



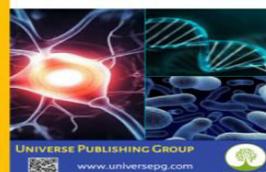
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## Assessment of Serum Levels of Free Tri-iodothyronine, Free Thyroxine and Thyroid Stimulating Hormone in Patients with Acne Vulgaris

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### ABSTRACT

The aim of the study was to assess serum levels of free tri-iodothyronine (FT<sub>3</sub>), free thyroxine (FT<sub>4</sub>) and thyroid stimulating hormone (TSH) in patients with acne vulgaris and apparently healthy subjects, to compare all the above-mentioned parameters between the two groups and to correlate severity of acne vulgaris with serum levels of free tri-iodothyronine (FT<sub>3</sub>), free thyroxine (FT<sub>4</sub>) and thyroid stimulating hormone (TSH). A total number of 120 participants of both genders were selected with age ranged from 15 to 45 years based on inclusion and exclusion criteria. Among them, 60 subjects with acne vulgaris were considered as Group A who were further sub grouped into mild, moderate and severe according to severity. Sixty age matched apparently healthy subjects were included in Group B for comparison. The level of serum free tri-iodothyronine (FT<sub>3</sub>), free thyroxine (FT<sub>4</sub>) and thyroid stimulating hormone (TSH) were estimated. The study revealed significant lower levels of FT<sub>4</sub> ( $p=0.02$ ) and significant higher levels of TSH ( $p < 0.001$ ) of group A when compared with group B. The difference of FT<sub>3</sub> level between group A and group B was not statistically significant ( $p=0.343$ ). FT<sub>3</sub>, FT<sub>4</sub> and TSH were positively correlated with the severity of the acne but statistically not significant.

**Keywords:** Acne vulgaris, Free tri-iodothyronine, Free thyroxine, FT<sub>4</sub>, FT<sub>3</sub>, and Thyroid stimulating hormone.

### INTRODUCTION:

Acne vulgaris, simply known as acne is a widespread chronic inflammatory and the widely prevalent human skin condition of the pilosebaceous unit that affects millions of people worldwide (Hareedy *et al.*, 2021; Shah *et al.*, 2021; Kaur *et al.*, 2021 & Suva *et al.*, 2014). It is externally visible one of the most experienced skin diseases in dermatology (Franik *et al.*,

2018). It accounts for the roughly 20% of all appointments of the dermatologists' practice (Federman & Kirsner, 2000). It is estimated that 85% of individuals between the age of the 12 and 24 experience acne (Alsulaimani *et al.*, 2020 & Franik *et al.*, 2018). It frequently persists throughout adulthood (Franik *et al.*, 2018) and frequently makes its first appearance during this time (Kamangar & Shinkai, 2012). Acne is widely

considered as a self-limited disorder that resolves naturally (Rahman *et al.*, 2012; Vergou *et al.*, 2011). Acne lesions are typically restricted to skin regions with a high density of the well-developed sebaceous follicles (Utami *et al.*, 2019; Hassan *et al.*, 2015). It predominantly affects face. Neck, upper trunk and back can be involved to a lesser extent (Utami *et al.*, 2019; Rahman *et al.*, 2012; Shahen *et al.*, 2019).

About 9.4% of the world's population are affected by acne vulgaris (AV), making it the eighth most common disease globally (Nada *et al.*, 2021). Acne is consistently estimated as the third most prevalent skin disease in the world (Lynn *et al.*, 2016). Globally, epidemiological studies demonstrate that acne can take place at any age. But it usually appears all round puberty and provokes during adolescence (Endres *et al.*, 2021). Significant acne lesions persist in 12% of women and in 3% of men by the time they reach their 40s (Bogino *et al.*, 2014). Recent epidemiological research has revealed that post adolescent acne is the increasing (Skroza *et al.*, 2018; Vergou *et al.*, 2011). This condition lasts longer & treatment is necessary (Vergou *et al.*, 2011). Acne vulgaris is identified by areas of the comedones, greasy skin (seborrhea), nodules, pustules, papules and scarring in some circumstances (Kaur *et al.*, 2021; Shah & Parmar, 2015; Adityan *et al.*, 2009). The causes of acne vulgaris development is multifactorial (Islami *et al.*, 2021; Shah *et al.*, 2021). Both experimental data and clinical observation support the significance of hormones in the etiology of the acne (Zouboulis, 2004). The development of acne requires functional sebaceous glands (Khondker *et al.*, 2012). Acne vulgaris results from overproduction of sebum, inflammation, hyper keratinization of the follicle and bacterial proliferation of hair follicles by *Propionibacterium acnes* (Sobhan *et al.*, 2020).

The epidermal homeostasis is significantly regulated by thyroid hormone (Kasumagic-Halilovic & Begovic, 2012). On skin, the thyroid hormone receptor (TR) attributes direct thyroid hormone action (Safer, 2011). The general population frequently experiences thyroid problems (Ogbonna and Ezeani, 2019). It has been calculated that about 1.5 billion people worldwide are at risk for thyroid problems (Mounika *et al.*, 2013). In patients with thyroid dysfunctions, acne accounts for 5% of skin abnormalities (Shrestha, 2018). Thyroid

hormones cause acne by affecting the sebaceous gland. Thyroxine has been demonstrated to promote lipid production & decrease mitotic activity. TSH increases the activity of sebaceous gland cells (Vergou *et al.*, 2011). Evidence suggests that the role of T<sub>4</sub> and TSH have similar effects on secretion of sebum. But they appeared to act in opposite ways (Ebling *et al.*, 1970). It is more likely that TSH stimulates the thyroid gland directly than indirectly by increasing the sebaceous gland response (Ebling *et al.*, 1970).

## **MATERIALS AND METHODS:**

### **Study place**

The study was carried out at the Department of Physiology, Dhaka medical college, Dhaka, Bangladesh from January 2022 to December 2022.

### **Ethical approval of the study protocol**

The study protocol was approved by Research Review Committee and Ethical Review Committee of Dhaka Medical College, Dhaka, Bangladesh.

### **Selection criteria of subjects**

#### **Inclusion criteria for case**

The inclusion criteria of case were clinically diagnosed case of the acne vulgaris (Seborrhea i.e., greasy skin, pustules, papules, nodules, comedones and scarring) with age range between 15 to 45 years and normal BMI of the both genders.

#### **Inclusion criteria for control**

Age, gender and BMI matched healthy persons were in inclusion criteria for control.

#### **Exclusion criteria**

Patients not willing to participate, pregnant women, lactating women, patients with systemic diseases like diabetes, malignancy, the chronic liver disease, chronic renal disease, diagnosed case of the thyroid disorders, patients with any other autoimmune disorders and the patients taking drugs (Lithium, amiodarone, dopamine, levodopa, bromocriptine and oral contraceptive pill) for the last three months that interfere with thyroid functions, alcohol users and smokers were rejected from the study.

### **Study population and design**

A cross sectional study was conducted in the Department of Physiology, Dhaka medical college, Dhaka, Bangladesh from January 2022 to December 2022. At

the beginning of the study, a total 120 number of the subjects were selected with age ranging from 15 to 45 years including both genders. Among the enrolled subjects, 60 patients with acne vulgaris were included in study group (Group A) and 60 age matched apparently healthy subjects who were not related to acne or any hormonal conditions with skin manifestations were considered as control group (Group B) for comparison. Group A was selected based on exclusion & inclusion criteria, from Department of Dermatology & Venerology, Dhaka Medical College Hospital & by personal contact from different areas of Dhaka city. Group B was selected from the personal contact from different areas of Dhaka city based on exclusion & inclusion criteria. Thyroid function results were categorized as normal, hypothyroidism, hyperthyroidism, subclinical hypothyroidism and subclinical hyperthyroidism. Complete dermatological assessment was performed to ascertain the extent of involvement of acne vulgaris in group A. Again, based on the severity of acne vulgaris, Group A was further divided into three subgroups: mild acne, moderate acne & severe acne. Using clinical criteria based on acne severity, acne was diagnosed dermatologically as follows: Mild acne - determined by the existence of either non-inflammatory lesions, inflammatory lesions (papulopustular) or both; Moderate acne - referred to as coincidental nodules, inflammatory lesions, mild scarring or combined; Severe acne - characterized by nodules, massive inflammatory lesions or both, together with injuries, moderate acne that still persists after six months of medication and acne determining a serious psychological impact. With all aseptic precautions, venous blood was drawn from antecubital vein for determination of serum free tri-iodothyronine (FT<sub>3</sub>), free thyroxine (FT<sub>4</sub>) and thyroid stimulating hormone (TSH). Thyroid profile was analyzed in Department of Nuclear Medicine, Dhaka Medical College Hospital, Dhaka in Siemens machine from Germany using an ADVIA Centaur XPT system.

### Statistical analysis

**Table 1:** Distribution of sociodemographic characteristics between two groups (N=120).

Variables	Overall (N=120)	Group A (n=60)	Group B (n=60)	p-value
<b>Age group (years)</b>				
Mean±SD	30.6±7.8	29.32±7.60	31.80±7.79	0.08 <sup>ns</sup>
<b>Gender</b>				

Statistical analysis was performed by using a computer based statistical program SPSS (Statistical Package for Social Science) version 26. All parameters were the expressed as mean ± standard deviation (mean ± SD). Unpaired student's t test was performed to compare the mean of hormonal parameters between study group & control group. For comparing the mean of hormonal parameters between two groups in skewed distribution, the Mann-Whitney test was performed. To assess the relationship between two qualitative variables, the chi-square test was performed. Kruskal-Wallis rank sum test was performed to compare hormonal parameters among three subgroups of patients with acne vulgaris. Spearman's rho correlation coefficient test was performed to assess the relationship between hormonal parameters & acne vulgaris severity. For all statistical tests, *p* <0.05 was taken into consideration as statistically significant.

### RESULTS:

One hundred and twenty participants, aged between 15 to 45 years, who satisfied the inclusion criteria were enrolled in the study. Among the enrolled subjects, 60 patients of acne vulgaris were included in Group A (study group) and 60 age matched apparently healthy subjects who were not related to acne or any hormonal conditions with skin manifestations were considered as Group B (control group) for comparison. The mean age of group A and group B were 29.32±7.60 years and 31.80±7.79 years respectively (**Table 1**). In group A, there were the 24/60(40%) male and 36/60(60%) female and in group B, there were 25/60(41.7%) male and 35/60(58.3%) female. Male and female ratio in group A and group B were 1:1.5 and 1:1.4 respectively. Male and female ratio in both group A and group B was 1:1.4 (**Table 1**). The mean body mass index (BMI) of group A and group B were 22.14±1.44 kg/m<sup>2</sup> and 21.64±1.54 kg/m<sup>2</sup> respectively (**Table 1**). There was no statistical difference observed for age, gender and BMI between the two groups. Therefore, the two groups were matched for age, gender and BMI.

Male	49(40.0%)	24(40.0%)	25(41.7%)	0.853 <sup>ns</sup>
Female	71(60.0%)	36(60.0%)	35(58.3%)	
Male: Female	1:1.4	1:1.5	1:1.4	
<b>Body mass index, BMI (kg/m<sup>2</sup>)</b>				
Mean±SD	21.89±1.51	22.14±1.44	21.64±1.54	0.069 <sup>ns</sup>

Results were expressed as mean ± SD for continuous variables and percentage for all categorical variables. Group A = study group and Group B = control group. *p*-values were based on the unpaired t-test for all continuous variables and chi-square test for categorical variables. The mean FT<sub>3</sub> level of group A and group B were 4.56±1.14 p mol/L and the 4.39±0.82 p mol/L respectively. The mean FT<sub>3</sub> levels were almost similar in group A and group B. In this study, the difference between the two groups were not the statistically

significant (*p*-0.343) (Table 2). The mean FT<sub>4</sub> level of group A and group B were 14.14±7.83 p mol/L and 15.16±3.22 p mol/L respectively. In the current study, statistically significant (*p*-0.020) difference was observed between the two groups (Table 2). Group A had a significant higher mean TSH level (9.28±8.74 mIU/L) compared with that of group B (4.33±2.44 mIU/L). In this study, TSH levels were observed statistically significantly (*p* < 0.001) increased in Group A compared with that of group B (Table 2).

**Table 2:** Comparison of hormonal parameters between two groups (N=120).

Variables	Group A (n=60)	Group B (n=60)	<i>p</i> -value
<sup>a</sup> FT <sub>3</sub> (p mol/L) Mean	4.56±1.14	4.39±0.82	0.343
Range	(2.11–7.46)	(1.56–5.94)	
Median	4.87	4.44	
<sup>b</sup> FT <sub>4</sub> (p mol/L) Mean	14.14±7.83	15.16±3.22	0.020*
Range	(4.80–64.43)	(4.66–26.37)	
Median	13.8	15.6	
<sup>b</sup> TSH (mIU/L) Mean	9.28±8.74	4.33±2.44	<0.001*
Range	(0.01–44.12)	(0.01–11.40)	
Median	8.19	4.31	

Results were expressed as mean ± SD for continuous variables. Group A= study group, and the Group B = control group. <sup>a</sup>*p*-value reached from unpaired t-test, <sup>b</sup>*p*-value reached from Mann-Whitney test. The mean subclinical hypothyroidism of the group A was 24/60 (40.0%) and group B was 13/60(21.6%) (Table 3 and Fig. 1). The mean hypothyroidism of group A and

group B were 18(30.0%) and the 4(6.7%) respectively. Out of the 60 acne vulgaris patients, two-third were observed subclinical hypothyroidism 24(40.0%) and the hypothyroidism 18(30.0%). There was a significant difference in the prevalence of thyroid disorder between groups A and the B (*p*- <0.001) (Table 3).

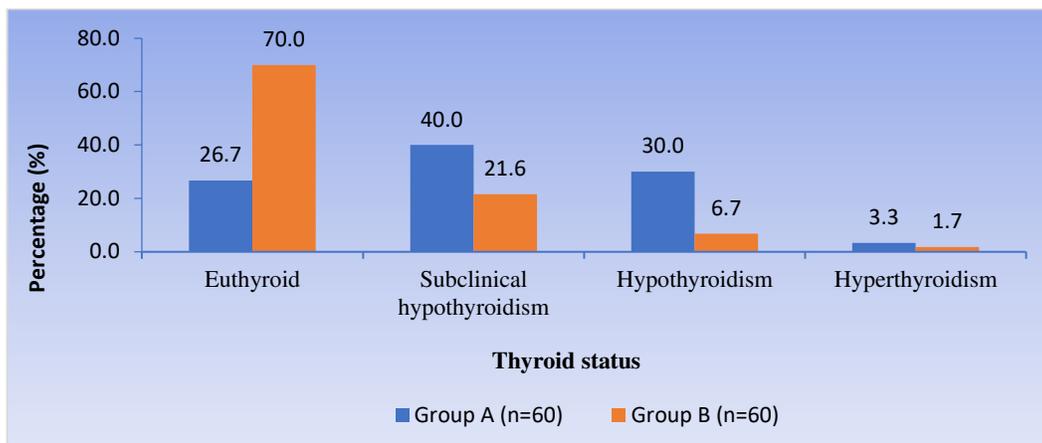
**Table 3:** Distribution of thyroid status among both groups (N=120).

Thyroid status	Overall (N=120)	Group A (n=60)	Group B (n=60)	<i>p</i> -value
Euthyroid	57(47.5%)	16(26.7%)	42(70.0%)	<0.001*
Subclinical hypothyroidism	37(30.8%)	24(40.0%)	13(21.6%)	
Hypothyroidism	23(19.2%)	18(30.0%)	4(6.7%)	
Hyperthyroidism	3(2.5%)	2(3.3%)	1(1.7%)	
Total	120(100%)	60(100%)	60(100%)	

Results were expressed as frequency, percentage for categorical variables. Group A = study group and the Group B = control group. *p*-value was based on Chi-square test for categorical variables.

The median value of FT<sub>3</sub> level were higher in severe (4.88 p mol/L) and moderate (4.88 p mol/L) type of acne vulgaris followed by mild type (4.25 p mol/L) of acne vulgaris but was not statistically significant (*p*-0.909) (Table 4). The median value of FT<sub>4</sub> level was

lower in mild type (11.98 p mol/L) of acne vulgaris followed by the severe (13.86 p mol/L) and moderate (14.37 p mol/L) type of acne vulgaris which was not statistically significant ( $p=0.424$ ) (**Table 4**).



**Fig. 1:** Bar diagram showing the distribution of thyroid status in two groups (N=120).

N = Total number of subjects

n = Number of subjects in each group

■ = Group A (Study group)

■ = Group B (Control group)

The median value of TSH level was higher in the moderate (9.16 m IU/L) type of acne vulgaris followed by severe (8.18 m IU/L) and mild (6.01 m IU/L) type of acne vulgaris but was not statistically significant ( $p=0.422$ ) (**Table 4**).

**Table 4:** Comparison among different subgroups of AV based on severity regarding FT<sub>3</sub>, FT<sub>4</sub> and TSH in Group A (n=60).

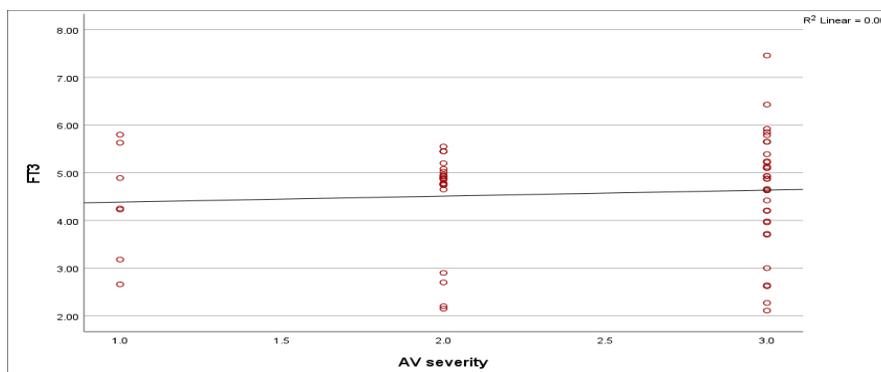
Characteristics	Severity of AV			p-value
	Mild (n <sub>1</sub> =7) Median (IQR)	Moderate (n <sub>2</sub> =21) Median (IQR)	Severe (n <sub>3</sub> =32) Median (IQR)	
FT <sub>3</sub> (p mol/L)	4.25 (2.66-5.80)	4.88 (2.15-5.55)	4.88 (2.11-7.46)	0.909
FT <sub>4</sub> (p mol/L)	11.98 (7.21-16.61)	14.37 (4.80-18.50)	13.86 (6.30-64.43)	0.424
TSH (mIU/L)	6.01 (0.49-12.86)	9.16 (0.21-44.12)	8.18 (0.01-42.02)	0.422

$p$ -value was based on Kruskal-Wallis rank sum test for continuous variables FT<sub>3</sub>, FT<sub>4</sub>, and the TSH displayed positive correlation with the severity of acne vulgaris ( $r= +0.057, +0.099$  and  $+0.063$  respectively) but was statistically not significant ( $p=0.667, p= 0.451$  and  $p=0.632$  respectively) (**Table 5** and **Fig. 2, 3** and **4**).

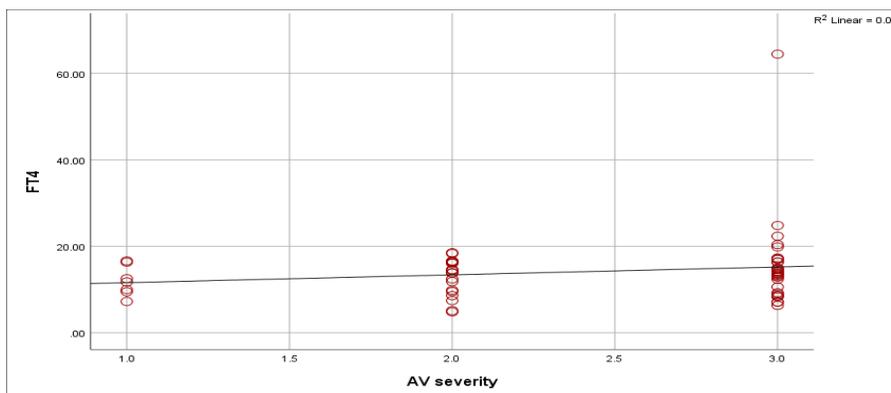
**Table 5:** Correlation between AV severity and thyroid profile in Group A (n=60).

	Correlation coefficients	
	rho-value	p-value
FT <sub>3</sub> (p mol/L)	+0.057	0.667
FT <sub>4</sub> (p mol/L)	+0.099	0.451
TSH (mIU/L)	+0.063	0.632

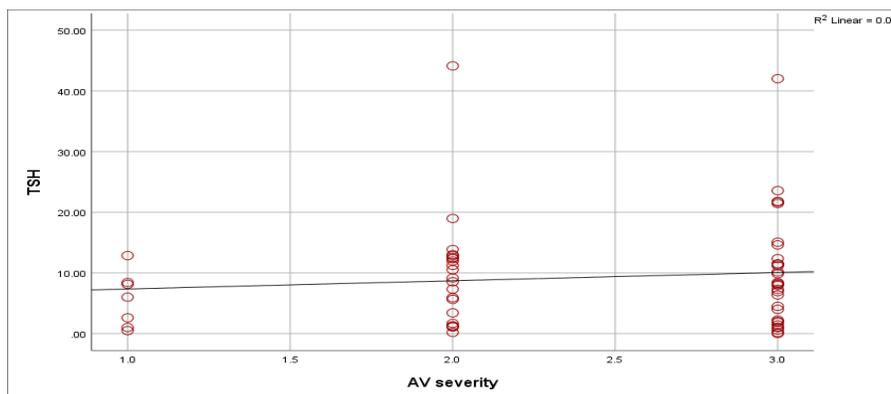
Spearman’s rho correlation test



**Fig. 2:** Correlation of acne vulgaris severity with FT<sub>3</sub> in group A (study group) (Spearman’s rho correlation coefficient test).



**Fig. 3:** Correlation of acne vulgaris severity with FT<sub>4</sub> in group A (study group) (Spearman’s rho correlation coefficient test).



**Fig. 4:** Correlation of acne vulgaris severity with TSH in group A (study group) (Spearman’s rho correlation coefficient test).

**DISCUSSION:**

In the present study, the mean age of acne and control were 29.32±7.60 years and 31.80±7.79 year’s respectively. The age range of the participants was from 15 to 45 years. In the present study, the most common age group was adolescents which was consistent with the study of the (Skroza et al., 2018); Perkins et al., 2012; Cunliffe & Gould, 1979). The age range varies in

different studies because acne vulgaris may appear at any age (Cunliffe & Gould, 1979). During the last 10 years, a growing body of literature suggests that an increasing number of patients suffer from acne after adolescence (Vergou et al., 2011). This might suggest either a genuine increase in the prevalence and severity of the disorder or a rising awareness of skin department’s interest in acne (Cunliffe & Gould, 1979). In

the current study, among the respondents, frequency of acne vulgaris was more prevalent in female 36/60 (60%) than the male 24/60(40%). The finding was in agreement with the study of many researchers of different countries. Skroza *et al.* (2018), Srinivasan & Thomas, (2019), Lynn *et al.* (2016), Suva *et al.* (2014) & Collier, (2008) demonstrated a female preponderance of acne vulgaris. In the present study, regarding the severity of acne vulgaris, more than half of the population 32/60(53.3%) had severe acne.

The least frequent form was mild acne. The prevalence of acne vulgaris (AV) was close to the prevalence of mild acne 7/60(11.7%). Around 35.0% (n= 21) of the population had moderate acne. In a previous study, Endres *et al.* (2021) found that significantly more people with severe acne were in the auto-immune thyroiditis (AIT) group ( $p < 0.001$ ). On the contrary, in previous studies by (Skroza *et al.*, 2018; Vergou *et al.*, 2011) the evaluation of acne severity showed that mild acne was the most frequent form of acne. This difference was might be due to the variation in sample size & methodology used. Burton *et al.* (1971) found a peak severity of acne in the teen-age group (Kaur *et al.*, 2021). Perkins *et al.* (2012) recruited 2895 women aged between 10 - 70 from the general population. Among them, 28% had mild acne and the 27% had clinically severe acne.

The present study demonstrated the mean FT<sub>3</sub> was almost similar in acne patients and control. Difference in FT<sub>3</sub> between the two groups was not statistically significant ( $p=0.343$ ). The finding was in agreement with the study of many researchers of the different countries. In a previous cross-sectional study, the Srinivasan & Thomas, (2019) in India, displayed that all the enrolled 50 acne vulgaris patients exhibited normal FT<sub>3</sub>. Stewart & Bazergy, (2017) in a case-control study evaluated that 116/130 (89%) cases and 60/65 (92%) controls revealed normal thyroid function tests. On the contrary, Vergou *et al.* (2011) in Greece, reported that acne patients seemed to have significantly lower levels of FT<sub>3</sub> ( $P=0.02$ ). This difference was might be due to the variation in sample size, ethnicity and methodology used. In tissue culture studies, using surrogates for DNA expression, triiodothyronine (T<sub>3</sub>) has been displayed to enhance the proliferation of both dermal fibroblasts and epidermal keratinocytes (KasuniversePG | [www.universepg.com](http://www.universepg.com)

magic-Halilovic & Begovic, 2012). In this study, the mean FT<sub>4</sub> of group A and group B were 14.14±7.83 p mol/L and 15.16±3.22 p mol/L respectively. There was statistically significant ( $p=0.020$ ) difference observed in FT<sub>4</sub> between the two groups. This is may be caused by the increased lipid synthesis and decreased mitotic activity of FT<sub>4</sub> (Vergou *et al.*, 2011). In a previous cross-sectional study, (Srinivasan & Thomas, 2019; Vergou *et al.*, 2011) displayed that reporting values for FT<sub>4</sub> did not significantly differ between the two groups. This difference was might be due to the difference in ethnicity, variation in sample size and methodology used. In a case-control study by Stewart & Bazergy, (2017) in Australia, observed statistically no significant difference of FT<sub>4</sub> between the two groups. The study was limited by its variation in the sample size and retrospectivity (Stewart & Bazergy, 2017). T<sub>4</sub> can influence production of thyroid antibody by either acting on the thyroid gland or directly on the B lymphocytes. The B lymphocytes that create antibodies against TSH receptors may be directly affected by T<sub>4</sub>, T<sub>3</sub> or both. In several tissues, T<sub>4</sub> is known to alter the activity of enzymes that promote the creation of phospholipids for cell-surface membranes in various tissues. Furthermore, the thyroid gland comprises thyroid hormone nuclear receptor which indicates that it might be a target tissue for thyroid hormones (Rieu *et al.*, 1994). The current study revealed that group A had a significant higher mean TSH level (9.28±8.74 mIU/L) compared with that of the group B (4.33±2.44 mIU/L). Statistically significant difference was observed in TSH level between the two groups ( $p < 0.001$ ). The difference was may be due to the TSH stimulates activity of sebaceous gland cell (Vergou *et al.*, 2011). In a previous study, in Nepal, Shrestha, (2018) observed that TSH was altered in 15.4% patients (Kaur *et al.*, 2021). Their study concluded with the comments that alteration in TSH levels may be responsible for the pathogenesis of acne. In India, Srinivasan & Thomas, (2019) recorded that one-fifth 9/50 (18%) of the acne vulgaris patients exhibited an elevated TSH level. In the study of some researchers of different countries, (Stewart & Bazergy, 2017 & Vergou *et al.*, 2011) the role of TSH in the acne vulgaris has been proven insignificant. This difference in results may be because of variation in sample size and different methods used in the statistical analysis. TSH stimulates activity of

sebaceous gland cell (Vergou *et al.*, 2011). Although the role of T<sub>4</sub> and TSH are similar in their effects on secretion of sebum, they appeared to sebaceous mitosis in opposite ways. TSH action in the amplifying the sebaceous gland response is more possibly to be direct rather than indirect concerning with the stimulation of the thyroid gland (Ebling *et al.*, 1970). Thyroid stimulators, such as the thyroid stimulating hormone (TSH) and TSHRAb cause the thyroid cell plasma membrane preparations in vitro to release thyroid cell-surface components, including TSH receptors, which may be proved antigenic (Rieu *et al.*, 1994).

#### CONCLUSION AND RECOMMENDATIONS:

The study revealed significantly higher levels of TSH and significantly lower levels of FT<sub>4</sub> in acne vulgaris. Subclinical hypothyroidism was more prevalent in the acne vulgaris which was also statistically significant. Levels of FT<sub>3</sub>, FT<sub>4</sub> and TSH were positively correlated with the severity of the acne but statistically not the significant. We recommend the assessment of thyroid auto antibodies and urinary iodine concentration.

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#### CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest.

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