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Serum Dicckopf-1 Level in Patients of Cicatricial and Non- Cicatricial Alopecia

[1]M.R.Mohamed, [1]A.A.Ebrahim, [1]D.M.Elhabak, [2]R.A.Kashaba
[1]Dermatology Venereology and Andrology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
[2]Clinical and Chemical Pathology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
Email: Maram.oraby77@gmail.com

Abstract

Hair loss disorders are divided into 2 major groups: Cicatricial and non-Cicatricial Alopecies. Cicatricial alopecies are disorders in which the hair follicle is replaced by fibrous scar tissue leading to permanent hair loss. In Non-cicatricial alopecies hair follicles are preserved and hair growth can resume when the cause of the problem is eliminated [1].Dickkopf-1 encodes a potent and specific endogenously-secreted Wnt antagonist that binds and inhibits LRP (Lipoprotein Receptor-Related Protein)5/6 receptors that are involved in canonical Wnt signaling during hair induction and growt[2] **Aim**: To evaluate the serum levels of DKK-1 in patients with cicatricial alopecia and non-cicatricial alopecia to determine its role in the pathogenesis of both disorders. **Patients and methods:** This prospective case-control study included sixty patients with cicatricial alopecia, sixty patients with non-cicatricial alopecia and twenty persons of apparently healthy subjects as a control group of the same age and sex. Patients were selected from the Dermatology outpatient clinic of Benha University Hospitals and Benha Teaching Hospital in the period between December 2019 and June 2020.**Results**: There was an overall significant difference in serum DKK-1 between the studied groups .Post-hoc analysis revealed significantly higher DDK-1 in the non-cicatricial group (median = 14.6, range = 11.4 – 57) than in the cicatricial group (median = 9.85, range = 3.4 – 67.3) and the control group (median = 4.93, range = 1.86 – 40.6).**Conclusion:** DKK-1 has important role for the pathogenesis of alopecia through documenting higher serum DKK-1 levels in patients with hair loss disorders compared to controls .Serum DKK-1 was a non-significant predictor of cicatricial alopecia, while it was a significant predictor for non-cicatricial alopecia.

Keywords: Dickkopf-1, cicatricial alopecia, non-cicatricial alopecia.

1. Introduction

Hair loss is often distressing and can have a significant effect on the patient's quality of life. Hair loss disorders are divided into 2 major groups: scarring and non-scarring alopecies. Scarring alopecies (Cicatricial alopecies) are disorders in which the hair follicle is replaced by fibrous scar tissue, a process that leads to permanent hair loss. In nonscarring alopecies (Non-cicatricial alopecies), hair follicles are preserved and hair growth can resume when the cause of the problem is eliminated[2].The hair follicle (HF) undergoes dynamic cycles of growth and regression throughout life[3].These cycles are repeated transits from a phase of active fiber production (anagen) to a resting phase (telogen), through rapid phases of tissue regression (catagen) and regeneration (neogen)[4].The reciprocal interactions between epithelial and mesenchymal tissues are essential for the growth and development of HFs. The dermal papillae (DP) is a cluster of specialized fibroblasts enveloped by hair matrix keratinocytes in the bulb of anagen hair that activates the keratinocytes to maintain and regenerate the hair growth cycle[5]. The Wnt/β-catenin pathway is one of the most important elements of hair growth regulation. β-catenin is markedly activated in DPCs during anagen and is important for regeneration of the hair cycle[6] **.** Dickkopf-1 (DKK-1) is involved in anagen-to-catagen transition in the hair cycle by regulating the activity of follicular keratinocytes[7]**.** Dickkopf-1 encodes a potent and specific endogenously-secreted Wnt antagonist that binds and inhibits LRP (Lipoprotein Receptor-Related Protein)5/6 receptors that are involved in canonical Wnt signaling during hair induction and growth[2]. It also activates the proapoptotic protein Bax and increases the release of DNA histone complex into the cytoplasm resulting in apoptosis in ORS keratinocytes[**.** Overexpression of DKK-1 results in failure to develop hair follicle [9].

2. Aim of the work

To evaluate the serum levels of DKK-1 in patients with cicatricial alopecia and non cictricial alopecia to determine its role in the pathogenesis of both disorders.

3. Patients and methods:

This prospective case-control study included sixty patients with cicatricial alopecia, sixty patients with non-cicatricial alopecia and twenty persons of apparently healthy subjects as a control group of the same age and sex.Patients were selected from the Dermatology outpatient clinic of Benha University Hospitals and Benha Teaching Hospital in the period between December 2019 and June 2020.

- **Inclusion criteria:** Newly diagnosed cases of hair loss of different clinical types who did not receive any topical or systemic treatment before.
- **Exclusion criteria:** Pregnancy, lactation and patients with other autoimmune cutaneous or systemic disease eg. psoriasis, vitiligo and oral lichen planus.

Methods

Every participant was subjected to the following:

1- **Complete history taking.**
2- **General examination:** Complete clinical examinations were done to exclude other autoimmune or systemic diseases.Also

Assessment of vital signs, and anthropometric measurements was done.

3- Clinical assessment of the hair (Local examination): Diagnosis of the condition by dermoscopy. Pattern of hair loss ,scarring or non-scarring lesion, site and number of lesions, morphology of the lesion, degree of severity and recurrence were assessed.

Grading and assessing severity of the lesions according to every condition as follows:

- **Androgenetic Alopecia:** grade and severity were assessed according to the Hamilton-Norwood classification system for males and the Ludwig system for females [10].
- **Alopecia Areata**: Severity of Alopecia Tool (SALT) Score was used to find out the quantitative assessment of scalp hair loss [11].
- **Telogen Effluvium**: assessed by Hair shedding scale (HSS) [12].
- **Discoid Lupus Erythematosus:** By Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI)[13].
- **Frontal Fibrosing Alopecia:** By Frontal Fibrosing Alopecia Severity Index (FFASI)[14].

Methods:

Blood Sampling: Five ml venous blood was collected from each participant under complete aseptic condition and put in a serum separating tube then was left for 30 mins till clotting then centrifuged (at 1500 rpm for 15 minutes). The separated serum was aliquoted and stored at -20°C for further testing.

120 ng/ml	**Standard No.5**	**120µl Original Standard + 120µl Standard diluents**
60 ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
30 ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
15 ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
7.5 ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

Determination of serum Dickkopf-1 level: Enzyme linked immune sorbent assay (ELISA) technique was used to detect serum level of Dickkopf -1

Assay procedure

1. Standard dilution: The test kit (Human Dickkopf-1) (DKK1) ELISA Kit ELISA Kit, (Technical MSN service online, email; sunredbio@msn.cn) supplied one original standard reagent which was diluted according to the instructions below.

2. Final measurement: Take blank well as zero, measure the optical density of (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.

3. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.

Calculation of Results Take the standard density as the horizontal the OD value for the vertical draw the standard curve on graph paper. Find out the corresponding density according to the sample OD value by the sample curve (the result is the sample density) or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density.

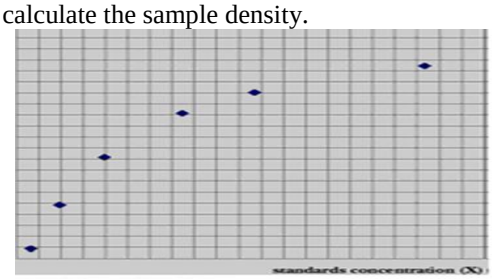

Fig. (1) Standard cure of Dickkopf-1

Statistical Analysis

Data management and statistical analysis were done using SPSS version 28 (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using Kolmogorov–Smirnov test, the Shapiro-Wilk test, and direct data visualization method. Quantitative data were summarized as medians and ranges.

4. Results

The study included 60 patients with non-cicatricial alopecia, 60 with scarring hair loss, and 20 healthy individuals with matched age and sex as controls.

Post-hoc analysis revealed significantly lower age in the non-cicatricial group (median =25, range = 12 – 55) than in the cicatricial group (median = 40, range = 16 – 70), with no significant difference between the cicatricial or the non-cicatricial groups and the control group. No significant difference was reported regarding gender **(Table 1).**

Table (1) Demographic characteristics of the studied groups

	Cicatricial (n = 60)	**Non-cicatricial (n = 60)**	**Controls (n = 20)**	**P-value**
Age (years)	30 (16 - 50)[a]	25 (12 - 55)[b]	30 (16 - 51)[a,b]	0.081 *
Sex				
Males	34 (56.7)	22 (36.7)	8 (40.0)	0.076
Females	26 (43.3)	38 (63.3)	12 (60.0)	

The most frequent diagnosis in the cicatricial group was post-inflammatory scarring alopecia (35%),

followed by frontal fibrosing alopecia (FFA) (33.3%) and discoid lupus erythematosus (DLE) (31.7%). In the non-cicatricial group, the diagnoses were alopecia areata (AA), androgenetic alopecia (AGA), and telogen effluvium (TE) (33.3% for each) **.**

There was an overall significant difference in serum DKK-1 between the studied groups ($P < 0.001$). Post-hoc analysis revealed significantly higher DDK-1 in the non-cicatricial group (median = 14.6, range = 11.4 – 57) than in the cicatricial group (median = 9.85, range = 3.4 – 67.3) and the control group (median = 4.93, range = 1.86 – 40.6) .

Serum DDK-1 showed a significant positive correlation with age in the cicatricial ($r = 0.256$, $P = 0.456$) and the non-cicatricial groups ($r = 0.370$, $P = 0.004$), while it showed non-significant correlations with disease duration in both groups **.**

5. Discussion

Alopecia or hair loss is a common complaint seen in the dermatologic practice. It is divided into scarring and non-scaring hair loss. Androgenetic alopecia (AGA), or patterned alopecia, is the commonest form of hair loss, [15]. seen in about 70% of the population

Androgenetic Alopecia is characterized by vellus transformation of scalp hairs, which corresponds to miniaturization of hair follicles by repeated hair cycles with progressively shortened anagen phase, and increased telogen/anagen ratio[16].

Alopecia areata is a non-cicatricial loss of hair in any area of the body, with a multifactorial autoimmune pathogenesis and an unknown etiology [17].

Despite the different etiology, AGA and AA have common effects on the hair follicles: hair follicle miniaturization and increased number of catagen/telogen hairs[18].

Telogen effluvium is one of the most common causes for diffuse non scarring hair loss affecting the scalp. It is an abnormality of hair cycling that result in excessive loss of telogen hairs occurring as a reaction pattern to various physical or mental stresses around 3 months after triggering event [19].

Discoid lupus erythematosus (DLE) DLE lesions are typically erythematous indurated plaques with keratotic scale. Follicular plugging (dilated follicles plugged with keratin) is also characteristic. When lesions heal, they classically leave behind atrophic scars (scarring alopecia on the scalp) and dyspigmentation [20].

Frontal Fibrosing Alopecia (FFA) Clinically is characterized by recession of the frontal hairline, frequently with involvement of the parietal and occipital region. At the periphery of the hairline, there is frequently perifollicular erythema and hyperkeratosis. Around 50 % to 95 % of patients experience rarefaction or loss of eyebrows, which may precede the frontal hairline recession by several years [21].

(DKK-1) is a powerful suppressor of the Wnt/b-catenin signaling pathway, an important hair cycle signal involved in hair follicle morphogenesis[6][22]. This signaling is involved in regulating the telogen to anagen transition and maintaining anagen phase characteristics in dermal papillae cells (DPCs) [23].

In this work, we aimed at evaluation of the serum levels of DKK-1 in patients with cicatricial alopecia and non-cicatricial alopecies in comparison to healthy controls, in an attempt to know its role in the pathogenesis of both disorders

This prospective case-control study was included sixty patients with cicatricial alopecia, sixty patients with non-cicatricial alopecia and twenty persons of apparently healthy subjects as a control group of the same age and sex.

Our results are supported by previous animal studies which reported that mice expressing high DKK-1 levels in the skin show early complete block in hair follicle development, suggesting an inhibitory role of DKK-1 on hair growth [9].

Similarly, **Mahmoud et al., (2019)** showed that the DKK-1 level is higher in the bald scalp compared with the haired scalp of AGA patients, and this may support the previous in vitro findings [24].

There were also previous animal studies that support our results, **Andl et al.** demonstrated complete block in HF development in the skin of mice expressing high levels of DKK-1 levels and so, hair growth can be inhibited by DKK-1[9][25].

6. Conclusion

DKK-1 has important role for the pathogenesis of alopecia through documenting higher serum DKK-1 levels in patients with hair loss disorders compared to control. Serum DKK-1 was a non-significant predictor of cicatricial alopecia, while it was a significant predictor for non-cicatricial alopecia.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

The study was approved by ethical committee of research involving human subjects of Benha faculty of medicine. An informed consent was obtained from all participants before being enrolled in the study.

Authors contribution

All authors are equally contributed.

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