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Serum Progranulin and Insulin Resistance Evaluation in Male Androgenetic Alopecia Patients

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Abstract

Background: AGA is a common hair disease with genetic predisposition and is characterized by non-scarring progressive miniaturization of the hair follicle. IR is a state in which a given concentration of insulin produces a less-than-expected biological effect. Obesity is the most common cause. Insulin was suggested to play a role in the regulation of cutaneous androgen metabolism and hair-growth cycle, which are relevant to AGA. The newly regulatory role of PGRN on energy homeostasis and chronic inflammation raised the possibility that PGRN may contribute to the progression of IR and metabolic dysfunction. The aim of this study was to evaluate serum PGRN, FBG and fasting insulin levels in patients with AGA, assessment of its clinical significance and its relation to IR and MetS. Methods: This study included 60 AGA male patients. In addition to 20 healthy individuals of matched age and sex served as controls. All participants were recruited from outpatient clinic of Dermatology, Venereology and Andrology department of Benha University Hospitals. Results: There was a non-significant difference between patients and control groups regarding weight, height and BMI. AGA patients showed significantly higher fasting insulin level and HOMA-IR, as compared to the control group. Serum PGRN was higher significantly in AGA group as compared to control group. Conclusion: Serum PGRN level and HOMA-IR were significantly higher in AGA patients when compared to control group. PGRN has a critical role which contributes to IR and MetS. AGA patients should be closely followed-up in the long term. Early detection of IR and MetS might help in good management of AGA patients.

Key words: Serum Progranulin, Insulin Resistance, Male Androgenetic Alopecia.

1. Introduction

Androgenetic alopecia (AGA), the most common nonscarring alopecia, is an androgen-induced disorder characterized by hair loss in genetically predisposed men. In AGA, androgens induce miniaturization of hair follicles, especially in the frontotemporal area and vertex of the scalp[1]. AGA patients were found to be more susceptible to cardiovascular diseases, insulin resistance (IR), metabolic syndrome (MetS), diabetes mellitus and hypertension[2].

IR plays a major role in the pathophysiology of MetS and hyperinsulinemia is an independent risk factor for coronary artery disease (CAD), wherein it accelerates the development of atherosclerosis and prevents the resorption of atherosclerotic plaque(3). Association between IR and AGA have been reported and hyperinsulinemia plays a pathogenic role in local androgen production and miniaturization of hair follicles[4].

Progranulin (PGRN) has emerged as an important regulator for IR. Studies showed that PGRN-deficient mice exhibited resistance to diet-induced obesity and insulin insensitivity through the modulation of inflammation, whereas adipocytes exposed to PGRN have increased susceptibility to be insulin-resistant, susceptibility to be insulin-resistant and those effects can be normalized with treatment of pioglitazone, an insulin-sensitizing agent[5]. In particular, PGRN is more highly expressed in visceral adipose tissue of the insulin-resistant patients with morbid obesity than in their age-, sex- and body mass index-matched insulin-sensitive counterparts suggesting that PGRN can be a key adipokine to mediate IR[6].

The aim of this study is to evaluate PGRN serum level and IR in male AGA patients and compare their levels in healthy controls.

2. Subiects and Methods

Subjects

This case control study was conducted on sixty AGA patients and twenty age and sex matched healthy volunteers served as controls. They were recruited from the outpatient clinic of Dermatology, Venereology and Andrology Department of Benha University Hospitals, during the period from May to December 2020.

Inclusion criteria

- Male patients with age ≥ 18 years.
- Cases who agreed to participate in the study.

Exclusion criteria

- known septic focus.
- Presence of associated chronic diseases as diabetes mellitus, cardiovascular diseases.
- History of taking immunosuppressive treatment.
- History of other dermatological diseases.
- Patients on systemic therapy for AGA for more than one month prior to the study.

Administrative design

This study was approved by the Research Ethical Committee of Benha Faculty of Medicine, and was carried out according to the guidelines of the Helsinki declaration principles.

Ethical consideration

Before taking blood samples, written informed consent was taken from each participant.

- Confidentiality and personal privacy were respected in all levels of the study.
- Patients felt free to withdraw from the study at any time without any consequences.
- Collected data were not used for any other purpose.

Methods

Every participant was subjected to

Complete history taking

1. Personal history including: name, age, marital status and special habits of medical importance (e.g. smoking).
2. History of AGA including; onset, course, duration.
3. Past history of other medical illness (endocrine disorders).
4. Family history of AGA.
5. Drug history including previous treatment of AGA.

General and local examination

General examination

Clinical examination of patients for any signs of systemic diseases.

Local examination

The AGA grade and severity was assessed according to Hamilton and Norwood classification[7; 8]. The Hamilton-Norwood scale **(Figure 1)** provided a helpful guide to classify AGA. The scale divided the clinical findings into seven stages and offered a visual depiction of the sequential stages of balding. The scale also described a less common type A variant of hair loss in which men demonstrate only the progressive movement of the anterior hairline posteriorly [8].

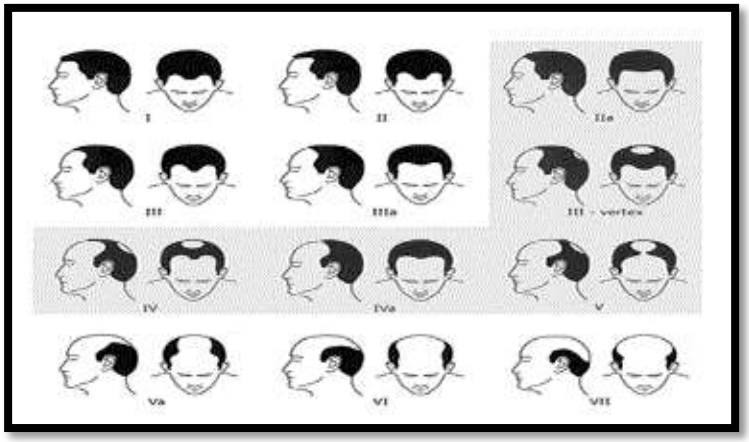

Fig. (1) Hamilton-Norwood classification of male balding [8].

Hamilton-Norwood classification of male balding [8]

- **Class I:** No or very minimal hairline recession along the anterior border in the fronto-temporal region.
- **Class II:** The anterior border of the hair in the fronto-temporal region has symmetric triangular areas of recession which extend no further posteriorly than 2 cm anterior to a line drawn in a coronal plane at the level of the external auditory meatus.
- **Class IIA:** The hairline is anterior to the coronal plane 2 cm anterior to the external auditory meatus.
- **Class III:** The triangular areas in type III extend posterior of the coronal plane which is 2 cm anterior to the external auditory meatus. This is the minimal level considered to represent baldness.
- **Class IIIA:** The hairline has receded back to a point between the limit of type IIA and the level of the external auditory meatus.
- **Class III Vertex:** Most of the hair loss is seen on the vertex. Frontal hair loss may be similar to type I or II but should not exceed type III. This type is most commonly seen with advancing age.
- **Class IV:** Hair loss on the vertex associated with frontal loss more severe than type III, but the frontal and vertex areas are separated by a distinct band of hair.
- **Class IVA:** The hairline has receded beyond the external auditory meatus but has not reached the vertex.
- **Class V:** Greater hair loss than type IV with only a sparse band of hair separating the frontal and vertex areas. The hair left on the occipital and parietal areas begins to form the shape of a horseshoe when viewed from above.
- **Class VA:** The area of denudation includes the vertex. Hair loss more severe than type IVA, cannot be distinguished from type VI or VII.
- **Class VI:** The frontal and vertex areas of hair loss are contiguous with greater lateral and posterior areas of denudation.
- **Class VII:** The most severe form of male pattern baldness. Only a narrow sparse horseshoe-shaped band of hair is left extending from the ears posteriorly to the occiput.

Laboratory investigations

All participants were tested for determination of fasting insulin, fasting blood sugar and PGRN levels.

Sampling

1. A 5 ml of venous blood sample was obtained from each participant after an overnight fasting (6-8 hours).
2. The blood sample was left at room temperature for about 20-30 minutes to coagulate, then centrifuged for about 10 minutes at 1300 rpm.
3. The separated serum was stored at -40°C till analysis.
4. Fasting glucose was measured by enzymatic colorimetric method.

1-Measurement of serum insulin levels

Fasting insulin levels were determined by the enzyme-linked immunosorbent assay (ELISA) method using the commercially available specific kit.

- IR status was assessed by HOMA-IR index, calculated as fasting insulin (mIU/mL) × fasting plasma glucose (mmol/L)/22.5.
- A normal HOMA-IR was set at ≤ 2.5 because a higher value has been associated with an increase in cardiovascular risk in the Mexican-American population [9].

Assay principle

Immunospec Insulin is a solid phase ELISA. The wells were precoated with monoclonal antibody with higher activity for insulin. The samples and controls were incubated in the wells with enzyme conjugate. The antibodies linked to horseradish peroxidase to form a sandwich complex bound to the well and the unbound conjugate was then washed off with wash buffer. The amount of bounded peroxidase was proportional to the concentration of the insulin present in the sample. Upon addition of the substrate and chromogen, the intensity of color developed is proportional to the concentration of insulin in the samples.

Assay procedure

1. All reagents and samples were put at room temperature (20-25°C) and mixed gently before beginning the test.
2. A 25 ul of serum sample, controls and reference were dispensed into the assigned wells.
3. A 100 ul of enzyme conjugate was dispensed into each well and mixed for 5 seconds, then incubated for 30 minutes at 25°C.
4. Incubation mixture was removed and the wells were rinsed five times with washing buffer.
5. A 100 ul of solution A (buffer solution containing hydrogen peroxide) was dispensed and then 100 ul of solution B (Tetramethyl benzidine) was added into each well.
6. Samples were incubated for 15 minutes at room temperature.
7. The reaction was stopped by adding 50 ul of 1N sulfuric acid or 2N HCl to each well and optical density (O.D.) at 450 nm was detected with a microwell reader.

2-Measurement of serum PRGN levels

The determination of serum PRGN was carried by ELISA according to the manufacturers' instructions Human PRGN ELISA Kit.

Assay princible

The kit used a double-antibody sandwich ELISA to assay the level of human PGRN in samples.

PGRN was added to monoclonal antibody enzyme well which was pre-coated with human PGRN monoclonal antibody.

PGRN antibodies labeled with biotin were added after incubation and combined with Streptavidin-HRP to form immune complex.

Incubation was carried out and washing again to remove the uncombined enzyme.

The chromogen solution A and B were added and the color of the liquid changed into blue, and after the effect of acid, the color finally became yellow.

The chroma of color and the concentration of the human substance PGRN of sample were positively correlated.

Assay procedure

1. Standard dilution: The standard reagent was diluted according to the instruction.
2. Inject samples:
   - Blank well: Samples and PGRN-antibody labeled with biotin, Streptavidin-HRP were not added, only chromogen solution A and B, and stop solution were allowed; other operations were the same.
   - Standard wells: A 50µl of standard solution was added, Streptavidin-HRP 50µl (since the standard already had combined biotin antibody, it was not necessary to add the antibody.
   - Test wells: 40µl sample was added, and then both PGRN-antibody 10µl and Streptavidin-HRP 50µl.

   Then the sealing memberance was sealed, and gently shaked, incubated 60 minutes at 37◦.
3. Confection: The 30×washing concentrate was diluted 30 times with distilled water as standby.
4. Washing: The memberance was carefully removed, and the liquid was drained, shaked away the remaining water.
5. A 50µl of chromogen solution A was added, then 50µl of chromogen solution B to each well. Gently mixed, incubated for 10 min at 37℃ away from light.
6. Stop: A 50µl stop solution was added into each well to stop the reaction (the blue changed into yellow immediately).
7. Final measurement: The blank well was taken as zero, the optical density was measured (OD) under 450 nm wavelength within 15 min after adding the stop solution.
8. According to standards' concentration and the corresponding OD values, the standard curve linear regression equation was calculated, and then the OD values of the sample was applied on the regression equation to calculate the corresponding sample's concentration. It was acceptable to use kinds of software to make calculations.

Statistical analysis

Data management and statistical analysis were done using SPSS version 25. (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using Kolmogorov–Smirnov test (for cases), the Shapiro-Wilk test (for controls), and direct data visualization methods (for both), and numerical data were summarized as means and standard deviations or medians and ranges. Categorical data were summarized as numbers and percentages. Quantitative data were compared between study groups using independent t-test or Mann-Whitney U test for normally and non-normally

distributed numerical variables, respectively. Categorical data were compared using the Chi-square test. ROC analysis was done for using serum PGRN in differentiating alopecia patients. Area Under Curve (AUC) with 95% confidence interval, best cutoff point, and diagnostic indices were calculated. Correlations between serum PGRN and different parameters were done using Spearman's correlation. Serum PGRN was compared according to different parameters using Mann Whitney U test. Logistic regression analysis was done for the prediction of alopecia. The odds ratio and the 95% confidence interval were calculated. All statistical tests were two-sided. P values less than 0.05 were considered significant.

3. Results

As shown in **Table (1),** there were non-significant differences between both groups regarding age (***P*** = 0.606), smoking (***P*** = 0.354), weight (***P*** = 0.084), length (***P*** = 0.319), BMI (***P*** = 0.149) and obesity (***P*** = 0.187).

Table (1) General characteristics of the study groups

		Cases (n= 60)	Controls (n = 20)	Test	*P*
Age (Years)	Mean ±SD	40 ±14	38 ±7	t = - 0.518	0.606
Smoking	n (%)	15 (25.0)	3 (15.0)	X^2 = 0.860	0.354
Weight (kg)	Mean ±SD	80 ±14	74 ±13	t = - 1.752	0.084
Length (cm)	Mean ±SD	171 ±7	169 ±7	t = - 1.003	0.319
BMI	Mean ±SD	27.4 ±4.2	25.8 ±4	t = - 1.459	0.149
Obesity	n (%)	18 (30.0)	3 (15.0)	X^2 = 1.743	0.187

t: Independent t-test was used for numerical data.
X^2: Chi-square was used for categorical data
BMI = Body mass index

The mean insulin level showed a significant difference between AGA patients and control group (Mean ±SD**:** 17.6 mIU/ml vs. 6.7 mIU/ml respectively**;** ***P***< 0.001). HOMA-IR was significantly higher in cases than controls (Mean ±SD**:** 3.92 vs. 1.53 respectively**;** ***P***<0.001) **(Chart 1)**, although there was a non-significant difference (***P*** = 0.333) between two groups regarding fasting blood glucose **(Table 2)& (Chart 1).**

Table (2) FBG, insulin level, and HOMA-IR in the study groups.

		Cases (n = 60)	Controls (n = 20)	T	*P*
FBG (mg/dL)	Mean ±SD	90 ±11	93 ±11	0.974	0.333
Insulin level (mIU/ml)	Mean ±SD	17.6 ±5.8	6.7 ±1.7	-12.850	<0.001*
HOMA-IR	Mean ±SD	3.92 ±1.46	1.53 ±0.44	-11.229	<0.001*

t: Independent t-test was used
FBG = Fasting blood glucose
* P<0.05 is significant

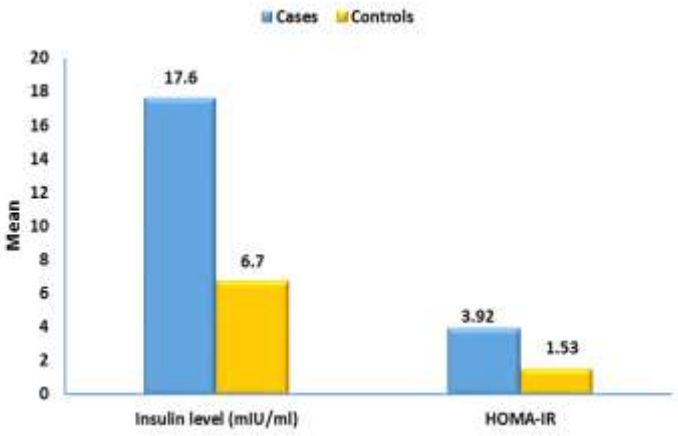

Chart (1) Insulin level and HOMA-IR of the study groups.

As shown in **Table 3**, the mean age of AGA onset was 23 years. The mean disease duration was 17 years. Regarding Hamilton-Norwood classification, the most frequent grade was grade III (21.7%), and the least frequent was grade II (11.7%).

Table (3) Clinical characteristics in AGA patients.

Age of AGA onset (Years)	Mean ±SD		23 ±5
AGA duration (years)	Mean ±SD		17 ±13
Hamilton-Norwood classification	II	n (%)	7 (11.7)
	III	n (%)	13 (21.7)
	IV	n (%)	12 (20.0)
	V	n (%)	10 (16.7)
	VI	n (%)	9 (15.0)
	VII	n (%)	9 (15.0)
Family history	n (%)		49 (81.7)
History of previous treatment	n (%)		9 (15.0)

The mean serum PGRN level was significantly higher in cases compared to controls (80.7 ng/ml vs. 12 ng/ml, ***P***<0.001) **(Table 4).**

Table (4) Serum PGRN of the study groups.

		Cases(n = 60)	**Controls(n=20)**	**Z**	***P***
Serum PGRN (ng/ml)	Median (range)	80.7 (11-258.9)	12 (8 - 215.7)	-3.7	<0.001*

Z:Mann Whitney U test was used *
P<0.05 is significant

4. Discussion

In this study, 81.7% of patients with AGA had positive family history. This was in agreement with Arias-Santiago et al.[10], who showed that among patients with AGA included in his study, 84.11% had a family history of AGA versus 19.1% of the control subjects (P=0.0001). In the study done by Radhakrishna,[11], a positive family history was significantly higher in the participants in the AGA group than the control group (P=0.007). In the study conducted by Kaya Erdogan et al. [11], family history was present in 66.7% (22 out of 33) of patients. In the same line, Abdelmawla et al.[12], showed that AGA was significantly associated with a positive family history.

In the current study, there was non-significant difference between patients and control groups regarding FBG level (*P*=0.333). This agreed with Abdelmawla et al.*(13)*, who reported that there were non-significant differences between patients and control group regarding FBG and postprandial glucose.

The current study results were in disagreement with Arias-Santiago et al.[14], who reported a significant difference between AGA patients and controls as regards FBG levels. These results could be explained by higher range of ages (46-60 years old) in their study. Hirsso et al.[15], found that 21% of patients with AGA had diabetes compared with 12% of controls. On the other hand, Agamia,[16], reported that FBG values were higher in AGA patients than in controls; that was related also to peripheral IR. The difference between these studies and the results of the current study may be explained by the exclusion of patients sufferring from diabetes and impaired glucose tolerance in the current study.

The relationship between AGA and hyperinsulinemia and cardiovascular-related disorders was first suggested by Matilainen et al.[17], who suggested that hyperinsulinemia caused by increased resistance to the peripheral action of insulin explains the association between AGA and CVDs. Insulin has also been shown to favor vasoconstriction and nutritional deficiency in the follicles of the scalp[16]. And so, hyperinsulinemia plays a role in local androgen production, whether de novo from cholesterol or by locally converting testosterone to DHT. DHT inhibits adenylcyclase activity, and is able to curtail the anagen cycle and could be responsible for the miniaturization of follicles in AGA[13].

Hirsso et al.[18] demonstrated a reduction in insulin sensitivity in males with AGA. Results of the present study also were similar to the results of González-González et al.[19], who found that a relationship exists between IR and early baldness. The HOMA-IR index was found to be significantly higher among AGA patients compared to the controls. Going with that, it was found that a relationship exists between IR and early baldness[20]. In their work, Bakry et al.[4], showed that 35% of cases and 19% of controls had IR with significant difference between both groups. Similar results were also shown by Kartal et al.[21], who reported that serum fasting insulin level and HOMA-IR score were significantly higher in the patients with AGA than in the control group. In the current study, there was a significant difference between patients and controls as regards HOMA-IR index values (P<0.001). This results also agreed with Sorour et al.[22], who found that AGA group showed significantly higher HOMA-IR index when compared to control group.

Conversely, Nabaie et al,[23], could not demonstrate any significant difference between AGA patients and control group with respect to levels of fasting insulin and IR. Abdel Fattah and Darwish,[24], concluded that there is no true association between AGA and IR, but their

coexistence with MetS could contribute to worsening of AGA.

IR plays a pathogenetic role in the miniaturization of hair follicles. Vasoactive substances associated with endothelial dysfunction in IR lead to microcirculatory disturbance, perifollicular vasoconstriction, and proliferation of smooth muscle cells in the vascular wall. This condition leads to microvascular insufficiency, local tissue hypoxia, and progressive miniaturization of hair follicles[17, 25].

IR also leads to the generation of inflammatory mediators and endothelial dysfunction. Insulin increases the release of nitric oxide from the endothelium at the physiological levels. An increased risk of atherosclerosis in IR cases is thought to be related to the loss of insulin's effect on nitric oxide expression[26].

In our study, the mean serum PGRN in the AGA group was 80.7 ng/ml that was significantly higher as compared with the control group 12 ng/ml ($P<0.001$). In an animal study, Kato et al.[27], investigated the consequences of PGRN overexpression in vivo by generating keratinocyte-specific PGRN transgenic mice model. The authors showed that overexpression of PGRN in keratin 5 (K5)+ cells decreased hair growth as well as the diameter of the hair shaft in mice.

5. Conclusion

Serum PGRN level and HOMA-IR were significantly higher in AGA patients when compared to control group. PGRN has a critical role which contributes to IR and MetS. IR and MetS are more common among AGA patients especially in patients with severe AGA. AGA patients should be closely followed-up in the long term. Early detection of IR and MetS might help in good management of AGA patients**.**

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