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Correlation between serum P450arom and sex hormones in males with late-onset hypogonadism: a pilot study

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Abstract. – OBJECTIVE: The research aimed to investigate the correlation between serum P450arom and sex hormones in males with late-onset hypogonadism (LOH).

PATIENTS AND METHODS: A total of 97 LOH patients and 301 matched healthy males of same age underwent androgen deficiency in the aging males (ADAM) and aging males' symptoms (AMS) scales as well as basic questionnaire survey. Serum P450arom, sex hormones, fasting blood glucose and lipid profiles were tested. General information, P450arom and sex hormone levels were compared between the LOH group and the control group. Pearson correlation analysis was used to analyze the correlation between serum P450arom concentration and AMS score, blood glucose, lipid profiles, body mass index (BMI) and sex hormones.

RESULTS: Compared with the control group, the fasting blood glucose, body mass index (BMI), and Estrogen/Total Testosterone ratio (E2/TT) were significantly increased in LOH group ($p<0.05$), while TT, E2 and testosterone secreting index (TSI) were significantly decreased ($p<0.05$). No significant difference in P450arom concentration was observed between the two groups ($p>0.05$). The serum P450arom concentration was not related to TT, E2/TT, AMS score, and BMI.

CONCLUSIONS: These findings suggested that the serum P450arom concentration is unrelated to LOH symptom score and sex hormone levels and could not be used as an observation index and diagnostic basis for LOH.

Key Words:
Cytochrome P450 aromatase (P450arom), Late-onset hypogonadism (LOH), Sex hormone, Symptoms score, Testosterone secreting index (TSI).

Introduction

Cytochrome P450 aromatase (P450arom) is the only rate-limiting enzyme that converts androgens to estrogen and plays an important role in regulating the balance of estrogen and androgen in the body[1]. An abnormal expression of P450arom in tissues is associated with many diseases. Nevertheless, there are only a few research studies on serum P450arom, and still lacked specific data on serum concentration of P450arom. Late-onset hypogonadism (LOH) is a disease that seriously affects the health of middle-aged and elderly men, accounting for an incidence of about 20.0% in males over 45 years old in China[2]. Aromatase Inhibitor has been shown to have certain curative effect for the treatment of LOH[3]. However, further investigation is needed to determine whether P450arom is related to the pathogenesis of LOH, the relationship between serum P450arom concentration and LOH symptom score and sex hormones, and whether the serum P450arom concentration can serve as an indicator for guiding the use of aromatase inhibitors. Hence, this study for the first time analyzed the relationship between serum P450arom and sex hormones and symptom scores in LOH patients, as well as explored the correlation between serum P450arom concentration and LOH.

Patients and Methods

In this case-control study, a total of 97 LOH patients aged 45-80 years and seen at the Department of Urology and Andrology Clinic, No. 1 Hospital of Shanxi Medical University between August 2016 and August 2017 were included. Meanwhile, 301 healthy men undergoing health checkup during the same period were selected as controls. These two groups had a matching factor of age (±3). The diagnosis of LOH was made according to the standard as stated in the *Diagnosis*

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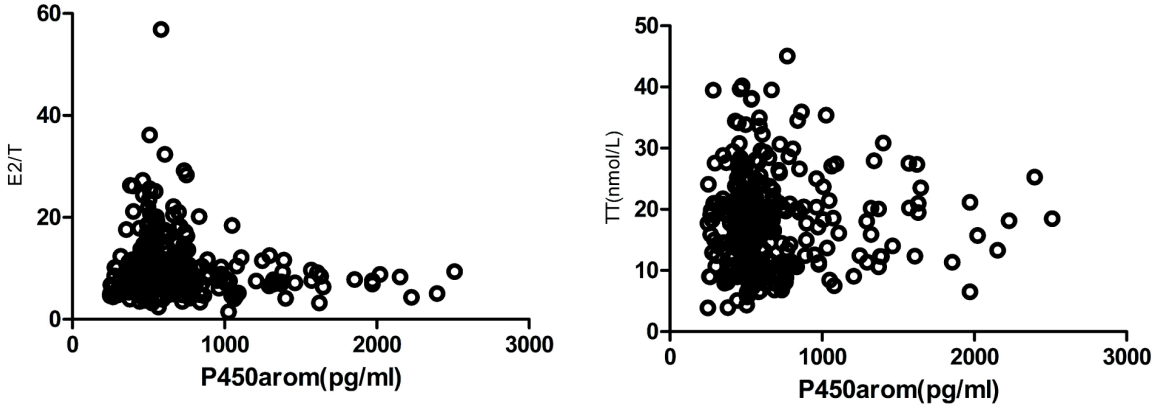

Figure 1. Correlation between P450arom and E2/TT or TT in all participants.

and Treatment Guidelines for Andrological Diseases in China: Symptom score positive, and total testosterone (TT) ≤11.5 nmol IU^{-1}. The inclusion criteria were as follows: (1) Patients who were initially diagnosed with LOH. (2) Patients who were diagnosed as LOH and did not receive treatment within the last 3 months. (3) Male patients aged 45-80 years. Exclusion criteria were as follows: (1) Patients with serious heart, brain, liver and kidney diseases. (2) Patients with hypothalamus and pituitary diseases. (3) Patients taking androgen, prednisone and other steroid drugs. (4) Patients having GnRH analogs, androgen antagonist drugs and estrogen antagonist drugs. (5) Patients having chorionic gonadotropin, menotrophin and other drugs. (6) Patients who had long-term administration of spironolactone and other antihypertensive drugs. (7) Patients who had long-term administration of psychotropic drugs. (8) Patients who were diagnosed as LOH and received drug treatment within the last 3 months. All patients signed the informed consent form. The study was approved by the human study Ethics Committee at the No. 1 Hospital of Shanxi Medical University. The subjects enrolled in the control group were in line with the following criteria: males of age 45-80 years and willing to be eugonadal healthy subjects. The exclusion criteria for the control group was the same as the experiment group.

Methods

All the participants underwent a survey of basic information and relevant risk factors, including age, BMI, education, marriage status, diet, living habits, smoking and drinking, exercise. For LOH diagnostic scale, the universal ADAM and AMS scales were adopted. Fasting blood samples were taken between 8:00-9:00 in the morning. The fasting blood glucose and lipid profiles were determined using an automatic biochemical analyzer (Hitachi 7180, Tokyo, Japan). Serum total testosterone (TT), estradiol (E_2), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using a spectrochemical method (Roche cobas6000, Mannheim, Germany), followed by calculation of E_2/TT, testosterone secreting index (TSI) (TSI = TT / LH). The serum concentration of P450arom was detected by using enzyme-linked immunosorbent assay (ELISA) (Shanghai Fanke Biotechnology Co. Ltd, Shanghai, China) according to the manufacturer's instructions. The correlation coefficient 'R' of linear regression and expected concentration was above 0.990, and the differences within the batches and between the batches were less than 9% and 11%, respectively.

Statistical Analysis

Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS Inc., Chicago, IL, USA). *t*-test was used to compare the levels of blood glucose, lipid profiles, BMI, T, E2, FSH, LH, E2/T, TSI levels and P450arom between the two groups, while chi-square test was used to compare the percentages between the two groups. Pearson correlation analysis was used to analyze the correlation between P450arom and TT, E_2/TT, BMI, AMS scores. A significance level of α=0.05 was adopted for all the tests.

Results

General Information

Statistical analysis demonstrated that fasting blood glucose level, triglycerides, low density

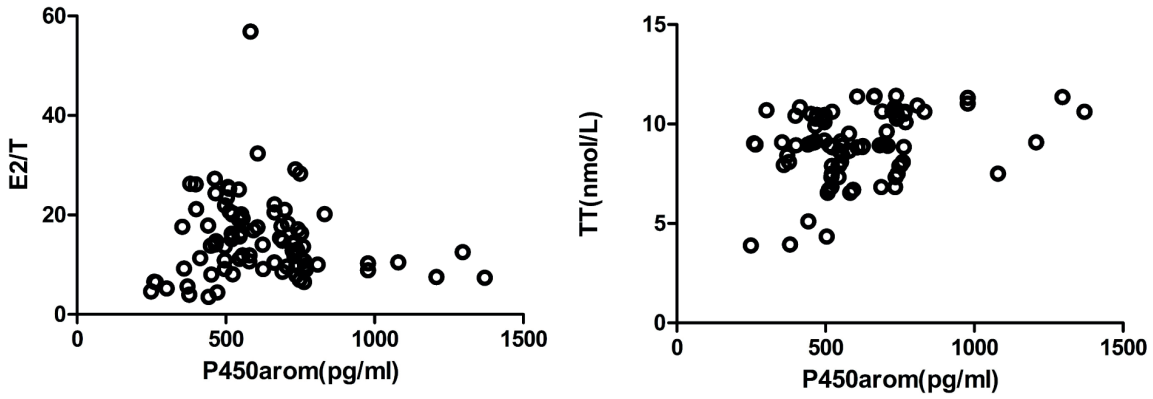

Figure 2. Correlation between P450arom and E2/TT or TT in the LOH group.

lipoproteins and BMI in LOH group were significantly higher than that in the control group (p=0.00, 0.02, 0.00), while there were no significant differences in smoking, drinking, total cholesterol and high-density lipoproteins between the LOH and the control groups (p=0.07, 0.34, 0.79, 0.06) (Table I).

Comparison of Sex Hormones and P450arom

Compared with the control group, AMS score and E_2/T ratio in the LOH group were significantly increased (p=0.00), the TT, E_2 and TSI levels were significantly decreased (p=0.00, 0.00, 0.00), and there was no significant difference in FSH and LH levels between the LOH group and the control group (p=0.54, 0.81). Meanwhile, the serum concentration of P450arom was dispersed greatly in all the participants, ranging from 107.97 pg ml^{-1} to 2622.51 pg ml^{-1}. There was no significant difference in P450arom levels between the LOH group and the control group (p=0.61) (Table II).

Relationship Between P450arom and AMS Score as well as P450arom and Sex Hormones

Pearson correlation analyses revealed that P450arom concentration was not significantly correlated with AMS score, TT, E_2, E_2/TT, FSH, LH and TSI in all participants (Rs were respectively -0.07, -0.01, -0.09, -0.07, -0.01, -0.05 and 0.02, p>0.05) (Figure 1). Also, in LOH group, the serum concentration of P450arom was not significantly correlated with AMS score, TT, E_2, E_2/TT, FSH, LH and TSI (Rs were respectively -0.03, 0.38, 0.15, -0.04, -0.16, -0.16, 0.12, p>0.05) (Figure 2).

Discussion

P450arom is the only rate-limiting enzyme that regulates androgen and estrogen levels in the body and plays a crucial role in maintaining the balance of androgens and estrogens[4,5]. The adipose tissue and testis are the main androgen-to-estrogen con-

Table I. Patients characteristics.

Variable	LOH group (n=97)	Control group (n=301)	p
Age (year), mean±SD	56.64±6.55	56.36±8.70	0.837
Smoking, n (%)	80(82.4%)	207(69.9%)	0.069
Drinking, n (%)	63(73.8%)	199(66.6%)	0.344
BMI (kg m^{-2}), mean±SD	27.13±3.14	25.18±2.98	0.000
Blood glucose (mmol L^{-1}), mean±SD	6.76±2.28	6.06±1.59	0.012
Total cholesterol (mmol L^{-1}), mean±SD	5.06±0.87	5.08±1.02	0.786
Triglycerides (mmol L^{-1}), mean±SD	2.66±1.58	1.79±1.06	0.000
Low density lipoproteins (mmol L^{-1}), mean±SD	3.67±1.08	3.04±0.84	0.024
High density lipoproteins (mmol L^{-1}), mean±SD	1.25±0.37	1.33±0.48	0.056

LOH: late-onset hypogonadism; BMI: body mass index

Table II. Comparison of sex hormone and P450arom between LOH group and control group.

Variable	LOH group (n=97)	Control group (n=301)	p
AMS (point), mean±SD	34.46±8.56	29.01±7.32	0.000
TT (nmol L^{-1}), mean±SD	9.07±2.09	19.62±6.35	0.000
E2 (pmol L^{-1}), mean±SD	96.36±43.40	145.68±48.87	0.000
E2/T, mean±SD	11.00±5.53	7.86±3.10	0.000
FSH (mIU ml^{-1}), mean±SD	8.66±10.72	7.98±6.11	0.537
LH (mIU ml^{-1}), mean±SD	5.83±4.36	5.98±3.77	0.810
TSI, mean±SD	2.06±1.02	4.04±2.21	0.000
P450arom (pg ml^{-1}), mean±SD	626.53±282.08	657.28±356.25	0.613

LOH: late-onset hypogonadism; AMS: aging males' symptoms; TT: total testosterone; E2: estradiol; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TSI: testosterone secretion index

verting sites in the body. Studies on infertile and obese men have found that elevated levels of E_2/TT were associated with high P450arom expression in testis and adipose tissue, and is indirectly confirmed by the fact that aromatase inhibitor can reduce E_2/TT and increase serum TT levels[6,7]. The questions of whether the serum P450arom concentration is associated with P450arom expression in the tissues, and whether it can indirectly reflect the P450arom expression in the tissues are still unknown. Studies on serum P450arom have been rarely reported, and still lacked a reference standard of the serum P450arom concentration in the normal population. There were limited studies available regarding the effect of serum P450arom on the risk factors of coronary heart disease. It was found[8] that serum E_2/TT and P450arom in male patients with coronary heart disease were lower than those in the control group. Guo et al[9] on risk factors for coronary heart disease in postmenopausal women also found that the serum P450arom concentration and serum TT were negatively correlated and suggested that P450arom was decreased with the conversion of TT to E_2, which was the leading cause of the decrease of serum E_2/TT ratio.

LOH is characterized by a series of symptoms of androgen deficiency. The relationship between P450arom and LOH is not yet clear, but aromatase inhibitors have achieved some curative effects in the treatment of LOH[3]. In addition, a number of previous studies[10,11] found that the serum E_2/TT level was significantly higher in LOH patients than that in the controls, suggesting that P450arom may be involved in the pathogenesis of LOH. Wang et al[12] believed that the mutation of P450arom upstream gene CYP19 led to a decrease of TT and an increase of E_2/TT levels, which might be the pathogenesis of LOH. Nevertheless, the correlation between serum P450arom and LOH has not been reported yet. In this work, we analyzed the relationship between serum P450arom and sex hormones and AMS in 97 LOH patients and 301 normal controls. The results showed that the TT and E_2 in LOH patients were significantly lower than those in the control group, while E_2/TT in LOH group were significantly higher than those in the control group and were consistent with the results of most of the previous studies[13]. It is believed that a decreased TT is associated with decreased number and function of testicular interstitial cells, as well as hypothalamus and pituitary secretion abnormalities. Then, it is necessary to understand whether the abnormal increase of E_2/TT level is associated with abnormal expression of P450arom and whether the serum P450arom concentration is associated with sex hormone level and AMS score[14]. Our results showed that there was no difference in serum P450arom concentration between the LOH group and the normal control group. The serum P450arom concentration was not correlated with the AMS score, TT, E_2, E_2/TT in all patients, and the serum P450arom concentration in LOH group did not change with the decreased serum TT and the increased E2/TT. Hence, we suggested that the serum P450arom concentration was unrelated to the severity of LOH, and was not associated with the decreased serum TT and estrogen-androgen imbalance in LOH patients. Hence, serum P450arom concentration cannot be used as an index for the diagnosis of LOH and guiding use of aromatase inhibitors. However, serum P450arom concentration may not represent the P450arom expression in tissues and organs, which are the main sites for conversion of androgen to estrogen. The adipose

tissue is the main place for producing estrogen in men. The estrogen produced in testes accounts for 20% of the total estrogen and 60% of the circulating estrogen. Changes in P450arom concentration and activity may occur only in adipose tissues, testes and other tissues in which P450arom plays the main action[15]. It remains to be determined whether there are any changes in P450arom concentration and activity in tissues and organs of LOH patients, whether the abnormal expression and activity changes in P450arom are involved in the pathogenesis of LOH, and whether the P450arom in tissues and organs is correlated to the serum P450arom concentration.

Conclusions

We showed that the serum P450 arom was unrelated to LOH. This work only investigated the relationship between serum P450arom and LOH symptom scores as well as sex hormones. The investigation did not involve the changes in P450arom expression and activity in tissues and organs of LOH patients (such as adipose tissue and testes), and did not investigate the relationship of serum P450arom, sex hormones and symptom scores with P450arom in tissues.

Conflict of Interest

The Authors declare that they have no conflict of interest.

Aknowledgements

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