RESEARCH ARTICLE

Clinical and Immunological Response to Doxycycline Versus Doxycycline Plus Vitamin C in Patients with Acne Vulgaris

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ABSTRACT

Objective: Study the influence of doxycycline versus doxycycline with vitamin C drugs in the management of acne vulgaris on some immunological parameters which include [Interleukin 1 beta (IL-1β), interleukin 1 (IL-8), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and toll-like receptor 2 (TLR2)] and the following objectives were adapted: 1-Selection of population for the study (control, patients) groups and follow up the patients after one month of treatment, the first group receive doxycycline and the second group receive doxycycline with vitamin C,2- Blood samples collection and separation of serums for immunological analysis, 3- Statistical analysis.

Methods: This study is a randomized clinical trial carried out in clinical dermatology in Merjan Medical city in Babylon from September 2018 to March 2019. The number of subjects enrolled in the present study was 60; their age was between (14–30 years), among whom 30 were acne patients, the remaining 30 subjects were apparently healthy individuals, and they were served as control**.** A dermatologist diagnosed a total of 30 acne patients to having moderate to severe acne and divided into two groups (15 patients in each group). Patients in the first group were treated with doxycycline (100mg) once daily after meal for 30 days, while in the second group patients were treated with doxycycline (100mg) capsule once daily after a meal in combination with vitamin C (500mg) chewable tablets once daily. After 1-month of therapy, the response was evaluated clinically and immunologically by measure the concentration of pro-inflammatory cytokines (IL-8, IL-1β, IFN-γ, TNF-α) and (TLR-2) by using enzyme-linked immunosorbent assay (ELISA) and the results were compared to their levels before treatment and that in the control group.

Results: Significant elevation in the serum levels ($p \leq 0.001$) of immunological parameter IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2 among acne patients in comparison to the control. The clinical response in the first group was good, moderate and poor in 5 (33%), 7(47%) and 3(20%) respectively, while in the second group was good, moderate and poor in 7 (47%), 7(47%) and 1(6%) respectively, the immunological results showed that the serum levels to the (IL-1β, IL-8, IFN-γ, TNF-α) and (TLR-2) were more reduced in the second group compared to their levels in the first group.

Conclusion: Significant elevation ($p < 0.001$) in the serum levels of (IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2) among moderate to severe acne patients in comparison to control group. Clinically the combination of doxycycline plus vitamin C was more efficient as therapeutically in comparison to doxycycline alone. Immunologically doxycycline plus vitamin C was more effective in reducing serum levels of (IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2) in comparison to doxycycline alone.

Keywords: Acne vulgaris**,** Doxycycline, Immunological parameter IL-8, IL-1β, TNF-α, IFN-γ, TLR-2, Vitamin C.

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INTRODUCTION

Acne vulgaris is a common disease that affects the pilosebaceous units in the skin and may produce non-inflammatory or inflammatory lesions.[1,2] It starts to appear after the onset of puberty; it can affect about 80% of young adults and adolescence.[2] Acne vulgaris can cause a clear detrimental psychosocial effect and may cause permanent scarring.[3]

Regarding the pathogenesis, initially, it is produced from the overproduction of sebum in the follicles of sebaceous glands of the skin, which are more present in the upper back and face. The hyperkeratinization of the follicle contributes to acne development, also gram-positive anaerobe bacteria Propionibacterium acnes (P.acnes), which colonizes within the sebaceous follicles releases enzymes (proteases, lipases),

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which was associated with the development of inflammation and induction of inflammatory mediators, that can plugged follicles and become inflammatory lesions which appear like papules, pustules, and nodules.[2,4-6]

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.[7] These cells have pattern recognition receptors (PRRs), which can recognize a large variety of pathogenic organisms 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),[8] 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 pro-inflammatory cytokine included in acne lesions,[9] like interleukins (IL-1β, IL- 1α, IL-12I, and L-8), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ).[10-12]

The main goal in the treatment of acne is to treat and control the present acne lesions, to prevent scare formation, decrease the duration of acne disease and to reduce morbidity.[2] Systemic antibiotics (oral) are common therapies to moderate the severe acne such as tetracycline, minocycline, doxycycline, erythromycin, and trimethoprim/sulfamethoxazole combination; antibiotic decrease P. acnes and some have anti-inflammatory effects.[5] Also, oral isotretinoin used in patients with moderate to severe acne, which affects almost all factors in acne pathogenesis.[13] Moreover, antioxidants are necessary for neutralizing the reactive oxygen species (ROS) formed due to inflammation, and vitamin C is an important antioxidant that can protect the skin from ROS. Also, it has an effect on stimulating collagen production, decreases post-inflammatory hyperpigmentation, enhances wound healing, and decrease the activation of various pro-inflammatory cytokines like TNF-α, IL-1, IL6, and IL8.[14,15]

Aim of Study

The comparison of the clinical and immunological response in patients with acne to doxycycline and doxycycline with vitamin C through the influence on some immunological parameters including IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2.

MATERIALS AND METHODS

Study design

The present study is a clinical trial carried out in clinical dermatology in Merjan Medical City in Babylon from September- 2018 to March – 2019. 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 a mean (19.60 ± 3.819), regarding the gender the nine females were included, and six patients were male suffering from moderate to severe acne. Blood samples were taken from all patients; then they were divided into two groups types the response were evaluated clinically and immunologically, (15 patients in each group) as followers :

- *A-Group 1*

Patients were treated with doxycycline (100mg) once daily after meal.

- *B-Group 2*

The patients were treated with doxycycline (100mg) capsule once daily after meal for in combination with vitamin C (500mg) chewable tablets once daily.

The response to these two types of treatment was evaluated clinically and immunologically after one month of therapy.

Control Group

Included 30 subjects who appeared healthy and their age were between (14-30 years) with a mean (19.65±3.799), subjects in the control group include nine females, and six males, blood samples were taken from each subject. The control and patients groups were matched in age and sex.

METHODS

Blood Collection

About (5 mL) were taken from venous blood of healthy and patients who were enrolled in this study through venipuncture via using a disposable syringe. Blood samples were placed in gel tubes (8 mL) after that allowed the blood to be clotted, then was centrifuged at speed (3000 rpm) for about 10 minutes to obtain the serum.[16]

The serum was put in an Eppendorf tube and immediately frozen at (-80C) until thawed for assay.[17]

Follow up 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, also measured the concentrations of cytokines in the serum (IL-1β, IL-8, IFN-γ, TNF-α) and (TLR-2) by using enzyme-linked immunosorbent assay (ELISA), and compared to their concentration before the initiation of the treatment.

Also, serum levels of (TNF-α, IL-1β, IL-8, IFN-γ) and TLR-2 measured in the control group and were compared to their concentration in the serum samples taken from the patients.

Laboratory Investigations

Cytokines serum levels (IL-8, IL-1β, TNF-α, IFN-γ) and TLR-2 measured by using cytokines ELISA kits (Elabscience company, china).

Ethical Approval

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

Statistics Analysis

Data analysis by using (version 21) of SPSS. Variables that were presented as percentages and frequencies. Variables that

presented as (means ± SD). Student t-test used for comparing the means of two groups. Pearson's chi-square (X2) that used to find the association between groups. The significant p-value was (≤0.05).

RESULTS

Clinical Response

In group 1, the clinical response was good, moderate and poor in 5 (34%), 7(47%), and 3(19%), respectively, in patients who treated with doxycycline (Table 1).

In group 2, the clinical response was good, moderate and poor in 7 (47%), 7(47%), and 1(6%), respectively, in patients who treated with doxycycline plus vitamin C.

The Immunological Response

Serum levels of (TNF-α, IL-8, IL-1β, IFN-γ, and TLR-2) among subjects of the study groups (Table 2).

Table 2 shows the mean values of the studied immunological parameters (IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2) according to the study groups (patients with acne before treatment and control group). The means of cytokines, according to study groups, were high significant (p ≤0.001) differences between means of all measured cytokines according to the study groups.

Serum Levels of (IL-8, IL-1β, IFN-γ, TNF-α, and TLR-2) According to the Type of Treatment

Serum Levels of TLR-2

Figure 1 shows that the serum levels of TLR-2 were significantly lower (p-value ≤ 0.05) in the group treated with doxycycline plus vitamin C in comparison to the group which was treated with doxycycline alone after one month of therapy.

Serum Levels of IL-1β

Figure 2 shows that there was no significant different (p-value ≥ 0.05) between doxycycline and doxycycline plus vitamin C, but the mean level in second group less than first group and more near to the mean of control group (1.092 ± 0.07)after one month of treatment.

Serum Levels of IL-8

Figure 3: Shows there was no significant difference (p ≥ 0.05) between doxycycline and doxycycline plus vitamin C, but the mean level in the second group less than the first group and more near to the mean of a control group (9.30 ± 2.44) after 1-month of treatment.

Serum Levels of TNF-α

Figure 4 shows that there was no significant difference (p ≥ 0.05) between doxycycline and doxycycline plus vitamin C, but the mean level in the second group less than the first group

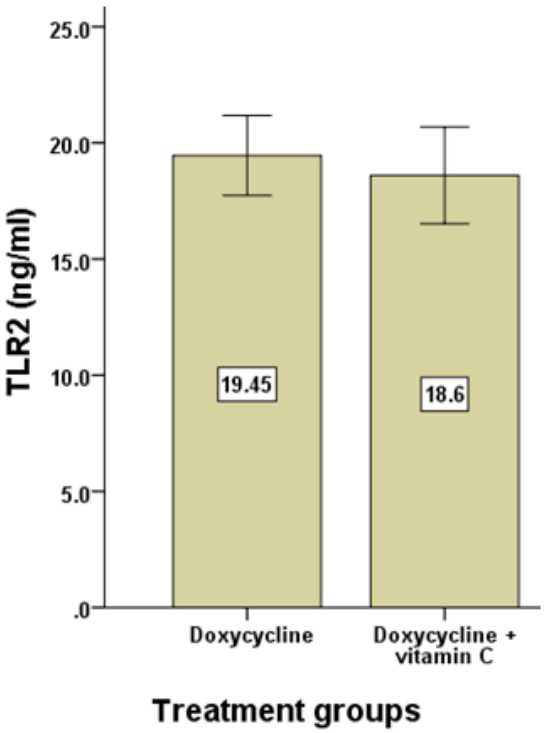

Figure 1: Mean serum levels of TLR-2 after one month of therapy with doxycycline and doxycycline plus vitamin C.

Table 1: Clinical response to the treatment after one month therapy with doxycycline alone and doxycycline plus vitamin C.

Treatment	*Good response*	*Moderate response*	*Poor response*	*Total*
1-Doxycycline	5(33%)	7(47%)	3(20%)	15(100%)
2-Dxycycline + Vitamin C	7(47%)	7(47%)	1(6%)	15(100%)

Table 2: Serum levels of the cytokines in patients and control groups.

Markers	*Groups*	*N*	*Mean*	*SD*	*t-test*	*P-value*
TLR-2	Patients	30	23.55	1.16	30.99	<0.001*
	Control	30	16.343	1.37		
IL-8	Patients	30	16.70	4.73	10.74	<0.001*
	Control	30	9.30	2.44		
IL-1β	Patients	30	4.027	0.54	41.5	<0.001*
	Control	30	1.092	0.07		
TNF-α	Patients	30	6.226	1.15	16.501	<0.001*
	Control	30	2.778	1.12		
IFN-γ	Patients	30	16.44	5.20	8.53	<0.001*
	Control	30	10.404	1.69		

*p ≤0.001

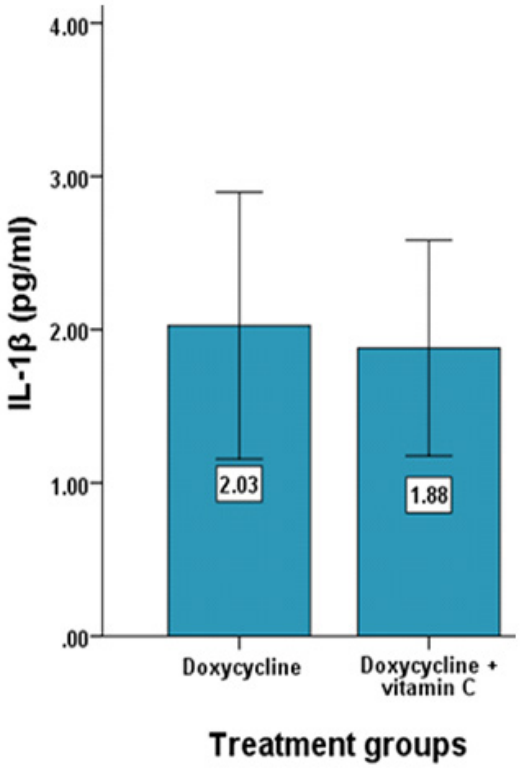

Figure 2: Mean serum levels of IL-1β after one month of therapy with doxycycline and doxycycline plus vitamin C.

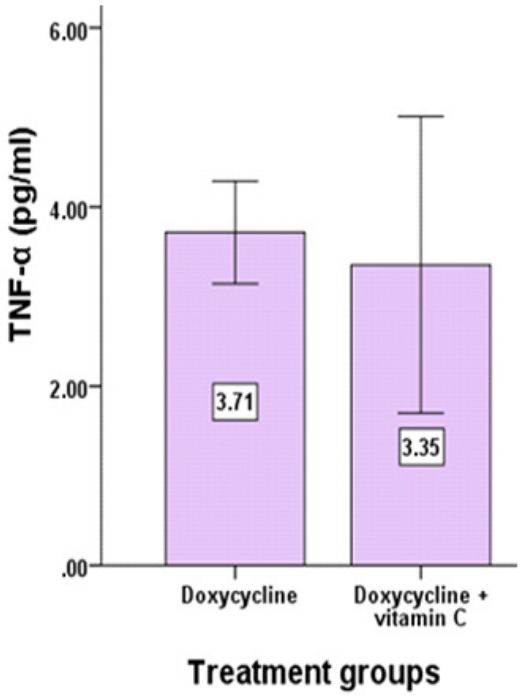

Figure 4: Mean serum levels of TNF-α after one month of therapy with doxycycline and doxycycline plus vitamin C

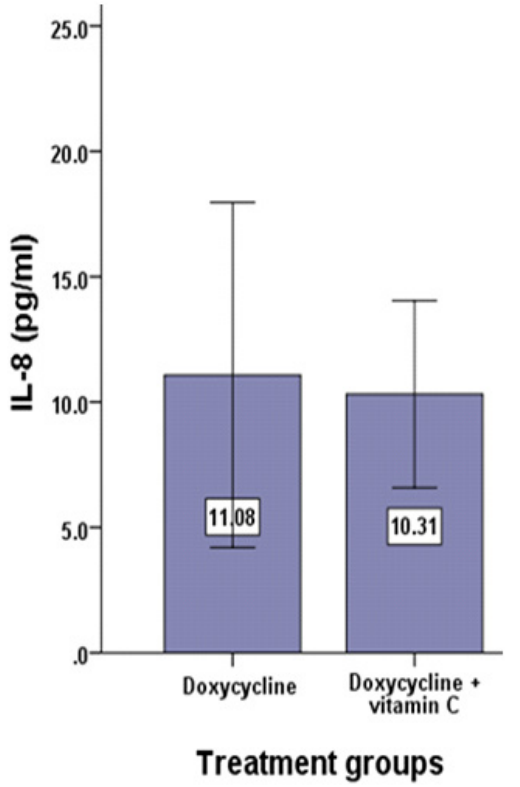

Figure 3: Mean serum levels of IL-8 after 1-month of therapy with doxycycline and doxycycline plus vitamin C.

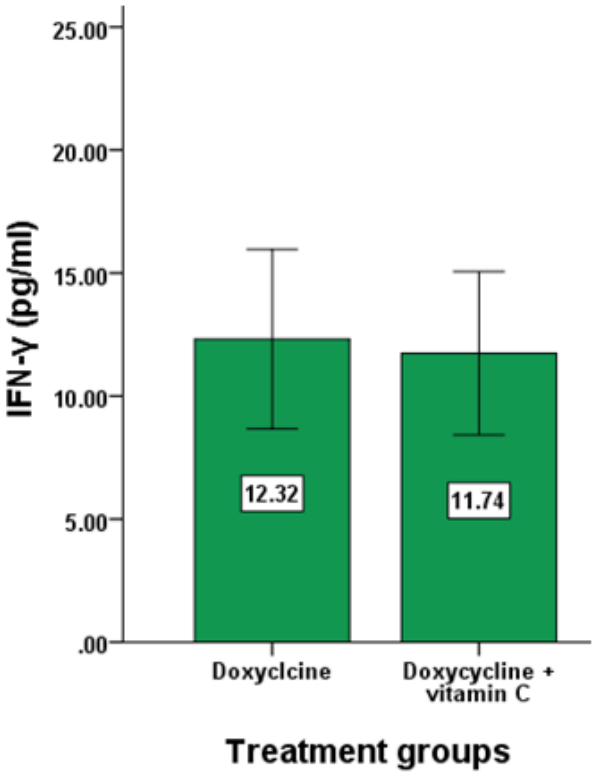

Figure 5: Mean serum levels of IFN-γ after one month of therapy with doxycycline and doxycycline plus vitamin C.

and more near to the mean of a control group (2.778 ± 1.12) after 1-month of treatment.

Serum Levels of IFN-γ

Figure 5 shows that there was no significant difference ($p \geq 0.05$) between doxycycline and doxycycline plus vitamin C, but the mean level in the second group less than the first group and more near to the mean of a control group (10.404 ± 1.69) after one month of treatment.

DISCUSSION

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

The TLR-2 (trans-membrane receptors), which are associated to the response of the innate immune system, TLR-2 is present in sebocytes, keratinocytes, dendritic cells, monocytes and macrophages that play an important role in the development of comedones and inflammatory lesions by stimulation of nuclear factor kappa B (NFkB) cause stimulation of different pro-inflammatory cytokines such as (IL-1α, IL-8, IL-12) and by stimulation several cells, like natural killer (NK), macrophages and neutrophils also the adaptive immune response is stimulated.[19]

In the present study, the level of TLR-2 (23.55 ± 1.16) was highly significantly increased ($p \leq 0.001$) in acne patients group as compared to the control group (16.343 ± 1.37). This result indicates a positive correlation between TLR-2 level and development of acne and this may be attributed to the ability of P. acnes to express particular pathogen-associated molecular patterns (PAMPs) which are recognized via TLR-2 and cause upregulation of TLR-2, which consequently releases cytokines which are responsible for the cause inflammatory response.[18-20]

IL-8 is a potent chemotactic factor that is released by different types of cells (e.g., monocytes, macrophages, and epithelial cells), and IL-8 is main pro-inflammatory mediators that induced in monocytes by the presence of a pathogen; thus, in the innate immunity, the key pro-inflammatory mediator against pathogen is IL-8 by inducing neutrophil chemoattraction.[21]

The serum level of IL-8 was highly significant($p \leq 0.001$). The increase, demonstrated in the present study in acne patients as a comparison to control group (16.70 ± 4.73 versus 9.30 ± 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.[21]

The highly significant($p \leq 0.001$) increase in serum level of IL-1β in acne patients group in comparison to the control group (4.027 ± 0.54 versus 1.092 ± 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-1β that observed as a response to P. acnes lead to the development of inflammatory acne lesions by activation of inflammasome in the monocyte-macrophage.[22]

The P. acnes triggers the innate immune response by activation of TLR-2 and the inflammasome. Thus the response of monocytes to P. acnes causes an up-regulation to caspase-1, which is required to proteolytic cleavage and activation of IL-1β.[11]

Results reached throughout this study regarding the highly significant ($p \leq 0.001$) increase in the serum level of TNF-α in acne patients group as a comparison to control group (6.226 ± 1.15 versus 2.778 ± 1.12) is confirmed by Al-hilali *et al.* (2014) study which compared the level of TNF- α of acne patients with different sex and age with a healthy control group and found that TNF-α 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-α level as one of the major pro-inflammatory cytokines, which play an important role in the inflammatory process in acne.[23] Also high level and role of TNF-α in patients with acne of the present study is in agreement with another previous study performed by Baz *et al.* (2008), which found that TNF-α was significantly increased($p \leq 0.001$) in patients with acne as compared to controls (healthy) group. Also, it revealed that there was no association between acne development and gender. Also, it's pointed that P. acnes cause to trigger the production of cytokines from monocytes, keratinocytes, and lymphocytes, including IL-1α, IL-1β, IL-8, and TNF-α, which are the responsible mediators for the development of inflammatory acne lesions.[24]

In the present study, IFN-γ serum level was highly significant ($p \leq 0.001$) increase in the acne patients as a comparison to the control group (16.44 ± 5.20 versus 10.404±1.69), is in agreement with Sugisaki *et al.* (2009) study which found that in acne patients, IL-12, IFN- γ, 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 (pro-inflammatory cytokines) such as IL-1β, TNF-α 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-γ), also IL-8 which is neutrophil chemotactic that contributes for the development of the inflammatory acne lesions by accumulates neutrophils in the follicules and subsequently, generated the pustules with the destruction of follicular walls by proteases which are secreted by accumulated neutrophils.[25]

According to results of the present study, doxycycline represent the first-line oral antibiotics therapy and more frequently used for the treatment of moderate to severe acne vulgaris as compared to tetracycline because it needs less frequent daily dose, has greater lipophilicity and in addition, doxycycline is favorable in long-term treatment due to its high efficacy and safety.[26]

A recently performed study confirmed the sensitivity of P. acnes to doxycycline as an antibacterial agent, which acts as inhibiter to bacterial protein synthesis and also as an anti-inflammatory agent, that can reduce neutrophil chemotaxis and their proteolytic enzymes ((polymorph nuclear leukocyte–derived collagenase MMP-8) and decrease the secretion of the pro-inflammatory cytokines such as IL-8, TNF-α.[27]

Results about the effect of doxycycline plus vitamin C treatment in the present study showed that the clinical response was more good with bright-looking patients face when compare to doxycycline alone because, in addition to the effect of doxycycline, vitamin C has an important role in the improvement of acne lesions. This finding could be attributed to the effect of vitamin C as an antioxidant agent because it can reduce the oxidative damage in the skin, scavenge (ROS), interact with active site (copper) at tyrosinase lead to inhibit the second messengers which stimulate melanogenesis cause decrease in post inflammatory hyperpigmentation because, with the presence of acne, melanocytes can be stimulated by cytokines (inflammatory mediators) lead to increase the synthesis of melanin and cause deposition of the pigment nearby keratinocytes that cause post-inflammatory hyperpigmentation that can be decreased by the use of antioxidant agents such as vitamin C thus can improving acne lesions.[28]

Moreover, an Indian study found that vitamin C has multiple effects with safety profile; therefore, it can use to reduce photo-aging, hyperpigmentation, tissue inflammation, and enhance wound healing, which improves vitamin C delivery to the dermis layer to stimulate collagen production with scavenging free radicals.[29]

Results of the present study about the use of vitamin C as adjunctive therapy for acne beside traditional acne treatment revealed that the use of vitamin C with doxycycline results in a more reduction in the levels of cytokines as compared to the group treated with doxycycline alone. This variation in the reduction of cytokines levels might be attributed to the antioxidant effect of vitamin C against ROS, which has an essential role in the development of inflammatory lesions of acne. Because, the ROS is generated by the epidermal cells following hyperkeratinization and proliferation of P. acnes that can result in an oxidative burst and the production of IL-8 is associated with this phenomena and cause the epidermal cells death, therefore, ROS that are crucial in the development of inflammatory acne lesions and can be abrogated by using efficient treatments against P. acnes.[30]

The higher level of ROS induces cellular damage by attacking lipids, proteins, and DNA; the antioxidant limits this damage by detoxifying ROS.[31] Morris was the first who added vitamin C and citrus juices to the routine acne vulgaris therapy, and he found that this addition results in a dramatic improvement.[32] Likewise, the P. acnes secret lipases cause irritative effect, and the free fatty acids present in sebum components are cytotoxic. Also, neutrophils that arrive into the follicles are released several inflammatory factors like ROS and lysosomal enzymes, which damage the follicle wall and exacerbate the inflammation.[33]

Regarding the ability of vitamin C to decrease the level of the immunological parameters tested by the present study it's inconsistency with that found by Telag (2013) who confirmed that vitamin C could inhibit (NFkB), which stimulate several pro-inflammatory cytokines like IL-1, IL-6, IL8 and TNF-α thus, vitamin C has anti-inflammatory activity that can be used in many diseases like rosacea and acne vulgaris also it can decrease the post-inflammatory hyperpigmentation and promote wound healing.[29]

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