ISSN NO: 2770-2936
Date of Publication:20-09-2022

Evaluation of the Serum Level of Complement Proteins C3, C4 and Immunoglobulins IgG, IgM for some Patients with Acne Vulgaris in Baquba City

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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 factors IgM, IgG immunoglobulins and C4, C3 complement proteins by using single Radio immunodiffusion method.

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 results of the levels of complement proteins C4 and C3 showed that the level of C3 in the serum of acne patients had a significant and statistically significant increase compared to its level in the serum of healthy subjects (P=0.001)) and the mean was ± standard deviation (138.51 ±85.50 and 49.73 ±28.23) on the Consecutively, while the increase in C4 concentration was highly statistically significant compared to the healthy group (P = 0.005), and the mean ± standard deviation was (76.32 ± 20.53 and 42.01 ± 19.28), respectively.

The results of the immunological study with regard to the levels of IgG and IgM immune antibodies indicated that the level of IgG increased significantly in patients serum (821.53 ± 59.37 mg/dl) compared to the control group (179.35 ± 36.91) mg/dl, while the levels of IgM recorded an increase in the patients serum, but this increase was Not significant compared to the healthy group (203.95 ± 190.80) (103.61 ± 89.91) mg/dl, respectively.

Keywords: Acne Vulgaris ,Complement Protein C3 C4 ,Immunoglobulins IgG IgM

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).

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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).

Also, (IgG,IgM) antibodies enhance inflammatory disorders of the skin (acne) after identifying the etiology, as they have an important role in the development of the disease (Cipriani *et al*, 2014; Hammers and Stanley, 2016).

There is evidence that activation of the complement system can be one of the primary triggers in the inflammatory response to acne, Puhvel et al. 1966 revealed higher levels of complement proteins present in patients with severe acne compared with patients with mild acne ((Puhvel et al, 1966)

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 immunoglobulins (IgG, IgM) and complement proteins (C3, C4) by using single Radio immunodiffusion method:-

First: The working principle:

This test was used to measure the level of immunoglobulins (IgG, IgM) and the level of complement proteins C3, C4 (Mancini et al, 1965). The principle of which depends on the quantification of antigen by measuring the diameters of the precipitated circles surrounding the antigen samples that distinguish the boundaries between antigen and The antibodies are suspended in the center of the agarose gel containing the specific antibodies.

Second:- The method of work:

1- The samples were extracted and left at room temperature for 15 minutes.

2- The plate cover was removed and placed on a stable stand and left at room temperature for a few minutes to allow the condensed water droplets in the holes to evaporate.

3- (5) microliters of samples were added to the pits in equal volumes and left until absorbed by the pits without moving.

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4- The plate was covered and transferred to the Moist chamber and left for (72) hours for (IgG) and (C3 , C4), and for (96) hours for (IgM)

5- The sedimentation diameters were measured by means of a magnifying glass and these results were compared with the standard tables of the company that prepared for the extraction of concentrations of immunoglobulins (IgG, IgM) and concentrations of complement proteins (C3, C4)

Resuls and Discussion :-

1- Results of measuring the level of (IgG and IgM) in the serum of the two groups of patients and healthy ones

The results of the current study showed that the level of IgG in the serum of acne patients was significantly increased with a statistical significance compared to its level in the serum of healthy controls (821.53 ± 59.37 and 179.35 ± 36.91), respectively. As for IgM levels, an increase was recorded in the serum of patients compared to the healthy group, but it was not significant. Statistics (203.95 ± 190.80 and 103.61 ± 89.91), respectively, as shown in Table (1)

Table (1) Comparison of the level of IgG and IgM in the serum of the two groups of patients and healthy ones

Mg/dl		Two study group		P – Value
		Patients (58)N	Control (32)N	
IgG	Mean	821.53	179.35	0.001*
	S.D	59.37	36.91	
IgM	Mean	203.95	103.61	0.005
	S.D	190.80	89.91	
* = There is a significant statistical difference				P ≤ 0.001

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 most of the acne patients had varying degrees of changes in immune rates. Systemic - the potential decrease in the relative and absolute number of total lymphocytes, T lymphocytes and their subpopulations versus the increased number of B lymphocytes and levels of IgM and IgG, which are intensified by activation of humoral immunity in response to the development of inflammation compared with healthy subjects. While in the study of Hurtado-Nedelec *et al* (2008) the levels of IgG and IgM in Britain in the sera of patients and healthy people were within the normal range.

The results of the current study also agreed with the results of the study of Yue-Xian *et al* in China in (2018), which was conducted on 300 cases of acne, where the cases were divided into three groups. It was found that there was an increase in the levels of both IgG and IgM significantly in the group of acne patients. Young men compared to their levels for the healthy group, but at different levels within the groups, where the levels of IgG and IgM in group (A) were lower than the levels found in both groups (B) and (C).

The results of the current study also agreed with the results of the study of Syzon *et al* in (2019), which was conducted on a group of patients, where the results showed that there is a significant increase in the levels of both IgG and IgM in the group of acne patients compared to their levels in the healthy group.

In another study conducted by Taro Horino *et al* (2021) in Japan on a patient with acne, the results showed elevated levels of IgG and IgM in the patient's serum. In another study conducted by Chiyoko *et al* in (2017) on a patient with acne Synaptic (AC) with abscesses on the face and neck and also with T13 disease (pigmentation trimester) The results showed a decrease in the level of IgM in the serum of the patient and an increase in the level of IgG where the clinical features and laboratory data of the patient support the

ISSN NO: 2770-2936
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diagnosis of selective IgM deficiency and risk of developing immunodeficiency which includes T13-induced SIgMD in this patient as T13 may represent a syndromic disorder associated with multiple organ malformation and risk of developing immunodeficiency including SIgMD.

The results of this study did not agree with the results of another study conducted by Mohanty and Kumar in India (2019) on a 20-year-old girl who complained of inflammatory lesions in the face, neck, back and abdomen for 8 months and was diagnosed with second-degree acne. The results of the study that the test of both IgG and IgM levels were negative.

The differences in the levels of these immunoglobulins from one study to another may be due to the different numbers of patients included in the study, as well as the difference in the degree of disease activity for these groups, or it may be that some patients included in the study have taken a specific type of immunomodulatory therapy. The reason may be that these differences may be racial or ethnic, or there may be a specific factor that causes the level of different immunoglobulins to vary (Singh *et al*, 2012).

Antibodies specifically target the acne-causing bacteria, so the level of these antibodies increases in the blood serum. This is done after killing and cutting the bacteria by macrophages. The macrophages inject toxic molecules and enzymes into the vacuole after the phagocytic cell of the bacteria is isolated inside this vacuole. These molecules and enzymes kill the bacteria and digest them into small pieces. These pieces are then presented to the immune system, which uses them to design specific antibodies (and many other things) (Buman.2011; Gladwin, 2011)

Immunoglobulin levels depend on several factors, including genetic factors, environmental factors, chronic disease, recurrent infections, aging, and gender, as well as geographical factors. And a general reduction in the immune response (AL-Dabbagh *et al*, 1988). Janeway *et al* (2016) indicated that B-cells are responsible for the production of immunoglobulins and that the amount and type of antibodies secreted depends on the physical interaction between antigen presenting cells (APC) and T- and B-cells. and other factors, including the production of cellular kinetics and chemical kinetics (chemokines)

2- The results of measuring the levels of complement proteins C3 and C4 in the serum of the two study groups:-

The results of the current study showed that the level of C3 in the serum of acne patients had a significant and statistically significant increase compared to its level in the sera of the healthy ones, where the P value was 0.001 and the mean mean ± standard deviation was (138.51 ± 85.50 and 49.73 ± 28.23), respectively, while the increase In the C4 concentration, it was highly statistically significant compared to the healthy group, as the P value was 0.005 and the mean ± standard deviation was (76.32 ± 20.53 and 42.01 ± 19.28) as shown in Table (4-13). (138.51 ± 85.50 and 49.73 ± 28.23) (76.32 ± 20.53 and 42.01 ± 19.28) as shown in Table (2)

The results of the current study are in agreement with the results of the study of Yue-Xian *et al* in China in (2018), which was conducted on 300 cases of acne, where the cases were divided into three groups. It was found that there was an increase in the levels of complement proteins, both C3 and C4, significantly in a group of patients. Acne compared to the levels of the healthy group, but at different levels within the groups.

Table (2) Comparison of C3 and C4 complement proteins between the two groups of patients and healthy controls

Mg/dl		Two study group		P – Value
		Patients (58)N	Control (32)N	
C3	Mean	138.51	49.73	0.001*
	S.D	85.50	28.23	
C4	Mean	76.32	42.01	0.005
	S.D	20.53	19.28	
* = There is a significant statistical difference				P ≤ 0.001

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The results of the current study also agreed with the results of the study of Faraj in Baghdad in (1991), which was conducted on 200 cases of acne, and it was found that there is an increase in the levels of complement proteins of C3 and C4 significantly (P value <0.005) in the group of acne patients compared to levels in the healthy group. Also, in the study of Cree et al. (1979), the activity of C3 and C4 complement proteins was determined in 11 patients with acne.

The results of this study did not agree with the results of another study conducted by Mohanty and Kumar in India (2019) on a 20-year-old girl who complained of inflammatory lesions in the face, neck, back and abdomen for 8 months and was diagnosed with second-degree acne. The results of the study that both C3 and C4 levels tested were negative.

Also, the results of this study did not agree with the results of another study conducted by Gregor and Ida Rasmussen in America, where the levels of C3 and C4 complement proteins were normal in patients.

There is evidence that complement activation proteins C3 and C4 are one of the primary catalysts in the inflammatory response to acne. In 1966, Puhvel *et al*. reported higher levels of complement-fixing antibodies in patients with severe acne compared to patients with mild or no acne (Puhvel *et al*, 1966).

Scott *et al* conducted a statistical analysis of non-inflamed (white and black heads) and inflamed (papules and nodules) acne lesions. The results of the study indicated that the most common area for the deposition of complement proteins is the basement membrane of the linear fatty granular basement membrane (of the affected units) where it was proven that there is a deposition of complement. C3b is seen in both inflamed and non-inflamed acne lesions.

Similar results have been reported by Dahl *et al* in early or non-inflamed acne lesions where the most common area of complement protein deposition was also the basement membrane region.

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