, A., & Egelrud, T. (2006). Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. Journal of Investigative Dermatology, 126(7), 1609-1621..