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Comparing the Biochemical Effects of Oral Isotretinoin Vs Azithromycin in The Treatment of Moderate to Severe Acne Vulgaris

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Abstract

Background: The treatment selection in acne vulgaris (AV) is difficult due to challenging assessing severity; therefore, it should depend on a proper history, physical examination, previous treatment and response to treatment.

Aims: The aim of this study is to investigate the differences in sebum composition and skin pH in patients with moderate to severe acne vulgaris to identify lipid markers in comparison with the apparently healthy group and to study the effects of isotretinoin or azithromycin on the level lipid profile, liver enzymes, in addition to sebum composition.

Methods and Material: This study was conducted on sixty women patients with AV and thirty, apparently healthy individuals as a control group. The patients were allocated into two groups, A and B, group A was given 500 mg of azithromycin taken on alternative days for 3 months and group B was given 40mg/day isotretinoin for 3 months. Before and after therapy serum cholesterol, TG, ALP, ALT, GGT, creatinine additional to sebum cholesterol and squalene levels were measured. In the healthy control group, these parameters were assessed only once.

Results: The results of the study showed significant differences ($p<0.05$) in serum levels of cholesterol, TG, sebum cholesterol, Squalene and skin pH in patient group when compared to control group. A statistically significant increase was detected in isotretinoin group post-treatment liver enzymes, lipid profile and sebum cholesterol, while sebum squalene was a significant decrease. While the results detected there was no significant effect of azithromycin on TG concentration.

Conclusions: Both isotretinoin and azithromycin has side effects but isotretinoin has more effects on squalene concentration.

Keywords: Acne vulgaris; Isotretinoin; Azithromycin; Squalene.
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Introduction

Acne is one of the chronic inflammatory diseases for pilosebaceous units. It can be characterized by seborrhea, erythematous pustules and papules and in more cases of severe nodules, the closed and open comedones formation, pseudocysts and deep pustules. Inflammatory lesions are followed by scarring [1]. In latest years, therapy of combination has become a vital part of acne treatment. There are different treatment modalities to acne vulgaris including systemic and topical drugs, depending on severity [2].

Isotretinoin (13cis-retinoic acid) Fig. 1-7 is a vitamin A derivative frequently given over a 20-week course (16–24 weeks), with a dosage of 0.5–1 mg/kg/day, depending on clinical response and side effects [3]. It has significant results in sebum production reduction, decreasing in surface and ductal *PA,* influences comedogenesis and shows properties of anti-inflammatory [4]. Azithromycin is one of the antibiotics that have been prescribed, for treatment of acne which is as effective as doxycycline and minocycline [5]. Azithromycin belongs to the azalide group of antibiotics and is closely related structurally to macrolides like erythromycin [6].

Subjects and Methods

This is a randomized, case- control study that was carried out in the dermatology unit at Al Imamain Alkadhimain Teaching Medical City/Baghdad to comparison the side effects between 500 mg of azithromycin taken in alternative days and 40mg/day isotretinoin for 12 weeks of treatment. All patients were clinically diagnosed having moderate to severe acne as categorized by the Global Acne Grading Score (GAGS) were included. The samples were collected within eight months starting from September 2016 until the end of May 2017. Serum samples were collected from 60 Iraqi women patients aged between (15 -35 years old) were investigated with moderate to severe acne. Control group was consisting of 30 apparently healthy individuals. Cases were excluded if they had any of the following criteria: azithromycin hypersensitivity, renal disease, liver disease,

diabetes mellitus, metabolic impairment pregnant women or under multi-vitamin supplementation. The patients were allocated into two groups, A and B, group A was given 500 mg of azithromycin taken on alternative days for 3 months and group B was given 40mg/day isotretinoin for 3 months. Together with systemic drug, patients was given 5% topical benzoyl peroxide gel to apply to the affected areas of the face and trunk once daily after the skin had been cleansed and dried. Patients were examined at baseline, 6 weeks and 12 weeks, in order to evaluate their clinical improvement and to measure several biochemical parameters. Patients were educated concerning compliance, side effects, constant follow up. The study was accomplished with the institutional review board agreement and written consents from all patients were obtained.

Venous blood about 5 ml was drawn from the patients each time before starting treatment, at 6 weeks and at the end of 12

weeks of treatment. The blood was allowed to clot for at least 10-15 min at room temperature, centrifuged for 10 min at 4000xg. Serum was taken to measurement liver enzymes, cholesterol, triglyceride and creatinine. The baseline and post treatment sebum production was collected using a Sebutape (CuDerm Corporation). Before sample collection, the region was cleansed with 70% ethanol solution wipe. Samples were taken from the forehead regions of patients. The Sebutape was removed, placed into a glass vial. The samples were stored at -20 C until sample extraction and analysis. Serum gamma-glutamyl transferase (GGT), glutamic--pyruvic transaminase (GPT), alkaline phosphatase (ALP) and creatinine concentrations were measured by commercial kits (Human, England). Serum cholesterol and triglyceride concentrations were measured by commercial kits (Spinreact, Spain). Sebum squalene and cholesterol were measured by HPLC method [7].

Statistical analysis used

All statistical analyses in study were performed usings Statistical Package for the Social Sciences (SPSS) version 20.0 for statistical software (SPSS Inc., Chicago, IL, USA).

Results

The results showed that were significant differences ($p<0.05$) in serum levels of cholesterol, TG, skin pH, sebum cholesterol and sebum squalene in patients with acne vulgaris (AV) when compared with the apparently healthy control group, while there were no significant difference in serum levels of GGT, ALT, ALP and creatinine in patients with acne vulgaris (AV) when compared with the apparently healthy control group as listed in table 1.

Table 1. Evaluation of Serum GGT, ALT, ALP, Cholesterol, TG, Creatinine, sebum cholesterol, squalene and skin pH in the Patients with Acne in Comparison with the Control Group.

Characteristic	Patients' group before treatment [n=60] (Mean± SD)	Control group [n=30] (Mean± SD)	*p* Value
GGT (IU/l)	5.97±1.9	5.62±1.82	0.416
ALT (IU/l)	16.41± 5.0	15.62± 2.82	0.426
ALP (IU/l)	98.01± 27.63	96.96±13.04	0.844
Cholesterol(mg/ml)	130.98±28.40	103.93 ±43.27	<0.001*
TG (mg/ml)	72.32±24.2	60.49 ±22.82	0.005*
Creatinine(mg/dl)	0.92 ± 0.62	0.82±0.56	0.736
Sebum cholesterol	57.07±30.6	34.12± 22.33	<0001*
Sebum squalene	0.056± 0.035	0.042± 0.026	0.04*
Skin pH	6.15 ±0.29	5.85±0.22	<0.001*

*Significant at *P*< 0.05 level of significance

Table 2. Effects of azithromycin on biochemical markers

Characteristic	Pre-treatment [n=30] (Mean± SD)	Post-treatment [n=30] (Mean± SD)	*p* Value
GGT(IU/l)	6.34±1.54	8.07±0.47	<0.001*
ALT(IU/l)	17.13± 5.39	19.91± 3.20	0.05*
ALP(IU/l)	99.35±20.09	125.78±16.12	<0.001*
Cholesterol(mg/ml)	123.08 ±30.47	151.41 ±20.15	<0.001*
TG (mg/ml)	70.22±27.01	69.03±5.18	0.624
Creatinine (mg/dl)	0.88±0.57	0.615±0.14	0.017*
Sebum cholesterol	52.10±21.88	524.81±216.17	<0.001*
Sebum squalene	0.056±0.016	0.034±0.20	<0.001*
Skin pH	6.12 ±0.23	6.14 ±0.14	0.848

*Significant at P< 0.05 level of significance

The results showed there were significant difference- in the two groups- ($p<0.05$) when compared with baseline readings (pre-treatment) of ALP, GGT, ALT, cholesterol, sebum cholesterol and squalene and those after 12 weeks of treatment (post-treatment) as listed in the table 2 and table 3.

Table 3. Effects of isotretinoin on biochemical markers

Characteristic	Pre-treatment [n=30] (Mean± SD)	Post-treatment [n=30] (Mean± SD)	*p* Value
GGT (IU/l)	5.6±2.18[a]	7.33±2.4[b]	<0.001*
ALT(IU/l)	15.69±4.56[a]	21.44±8.22[b]	0.009*
ALP (IU/l)	96.68±33.85[a]	167.94±72.94[c]	<0.001*
Cholesterol (mg/ml)	138.87±24.17[a]	158.17±30.48[b]	0.05
TG (mg/ml)	74.43±21.27[a]	107.28±39.79[b]	<0.001*
Creatinine(mg/dl)	0.96±0.68[a]	1.04±0.39[a]	0.791
Sebum Cholesterol	62.04±37.20[a]	319.67±130.67[c]	<0.001*
Sebum squalene	0.055±0.048[a]	0.014 ± 0.012[b]	<0.001*
Skin pH	6.18±0.34[a]	6.12 ±0.23[a]	0.449

*Significant at *P*< 0.05 level of significance

Table 4. Comparative effects of isotretinoin and azithromycin on biochemical values.

Characteristic	Azithromycin (Mean± SD)	Isotretinoin (Mean± SD)	*p* Value
ALP (IU/l)	125.78±16.12	167.94±72.94	0.003*
TG (mg/ml)	69.03±5.18	107.28±39.79	<0.001*
Creatinine (mg/dl)	0.615±0.14	1.04±0.39	<0.001*
Sebum Cholesterol	524.81±216.17	319.67±130.67	<0.001*
Sebum squalene	0.034±0.02	0.014 ± 0.012	<0.001*

*Significant at P< 0.05 level of significance

Comparison between azithromycin and isotretinoin after 12 weeks of treatment in the two groups (A and B) was listed in table 4.

Discussion

The current study results revealed that serum level of cholesterol and TG was significantly elevated in patients with acne vulgaris (AV) when compared with the apparently healthy control group. This result agrees with [8,9,10] they found that a significant increase in total cholesterol and triglyceride in acne patients as compared to healthy controls. Another study showed there was no significant difference between them [11]. The total levels of cholesterol of the increased serum may affect acne vulgaris development by rising androgens, as both gonadal and adrenal androgens are, synthesized from cholesterol of plasma [12]. Fatty acids of exogenous from lipoproteins can be released inside cells by lipase of lipoprotein, that has been expressed in the glands of sebaceous at, mRNA level, synthesis of de novo occurs to a lesser extent [13]. The results of the present study revealed that the mean levels of skin pH, sebum cholesterol and sebum squalene were a significant increase in the patient group when compared to control group. Skin pH reveals the condition of the sweat or sebum secretion, stratum corneum, exogenous materials applied on the face and living microorganism. The factors that influencing the skin pH are classified as exogenous and endogenous factors. Exogenous factors contain products of skin care, occlusive dressings, skin irritants and topical medications. Endogenous factors contain age, gender differences or ethnic, anatomic sites, sweat, sebum and diseases of the skin [14]. Regarding acne, changes in the quantity of *Propionibacterium acnes* (PA) colonization and sebum secretion likely affect the skin pH. The excreted sebum onto the skin surface effects moderately on the skin pH [15]. *Propionibacterium acnes* products acetic acid, free fatty acids and propionic acid, these acids may alter the skin pH. Simultaneously, especially in inflammatory acne, follicles of damaged hair may cause the buffers loss in the tissue which change skin pH. Hence, the total PA effect on the pH of the skin cannot be measured [16]. The sebum pH and secretion in subjects without and with acne are studied by Kim et al.. The acne patients had a slightly higher skin pH than without acne subjects [17]. The current study showed that sebum production and composition of acne patients was significantly higher than apparently healthy group. Apostolos et al., has been found that the subjects with acne had more sebum (59%) than the control subjects [18] and Costa et al., demonstrated that squalene is considered the principal lipid that is increased in the sebum of patients with AV, and the production of squalene is performed directly by sebocytes. The presence of squalene is a marker of the likelihood and severity of AV because this compound enhances the comedogenicity and proliferation of PA [19]. Also, squalene seems in sebum only and this is the reason of using it as a marker that permits for telling the difference between epidermal lipids and sebaceous lipids [20]. The very interesting thing is that the fact a sebaceous gland cannot convert squalene into cholesterol because of an incomplete system of enzymes. Thus, it might imply that cholesterol in isolated sebum comes from epidermis rather than from sebaceous glands [21]. It allows for differentiating between lipids secreted by the sebaceous glands and epidermal lipids. The environment of lipid inside the follicle of the hair and using a base that would be well-matched with the excretion, improve the action of the medicines applied to the skin that they can be transported through the follicle. The drug administration that dissolves in oil phase using the solvents that react with the facilitate of sebum the drug accumulation inside the, follicle, which is easier in certain disease treatment, e.g. acne [20]. The present study result showed that serum level of GGT, ALT, ALP and cholesterol was significantly higher in patients at the end of three months treatments with azithromycin when compared with patient pre-treatment values while there was a significant decrease in creatinine level after treatment these results may be due to one of the most important features of azithromycin is the fact that high tissue concentrations in compared to the serum concentration dropping to very low levels. Azithromycin has high affinity against tissues, it may due to incidence of tertiary group of amines that give it both hydrophobic and hydrophilic properties [22]. The primary azithromycin metabolism is via hepatic demethylation, a process resulting in breakdown drug's chemical structure with the aid of enzymes [23]. The current study showed that significant increase in liver enzymes after 12 weeks of treatments and these results disagree with [24] in which there were no substantial difference in liver enzymes after 12 weeks of treatment with azithromycin. There were a number of research echoing its potential for changing the enzyme levels throughout azithromycin therapy [25,26,27,28]. Ebenezer suggested that the hepatotoxic effects of azithromycin appear to progress through generation of free radicals (ROS) as evidenced by a reduction in the levels of enzymic and non-enzymic antioxidants [29].

The current study also demonstrates that sebum cholesterol was elevated while sebum squalene was decreased after azithromycin treatment versus the pre-treatment.

The current study was in continuance of some other studies into the effect of isotretinoin on several biomarker in the acne patients and found that the levels of serum GGT, ALT, ALP, triglycerides, cholesterol were elevated after 3 months of the treatment and this result agrees with other studies [30,31,32,33]. Although many studies reported alterations in serum transaminase and lipid levels, other studies reported no significant change in the level of these parameters, such as Brito et al., found no statistically significant changes in liver transaminase, TG levels following treatment with isotretinoin [34]. The increase in the levels of triglyceride in patients who are treated with oral isotretinoin might be related to a removal rate reduction of these lipids in plasma [35]. It also seems to be influenced by the rise in expression of the gene for Apo E. An increase in triglyceride level is the most commonly seen side effect of isotretinoin [36]. The current study also demonstrates that sebum cholesterol was elevated while sebum squalene was decreased after isotretinoin treatment versus the pre-treatment values and this results

agree with other results [37,38]. There is only a small proportion of cholesterol in sebaceous lipids and a high proportion in epidermal lipids. Thus, when there is a small sebaceous component, as in the pre-pubertal child, or after the glands are greatly inhibited, as with isotertinoin, the cholesterol concentration in the lipids of skin surface increases [37]. Pochi thinks it may represent a change in synthetic pathways within the gland resulting in a preferential preservation of squalene synthesis [39]. The current study showed a significant increase in serum ALP, TG and creatinine in patients under isotretinoin treatments when compared to those under azithromycin treatment after the same duration (12 weeks), while there was a significant decrease in sebum cholesterol and sebum squalene, this result can indicate a wide side effects range of isotretinoin when compared with azithromycin, however the effect of isotretinoin on acne lesion is greater than the effect of azithromycin.

Conclusions

Serum cholesterol, TG, sebum cholesterol, squalene and skin pH were significantly elevated in the patients with moderate to severe acne in comparison with the apparently healthy group. Both isotertinoin and azithromycin were able to decrease sebum squalene and increase sebum cholesterol significantly in comparison with pre-treatment data.

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