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Serum Levels of Androgen-Associated Hormones Are Correlated with Curative Effect in Androgenic Alopecia in Young Men

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Background: Androgenic alopecia (AGA) is the most common type of hair loss in men. However, the pathogenesis is not yet fully understood and therapeutic approaches are limited. This retrospective study investigated the association between levels of androgen-associated hormones and curative effect in androgenic alopecia in young male AGA patients.





Material/Methods: By using chemiluminescence immunoassay, serum levels of androgens and upstream regulated hormones were measured in 178 young male patients with AGA and in 61 normal controls before therapy, 1 and 2 weeks after administration of finasteride.

Results: Before oral finasteride therapy, we found significantly higher levels of serum free testosterone (FT) and dihydrotestosterone (DHT) in AGA patients than in normal controls. The levels of serum sex hormone-binding globulin (SHBG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were similar in the 2 groups. There were no significant differences in serum androgen levels, including DHT and FT, among AGA patients with different grades of hair loss severity ($p > 0.05$). After finasteride therapy, the levels of DHT decreased significantly ($p < 0.05$). Increased serum levels of LSH or LH were also observed in 55 patients after therapy ($p < 0.05$). The levels of SHBG did not change significantly after therapy ($p > 0.05$). Patients with lower levels of serum FT and DHT than before who accepted finasteride therapy had a higher ratio of curative effect manifested by improved severity grade ($p < 0.05$). Patients with higher levels of LSH or LH had a lower curative rate compared to those without change after therapy ($p < 0.05$).

Conclusions: We confirmed the role of the androgens hypothalamus-hypophysis-sexual gland axis in the pathogenesis of AGA and the treatment effect of oral anti-androgen therapy in young male Chinese patients.

MeSH Keywords: **Alopecia • Androgens • Finasteride**

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Background

Androgenetic alopecia (AGA) – also termed male-pattern alopecia, common baldness, and male-pattern hair loss – is the most common type of alopecia occurring after puberty. It is typically manifested as progressive hair thinning and shortening in affected areas. Although regarded as a minor dermatological condition, it affects self-image and is a great cause of anxiety and depression in some patients, particularly younger ones [1–3]. Many strategies and drugs have been developed to treat AGA in clinical practice. Many oral drugs for AGA are available. Currently, minoxidil and finasteride are approved by the Food and Drug Administration (FDA).

Some drugs for AGA have anti-androgen effects, which also results in psychological burden in younger patients, such as hyposexuality, mastalgia, gastrointestinal disorders, and hepatic dysfunctions. The best dose should be chosen for the minimal inhibition of normal sexual function and elimination of adverse effect on folliculus pili and hair growth. Dermatologists should consider the psychological well-being and physiological adverse effects of inhibiting sexual hormones of AGA patients in determining the appropriate treatment choice, such as adverse effects due to anti-androgen therapy, including erection dysfunction and sexual hypoactivity [4,5]. Therefore, we should re-evaluate the influence of oral anti-androgen drug on the secretion of sexual hormones, regulation of sexual hormones, and the hypothalamus-hypophysis-sexual gland axis.

The pathogenesis of AGA has been partly explained and is closely associated with the aberrant regulation of androgen, the androgen receptor (AR) and androgen metabolism-related enzymes [5,6]. High levels of the potent androgen dihydrotestosterone (DHT) and increased expression of the AR gene were found in patients with balding scalps in a previous study; an androgen-dependent gene sets and related signaling pathway-induced biological process were believed to be involved in the underlying mechanisms [1]. The key phenotypic features of AGA included alteration of the hair cycle, and follicular micro-inflammation and miniaturization [7]. DHT is a major cause of AGA, and 5 α -reductase (5 α -R) converts testosterone (T) into DHT, so routine measurement of serum androgens in young patients with AGA may be reasonable. Here, we randomly selected 178 young male AGA patients and 61 normal controls. Because it has been reported that a daily oral finasteride dose of 1 mg has been shown to reduce scalp DHT by 64% and serum DHT by 68% [8], we also measured serum androgen levels before and after finasteride treatment and explored the correlation between hormones response and curative effect. We measured levels of serum T, free testosterone (FT), and SHBG by chemiluminescence immunoassay. FSH, LH, and DHT were measured by radioimmunoassay to further study the interrelationship between sexual hormones levels and AGA in young

patients, including the severity grade and curative response to anti-androgen drug treatment.

Material and Methods

Patient enrollment

This study enrolled 178 male patients with AGA and 61 male normal controls from Zhejiang University Hospital. The inclusion criteria included were: 1) 20–40-year-old men; 2) without administration any anti-androgen drug and other medical intervention for alopecia before enrollment; and 3) without any systemic disease and endocrine diseases, including hyperthyroidism and Cushing syndrome. The diagnosis of AGA was clinically established, and patients were separated into 3 groups based on hair loss severity, from type II to IV, according to Hamilton Classification criteria. This observational study conformed to the Declaration of Helsinki and all participants gave written informed consent. The whole study was approved and monitored by the Institutional Review Committee of Zhejiang University.

Measurement of serum levels of androgens and other related hormones

Serum T, FT, and SHBG levels were measured by chemiluminescence immunoassay, and DHT was measured by radioimmunoassay. Test kits were purchased from Belgium Biotechnology (Ezilon Europe, Belgium). Testing was conducted per the test kits' protocols. Clinical assessment was conducted according to the Hamilton Classification criteria, and each patient was assessed by 3 different dermatologists. Three independent tests were carried out for each sample.

The evaluation of curative effect

The curative effect was evaluated by improvement of hair loss according to the Norwood-Hamilton scale. After treatment with oral anti-androgen drug, the degradation of hair loss severity based on the Norwood-Hamilton classification criteria was assessed by 2 independent dermatologists and then recorded. If the 2 dermatologists reached different conclusions, the third dermatologist would be asked for reevaluation of the hair loss status after therapy. Patients with a decreased hair loss severity score were considered to have an obvious and unequivocal curative effect.

Statistical analysis

SPSS version 20.0 (SPSS, Inc, Chicago, IL, USA) software was used for all statistical analyses. The comparison of quantitative data between the patients and healthy controls were analyzed

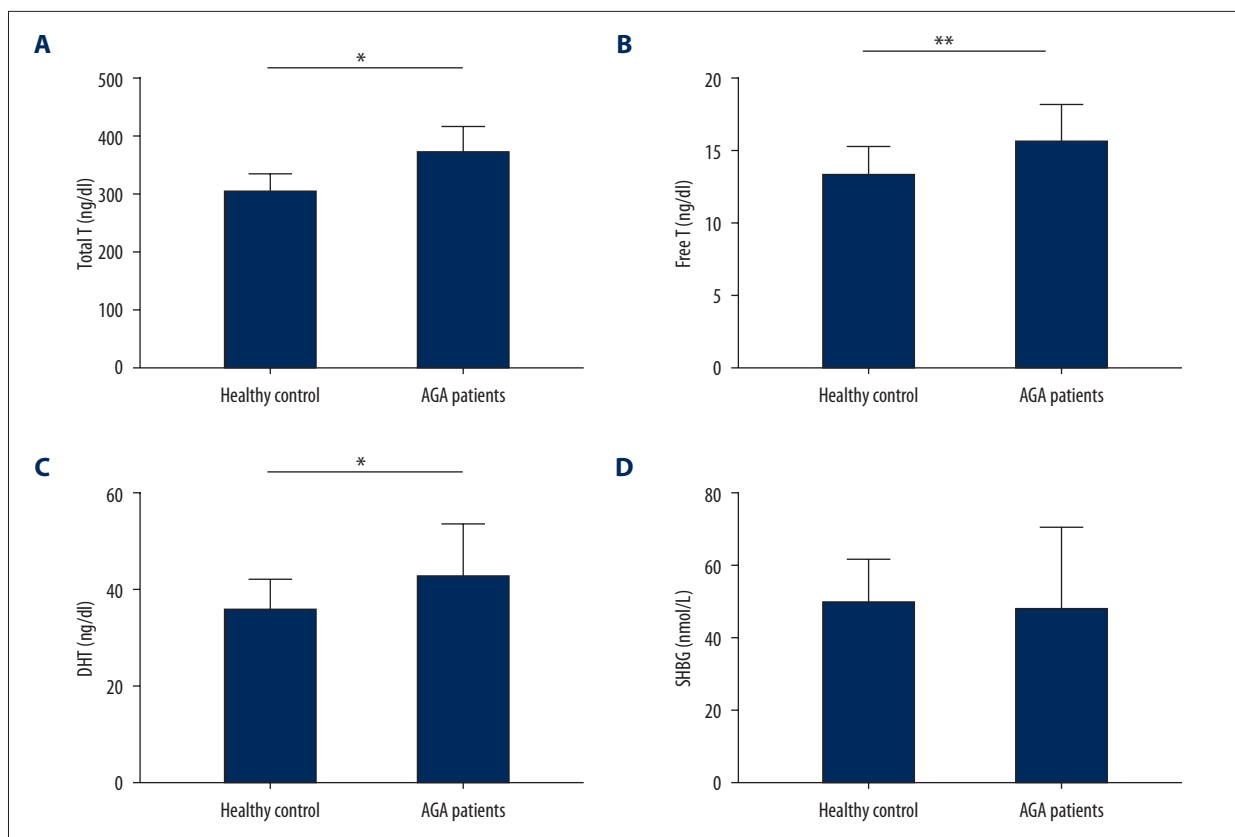


Figure 1. Comparison of serum androgen levels including the total testosterone (T) (A), dihydrotestosterone (DHT) (B), free testosterone (FT) (C) and sex hormone-binding globulin (SHBG) (D) between the alopecia group and normal controls. The *t* test was used for statistical analysis. $P < 0.05$ indicates a statistically significant difference. * $P < 0.05$, ** $P < 0.01$.

t test. The differences among the 3 groups according to the severity grade were examined by analysis of variance (ANOVA). The enumeration data were analyzed by chi-square test. $P < 0.05$ indicated a significant difference according to the 2-sided test.

Results

Patient baseline data

The alopecia patients were grouped into 3 subgroups according to the severity grade. The 78 men with AGA aged 23–30 (25.43 ± 3.59) years were subdivided into type II ($n=29$), type III ($n=41$), and type IV ($n=8$). There were 61 subjects aged 20–30 years without alopecia (24.45 ± 3.34). There was no significant difference between the ages of the 2 groups ($P > 0.05$).

Comparisons of serum levels of androgen

There were significant differences in the serum androgen levels between the alopecia group and the normal controls for all measured levels except for serum testosterone. Total T, FT and DHT were higher in the AGA group (Figure 1A–1C), while

SHBG was similar between the AGA group and normal controls (Figure 1D), but there were no significant differences among the 3 patient subgroups (Figure 2A–2C). This indicated that the severity of AGA was independent of sexual hormones levels and was affected by other factors, such as the duration of alopecia or the sensitivity of hair follicle cells to androgens. This result should be elucidated by further investigation. Furthermore, we assessed the serum levels of FSH and LH in these patients, and did not find any significant differences between the patients and healthy controls (Figure 3A, 3B), which demonstrated that the negative feedback mechanism involved in the production of T and DHT might be impaired in the alopecia patients.

Change of androgens levels after anti-androgen therapy

Post-treatment serum DHT, T, and FT levels were significantly decreased compared with pre-treatment levels (Figure 4A–4C). There was no significant difference in the serum SHBG levels before and after therapy (Figure 4D). There were 136 of all patients who received therapy whose serum levels of DHT decreased after therapy compared to the baseline value. In these patients, there was a higher curative rate compared to those with unchanged serum levels of DHT (Table 1). However, in

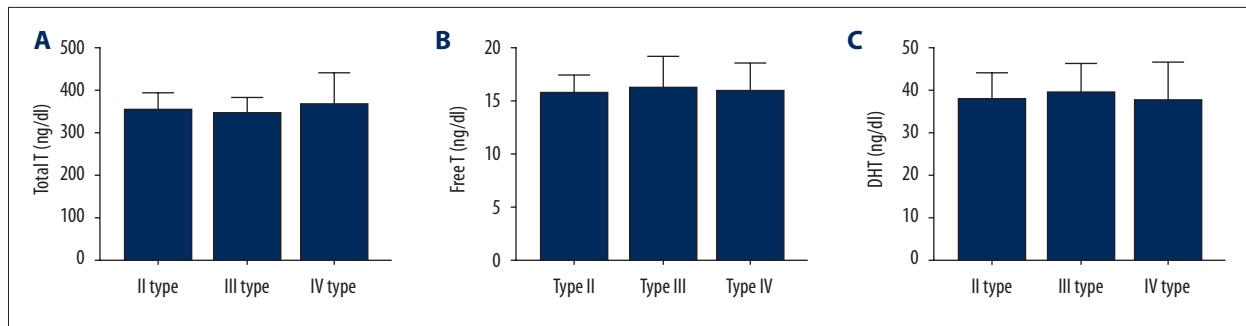


Figure 2. The comparison of the levels of serum androgens in AGA subgroups according to hair loss severity. Total testosterone (T) (A), free testosterone (FT) (B), and dihydrotestosterone (DHT) (C) were compared between different patient subgroups. ANOVA was used for the statistical analysis. $P < 0.05$ indicates statistical significance.

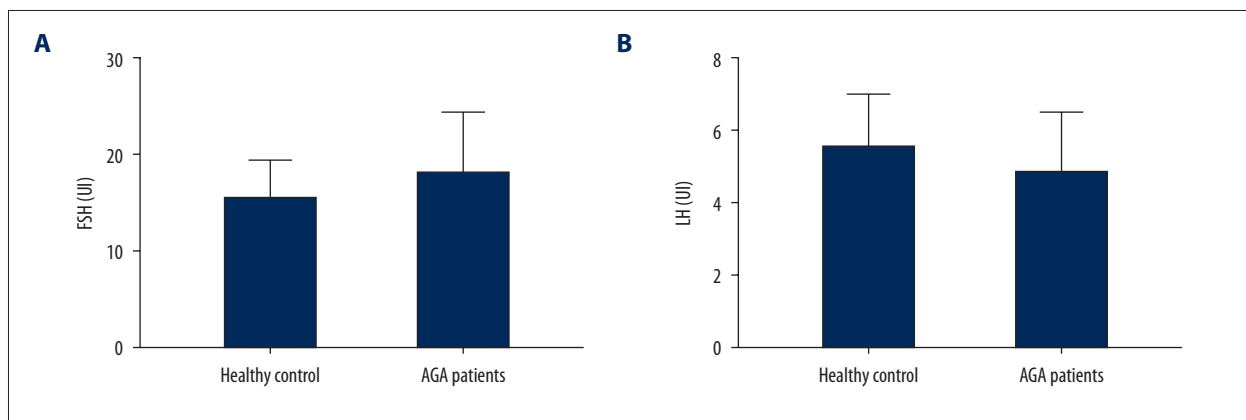


Figure 3. Comparison of follicle-stimulating hormone (FSH) (A) and luteinizing hormone (LH) (B) between the alopecia group and normal controls. The *t* test was used for statistical analysis. $P < 0.05$ indicates statistical significance.

patients with decreased serum levels of DHT, the difference in curative rate was not significantly different between patients with or without a decreased percentage over 50% (Table 2). Our results reveal that the decreased levels of serum levels of DHT could be necessary to the improvement of hair loss, but the excessive decrease in androgen did not bring more benefits for patients. The serum levels could be potential biomarkers for the selection of drug dose and precise therapy in AGA patients. Our data suggest that the decreased percentage of serum androgen levels after therapy compared to baseline should not be over 50%. Our results provided novel findings showing that the extent of DHT decrease should be controlled in the treatment of AGA to the minimize adverse effects of anti-androgen therapy.

Change in the androgen hypothalamus-hypophysis-sexual gland axis after anti-androgen therapy

There was a slight increase in the levels of LH and FSH after anti-androgen therapy, but the levels of these hormones were not significantly increased after administration of oral anti-androgen drug compared to before therapy (Figure 5A, 5B). In 55 of these patients who received an oral anti-androgen drug, we observed increased levels of FSH or LH after therapy. More

intriguingly, patients with increased FSH or LH levels had a lower curative rate in the follow-up (Table 3). The data demonstrated that the hypothalamus-hypophysis-sexual gland axis hyperresponsiveness to drug-induced decrease of androgens might be associated with the drug resistance in those androgenic alopecia patients who have no improvement of symptoms.

Discussion

Various hormones are involved in the regulation of hair growth and follicle function, and the effect of androgens is the most crucial, but there are still many unanswered questions about the androgen metabolism and regulation after anti-androgen therapy. The effects of androgens on follicles vary depending on the body site. Before puberty, only silky vellus hair grows in the pubic and axillary areas, but terminal hair growth with larger, curlier, and darker hair shafts only develops when production of androgens starts to increase. Although androgens stimulate beard growth and body hair in other sites in males, they suppress hair growth in AGA. This reciprocal effect has been described as the androgen paradox [9].

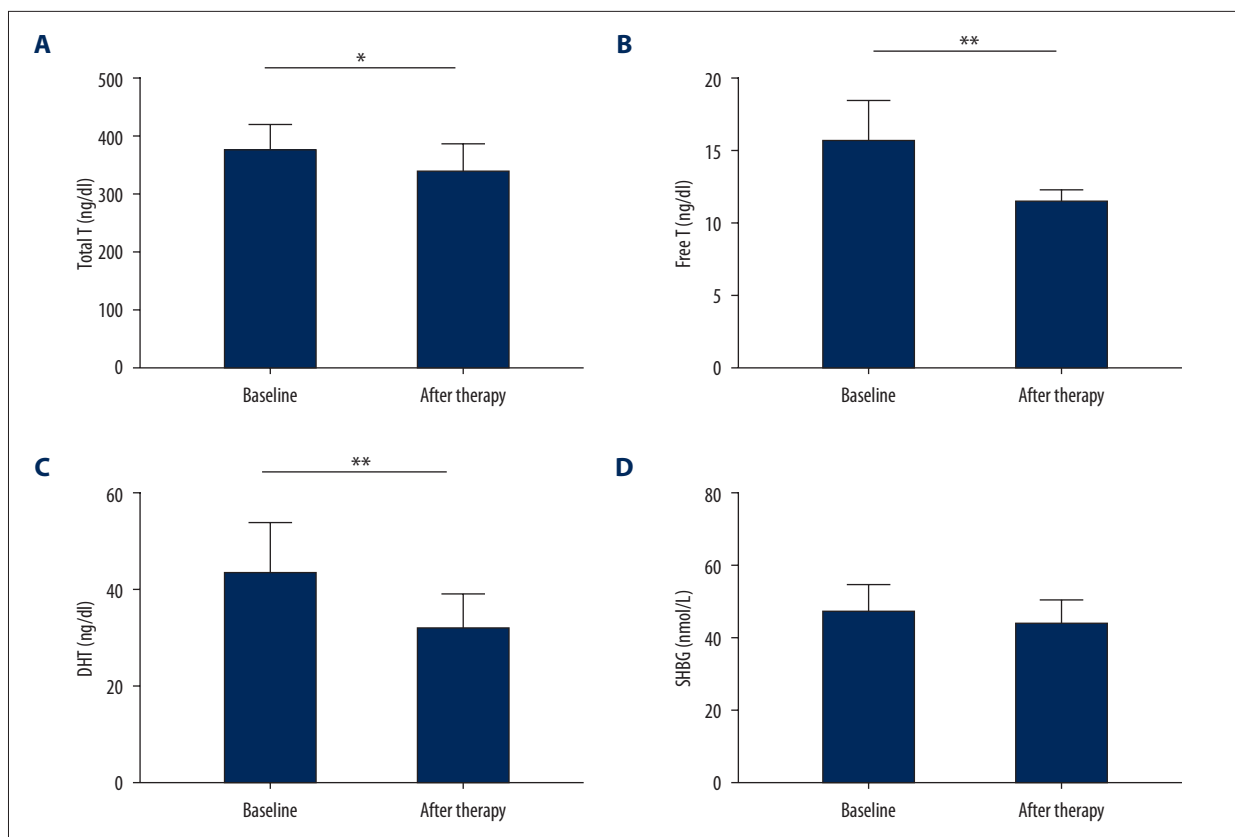


Figure 4. Total testosterone (T) (A), free testosterone (FT) (B), dihydrotestosterone (DHT) (C), and sex hormone-binding globulin (SHBG) (D) were compared before and after anti-androgen therapy. The *t* test was used for statistical analysis. *P*<0.05 indicates statistical significance. * *P*<0.05, ** *P*<0.01.

Table 1. Result of chi-square test for the comparison of curative rate between patients with or without decreased DHT levels.

Patients	With DHT decrease	Without DHT decrease	Total	χ^2 value	P value
Positive response	96	19	115	9.018	0.005
No response	40	23	63		
Total	136	42	178		

Table 2. The result of chi-square test for the comparison of curative rate between patients with or without a decrease percentage over 50% compared to baseline levels.

Patients	Decreased percentage $\geq 50\%$	Decreased percentage $< 50\%$	Total	χ^2 value	P value
Positive response	42	54	96	0.018	>0.99
No response	17	23	40		
Total	59	77	136		

Human 5 α -R1 and 5 α -R2 are 2 isoform zymoproteins encoded by the steroid 5 α -reductase 1 and 2 genes, respectively. Both play an important role in the promotion of conversion from

testosterone to DHT in target cells. It has been reported that 5 α -R1 is expressed in various androgen-independent organs (e.g., the liver, brain, sebaceous glands, epidermis,

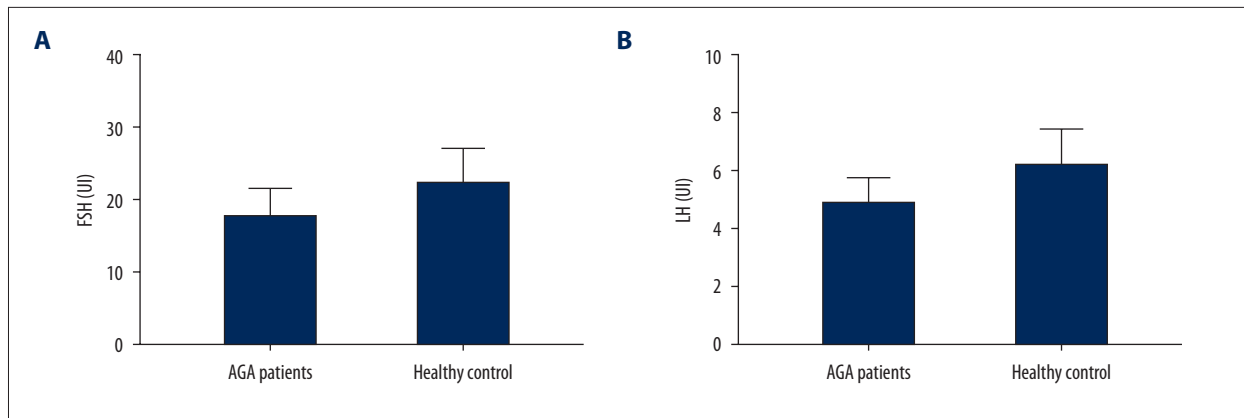


Figure 5. Comparison of follicle-stimulating hormone (FSH) (A) and luteinizing hormone (LH) (B) before and after anti-androgen therapy. The *t* test was used for statistical analysis. $P < 0.05$ indicates statistical significance.

Table 3. Result of chi-square test for the comparison of curative rate between patients with or without increased levels of FSH/LH.

Patients	With increase of FSH/FH	Without increase of FSH/FH	Total	χ^2 value	P value
Positive response	10	136	146	19.11	<0.001
No response	11	21	32		
Total	21	157	178		

sweat glands, hair follicles, and endothelial cells and Schwann cells in the myelin sheaths of nerves) [1], whereas 5 α -R2 is primarily expressed in androgen-dependent organs, including the epididymis and hair follicles [10]. The growth of axillary and female-pattern pubic hair is not affected in male pseudohermaphrodites because of 5 α -R2 deficiency. But the 5 α -R2 deficiency can lead to impairment of beard growth and eradication of AGA in those patients. This suggests that 5 α -R2 plays an important role in beard growth and AGA development, but has no effect on pubic and axillary hair growth. Increased androgens are responsible for up to 80% of female hirsutism, indicating that androgens play an important role in regulating hair growth in both males and females. Oral anti-androgenic contraceptives have been considered as standard therapy for AGA [9].

The increased affinity of testosterone binding to SHBG results in the reduction of free testosterone and incremental production of binding protein. The binding type of testosterone has no biological activity compared to the free types and cannot transform into DHT. In males, approximately 70% of DHT results from testosterone conversion. Elevated DHT level is observed in men with AGA. Increased levels of DHT were also found in about 40% of women with idiopathic hirsutism and 35% with PCOS, whereas in male patients with azoospermia and anorchia, the level of DHT is significantly reduced. Therefore, the measurement of DHT is useful for evaluating response to anti-androgen drugs in AGA patients, as previously described in prostate

cancer [11]. Furthermore, previous studies demonstrated significantly higher expression of androgen receptor (AR) in dermal papilla (DP) cells located in the hair loss area compared with non-bald occipital DP cells in AGA patients, suggesting that AR regulates androgen sensitivity in DP cells [12,13].

The action of androgens is mediated by activation of intracellular nuclear receptors, which promote the expression inducible transcription factors [9]. A recent study found increased DNA methylation of the AR promoter in occipital scalp hair follicles compared with vertex scalp follicles in AGA, which indicates that DNA methylation prevents AR expression in occipital follicles [14]. A recently promoted idea is that there is crosstalk between the androgens-associated pathway and Wnt/ β -catenin signaling involved in the pathogenesis of AGA. The Wnt signaling pathway is indispensable for the hair-inducing activity of DP cells [9], as well as for hair follicle development and regeneration [15]. The administration of DHT obviously suppressed the proliferation of keratinocytes stimulated by Wnt-3a by co-culture [9]. Mechanically, DHT suppressed Lef/Tcf-mediated transcriptional activity in DP cells from AGA patients. However, the DHT-induced suppression of proliferation and blocking of Wnt signaling were not observed in DP cells from non-AGA males.

Our results suggest that crosstalk between Wnt/ β -catenin and androgen receptors is involved in the effects of androgens on

hair growth. Recently, a phase I clinical trial demonstrated that intradermal administration of a 'hair-stimulating complex' containing Wnt and follistatin successfully promoted hair growth in AGA patients [16]. Few studies have investigated the correlation between AGA and serum DHT concentration [11]. One study of AGA patients showed no increase in DHT or T concentrations, but found an elevated DHT/T ratio [17]. Other studies have found significantly higher DHT levels in men with AGA, suggesting that measurement of DHT should be performed before anti-androgen therapy, but other studies have demonstrated that some genetically susceptible individuals are still subjected to hair loss even if the serum androgen levels are normal [18]. There is sufficient evidence that DHT plays an important role in the pathogenesis of AGA. DHT has a 5 times greater binding affinity for the androgen receptor than FT [19]. After binding to receptors, the AR-mediated signaling is transferred into the nucleus to regulate expression of genes related to hair growth, silencing the hair growth period and causing hair loss [12]. Our study showed that serum FT and DHT levels in the AGA group were higher than in controls, but SHBG was similar between 2 groups ($P>0.05$). Our data demonstrate that increased bioactivity dependent on conversion of binding testosterone to free testosterone might be irrelevant to the pathogenesis of AGA and that the elevated levels of free testosterone are not directly involved in hair loss. Therefore, it might be more important to measure the level of DHT in AGA patients for earlier diagnosis and therapeutic evaluation. We divided AGA patients into 3 subgroups based on the Hamilton Classification criteria, and there was no relationship found in this study between serum levels of androgen and disease severity. This suggests that it is not necessary to adjust the dose of medicine for disease severity in clinical treatment.

Many clinical studies have indicated that finasteride at a dose of 1 mg/day effectively controls AGA [8]. An even lower dose

of finasteride might still decrease the level of DHT; however, to achieve an optimal clinical result and effectively maintain the level of DHT, a minimum of 1 mg/day finasteride is necessary [20]. We found that the decreased level of DHT was correlated with a higher curative rate, and the minimum decreased percentage after anti-androgen therapy necessary to an obvious curative rate was not over 50%, because those with a decrease of over 50% do not have a significantly higher curative rate. These data provide a novel strategy for the designation of precise drug dose and personal therapy to avoid the adverse effects of anti-androgen drugs. We also compared the regulatory function of the hypothalamus-hypophysis-sexual gland axis in patients who had anti-androgen effects, such as erectile dysfunction, sexual hypoactivity, and gynecomastia. We found secondary regulation by the hypothalamus-hypophysis-sexual gland axis following the inhibited production of androgens has a negative influence on the curative effect. This feedback regulation causes the increase of FSH and LH levels, which in return stimulate the hormonal secretion and function of sexual glands. Our study shows that finasteride therapy causes decreased levels of DHT, T and FT, which is consistent with the mechanism of AGA. Although T levels were found to be slightly increased after treatment, the change was not statistically significant. However, DHT showed an inhibitory biological effect on the hypothalamus-hypophysis-sexual gland axis through negative feedback.

Conclusions

Genetically-determined hair follicle sensitivity to DHT and reactions to different androgen concentrations are involved in the pathogenesis of AGA. Finasteride reduces serum DHT level in patients, which was effective in treatment. Routine measurement of DHT is recommended during treatment.

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