Type of the Paper (Research article)

The Molecular Association of IL19 Gene Polymorphism in Acne Vulgaris Egyptian Patients: A Case-Control Study

Nesreen M. Aboraia[1], Samar El-Tahlawi[2], Dina Abdel Fatah[3], Alia A. Mohammed[1*]

[1]Department of Dermatology, STDs & Andrology, Faculty of Medicine, Fayoum University, Fayoum 63511, Egypt.

[2]Department of Dermatology, Faculty of Medicine, Cairo University, Giza, Egypt.

[3]Department of Medical Biochemistry & Molecular Biology Faculty of Medicine, Cairo University, Egypt.

Correspondence: Alia A. Mohammed, aa7582@fayoum.edu.eg, Tel: (002) 01096505417.

Received: 15 May, 2024 Reviewed: 1 June, 2024
Accepted: 1 July, 2024 Published online: 30 Sep, 2024

Abstract:

Introduction: Acne vulgaris is a widespread dermatological condition that affects the pilosebaceous unit and is characterized by chronic inflammation. In addition, its elongated course, the pattern of returns and relapse, and manifestations including acute outbreaks or slow progression classify it as a chronic disease. Furthermore, acne exerts numerous adverse psychological and social impacts that significantly diminish the quality of life experienced by affected cases.

Aim of the study: To discuss the potential association between the Interleukin 19 gene polymorphism and acne vulgaris in Egyptian patients.

Subjects and Methods: This research involved one hundred cases with acne and 100 normal individuals as a group control, enrolled from the Dermatology Clinic, Faculty of Medicine, Fayoum University, with acne vulgaris from 16 to 30 years old, not receiving treatment in the last two weeks or systemic treatment in the last month. Two ml of blood were collected from every case; two ml in EDTA tubes for genetic analysis via DNA extraction.

Results: The TT and TC genotypes significantly increase the risk of the occurrence of acne (p <0.001). The occurrence of allele T increases the risk of acne in comparison to allele C (p <0.001).

Conclusions: The occurrence of allele T increased the risk of acne in comparison to allele C.

Keywords: Acne Vulgaris; Interleukin 19; Allele T; Allele C; gene polymorphism.

1. Introduction

Acne vulgaris is a pathological condition caused by various factors affecting the pilosebaceous follicles, resulting in both inflammatory and non-inflammatory lesions, which are classified as open and closed comedones. Acne is caused by increased sebum production, inflammation, follicle hyperproliferation, and the presence of *Propionibacterium acnes* [1]. Inflammation is believed to be responsible for both inflammatory and non-inflammatory lesions, and it also contributes to varying degrees of scarring [2]. It is crucial to identify the underlying factors contributing to this disease, as it affects 85% of young adults between the ages of 12 and 25, according to the Global Burden of Disease (GBD) [3]. Hospital-based dermatology registries in the United States report that this condition affects around forty to fifty million individuals. More adolescent boys are affected by acne vulgaris than girls; conversely, among adults, the condition appears to impact a greater proportion of women compared to men [2].

The disease mechanism involves four processes that lead to the formation of acne lesions. The first process is the secretion of inflammatory mediators into the skin. Second, comedones form as a result of changes in keratinization. Third, these changes not only alter sebum production but also increase it along with androgen receptor sensitivity, which encourages *Propionibacterium* to further colonize the follicles [2]. Family and genetic studies involving heterozygous and homozygous twins have accumulated evidence supporting the concept that hereditary factors contribute to the development of acne [5]. The inflammation associated with this condition stimulates toll-like receptors on keratinocytes, leading to the production of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-10, and IL-12 [1].

A pro-inflammatory cytokine called IL-19 is produced by epithelial cells. Different types of cells, including keratinocytes, T cells, and monocytes, secrete IL-19 when activated, mainly by IL-1β. The primary function of IL-19 is to facilitate communication between inflammatory tissues in both hematopoietic and non-hematopoietic tissues. IL-19 promotes the formation of CD4+ T lymphocytes of the Th2 type and helps keratinocytes proliferate, along with IL-20 and IL-24. This is particularly significant

because both IL-19 and IL-20 bind to the IL-20 receptor, which also functions as the receptor for IL-19 [1, 6]. IL-19 is characterized by its ability to generate positive feedback loops to amplify its effects. Once stimulated during the inflammatory response, it will release cytokines in an ongoing process [1].

Due to its prolonged duration, tendency to recur and relapse, and symptoms such as severe eruptions or sluggish onset, acne is regarded as a chronic condition. Additionally, patients' quality of life is significantly negatively impacted by acne's psychological and social effects [7]. In this current study, we aim to determine whether variations in IL-19 serum concentrations affect acne vulgaris severity and investigate the potential correlation between IL-19 gene polymorphism and acne vulgaris in Egyptian cases.

2. Subjects & Methods

2.1. Subjects

The present case-control prospective research involved 100 cases who have acne and 100 normal individuals represented as a control group, enrolled from the Dermatology Clinic, Faculty of Medicine, Fayoum University, after obtaining notified consent from every case and control. The research was conducted over 12 months. Study protocol and written informed consent were approved by the Local Ethical Committee of Fayoum Faculty of Medicine. All patients (or legal guardians) signed a written informed consent before participation and after illustration of all study procedures and possible risks and benefits.

Inclusion and exclusion criteria

We included all patients between the ages of sixteen to thirty patients included are with any type of acne vulgaris. However, cases who received topical therapy within the previous week or systemic treatment in the last month were excluded. Also, cases that have additional medical conditions that are associated with elevated levels of IL-19 in their serum or skin lesions, including asthma, psoriasis, and atopic dermatitis were excluded.

2.2.Methods

The categorization of clinical manifestations of acne vulgaris is mild, moderate, or severe depending on the

presence of comedones, papules, pustules, nodules, and cysts [8].

Samples collection and DNA preparation

Two milliliters of intravenous blood were collected from each participant; 2 mL in EDTA tubes for DNA extraction and subsequent genetic analysis. Utilizing a nucleic acid extraction reagent (NucleoSpin®) acquired from Macherey-Nagel GmbH & Co. KG, Germany, DNA was obtained as follows:

A volume of 350 microliters (µL) of DA1 buffer and 3.5 µL of β-mercaptoethanol were mixed vigorously with the blood mononuclear cells. To clear the lysate and reduce viscosity, the mixture was filtered using a NucleoSpin® Filter (violet ring) placed in a 2 mL collection tube and centrifuged for 1 minute at 11,000 rpm. After obtaining the NucleoSpin® Filter, a volume of 300 µL of 70% ethanol was added to the homogenized lysate and mixed by pipetting up and down five times. The NucleoSpin® DNA Column (light blue ring) was placed in a collection tube, and the lysate was added to the column and centrifuged for 30 seconds at 11,000 rpm. Next, 350 µL of membrane desalting buffer (MDB) was added and centrifuged at 11,000 rpm for 1 minute to dry the membrane. Then, 600 µL of buffer DA3 was added to the NucleoSpin® DNA Column and centrifuged at 11,000 rpm for 30 seconds. The flow-through was discarded, and the column was placed back into the collection tube. A second wash was performed by adding 250 µL of buffer DA3 to the NucleoSpin® DNA Column and centrifuging at 11,000 rpm for 2 minutes to fully dry the membrane.

DNA Elution

Following this, DNA was eluted in 60 microliters (µL) of DNase-free H_2O and centrifuged for 1 minute at 11,000 rpm. Spectrophotometry was employed to determine the concentration and integrity of the DNA (A260/A280 ratio) using a double-wavelength Beckman spectrophotometer manufactured in the United States. The isolated and purified DNA specimens were preserved at -80 degrees Celsius for subsequent use.

IL-19 Gene Mutation Analysis

The presence of an IL-19 gene mutation was verified using polymerase chain reaction (PCR) of genomic DNA isolated from peripheral blood mononuclear cells. Real-time PCR primers and TaqMan probes were designed using Primer Express v2.0 software (Applied Biosystems). In the

ABI PRISM DNA sequencing system (Applied Biosystems), PCRs were conducted for each SNP in a single reaction tube, using a thermostable 96-well plate containing both wild-type and mutant alleles. The missense coding SNP for the IL-19 gene was assessed via Assays-on-Demand SNP Genotyping Products (Applied Biosystems) according to the TaqMan Real-Time PCR technique. A missense coding mutation occurs when a codon, which is a sequence of three consecutive nucleotides in mRNA, is altered, resulting in the encoding of a different amino acid.

The TaqMan PCR technique employs two distinct types of TaqMan probes, each corresponding to a segment of DNA: a target SNP site containing different alleles, and the 5' to 3' nuclease activity of Taq polymerase, which is essential for PCR. The 5' ends of the TaqMan probes are equipped with fluorescent dyes, while the 3' ends contain a quencher. During the PCR cycles, the TaqMan probes anneal to the target DNA and are subsequently cleaved by the 5' to 3' nuclease activity of Taq polymerase, provided that there is no mismatch between the probes and the target sequences. The fluorescence detector ABI PRISM 7000 (Applied Biosystems) is then used to measure the intensity of the fluorescent dyes as they are released from the probes.

2.3.Statistical Analysis

The data were entered and coded using version 22 of the SPSS statistical package. For quantitative data, the mean and standard deviation were calculated; for qualitative data, frequencies (number of patients) and relative frequencies (%) were used. A Chi-square (χ^2) test was performed to compare categorical data. The risk of acne-causing genotypes was determined using multivariate logistic regression. Odds ratios (OR) and their 95% confidence intervals (CI) were also calculated. A p-value of less than 0.05 was considered statistically significant.

3. Results

As shown in **Table 1**, no statistically significant variance was observed according to gender and age among the control group and the acne patient (p =0.11 and 0.08, respectively).

Table 1: Age, Sex, and Grade of acne.

Variable		Group I (Control, n=100)	Group II (Acne, n=100)	*P*-value
Age (years)		27.24 ±5.25	25.73 ±7.84	0.11
Sex	**Females**	58 (58%)	46 (46%)	0.089
	Males	42 (42%)	54 (54%)	
Severity	**Mild**		41 (41%)	0.18
	Moderate		33 (33%)	
	Severe		26(26%)	

Among the genotypes, the CC genotype had 66 (66%) controls and 13 (13%) acne patients. The TC genotype had 18 (18%) control and 38 (38%) acne patients. The TT had 16 (16%) controls and 49 (49%) acne patients. A statistically significant reduction was observed in the frequency of CC genotype in acne patients contrasting to the control group (p <0.001). A statistically significant rise in the frequency of TT and TC genotypes in acne patients contrasted to the control group (p <0.001), as shown in **Figure 1**.

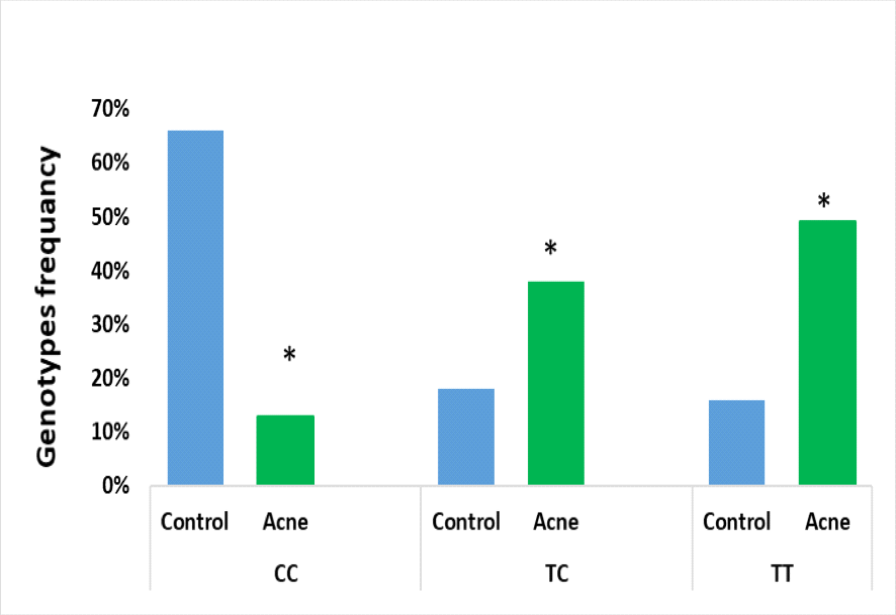

Figure 1: Different IL-19 genotype frequencies among different studied groups. Data were expressed as a percentage p <0.05 was significant. (*) Denotes significance.

Among the alleles, the count of allele C was 150 (75%) in the group control and 64 (32%) in acne cases. The count of allele T was 50 (25%) in the group control & 163 (68%) in acne cases. The TT and TC genotypes statistically significantly increased the risk of the occurrence of acne (p <0.001, Odd ratio 0.064, 0.093, respectively). The occurrence of allele T increased the risk of acne in comparison to the C allele (p <0.001, odd ratio = 0.308).

Table 2: Regression analysis for prediction of occurrence of acne among different IL-19 genotypes.

Variables			Groups		Sig.	Odd ratio	CI 95%
			Control n= 100	Acne n= 100			
Genotype	CC	Count	66	13			
		%	66.0%	13.0%		R	
	TC	Count	18	38			
		%	18.0%	38%	<0.001*	0.093	(0.041 -0.211)
	TT	Count	16	49			
		%	16.0%	49.0%	<0.001*	0.064	(0.028 -0.146)
Allele	C	Count	150	64			
		%	(75%)	(32%)		R	
	T	Count	50	136	<0.001*	0.308	(0.201 -0.470)
		%	(25%)	(68%)			

*significant.

In the mild acne patients, the count of TT was 12 (25%), the count of TC was 20 (51.3%) and the count of CC was 9 (69.2%). In the moderate acne patients, the count of the TT was 17 (35.4%), the count of the TC was 12 (30.8%) and the count of the CC was 4 (30.8%). In the severe acne patients, the count of the TT was 19 (39.6%), the count of the TC was 7 (17.9%), and the count of the CC was 0 (0%). A statistical variance in the genotype frequencies of various grades of acne patients ($p < 0.006$). The highest TC and CC genotypes were exhibited in mild acne patients, while the highest TT genotype was exhibited in severe acne patients.

The incidence of the TT genotype was significantly higher in severe acne while CC and TC genotypes were more common in mild and moderate acne (p =0.006) as shown in **Figure 2.**

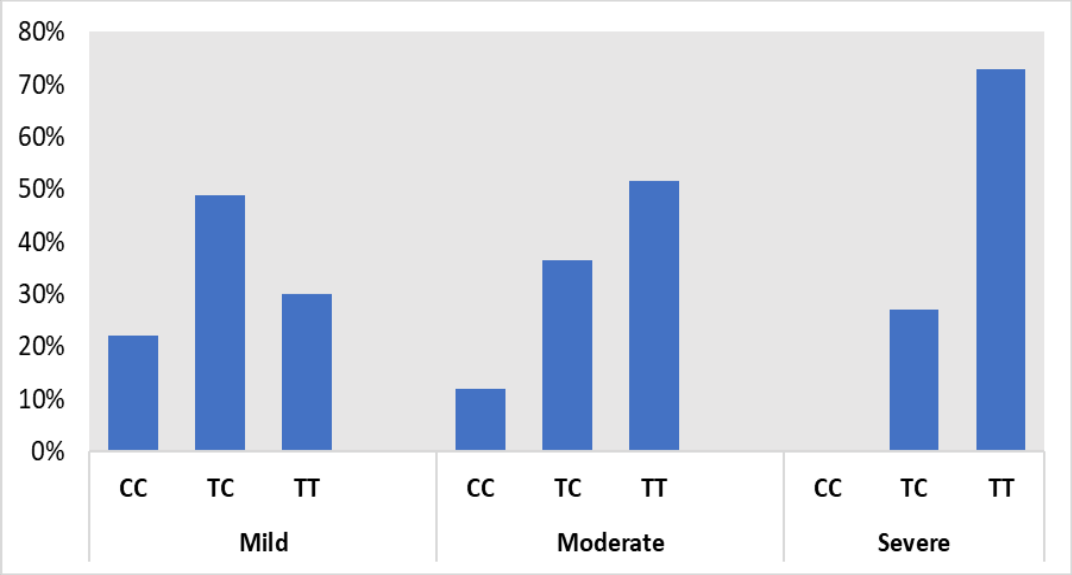

Figure 2: Genotype frequency in different grades in acne patients.

4. Discussion

Acne is a frequently occurring, predominantly chronic inflammatory condition of the pilosebaceous unit that impacts an estimated 85% of young adults and adolescents. The prevalence of acne vulgaris varies according to ethnicity and age. It commonly affects areas with an abundance of sebaceous glands, including the back, face, neck, and upper thorax. However, the incidence of acne in men and women is approximately the same [10, 11].

Many different cell types secrete cytokines, which influence the functions of those cells. In multicellular organisms, they play a crucial role in both physiological and pathological processes. Generally, interleukins, a type of cytokine, are used for communication, especially between immune cells [12]. The initial identification and cloning of interleukin-19 (IL-19) occurred through a search of Expressed Sequence Tag (EST) databases for IL-10 homologs. The IL-19 gene is situated within the "IL-10 cluster" on chromosome 1q32, which also contains the genes encoding multiple other members of the IL-10 family. IL-19, which is part of a subfamily of the IL-10 family of interleukins, is categorized as a class II cytokine [13]. This category also includes types I, II, and III interferons and members of the IL-10 family. Certain research supports the notion that IL-19 and IL-20

play a crucial role in inflammatory skin diseases [14-16].

The current study is a case-control study that enrolled 100 cases of acne vulgaris and 100 normal individuals, who were age- and gender-matched healthy controls, recruited from Fayoum University Hospital's dermatology outpatient clinic. The objective of this research was to determine the molecular association of IL-19 gene polymorphism and its role in acne vulgaris patients. In our study, a significant reduction was observed in the frequency of the CC genotype in acne cases compared to the control group, while a significant increase in the frequency of the TT and TC genotypes was found in acne patients compared to the control group. Additionally, the TT and TC genotypes showed a significant increase in the risk of developing acne ($p < 0.001$, odds ratio = 0.064, 0.093, respectively), and the occurrence of the T allele increased the risk of acne in comparison to the C allele ($p < 0.001$, odds ratio = 0.308).

To our knowledge, no other studies have examined the association between IL-19 genotype frequency and acne vulgaris. However, other studies have discussed the role of IL-19 in other dermatological conditions. For example, a study discussed the role of IL-19 in psoriasis and showed that IL-19 exhibited the greatest variation in expression between psoriatic lesions and normal skin among 30 separately quantified cytokines [17]. Blood IL-19 concentration, associated with the severity of psoriasis, indicated excessive cutaneous IL-19 production. Consequently, systemic and dermal IL-19 levels were significantly reduced by anti-psoriatic medications. On the other hand, non-dermatological studies have concluded that IL-19 polymorphisms (rs2243188 and rs2243193) may play a protective role in the progression of ulcerative colitis in the Mexican population [18].

No investigations are known to discuss the function of IL-19 polymorphisms in acne vulgaris, but other studies have investigated other types of IL polymorphisms in acne vulgaris. A study conducted in Alexandria, Egypt, investigated the interleukin (IL)-6 572 polymorphism in cases with acne vulgaris and its association with the gender of each case and the severity of the acne. The results showed a significant correlation between IL-6 572 variant genotypes and acne (93%) compared to the control group (45%). There were no significant associations between the

IL-6 572 variant genotypes and either the gender of cases or the severity of acne [19]. In another study, the recurrence of the TT genotype in cases was significantly higher than in the control group, while the CT genotype was significantly more frequent in the control group compared to the cases [20].

5. Conclusion

According to the results of our study, the CC genotype was significantly less prevalent among acne cases compared to the control group (p = 0.001), while the frequency of the TT and TC genotypes was significantly higher than that of the control group (p = 0.001). Additionally, the TT and TC genotypes were strongly associated with an increased risk of developing acne (p = 0.001). In conclusion, the presence of the T allele raises the risk of acne compared to the C allele.

We recommend further studies on a larger population to investigate potential interactions between IL-19 and other interleukins and their relationship with acne vulgaris. The collected information and suggested recommendations will help avoid many acne complications, such as scarring and hyperpigmentation, and in planning lifestyle programs for acne patients who are genetically prone to be affected (TT-TC genotype).

Ethics approval and consent to participate: The Research Ethical Committee approved the study and informed consent at the Faculty of Medicine, Fayoum University, Egypt.

Funding: This study is not funded.

Conflicts of Interest: All authors declare they have no conflicts of interest.

References

1. Mochtar M, Prakoeswa CRS, Mahmoud M, Oktarina VD, Permatasari F. The difference in interleukin-19 serum on degrees of acne vulgaris severity. Int J Inflamm. 2018;2018:1-6. doi: 10.1155/2018/7264590
2. illiams HC, Dellavalle RP, Garner S. Acne vulgaris. Lancet. 2012;379(9813):361-372. doi: 10.1016/S0140-6736(11)60321-8
3. Lynn DD, Umari T, Dunnick CA, Dellavalle RP. The epidemiology of acne vulgaris in late adolescence. Adolesc Health Med Ther. 2016;7:13-25. doi: 10.2147/AHMT.S55832
4. Bhate K, Williams HC. What's new in acne? An analysis of systematic reviews published in 2011–2012. Clin Exp Dermatol. 2014;39(3):273-278. doi: 10.1111/ced.12293
5. Das S, Reynolds RV. Recent advances in acne pathogenesis: implications for therapy. Am J Clin Dermatol. 2014;15(6):479-488. doi: 10.1007/s40257-014-0099-z
6. embic Z. Cytokines of the immune system: interleukins. The cytokines of the immune system: The role of cytokines in disease related to immune response. San Diego: Mica Haley; 2015. p. 143-239.
7. Gollnick H. From new findings in acne pathogenesis to new approaches in treatment. J Eur Acad Dermatol Venereol. 2015;29:1-7. doi: 10.1111/jdv.13186
8. Makrantonaki E, Ganceviciene R, Zouboulis CC. Acne vulgaris. Nat Rev Dis Primers. 2015;1:15029. doi: 10.1038/nrdp.2015.29
9. Chan Y. Biostatistics 102: Quantitative data–parametric & non-parametric tests. Blood Press. 2003;140(24.08):79. doi: 10.1161/01.CIR.0000074208.72523.30
10. Lichtenberger R, Simpson MA, Smith C, Barker JN, Navarini AA. Genetic architecture of acne vulgaris. J Eur Acad Dermatol Venereol. 2017;31(12):1978-1990. doi: 10.1111/jdv.14416
11. Degitz K, Placzek M, Borelli C, Plewig G. Pathophysiologie der Akne. J Dtsch Dermatol Ges. 2007;5(4):316-323. doi: 10.1111/j.1610-0387.2007.06311.x
12. Kunz S, Wolk K, Witte E, Witte K, Doecke WD, Volk HD, et al. Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs. Exp Dermatol. 2006;15(12):991-1004. doi: 10.1111/j.1600-0625.2006.00486.x
13. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol. 2011;29:71-109. doi: 10.1146/annurev-immunol-031210-101312
14. Blumberg H, Conklin D, Xu WF, Grossmann A, Brender T, Carollo S, et al. Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell. 2001;104(1):9-19. doi: 10.1016/S0092-8674(01)00185-X
15. Liu L, Wesselschmidt R, Lu H, Borok Z, Zhou Q, Minoo P. Selective enhancement of multipotential hematopoietic progenitors in vitro and in vivo by IL-20. Blood. 2003;102(9):3206-3209. doi: 10.1182/blood-2003-02-0393
16. Ghoreschi K, Thomas P, Breit S, Dugas M, Mailhammer R, van Eden W, et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. Nat Med. 2003;9(1):40-46. doi: 10.1038/nm808
17. Gallagher G, Eskdale J, Jordan WJ. Human interleukin-19: structure, function and disease

associations. Anti-Inflamm Anti-Allergy Agents Med Chem. 2006;5(3):233-242. doi: 10.2174/187152306778019524

18. Fonseca-Camarillo G, Furuzawa-Carballeda J, Llorente L, Yamamoto-Furusho JK. Expression of interleukin (IL)-19 and IL-24 in inflammatory bowel disease patients: a cross-sectional study. Clin Exp Immunol. 2014;177(1):64-75. doi: 10.1111/cei.12310
19. Ragab M, Shohieb ZS, Eltokhy MA, Abd Elnaby AG. Association of interleukin-6 gene promoter polymorphism with acne vulgaris and its severity. Clin Exp Dermatol. 2019;44(6):637-642. doi: 10.1111/ced.13951
20. Ibrahim AA, Saeed SA, El-Refaey AM, Hassanein SM, Emara AM. IL 1A (-889) gene polymorphism is associated with the effect of diet as a risk factor in acne vulgaris. J Cosmet Dermatol. 2019;18(1):333-336. doi: 10.1111/jocd.12841
21. Al Robaee AA, AlZain TM, Alzolibani AA, AlShobaili HA, Al Marshood AA. Association of interleukin 4 (-590 T/C) and interleukin 4 receptor (Q551R A/G) gene polymorphisms with acne vulgaris. Ann Saudi Med. 2012;32(4):349-354. doi: 10.5144/0256-4947.2012.349.