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Original Article

Prevalence of Non-classical Congenital Adrenal hyperplasia among Patients Managed as A polycystic Ovarian Syndrome using 17-Hydroxy-progesterone as A Biomarker

Mahmoud Farid Abdelmonem [*1] **Ahmed Mohammed Saba** [1] **Ahmed Mohammed Rammah** [2]

[1] Department of Medical Biochemistry, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

[2] The International Islamic Center for Population Studies and Research, Al-Azhar University, Cairo, Egypt.

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ABSTRACT

Background: Women with non-classical congenital adrenal hyperplasia (NCAH) is presented with clinical manifestations similar to that of polycystic ovary syndrome (PCOS). Thus, the clinical differentiation between two conditions is challenging. This study aimed to estimate the prevalence of NCAH in women managed as a polycystic ovarian disease.

Patients and Methods: This work was conducted at the Assisted Reproduction Unit (International Islamic Center for Population Studies and Research, Al-Azhar University). It includes 120 women, 100 of them representing the patient group. Those women asked the medical advice for treatment of anovulation infertility by the Intracytoplasmic sperm injection (ICSI). The other 20 females represented the control group. They were treated for male-factor infertility. The prevalence of NCAH among female treated as PCOS was estimated.

Results: The diagnosis of NCCAH was confirmed for 5% of women treated as PCOS in this study. The study also confirmed the clinical and biochemical similarities between the two conditions. However, the differentiation was possible by the significant difference of 17-hydroxyprogesterone between the two groups. A cutoff value of 3.98 ng/ml provided an excellent diagnostic power for NCAH. The area under the curve (AUC) was 0.981, sensitivity was 100.0% and specificity was 97.89%.

Conclusion: The measurement of 17-hydroxyprogesterone can be used as a screening tool to differentiation PCOS from NCAH, permitting proper treatment planning.

Keywords: Non-classical; Congenital; Adrenal hyperplasia; Polycystic Ovarian Syndrome.

* Corresponding author
Email: mahmoudfarid2014a@gmail.com

INTRODUCTION

The polycystic ovary syndrome (PCOS) is the commonest endocrine disorder among women in their reproductive age. The estimate prevalence of PCOS is ranged between 2 and 18%, and its global prevalence is 9.2% as reported recently. The use of different diagnostic criteria explains the variability of the prevalence [1-3]. The presence of polycystic ovary, hyper-androgenism, ovulatory dysfunction (two of these three manifestations) and exclusion of other possible causes of hyperandrogenism or ovulation dysfunction are the recommended Rotterdam diagnostic criteria of PCOS. These criteria are recommended by the Guidelines of the Endocrine Society for diagnosis of PCOS [4, 5].

Hyperandrogenism can be manifested clinically by hirsutism, acne or balding of the male pattern. On the laboratory levels, it may be manifested by significant elevation of testosterone (total or bioavailable) or dehydroepiandrosterone sulfate (DHEAS). However, the sensitivity of these assays is questioned even in the present of clinical manifestations of hyperandrogenism. It must be noted that, when there was a rapid and progressive hirsutism/acne, with enlargement of clitoris (i.e., clitoromegaly), the suspicion of malignancy must be raised [6-8].

The transvaginal ultrasound is the imaging modality of choice for detection of polycystic ovaries. The diagnosis is confirmed when ultrasound detected 12 or more follicles of diameter (2-9 mm) or increased ovarian volume over 10 cm^3 in at least one of the two ovaries and no other ovarian lesions were recognized [4].

The congenital adrenal hyperplasia (CAH) is a genetic diseases of recessive nature. It is due to mutations in genes encoding many enzymes (mainly 21-hydroxylase, 11β-hydroxylase or 3β-hydroxy-steroid dehydrogenase). The commonest is the mutations of the gene encoding 21-hydroxylase and other genetic mutations are less frequently reported. However, the disease had many phenotypes with wide clinical presentations. This depending on the mutations severity and the frequency of affected alleles [9-11].

Regardless of the affected enzymes, the deficiency leading to decreased production of two main hormones secreted by adrenal cortex, aldosterone and cortisol. This is associated with the reduction of the negative feedback of cortisol on pituitary gland. This stimulates compensatory increase of the pituitary adrenocorticotrophic hormone (ACTH). This subsequently associated with adrenal cortex hyperplasia [12]. CAH are presented in two types, the classical and non-classical types. The classical type is rare (affecting 1 from 15000 live births). It is recognized by a neonatal ambiguous genitalia. However, the diagnosis is crucial, as 75% of these patients are prone to salt-wasting [12-15]. On the other side, the non-classical type of CAH must be expected in any women with classical manifestations of PCOS (mainly, alopecia, acne, hirsutism or menstrual irregularities). This is due to the overlapping of clinical manifestations between the two conditions [9]. However, infertility is uncommon association with non-classical, but prevalent in classical CAH [12].

This study aimed to estimate the prevalence of NCCAH in women with received treatment for PCOS.

PATIENTS AND METHODS

This study was conducted at the Assisted Reproduction Unit of the International Islamic Center for Population Studies and Research (Al-Azhar University). It included 120 women with PCOS. They were 100 patients treated by the assisted reproduction (IUI or ICSI) due to anovulation infertility. The other twenty patients (assigned as a control group) were treated for male-factor infertility. Their age ranged between 20 and 35 years (reproductive age). The diagnosis of PCOS was done according to the revised Rotterdam criteria (ESHRE2003) [4].

Exclusion criteria includes pregnancy, thyroid dysfunction, hyper-prolactinemia, neoplasms secreting androgens, amenorrhea due to hypo-thalamic causes, Cushing's syndrome and age less than 20 years or more than 35 years.

All women were clinically evaluation by history taking and detailed clinical examination. Then, all were submitted to transvaginal ultrasound (to confirm the diagnosis or detect any other abnormalities) and laboratory investigations (e.g., random blood sugar, prolactin estradiol, Antimullerian hormone and 17-OH progesterone by ELISA, luteinizing hormone (LH), Follicle

stimulating Hormone (FSH) by automated Vidas Apparatus). According to levels of 17- OH progesterone determined at the follicular stage, women in each group were categorized into those with increased values of 17- OH progesterone, or those with normal values of 17- OH progesterone.

Sample used for laboratory investigations were collected from women attending Al-Azhar University Hospitals and related research centers. The samples were treated to collect serum, which was stored at the Central Laboratory of the department of medical biochemistry (Al-Azhar Faculty of Medicine) at -70°C until the time of assay.

The DRG 17-α-OH Progesterone ELISA Kit, a solid phase enzyme-linked immunosorbent assay (ELISA) was used for determination of 17-α-OH as described by the manufacturer. Briefly, the microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the 17-α-OHP molecule. Endogenous 17-α-OHP of a patient sample competes with a 17-α-OHP-horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of 17-α-OHP in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of 17-α-OHP in the patient sample. Chromate ELISA microplate readerl2 was used to perform all analysis.

Statistical analysis of data: The collected data were coded and fed to the statistical package for social sciences (SPSS) for analysis. Continuous numerical data were summarized by their means, medians, and ranges. Each two groups were compared by independent samples students or Mann-Whiteny tests as appropriate. P value < 0.05 was set as the margin for significance to interpret our results. Receiver operation characteristic (ROC) curve was plotted to test the diagnostic power of 17-OHP for NCAH. The area under the curve (AUC) reflected the power of the test, with determination of the best cutoff value, sensitivity and specificity of the test.

RESULTS

When comparing patients with PCOS to controls, both were comparable regarding patient age, LH, FSH, Prolactin (PRL), thyroid stimulating hormone (TSH), random blood sugar (RBS), and antimullerian hormone. However, patients with PCOS had significantly higher body mass index (BMI), E2, hirsutism score and 17-OH progesterone (Table 1). Five of the 100 patients presented with PCOS had increased levels of 17-OH progesterone more than (0.2 – 1.0 ng/dl) and they were NCAH. Comparing the patients with NCAH to those with PCOS revealed that, both were comparable for all studied variables except significant decrease of E2 and random blood sugar. The values of 17-OH progesterone were increased in NCAH than PCOS. However, the difference did not reach statistical significance (Table 2). Females with NCH had significantly higher values of body mass index, hirsutism score and 17-OH progesterone than the control subjects. However, other variables showed non-significant differences (Table 3). After building the receiver operative characteristics (ROC) curve, 17OHP at a cutoff value of 3.98 had an excellent predictive power for NCAH. The AUC was 0.981, sensitivity was 100.0% and specificity was 97.89 (Table 4, figure 1).

Table (1): Comparison between PCOS and healthy (control) study groups

	PCOs (n = 100)	Control (n = 20)	P Value
Age (years)	26.79 ± 3.6	25.3 ± 6	0.12
BMI (Kg/m^2)	31.3 ± 7	26 ± 3.0	**< 0.001***
LH	10.3 ± 5.1	9.4 ± 3.0	0.25
FSH	8.2 ± 7.5	8.0 ± 1.0	0.9
E2	55 ± 1	49 ± 1	**< 0.001***
PRL	17 ± 5	15 ± 4	0.09
TSH	3.2 ± 3	3.9 ± 1	0.3
RBS (mg/dl)	92.5 ± 10.1	92.57 ± 10	0.97
AMH	13.2 ± 16	12.8 ± 15	0.9
Hirsutism Score	12.1 ± 3	6 ± 1.6	**< 0.001***
17OH Progesterone	2.5 ± 2	0.5 ± 0.3	**< 0.001***

Table (2): Comparison between NCAH and PCOs in demographic and biochemical markers

	NCAH (n = 5)	PCOS (n = 95)	P Value
Age (year)	25 ± 4	26.79 ± 3.6	0.3
BMI (Kg/m^2)	30.8 ± 6.2	31.3 ± 7	0.8
LH	10.3 ± 15	12 ± 5	0.5
FSH	8.2 ± 7	7 ± 2	0.28
E2	50.7 ± 14	55 ± 1	**< 0.001***
PRL	16.2 ± 4	17 ± 5	0.726
TSH	3.1 ± 4	3.2 ± 3	0.9
RBS (mg/dl)	89 ± 3	99.5 ± 6	**< 0.001***
AMH	12 ± 18.5	13.2 ± 16	0.87
Hirsutism Score	13.5 ± 4	12.1 ± 3	0.3
17OH Progesterone	4.134 ± 2.8	2.5 ± 2	0.08

Table (3) Comparison between healthy (control) and NCAH patients in demographic and biochemical markers

	NCAH (n = 5)	Normal (n = 20)	P Value
Age (year)	25 ± 4	25.3 ± 6	0.25
BMI (Kg/m^2)	30.8 ± 6.2	26 ± 3	**0.017***
LH	10.3 ± 15	6.4 ± 3	0.264
FSH	8.2 ± 7	8 ± 1	0.89
E2	50.7 ± 14	49 ± 1	0.5
PRL	16.2 ± 4	15 ± 4	0.5
TSH	3.1 ± 4	3.9 ± 1	0.4
RBS (mg/dl)	89 ± 3	92.57 ± 10	0.5
AMH	12 ± 18.5	12.8 ± 15	0.9
Hirsutism Score	13.5 ± 4	6 ± 1.6	**< 0.001***
17OH Progesterone	4.134 ± 2.8	0.5 ± 0.3	**< 0.001***

Table (4): Predictability of 17OHP for diagnosis of NCAH among PCOS patients

Variable	Value
AUC	0.981
Cut off point	3.98
Sensitivity	100.0%
Specificity	97.89
P value	<0.001*

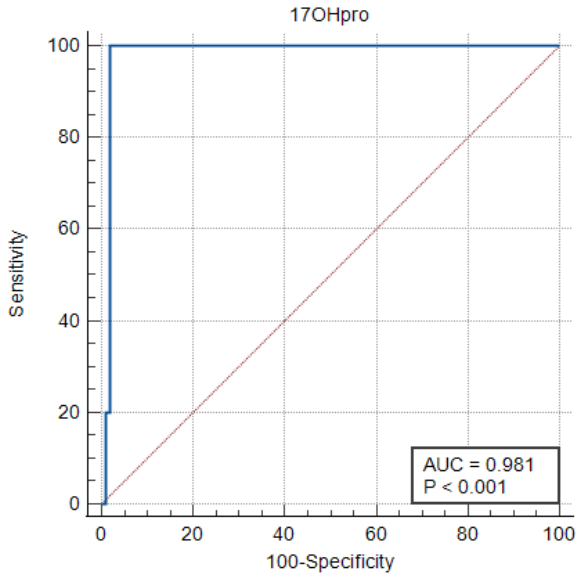

Figure (1): ROC curve analysis for basal serum 17-hydroxyprogesterone (17OHP) levels for the diagnosis of NCAH among women with PCOS.

DISCUSSION

The prevalence of NCAH among women with PCOS in the current work was 5%. This study is one of the earliest studies to determine prevalence of NCAH among Egyptian females with PCOS. This is slightly lower than the reported prevalence among Turkish women as reported by **Sahin and Keleştimur** [16].

In addition, **Fanta *et al.*** [17] reported that, the NCAH prevalence among hyperandrogenic women was 2.68%. The leading clinical manifestations were oligomenorrhea. But alopecia were a minor clinical problem. However, the prevalence of NCAH in PCOS women in the current work is close to the worldwide prevalence reported value by **Carmina *et al.*** [18] who conducted a systematic review and demonstrated that, the worldwide prevalence of NCAH in women with clinical manifestations of androgen excess (as those with PCOS) was 4.2%. They added that, NCAH lead to acceleration in growth (premature pubarche), cutaneous manifestations, oligoovulation and PCOS. As the current study, the same authors reported that the mainstay in diagnosis of NCAH is based on the serum concentrations of 17-OHP.

Our study confirmed the clinical and biochemical similarities between NCAH and PCOS. Thus, it is difficult to differentiate both conditions. However, increased values of 17-hydroxyprogesterone may be the only biochemical differentiating chemical indicator among those group of patient. Within this context, **Lidaka *et al.*** [19] reported non-significant differences in the prevalence of NCAH-causing genetic variations in women with PCOS. It means that, irrespective of genetic disturbances in NCAH, no genetic polymorphism could diagnose the condition. Hence, the importance and value of the 17-hydroxyprogesterone in prediction of NCAH, reported in the current work.

We also tried to establish a cutoff point to distinguish NCAH among women presenting with hyperandrogenic signs and symptoms. Our cutoff point is 3.98 ng/ml. However, **Carmina *et al.*** [18] after a systematic review reported that, the basal 17-OHP ≥2 ng/ml should be used as an indicator of probable diagnostic marker of NCAH. However, they elevated the concentration to be ≥10 ng/ml to have definitive diagnosis of NCAH. They proposed the use of genetic analysis of CYP21A2 gene (the gene encoding 21OHP activity) as a confirmatory tests.

The reported cutoff point in the current work is also higher than that reported by **Escobar-Morreale *et al.*** [20] who suggested 1.72 ng/ml as the best cutoff point with sensitivity of 100% and specificity of 88.6%. However, the same authors defined three cutoff points. The first and most sensitive was 1.7 ng/dl, followed by 2 ng/ml (sensitivity 83.3% and 93.6% specificity) and the lease sensitive cutoff value was 3 ng/ml (sensitivity of 66.7%, and specificity of 98.9%).

It must be noted that, with increased value of cutoff point, the sensitivity was reduced with increased specificity. However, in the current work, our value had the best sensitivity and specificity. In addition, we are able to provide an accurate estimation of 17-OHP performance as a screening tool for diagnosis of NCAH, as we measured values in all patients (hyperandrogenic) and healthy controls.

Our result are in line with **Kang *et al.*** [21] who suggested a single early morning assessment of the basal serum 17-OHP as an accurate screening tool. This permits the proper use and restriction of glucocorticoid treatment of NCAH. They recommended establishment of the best cutoff for each laboratory. But, if it is impossible to do that, they recommended the use of 3.98 ng/ml as the best cutoff, irrespective of the current recommendations of 2ng/ml as the upper limit of the normal 17OHP concentrations.

In conclusion, NCAH is a potential rate condition in PCOS patients (with hyperandrogenism). However, it must be differentiated to permit proper treatment. A single early morning concentration of the serum 17-OHP can be used as an accurate screening tool for diagnosis of NCAH. However, confirmation by genetic counseling is crucial to properly treat the condition. Different cutoff values of 17-OHP were reported in literature originating the need to establish a cutoff for each laboratory. However, results of the current work cannot be generalized due to its limitation of small number of patients. Thus, future large-scale studies are recommended.

Conflict of interest and financial disclosure:

None to be disclosed.

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