Original Article

Evaluation of the Serum Level of Cytokines IL-1B, IL-17 for some Patients with Acne Vulgaris in Baquba City

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DOI: 10.47750/pnr.2022.13.S01.89

Abstract

This study was conducted in Baqubah Teaching Hospital/Consulting Clinic which lasted from the beginning of September 2021 to the end of February، 2022, as the study aimed to investigate the serum level of some immunological parameters in patients with acne in Diyala Governorate from these This study was conducted in Baqubah Teaching Hospital/Consulting Clinic which lasted from the beginning of September 2021 to the end of February، 2022, as the study aimed to investigate the serum level of some immunological parameters in patients with acne in Diyala Governorate from these factors Interleukin 1 beta and Interleukin 17 (IL-17, IL-1B) by using ELISA technique,

The study included 90 individuals, where 58 blood samples were collected from acne patients after their diagnosis by the dermatologist specialized in the Dermatology Division at the Consulting Clinic. The number of males was (46) and the percentage of (79.4%) and the number of females was (12) and the percentage (20.6%), where the results showed a significant increase in males than in females, as well as the study included (32) apparently healthy individuals, 27 males and 5 females were considered as the control group. The results showed that the average age of acne sufferers was 18.879 years. The study also showed that the highest age group affected by acne for males and females is (15-20) years.

The study showed that the levels of cellular kinetics were represented in that the concentration of IL-1B in the serum of acne patients was higher than its concentration in the serum of the healthy group, as the mean ± standard deviation was (128.56 ± 71.06 and 114.86 ± 47.81) pg/ml respectively.

The study also showed an increase in the concentration of IL-17 in the serum of acne patients than in the serum of the healthy group, and this increase was not statistically significant, and the mean ± standard deviation of the group of patients was (37.86 ±23.24) Pg/ml and the healthy group was (26.03 ±15.58) Pg/ml .

Keywords: Acne Vulgaris, Level of Cytokines IL-1B , IL-17.

INTRODUCTION

Acne Vulgaris is a chronic inflammatory disease that is widely spread associated with the pilosebaceous unit of the hair, which includes (hair follicles, hair shaft and sebaceous gland), especially in the face, neck, upper trunk (shoulders) and back due to the large number of sebaceous glands in these places (Williams et al, 2012) .

It is among the most common skin diseases worldwide, with an estimated 650 million people affected (Vos et all, 2012). Acne is considered a chronic disease due to its long course, pattern of recurrence and relapse, acute manifestations and outbreak or slow onset of the disease. Moreover, acne causes profound psychological effects that socially affect the quality of life of patients (Gollnick & Finlay, 2008).

Globally, acne ranks eighth in overall prevalence, with the highest rates reported in Western Europe, North America "high-income", and southern Latin America (Hay et al, 2014).

The onset of acne disease depends on several factors, which is the increased production of sebum from the sebaceous glands and the follicular keratinization of the sebaceous ducts (Dreno, 2017). In addition to these factors, a third major factor in the development of acne was recently revealed, which is the microbiome and its interactions with the Innate immunity system (Hall et al, 2018).

Together, these factors constitute the pathology of the sebum unit of the hair, thus the formation of acne, which begins with the obstruction of the duct of the sebaceous glands (Obstruction_of_Pilosebaceousduct) and then increases its secretion of sebum

production, hormones, microorganisms, and inflammation (Williams, 2012). The appearance of acne is usually associated with the onset of puberty, When sebum production increases. As such, the prevalence of acne increases with age in the age range (10-30) years, with the highest incidence seen in adolescents (14-19) years and a relatively low incidence in prepubertal children (Bhate & Williams, 2013).

After reaching the late teenage years or adulthood at the end of twenty years, acne prevalence rates follow a declining trend with increasing age (Bhate & Williams, 2013; Janani & Sureshkumar, 2019).

The immune system provides protection to the host in the early stage of infection challenges and relies on a set of germline-encoded receptors and molecules that recognize conserved molecular patterns found primarily in microorganisms (Fitzpatricks, 2008).

Cytokines in the immune system have a strong relationship with the pathology of acne, as the level of IL-1B and IL-17 secretion increases in acne patients. And other evidence, which indicates that these cellular kinetics have a major role in the pathogenesis of Acne disease (Choi et al, 2012; Agak et al, 2018).

Material and methods: -

This study was conducted for the period from the beginning of October 2021 to the end of February 2022, as (50) blood samples were collected from patients with acne disease after diagnosis by a specialist doctor in the consulting clinic at Baquba Teaching Hospital in Diyala Governorate. As the number of males was (46) and the number of females was (12) within my age range between (15-30) years, and (32) blood samples were collected from apparently healthy people of both sexes and used as a control group, and the number of males was (27) and Females (5), within my age range between (19-61) years, and did not suffer from any chronic or acute disease. Where (5 ml) of blood was withdrawn through the use of medical plastic syringes and wine, and the drawn blood was placed in test tubes and left for (30) minutes at room temperature for coagulation, then the serums were separated by a centrifuge for (5) minutes at a rate of (3000) cycle/min), and the serum was divided into equal amounts (250) µl in small tubes (Eppendroff) and stored at a temperature of (-20C) until use, and each section of the preserved serum was used once to avoid repeated thawing and freezing of the sample.

Measurement of serum level of cytokines IL-17 , IL-1B

The levels of serum Interleukine (IL-17, IL-1B) were quantitatively determined by using the Sandwich ELISA test for (58) acne patients and (32) healthy people according to the instructions contained in the examination kit manufactured by (Korain Company). and the principles of testing shown in Figure (1)

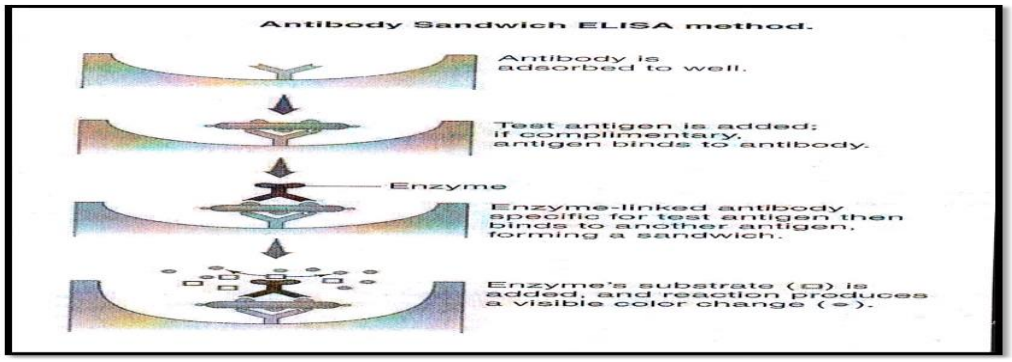


Figure (1) ELISA sandwich test principle (Talaro,2005)

First:- Test working principle

This test is used in a quantity of ready-made enzymes and according to the immuno assay technique, as the specialized antibodies for Interleukine 1B and Interleukine 17 are lined for accurate calibration holes, standards and samples were placed in these holes and in the presence of (IL-17, IL-1B) A link occurs with the consumption of antibodies, then the unlinked materials are removed, by adding biotin, as it works to bind specialized antibodies (IL-17, IL-1B) when Add it to the drill, after the washing process avidin is added to the drill , avidin that binds an enzyme (HRP) and an enzyme (HRP) is added to the drill, and then the washing process was carried out to remove any unbound substances and any obstruction that may hinder the reagents (avidin-enzyme reagent) and the base material is added Substrate Tetramethyl Benzinide (TMB) to the pits, and after adding these solutions, the color ratios begin to change according to the amount of (IL-17, IL-1B) associated in the first step, then the color change stops by adding the sulfuric acid to the reaction, so the blue color turns to yellow The optical density (O.D) is measured and the result is read by an ELISA reader at a wavelength of 450 nanometers (Talaro, 2005).

Second: Kit, components:

1- Micro Elisa Plate

2- Reference Standard

3- Concentrated Biotinylated Detection Ab

4- Concentrated HRP Conjugate

5- Reference Standard & Sample Diluent

6- Biotinylated Detection Ab Diluent

7- HRP Conjugate Diluent

8- Concentrated Wash Buffer

9- Substrate Reagent

10- Stop Solution

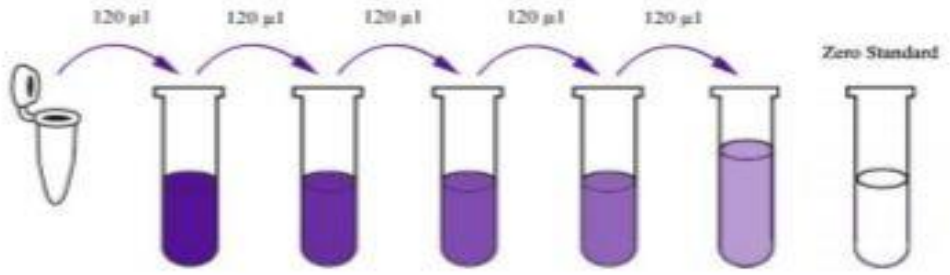
11- Plate sealers

Third: Preparation of IL-17 and IL-1B solutions.

All solutions were placed at room temperature 30 minutes before use. The solutions were prepared immediately during the use of each test according to the manufacturer's instructions, taking into account avoiding foaming and using mixing and continuous movement until the insoluble crystals are dissolved and a series of dilutions are prepared using distilled water working from it. Dilution to obtain the stock measurement solution (320) ng/L.

1- The standard standard whose concentration is (640) nanograms / liter using distilled water, dilution works from it to obtain the standard stock solution (320) nanograms / liter

2- Standard solutions (S1, S2, S3, S4, S5) were prepared by using serially diluting the Standard Stock Solution (320) with standard diluent in order to generate the following concentrations (S1 = 20 , S2 = 40). S3 = 320 = 320 S4 = 320 . In the case of IL-1B, in the case of IL-17 it is (S1 = 50, S2 = 100, S3 = 200, S4 = 400, S5 = 800) pg / Ml



3- Wash buffer:

20 ml of washing buffer (its concentration 25 X) was diluted to 480 distilled water to yield 500 ml (1 X) concentration.

If crystals are generated in the concentrate, mix gently until completely dissolved.

Fourth:- Method of work , IL – 1B , IL-17 \ Assay Procedure

1- After preparing all the solutions, they are left at room temperature in order to complete the method of work at this temperature

2- 50 µl of standard (S5, S4, S3, S2, S1, S0) were added to the standard bore.

3- 40 µl of samples were added, then 10 µl and the specific antibodies according to the type of cellular kinetics (IL-1B , IL-17) were added.

4- 50 µl of Conjugate (Streptaviden-HRP . conjugate) was added

5- Mixed well, covered the dish, and then incubated it at 37°C for 60 minutes

6- The cover was removed and then the dish was washed 5 times with a washing buffer

7- Leave the plate to dry

8- 50 microliters of substrate solution (A) were added to each hole, and then 50 microliters of substrate solution (B) was also added to each hole.

9- The dish was covered with a new lid and incubated for 10 minutes at a temperature of 37 °C and in a dark place

10- 50 microliters of the stop solution of sulfuric acid were added, and the blue color turns to yellow immediately

11- The optical density (O. D) was read for each hole within 10 minutes of adding the stop solution at a wavelength of 450 nm.

12- The concentrations of IL-1B, IL-17 were found from the following curves

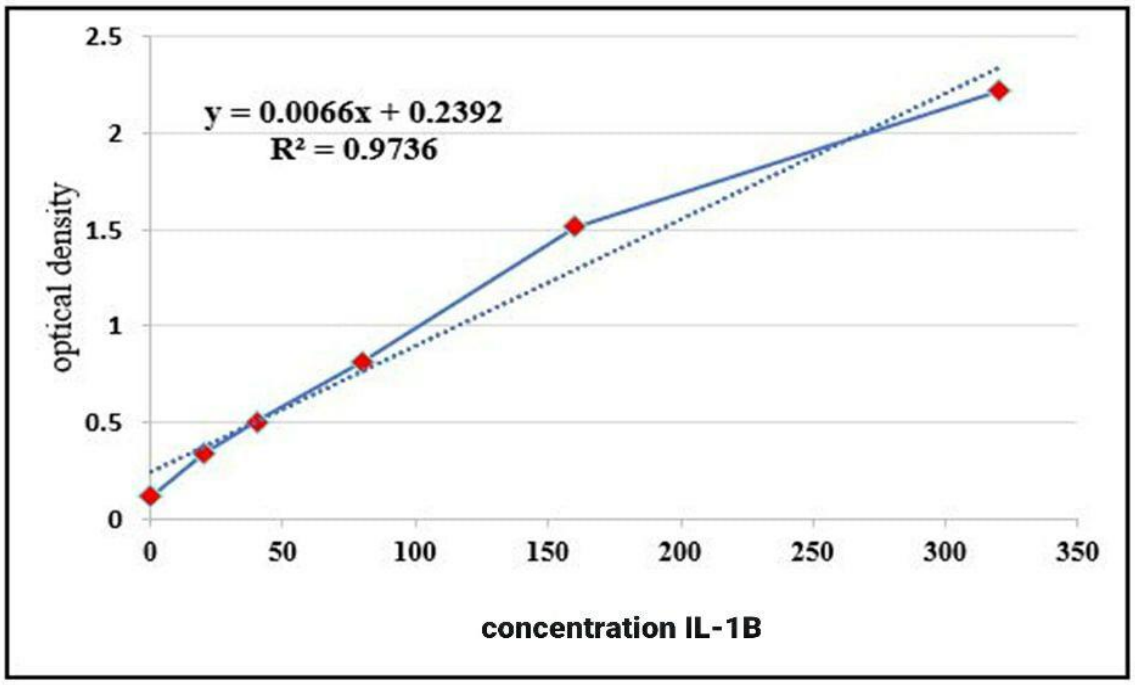


Figure (2): Standard curve for Interleukine first beta (IL-1B).

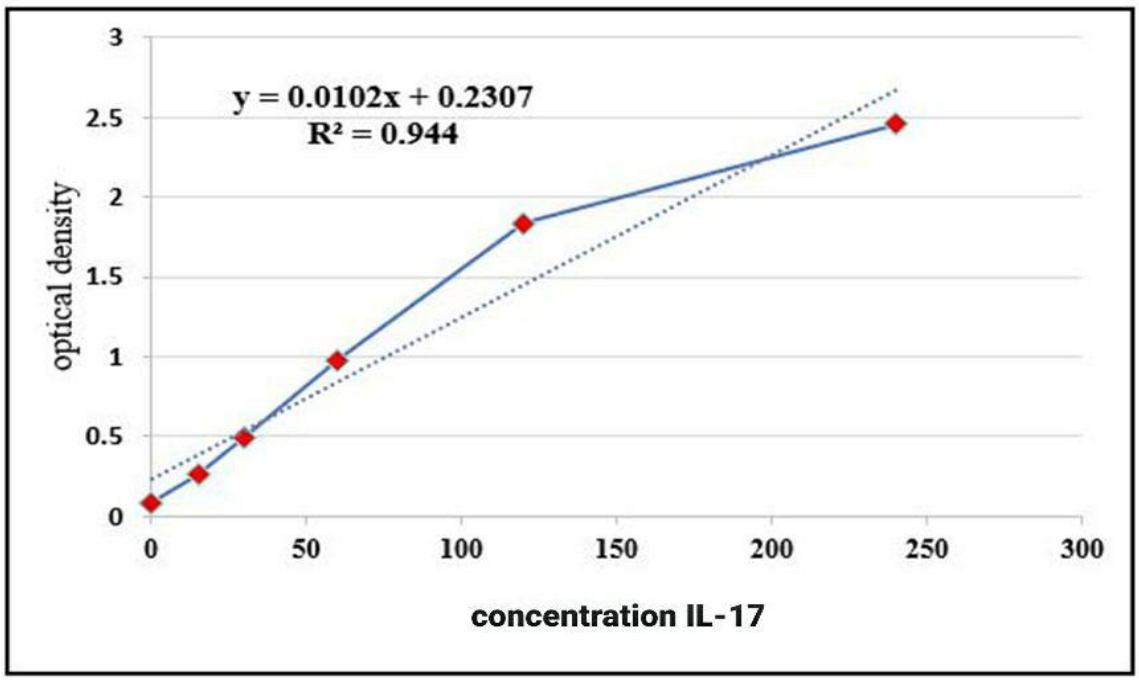


Figure (3): Standard curve for Interleukine 17 (IL-17).

Resuls and Discussion: -

1- Results of measuring IL-1B concentration in serum of acne patients and healthy group

Table (1) shows that the concentration of IL-1B in the serum of acne patients was higher than its concentration in the sera of the healthy group, as the mean ± standard deviation in the group of patients was 128.56 ± 71.06 and 114.86 ± 47.81 Pg/ml in the healthy group and this is The increase in focus was unimaginable Significantly, the value of P = 0.222.

Table (1) Measurement of IL-1B concentration in serum of Acne patients and healthy group

Mg/dl		Two study group pg/ml		P – Value
		Patients (58)N	Control (32)N	
IL-1B	Mean	128.56	114.86	0.222
	S.D	71.06	47.81	
N.s: insignificant *				

The results of the current study are in agreement with the results of the study conducted by Dashko et al (2018) in Poland on 114 acne patients aged between 18 and 35 years, where the results indicated an increase in IL-1B concentration in most of the patients with acne compared to the control group.

This is reinforced by what was stated in the study conducted by El-Attar et al in (2022) on a group of acne patients (60 patients) and twenty healthy volunteers as controls, which showed an increase in the concentration of IL-1B in the sera of acne patients compared to the healthy ones. Statistically significant increase in the expression of IL-1β in acne vulgaris compared with controls ($P < 0.001$) for both. IL-1β expression was positively correlated with both clinical severity of acne ($P = 0.022$) and severity of tissue inflammation ($P = 0.011$).

Since the association of increased expression or concentration of Interleukin-1β with acne is a significant positive association with clinical and pathological severity of acne, IL-1β could therefore be a major player in the pathogenesis, severity and development of post-acne scarring (El-Attar et al, 2022)

The results also agreed with the results of the study conducted by Kelhala et al (2014) in Germany on a group of acne patients (56 patients) which showed an increase in the concentration of IL-1B in the sera of acne patients compared to the healthy controls.

On the same line, Kistowska et al found elevated levels of IL-1B mRNA in skin biopsies from papular pustular acne (50-fold) when compared to mild pimples where C.acne secretes chemicals that stimulate IL-1B cytokine secretion by monocytes. IL-1B secretion is the main driver of inflammatory responses in acne and is mediated by activation of the inflammasome (Kistowska et al, 2014).

In another study conducted by Thanh et al in Vietnam in (2022) including 20 patients with acne, this study shows elevated immune activity of IL-1B in papular biopsies of inflammatory acne. Increasing the severity of the disease. This cytokine IL-1B could be a good candidate for targeting the treatment of acne vulgaris.

The results of the study conducted by Askari et al in Iran in (2017) on a group of patients with acne showed that the average levels of IL-1B are significantly higher in patients with acne than those who do not suffer from acne, where the study showed a relationship Positive correlation between serum levels of pro-inflammatory cytokines (IL-1β, IL-8, and IL-12) and acne vulgaris.

2- Results of measuring IL-17 concentration in serum of acne patients and healthy group

Table (2) shows an increase in the concentration of IL-17 in the serum of acne patients than it is in The serum of the healthy group and this increase was not significant as the P-value = 0.545 and the mean ± standard deviation of the group of patients was 37.86 ± 23.24 and the group of the healthy group was 26.03 ± 15.58 Pg/ml.

Table (2) Measurement of IL-17 concentration in serum of Acne patients and healthy group

Mg/dl		Two study group pg/ml		P – Value
		Patients (58)N	Control (32)N	
IL-17	Mean	37.86	26.03	0.222
	S.D	23.24	15.58	
N.s: insignificant *				

The results of the current study are in agreement with the results of the study conducted by the researcher Dashko et al (2018) in Poland on 114 patients with acne, where the results indicated an increase in the concentration of IL-17 in most patients with acne compared to the control group. Also, the results of the study conducted by Kelhala et al (2014) in Germany on a group of acne patients (56 patients) showed an increase in the concentration of IL-17 in the sera of acne patients.

The results of the current study are also in agreement with the results of Murlistyarini et al 's study in Indonesia in (2018), which was conducted on 68 patients with acne. The results indicated an increase in the concentration of IL-17 in patients compared with healthy subjects, but there was a difference in the average levels of IL-17 concentration in Serum between groups of patients with different severity of acne (AV Acne Vulgaris) means that the concentration levels of the cytokine IL-17 in the patients' serum increased in line with the increase in the severity of their acne.

Also, the results of the current study are in agreement with the results of the study of Agak et al in America in (2014) which was conducted on a group of patients with acne. The results indicated an increase in the concentration of IL-17 in the patients' sera compared with the control group.

IL-17 plays a role in the development of inflammatory lesions as C.acne is believed to be able to stimulate the production of IL-17 in the peripheral region, in addition to its IL-17 receptors IL-17RA and IL-17RC-IL-,2014) (Kelhala et al., 2014).

Agak et al. reported that the immunostimulatory protein molecules secreted by C.acne in the follicle are processed by Langerhans cells and presented to CD4+ T cells. Secretion of the cytokine would cause the differentiation of CD4+ T cells into Th-17 cells, which would then act to produce the cytokine IL-17 leading to the formation of inflammation and this is one of the characteristics of AV (Agak et al, 2014).

Agak et al reported that in (2014) when C.acnes was isolated from acne patients the bacterium was able to significantly stimulate IL-17 secretion ($p < 0.001$) in human peripheral blood mononuclear cells (PBMCs) with an average value of 500 - 700 pg/ml.

Moreover, IL-17 plays a role in proliferation and differentiation of keratinocytes as Lai et al (2012) showed that after binding of IL-17 to the IL-17RA receptor in keratinocytes, keratinocytes were sensitized to expression of the regenerating islet-derived protein. 3-alpha (REG3A) This protein modulates keratinocyte feedback to inhibit the termination of differentiation processes, and increases cell proliferation by binding to (EXTL3) exostosin-like 3, followed by activation of phosphatidylinositol 3-kinase. Whereas in other studies the roles of IL-17 have been determined by stress-activated protein kinase (CIKS) signaling and thus cause hyperproliferation and disturbed differentiation of keratinocytes.

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