

The frequency of 21-alpha hydroxylase enzyme deficiency and related sex hormones in Iraqi healthy male subjects versus patients with acne vulgaris

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ABSTRACT

الأهداف: معرفة تكرار الأشخاص الأصحاء العراقيين المصابين بمرض فرط إنتاج خلايا الغدة الكظرية (CAH) الخلقي الغير تقليدي بسبب نقصان الأنزيم 21- هيدروكسيليز مقارنة مع مرضى حب الشباب .

الطريقة: أجريت دراسة الحالة في مركز لفحص مستويات الهرمون للمتعرجين. تم إجراء الدراسة قسم الأمراض الجلدية - بغداد - المستشفى التعليمي و قسم الكيمياء الفيزيولوجية - كلية الطب - جامعة بغداد - خلال الفترة من سبتمبر 2007 حتى فبراير 2008.

النتائج: أن تكرار الأشخاص الأصحاء المصابين بنقص الأنزيم 21- هيدروكسيليز هو 1:43 (2.3%)، وفي مرضى حب الشباب هو 6:43 (13.95%) الذي قد قدر بواسطة قياس مستوى 17 هيدروكسيبروجيسترون الذي قد ارتفع بصورة معنوية في أمصال مرضى حب الشباب مقارنة مع أمصال الأشخاص الأصحاء ($p=0.020$). وأن مستوى الكورتيزول الكلي قد قل بصورة معنوية في أمصال مرضى حب الشباب مقارنة مع أمصال الأشخاص الأصحاء ($p=0.022$).

خاتمة: تبين هذه النتائج أهمية الفحص الكشفي لنشاط الأنزيم 21- هيدروكسيليز (17- هيدروكسيبروجيسترون) في أمصال مرضى حب الشباب .

Objectives: To find out the frequency of nonclassical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency among Iraqi healthy male individuals versus male patients with acne vulgaris.

Methods: This case-control study and single-center examination of hormone levels in a cohort of volunteers was conducted in the Department of Dermatology, Baghdad Teaching Hospital, and in the Physiological Chemistry Department of the College of Medicine, Baghdad University, Baghdad, Iraq, from September 2007 to February 2008.

Results: The frequency of 21-hydroxylase enzyme deficiency in healthy male subjects was 1:43 (2.3%), while in male patients with acne vulgaris, this was 6:43 (13.95%). Serum 17-hydroxyprogesterone (OHP) levels were statistically and significantly elevated in male patients with acne vulgaris compared with healthy male controls ($p=0.020$). The serum total cortisol level was significantly reduced in patients with acne vulgaris in comparison with that of healthy controls ($p=0.022$).

Conclusion: These results support the necessity of inclusion of the 21-alpha hydroxylase enzyme activity (serum 17-OHP level) screening test in acne patients.

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Acne vulgaris is a chronic inflammatory self-limiting disease of the pilosebaceous unit that is mainly seen among adolescents, but it might continue for several years.^{1,2} Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from the deficiency of one of the 5 enzymes required for the synthesis of cortisol from cholesterol in the adrenal cortex. In a person with normal adrenal function, the adrenal gland produces both cortisol and androgens.³ The 21-hydroxylase enzyme hydroxylates 17-hydroxyprogesterone (17-OHP) at the 21-position,

producing 11-deoxycortisol, and a key step in the production of cortisol.⁴ In 21-hydroxylase deficiency (21-OHD), which is responsible for 90-95% of CAH cases, there is an accumulation of this substrate (17-OHP) that is shunted into the androgenic hormones pathway with a resultant cortisol deficiency.⁵ The 21-OHD occurs in 2 forms; classical and non-classical. Non-classical 21-OHD refers to the condition in which partial deficiencies of 21-hydroxylation permits a late onset, a less extreme hyperandrogenism, and milder clinical syndrome, or even no symptoms at all (cryptic form).^{6,7} Non-classic 21-OHD has a much higher frequency than the classic forms of CAH. The non-classic form occurs in approximately 0.2% of the general white population, but is more frequent (1-2%) in certain populations, such as Jews of Eastern European origin. The lower general frequency is similar to that estimated on the basis of CYP21 genotyping of newborns in New Zealand (0.3%).⁴ Therefore, the aim of the present study is to evaluate the frequency of the non-classical form of CAH due to 21-OHD in Iraqi male patients with acne vulgaris versus healthy male controls. This study was also designed to assess the status of serum cortisol and androgenic hormones concentrations in these 2 groups.

Methods. This case controlled study was carried out from September 2007 to February 2008 in the Department of Dermatology and Venereology of Baghdad Teaching Hospital, and in the Department of Physiological Chemistry, College of Medicine, and University of Baghdad, Iraq. The study consisted of 43 male patients with acne vulgaris, with an age range between 18-30 years. Patients were divided into 3 groups according to severity of their acne:⁸ a mild acne group that included 15 patients, a moderate group of 15 patients, and a severe group of 13 patients. Full history and examination was carried out for each patient including age, weight, height, duration of the disease, occupation, aggravating factors, relieving factors, past medical history, family history, drug history, type of food, and site of lesions. Exclusion criteria were those patients younger than 18, or older than 30 years, and those who underwent recent intake of systemic glucocorticoids, or any hormonal therapy (less than 3 months before inclusion). Forty-three healthy male controls (18-30 years) without acne were included as a control group. Formal consent was taken from each patient, after full explanation regarding nature of disease, course, prognosis, and its complication. Ethical approval was received from the Scientific Committee of the Department of Physiological Chemistry, College of Medicine, and University of Baghdad, Iraq.

Five milliliters of peripheral venous blood was aspirated between 8:00-9:00 AM from each patient and

control subject. Blood samples were collected in plain test-tubes and centrifuged after 30 minutes, the serum was separated and stored at -18°C until the time of assay. The biochemical investigations included in the present study were the measurement of serum concentration of 17- α hydroxyprogesterone (17- α OHP) as an indicator of 21- α hydroxylase enzyme activity, androstenedione, dehydroepiandrosterone sulphate (DHEA-S), total testosterone, and total cortisol. The serum 17- α OHP level was assessed by the enzyme-linked immunosorbent assay (ELISA) technique based on the methods reported by Demers.⁹ The serum concentrations of androstenedione and DHEA-S were also measured by the ELISA procedure based on the methods reported by Kicman et al,¹⁰ and Diagnostic Systems Laboratories.¹¹ The serum concentration of total testosterone was assessed by the enzyme immunoassay technique based on the method reported by the College of American Pathologists.¹² The total serum cortisol concentration was measured by the ELISA technique according to the methods reported by Demers.⁹

The Statistical Package for Social Sciences (SPSS) version 15 (SPSS Inc., Chicago, IL., USA), and Minitab analysis programs were used for all statistical analyses. Statistical significance was assessed by ANOVA and student t-tests. The linear regression test was applied for the correlation between different parameters, and the significance of the r-value was checked using t-test. *P*-values of <0.05 were considered significant.

Results. The frequency of 21-OHD as evaluated by its immediate substrate (17-OHP serum level) was 1:43 (2.3%) in Iraqi healthy individuals, and 6:43 (13.95%) in Iraqi male patients with acne vulgaris on the basis that the cutoff level of serum 17-OHP was >3 ng/ml.⁷ Table 1 shows the clinical and biochemical data for healthy controls and patients with acne vulgaris. This table shows that there was no significant difference in body mass index between healthy controls and patients with acne vulgaris. The mean (\pm standard deviation [SD]) value of serum 17-OHP level was significantly increased in patients with acne vulgaris compared with that of healthy controls, and the mean (\pm SD) value of serum total cortisol was significantly lower in acne patients than in healthy controls. There was no significant difference in serum androstenedione, DHEA-S, and total testosterone levels between healthy controls and patients with acne vulgaris. The results of a comparison of mean (\pm SD) serum values of 17-OHP and total cortisol concentrations in healthy controls and in mild, moderate, and severe types of acne revealed that the mean (\pm SD) value of serum 17-OHP levels of the severe type of acne (3.16 ± 0.77 ng/ml) were significantly higher than that of healthy controls (1.68 ± 0.73 ng/ml, $p=0.0001$), mild type (1.37 ± 0.23 ng/ml,

Table 1 - Clinical and biochemical data for healthy controls and patients with acne vulgaris.

Parameters	Controls (n=43)	Patients (n=43)	T-test	P-value
BMI (kg/m ²)*	23.06 \pm 1.4	22.75 \pm 1.31	1.04	>0.05
17-OHP (ng/ml)	1.68 \pm 0.73	2.09 \pm 0.87 [†]	6.29	0.013
Androstenedione(ng/ml)*	2.83 \pm 1.09	2.98 \pm 0.94	0.75	>0.05
DHEA-S (μ g/ml)*	2.78 \pm 1.31	2.92 \pm 1.04	2.16	>0.05
Total testosterone(ng/ml)	5.49 \pm 1.02	5.67 \pm 1.07	3.6	>0.05
Total cortisol (ng/ml)	87.42 \pm 45.94	68.33 \pm 28.54 [‡]	5.44	0.021

BMI - body mass index, OHP - hydroxyprogesterone, DHEA-S - dehydroepiandrosterone sulphate,
**t*-test, no significant difference between control and patient groups, [†]*t*- test for 17-OHP between
controls and patients with acne vulgaris, *p*=0.020, [‡]*t*-test for serum total cortisol between controls and
patients with acne vulgaris, *p*=0.022

p= 0.014), and moderate type of acne (1.88 \pm 0.16 ng/ml, *p*=0.032) (*p*=0.0001). While the mean (\pm SD) value of serum total cortisol levels of the severe type of acne (37.00 \pm 14.1 ng/ml) was significantly lower than that of healthy controls (68.32 \pm 28.52 ng/ml, *p*=0.026), mild type (139.5 \pm 23.87 ng/ml, *p*= 0.0001), and moderate type of acne (79.04 \pm 15.79 ng/ml, *p*=0.0001). Furthermore, the results of the present study revealed that in patients with acne vulgaris, there was a significant positive correlation between serum levels of 17-OHP and serum concentrations of androstenedione (*r*=0.912, *p*=0.0001), DHEA-S (*r*=0.925, *p*=0.0001), and total testosterone (*r*=0.911, *p*=0.0001). While there was a significant inverse correlation between serum levels of total cortisol and serum concentration of 17-OHP (*r*=-0.868, *p*=0.0001), androstenedione (*r*=-0.966, *p*=0.0001), DHEA-S (*r*=-0.855, *p*=0.0001), and total testosterone (*r*=-0.819, *p*=0.0001).

Discussion. The results of this study revealed that the frequency of 21-OHD in Iraqi healthy male individuals was 2.3% (1:43), and in Iraqi male patients with acne vulgaris was 13.95% (6:43). This finding is similar to that recorded by Placzek et al¹³ in Germany who reported that the frequency of 21-OHD in male patients with acne vulgaris was 14.6% (12:82). The present study shows that serum 17-OHP levels were significantly increased in patients with acne vulgaris versus healthy controls. This finding is similar to that observed by Placzek et al¹³ in Germany who reported that serum 17-OHP levels were significantly higher in acne male patients than in controls.¹³ Degitz et al⁷ reported that affected men with acne vulgaris had significantly higher mean value of serum 17-OHP than healthy male controls. The present study also has shown that serum 17-OHP levels of the severe type of acne were significantly higher than that of healthy controls, mild, and moderate types acne. This data agree with the results displayed by Placzek et al,¹³ who observed

that serum 17-OHP level was increased in severe acne patients compared to healthy controls. This does indicate that as the severity of acne increases, there is a rise in serum 17-OHP levels, which reflects the severity of 21-OHD. Although acne vulgaris is a self-limiting disease, it might be severer and persistent, and delayed in onset. In these cases, one should look for causes; one of these is non-classical 21-OHD (CAH), which accounts for 90-95%. This indicates the necessity to investigate this enzyme in patients with persistent and delayed acne. Regarding androgenic hormones, the results of the present study showed that serum androstenedione, DHEA-S, and total testosterone levels were increased in acne patients compared to those of healthy controls, but these differences did not reach a significant level. These results were again in harmony with that noticed by Placzek et al,¹³ who observed that there was an increase in serum androstenedione level in male patients with acne than in healthy controls, but this difference was not significant.¹³ Degitz et al⁷ also confirmed that serum androstenedione levels were more elevated in acne patients than in healthy controls without reaching a significant level.

The important significant correlations observed in the present study may indicate that when the serum 17-OHP level increases (which reflects the severity of 21-OHD), other hormones like serum androstenedione, DHEA-S, and total testosterone levels will increase, and this reflects the utilization of 17-OHP (the immediate substrate for 21-hydroxylase enzyme) by the minor pathway for the production of various androgens. Furthermore, the present study revealed that the serum total cortisol level was significantly decreased in patients with acne compared to healthy controls.

In the present work, one limitation represents the inability to determine the immediate substrate for 21-hydroxylase (17-OHP) after ACTH stimulation (Synacthen test). Another point is that molecular

screening techniques for 21- hydroxylase also exist, but presently they are not easy available.

In conclusion, the frequency of 21-OHD enzyme deficiencies in Iraqi healthy male subjects was 1:43 (2.3%), while in male patients with acne vulgaris was 6:43 (13.95%). The results of this study encourage the necessity of inclusion of serum 17-OHP (21-OHD) as a screening test in patients with acne vulgaris, especially in the delayed and severe type. Further studies with performing ACTH simulation test and gene analysis for example, PCR and HLA typing, are recommended.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.