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ADT-G as a promising biomarker for peripheral hyperandrogenism in adult female acne

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ABSTRACT

Acne vulgaris is a chronic inflammatory disease that affects the pilosebaceous unit. Recent studies have shown an increasing number of cases of acne in adult women. These cases are predominantly normoandrogenic and present some clinical differences compared to adolescent acne. Local glandular metabolism turns some weak hormonal precursors into more active substances that increase the production of sebum, leaving these areas more prone to an increasing the colonization by Propionibacterium acnes (P. acnes). Our objective was to evaluate the usefulness of an androgenic metabolite as an adult female acne biomarker. The study population consisted of 38 adult women with acne without any features of hyperandrogenism and a control group. They were recruited from the clinic of Dermatology Hospital Division of São Paulo, Federal University of São Paulo from January 2012 to September 2014. After the first hormonal dosages, patients with acne were randomized into two different groups: one receiving a combined oral contraceptive (COC) containing 0,02 mg of ethinylestradiol and 3 mg drospirenone in a regimen of 24 days of medication, and the other group was treated with a topical gel containing 15% azelaic acid (AA), twice daily, both for six months. With the end of treatment new dosages were performed. Regarding the hormones, total and free testosterone and dehydroepiandrosterone sultate were quantified. In addition, the detection and quantification of androsterone glucuronate (ADT-G), an androgenic metabolite, has been developed. Only ADT-G was sensitive in detecting differences between the control and acne groups, and presented reduction of their values with systemic treatment. Therefore, only ADT-G was able to analyze the peripheral hyperandrogenism in cases of adult female acne.

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Introduction

Researchers analyzing epidemiological data of adult acne showed prevalence increased in women beyond 26 years old, indicating a statistically significant difference when compared to men. The prevalence in women ranges from 14–20% compared to 3–5% for men.[1-5] Furthermore, lesions in adult women are more intense. Clinically, they have moderate acne involving the lower face and lateral neck. In contrast, the typical presentation in adolescents involves the frontal area, nose, and malar area.[6] Not all adults have this pattern of distribution, and the presence of a large number of comedones was reported in smokers.[7,8] Usually, these patients have no hormonal abnormalities. They have a prolonged evolution with chronic skin inflammation.[9]

Sebaceous gland, among other peripheral tissues, is able to transform weak hormone precursors into potent androgens [testosterone (T) and dihydrotestosterone (DHT)]. Its cytoplasm contains all the six enzymes needed for androgenic amplification. This function is known as peripheral conversion and in the end stimulates increased sebum production.[10,11] A minimal amount of the potent intracellular androgenic products reach the circulation, therefore the major action is restricted to the sebocyte. Increased sebaceous production favors colonization by P. acnes.[12]

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Androgens need to be metabolized in water-soluble substances to be excreted. Thus, all androgens, regardless of the location of their production, are eliminated by sulfation and glucuronidation processes.[13] Androsterone glucuronide (ADT-G) accounts for more than 70% of these metabolites.[14] Although, most of the time, its dosage is performed by radioimmunoassay, this technique presents low specificity and sensitivity when compared to new laboratory analytical methods such as liquid chromatography associated with mass spectrometry.[15-17]

Another androgenic metabolite, known as 3 alpha-androstanediol glucuronide (3 alpha diol-G) of molecular weight very close to ADT-G; 468,58 g/mol and 466,56 g/mol respectively; was studied in acne cases but has proved useful as a biomarker only when hirsutism is present in association.[18-19]

Objective

The objective of this study was to develop a precision laboratory analysis to verify if the ADT-G would serve as a biomarker of peripheral hyperandrogenism in cases of patients with adult female acne.

Methods

Women between 26 and 44 years old were the study population. The acne group had diagnosis of mild to moderate acne, affecting the face and, healthy women with the same age range composed the control group.

The Research Ethics Committee – Federal University of São Paulo, approved the project / Hospital São Paulo, on November 4, 2011, under no. 1622/11 and was enrolled in Clinicaltrials.gov – Identifier: NCT01850095.

Patients with signs and symptoms of clinical hyperandrogenism such as hirsutism, androgenetic alopecia and irregular menses, were excluded.

Transvaginal ultrasonography and free / total testosterone (FT/TT) and dehydroespandrosterone sulfate (S-DHEA) dosages were performed in all women. Only the subjects with the exams within the reference range remained in the study.

The acne group (38 women) was randomized into two groups (software: random.org) in order to be treated with two distinct medications. One group was treated with azelaic acid (AA) 15% in gel, bid, for 6 months and the other group with combined oral contraceptive (COC) containing ethinylestradiol 0.02 mg and drospirenone 3 mg at the same time. Ten healthy women were included in the control group.

New blood samples were collected before and after the treatment. Inflammatory lesions count and photographic records were made to analyze the treatment evolution.

The ADT-G dosage was performed with a new "in-house" methodology using solid phase extraction and quantification by liquid chromatography associated with sequential mass spectrometry (LC-MS/MS).

Detection and quantification of ADT-G

The ADT-G analysis was performed by LC-MS/MS, operating in the electrospray-negative mode. On the analysis's day, calibrations with standards, ranging from 1 to 100 ng / ml were prepared.

Extraction of samples

Briefly, 200 μL of each plasma sample was mixed with 200 μL of acetonitrile (ACN) and 20 μL of a solution containing the deuterated internal standard (ADT-G-d4). This mixture was stirred in individual tubes for 2 minutes and then centrifuged at 14000 rpm for 10 minutes.

The samples were transferred to the solid phase extraction columns (SAX column – strong anion exchange) after being activated with ACN and equilibrated with water. After application of the samples, the columns were washed with 1 mL of water. The analytes of interest were eluted with 1 mL of a solution containing 95% ACN and 1% formic acid. The eluates were evaporated in vacuum for 2 hours. The dried residue was reconstituted in 50 μL solution of 50/50 mix methanol/water.

Analysis by liquid chromatography tandem-mass spectrometry (LC-MS/MS)

The derivative was analyzed by LC-MS/MS operating in the negative electrospray mode using a system composed of an Acquity binary pump and XEVO TQS (Waters) mass spectrometer with CTC 2777 injector. It was used a column Gemini NX 3 μm C18 150 $\times$ 2 mm. Total running time was 2 minutes. ADT-G and ADT-G-d4 were detected by monitoring selected reactions (MRM) using two transitions for each analyte. Data were processed and quantified by TargetLynx software (Waters). The method was validated according to the CLSI guidelines and

parameters such as linearity, precision, limit of quantification, and recovery were evaluated.

Statistical analysis

Qualitative variables were described as number and percentage, and quantitative variables were described by means of central tendency (mean and median) and variability measures (standard deviation, standard error and inter-quartile interval).

A model of analysis of variance (ANOVA) with repeated measures was used to compare the number of inflammatory lesions counted by the investigator before and after the treatments (COC and AA).

For the comparison between the two treatments regarding the general appearance of the skin performed by the independent examiners in the photographic evaluation, the Fischer exact test was used.

All statistical analyzes were performed with SPSS® software version 16.0 (SPSS® Inc., Illinois, USA). All tests were two-tailed and p value <0.05 was considered statistically significant.

Results

Table 1 shows the initial population characteristics. The statistical analysis proved the homogeneity of the studied groups.

Clinical evolution

The clinical evolution, analyzed by the two independent dermatologists, was considered efficient with both treatments. However, for the examiner 1 there was no significant difference between the groups (Fisher's exact test p = 0.180) and for the examiner 2, the group treated with COC presented a superior improvement with statistical significance in relation to the group treated with azelaic acid (p = 0.002). A significant decrease in mean number of lesions after treatments was observed in both groups (p < 0.001).

Table 1. Clinical and demographic data according to the treatment subgroups.

	TREATMENT		
	COC(n = 20)	AA(n = 18)	p-valor
Age[a] (years)	33,7 ± 5,5	33,1 ± 5,3	0,695[#]
Smoking	1 (5,0%)	2 (11,8%)	0,584[##]
"U type" lesion distribution	14 (70,0%)	13 (72,2%)	>0,999[##]
Onset of acne			0,516[&]
adolescence	9 (45,0%)	10 (55,6%)	
adult	11 (55,0%)	8 (44,4%)	
Intensity			0,132[###]
none	—	—	
comedones and rare papules	1 (5,0%)	1 (5,6%)	
predominance of papules and pustules	18 (90,0%)	12 (66,7)	
nodules	1 (5,0%)	5 (27,8%)	

[a]amean ± standanrd deviation.
[#]Student T test /.
[##]Fisher's exact test /.
[###]generalization of Fishers's exact test.
[&]chi-square.

Hormones and ADT-G

Before the treatment

Statistical analyses proved that the subgroups were homogenous for ADT-G values, before the treatments (p = 0.559 – Student's t-Test). Figure 1 shows a significant difference between the groups (p = 0.013), with a mean value of 10.4 ± 4.0 ng / ml (95% CI – [2,3]), higher in acne group. No difference was found with the other hormones analyzed.

Treatment influence

According to the Fig. 2, the ADT-G levels at the AA group did not present significant variation from before and after the treatment. However, the COC group presented a significant decrease in the mean ADT-G from before to after the treatment (p = 0.029).

According to the Fig. 3, at the AA subgroup, the FT mean levels did not change with the treatment. On the other hand, in the COC group, a significant difference was observed ($p < 0.001$).

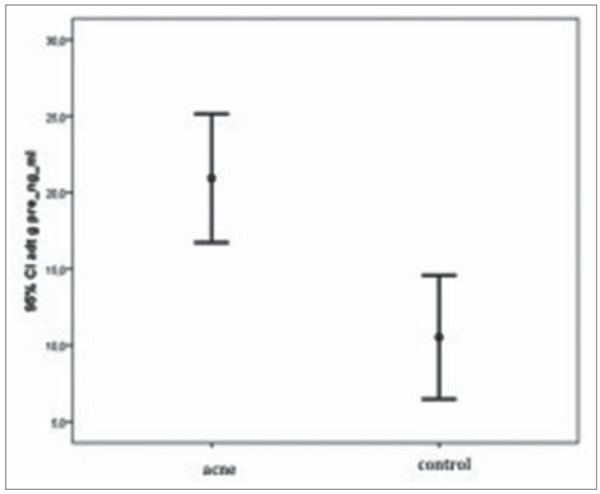

Figure 1. Error bar graphs representing mean ± standard deviation values obtained in ADT-G levels in acne and control groups, before treatment.

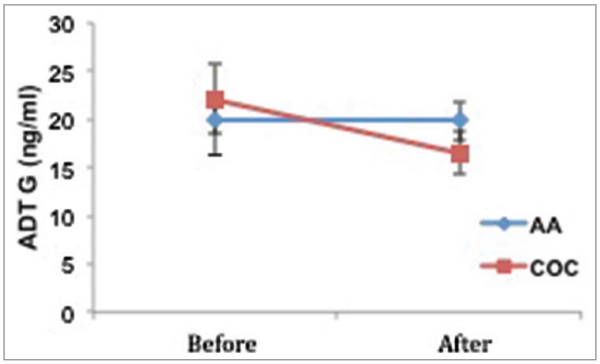

Figure 2. Profile chart of subgroups, ADT-G levels before and after treatments.

Total testosterone and S-DHEA mean levels showed no significant variation with treatment.

Discussion

In general, almost all patients with acne present increased sebaceous secretion. Some studies have demonstrated an increase in 5α-reductase activity and higher androgen receptors expression in sebocytes.[14,20]

However, even with normal androgenic levels, sebaceous glands may be hyperactive. Sebocytes have all the necessary enzymes for conversion weak hormonal precursors in more potent ones, which act on intracellular receptors.[21]

In this study, we sought to indirectly verify the androgenic peripheral activation, performed by many tissues including the skin, by the dosage of the most frequent androgenic metabolite, ADT-G. The mean values of the laboratory dosages were used to verify the existence of significant differences between the studied groups.

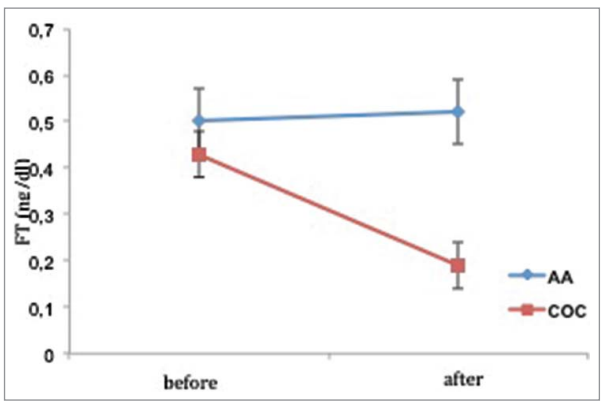

Figure 3. Profile graph of subgroups, free testosterone (FT) levels before and after treatments.

No differences were observed regarding the levels of TT, FT and DHEA-S among the two groups before the treatment. This is consistent with most previous studies that classify these patients as "normoandrogenic".[22]

However, we believe the best term would be "normoadrogenemic," which means: with no plasma androgen elevation.

Thus, TT / FT and DHEA-S were not useful as androgenic markers in these patients with isolated acne. Previous studies have confirmed that TT and FT are not capable of analyzing the total androgen activity and that they would be helpful only in monitoring patients with hirsutism associated or not with acne.[23]

The ADT-G levels, obtained by a high specificity and sensitivity analytical chemistry technique (liquid chromatography tandem-mass spectrometry) showed a significant increase in mean values, 10.4 $\pm$ 4 ng / ml, in the comparison between the acne and the control, allowing to conclude that this biomarker was able to differentiate the groups. The levels of ADT-G can reflect the total androgenic metabolism.

Labrie et al. (2006) analyzing childbearing and postmenopausal women also suggested the use of ADT-G as an androgenic biomarker rather than testosterone.[24] Carmina et al. (2002), found that in women with only acne the best biomarker would be the levels of ADT-G, even though at the time he used a less sensitive method (radioimmunoassay).[25]

Regarding the treatment effect, it was observed that the use of COC significantly reduced the levels of ADT-G, proving the antiandrogenic effect of ethinylestradiol plus drospirenone. Thus, ADT-G, besides a hormonal biomarker, was also useful in the treatment follow-up, which was compatible with the clinical improvement of the lesions.

As expected and already revealed by others studies the free testosterone levels were significantly reduced by treatment with COC. The mechanism is in part due to the increase in hepatic synthesis of SHBG (sex hormone-binding globulin), induced by the estrogen contained in the COC.[26] The total testosterone and DHEA-S mean levels showed no alterations with the treatments.

As was expected, the topical use of AA did not produce any change in hormone levels, since its systemic absorption is negligible, less than 4%.[27] Therefore is possible to conclude that although this medication may block the 5 alpha-reductase, as suggested by

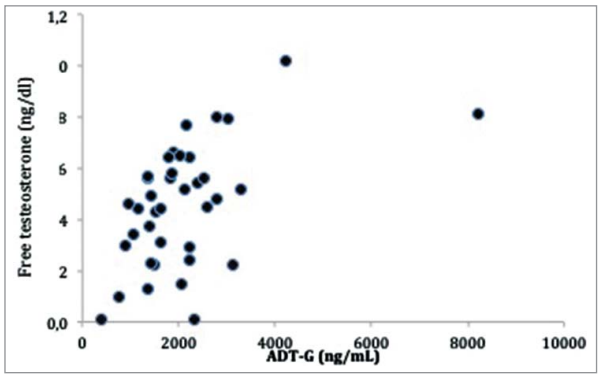

Figure 4. Correlation between FT and ADT-G, before treatment – Spearman Correlation Coefficient: r = 0.43 – 95% CI [0.143; 0.668].

Stamatiadis, this action is not sufficient to alter plasma concentrations.[28]

Before the treatment, the cross analysis between FT and ADT-G levels (Fig. 4), revealed a low coefficient ($r = 0.44$), proving a weak correlation among the hormone level (plasmatic) and its main metabolite.

Therefore, in cases of adult female acne, without other clinical manifestations of hyperandrogenism, free testosterone should not be considered as a biomarker of hormonal activity, since this dosage certainly underestimates the peripheral conversion performed by several tissues.

Conclusion

The levels ADT-G were more sensitive in detecting differences between the control and acne groups, thus more fully analyzing the androgen metabolism, especially the peripheral system.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest.

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