Aswan University Medical Journal, volume 5 / No.3/ September 2025 (68-79) Online ISSN: **2735-3117**

ORIGINAL ARTICLE

Serum homocysteine level in Acne patients before and after oral Isotretinoin.

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ABSTRACT

Keyword:Acne, Homocysteine (Hcy), Isotretinoin.

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Background and Aim: Acne vulgaris (AV) is a chronic inflammatory skin lesion of the pilosebaceous unit. Whatever the dose of prescribed Isotretinoin (Iso), follow-up of the homocysteine (Hcy) level is beneficial for the patients to prevent hyperhomocysteinaemia and associated complications. This study is aimed at measuring the serum level of Hcy in AV cases pre- and post-treatment of oral Iso. **Methodology:** This is a cross-sectional case control study that conducted on 60 individuals, 30 patients diagnosed with acne vulgaris, and 30 age-matching healthy volunteers as control group. The chosen patients were allocated to receive 20 mg of Iso every day for three months. **Results:** There was insignificant difference in serum levels of Hcy, liver function test (LFT) (SGOT, SGPT), total cholesterol and triglycerides (TGs) between case and control groups before isotretinoin treatment. While, there was significant increase in serum level of Hcy, liver function test (SGOT, SGPT), total cholesterol and triglycerides after three months of treatment with Iso among acne cases. **Conclusion:** Serum level of Hcy increased after 3 months of Iso treatment, which could be due to liver dysfunction. So, evaluation of Hcy was useful for acne cases prior to the initiation of Iso therapy.

INTRODUCTION

Acne vulgaris (AV) is a skin lesion characterized by the development of papules, pustules, comedones, and nodules. AV cases are often associated with a bad quality of life. It occurs secondary to blockade of the hair follicles and follows the next processes: a greater than normal amount of sebum formation (influenced by androgens), extreme accumulation of keratin causing comedo formation, hair follicles' colonisation by Propionibacterium acnes (P. acnes), and the discharge of proinflammatory cytokines. In addition, androgens have a central function in AV pathogenesis from increased levels or aggravated responses [1].

In 2015, AV affects about 633 million subjects all over the world, making it the 8th most frequent disease all over the world. Acne typically manifests during adolescence, affecting about 85% of teenagers. However, teenagers can also be affected before and after puberty [2].

A lot of therapeutic modalities for AV are available, such as lifestyle modification; which include healthful life style, drugs and medical approach. In spite of the availability of several therapeutic modalities, none of them has confirmed to be optimum [3].

Oral isotretinoin has the ability to counteract the pathogenetic mechanisms that participate in AV development via its wide actions on cellular differentiation, apoptosis, inflammation, and sebaceous gland activity. It causes a marked decrease in sebum formation, affects comedogenesis, lowers surface and ductal Propionibacterium acnes, and demonstrates anti-inflammatory characteristics [4].

Together with various clinical adverse events, isotretinoin could trigger hyperlipidemia, elevated liver enzymes, and reduction of biotinidase activity, which can lead to elevation of serum homocysteine (Hcy) level, which is associated with vascular, neurological, renal, cardiac, and gastrointestinal disorders [5]. So, this work aimed to measure serum level of Hcy in AV cases pre- and post-treatment of oral Iso therapy, as well as, to correlate between serum level of Hcy and severity of acne vulgaris patients.

PATIENTS AND METHODS

The study approved by the local Ethics Committee of the Faculty of Medicine, Aswan University, Aswan, Egypt, and informed written consent was obtained from all patients. The study was conducted by Helsinki standerds as revised in 2013.

This is a clinical based cross-sectional case control study that was conducted on 60 individuals, 30 patients diagnosed with acne vulgaris as case group, and 30 age-matching healthy volunteers as control group. The selected patients were assigned to receive 0.5mg/kg/day of isotretinoin once daily with heavy meal. The treatment was continued for at least three months. All subjects selected from those attending the outpatient clinic of Dermatology, Venereology and Andrology Department, Aswan University Hospital, between December 2020 and December 2021.

Patients with history of systemic isotretinoin treatment in last 3 months, pregnant, lactating or female willing to conceive during the study period, patients with history of allergic reaction to isotretinoin, patients with history of hyperlipidemia, endocrine disorders, cardiac disorders, renal disorders, liver disorders, hematological disorders, patients with history of malignancies, patients with recent history of alcohol abuse or psychiatric illness, marked depressive manifestations, and patients not giving consent or unable to come for three months follow-up, were ruled out.

The patients were subjected to full history taking include: Personal history as name, age, sex, residence, and occupation; Present history as onset, course, duration and type of acne; Past history of any medical ailments like endocrine disorders, cardiac disorders, renal disorders, liver disorders, hematological disorders or psychiatric illness; Family history of acne or other general disorders; and drug history of any systemic or topical therapy for acne vulgaris.

Examination of patients were done and included: General examination for assessment of general condition of the patients, and dermatological evaluation of cases in order to define type of and severity of acne.

As regard biochemical investigations, two blood samples (5 cc) were acquired by venipuncture of the antecubital veins of cases and after a 12-hour fasting. One sample collected in EDTA blood to measure homocysteine, the other sample collected in Serum

separator tubes (SST) to measure Liver function test (LFT) (SGOT, SGPT), Cholesterol and Triglycerides. The blood samples were permitted to clot for half an hour at 22C prior to centrifugation for fifteen min at 1000 x g [6].

Liver function test (SGOT, SGPT), Cholesterol and TGs were measured spectrophotometrically using commercial kits. The kits were from **Diasys Company (Germany)**. SGOT and SGPT were measured by Auto Analyzer Technical RA 1000 in patient's plasma. Cholesterol was measured using the cholesterol oxidase approach (CHOD). Measurement of TGs was conducted by colorimetric enzymatic test using glycerol-3-phosaphate oxidase (GPO).

Total plasma homocysteine levels were measured according to the Biotin double antibody sandwich technology, by using Human Homocysteine (Hcy) enzyme-linked immune sorbent assay (ELISA) Kit **(Shanghai Crystal Day Biotech Co., Shanghai, China)**.

Screening for biochemical parameters was conducted pre and 3 month post-treatment of isotretinoin. These parameters included serum levels of Hcy, LFT (SGOT, SGPT), total cholesterol and triglycerides.

Statistical methods: The collected data were coded, and analyzed using the SPSS (IBM-SPSS/PC/VER 24.0) program for windows. Descriptive data: mean±SD, median, range, frequencies, percentage were measured. Chi square and Fisher Exact tests were used to compare the difference in distribution of frequencies among various groups. U test was calculated to test the median differences in continuous variables between groups and related-samples The Wilcoxon test was used to compare the median Pre- vs. Post-treatment. Multivariable logistic regression was conducted to assess the independent effect of HC on AV. ROC curve was showed the diagnostic performance of HC for diagnosis of AV. Validity statistics were also conducted. Significant difference was set at $p < 0.05$.

RESULTS

There was insignificant difference between both groups as regard age and sex (p= 141, 318) respectively. Regarding the serum level of homocysteine: the mean level was lower in acne cases (8.2 ± 3.4 mcmol/L) in comparison with controls (p=0.008), as shown in **(Table 1)**.

As regard sociodemographic characteristics of the studied acne patients, their age ranged between 16 and 29 years with a mean of 20.8 ± 3.6 years. Also, about three-quarters of cases (n=22) were females and one-quarter (n=8) was males. Moreover, the patient's weight ranged between 47 and 78 with a mean of 63.6 ± 8.8 and a median of 63.5 kilograms. For the acne severity, about 13% of cases (n=4) had mild severity, one-quarter (n=8) had moderate severity and 60% had severe disease, as shown in **(Table 2)**.

As regard the effect of treatment on the SGOT, SGPT, TG, Cholesterol and homocysteine Levels, the mean SGOT SGPT, TG, Cholesterol and homocysteine Levels was significantly (p<0.001) increased after treatment Levels was significantly (p<0.001) increased after treatment, as shown in **(Table 3)**.

As regard correlation between homocysteine level and the other parameters before and after treatment with isotretinoin among acne cases, the level of homocysteine before treatment showed negative moderate significant correlation with SGOT before and after

treatment, SGPT and TG after treatment and this was statistically significant ($p<0.05$), as shown in **(Table 4)**.

As regard the relationship between Hcy Level and disease severity in AV cases. There was non-significant association between grades of severity regarding Hcy level before treatment (p=0.844). Likewise, there was non-significant association between grades of severity regarding Hcy level after treatment (p=0.083). Moreover, plasma Hcy levels were significantly increased in the moderate and severe cases after 3-months of Iso therapy (p=0.003 and <0.001, respectively). However, non-significant elevation was found in the mild cases after 3-months of Iso therapy (p=0.055), as shown in **(Table 5)**.

Tables

Table 1: Socio-demographic Data Differences between both groups.

	Control	Case	P-value
Age in years	**23.20 ± 4.9**	**20.83 ± 3.6**	= 0.141*
Sex			
• **Male**	11 (36.7%)	8 (26.7%)	= 0.318**
• **Female**	19 (63.3%)	22 (73.3%)	
Homocysteine Level (mcmol/L)			
• **Mean±SD**	11.77 ± 9.8	8.23 ± 3.4	= 0.008*

***T-test was utilized to compare the mean differences between cases and controls**
****Chi-square test was utilized to compare the ratios among groups**

Table 2: Socio-demographic characteristics of the studied Acne Cases.

Variable	Category	n = 30
Age in years	• **Mean±SD**	20.83 ± 3.6
	• **Median(Range)**	20 (16 - 29)
Sex	• **Male**	8 (26.7%)
	• **Female**	22 (73.3%)
Weight in Kg	• **Mean±SD**	63.57 ± 8.8
	• **Median(Range)**	63.5 (47 - 78)
Acne Severity at	• **Mild**	4 (13.3%)

Baseline	• **Moderate**	8 (26.7%)
	• **Severe**	18 (60%)

Table 3: Effect of Isotretinoin on the SGOT, SGPT, TG, Cholesterol and homocysteine Levels.

Variable			**n = 30**	**P-value**
SGOT Level	**Before Treatment**	• **Mean±SD**	**20.01 ± 6.5**	**< 0.001***
		• **Median(Range)**	**19 (12 - 33)**	
	1.5 month After Treatment	• **Mean±SD**	**23.77 ± 7.1**	
		• **Median(Range)**	**21.5 (14 - 37)**	
SGPT Level	**Before Treatment**	• **Mean±SD**	**18.95 ± 5.1**	**< 0.001***
		• **Median(Range)**	**19.5 (9.5 - 27)**	
	1.5 month After Treatment	• **Mean±SD**	**24.47 ± 6.3**	
		• **Median(Range)**	**25.5 (12 - 35)**	
TG Level	**Before Treatment**	• **Mean±SD**	**73.37 ± 25.7**	**< 0.001***
		• **Median(Range)**	**67 (39 - 146)**	
	1.5 month After Treatment	• **Mean±SD**	**79.31 ± 27.3**	
		• **Median(Range)**	**71.5 (40 - 151)**	
Cholesterol Level	**Before Treatment**	• **Mean±SD**	**141.09 ± 28.5**	**< 0.001 ***
		• **Median(Range)**	**137 (89 - 192)**	
	1.5 month After Treatment	• **Mean±SD**	**156.10 ± 35.2**	
		• **Median(Range)**	**146 (101 -**	

			233)	
Homocysteine Level	**Before Treatment**	• **Mean±SD**	**8.23 ± 3.4**	**< 0.001 ***
		• **Median(Range)**	**7.4 (4.3 - 23)**	
	3 months After Treatment	• **Mean±SD**	**11.63 ± 4.4**	
		• **Median(Range)**	**11 (4.6 - 25)**	

Table 4: Correlation between Homocysteine and other Parameters before and after treatment with isotretinoin among Acne cases.

	Homocysteine BT		**Homocysteine AT**	
	rho*	**P-value****	**rho**	**P-value**
Homocysteine BT	**1**		**0.771**	**<0.001**
SGOT BT	**-0.374**	**= 0.023**	**-0.322**	**= 0.044**
SGOT AT	**-0.411**	**= 0.013**	**-0.363**	**= 0.026**
SGPT BT	-0.156	= 0.058	-0.032	= 0.867
SGPT AT	**-0.419**	**= 0.024**	-0.068	= 0.726
TG BT	-0.215	= 0.262	**-0.334**	**= 0.038**
TG AT	-0.131	= 0.499	-0.095	= 0.626
Cholesterol BT	-0.100	= 0.605	-0.107	= 0.582
Cholesterol AT	-0.032	= 0.868	-0.067	= 0.729

Table 5: Relationship between Homocysteine Level and Disease Severity in AV cases.

Variable	**Mild (1)**	**Moderate (2)**	**Severe (3)**	**P-value***
Homocysteine Level				
Before Treatment				= 0.844

• **Mean±SD**	**8.02 ± 2.3**	**7.80 ± 2.7**	**8.25 ± 3.9**	
• **Median(Range)**	**9.1 (4 – 9.5)**	**8.1 (4 - 12)**	**6.9 (5 - 23)**	
• **P-value****	1 vs. 2=0.917	1 vs. 2=0.766	1 vs. 2=0.909	
After Treatment				
• **Mean±SD**	**11.01 ± 3.7**	**14.20 ± 4.8**	**10.77 ± 4.1**	= 0.083
• **Median(Range)**	**11.6 (6 - 15)**	**14.7 (4.5 - 19)**	**10 (6 - 25)**	
• **P-value****	1 vs. 2=0.142	1 vs. 2=0.081	1 vs. 2=0.714	
P-value***	= 0.055	**= 0.003**	**< 0.001**	**< 0.017*[4]**

DISCUSSION

It has been demonstrated that Hcy values increase following the use of Iso therapy [6, 7]. Therefore, we aimed to measure the serum level of Hcy in AV cases pre- and post-treatment of oral Iso. As well, to correlate between serum levels of homocysteine and clinic-demographic data in AV cases pre- and post-treatment of oral Iso therapy.

This study conducted on 60 individual, 30 patients presented with acne, and 30 as control, at the beginning of the study we found significant difference between case and control groups as regard age and gender, as we found that, case group was younger and the majority of patients were female (the cases were 22 (73.3%) female and 8 (26.7%) male with a mean age of 20.83 ± 3.6 years).

This finding matches with Karadag et al. [6], Kamal and Polat, [7], Dursun et al. [8], Roodsari et al. [9], and Polat et al. [10], as they found female predominance with a group mean age of 21.0±2.7; 21.0 ± 6.0; 22.3 ± 4.7; 21.4 ± 3.4; 21.72 ± 3.76 years in their studies respectively.

The previous finding may be due to the fact that, AV is a common disease in adolescence with female preponderance [11]. As well Goulden et al. [12], found presence of hormonal alterations in acne female patients. Additionally, Yang et al. [13], reported that, female preponderance may be due to the fact that not all male cases with AV present and seek medical advice.

At the beginning of this study, we found insignificant difference in homocysteine level between both groups before isotretinoin treatment.

In accordance to our results, Kamal and Polat, [7], made a case-control study on 124 individuals (62 cases and 62 control), and reported that, the differences between the baseline values of homocysteine, was not statistically significant between the groups.

Moreover, Schulpis et al. [5], conducted a study on 28 cases with cystic acne, and 30 subjects as control, and they reported no statistical differences were observed in Hcy levels among studied groups before isotretinoin treatment. As well, Polat et al. [10], reported the same results. This can be explained by that, the randomization process encouraged a balance between both groups (similar groups).

On the other hand, Jiang et al. [14], made a study on 124 AV cases and 70 healthy subjects, matched regarding age and sex in which serum Hcy levels were measured. At the end of the study they found that, Hcy levels in AV cases were higher than in normal subjects. Also, Arora et al. [15], made a study on 60 females with severe AV and compared their Hcy levels with healthy subjects, and they found that, serum Hcy levels were displayed to be high in females with severe AV compared to controls. This variation between previous study and ours, might be due to alteration in sample size, type of study, different ethnic groups, additionally, Jiang et al. [14], study had limitation, as the serum Hcy level was not detected with different durations in the same subject [14]. As well, Arora et al. [15], study had limitation, as the serum Hcy level was measured in female gender only and in one type of acne.

In the present study, we demonstrated significant increase in liver enzymes (SGOT and SGPT) as well as increase in lipid profile (triglyceride and cholesterol), after isotretinoin treatment.

Our finding goes in line with, Sallam et al. [16], conducted a prospective study on 44 cases (22 cases with moderate AV and 22 cases with severe AV). Iso was initiated in a dose of 0.5, and one mg/kg/day in moderate and severe AV cases correspondingly. They found a significant elevation in lipid profile and LFT that was detected in entire patients.

This finding matches with Zane et al. (2006) [17], who studied 13772 cases with AV undergoing oral Iso treatment. The authors found increased liver enzymes (ALT and AST) and serum lipid levels [17].

In the study of Kızılyel et al. [18], they displayed that there were significant increases in TG values in cases undergoing Iso therapy. Also, Vieira et al. [19] conducted there study on a total of 130 cases managed by Iso and observed an increase in LFT, and TG levels. Additionally, Sarkar et al. [20], found that, hypertriglyceridemia and hypercholesterolemia was present in most of patients.

In addition, Leelambika and Sarkar, [21] Bershad et al, [23] have recorded that Iso increases cholesterol and TGs, comparable to the current study. The laboratory changes were noticed in the serum values throughout and following the therapeutic duration in comparison to the basal value.

Some studies suggest that, Iso raises the TG level in fifty percent and cholesterol in 30% of cases [24]. The actual etiology of the increase in lipid by Iso isn't identified. On the other hand, the retinoids typically bind to the plasma albumin and may affect certain important groups in the active site of the proteins or enzymes (during lipid metabolism) [25]. It is theorized that retinoid-albumin interaction could displace the TG from albumin, inducing its increase. Another suggested theory is that Iso affects the levels of ALT and AST, possibly by hepatic inflammatory changes [26, 27].

Although much research recorded changes in serum transaminase and lipid levels, in contrast to our findings, other studies recorded no effect. Brito et al. [28] conducted their study on 150 subjects and displayed insignificant changes in LFTs, TG, or cholesterol levels following Iso treatment. In addition, Alcalay et al, [29] conducted their study on a total of 1292 subjects and demonstrated that serum values of liver enzymes weren't increased to a degree requiring cessation of Iso therapy. Also, Baxter et al. [30] conducted their study on 30 subjects and recorded insignificant difference in TG or cholesterol levels measured at pre- or throughout treatment with Iso. Also, in the study of Kızılyel et al. [18], liver enzymes were slightly affected compared to lipid profile in cases using Iso treatment. This variation between previous studies and ours could be secondary to alteration in sample size, type of study, follow-up period, and different ethnic groups.

We demonstrated a significant increase in homocysteine (Hcy) level after three months of treatment with Iso. This result was in the same line with a lot of previous studies that displayed the impact of Iso on Hcy values.

Recently, Kim et al. [31], assessed ten studies that evaluated plasma values of Hcy during Iso therapy in acne cases, and they revealed significant increase in Hcy levels in the Iso treatment group.

Also, Sallam et al. [16], conducted a prospective study on 44 patients (22 patients with moderate AV and 22 cases with severe AV). Iso was initiated in a dosage of 0.5 mg/kg/day and 1.0 mg/kg/day in moderate and severe AV cases, correspondingly. They found a significant difference in Hcy level among all cases. In addition, Hcy serum level in the patient's group receiving a high dose of Iso (one mg/kg/d) displayed highly significant results ($p=0.001$) in contrast to the patient's group receiving a low dose of Iso (0.5 mg/kg/d) ($p=0.003$).

This matches with Yosef et al. [32], who made a study on 60 cases with moderate to severe AV. Using a chemiluminescent immunoassay, Hcy, levels were measured at baseline and after four months of Iso therapy. At the end of the study they demonstrated that, Iso-treated cases have to be followed up carefully for Hcy level, as they found that, Hcy was significantly higher after 4 months of Iso treatment compared with the baseline values.

Moreover, in a study of Kamal and Polat [7], who made a case-control study on 124 individuals (62 cases and 62 controls), they reported that Hcy levels were significantly elevated in both groups taking 0.5 and 1.0 mg/kg/day isotretinoin.

In accordance with previous results, Karadag et al. [6], made a study on 66 cases with moderate or severe AV and found that Hcy levels increased after the termination of four months of Iso therapy.

In a similar Roodsari et al. [9], had 47 cases with moderate or severe AV receiving Iso, and they reported that, Hcy level increased after treatment.

Similarly, Polat et al. [10], comprising 74 patients on Iso therapy with nodulocystic acne, and found that plasma Hcy levels increased after treatment.

As well, Schulpis et al. [5], they assessed Hcy values in 28 cases on Iso therapy for AV, and demonstrated that plasma values of Hcy, was elevated after 45^{th} day of the treatment.

The possible explanation with supporting the previous findings is that, increased Hcy values may be secondary to the drug effect on the enzyme cystathionine-beta-synthase (CBS) with no impairment of hepatic functions [7]. Furthermore, increased Hcy was linked to diminished levels of folate and vitamin B12 during Iso therapy. This indicates that Iso could be linked to deficiencies in these vitamins, complicating the recycling of Hcy [31].

On the other hand, Chanson et al. [33] conducted a study on 20 cases with acne and 20 elderly healthy male volunteers and reported that Iso therapy didn't cause significant changes in the plasma Hcy value. This variation between the previous study and ours could be due to alteration in sample size (20 patients), gender (male only), and different ethnic group (France).

In this study, we found no significant correlation between homocysteine and other biochemical parameters (SGOT, SGPT, total cholesterol and triglycerides) before and after treatment with isotretinoin among acne cases.

This goes in line with Roodsari et al. [9], as they didn't detect any association between Hcy and the plasma values of other biochemical parameters pre- and post-treatment with Iso, indicating that increased Hcy values may be secondary to the action of Iso on the enzyme CBS.

In contrast, Schulpis et al. [5], they assessed plasma Hcy values in 28 cases on Iso treatment for nodulocystic acne, and found significant association between Hcy levels, vitamins, and both ALT and AST. This variation between the previous study and ours could be secondary to change in sample size (28 patients), type of acne (nodulocystic only) and different ethnic group (Greece).

Limitations

Our cases were limited to cases living in Upper Egypt that may stress the significance of geographic and ethnic background in the clinical presentation of acne. Second, a small sample size and a relatively short period of study suggest significant differences in our findings compared with the other studies. Finally, the study design and the possibility that selection bias was introduced by including patients already attending our outpatient clinic.

RECOMMENDATIONS

We recommend that, the inclusion of a higher number of high-quality randomized controlled trials, long period of study and follow-up, and selection of different ethnic groups would be required in upcoming studies.

CONCLUSION

In conclusion, the serum level of Hcy increased after 3 months of isotretinoin treatment. Evaluation of Hcy, liver function test (SGOT, SGPT), total cholesterol, and TGs was valuable for acne cases prior to the initiation of isotretinoin treatment. Moreover, it is suggested that the increased Hcy levels in cases after 3 months with isotretinoin treatment may be due to the suppression of CBS by the drug and/or their impaired liver functions.

Funding support: None.

Conflicts of interest: None.

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