Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2021.68379.2176 Volume 30, Issue 1.3, April 2024, Supplement Issue
Manuscript ID ZUMJ-2103-2176 (R4)
DOI 10.21608/ZUMJ.2021.68379.2176
ORIGINAL ARTICLE

Association between Interleukin-19 Concentration and Degree of Severity of Acne Vulgaris

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Submit Date 27-05-2021
Revise Date 13-07-2021
Accept Date 2021-07-28

ABSTRACT

Background: Acne vulgaris is a multifactorial disease associated with pilosebaceous follicle and results in inflammatory and non-inflammatory lesions. Interleukin-19 was identified for the first time in 2000 by analysis of genomic sequences for IL-10 homologues, the genetic location for IL-19 on human chromosome 1q32, and is closely linked to IL-10 as part of a gene cluster with IL-20 and IL-24**.** The study aimed to measure serum IL-19 levels in different degrees of severity of acne vulgaris patients for better understanding of its role in the etiopathogenesis of acne, and to prove if there is a possible correlation between serum Interleukin 19 level and the degree of severity of the acne. **Methods:** Our study was a case control study done at out-patient clinic of Dermatology, Venereology and Andrology Department at Zagazig University Hospitals. The study included 48 individuals divided into 2 groups: Patient group: 36 patients with acne vulgaris (12 mild, 12 moderate, 12 severe) Control group: 12 healthy persons. All subjects in this study were subjected to history taking and assessment of the severity of acne using acne staging and laboratory investigation (IL-19). **Results:** There was statistically significant difference in serum IL-19 between the different grades of the disease severity. Analysis revealed significant difference in IL-19 serum concentration between (mild and moderate group) and (moderate and severe group), While highly significant difference was found between IL-19 serum concentration of mild cases group and severe cases group. Serum IL-19 level was not statistically related to the age of onset, family history, gender, and previous treatment. **Conclusions:** From this study we could prove that IL-19 is related to the inflammatory etiopathogenesis of mild, moderate and severe acne vulgaris. Also, Serum IL-19 level has significant statistical difference between the different grades of acne vulgaris severity.
Key words: Interleukin-1; Acne Vulgaris severity; Evaluation

INTRODUCTION

Acne is a disease related to the pilosebaceous follicle and lead to both inflammatory and non-inflammatory lesions [1]. The American Academy of Dermatology (AAD) defines acne vulgaris as a chronic inflammatory dermatosis with open or closed comedones (blackheads and whiteheads) and inflammatory lesions, including papules, pustules, or nodules [2].

There are four pathological processes responsible for the development of acne which are cutibacterium acnes proliferation and colonization, increased sebum production, abnormal follicular hyperkeratinization, and inflammatory mechanisms (innate and acquired immunity) [3].

Inflammation has a central role in both inflammatory and non-inflammatory lesions in acne vulgaris. inflammation in acne is linked to propionibacterium acnes which stimulates the keratinocytes by Toll-like receptors (TLRs) stimulation to produce proinflammatory cytokines such as interleukin- 1 and other cytokines such as as IL-6, IL-8, IL-10, and IL-12 [4].

Cytokines are proteins secreted by different cells and affect many functions. They are important in pathological and physiological processes in multicellular organisms. Interleukins are cytokines

that serve to establish communication particularly between immune cells [5].

In 2000, Interleukin-19 was identified for the first time through analysis of sequences of genes for IL-10 homologues, the genetic location for IL-19 on human chromosome 1q32, and is closely linked to IL-10 as part of a gene cluster with IL-20 and IL-24 [6].

Interleukin 19 is a member of the IL-10 family of interleukins, with the prototypic members IL-10, IL-20, IL-22, IL- 24, IL-26, IL-28A, IL-28B and IL-29 [7].

Interleukin 19 is a cytokine secreted by epithelial cells in addition to proinflammatory stimulation [4].

However, Interleukin 19 relation to the pathogenesis of acne vulgaris remains unclear. There are many factors of proinflammatory cytokines contributed to acne vulgaris, but only the study of **Mochtar et al.,** was the first to study the relation between serum IL-19 and acne vulgaris. They reported similarly statistically significant difference between IL-19 serum concentration of mild acne vulgaris patients group and severe acne vulgaris patients group [4].

This study aimed to measure serum IL-19 levels in different degrees of severity of acne vulgaris patients for better understanding of its role in the etiopathogenesis of acne, and to prove if there is a possible correlation between serum Interleukin 19 level and the degree of severity of the acne.

METHODS

Our study was a case control study which was done at out-patient clinic of Dermatology, Venereology and Andrology Department at Zagazig University Hospitals.

Sample Size: assuming that mean ± SD of IL-19 concentration in mild acne vulgaris is 18.38 ± 9.59 versus 31.19 ± 20.36 in severe acne vulgaris so the sample size is 48 (12 in each group) using OPEN EPI at power 80% and C.I 95%.

Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Patients:

The study included 48 pateients divided into 2 groups: Patient group are 36 patients with acne vulgaris (12 mild, 12 moderate, 12 severe) and Control group are 12 healthy person were enrolled to assay serum level of Interleukin-19 using ELISA kits.

Ages included in the study were between 16 and 30 years. We included clinical cases diagnosed with acne vulgaris who were able to participate in the research, fill in a questionnaire and a statement of willingness and didn't take any systemic or topical treatment in the last two weeks. We excluded acne vulgaris patients undergoing systemic and topical treatment in the last two weeks and other conditions that IL-19 plays a role in their pathogenesis such as psoriasis, atopic dermatitis and asthma.

Methods:

Sample size was determined using unpaired numerical analytical test and informed consents were taken from patients and controls before starting the work.

All patients in this study were subjected to detailed history taking including personal history as name, age, and gender. Present history including onset, course, duration of acne and aggravating factors were assessed. Family history of acne vulgaris and previous treatment of acne or other drugs either topical or systemic in the last two weeks were considered as well. Past history of medical illnesses such as diabetes, hypertension, liver, or renal disease and general health as diet, special nutrition regimens or menstrual irregularities were assessed.

Clinical examination was done including general examination: blood pressure, pulse, heart rate, respiratory rate and temperature. Dermatologic examination including types of acne lesions, distribution, post inflammatory hyperpigmentation (PIH), active inflammatory lesions or scarring were also recorded.

Assessment of the severity of acne was done using acne staging by Lehmann et al. [8] as following; Mild acne: <20 comedones, <15 inflammatory lesions, or, total lesion count <30, Moderate acne: 20–100 comedones, 15–50 inflammatory lesions, or, total lesion count 30–125 and Severe acne: >5 pseudocysts, total comedo count >100, total inflammatory count >50, or total lesion count >125.

Laboratory examination was dine using five milliliters of venous blood sample collected from each subject in clot activator and gel containing tubes for serum separation for IL-19 serum concentration examination. The blood samples were centrifuged at 3000 rpm (CENTRIC 322A® centrifuge) for 15 minutes to separate the serum then stored at -20°C before assaying (Figure 1).

Human IL-19 immunoassay procedure which applied was done by using a double_antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Interleukin 19 (IL-19) in samples (Figure 1).

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Interleukin 19 (IL-19) in samples. Interleukin 19 was added (IL-19) to monoclonal antibody enzyme which is pre-coated with Human Interleukin 19(IL-19) monoclonal antibody, then, Interleukin 19(IL-19) antibodies labeled with biotin was added, and combined with Streptavidin-HRP to form immune complex; then carried out incubation and washing again to remove the uncombined enzyme. Then Chromogen Solution A, B was added and as a result the color of the liquid changed into the blue, and at the effect of acid, the color finally became yellow. The Chroma of color and the concentration of the Human Substance Interleukin 19(IL-19) of sample were positively correlated.

Assay procedure

All reagents were brought and prepared in room temperature before use as the number of strips required for the procedure was detected. The unused strips stored at 2-8°C, 50µl standard solution was added to standard well, 40µl sample was added to sample wells, 10µl anti-IL-19 antibody, then 50µl streptavidin-HRP was added and well mixed. The plate was covered with a sealer, then incubated for 60 minutes at 37°C.

The sealer was removed and the plate was washed 5 times by wash buffer, 50µl substrate solution were added to each well then 50µl substrate solution B were also added. The plate incubated was covered with a new sealer for 10 minutes at 37°C in the dark and 50µl Stop Solution was added to each well, the blue color was changed into yellow immediately.

The optical density (OD value) of each well was determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding stop solution.

Administrative design: Approval was obtained from Zagazig University Institutional Review Board (IRB).

Statistical analysis

Data were analyzed using IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for windows (NCSS LCC., Kaysville, UT, USA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

Analysis of variance ANOVA F test of significance was used when comparing between more than two means Pearson's correlation coefficient (r) test was used for correlating continuous data.Probability (P-value): P-value $\leq$0.05 was considered significant, P-value $\leq$0.001 was considered as highly significant and P-value >0.05 was considered insignificant.

RESULTS

- This table shows that there was no statistically significant difference among both studied groups as regarding age, gender and occupation **(Table 1)**. In addition, all studied acne vulgaris cases had face lesions, only 16.7% of them that had also chest or back lesions and 13.9% had arms lesions, 55.6% of them had positive family history and 52.8% received previous treatment **(Table 2)**. **Table (3)** shows that level of IL-19 was significantly increased among moderate and severe cases of acne vulgaris than mild cases.
- This table shows a statistical significant increase of serum IL-19 level among acne vulgaris cases with multiple site lesions than those having only one site lesion, while no significant difference regarding other variables **(Table 4)**.

Table 1: Demographic characteristics among both studied groups.

		Cases N=36		Controls N=12		t-test	P
Age\ years Mean ±SD Range		18.9 ± 2.91 16 - 25		19.9 ± 3.32 17 - 30		0.97	0.35 NS
		N	**%**	**N**	**%**	**X^2**	**P value**
Gender	**Male**	14	38.9	3	25.0	Fisher	0.38 NS
	Female	22	61.1	9	75.0		
Occupation	**Student**	28	77.8	9	75.0	3.68	0.159 NS
	Nurse	5	13.9	0	0.0		
	Housewife	3	8.3	3	25.0		

NS: P-value>0.05 is not significant

Table 2: Clinical data and disease history among studied cases:

	Cases N=36	
	N	**(%)**
Site of lesion: one site	26	(72.2%)
Many sites	10	(27.8%)
Site of involvement: Face	36	(100%)
Chest	6	(16.7%)
Back	6	(16.7%)
Arms	5	(13.9%)
Previous treatment	19	(52.8%)
Positive family history	20	(55.6%)

Table 3: Relation between studied biomarker and disease severity among studied acne vulgaris cases.

	Mild N=12	**Moderate N=12**	**Severe N=12**	**F test**	**P**
	Mean ±SD Range	**Mean ±SD Range**	**Mean ±SD Range**		
IL-19 (pg\ml)	162.6 ± 54.7 57.8 – 220	213.4 ± 8.45 200 – 220	258.7 ± 61.3 200 – 369.66	12.21	<0.001 HS

HS: P-value <0.001 is high significant

Table 4: Relation between studied biomarker and other clinical data of studied cases.

Variables		**N**	**Mean± SD IL-19**	**t-test MW***	**P value**
Received previous treatment	**Yes**	19	204.9 ± 66.4	0.69	0.49 NS
	No	17	219.1 ± 55.5		
Family history	**+ve**	20	213.1 ± 41.6	0.73*	0.46 NS
	-ve	16	209.6 ± 80.5		
Site of lesion	**One**	26	192.9 ± 49.98	3.37	0.002 S
	Multiple	10	260.2 ± 62.7		
Gender	**Male**	14	211.1 ± 39.1	0.043	0.966 NS
	Female	22	211.9 ± 72.5		

NS: P-value>0.05 is not significant, S: P-value <0.05 is significant

Fig. 1: The centrifuge device that used in our study (Centric 322A®, Slovenia)

DISCUSSION

Acne vulgaris is a multifactorial disease which is associated with pilosebaceous follicle and results in inflammatory and non-inflammatory lesions. Inflammation has a central role in both inflammatory and non-inflammatory lesions in acne vulgaris.

Our study proved, there is statistically significant difference in serum IL 19 between the different grades of acne vulgaris severity.

The mean serum level of IL19 (pg/mL) among mild, moderate and severe cases were 162.6 ± 54.7 and 213.4 ± 8.45 and 258.7 ± 61.3 respectively with increased significant difference among moderate and severe cases than mild cases.

That means that the increase in serum interleukin 19 levels was proportional to the increase in the severity of acne vulgaris with a statistically significant difference between the four study groups (control, mild, moderate, and severe acne patients groups).

These finding is in agreement with the finding of Mochtar et al., [4] that showed a significant difference between the IL-19 serum concentration of moderate acne vulgaris patients group and severe acne vulgaris patients group. While no significant difference was found between the concentration of IL-19 of mild acne vulgaris patients group and moderate acne vulgaris patients group. The mean of IL-19 serum concentration of group of patients with mild acne vulgaris is 18.38±9.59pg/ml, that of moderate acne vulgaris patients is 21.23 ± 11.99 pg/ml, and that of severe acne vulgaris patients is 31.19 ± 20.36 pg/ml, the mean of IL-19 serum concentration of all groups is 23.60 ± 15.51 pg/ml.

Kunz et al. [9] stated that IL-1β could induce expression of IL-19 in keratinocytes both in vitro and in vivo and was in agreement with Bao et al. [10] who found that the increase of proinflammatory IL-1β results in the release of IL-19 although the unknown signal of activation between IL-19 and IL-1β. These cytokines lead to inflammatory processes and tissue damage in acne vulgaris patients and may lead to an increase in incidence and severity of acne vulgaris. Such cytokines may relate and affect the acne vulgaris severity [11].

On the other hand, our results proved significant difference between serum concentration of IL-19 of moderate acne vulgaris patients group and severe acne vulgaris patients group, and significant difference between serum concentration of IL-19 of mild cases group and moderate cases group. While highly significant difference was found between IL-19 serum concentration of mild cases group and severe cases group.

So, these findings prove that inflammatory response has a central role in both inflammatory and non-inflammatory lesions of AV. The inflammation is related to P. acnes as a stimulating factor of inflammatory responses which affect the severity of Acne vulgaris through pathways including cytokines underlying the formation of papules, pustules, and nodulocystic acne. Therefore, IL-19 might potentially be related to the severity of acne vulgaris.

While Mochtar et al., [4] only found the difference in serum levels of IL-19 between moderate and severe acne vulgaris patient groups, our results showed the difference not only between different acne vulgaris patient groups, but also between control subjects and acne vulgaris patients.

Moreover, our mean IL-19 serum level was higher (in all study groups) than the level in Mochtar et al., [4] study. This may be related to racial differences or may be due to higher acne vulgaris severity in Egypt.

On the other hand, our study showed that, serum IL-19 level was not statistically related to neither the age of onset nor family history, gender, previous treatment. This indicates that IL-19 is contributed directly to the inflammatory process of acne and has no correlation with the change in demographical data of the subjects.

CONCLUSIONS

From this study we could prove that IL-19 is related to the inflammatory aetiopathogenesis of mild, moderate and severe acne vulgaris. Also, Serum IL-19 level had significant statistical difference between the different grades of acne vulgaris severity.

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To Cite:

Albalat, W., Elsaid, H., Ibrahim, H. Association between Interleukin-19 Concentration and Degree of Severity of Acne Vulgaris. *Zagazig University Medical Journal*, 2024; (232-237): -. doi: 10.21608/zumj.2021.68379.2176