

## Original Research Article

# Secretors Frequency and H-Substance Level in Polycystic Ovary Syndrome Patients and in Apparently Healthy Controls

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## ABSTRACT

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Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by hormonal imbalances. The syndrome is suspected as autoimmune disease. The process of secretion mediated by Fucosyltransferase2 is an essential step in generating gonadotropins hormones, like Follicle stimulating hormone (FSH) that is central to the process of cystic change in the ovary. In this study, one hundred hormonally and clinically characterized polycystic ovary syndrome (PCOS) patients ranging in age from 18-44 years and fifty apparently healthy controls were enrolled. The patients and controls were saliva typed for Lewis secretors and non-secretors. The results emerged from this study reveal the followings: Demographic data indicated an incidence age of  $26.5 \pm 4.53$  in patients versus  $28.54 \pm 6.07$  in controls ( $p=0.022$ ). FSH/LH ratio was  $0.91 \pm 0.61$  in patients and was  $2.18 \pm 2.35$  in controls. Overall-patients and controls secretors gave about identical frequency approaching 87 and 90% in patients and controls respectively. The level of H-substance in saliva (*FUT2* enzyme activity) didn't differ in patients and controls ( $43.6 \pm 70.5$ ) in patients versus  $43.95 \pm 62.64$  in controls ( $p=0.98$ ). Sorting out secretors to Leb and Lea-b- secretors gave a decreased 28.65 mean titer in patients versus 40.12 titer in controls. The conclusion drawn from the findings presented in this communication is that there is no role of Fucosyltransferase2 gene product that is associated with polycystic ovary syndrome.

## Introduction

Polycystic ovary syndrome is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism which generate short and long term consequences on female health (1).

Infertility is one of the most alarming

associated morbidities, as it currently affects approximately 48.5 million women aged 20–44 years (2), with PCOS accounting for 6–15% of these cases (3).

The manifestations of PCOS are not confined to the gynecological sphere ; women afflicted by this disease show an increased prevalence of several

comorbidities, including obesity, dyslipidemia, hypertension, metabolic syndrome (MS), and type 2 diabetes mellitus (T2D) in comparison with women without PCOS. These features, along with other alterations such as endothelial dysfunction and a chronic low grade inflammatory state, underlie the greater risk of developing cardiovascular disease and increased mortality observed in these subjects(4).

Polycystic ovary syndrome is a common endocrine system disorder among women of reproductive age. Women with PCOS may have enlarged ovaries that contain small follicles located in each ovary as seen during an ultrasound exam(5). Symptoms include: irregular or no menstrual periods, heavy periods, excess body and facial hair, acne, pelvic pain, trouble getting pregnant, and patches of thick, darker, velvety skin. PCOS is due to a combination of genetic and environmental factors (6).

Glycosylation of gonadotropins, especially FSH is an essential step in FSH-FSH receptor binding and this step is mainly controlled by secretor gene (Fucosyltransferase2) (7). A casual effect of aberrant *FUT2* could brought about decreased FSH function and subsequent effects including cystic formation in the ovary as well as aberrant glycosylation-based immune dysfunction. The present study aimed at evaluating the level of *FUT2* enzyme activity product in PCOS patients and healthy controls.

## **Materials and Methods**

### **Subjects**

One hundred polycystic ovary cases were enrolled in this study. They are ranging from 18 to 44 years old, besides, fifty apparently healthy control women were included

(control-1) that ranged from 18 to 44 years old.

### **Saliva Samples**

Saliva of 100 patients were collected according to (8). A piece of Arabic gum was chewed for (5 to 4) minutes to stimulate the secretion of saliva. One ml saliva was collected in a cleaned test tube. Saliva was diluted 1: 2 with normal saline. Centrifugation was followed at 4000 rpm for 10 minutes, The supernatant was placed in another clean test tube. Saliva was boiled at 100 °C in a water bath for 10 minutes and preserved at -40 °C until being used.

### **Determination of Secretors and Non Secretors of Lewis Groups (Haemagglutination Inhibition Test)**

This test was used to investigate secretors and non-secretors individuals. It was done according to (9), using 96 U shape wells microtiter plate and as follows:

Fifty microliters of saliva prepared as above was placed in all wells of microtiter plate. 50 µl of diluted Anti-H lectin (1:25) (titrated in advance), was added to all wells of microtiter plate. Incubation 15 minutes at room temperature was followed and 50 µl of human red blood cell (blood group O) suspension (0.05%) was added to all wells of the microtiter plate. The plate was incubated at 4 °C for 24 hours, agglutination was recorded as follows: Inhibition of agglutination indicates secretor status, while agglutination indicates a non-secretor sample.

### **Titration of H Substance**

This test was used to evaluate the titer of H Antigen in saliva, using anti-H lectin. It was done according to the procedure adopted as follows:

Fifty microliters of diluent bovine serum albumin (BSA) 0.2% in saline was added to all wells of microtiter plate, then 50 µl of saliva prepared previously was added in first row only, (1-12) wells of the plate. Serial dilution of saliva was followed and 50 µl of diluted Anti-H lectin (1:25), was added to all wells of microtiter plate. The plate was incubated for 15 minutes at room temperature, then 50 µl of human red blood cell suspension O- blood group 0.05%, was added to all wells. The plate was incubated at 4 °C for 24 hours. The last row of microtiter plate served as negative control, and contains diluents and saliva without anti-H. Titer of H-substance in the sample represent the reciprocal of the highest dilution of saliva giving inhibition of anti-H mediated agglutination.

Statistical analysis was performed using t-test or Chi-square to test statistical differences between test treatment and control treatment using their means values.

## **Results and Discussion**

### **Characterization of Polycystic Ovary Syndrome Patients**

The data demonstrated in table (1) showed age mean, (Body Mass Index), FSH, LH levels as well as FSH/LH ratio in patients and controls. The mean age of patients was  $26.5 \pm 4.53$ , compared with  $28.5 \pm 6.07$  in controls, (P-value was 0.022, 95% confidence interval of difference = 0.2972-3.7828). In this study, PCOS affects women in different ages. PCOS is the most common endocrine disorder among women at the ages of 18 and 44(1).

On the other hand BMI mean of patients was  $32.74 \pm 6.83$ , they were very obese, compared with  $28.37 \pm 5.12$  for controls (P-value was 0.00, 95% confidence interval of difference = 0.2084-6.5316).

The finding outlined above agree with Teede *et al.*, (2010) (1). Signs and symptoms of PCOS appear as a tendency towards central obesity and other symptoms associated with insulin resistance. The FSH mean of patients was  $4.51 \pm 1.99$ , compared with  $7.12 \pm 4.95$  for controls (P= 0.000, 95% confidence interval of difference = 1.4872-3.7328).

A diminished secretion of FSH which can result in failure of gonadal function (hypogonadism). Association with this females cessation of reproductive cycles is commonly observed. PCOS is associated with low FSH level (10). Low FSH value reported in literatures and in our study could be due to many reasons among them the presence of anti-FSH auto antibody.

The LH mean value of patients was  $4.95 \pm 3.23$ , compared with  $3.26 \pm 2.1$  for controls (P= 0.001, 95% confidence interval of difference = 0.6957-2.6843). This result is in agreement with (11) which reported Persistently high LH levels indicative of situations where the normal restricting feedback from the gonad is absent, leading to a pituitary production of both LH and FSH. While this is typical in the menopause, it is abnormal in the reproductive years.

The FSH/LH ratio mean of patients was  $0.91 \pm 0.61$ , compared with  $2.18 \pm 2.35$  for controls (P= 0.00, 95% confidence interval of difference = 0.6957-2.6843).

### **Frequency Distribution of Secretors and Non Secretors in Polycystic Ovary Syndrome Patients and Healthy Controls**

As depicted in figure (1), the percentage of secretors in patients was 87%, while the percentage of secretors in controls was 90% (P= 0.506, 95% confidence interval of difference = 0.28-1.93). However, the percentage of non-secretors in patients was

13%, while the percentage of non-secretors in controls was 10% (P= 0.506, 95% confidence interval of difference = 0.52-3.52). There are no data related to the

activity of secretor gene in PCOS, and it seems that this gene didn't contribute to a differences in all PCOS patients secretors compared to apparently healthy controls.

**Table.1** Demographic Data of Polycystic Ovary Syndrome Patients and Healthy Controls

Patient (n=100)	Mean±SD	P-value
Age	26.5±4.53	0.022
BMI	32.74±6.83	
FSH	4.51±1.99	
LH	4.95±3.23	
FSH/LH ratio	0.91±0.61	
<b>Control (n=50)</b>		
Age	28.54±6.07	0.001
BMI	28.37±5.12	
FSH	7.12±4.95	0.00
LH	3.26±2.1	
FSH/LH ratio	2.18±2.35	

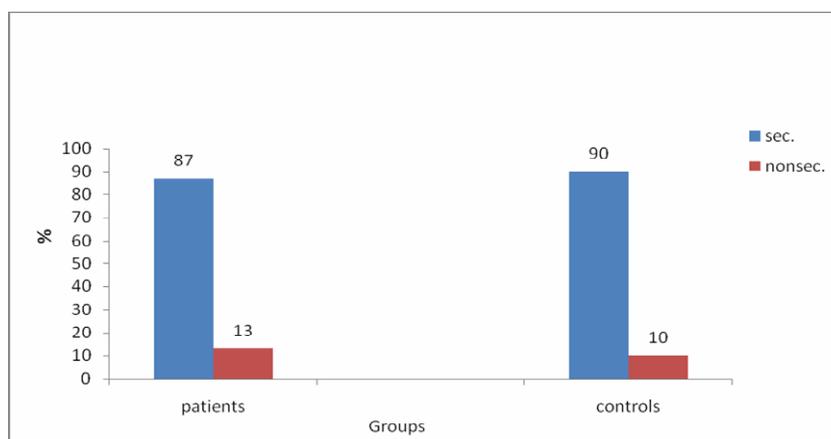
Significant if p-value ≤ 0.05 Highly significant if p-value ≤ 0.01

**Table.2** Titer of H-Substance in Groups of PCOS Patients and Controls

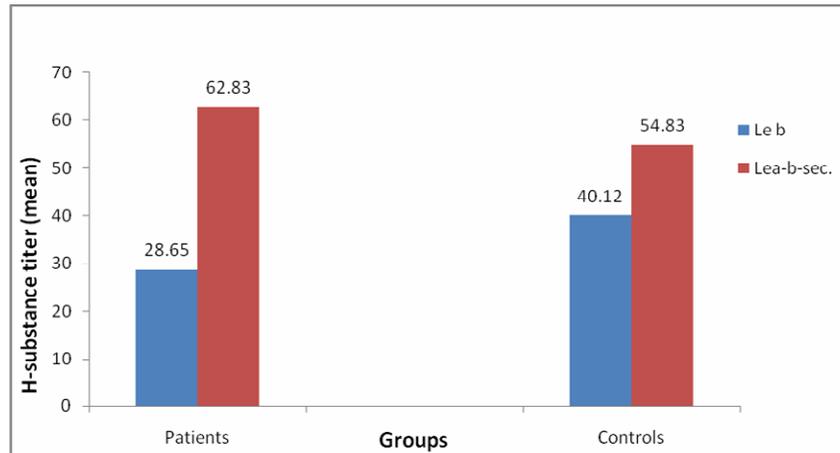
Groups	n	H-substance titer (mean±SD)	p-value
Patient			
Secretors	87	43.6±70.5	0.980
Control			
Secretors	45	43.95±62.64	

Significant if p-value ≤ 0.05 Highly significant if p-value ≤ 0.01

**Figure.1** Frequency Distribution of Secretors and Non Secretors in Polycystic Ovary Syndrome Patients and Healthy Controls



**Figure.2** Titration of Saliva H Substance of PCOS Patients and Healthy Controls of Le b and Lea-b-Secretors



### Titration of Saliva H-Substance

As seen in table (2) the mean value of saliva H- substance in secretors patients was  $43.6 \pm 70.5$  versus  $43.95 \pm 62.64$  in secretor controls ( $P = 0.980$ , 95% confidence interval of difference =  $26.6133 - 27.3133$ ).

This result indicates that the level of Se (*FUT2*) enzyme activity is not a factor in all PCOS secretors patients. Also, this coincide with the distribution of secretors and non secretors seen in table 2 .

Secretor gene is responsible for building up Le b structure as well as Lea-b- secretor structure.

### Titration of Saliva H substance in (Le b, Lea-b-sec.)

To sort-out the above groups, the findings presented in figure (2) showed that H-substance level is different among Le b secretor groups in patients and controls. The mean level of H-substance was  $28.65 \pm 54.38$  in patients versus  $40.12 \pm 66.82$  in controls and was statistically non significant ( $P = 0.483$ , 95% confidence interval of difference

=  $- 21.0076 - 43.9476$ ). Indeed differences in secretors H-substance (Le b and Lea-b-sec.) patients was seen Le b gave  $28.65 \pm 54, 38$ , while Lea-b- gave  $62.83 \pm 81, 17$ . This is interesting, and might involve additional contributing factor involved in this differences.

The findings presented urge for no role of secretor gene in PCOS pathogenesis, but other glycosylation-defective step could contribute in lewis secretors subgroups. This requires indepth investigation.

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