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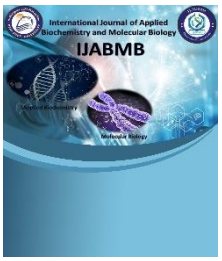

Serum Epidermal Growth Factor Level and its Relation to Severity of Alopecia Areata

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Abstract:

Background: Epidermal growth factor (EGF) is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to epidermal growth factor receptor (EGFR). The roles of EGF and EGFR on the pathogenesis of alopecia areata (AA) are uncertain. However, several reports suggest the association between EGF signaling and AA.

Objective: To estimate the serum level of EGF in patients with Alopecia Areata and its correlation with disease severity.

Patients and Methods: This is a case control study, was conducted at the outpatient clinic in Beni-Suef University hospital, on Sixty persons divided into 2 groups: (Group A): included 30 patients having alopecia areata, (Group B): included 30 apparently healthy age- and sex-matched individuals as controls, from January 2020 to July 2022.

Results: There was strong correlation between EGF and SALT score with high significance

Conclusion: The EGF is significantly elevated in AA patients when compared to the control subjects. It is also positively correlated with the number and the extent of the disease, making it a possible marker of AA severity. High levels of EGF are present in patients with AA and positively correlate with severity, which may indicate a causal role of EGF in pathogenesis of AA.

Keywords: Alopecia Areata, EGF

Introduction:

Alopecia areata (AA) is a chronic inflammatory non-scarring condition affecting the hair follicle (HF) that leads to hair loss ranging from small well-defined patches to complete loss of all body hair. The main theory of AA pathogenesis is that it is an autoimmune phenomenon resulting from a disruption in HF (1). Alopecia areata may be the second-most incessant non-scarring alopecia following male What's more female design alopecia. Clinical designs for hair passing on alopecia areata need aid Typically precise unique (2). The trigger element influences the methodology of keratinocytes division (3). It is known that there may be an expansive number for sign particles included in regulation of a hair cycle and recovery of a follicle. The individuals incorporate specific genes, a portion sorts for Growth factors, atomic receptors, cytokines, subcellular indicating pathways (4).

A few development Components show up to control the advancement and the cycle of a hair follicle for example, epidermal Growth component (EGF), transforming Growth element (TGF), keratinocyte development element (KGF), vascular endothelial development (VEGF) (5). Epidermal Growth component relatives initiate intracellular indicating course upon tying should their receptors, all things considered known as those ErbB crew. The ErbB gang comprises ErbB1 (or EGFR), ErbB2, ErbB3 what's more ErbB4 (6). Epidermal Growth figure (EGF) may be a Growth component that assumes a paramount part in the regulation from claiming cell growth, proliferation (7). So, this study aimed to estimate the serum level of EGF in patients with Alopecia Areata and its correlation with disease severity.

Patients and methods:

This case control study was conducted from Dermatology outpatient clinic in Beni Suef University hospital from January 2020 to July 2022. An informed verbal consent

from the participants was taken and confidentiality of information was assured. An official written administrative permission letter was obtained from dean of faculty of medicine, Beni-Suef University hospital manager, Head of dermatology and venereology department in the same university. The title and objectives of the study were explained to them to ensure their cooperation. Ethical committee permission from the faculty of medicine ethical committee was also obtained with approval No: FMBSUREC/08032020/El Metwally

Sixty persons were recruited from Dermatology outpatient clinic. Group A: Comprising 30 patients having alopecia areata. Diagnosis of alopecia areata was confirmed based on the typical clinical picture of alopecia. Group B: Comprising 30 apparently healthy age- and sex-matched individuals as controls, giving no personal or family history of alopecia. Control subjects were recruited from among medical students, health care personnel and patients presenting at the dermatological outpatient clinic. They were selected not to have any autoimmune illness and on no interfering medications.

Inclusion criteria included all patients with alopecia areata regardless of their age and sex. Patients were selected by confirmed diagnoses of AA given by a certified dermatologist, healthy control group were age and sex matched with our patients.

Exclusion criteria were Patients with alopecia areata on systemic treatment within 2 months or patients on topical treatment within 2 weeks before the start of the study, Patients with scarring and other non-scarring alopecia, Patients suffering from thyroid diseases., Patients suffering from autoimmune diseases, Patients suffering from infectious diseases, Patients suffering from cutaneous tumors, and Patients suffering from hyperandrogenism.

Full history was taken with stressing on drug intake, systemic illness, or weight loss. Further, a family history of hair loss is characteristic in androgenetic alopecia and alopecia areata. Laboratory tests should evaluate iron and vitamin D levels and thyroid function, Complete general examination was done. Clinical evaluation should establish if hair density is normal or decreased and if the scalp shows diffuse or patchy alopecia. The severity of hair shedding should be assessed by a pull test. The pull test begins with gentle traction on a group of hairs (approximately 40–60) on three separate areas of the scalp. The hairs extracted by this gentle traction are examined microscopically and quantified. A normal or negative pull test extracts three or fewer hairs and a positive pull test extracts six or more hairs from a single area. Microscopically, the hair roots should be identified as being in the telogen or anagen phase and checked for dystrophy. The telogen roots, which have a club-shaped bulb and an absence of an inner root sheath, are the most seen in a variety of hair disorders. Dystrophic, broken hairs are seen in diseases that interrupt the mitotic activity of anagen follicles, including alopecia areata and systemic chemotherapy. Anagen roots, which are darkly pigmented and triangular or delta-shaped, are typically seen in lymphocytic cicatricial alopecia.

Clinical assessment of the patients by alopecia areata (SALT) score; Severity of Alopecia Tool Score. In which the scalp surface was divided into four quadrants. Hair loss in each quadrant was scored based on the percentage of hair lost and clinical findings. SALT score is used to determine the quantitative assessment hair loss of the scalp, where the scalp is divided into 4 parts, top (40% - 0.4), posterior (24% - 0.24), right side (18% - 0.18), and left side (18% - 0.18). The hair loss percentage is determined independently in each area and is multiplied by the percentage of scalp covered in that area of the scalp, and adding together the products of each area will give the SALT score. SALT score is easily calculated and validated. But it does not include hair pigmentation, body hair, and nail involvement (8).

The kit which was used in this study is MBS2709586 96 Wide-range Enzyme-linked Immunosorbent Assay Kit for Epidermal Growth Factor (EGF). The microplate provided in this kit has been pre-coated with an antibody specific to EGF. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to EGF. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain EGF, biotin-conjugated antibody and enzyme-conjugated Avidin exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of EGF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

All reagents were prepared, samples and standards; 100µL standard or sample was added to each well and incubated for 1 hour at 37oC; 100µL prepared Detection Reagent A was aspirated and added. Then was incubated for 1 hour at 37oC; then aspirated and washed 3 times; 100µL prepared Detection Reagent B was added and incubated for 30 minutes at 37oC, then was aspirated and washed 5 times; 90µL Substrate Solution was added and incubated for 10-20 minutes at 37oC; 50µL Stop Solution was added and read at 450nm immediately.

Statistical analysis

Analysis of data was done using Statistical Program for Social Science version 20 (SPSSInc, Chicago, IL, USA). Quantitative variables were described in the form of mean and standard deviation. Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student t test was performed. Qualitative variables were compared using chi-square (X2) test or Fisher's exact test when frequencies were below five.

Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, A P value < 0.05 is considered significant.

Results:

There was no statistically significant difference between the studied groups as regard Demographic data (Table 1).

Table 1: Comparison between studied groups as regard demographic data

	Cases (n = 30)		**Control (n = 30)**		**Test of sig**	**p**
Age (years)						
Min. – Max.	6 – 50		6 – 51		t= 0.112	0.911
Mean ± SD.	30.03 ± 13.79		30.43 ± 13.86			
Sex	**No.**	**%**	**No.**	**%**		
Male	22	73.3	20	66.7	χ^2= 0.317	0.573
Female	8	26.7	10	33.3		

*χ^2: **Chi square test** **t: student-t test***

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

Among the cases there were 5 (16.7%) with positive family history of alopecia, 17 (56.7%) with disease duration less than 1 month and 13 (43.3%) more than 1 month, 10 (33.3%) who previously used treatment among them there were 7 (70%) with positive response to that treatment and the mean SALT score was 51.73 (±24.07 SD) with range (7-89) %. As shown in Table 2.

Table 2: Distribution of studied cases according to alopecia characteristics

	Cases (n = 30)	
Family history	No.	%
No	25	83.3
Yes	5	16.7
Disease duration		
<1 month	17	56.7
>1 month	13	43.3
Previous treatment		
No	20	66.7
Yes	10	33.3
Response to previous treatment		
No	3	30.0
Yes	7	70.0
SALT score (%)		
Min. – Max.	7 – 89	
Mean ± SD.	51.73 ± 24.07	

There was statistically significant difference between the studied groups as regard EGF (Table 3), (Figure 1).

Table 3: Comparison between studied groups as regard EGF

	Cases (n = 30)	**Control (n = 30)**	**Test of sig**	**p**
EGF (ng/ml)				
Min. – Max.	78.1 – 941.9	94.8 – 283.2	U= 207.0	<0.001*
Mean ± SD.	362.02 ± 227.68	178.16 ± 59.08		
Median (IQR)	315.85 (185.58 – 469.6)	179.4 (130.9 – 209.43)		

U: Man-Whitney test

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

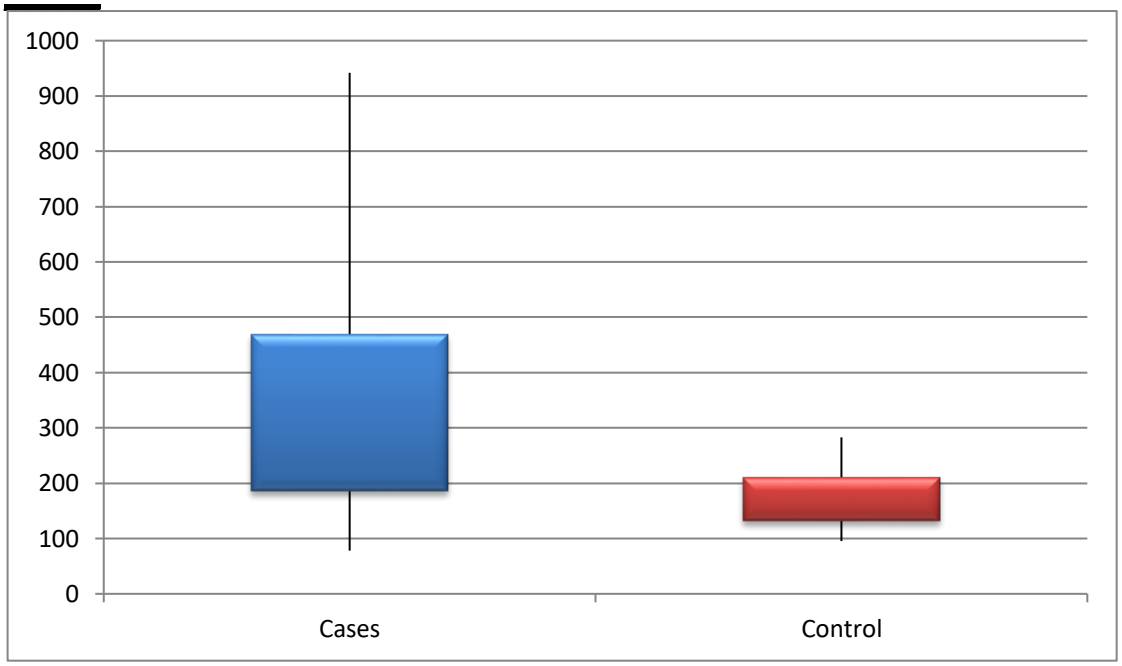

Figure 1: Comparison between studied groups as regard EGF

Table 4 showed no statistical relation between EGF and demographic data. Table 5 showed no statistical relation between EGF and alopecia history. There was significant positive correlation between EGF and SALT score with significance (Table 6), (Figure 2).

Table 4: Relation between EGF and demographic data in cases group

	EGF	**Test of sig**	**p**
Age			
<10 (n=3)	428.9 (285.8 – 482.9)	U= 37.0	0.845
>10.(n=27)	301.8 (189.75 – 468.7)		
Sex			
Male	265 (165.9 – 469.6)	U= 61.0	0.219
Female	391.4 (325.25 – 454.73)		

Data expressed as Median (IQR)

U: Mann-Whitney test

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

Table 5: Relation between EGF and alopecia history

	EGF	Test of sig	p
Family history			
No (n=25)	329.9 (181.4 – 470.5)	U= 58.0	0.829
Yes (n=5)	255.4 (204.2 – 466.9)		
Disease duration			
<1 month (n=17)	329.9 (204.2 – 448.4)	U= 108.0	0.934
>1 month (n=13)	274.6 (181.4 – 473.7)		
Previous treatment			
No (n=20)	341.25 (198.5 – 471.3)	U= 84.0	0.502
Yes (n=10)	233.55 (172.23 – 457.08)		

Data expressed as Median (IQR)

U: Mann-Whitney test

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

Table 6: Correlation between EGF and SALT score

	EGF	
SALT score	**r**	**p**
	0.825	**<0.001***

r: Pearson correlation

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

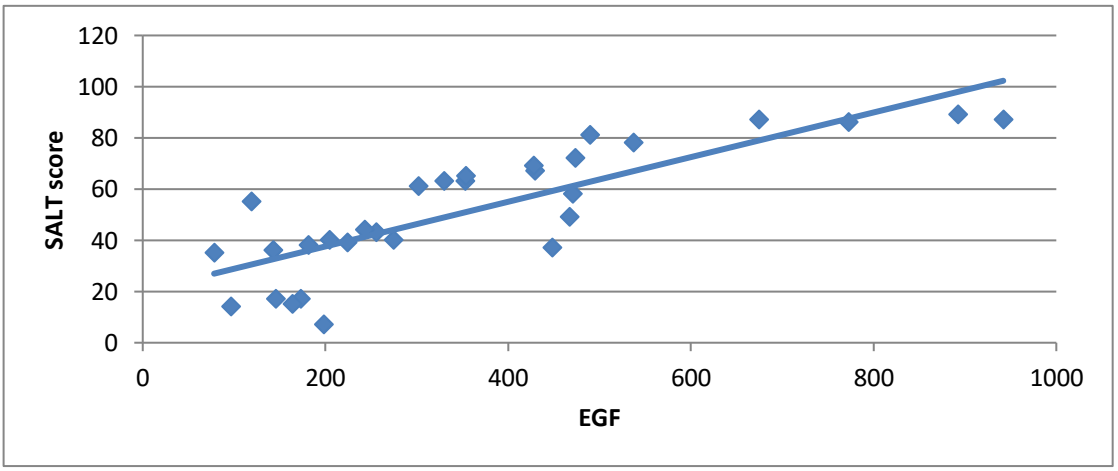

Figure 2: Correlation between EGF and SALT score

Discussion:

This study aimed to estimate the serum level of EGF in patients with Alopecia Areata and its relationship with disease severity. To obtain this aim, 30 patients with alopecia areata (AA) were recruited from outpatient clinic of the dermatology department of Beni-Suef University Hospital (Group A) and were compared with 30 apparently healthy age- and sex-matched individuals with no personal or family history of alopecia as controls (Group B). Control subjects were recruited from among medical students, health care personnel and patients presenting not known to have any autoimmune illness and on no interfering medications.

Patients on treatment, patients with cutaneous tumors, patients with other autoimmune diseases or other types of alopecia were excluded from the current study. All patients in the current study underwent full history taking, complete general examination, dermatological examination and clinical assessment of the patients by alopecia areata (SALT) score, and estimation of serum level of human epidermal growth factor using ELISA KITS.

In the present study we found that among the cases in group A there were 22 (73.3%) males and 8 (26.7%) females with mean age 30.03 (±13.79 SD) and range (6-50) years. Among the controls in group B there were 20 (66.7%) males, 10 (33.3%) females with mean age 30.43 (±13.86 SD) and range (6-51) years.

There was no statistically significant difference between the studied groups as regard Demographic data. In agreement with our results, El-Refaey et al. showed that there was no statistically significant difference between the studied groups as regard age and sex (2). Chu et al. showed that the percentage of females was higher in the AA group (P <. 01) (9). Bitan et al. showed that the average age in both AA patients and controls was 39.97 ± 13.61, with both groups showing a 62.9% male predominance (10).

There was no statistically significant difference between the studied groups as regard age and sex. El-Refaey et al. showed that alopecia areata patients were 38 males (63.3%) and 22 females (36.7%) with mean age was 27 years old with mean SD (27±12) years. Twenty-five healthy subjects served as control. There were 20 males (80%) and 5 females (20%). Their mean age was 30 years old with mean SD (30±10). There were no statistically significant differences between patients and control groups as regard to age and sex with ($p \geq 0.05$) (11).

According to the present study, EGF was found to be significantly higher among patients with AA when compared to controls (362.02 ± 227.68 vs 178.16 ± 59.08; P<0.001*). In agreement with our study, the mean serum EGF was statistically significantly higher in patients with AA than that of controls in El-Refaey et al.; El-Refaey et al. studies (2; 12). In contrast with our study, Brown et al. found that the EGFR downregulation has been linked with HF formation (13). 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 (14).

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 α, 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 (15).

Although the etiopathogenesis of AA is poorly understood, most research thinks that it relates to immune processes (Olsen et al.) (8). Several growth factors appear to control the development and cycle of hair follicle such as epidermal growth factor (EGF), transforming growth factor (TGB), keratinocyte growth factor (KGF), vascular endothelial factor (VEGF) (Kubanov and Yu) (16). Those EGF camwood Push development of the ORS compartment from claiming mink hair follicles done vitro and improve burgeoning Also movement of the ORS units by means of actuation of the Wnt/β-catenin indicating pathway (El-Refaey et al.) (2). The parts of EGF and EGFR in the pathogenesis from claiming AA are obscure. However, a few reports bring recommended cooperation between EGF indicating and AA (Won et al.) (17).

In the present study, EGF had no significant correlation with any of patients' age. In their study on healthy individuals, Meybosch et al. found that serum EGF was inversely correlated with age ($r = —0.873$; $P<0.001$) (18).

In the current study, EGF was lower in patients with previous treatment when compared to patients with no previous treatment. Yet, the difference was significant. This was coincident with results obtained by El-Refai et al., EGF was lower in patients with previous treatment (52 ± 65) than patients with no previous treatment (60.8 ± 65). Yet, the difference was not significant ($P > 0.05$) (2).

In the present study, EGF decreased with prolonged disease duration more than 1 month. Yet, the difference was not significant. This disagreed with results obtained by El-Refai et al., higher EGF level was reported in AA patients with disease duration >1 month (67.5 ± 74) than in patients with disease duration ≤1 month (47 ± 53.5). Yet, the difference was not significant ($P > 0.05$) (2).

In the present study, EGF had no significant correlation with gender. In agreement, (El-Refai et al.; El-Refaey et al.) reported no significant difference between males and females regarding EGF serum level among AA patients (2; 12). Moreover, Kubanov et al. 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 (19). On the contrary, Kubanov et al. study, men with androgenic alopecia had an increase in EGF growth factor. This abnormality was 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 (19).

In the current study, EGF was found to be positively correlated with SALT score ($r=0.825$, $P<0.001$*). In agreement with our finding, El-Refai et al. reported that EGF in patients of severe AA was statistically significantly higher than moderate AA patients, and moderate cases were higher than mild AA patients ($P = 0.0001$) (2). El-Refai et al. also found that the highest level of mean EGF level in Alopecia universals followed by Alopecia totalis then significantly higher number of lesions ($P = 0.003$) (2). Dias et al. showed that SALT score of 1 (S1) was seen in 64.2% of patients, SALT score of 2 in 10.4%, and 3,4,5 was seen in 9%, 4.5% and 11.9%. There was a significant relationship between vellus hair and lower SALT score. The study did not find any significant association between other trichoscopic features and SALT score (20).

Conclusion: EGF is significantly elevated in AA patients when compared to the control subjects. It is also positively correlated with the number and the extent of the disease, making it a possible marker of AA severity. High levels of EGF are present in patients with AA and positively correlate with severity, which may indicate a causal role of EGF in pathogenesis of AA.

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