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ANTI-FOLLICLE STIMULATING HORMONE ANTIBODIES IN POLYCYSTIC OVARY SYNDROME AND APPEARANTLY HEALTHY CONTROLS

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ABSTRACT

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by hormonal imbalances. The syndrome is suspected as autoimmune disease. In this study, one handered hormonally and clinically characterized polycystic ovary syndrome (PCOS) patients ranging in age from 18-44 years and fifty appearently healthy controls were enrolled. The patients and controls were sera-screened for anti-FSH antibody by established enzyme-linked immunosorbent assay (ELISA), besides the specificity of anti-FSH antibody was investigated. The results emerged from this study reveal that anti-FSH autoantibody detection in patients secretors and non-secretors compared to respective controls showed that both secretors in patients and controls gave anti-FSH antibody(0.2±0.145 versus0.192±0.113) in patients and controls secretors respectively, however non-secretors showed a decreased level of anti-FSH autoantibody in patients compared to controls non-secretors(0.119±0.036 versus 0.254±0.148) in patients and controls non-secretors respectively and the test was significant at p=0.006. Part of anti-FSH was hormone specific as demonstrated by free-hormone inhibition ELISA.

KEYWORDS: handered hormonally, appearently healthy, immunosorbent.

INTRODUCTION

Polycystic ovary syndrome (PCOS) 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 associated with PCOS 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] although up to 70% of women with PCOS may be undiagnosed.[4] Indeed, its optimal diagnosis is often hindered due to its apparent similarities with several other pathologies remarkably obesity as well as Cushing's syndrome, ovarian and adrenal neoplasms and congenital adrenal hyperplasia.[5]

The human ovary can be the target of an autoimmune attack in various circumstances, including several organ-specific or systemic autoimmune diseases. Clinically, the ensuing ovarian dysfunction often results in premature ovarian failure (POF), but other pathologies involving the ovaries, such as unexplained infertility, polycystic ovary syndrome (PCOS) and endometriosis have been associated with anti-ovarian autoimmunity.[6]

The diagnosis of an autoimmune mechanism in these pathologies has relied for a long time on the detection of antiovarian autoantibodies, but recently special attention has also been focused on the cellular component of the autoimmune response.[7] However, little is known about the molecular targets of the autoimmune effectors, and very few autoantigens have been formally identified.[8]

The most prominant hormonal disturbances in polycystic ovary syndrome is an increased androgen and progesterone production, an effect that is due to short supply of FSH and shortage in it´s effect on granulosa cells.[6]

The gathered experimental data of this study is aimed at shedding some light on the immunopathogenesis of PCOS, the mysterious suspected autoimmune disease.

MATERIALS AND METHODS

Subjects

One handerd polycystic ovary cases were enrolled in this study. They are ranging from 18 to 44 years old, besides, fifty appearently healthy control were included (control-1) also ranging from 18 to 44 years old. Another control group of weman were seeked (control-2).

Anti-FSH Antibodies detection by ELISA

This test was used to investigate the presence of Anti-FSH antibodies (Ab). It was done as follows (K.H. Ali personal communication).

200 µl of FSH coatig solution (200IU/ml, 100 µl of resuspended FSH was added to 35 ml of sodium bicarbonate buffer (0.05). was added to all wells of 96- flat bottom microtiter plate. Leave overnight at 4℃ .Washed three times with washing buffer. (The buffer was taken from HCV detection ELISA kit). 200 µl of bovin serum albumin 1% (diluted in sodium bicarbonate buffer) was added in all wells of plate. Incubate for one hour at 37℃. Re-washed three times with washing buffer. 100 µl of diluted sera (1:60) was replicated on coated plate. Leave overnight at 4℃ and re-wash three times with washing buffer. 100 µl of conjugate was added to all wells. (The conjugate was taken from HCV kit). Incubate for 30 min. at 37℃, and re-wash three times with washing buffer. 100 µl of substrate (HCV kit) was added to all wells. Incubate for 15 min. at 37℃. 50 µl of stop reaction solution was added to all wells. Finally, the optical density (O.D) for each well was determined at 450nm using a microplate ELISA reader. 16 wells (two columns) were left without sera to served as control and for cut-off value determination.

Cut−off value calculation

Included the mean optical density values of no antigen coated wells plus 2 SD.

Samples were divided depending on the value of the Cut-off.

Positive: if the optical density of the sample is greater or equal to the value of the Cut-off.

Negative: if the optical density of the sample is less than the value of the Cut-off.

Determination of specificity of anti-FSH antibodies (Inhibition experiment)

The procedure used for inhibition experiments seen below was adopted and as follows.

200 µl of FSH coatig solution was added to all wells of 96-flat microtiter plate. Leave overnight at 4℃ .Washed three times with washing buffer. 200 µl of of bovin serum albumin 1% (diluted in sodium bicarbonate buffer) was added to all wells of plate. Incubate for one hour at 37℃. Re-washed three times with washing buffer. To do inhibition experiment, different concentrations of human free FSH of 12.5, 25, 50 and 100 mIU/ml were used as inhibitors.

Control and test sera diluted 1:40 at 50 µl were mixed with equal volume of inhibitors, left at room temperature for 20 min. and added to coated plate. Saline replaced inhibitor and served for non inhibited values .The plates were left at 4℃ overnight and processed for ELISA as indicated above.

RESULTS AND DISCUSSION

The data demonstrated in table (1) showed the mean±SD of FSH antibodies level (measured as O.D of 1:60 diluted sera) in patients and controls. FSH autoantibody in Secretors patients was 0.2±0.145, while in Secretors controls was 0.192±0.113 (P= 0.747, 95% confidence interval of difference = 0.0410-0.0570). It was not significant.

The mean value of FSH autoantibody in non-secretors´s patients was 0.119±0.036, while in non-secretors´s controls was 0.254±0.148 (P= 0.006, 95% confidence interval of difference = 0.0454-0.2246). In this instance non-secretors demonstrated a significant decrease in auto-anti FSH antibodies in patients. Non-secretors didn´t have secretor gene activity, an effect that is linked to the decrease of anti-FSH antibody in those group of patients. The molecular events as how to this contribute to autoimmunity is also not clear. There are no data reported in this situation of PCOS patients. On the other hand however, anti-FSH autoantibody irrelevant to PCOS was reported in IVF patients.[9] Besides, anti-ovarian autoantibodies were demonstrated in premature menopause.[10]

Table (1) Mean±SD of FSH antibodies level in patients and controls.

Groups	n	+No./total %	FSH antibodies (mean±SD) (O.D 450 nM)	P-value
Patient	100	32		
Secretors	87		0.2±0.145	0.747
non-secretors	13		0.119±0.036	0.006
Control1	50	36		
Secretors	45		0.192±0.113	
non-secretors	5		0.254±0.148	
Control2	15		0.119±0.029	

Sera were diluted 1:60 and tested by ELISA Positive was based on cut-off value

Significant if p-value ≤ 0.05

Highly significant if p-value ≤ 0.01

Specificity of anti-FSH antibodies in polycystic ovary syndrome lewis (a) patients and healthy controls

As shown in table (2) the mean±SD of anti-FSH in the presence of inhibitors (Free FSH) and without inhibitors in Le a patients and controls, are shown. Free FSH at 100,50,25,12.5 mIU/ml was used to inhibit anti- FSH autoantibody reacting with coated FSH. In Le a patients, mean±SD of O.D of anti-FSH antibodies are shown. The results presented in these tables clearly showed that part of anti-FSH autoantibody in Le a groups approaching (10.1-18.3)percent inhibition is related to anti-FSH antibody in patients as well as in controls which approach 53.2% inhibition at free FSH antigen of 25mIU/ml.

Table (2) Specificity of anti-FSH autoantibodies in Le a patients and controls.

Free FSH concentration mIU/ml	Anti-FSH value (O.D 450 nM) (mean±SD)		Inhibition %	P-value
	Without inhibitors	With inhibitors		
Patient n=3				
12.5	0.136±0.035	0.161±0.058	18.3	0.557
25	0.163±0.029	0.144±0.054	11.6	0.620
50	0.18±0.074	0.154±0.026	14.4	0.597
100	0.167±0.039	0.15±0.037	10.1	0.613
Control n=3				
12.5	0.136±0.031	0.121±0.032	11.02	0.591
25	0.154±0.03	0.072±0.039	53.2	0.045
50	0.146±0.024	0.162±0.05	10.9	0.644
100	0.161±0.037	0.163±0.053	1.24	0.960

Sera were diluted 1:40 and tested by ELISA.

Significant if p-value ≤ 0.05.

Highly significant if p-value ≤ 0.01.

Based on the first reported results seen in table 1 and 2, it seems that both patients and controls showed anti-FSH antibodies, besides, there was a prevalence of such antibodies in non-secretors. The reason of the presence of such antibodies in non-secretors and their differences in control-1 and 2 and their ability of immune complex formation in these groups is under investigation.

The findings gathered in this report might be part of undefined ovarian autoimmunity process. In this instance, the ovary is targeted by the body's immune system leading to a pathological condition known as"ovarian autoimmunity". In most endocrine autoimmune diseases, an abnormal level of the regulatory hormone is a primary diagnostic indicator of

potential pathology. The diagnosis is confirmed by measurement of specific autoantibodies. Regardless of the mechanisms involved in autoimmune pathology, detection of specific autoantibodies seems to be the most practical clinical and research marker of most autoimmune diseases. Clinically, the ensuing ovarian dysfunction often results in premature ovarian failure (POF), but other pathologies involving the ovaries, such as unexplained infertility, polycystic ovary syndrome (PCOS) and endometriosis have also been associated with anti-ovarian autoimmunity.[6]

Platia' group suggested the presence of anti-gonadotropin and anti-gonadotropin receptor antibodies in women with resistant ovary syndromes.[11]

Presence of anti-FSH and anti-LH antibodies (by ELISA and IHC with human ovarian tissue) as reported by Meyer' group after immunization against exogenous gonadotropins resulted in poor responders to IVF in infertile women.[12] A15 kDa protein representing the β-FSH from a total human ovarian extract was demonstrated to be an auto-antigen when sera from women with POF/POI were used to screen by Western blot analysis. The region 78–93 of this sub-unit known to be involved in receptor binding was found to be a target by the serum AOA and thereby explained the cause of ovarian failure in these women.[13]

Further research is needed as to the anti-FSH antibodies contribution in ovarian autoimmunity and PCOS in particular.

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