

# Clinical and Immunological Evaluation of Isotretinoin in Patients with Acne Vulgaris

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## Abstract

**Objective:** To study the clinical and immunological influence of isotretinoin drug in the management of acne vulgaris by evaluation some immunological parameters which include ( IL-1 $\beta$ , IL-8, IFN- $\gamma$ , TNF- $\alpha$  and TLR2).

**Methods:** This study is a randomized clinical trial carried out in clinical dermatology in Merjan Teaching Hospital in Babylon from September- 2018 to March – 2019. The number of patients in the present study was 30, their age between (14- 30 years), who were diagnosed by dermatologist to having moderate to severe acne and measured the immunological parameters (IL-8, IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ ) and ( TLR-2 ) before treatment, then treated with isotretinoin (20mg) once daily for thirty days, the response were evaluated clinically and immunologically after one month of therapy, by measure the concentration of proinflammatory cytokines (IL-8, IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ ) and ( TLR-2 ) by using enzyme- linked Immunosorbent assay (ELISA) technic.

**Results:** Significant elevation in the serum levels (  $p \leq 0.001$ ) of immunological parameter (TLR-2, IL-8, IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ ) among acne patients were noted in comparison to the control.

After one months of treatment the clinical response in group treated with isotretinoin was good, moderate and poor in 9 (60%), 6(40%), 0(0%) respectively, the immunological results showed that significant reduced (  $p \leq 0.001$ ) in the serum levels of (IFN- $\gamma$ , IL-8, IL-1 $\beta$ , TNF- $\alpha$  and TLR-2) of pretreatment in comparison to post treatment after one month of therapy.

**Conclusion:** Significant elevation ( $p \leq 0.001$ ) in the serum levels of (TLR-2, IL-8, IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ ) among moderate to severe acne patients before treatment in comparison to control group. Clinically isotretinoin efficient in treatment of patients with acne after one month of therapy. Immunologically isotretinoin significant reducing ( $p \leq 0.001$ ) serum levels of (TLR-2, IL-8, IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$ ) of pretreatment patients in comparison to post treatment after one month of therapy.

**Key words:** Acne vulgaris, immunological parameter TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\gamma$ , isotretinoin.

## Introduction

Acne vulgaris is a disease of the pilosebaceous unit with high prevalence in adolescents characterized by comedones, papules, pustules, cysts and scars <sup>(1)</sup>. The pathogenesis of acne is multifactorial, it involves an increase in androgen hormone levels along with excess sebum production, hyperkeratinization, hyper colonization of P. acnes which lead to stimulation of the innate immune system and ultimately inflammation <sup>(2)</sup>.

The immune system protects the skin against harmful microbial, physical and chemical substances. P. acnes which can activate the innate immune reactions in sebocytes, keratinocytes and monocytes in the peripheral blood<sup>(3)</sup>. These cells have pattern recognition receptors (PRRs) which can recognize a large variety of pathogenic organism, that are responded to particular pathogen -associated molecular patterns (PAMPs) and cause activation of the innate immune system. The most

important group of PRRs is Toll-like receptors (TLR-2)<sup>(4)</sup>, that expressed in human sebocytes and keratinocytes, TLR-2 is activated by *P. acnes* and can change the content of lipid in sebum, then activates several proinflammatory cytokine included in acne lesions<sup>(5)</sup>, like interleukins (IL-1 $\beta$ , IL-1 $\alpha$ , IL-12 and IL-8), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ )<sup>(6,7,8)</sup>.

The systemic antibiotic (oral) are common therapy for moderate to severe acne such as tetracycline, minocycline, doxycycline, erythromycin, and trimethoprim/sulfamethoxazole combination; antibiotic decrease *P. acnes* and some have anti-inflammatory effects<sup>(9)</sup>. Also, oral isotretinoin which used in patients with moderate to severe acne which affects almost all factors in acne pathogenesis<sup>(10)</sup>.

**Aim of Study:** Study the clinical and immunological response of isotretinoin in the treatment of acne by used some immunological parameters including (TLR2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\alpha$ ).

## Materials and Methods

### Study design

The present study is a clinical trial carried out in clinical dermatology in Merjan Teaching Hospital in Babylon from September- 2018 to March – 2019.

### Methods:

#### 1-Choice of population

In this study the subjects enrolled were:

**patients group :** the thirty patients included in this study were among those who attended the clinical dermatology and their age was between (14- 30 years) with mean (19.60 $\pm$ 3.819), blood samples were taken from all patients to measure the immunological parameters before treatment then they were received isotretinoin (20mg) once daily, regarding the gender the (18) females were included and (12) patients were male suffering from moderate to severe acne, the response were evaluated clinically and immunologically after one month of treatment.

**Control group:** Included thirty subjects who were appear healthy and their age were between (14 -30 years) with mean (19.65 $\pm$ 3.799 ), subjects in the control group

include ( 18) females and (12) males, blood samples were taken from each subject to measure the immunological parameters and comparison with patients group (before treatment). The control and patients groups were matched in age and sex .

**2- Blood collection:** A bout (5 ml) were taken from venous blood of healthy and patients who were enrolled in this study through venipuncture via using disposable syringe. Blood samples were placed in gel tubes (8 ml), after that allowed the blood to be clotted, then was centrifuged at the speed (3000 rpm) for about 10 minutes to obtain the serum.

Serum was put in an eppendrof tube and immediately frozen at ( -80C ) until thawed for assay<sup>(11)</sup> .

**3- Evaluating of the patients:** Patients were followed up for one month of the initiation of treatment, and the response was evaluated clinically through the improvement of the lesions as that ( good response >75%, moderate response 75-50%, the mild response 50-25%, the poor response < 25%<sup>(12)</sup> ), also measured the concentrations of immunological parameters in the serum (TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$ , and IFN- $\gamma$ ) by using enzyme- linked Immunosorbent assay (ELISA), and compared to their concentration before the initiation of the treatment.

**4-Laboratory investigations:** Serum levels of ( TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\gamma$  ) measured by using cytokines ELISA kits (Elabscience company, china).

**5- Ethical approval:** In this study the ethical approval was obtained from research ethical committee in the college of medicine, University of Babylon-Iraq.

#### 6-Statistics analysis:

Data analysis by using( version 21) of SPSS. Variables that were presented as percentages and frequencies. Variables that presented as (Means  $\pm$  SD). The significant p-value was ( $\leq 0.05$  ).

## Results

### 1-Clinical response ( table 1):

The clinical response of isotretinoin was good, moderate and poor in 9 (60%), 6(40%), 0(0%)

respectively.

**Table (1): Clinical response of isotretinoin after one month of therapy.**

Treatment	Good response	Moderate response	Poor response	Total
Isotretinoin	9(60%)	6(40%)	0(0%)	15(100%)

## 2- The immunological response:

### A- Serum levels of (TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$ , and IFN- $\gamma$ ) among subjects of the study groups.

Table (2) The means serum levels of immunological parameters according to study groups were highly significant ( $p \leq 0.001$ ) differences between patients with acne before treatment and control group.

**Table (2) :Serum levels of immunological parameters in pretreatment patients and control group.**

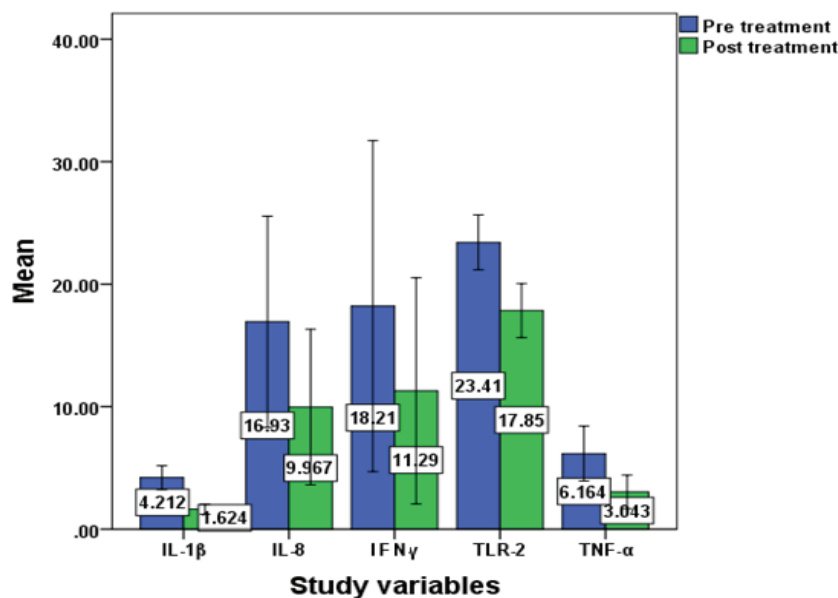
Markers	Groups	N	Mean	SD	P-value
TLR2 (ng/ml)	Patients	30	23.55	1.16	<0.001 *
	Control	30	16.343	1.37	
IL-1 $\beta$ (pg/ml)	Patients	30	4.027	0.54	<0.001 *
	Control	30	1.092	0.07	
IL8 (pg/ml)	Patients	30	16.70	4.73	<0.001 *
	Control	30	9.30	2.44	
TNF- $\alpha$ (pg/ml)	Patients	30	6.226	1.15	<0.001 *
	Control	30	2.778	1.12	
IFN- $\gamma$ (pg/ml)	Patients	30	16.44	5.20	<0.001 *
	Control	30	10.404	1.69	

\* $p \leq 0.001$

### B- Serum levels of (TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$ and IFN- $\gamma$ ) in pre and post treatment of isotretinoin after one month of therapy:

**Table (3): Serum levels of(TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\gamma$ ) according to isotretinoin**

Markers	N	Study groups	Mean	SD	P-value
TLR-2	30	Pre treatment	23.41	1.11	<0.001*
	30	Post treatment	17.84	1.10	
IL-8	30	Pre treatment	16.92	4.312	<0.001*
	30	Post treatment	9.96	3.17	
IL-1 $\beta$	30	Pre treatment	4.21	0.48	<0.001*
	30	Post treatment	1.62	0.20	
TNF- $\alpha$	30	Pre treatment	6.16	1.11	<0.001*
	30	Post treatment	3.04	0.68	
IFN- $\gamma$	30	Pre treatment	18.20	6.75	0.005*
	30	Post treatment	11.29	4.61	

**Figure (1):Serum levels of (TLR-2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\gamma$ ) according to isotretinoin.**

### Discussion

The current study revealed that there was a high significant ( $p \leq 0.001$ ) elevation in the levels of serum in (TLR- 2, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IFN- $\gamma$ ) among acne patients in comparison to their levels in control group ( table 2).

In present study the level of TLR-2 ( $23.55 \pm 1.16$ ) was highly significantly increased ( $p \leq 0.001$ ) in acne patients group as compared to control group ( $16.343 \pm 1.37$ ), this result indicates a positive correlation between TLR-2 level and development of acne and this may attributed to the ability of *P. acnes* to express particular pathogen-associated molecular patterns (PAMPs) which

are recognized via TLR-2 and cause up regulation of TLR-2 which consequently releases cytokines which are responsible for the cause inflammatory response<sup>(13, 14, 15)</sup>.

TLR-2 (trans-membrane receptors) which are associated to the response of innate immune system, TLR-2 is present in sebocytes, keratinocytes, dendritic cells, monocytes and macrophages that play important role in the development of comedones and inflammatory lesions by stimulation of nuclear factor kappa B (NF $\kappa$ B) cause stimulation of different proinflammatory cytokines such as ( IL-1 $\alpha$ , IL-8, IL-12) and by stimulation several cells, like natural-killer (NK), macrophages and neutrophils also the adaptive immune response is stimulated<sup>(14)</sup>.

The highly significant (  $p \leq 0.001$  ) increase in serum level of IL-1 $\beta$  in acne patients group in comparison to the control group ( 4.027 $\pm$ 0.54 versus 1.092  $\pm$  0.07) may be related to the ability of P. acnes in the skin to modulate immune responses as a result to the stimulation innate immune receptors, moreover in acne, the high levels of IL-1 $\beta$  that observed as response to P. acnes lead to development of inflammatory acne lesions by activation of inflammasome in the monocyte–macrophage<sup>(16)</sup>.

The P. acnes triggers the innate immune response by activation of TLR-2 and the inflammasome, thus the response of monocytes to P. acnes cause an up regulation to caspase-1 which is required to proteolytic cleavage and activation of IL-1 $\beta$ <sup>(7)</sup>.

The serum level of IL-8 was highly significant (  $p \leq 0.001$  ) increase that demonstrated in the present study in acne patients as comparison to control group (16.70  $\pm$ 4.73 versus 9.30  $\pm$  2.44) may be explained by the ability of P. acnes to stimulate the production of IL-8 in human monocyte by NF-kB which is the most important factor for IL-8 gene transcription<sup>(17)</sup>.

IL-8 is potent chemotactic factor that is released by different types of cells (eg. monocytes, macrophages, and epithelial cells), and IL-8 is main proinflammatory mediators that induced in monocytes by the present of pathogen; thus, in the innate immunity the key proinflammatory mediator against pathogen is IL-8 by inducing neutrophil chemo attraction<sup>(17)</sup>.

Results reached throughout this study regarding the highly significant (  $p \leq 0.001$  ) increase in the serum level of TNF- $\alpha$  in acne patients group as comparison to control group (6.226  $\pm$ 1.15 versus 2.778  $\pm$ 1.12) is confirmed by Al-hilali et al. (2014) study which compared the level of TNF-  $\alpha$  of acne patients with different sex and age with a healthy control group and found that TNF- $\alpha$  level was significantly increased in patients with acne when compared to the healthy control group, and no significant differences were present between the genders, also it demonstrated an association between susceptibility to acne and TNF- $\alpha$  level as one of the major proinflammatory cytokines which plays an important role in the inflammatory process in acne<sup>(18)</sup>. Also high level and role of TNF- $\alpha$  in patients with acne of present study is in agreement with another previous study performed by Baz et al. (2008) which found that TNF- $\alpha$  was significantly increased (  $P \leq 0.001$  ) in patients with acne as compared to controls (healthy) group, and also it revealed that there was no an association between acne development and gender, also it's pointed that P. acnes cause trigger the production of cytokines from monocytes, keratinocytes and lymphocytes including IL-1 $\alpha$ , IL-1 $\beta$ , IL-8, and TNF- $\alpha$ , which are the responsible mediators for the development of inflammatory acne lesions<sup>(19)</sup>.

In the present study, IFN- $\gamma$  serum level was highly significant (  $p \leq 0.001$  ) increase in the acne patients as comparison to the control group (16.44 $\pm$ 5.20 versus 10.404 $\pm$ 1.69), is in agreement with Sugisaki et al.(2009) study which found that in acne patients, IL-12, IFN-  $\gamma$ , and IL-8 were significantly elevated in the peripheral blood mononuclear cells (PBMC) as compared to that of healthy donors, and they explain their result by that, the inflammatory lesions of acne (microcomedone and comedone) which present in early stage, there are many of (CD4+ T cells) are exposed in the inflammatory lesions, also the P.acnes trigger inflammatory response via production of ( proinflammatory cytokines) such as IL-1 $\beta$ , TNF- $\alpha$  and IL-8 from monocytes and stimulates the secretion of IL-12 by TLR-2 from keratinocytes and monocytes, and under the effect of IL-12, the naïve T cells will be differentiated into ( Th1 cells) which secrete (IFN- $\gamma$ ), also IL-8 which is neutrophil chemotactic that contributes for development of the inflammatory acne lesions by accumulates neutrophils in the follicles and subsequently, generated the pustules with destruction

of follicular walls by proteases which are secreted by accumulated neutrophils<sup>(20)</sup>.

Results about isotretinoin treatment in this study showed that the patients treated with isotretinoin experienced good response and this treatment was more effective comparing with other drugs in this study with few side effects included mild xerosis in all patients and two patients with mild cheilitis. This finding was approximately matched with the previous Iraqi study done by<sup>(21)</sup> (Kubaisi, 2010) who found that the use of isotretinoin low dose regimen (20 mg/ day) showed a good response in (93.5%) at the end of two months of treatment with mild side effects (xerosis, cheilitis and/or epistaxis) also similar results were recorded by<sup>(22)</sup> (Amichai et al., 2006) in patients with moderate acne who were treated with low dose (20 mg/ day) in comparison to the standard dose (0.5 – 1 mg/kg/day).

The present study was in agreement with<sup>(23)</sup> (Läuchli, 2011) who mentioned that isotretinoin works by reducing sebum secretion, follicular plugging, and *P. acnes* proliferation with the common adverse effects like cheilitis and xerosis, which are treated by using frequent emollient applications.

Almost isotretinoin has a 85% cure rate, the high activity of oral isotretinoin used to treat acne may be explained by its ability to reduce about 90% of sebum production by affecting the G1-S of the cell cycle which reduce DNA synthesis that cause an apoptosis of sebocytes and sebaceous gland involution<sup>(24)</sup>.

The current results established that orally given isotretinoin can counter act all pathogenesis mechanism in acne lesions development because isotretinoin affects cellular differentiation which induce apoptosis and reduces the activity sebaceous glands and subsequently reduces both comedones formation and *P. acnes* colonization which in turn reduce the release of proinflammatory cytokines thus isotretinoin exhibit anti-inflammatory activities<sup>(25)</sup>. Isotretinoin has no direct antimicrobial action but it reduces the growth of *P. acnes* by reducing the size of the pilosebaceous duct, so it changes the microenvironment of duct making it less convenient for colonization of *P. acnes* which lead to reduces the inflammatory lesions of acne<sup>(26)</sup>, moreover it is found that the increase in colonization of *P. acnes* in lesions of acne can cause a hydrolysis of sebum

triglycerides by the effect of bacterial lipases result in the secretion of FFAs such as oleic acid that can activate TLR-2 and subsequently Th activation which in turn acts as a trigger for the inflammation<sup>(27)</sup>.

The good results including immunological and clinical responses in the group treated with isotretinoin may be attributed to the ability of isotretinoin to reduce the size and the levels of sebum secretion by inducing sebocytes apoptosis<sup>(28)</sup>, or could be attributed to the ability of isotretinoin to inhibits ROS production, induces keratinocyte apoptosis and reduce IL-8 release<sup>(29)</sup>.

Regarding the decreased levels of TLR-2 reported by the present study in the group treated with isotretinoin, it may be occurred due to the decreases in the expression of TLR-2 on monocytes as it has been mention by previous study which demonstrated that isotretinoin induces acne lesions remission by normalizing the immune response of these lesion to the *P. acnes* and decreasing TLR-2 express on the monocytes and these changes persist for about 6 months after cessation of therapy<sup>(30)</sup>.

**Acknowledgements:** The authors wish to thank all doctors in Dermatology Department in Merjan Teaching Hospital in Babylon who have given us valuable help in diagnostic and collecting the patients. Also, would like to express our deepest gratitude and respect to Al-Mustaqbal University college to contribute to the publication of this research.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

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