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# More is Not Always Better in Hair Growth Factors. Epidermal Growth Factor: Hair Growth Factor Involved in Alopecia Areata Pathogenesis

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## ABSTRACT

**Background:** Alopecia areata (AA) is the second most frequent nonscarring alopecia after androgenetic alopecia; the trigger factor induces changes in the growth plate of hair bulb and leads to premature termination of anagen phase. Epidermal growth factor (EGF) can be the key molecule that participates in initiation and suppression of normal hair growth cycle. The role of EGF in the pathogenesis of AA is still uncertain. **Aim of the Work:** This aim is to estimate the serum level of EGF in patients with AA trying to detect its role in AA pathogenesis and correlate it with the disease severity. **Subjects and Methods:** This case-control study included 60 clinically diagnosed patients with AA with different variants and severities and 25 age- and sex-matched healthy controls. EGF level was measured using ELISA. **Results:** The mean serum EGF was statistically significantly higher in patients than of controls ( $P < 0.0003$ ). The EGF level was higher in patients with disease duration  $>1$  month than patients with disease duration  $\leq 1$  month, and it was higher in patients with high recurrence than patients without recurrence ( $P > 0.05$ ). The EGF in patients of severe AA was statistically significantly higher than moderate AA patients, and moderate cases were higher than mild AA patients ( $P = 0.0001$ ). Furthermore, the level of EGF with scalp involvement was higher; the highest serum level of EGF marker in S4 (75%-99%) hair loss then S3 (50%-74% hair loss), illustrated in table (4) followed by different percentage of hair loss, difference statistically significant. **Conclusions:** Elevated hair-specific growth factor as EGF is not always a good sign for hair growth and functioning promotor inducing hair recovery, but it may be linked to the pathogenesis of hair disorders as AA.

**Key words:** Alopecia areata, growth factors and epidermal growth factor, hair growth cycle

## INTRODUCTION

There are a large number of signal molecules involved in the regulation of the hair cycle and regeneration of hair follicle (HF). Those include genes, growth factors, nuclear receptors, cytokines, and subcellular signaling pathways.<sup>[1]</sup> Growth factors that control the HF development and growth cycle are epidermal growth factor (EGF), transforming growth factor (TGF), keratinocyte growth factor (KGF), and vascular endothelial growth factor (VEGF).<sup>[2]</sup> Some of these growth factors responsible for anagen phase initiation (KGF& VEGF), others suppress growth and differentiation of HF in telogen and catagen phases (TGF  $\beta$ , EGF). Thus, growth factors can be key molecules that participate in the initiation and suppression of normal hair growth cycle.<sup>[3]</sup>

Although hair growth factors are important for HF physiological growth, its dysregulation may have a role in hair disorders. The roles of EGF and EGFR in the pathogenesis of alopecia areata (AA) are unknown. However, reports have suggested an association between EGF and its receptor (EGFR) signaling and AA.<sup>[4]</sup> The

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pathogenesis of AA is incompletely understood, though it is believed to result, at least in part, from a loss of immune privilege in the HF, autoimmune HF destruction, and upregulation of inflammatory pathways.<sup>[5]</sup> We can clarify the other face in AA pathogenesis is the dysregulation of hair growth factors as some are well approved as TGF  $\beta$ 1 and others not well known and need more work as EGF.

In AA, hair shedding occurs even before the anagen starts leaving the HF empty (kenogen). Thus, AA is generally a disorder of hair cycling and is considered to be a persistent state of kenogen.<sup>[6]</sup> However, the exact genes involved in hair loss are not clearly known, and some of the proposed genes responsible for hair growth are desmoglein, activin, EGF, fibroblast growth factor, lymphoid enhancer-binding factor-1, and sonic hedgehog.<sup>[7]</sup>

The EGF promotes the growth of the outer root sheath (ORS) compartment of mink HFs and enhances the proliferation and migration of ORS cells through the activation of the Wnt/ $\beta$ -catenin signaling pathway.<sup>[8]</sup> However, with the completion of follicular growth, normal expression of EGF and EGFR in ORS is downregulated, and continuous expression of EGF in HFs of transgenic mice arrested follicular growth and development at the final stage of morphogenesis.<sup>[9]</sup>

The polymorphism of EGF was correlated with the pathogenesis of AA as it caused the compensatory expression of EGF. The elevation of EGF inhibits the induction of anagen phase and decreases hair shaft elongation. The polymorphism of EGFR may cause functional deceleration of protein-like EGFR inhibitor and thus modulate the expression of immune molecules.<sup>[4]</sup> EGFR inhibitors lead to the disorganized formation of the HF and manifest as abnormalities such as finer, curlier, and more brittle hair and both scarring and nonscarring alopecia.<sup>[10]</sup> Hence, elevated EGF as a compensatory effect of receptor polymorphism, block, inhibition, or increased competitors as TGF may be involved in AA pathogenesis through its direct effect on HF, nonfunctioning hair growth factor, or through immunological mechanisms.

## SUBJECTS AND METHODS

### Study population

This case–control study included 60 clinically diagnosed patients with AA with different variants and severities and 25 age- and sex-matched healthy controls. Patients were selected from the outpatient clinic of the Dermatology

Department. Written informed consent was taken before the start of the study, which is approved by the Ethics Committee for Human Research. This protocol of research work was in accordance with the Helsinki Declaration of Human Rights 1975.

Patients with scarring and other nonscarring alopecia and patients suffering from thyroid diseases, autoimmune diseases, infectious diseases, tumors, and hyperandrogenism were excluded. Each patient was subjected to complete history taking, general examination, and clinical assessment of AA lesions to assess site, number, size, and severity. The severity of AA was assessed by the Severity of Alopecia Tool score.<sup>[11]</sup>

### Blood samples and quantification of epidermal growth factor

Blood samples were collected in complete aseptic condition by venipuncture, and then, blood was left to clot and then centrifuged for 15 min at 5000 rpm. The sera were separated and stored at  $-20^{\circ}\text{C}$  until the time of the assay. This ELISA kit uses a double-antibody sandwich ELISA to assay the level of EGF in samples, from Sun Red Biotechnology Company, made in Shanghai, China, Catalog no. 201-12-0145.

### Statistical analysis

All data were collected, tabulated, and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA, 2011). Quantitative data were expressed as the mean  $\pm$  standard deviation and minimum–maximum, and qualitative data were expressed as absolute frequencies (number) and relative frequencies (percentage). Continuous data were checked for normality using Shapiro–Wilk test. Mann–Whitney *U*-test was used for comparing two groups of not normally distributed variables. Kruskal–Wallis test was used to compare between more than two groups of nonnormally distributed variables. The percentage of categorical variables was compared using Chi-square test. Logistic regression analysis was used to determine the predictors of AA. All tests were two-sided.  $P < 0.05$  was considered statistically significant and  $P \geq 0.05$  was considered statistically insignificant.

## RESULTS

We found that the mean serum EGF was statistically significantly higher in patients ( $57.3 \pm 64.8$ ) than that of controls ( $18.9 \pm 16$ ) ( $P < 0.0003$ ) [Table 1].

The EGF level was higher in patients with disease duration >1 month (67.5 ± 74) than patients with disease duration ≤1 month (47 ± 53.5). Furthermore, EGF was lower in patients with previous treatment (52 ± 65) than patients with no previous treatment (60.8 ± 65), and it was higher in patients with no response to previous treatment (90.7 ± 98.3) than patients who gave a response to treatment (32.7 ± 28); it was higher in patients with high recurrence (68.4 ± 76.6) than patients without recurrence (50.8 ± 57) ( $P > 0.05$ ) [Table 2].

The EGF in patients of severe AA was statistically significantly higher than moderate AA patients, and moderate cases were higher than mild AA patients ( $P = 0.0001$ ) [Table 3]. There was a statistically significant difference of EGF level regarding the type of lesions. The highest level of mean EGF level in Alopecia universalis followed by Alopecia totalis then higher number of lesions, the difference was statistically significant ( $P = 0.003$ ). Furthermore, the level of EGF ng/ml was different with scalp involvement; the highest level in S4 hair loss was 221.8 ± 16.8 ng/ml. Then, S3 the EGF level was 113.4 ± 117.2 ng/ml, followed by different percentage of hair loss,

the difference was statistically significant ( $P = 0.007$ ). The EGF level of B0 was 60.0 ± 67.7 and B1 32.7 ± 24.7 with statistically insignificant difference ( $P = 0.27$ ) [Table 4].

## DISCUSSION

The results of this study showed that the serum level of EGF was significantly higher in patients with AA compared to healthy controls ( $P < 0.0003$ ). This was in agreement with a study conducted by Wilkening *et al.*<sup>[4]</sup> which suggested that EGF and EGFR could be associated with the pathogenesis of AA. As studied the association between EGF and EGFR gene polymorphisms and susceptibility to AA in the Korean population, their results were one SNP (rs11569017) of EGF which showed a significant difference between the AA group and the control group.

The effects of EGF on the homeostasis of the HF were matter of great debates. This study showed that EGF had a negative effect on HF growth and may be linked to AA pathogenesis. This may be explained as the EGF binds to ErbB1 and participates in regulating DNA synthesis rate of ORS cells and hair bulb cells of HF, so that the HFs enter regression period in advance from growth period.<sup>[12]</sup> In the early stages of HF growth, EGF and/or TGF- $\alpha$  can inhibit HF formation, which are greatly related with ErbB1.<sup>[13]</sup> In human HF culture, EGF and EGFR showed a capacity for inhibiting hair shaft elongation and changing the morphology to catagen growth pattern by suppressing mitotic regulators including Rcc2 and Stathmin 1.<sup>[14,15]</sup> The EGF receptor mediates the termination of the anagen stage.<sup>[10]</sup> The EGF injection in sheepskin produced hair

**Table 1: Comparison between alopecia areata patients and controls as regards epidermal growth factor level**

Item	Studied groups		MW	P
	AA patients (n=60)	Controls (n=25)		
EGF				
Mean±SD	57.3±64.8	18.9±16	4.3	0.0003
Minimum-maximum	1.8-232.1	0.18-77.1		(S)

AA – Alopecia areata; EGF – Epidermal growth factor; MW – Mann-Whitney U-test; S – Significant; SD – Standard deviation

**Table 2: Comparison between alopecia areata patients' epidermal growth factor level as regards duration, previous treatment, previous response, and recurrence**

Items	n (%)	EGF		MW	P
		Mean±SD	Minimum-maximum		
Disease duration (month)					
≤1	30 (50)	47±53.5	10.7-200.8	1.1	0.28 (NS)
>1	30 (50)	67.5±74	1.8-232.1		
Previous treatment					
Yes	24 (40)	52±65	10.7-232.1	1.5	0.14 (NS)
No	36 (60)	60.8±65	15-225.8		
Response to previous treatment					
Yes	16 (66.7)	32.7±28	10.7-127.4	0.7	0.49 (NS)
No	8 (33.3)	90.7±98.3	12.3-232.1		
Recurrence					
Yes	22 (36.7)	68.4±76.6	1.8-220.6	0.1	0.9 (NS)
No	38 (63.3)	50.8±57	12.6-225.8		

EGF – Epidermal growth factor; MW – Mann-Whitney U-test; S – Significant; SD – Standard deviation; NS – Insignificant

loss and epidermal thickening.<sup>[16]</sup> EGF, which produces hyperproliferation of the basal cell layers, causes arrest of the HF development.<sup>[17]</sup> Philpott and Kealey<sup>[18]</sup> studied the effect of EGF on cells of ORS of isolated human HFs and suggested that EGF may play a role in transition from anagen to catagen. Furthermore, Hansen *et al.*<sup>[19]</sup> suggested that the EGF receptor mediates the termination of the anagen stage.

By indirect way, trichomegaly was coincidence during treatment with the EGFR inhibitors cetuximab, erlotinib, and gefitinib. The sustained increases in hair growth suggested that decreased EFG can stimulate HF growth.<sup>[20]</sup>

It was also found that men with androgenic alopecia had a reduction in VEGF and the increase in EGF and TGF-β1 growth factors. This abnormality in the expression of growth factors is combined with the reduction in the total amount of hairs in the parietal region, change of terminal and vellus hair percentage ratio in the parietal region toward the increase of vellus hair, and violation of normal ratio of anagen and telogen hairs toward the increase of hairs that are in telogen.<sup>[2]</sup> However, Kubanov *et al.*<sup>[3]</sup> studied the expression of growth factors (VEGF, KGF, EGF, and TGF-β1) in the HF of patients with telogen effluvium and showed that EGF expression was equivalent in these patients and healthy women and did not differ statistically.

**Table 3: Comparison between alopecia areata patients epidermal growth factor level as regards severity of the lesion**

Severity	n (%)	EGF		KW	P
		Mean±SD	Minimum-maximum		
Mild	33 (55)	28.1±16.7	1.80-82	23	0.0001 (S)
Moderate	17 (28.3)	38.5±26.5	10.7-127.4		
Severe	10 (16.7)	185.6±56.3	30.6-232.1		

KW – Kruskal-Wallis test; SD – Standard deviation; EGF – Epidermal growth factor

**Table 4: Serum epidermal growth factor and severity of alopecia tool score**

	n (%)	EGF		KW	P
		Mean±SD	Minimum-maximum		
Number of patches					
1	31 (51.7)	31.24±30.7	10.70-187.3	23.23	0.003
2	9 (15.0)	55.18±58.93	1.80-161		
3	3 (5.0)	63.43±75.18	14.40-150		
4	3 (5.0)	66.50±52.86	32.50-127.4		
5	4 (6.7)	53.62±27.4	34.5-165		
6	4 (6.7)	80.10±57.98	15.80-80		
7	3 (5.0)	164.66±32.5	132-197		
Totalis	2 (3.3)	159.5±48.7	125-194		
Universalis	1 (1.6)	232.1	232.1		
Scalp involvement (S)%					
S0 (no hair loss)	8 (13.3)	31±26.4	1.8-82	14.1	0.007
S1 (<25% hair loss)	41 (68.3)	46.6±53	10.7-200.8		
S2 (25%-49% hair loss)	5 (8.3)	38.2±11.7	25.9-56.7		
S3 (50%-74% hair loss)	2 (3.3)	113.4±117.2	30.6-196.3		
S4 (75%-99% hair loss)	4 (6.7)	221.8±16.8	197-232.1		
S5 (totalis and universalis)	0	0.0	0.0		
Nail involvement (N)%					
No (no nail involvement)	54 (90)	45.2±52	1.8-200.8	MW=2.6	0.009
N1 (some nail involve)	6 (10)	165.8±71	20.8-232.1		
Body involvement (B)%					
B0 (no body hair loss)	48 (80.0)	60.±67.7	10.7-232.1	MW=1.1	0.27
B1 (some body hair loss)	11 (18.3)	32.7±24.7	1.8-82		
B2 (100% body hair loss)	1# (1.7)	232.1	232.1-232.1		
Total involvement					
SoBn	8 (13.3)	41.95±58.99	1.80-182	3.23	0.199
SnBo	48 (80)	78.23±123.97	10.70-800		
SnBn	4 (6.7)	74.30±105.36	17.50-232.10		

#Excluded from relation (n=1). KW – Kruskal-Wallis test; MW – Mann-Whitney U-test; EGF – Epidermal growth factor; SD – Standard deviation

Against this study, the EGFR downregulation has been linked with HF formation.<sup>[21,22]</sup> The use of EGFR inhibitors can cause skin inflammation and exacerbation of autoimmune diseases, and these immune-related effects of EGFR inhibitors are due to their direct effects on the expression of the major histocompatibility complex Class I and/or Class II molecules.<sup>[23]</sup> The roles of the EGF family ligands and receptors, as well as their interplay and physiological phases, have the potential to produce a revolution in the management of hair loss. The availability of topical EGFR blockers and the development of more specific molecules that will stimulate the hair growth pathways will build on the fact that EGFR blockade can produce long-term hair growth. Continuous expression of EGF, or TGF  $\alpha$ , although producing a wavy phenotype, impedes the growth of hair. However cyclic variations in the level of EGFR, may result in hair growth and produce new hair formation.<sup>[12]</sup>

The highest level of mean EGF level in alopecia universalis followed by alopecia totalis then increased number of lesions, the difference statistically significant ( $P = 0.003$ ). The highest level in S4 hair loss was  $221.8 \pm 16.8$  ng/ml. Highest serum level of EGF marker in S4 (75%-99%) hair loss  $221.8 \pm 16.8$  ng/ml then S3 (50%-74% hair loss) was  $113.4 \pm 117.2$  ng/ml, illustrated in table (4) followed by different percentage of hair loss, difference was statistically significant ( $P = 0.000$ ). The EGF level of B0 was  $60.0 \pm 67.7$  and B1  $32.7 \pm 24.7$  with a statistically insignificant difference ( $P = 0.27$ ), which disagrees with a study done by Wilkening *et al.*<sup>[4]</sup> who showed no significant difference associated with any of the SNPs of EGF in patchy or totalis alopecia, but one SNP (rs17337023) of EGFR showed significant differences between patchy-type AA and alopecia totalis ( $P < 0.05$ ).

## CONCLUSIONS

The normal level of EGF seems to modulate the natural physiological growth of the HF. However, increased EGF signaling intensity produces a reversed effect on HF growth. Elevated growth factors as EGF not always a good sign for hair growth and functioning promotor inducing hair recovery, but it may be linked to the pathogenesis of hair disorders as AA. EGF may be used as a marker of severity, prognosis, and recurrence.

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This was the authors' own work. Laboratory investigations were done in clinical pathology laboratory.

## Conflicts of interest

There are no conflicts of interest.

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