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Effect of isotretinoin treatment on the levels of serumhomocysteine, vitamin B_6, vitamin B_7, vitamin B_{12} and folic acid and on sebum composition in patients with moderate to severe acne vulgaris.

Alaa Sh. Abdulbari[1], Noor M.Ali[1]*, Ahmed R. Abu Raghif[2], Nadheer A. Matloob[3]

[1]Department of Biochemistry / College of Medicine /AL-Nahrain University/ Baghdad
[2]Department of Pharmacology /College of Medicine /AL-Nahrain University/ Baghdad
[3]Section of Dermatology & Venereology/College of Medicine /AL-Nahrain University/ Baghdad

Abstract : Background: AcneVulgaris(AV) is related to the pilosebaceous follicle. It is considered as adolescent disorder which is characterized by formation of open and closed comedones, papules, pustules, nodules and cysts.Isotretinoin is the only systemic therapy, which has advantages of cure, but causes significant side effects at higher doses.
Objective: The current study was conducted to evaluate serum homocysteine, vitamin B_6, vitamin B_7, vitamin B_{12} and folic acid and on sebum composition after treatment with isotretinoin.
Methods: This study was conducted on thirty patients with AV. Clinical and laboratory evaluations were conducted before the start of therapy, 6 weeks after, and 12 weeks after treatment.serumhomocysteine, vitamin B_6, vitamin B_7, vitamin B_{12} , folic acid liver function tests, serum cholesterol and triglyceride , creatinine levels were tested as well assebum cholesterol and sebum squaleneSPSS 20 was used for statistical analysis.
Results: The results of the study showed significant differences ($p<0.05$) in homocysteine, vitamin B_6, vitamin B_7, vitamin B_{12}, folic acid, GGT, ALP, ALT, serum cholesterol, triglyceride, sebum cholesterol and sebum squalene and skin moisture levels, while there were no significant differences in skin temperature ,skin skin pH and creatinine.
Conclusions: Evaluation of homocysteine, vitamin B_6, vitamin B_7, vitamin B_{12} and folic acid beside the routine tests were beneficial for the patients before they started isotretinoin treatment.
Keywords : Acne Vulgaris, homocysteine, vitamin B_6, vitamin B_7, vitamin B_{12} and folic acid.

Introduction

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that affects predominantly adolescents and young adults. It is characterized by noninflammatory, open or closed comedones and inflammatory papules, pustules, and nodules. It results from androgen‐induced increased sebum production,

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altered keratinization, inflammation, and bacterial colonization of hair follicles by *Propionibacterium* acnes. (Vidyadhar 2017).

Isotretinoin(ISO) is a naturally occurring substance introduced in 1982. It is the only anti-acne agent that affects the known major etiologic mechanisms: sebum production, comedogenesis ,Propionibacterium acne colonization of ductal and skin surface, and monocyte chemotaxis-induced inflammation. This explains its unique ability to sustain long-term remissions and, in some cases, permanent remission (Ahmad HM , 2015). Isotretinoin is the only systemic therapy, which has advantages of cure, but causes significant side effects at higher dosages (Chetan, 2014).In approximately 80% of people, a single course reports complete remission in more than 50% patients and 20% of patients need a second form. Adverse effects include dry skin, nosebleeds, muscle pains, increased liver enzymes and increased lipid levels in the blood (Manoj S.,2014).

The aims of the study: to investigate the effects of Isotretinoin on serum homocysteine, vitamin B6, vitamin B7, vitamin B12 and folic acid and on sebum composition in patients with AV.

Patients and Method

This is a randomized, case- control study that was taken out in the dermatology unit at Al ImamainAlkadhimain Teaching Medical City to assess the efficacy of oral isotretinoin for 12 weeks of treatment, Patients were followed up every 6 weeks for evaluation of the disease. Patients having moderate to severe acne as categorized by the Global Acne Grading Score, GAGS, were included. All patients were diagnosed clinically. Patients were educated regarding compliance, regular follow up, side effects, avoidance of other oral medication whilst on treatment. Written consent for the treatment was obtained from all patients.

The samples were collected within eight months starting from September 2016 until the end of May 2017. Serum and sebum samples were collected from 30 women Iraqi patients aged from (15 -35 years old) were investigated with moderate to severe acne.In addition to 30 apparently healthy individuals (as control group), who was age and gender matched to patient groups. All patients were given oral isotretinoin for 12 weeks. Patients were excluded if they have any of the following criteria: pregnancy, renal disease, patients with renal disease, liver disease, diabetes mellitus, metabolic impairment or patient under vitamins supplement also patient with chronic disease. Together with systemicIsotretinoin , patients were given 5% topical benzoyl peroxide gel to apply to the affected areas of the face and trunk once daily at night after the skin had been cleansed and dried. Patients were examined at baseline, 6 weeks, and 12 weeks (completion of treatment), in order to evaluate their clinical improvement and to measure several biochemical parameters.

Five ml of venous blood was drawn from (30) patients withAV ranging between (15-35) years old before starting treatment, after 6 weeks and after 12 weeksof treatment period . The blood was allowed to clot for at least 10-15 min at room temperature, centrifuged for (10) min at (4000xg). Serum was removed and divided into two parts the first to measure the biochemical parameters and the other part wasdeprotienzed and stored at - 18 ^{0}C until the time of HPLC assay to measure several vitamin concentrations.

The baseline and post treatment sebum production was collected using a Sebutape, Sebutape which is a hydrophobic adhesive film with innumerable tiny cavities which trap sebum as it issues from each follicular orifice,before sample collection, the region was cleansed with 70% ethanol solution wipe. Samples were taken from the right and left sides of 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 GGT ,GPT , AlK and creatinine concentrations were measured by commercial kits (Human, England). Serum cholesterol and triglyceride concentrations were measured by commercial kits (Spinreact , Spain).

Serum levels of vit.B_6, vit. B_7, vit.$B_{12}$and folic acid were measured by HPLC method (Sawsan , 2011). While serum homocystiene was measured by sandwich enzyme-linked immunosorbent assay (ELISA) kit (Kono Biotech. Co. China).

Sebum sequalene and sebum cholesterol were measured by HPLC method(Kazuo I. *et. al,* 1987).

Results:

The mean values (mean±SD) of GGT, ALT, ALP, Cholesterol, TG and Creatinine at baseline (before treatment) was 5.6±2.18, 15.69±4.56 , 96.68± 33.85 , 138,87± 24.17, 74.43±21.27 and 0.96±0.68 respectively,

and at 6 weeks of treatment was 8.26±2.88 , 16.70±8.96 , 134.13±55.73, 142.03±39.23, 69.36±43.23 and 1.05±0.63 respectively , and at 12 weeks of treatment was 7.33±2.4 , 21.44±8.22 , 167.94±72.94 , 158.7±30.48, 107.28± 39.79 and 1.04±0.39 respectively.

The results showed that there was a significant difference(p<0.05) between the baseline readings of serum GGT and serum ALP and after 6 weeks, and between the baseline and after 12 weeks.

The results showed that there was no significant difference(p>0.05) between the baseline readings of serum ALT , Cholesterol and TG and after 6 weeks of treatment , while there was a significant difference (p<0.05) in readings between baseline and after 12 weeks of treatments.

The current study showed that there was no significant difference(p>0.05)in serum creatinine among all the study as shown in the table (1)

Table (1): Effect of Isotritinoin on the GGT, ALT, ALP, Cholesterol, TG and Creatinine in the patient group before and after 6 and 12 week of treatment.

Characteristic	Patients' group before treatment [n=30] (Mean± SD)	After 6 week group [n=30] (Mean± SD)	After 12 week group [n=30] (Mean± SD)	*p* Value
GGT (IU/l)	5.6±2.18[a]	8.26±2.88[b]	7.33±2.4[b]	<0.001
ALT (IU/l)	15.69±4.56[a]	16.70±8.94[a]	21.44±8.22[b]	0.009
ALP (IU/l)	96.68±33.85[a]	134.13±55.73[b]	167.94±72.94[c]	<0.001
Cholesterol	138.87±24.17[a]	142.03±39.23[a]	158.17±30.48[b]	0.05
TG (mg/ml)	74.43±21.27[a]	69.36±43.23[a]	107.28±39.79[b]	<0.001
Creatinine	0.96±0.68[a]	1.05±0.63[a]	1.04±0.39[a]	0.791

*Significant at P< 0.05 level of significance
**different litters means significance.

Serum levels of homocysteine, folic acid, Vit. B12, Vit. B7 and Vit. B6 in patients before and after treatment are shown in table 2

The mean values (mean±SD) of homocysteine, folic acid, Vit. B12, Vit. B7and Vit. B6 at baseline (before treatment) was 0.88±0.65, 15.73±1.22, 448.47±30.37, 1.36±0.11 and 27.43±1.80 respectively, and at 6 weeks of treatment was 1.41±1.26, 8.46±0.55, 294.21± 13.94, 0.53±0.1and17.47±1.13 respectively , and at 12 weeks of treatment was 2.20±2.27, 2.76±0.30, 194.98±6.04 , 0.24±0.04, and 36.05±1.10 respectively.

The results showed that there was a significant difference(p<0.05) between the baseline readings of serum folic acid, Vit. B12, Vit. B7and Vit. B6 and after 6 , 12 weeks of treatment, while serum homocysteine has a significant difference (p<0.05) only between the baseline readings and after 12 weeks of treatment shown in the table (2)

Table (2): Effect of Isotritinoin on the homocysteine, Folic acid, Vit. B12, Vit. B7and Vit.B6 in the patient group before and after 6 and 12 week of treatment.

Characteristic	Patients' group before treatment [n=30] (Mean± SD)	After 6 week group [n=30] (Mean± SD)	After 12 week group [n=30] (Mean± SD)	*p* Value
Homocysteine	0.88±0.56[a]	1.41±1.26[a]	2.20±2.74[b]	<0.001
Folic acid	15.73±1.22[a]	8.46±0.55[b]	2.76±0.30[c]	<0.001
Vit. B12	448.47±30.37[a]	294.21±13.94[b]	194.98±6.04[c]	<0.001
Vit. B7	1.36±0.11[a]	0.53±0.1[b]	0.24±0.04[c]	<0.001
Vit. B6	27.43±1.80[a]	17.42±1.13[b]	36.05±1.10[c]	<0.001

*Significant at P< 0.05 level of significance
**different litters means significance.

Evaluation of skin characteristic (skin temperature ,skin moisture ,skin pH) and sebum content (sebum cholesterol and sebum squalene) in the patients withacne before and after treatmentare shown in table 3.

The mean values (mean±SD) of (skin temperature ,skin moisture ,skin pH), sebum content (sebum cholesterol and sebum squalene) and clinical score in the baseline patient36.62 ± 0.29 , 24.24±2.20, 6.18±0.34 , 62.04±37.20, 0.012±0.02 and 32.2 ± 3.15respectively , and after 6 weeks of treatment was 36.45 ± 0.40, 25.80±2.45, 6.21 ±0.25, 189.24±63.05, 0.044±0.02 and 22.33± 4.12 respectively, and after 12 weeks of treatment was 36.47 ± 0.39, 26.97 ±2.68, 6.12 ±0.23, 319.67±130.67, 0.044 ± 0.02, 11.23 ± 3.05 respectively.

The mean levels of skin moisture , clinical score ,sebum cholesterol and sebum squaleneshowed a significant difference (p<0.05) in patientsbefore and after treatment.The current study showed that there was no significant difference (p>0.05) in skin temperature and skin pH in the patient that taken isotritinoin along the study as shown in table (3).

Table (3):Evaluation of skin characteristic (skin temperature ,skin moisture ,skin pH) , sebum content (sebum cholesterol and sebum squalene) and clinical score in the patients with acne who treated with oral isotritinoin.

Characteristic	Patients group before treatment	Patients group after 6 weeks treatment	After 12 weeks group [n=30]	*p* Value
Skin temperature	36.62 ± 0.29[a]	36.45 ± 0.40[a]	36.47 ± 0.39[a]	0.142
Skin moisture	24.24±2.20[a]	25.80 ±2.45[b]	26.97 ±2.68[cb]	<0.001
Skin pH	6.18±0.34[a]	6.21 ±0.25[a]	6.12 ±0.23[a]	0.518
Sebum Cholesterol	62.04±37.20[a]	189.24±63.05[b]	319.67±130.67[c]	<0.001
Sebum squalene	0.055±0.048[a]	0.044±0.027[a]	0.014 ± 0.012[b]	<0.001
Clinical Score	32.2 ± 3.15[a]	22.33± 4.12[b]	11.23 ± 3.05[c]	<0.001

*Significant at P< 0.05 level of significance
**different litters means significance.

Discussion:

Moderate to severe acne vulgaris is difficult to treat and associated with psychological insult to patient. Although Isotretinoin was very effected in treatment of patient with acne, it associated with adverse effect in those patients.

The current study was in continuation of several studies on the effect of isotritinoin on several biomarkers in the acne patients and found level of serum GGT, ALT, ALP , triglycerides, cholesterol , homocysteine were elevated after 3 months of the treatment and this results agree with other studies [Muhammad 2015, Polat 2008,, Vieira 2012 ,Fusun 2017, Hilal 2014]Although many studies reported alterations in serum transaminase and lipid levels, other studies reported no significant in the level of these parameters , such as Brito et al found no statistically significant changes in liver transaminase, TG levels following treatment withIsotretinoin.In another study by Javanbakht et al, serum levels of homocysteine was not elevated through treatment withIsotretinoin. The results showed a decrease in the serum level of folic acid , vitamin B_{12} and vitamin B_7 after 3 months of treatment with Isotretinoin,this results in agreement with several studies[Muhammad 2015, Hilal 2014, Karadag 2011], while disagrees withPolat 2008.

The increase in triglyceride levels in patients treated with oral isotretinoin may be related to a reduction in the removal rate of these lipids from plasma(De Marchi 2006) It also appears to be influenced by theincrease in gene expression for Apo E. An increase in triglyceride level is the most commonly seen side effect of Isotretinoin(Rodondi 2002).

Vitamin B and folic acid, with their interrelated metabolism, are important vitamins in various metabolic pathways, including the homocysteine (Hcy) pathway. Vitamin B and folic acid are cofactors of the methyltransferase enzyme and with their deficiency, hyperhomocysteinemia is seen(Hilalet.al, 2014)

The previous study concluded that elevation of homocysteine levels despite the normal values of responsible vitamins for the metabolism of amino acid strongly suggested that cystathionine-β-synthase might be affected by the drug, acting as an inhibitor of the enzyme or the liver dysfunction caused by Isotretinoin (Muhammad 2015), Karadag et al.thinks that longer treatmentwith higher cumulative doses ofIsotretinoin might

havecaused subclinical vitamin B12 and folic acid deficiencieshavemore effect on the homocysteine level(Karadag 2011). The exact mechanism of action of Isotretinoin on folic acid reduction is still unknown .Javanbakht et al. suggest that is probable that Isotretinoin by interacts with some essential groups in the active site of important protiens or enzymes at folic acid metabolism.this interference might be initiated by the changes in intestinal absorption of folic acid, different steps of formation tetrahydrifolate from folic acid , induction of enzymes in te liver and finally depletion of folic acid(Javanbakht 2012), Hilalet.al, 2014, alsosuggest that long term use of Isotretinoin might decrease intestinal absorption of vitamin B and folic acid (Hilalet.al, 2014). This study stressed that the long term Isotretinoin therapy caused hepatic dysfunction, followed by vitamin B and folic acid deficiencies, due to its cumulative effects, suggesting an important role in homocysteine metabolism.

The results of current study show a decrease level of vitamin B_7 when compared withpretreatment value,supports the assertion that Isotretinoin lead to hair loss as its side effects.

Increase concentration of vitamin B_6 posttreatmentversus the pretreatment may be due to increase homocysteine level that lead to increase conversion of homocysteine to cystathionine in the presence of vitamin B_6 dependent enzyme cystathionine-β-synthase and then cystathionine convert to cysteine and α-ketobutyrate viacystathionine-γ-lyase (vitamin B_6 dependent enzyme) (Prem Gupta 2013).

To our knowledge, this is the first study investigatingchanges in serum vitamin B_7 and vitamin B_6 levels in patients receiving Isotretinoin treatment.

The current study also demonstrate that sebum cholesterol was elevated while sebum squalene was decrease after Isotretinoin treatment versus the pretreatment and this results agree with other results[Strauss JS et. al1980,Thomsen 1981]. Thomsen et.al suggestthese changes reflected aconversion of the surface skin lipid pattern from that characteristic of an adult to that characteristicof a child. Since approximately 95% of the surface skin lipids in the adult are ofsebaceous gland origin, theoretically a change in skin surface lipid composition resultingfrom inhibition of sebum production would require at least an 85% reduction in sebumproduction(Thomsen et.al 1981).

Pochi PE.et.althinksit may representa change in synthetic pathways within the gland resulting in apreferential preservation of squalene synthesis (Pochi PEet.al 1970). There is only a small proportion of cholesterol in sebaceouslipids and a high proportion in epidermal lipids. Thus, whenthere is a small sebaceous component, as in the prepuberalchild, or after the glands are greatly inhibited, as with Isotretinoin, the cholesterol concentration in skin surface lipidsincreases (Strauss JS et. al 1980).

Conclusion:

Administration of Isotretinoin for 12 weeks in patients with moderate to severe acne vulgaris cause significant elevation of serum GGT , ALP , ALT,homocystine, vitamin B_6, cholesterol, triglycerides and sebum cholesterol , while serum vitaminsB_7 , B_{12}, folic acid and sebum squalene was significant decrease.Oralisotretinoin is a very effective treatment foracne vulgariswith statistically significant differencein clinical score.Vitamin B_7/ Vitamin B_{12} /folic acid may be should be given under medical surveillance before and during Isotretinoin therapy. Supplementation of these vitamins should be recommended in cases of their deficiency.

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Ethical Approval:

All implemented steps in this work complied with the ethical standards of the University Research Committee and with the Helsinki declaration; no formal ethical review was required.

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