Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Original Article

Frequency of 21-Hydroxylase Enzyme Deficiency in a Female Population with Hirsutism in Yaounde

Fréquence du déficit enzymatique en 21 hydroxylase dans une population de femmes présentant un hirsutisme à Yaoundé

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Key words: Hirsutism, 21-hydroxylase deficiency, 17-hydroxyprogesterone, Frequency, Yaounde.

Mots clés: Hirsutisme, Déficit en 21-hydroxylase, 17-hydoxyprogestérone, Fréquence, Yaoundé.

Article history
Submitted: 19 February 2023
Revision requested: 7 March 2023
Accepted: 16 March 2023
Published: 30 March 2023

ABSTRACT

Introduction. Hirsutism is defined as excessive male-pattern hair growth in women. In our society the etiology of this clinical situation is currently unspecified. The aim of this study was to determine the frequency of 21-hydroxylase enzyme deficiency in females with hirsutism in Yaoundé. **Methodology.** We conducted a descriptive and analytical cross-sectional study. Participants were recruited by advertisement. Our study population consisted of women of childbearing age, with hirsutism, non-pregnant and non-breast feeding. After interrogation, we proceeded to physical examination of participants with evaluation of the Ferriman and Gallwey score. We drew blood at the beginning of the follicular phase at T0 and T60 minutes after stimulation with 0.25 mg of tetracosactide or synthetic ACTH. We performed ELISA enzyme immunoassays for 17-hydroxyprogesterone to determine the frequency of 21-hydroxylase deficiency. The threshold for defining this deficit was established at 10 ng/ml of 17-hydroxyprogesterone after stimulation. **Results.** We recruited 73 women aged 27.4 ± 6.9 years. The mean Ferriman and Gallwey score was 14 ± 4; 67%, 30% and 3% of participants had mild, moderate and severe hirsutism, respectively. Concerning the hormonal profile, the median serum level of 17-hydroxyprogesterone varied from 2ng/ml(0.3-25ng/ml) before stimulation to 4.3 ng/ml(1.6-25 ng/ml) after stimulation. The frequency of 21-hydroxylase enzyme deficiency was 3%. There was no significant correlation between severity of hirsutism and enzyme deficiency. **Conclusion.** Congenital adrenal hyperplasia due to 21-hydroxylase deficiency appears to be a rare etiology in our study. ACTH stimulation test is necessary for diagnosis. Confirmation of the diagnosis can be done with the search for mutations in the CYP21A2 gene if possible.

RÉSUMÉ

Introduction. L'hirsutisme est le développement chez la femme d'une pilosité excessive de type masculin. Ses données étiologiques ne sont pas élucidées dans notre sociéte. Le but de cette étude était de déterminer la fréquence du déficit enzymatique en 21-hydroxylase dans une population de femmes présentant un hirsutisme à Yaoundé. **Méthodologie.** Nous avons mené une étude transversale descriptive et analytique. Notre population d'étude était constituée de femmes en âge de procréer, présentant un hirsutisme, non enceintes et non allaitantes, cotées selon le score de Ferriman et Gallwey. Nous avons procédé aux prélèvements sanguins en début de phase folliculaire à T0 et T60 minutes après stimulation à 0.25 mg de tétracosactide (Synacthène), puis réalisé les dosages immuno-enzymatiques ELISA de la 17-hydroxyprogestérone et déterminé la fréquence du déficit en 21-hydroxylase. Le seuil de définition de ce déficit était établi pour une valeur de la 17-hydroxyprogesterone après stimulation supérieure à 10 ng/ml. **Résultats.** Nous avons recruté 73 femmes âgées de 27.4 ± 6.9 ans. Le score de Ferriman et Gallwey moyen était de 14 ± 4 avec 67% des participantes présentant un hirsutisme léger, 30% un hirsutisme modéré et 3% un hirsutisme sévère. Le taux sérique médian de 17-OHP variait de 1.2ng/ml (0.3-25 ng/ml) avant stimulation, à 4.3 ng/ml (1.6 - 25 ng/ml) après stimulation. La fréquence du déficit enzymatique en 21-hydroxylase était de 3%. Il n'y avait pas de corrélation significative entre la sévérité de l'hirsutisme et le déficit enzymatique. **Conclusion.** L'hyperplasie congénitale des surrénales due à un déficit en 21-hydroxylase en est une étiologie certes rare, mais présente dans notre contexte. Le test de stimulation à l'ACTH est nécessaire au diagnostic. La confirmation du diagnostic devra être faite si possible avec la recherche des mutations du gene CYP21A2.

HIGHLIGHTS

What is already known on this topic

The late onset of 21-OH deficiency causes hirsutism in adult women and is associated with menstrual cycle disorders and infertility. However, socio-anthropological considerations limit medical assessment of hirsutism in Africa, resulting in sparse data about its etiologies and specifically the role of 21-OH deficiency.

What question this study addressed

The frequency of 21-hydroxylase enzyme deficiency in females with hirsutism in Yaounde.

What this study adds to our knowledge

The frequency of 21-hydroxylase enzyme deficiency was 3%. Thus, congenital adrenal hyperplasia due to 21-hydroxylase deficiency appears to be a rare etiology of hirsutism in Cameroon.

How this is relevant to practice, policy or further research.

It's important to search other etiologies of hirsutism.

INTRODUCTION

Hirsutism is the development of excessive hair growth in women in areas where they are normally less hairy than males. It is a phenomenon resulting from androgen stimulation of hair follicles and sebaceous glands. The etiologies of hirsutism vary, including polycystic ovary syndrome (PCOS) at 82%, ovarian or adrenal neoplasia at 0.2%, 21-hydroxylase (21-OH) deficiency at 2.2% and idiopathic hirsutism at 4.7 % [1,2]. Congenital adrenal hyperplasias (CAH) are autosomal recessive disorders with 21-hydroxylase (21-OH) deficiency due to mutations in the CYP21A2 gene. The 21-OH deficiency is responsible of classic form of congenital adrenal hyperplasia which results in utero virilization of female fetus and a non classic form (late onset) which presents late in young adult female. Non classic congenital adrenal hyperplasia is a cause of hirsutism in adult women and is sometimes associated with menstrual cycle disorders and infertility [3]. Worldwide, the prevalence of non classic congenital adrenal hyperplasia varies by ethnic group. Among whites, the prevalence of the nonclassic form may be as high as 1 in 1000 to 1 in 100 births , with the prevalence being even higher among Mediterraneans, Hispanics, and Eastern European Jews [4,5] A study carried out in Turkey on 126 adolescent girls with hirsutism revealed a prevalence of 3.8% [6]. Hirsutism is a reality in our population. A study on the clinical, psycho-social and metabolic profile of hirsute women in Yaoundé, revealed that hirsutism was perceived as normal in 58.3% of participants, a sign of being a tomboy in 46.7% and a seductive feature in 36.7%[7]. These socio-anthropological considerations limit medical assessment, resulting in sparse data on possible etiologies of hirsutism in Africa. Our study aimed to evaluate the frequency of 21-hydroxylase deficiency in women with hirsutism in Yaoundé.

METHODOLOGY

Study design

This was a cross-sectional, descriptive, and analytical study. It took place in the Department of Endocrinology and Metabolic Diseases of the Central Hospital of Yaoundé over a period of eight months: from October 2015 to May 2016. The participants were recruited by advertisement through social networks and based on data from the study by Ekobena and al on the clinical, psychosocial and metabolic profile of women with hirsutism in Yaoundé[7].

Inclusion criteria: we included women aged at least 18 years with hirsutism regardless of the degree of severity, non-pregnant, non-breastfeeding, without recent intake of corticosteroids, antiandrogens, estrogen-progestogens or spironolactone and willing to participate in the study.

Exclusion criteria: We excluded postmenopausal women with hirsutism.

Sampling: Our sample size was calculated based on the following formula: $n= z2\alpha/2\ \pi\ (1-\pi)/ E2$ where:

n= minimum sample size to obtain significant results for an event and a fixed level of risk

$Z2\alpha/2$= confidence level (1.96)

π= estimated prevalence of the variable studied

E= error margin (5%).

For our study, we used the prevalence of nonclassic congenital adrenal hyperplasia due to 21-hydroxylase deficiency in patients with hirsutism in Turkey which was 3.8% [5]. This resulted in a minimum sample size of n= 56.

Clinical and biological sample collection

Participants were seen on the fourth day of their menstrual cycle if the woman is cycling regularly early in the morning between 6.am and 8.am for basal 17 OH progesterone (17-OHP). For women with amenorrhea or infrequent menses, the sample can be drawn on a random day. We used author-designed pretested questionnaire. After interrogation on the history of hirsutism, personal and family history, we proceeded to a physical examination including measuring anthropometric parameters, searching for signs of hyperandrogenism and virilization, and determining the Ferriman and Gallwey score. Hirsutism was classified according to the modified Ferriman and Gallwey score as follows [30]: mild: 8-16, moderate: 17-24, severe: >24. We drew 5 milliters of venous sample at T0 for basal 17 OH progesterone (17-OHP) levels and 60 minutes after injection of synthetic ACTH (Synacthene° 0.25 mg). The patient sample was left to clot at room temperature and then centrifuged at 1500 revolutions/minute for 10 minutes and the resulting serum was collected and stored at – 20 °C for 17OHP essay.

17-OHP measurement

The 17OHP was measured from serum samples through the competitive Elisa immunoenzymatic assay for 17-OHP supplied by IBL INTERNATIONAL GMBH (17-OH-Progesterone ELISA) according to manufacturer's procedure. Analysis were performed at the laboratory of the University Teaching Hospital of Yaoundé.

Judgment criterion

We considered a positive 21-hydroxylase deficiency if serum 17-OHP level was greater than 10ng/ml 60 minutes after ACTH stimulation [8].

Statistical analyzes

Data collected was entered using SPSS version 16.0 software. Comparison between groups was made using the Student's test. The association between categorical variables, and quantitative variables was assessed by Fisher's exact test and Spearman's nonparametric correlation, respectively. The threshold of statistical significance was set at $P < 0.05$.

Ethical considerations

All participants gave a written informed consent. We obtained authorization N°2016/04/752/CE/CNERSH/SP from the national ethics committee for human health research in Cameroon.

RESULT

Epidemiological data

We retained 73 women with hirsutism, 60% were students with an average age of 27.4+/-7.9 years. The average Ferriman and Gallwey score was 14 ± 4 with a minimum of 7 and a maximum of 25. A family history of hirsutism was found in 90% of participants and 15% presented with menstrual cycle disorders especially spaniomenorrhea. The average age of onset of hirsutism was 16 ± 6 years and the mean duration was 11 ± 6 years.

Hormonal profile of participants (Table 1)

The median basal serum 17-OHP level was 1.2 ng/ml, with a minimum of 0.3ng/ml and a maximum of 25ng/ml. The median serum 17-OHP level after ACTH stimulation was 4.3 ng/ml, with lower and upper limits of 1.6 ng/ml and 25 ng/ml, respectively. There was a significant association between basal hormone levels and those after ACTH stimulation ($p<0.05$).

Table 1: Characteristic of study population

Variables	N	Frequency	Percentage
Marital status	73		
Married		6	6.6
Unmarried		59	80
Concubine		6	10
Widow		1	1.7
Divorced		1	1.7
Educational level	73		
Primary		2	1.7
Secondary		17	23.3
Tertiary		54	75
Profession	73		
employed		3	5.0
Student		44	61.7
Government worker		12	15
Private sector employed		14	18.3
Family history of hirsutism	73		
Yes		66	90.4
No		7	9.6
Menstrual cycle disorders	73		
Yes		11	15.1
No		62	84.9
Mean age		27.4+/-7.9 ans	

Frequency of 21-hydroxylase deficiency

In our study, 3% of participants had serum 17-OHP levels after synacthen test >10ng/ml(Table 2).

Table 2: Hormonal profile of study population

Variables N=73	Number	Percentage (%)
Basal 17- OHP values		
<2ng/ml	56	76.7
>2ng/ml	17	23.3
17- OHP values after ACTH stimulation		
<10ng/ml	71	97
>10ng/ml	2	3

Table 3: Correlation between age, clinical parameters, severity of hirsutism and laboratory data.

Variables	N	Spearman's rho	P-value
Basal 17- OHP values	73		
Weight (kg)		-0.89	0.4
Systolic BP (mmHg)		-0.79	0.5
Diastolic BP (mmHg)		-0.08	0.4
Ferriman et Gallwey score		-0.12	0.9
Age (years)		-0.326	**0.005**
17- OHP values after ACTH stimulation	73		
Weight (kg)		-0.37	0.7
Systolic BP (mmHg)		-0.104	0.3
Diastolic BP (mmHg)		-0.303	0.09
Ferriman et Gallwey score		-0.045	0.7
Age (years)		-0.350	**0.002**

17-OHP : 17 hydroxyprogesteron, 21-OH : 21 hydroxylase, ACTH : Adrenal corticotropin, ACTH : Adrenocorticotropic hormon, BP : Blood pressure, CAH : Congenital adrenal hyperplasias, Mmhg : Millimeter of mercury, Ng/ml : Nanogram per milliliter, PCOS : Polycystic ovarian syndrom

This represented the frequency of 21-hydroxylase enzyme deficiency. Participants with 21-hydroxylase deficiency had mild hirsutism. We noted a significant correlation between age of participants and serum level of 17-OHP (Table 3). We din't note the significant correlation between clinicals parameters, Ferriman Gallwey score and level of 17-OHP.

DISCUSSION

The etiologies of hirsutism are diverse. However the frequency in our context is not known. We conducted a study whose main purpose was to determine the frequency of 21-hydroxylase enzyme deficiency in a female population with hirsutism in Yaoundé. To achieve this, we enrolled 73 women with hirsutism regardless of degree of severity who accepts to participate in the study. We obtained this sample size taking into account logistical limitations like the blood test constraint on the 4th of the menstrual cycle and early morning. As such, this may constitute a bias for our study because it is made up of volunteers. We used as diagnostic method the serum assay of 17-OHP by ELISA after stimulation with synthetic ACTH. It's a good screening test for nonclassic CAH. We used as endpoint a rate of 17OHP>10 ng/ml after the synacthen test. Due to the possibility of false positives, some authors suggest considering a rate of > 15 ng/ml [9]

However, gene expression is the best method to establish with certainty the diagnosis of 21-hydroxylase deficiency[4,9–11], although its high cost and unavailability limits its use in our context. The main strength in our study was in the hormonal diagnosis that we offered our participants and it's the first study which try to explore etiologies of hirsutism. The average age of our study population was 27 years. They were mainly single women with a university education. Which is similar to the results of Ekobena and al, probably because the study was carried out in the same population. The av erage age of onset of hirsutism was 16 years. This is higher than the result of Binay et al in Turkey which found an average age of 12 years. This is probably because the latter's study population consisted of children[6]. The average Ferriman and Gallwey score was 14/36. This score varies due to ethno-geographical differences found in studies on hirsutism[12]. We found a frequency of 21-hydroxylase enzyme deficiency of 3%. Our result is lower than that found by Arnaout and al in Jordan [13] Trakakis and al in Greece[14] and Carmina and al in Italy [8] who found 37%, 9.3% and 4.7% respectively. However our figure is higher than that of Romaguera and al in Puerto Rico who find 1% [15]. This difference could be explained by variations in sample size, population studied, ethnic differences and assay methods which differ. We noted a negative correlation between participants' age and serum 17-OHP levels. Thus, the younger the subject, the higher the level of 17-OHP probably because the ovarian production. The same observation was made by Carmina et al[8]. In our context, this study calls for vigilance in assessing women with hirsutism who come for consultation for any problem whatsoever. More importantly because they are unlikely to consult specifically for this sign.

Study Limitations

- Sample Size
- Lack of ovaries ultrasounds

CONCLUSION

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency appears to be a rare etiology in our study. Given that basal 17-hydroxyprogesterone tends to overestimate the frequency of congenital adrenal hyperplasia, ACTH stimulation test is necessary for diagnosis. Confirmation of the diagnosis can be done with the search for mutations in the CYP21A2 gene if possible. Therefore it's important to search others etiologies of hirsurtism by carrying out other etiological studies.

Acknowledgements

We acknowledge all the women who took part in this study.

Authors' Contributions

Francine Mendane and Martine Claude Etoa conducted the study and wrote the manuscript, Evarita Ebolo collected data and did statisticals analysis. Ama Moor Vicky supervised the biological analysis. Mesmin Dehayem and Ester Meka helped with data collection, analysis and interpretation of data. Eugène Sobngwi designed and supervised the study. All authors have read and agreed to the final version of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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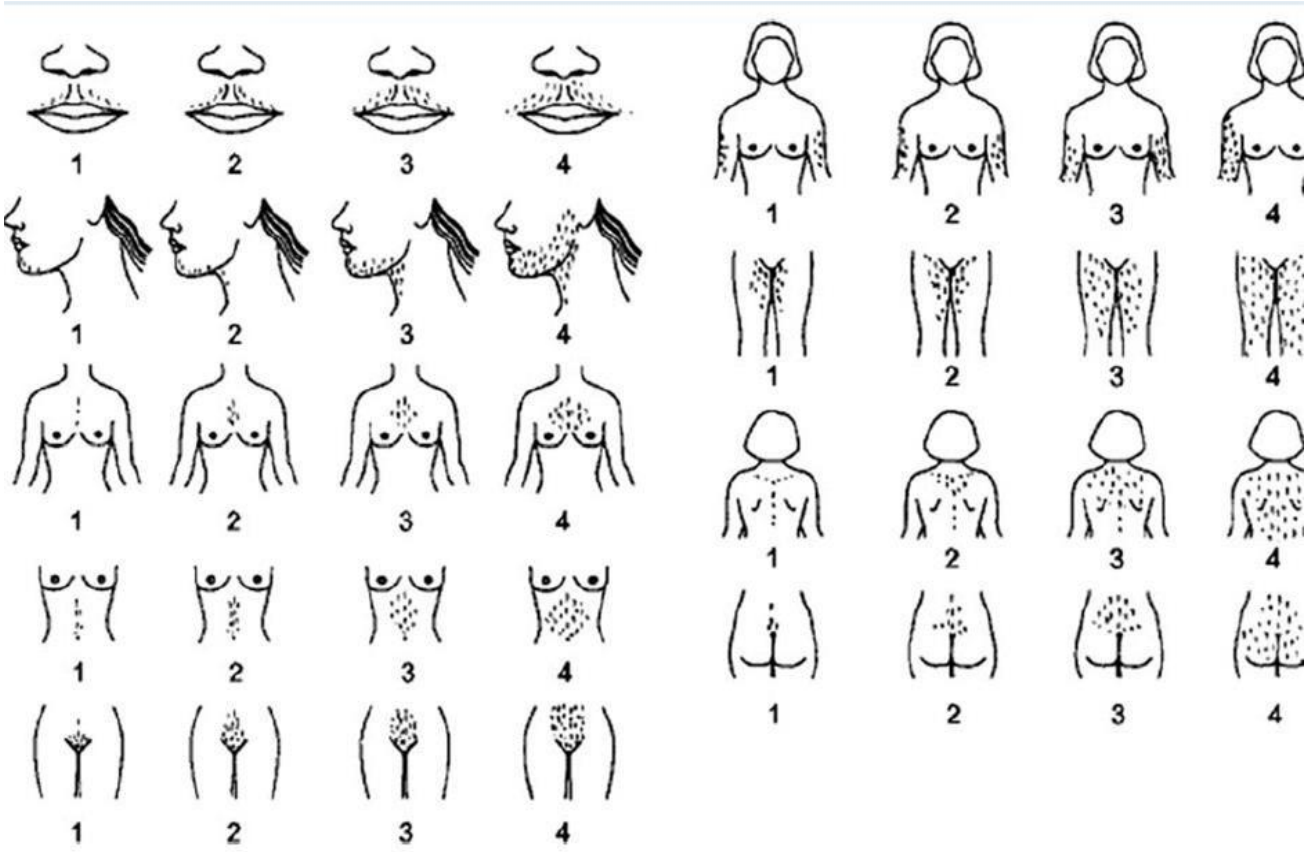

Modified Ferriman Gallwey Score