Global Scientific JOURNALS

GSJ: Volume 11, Issue 3, March 2023, Online: ISSN 2320-9186
www.globalscientificjournal.com

MOLECULAR STUDY OF ICAM-1 GENE IN IRAQI GIRLS SUFFERED WITH ACNE VULGARES

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Abstract:
Acne vulgaris is a long-lasting skin disorder that causes the pilosebaceous glands to become inflamed. Acne vulgaris develops as a result of four different pathogenesis theories, including sebum production, follicular skin, bacterial colonization by Propionibacterium acnes, and inflammatory mediators. The American Academy of Dermatology's categorization system must be used to identify lesions in order to make the diagnosis of acne vulgaris. Due to the complex origins of acne, there are many different approaches to managing it, such as monotherapy or combinations of different medications that work to decrease the anti-inflammatory and antibacterial activity. DNA was extracted from the blood of (71) patient who were subjected to this study, Determination of the genetic variation of the ICAM-1 gene byTetra–ARMS-PCR technology. **D**etermination of nucleotide sequencing of amplified pieces using DNA sequencing technology. . According to the study's results, the proportion of patients with Acne Vulgares, who observed the wild genotype AA was lowest (17%) and the proportion who observed the mutant genotype GG was largest (32%), and the proportion of observing for the heterogeneous AG was (51%) comparison to healthy group, who observed the wild genotype AA was largest (50%) and the proportion who observed the mutant genotype GG was lowest (20%), and the proportion of observing for the heterogeneous AG was (31%), The allelic observation rate in patients with Acne Vulgares was 58 percent for the mutant G allele and 42 percent for the normal A allele

Key words: ICAM-1 gene, Acne vulgares, T-ARMS- PCR, DNA sequencing and polymorphism

Introduction:

Approximately 80% of young adults and adolescents have acne vulgaris, a chronic inflammatory disease of the skin. Acne vulgaris is characterized by open and closed comedowns and lesions with inflammatory nodules, pustules, and papules, which typically affect the face, chest, and back [1_2] . Acne vulgaris is a chronic disease that

requires prolonged therapy for a satisfactory outcome. Treatment adherence in patients is a major problem, particularly for topical treatments, owing to side effects and the prolonged treatment time. Insufficient adherence leads to recurrence of acne, patient dissatisfaction, and increased medical costs. Numerous studies have reported low adherence rates for acne treatments [3]. Four key pathogenic processes associated with the formation of acne lesions, include: 1) Alteration of follicular keratinization that leads to comedowns. 2) Increased and altered sebum production under androgen control. 3) Bacterial colonization mainly by Propionibacterium acnes, and 4) Complex inflammatory mechanisms that involve both innate and acquired immunity [4]. Topical retinoid are used in the treatment of both non inflammatory and inflammatory acne. Currently, the Food and Drug Administration (FDA) has approved three topical retinoid: adapalene, adarotene, and tretinoin.these agents help normalize follicular keratinization and decrease keratinocyte cohesiveness, thereby reducing follicular occlusion and comedown formation [5]. The cause of acne is multifactorial, including genetics, hormones, and bacteria. It is known that the number of sebaceous glands that one has is an inherited trait, and that it is unlikely for 1 twin to develop acne of different severity than the other twin [6]. In some mechanistic studies it has been found that IL1, intercellular adhesion molecule 1 (ICAM-1), tumor necrosis factor alpha (TNF-α) induce differentiation of human sebocytes into keratinocytes like phenotype, resulting in hyperkeratosis in the infundibulum in acne vulgaris [7]. ICAM-1 over expressed by endothelial cells during inflammation, resulting in increased firm leukocyte endothelial cell adhesion and transmigration of leukocytes at sites of inflammation. Therefore, the loss of ICAM-1 inhibits wound healing, keratinocyte migration from the edges of the wound toward the center, and granulation tissue formation [8]. It primarily affects the pilosebaceous units and can take on various forms depending on the quantity of abnormalities, nodules, cysts, and abscesses [16]. The primary characteristics of acne are associated with an excessive sebum generation, an aberrant keratinization process, the colonization of Cutibacterium acnes in oily body sites (face, neck, chest, and back), and an inflammatory process. Similar to AD, the relationships and sequence of events that lead to the development of this illness are not well understood [17]. The link between nutrition and acne is becoming more and more clear. Diets with a high glycemic index, dairy intake, and whey protein consumption have all come under scrutiny. As more data mounts, dietary changes and natural remedies are expected to become more significant in the management of acne [18].

The study's objective, is to detect the abnormality change in sequence in ICAM-1 gene in girls with acne vulgares in Irqi population.

Case Study:

This study included (71) patient of an age group ranging from (20-45) years of reviews to the private pathological analysis labor atories in the city of Mosul for a period ranging

from June to September of 2022, and it was relied on these clinical cases of the disease to choose Samples . The samples were divided into four parts based on the biochemical results: The first group

Collection of Blood sample:

(5.0)ml of venous blood was withdrawn from these patient and divided into two groups, the first part was placed in tubes containing EDTA anticoagulant to extract DNA, and the second group was placed in tubes free of any anticoagulant. The tubes were left for one hour until the blood clotted, after which a centrifugation was carried out for a period of (10) ten minutes at a speed of (3000) cycles/minute to obtain the blood serum on which the biochemical tests were conducted.

Collection of Blood sample:

DNA was extracted from the blood of (71) patient who were subjected to this study, using the modified method presented by (Iranpur and Esmailizadeh., 2010).

Genotyping:

Tetra–ARMS-PCR Reactions:

The DNA concentration in all study samples is adjusted after being measured by biodrop by diluting them with TE buffer solution to obtain the required concentration for performing PCR reactions and was (25) ng/microliter for each sample.

Four primers are added for each primer reaction (F-outer and R-outer) for the whole gene, forward outer-reverse inner for the normal allele, forward outer-reverse inner) for the mutant allele.

The PCR reaction mixture is prepared by mixing the nucleic acid of each sample and the primer designated for the mutations under study with the components of the master-mix in a 0.2-ml PCR-tube produced by the English by Biolaps Company. Mix in the Microfuge for a period between (5-3) seconds to ensure that the reaction components are mixed, Then, the PCR tubes were inserted into the thermocycler within the special program for each mutation, then the reaction product is injected into the pits of the prepared agarose gel, at a concentration of 2%, with the addition the Ladder DNA prepared by Biolaps Company, in one of the first holes, after which the samples are migrated Running the electrophoresis device for a period of 45 minutes, after which the bands are imaged using a gel-documentation device.

Determination of the genetic variation of the ICAM-1 gene byTetra–ARMS-PCR technology

The presence of the A G mutation for **ICAM-1 gene** was detected by adding 4 µl (100 nanogram) of template DNA and 1 µl (10 picomol) of each mutation specific primer for PKD1 gene mutation supplied by the Korean company Macrogen to the contents of the master mix

The presence of the AG mutation of for **ICAM-1 gene** by adding 4 µl (100 nanogram) of template DNA and 1 µl (10 picompl) of each mutation specific primer , which was designed by the researcher using Pimer 3 software and used For the first time on this gene, it was prepared by the Korean macrogen company, and it was added to the contents of the master mix. The final reaction volume was 20 µl (Rizk *et al*., 2019).

Table 1: Shows the primers used to determine genetic variation at the locus (rs17467825) using PCR technology.

Primer	Sequence	Band size	Annealing
F-outer	CACCCACCTCCATGTCATCTCATCGTGT	490 bp	59
R-outer	CCCATTATGACTGCGGCTGCTACCACAG		
F-inner	GAGCACTCAAGGGGAGGTCACCCTCG	290 bp	
R-inner	TCACTCACAGAGCACATTCACGGTCACATT	390 bp	

Then the reaction tubes were inserted into the thermocycler to conduct the multiplication reaction using the special program for the reaction as shown in Table 2:

Table 2: Shows the program adopted in the ARMS-PCR technique to identify the mutation (rs17467825)**.**

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation.	95.0.	5.0. min.	1
2.	Denaturation.	95.0.	45.0. sec.	35
3.	Annealing.	67.0.	1.0. min.	
4.	Extension.	72.0.	1.0. min.	
5.	Final extension.	72.0.	7.0. min.	1
6.	Stop reaction.	4.0.	5.0. min	1

The optimal temperature for the primer bonding in this reaction was determined using the Gradient program on the thermocycler device; the gradient was (5) and the mean temperature was (59) C. The temperature of 59°C was used since it gave the best results. Then the PCR reaction was separated by 2% agarose gel**.**

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology:

The sequence of the nitrogenous bases of the gene was determined for the VDBP samples that were included in the study for the purpose of verifying the validity of the designed primer that was used in the ARMS-PCR technique and for the purpose of detecting the presence of any additional discrepancies in the gene. These genes were obtained using a 3130 Genetic Analyzer device supplied by the Japanese company Hitachi. These gene

sequences were matched with the gene sequences documented in the National Center for Biotechnology Information NCBI, and the results were analyzed using BLAST program.
Table 5: shows the primers belonging to the for **ICAM-1 gene** and on which the DNA sequencing test was conducted.

	Primer	Sequence
ICAM-1 gene	Forward	5′ GGCCAGCTTATACACAAGAACC 3′
	Reverse	5′ TGTCATCATACTGTGGTAGCA 3′

Biochemical test:
This study also include some biochemical parameters used as a Acne vulgaris markers like:
(FSH,LH,Prolactin,Testosterone,SHBG,Ca)

Result and Discussion
The results showed, as in Figure (1), the existence of a relationship between Iraqi people with Acne Vulgares and the mutation of ICAM-1 Gene, As shown in Table (4),

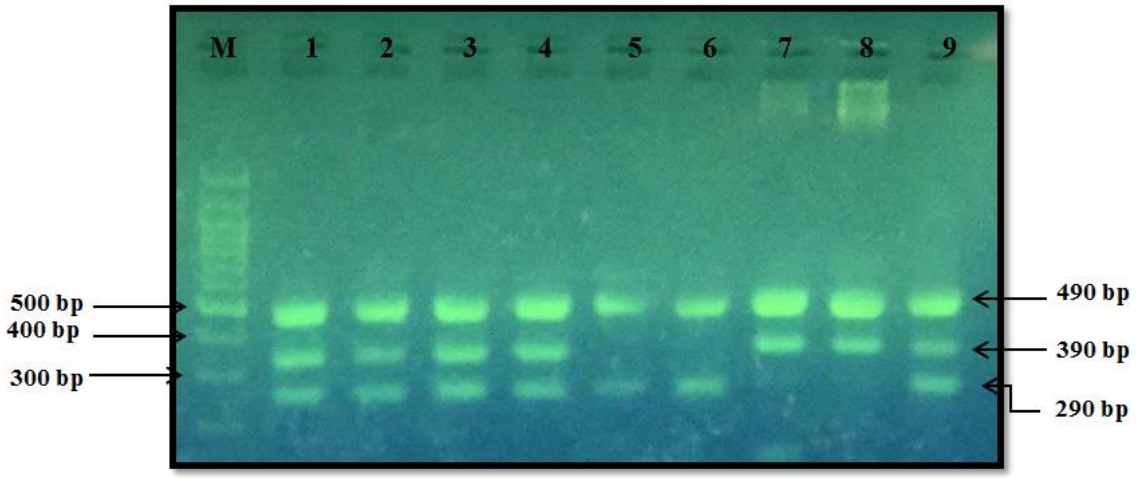

Figure (1: the relationship between girls with acne vulgares and the mutation of ICAM-1 Gene , with three band in PCR product 490 bp fpr universal band, 390 bp for wild type allele and 290 for mutant type allele, samples (1,2,3,4,9) have hetero-genotype AG, samples (7,8) wild genotype AA, samples (5,6) have mutant genotype GG.

Table (4): Distribution of the percentage of allelic observations and genotypes of the ICAM-1 gene mutation among a group of healthy groups and Iraqi people Iraqi people with Acne Vulgares, knowing that the A allele is the normal allele and the G allele is the mutant allele:

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
AA	12	17	10	50	P = 0.0278	4.5833	1.1808 to 17.7900
AG	36	51	6	30			
GG	22	32	4	20			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
A	60	42	26	65	P = 0.0150	2.4762	1.1922 to 5.1429
G	80	58	14	35			

According to the study's results, the proportion of patients with Acne Vulgares, who observed the wild genotype AA was lowest (17%) and the proportion who observed the mutant genotype GG was largest (32%), and the proportion of observing for the heterogeneous AG was (51%) comparison to healthy group, who observed the wild genotype AA was largest (50%) and the proportion who observed the mutant genotype GG was lowest (20%), and the proportion of observing for the heterogeneous AG was (31%), The allelic observation rate in patients with Acne Vulgares was 58 percent for the mutant G allele and 42 percent for the normal A allele.

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology: Figures (5) show the PCR reaction 520 bp, for ICAM-1 gene, on which the nucleotide sequence identification test was performed.

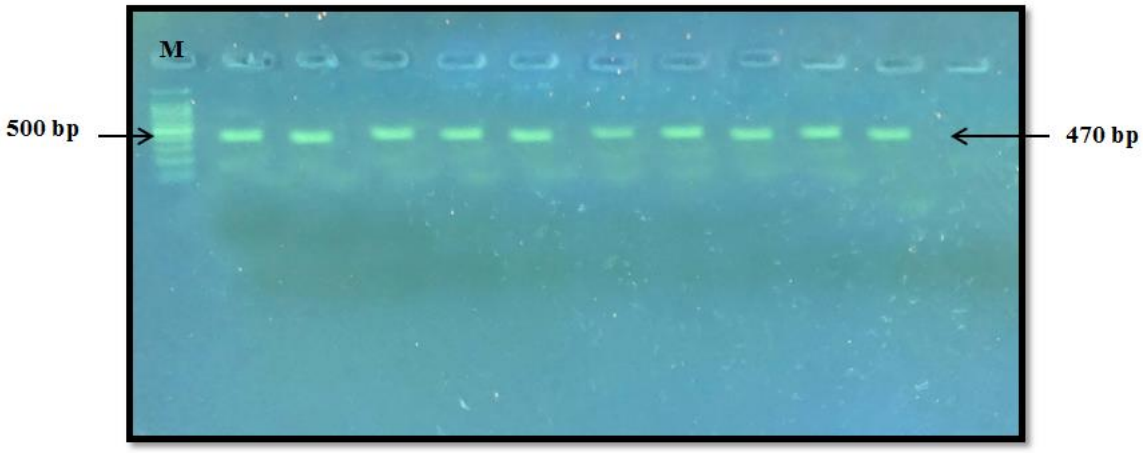

Figure 5: shows the PCR reaction product of ICAM_1 gene in girls with acne vulgares and the reaction product 470 bp, M is the Ladder with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel.

The results of a sequencing test for the amplified of ICAM-1 gene had differences in a number of nucleotides, however exon 5 had no differences. These findings are depicted in the following figures:

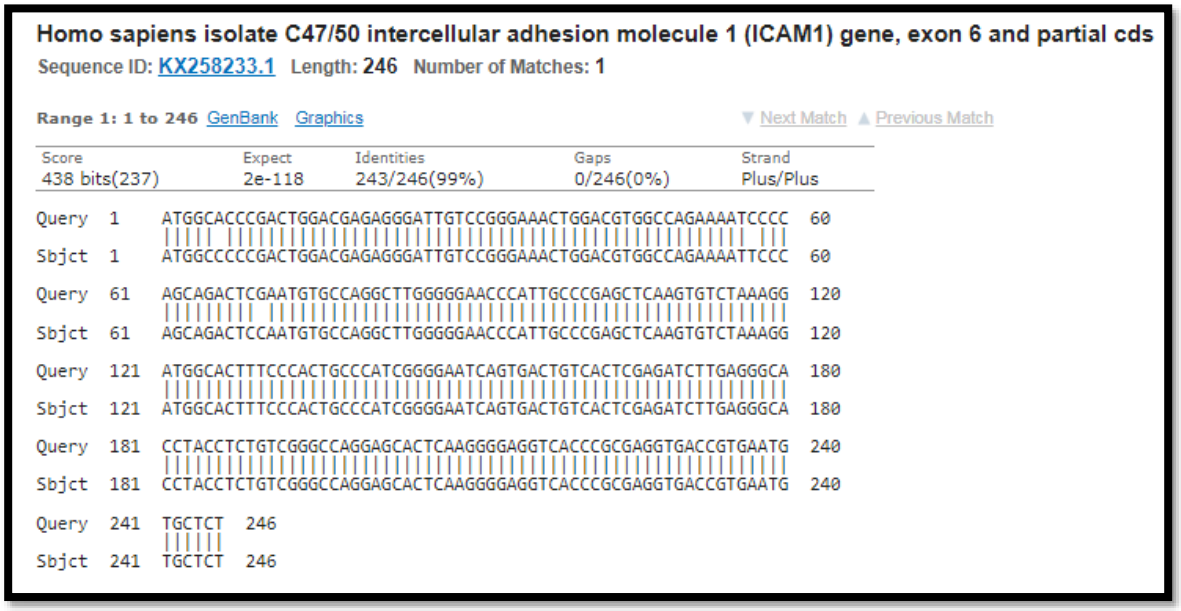

Figure 8: The result of matching with the nucleotide sequence of ICAM-1 gene was compared with the sequences of the original gene at the NCBI site

Table 8: illustrates the locations and kinds of alterations in the *ICAM_1* gene in breast cancer patients

ID sequince	Nucliotide	Location	Mutation type	Idendity	Gaps
Kx258233.1	C⟶A	6	(Trans version)	(99%)	(0)
Kx258233.1	T⟶C	57	(Transition)	(99%)	(0)
Kx258233.1	C⟶G	70	(Trans version)	(99%)	(0)
Kx258233.1	C⟶T	135	(Transition)	(99%)	(0)
Kx258233.1	T⟶C	172	(Transition)	(99%)	(0)
Kx258233.1	C⟶G	223	(Trans version)	(99%)	(0)
Kx258233.1	G⟶C	3	(Trans version)	(99%)	(0)
Kx258233.1	A⟶T	107	(Trans version)	(99%)	(0)
Kx258233.1	C⟶A	198	(Trans version)	(99%)	(0)

Kx258233.1	G ⟶ A	14	(Transition)	(98%)	(0)
Kx258233.1	G ⟶ C	86	(Trans version)	(98%)	(0)
Kx258233.1	G ⟶ T	109	(Trans version)	(98%)	(0)
Kx258233.1	T ⟶ C	191	(Transition)	(98%)	(0)
Kx258233.1	C ⟶ G	206	(Trans version)	(99%)	(0)

When the table 8 is observed, it shows the different types of genetic variations and their locations on the ICAM_1 gene after conducting a sequencing test and comparing them with the gene sequences at the NCBI site.

The results of this study shows an increase in the levels of biochemical parameters in Acne vulgaris patients, Prolactin (40 ng\ml), Testosterone (1.5ng\ml), LH (20 iu\l), FSH(25 in\l), Ca(9.5 mg\100l), compare with healthy group: Prolactin (5_35 ng\ml), Testosterone (<1 ng\ml), LH(1_15iu\l), FSH (1_20 iu\l), Calcium (8.5_10.5 mg\100ml).

Discussion

In the present study, there were significant associations between ICAM (241&496) polymorphism and acne patients' BMI where the highest BMI was among acne patients with GG genotype that was highly expresses in acne patients. This indicates that there is association between obesity and ICAM genotype expression as obesity enhances expression of ICAM-1. In accordance with a study, Lin et al., [9]. Aimed to investigate the effect of visfatin, that is known to act as a mediator in obesity, on the adhesion of THP-1 monocytes to human vascular endothelial cells through measurement of ICAM-1 and VCAM-1 expression in endothelial cells by western blotting concluded that visfatin promoted monocyte-endothelial cell adhesion by increasing ICAM-1 and VCAM-1 expression via the activation of p38/PI3K/Akt signaling and downstream ROS production and IKK/NF-κB activation. This indicates that obesity promotes ICAM-1 expression and consequently according to our results increase incidence of severity of AV.

Numerous inflammatory disorders' etiology and outcomes have been demonstrated to be significantly influenced by ICAM-1. Examples include type 1/type 2 diabetes, Graves' illness, multiple sclerosis, inflammatory bowel diseases, and Behçet's disease, where it is thought to be a genetic risk factor. [10]. The highest BMI was found in acne patients who had the GG genotype, which is strongly expressed in acne patients. The present investigation found substantial

relationships between the ICAM (241&496) polymorphism and the BMI of acne patients. As obesity increases ICAM-1 expression, this suggests a relationship between ICAM genotype expression and obesity. According to research by Lin et al., [11] aimed to investigate the effect of visfatin, that is known to act as a mediator in obesity, on the adhesion of THP-1 monocytes to human vascular endothelial cells through measurement of ICAM-1 and VCAM-1 expression in endothelial cells by western blotting concluded that visfatin promoted monocyte-endothelial cell adhesion by increasing ICAM-1 and VCAM-1 expression via the activation of p38/PI3K/Akt signaling and downstream ROS production and IKK/NF-κB activation. This indicates that obesity promotes ICAM-1 expression and consequently according to our results increase incidence of severity of AV. Regarding ICAM-1 gene polymorphism and its expression, it was found that that there were statistically significant differences among the studied groups as regard ICAM (241&496) polymorphism where the highest percentage of GG were among severe acne group and none of controls had GG genotype . In terms of gene expression, there were highly statistically significant differences across the tested groups, with the severe acne group having the greatest gene expression (2.070.6). ICAM (241&496) polymorphism and gene expression were highly statistically significant correlated in acne patients, with GG genotype being associated with the greatest levels of gene expression. This shows that ICAM-1 (469 and 241) gene polymorphism and expression are common in acne patients and they are mostly connected to the severity of the AV.

Su et alstudy .'s results [12] showed that the expression of TLR-2, NF-B, and its downstream factor ICAM-1 increased in the inflammatory skin tissue produced by P. acnes, supporting these findings. Pretsch et al[13] .'s study that included treating AV with T. wortmannii compounds showed that the most efficient therapeutic agents to treat acne disease had prominently down-regulated ICAM-1 expression in endothelial cells after TNF- stimulation and IL-8 secretion in keratinocytes after P. acnes treatment via the NF-B and MAPK pathways. ICAM-1 expression is related to the severity and type of inflammation. Increased ICAM-1 expression is thought to be a key starter in many processes, including leukocyte and keratinocyte contact. [14]. ICAM-1 was found to be universally expressed by basal keratinocytes, inflammatory cells, and cells in the interstitial dermis of acne lesions, according to a study that aimed to investigate the pattern of expression of adhesion molecules in evolving acne lesions. The study included 49 patients with moderate to severe acne who were divided into four groups based on the duration of inflammation: up to 6 h, from 6 to 24 h, from 24 to 48 h, and from 48 to 72 . Endothelial cells express ICAM-1 constitutively, and it has been demonstrated that both localized and generalized cutaneous inflammation exhibit up-regulated endothelial cell ICAM-1 expression. ICAM-1 expression in keratinocytes has been observed to be modest in normal skin, however it is reported that TNF-a and IFN-g, not IL-1, are the triggers. [15]. Studies have shown that NF-B controls ICAM-1, an essential adhesion molecule that regulates the adhesion reaction and encourages the adherence of inflammatory areas. Studies have shown that NF-B controls ICAM-1, an essential adhesion molecule that regulates the adhesion reaction and encourages the adherence of inflammatory areas.

Conclusion: According to this study, the observation of different genotypes and allelic frequency of ICAM-1 gene and detection some mutation by Sequence technique mutation in girls with acne vulgares in Iraqi population.

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