Evaluation of Serum Level of Zinc Alpha 2 Glycoprotein in Male Patients with Androgenetic Alopecia

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Abstract

Background: Androgenetic Alopecia (AGA) is a prevalent disorder in men that causes hair loss. One potential biomarker for AGA is Zinc Alpha 2 Glycoprotein (ZAG). The objective of this research was to compare the blood levels of insulin resistance and ZAG in male patients with AGA to those in healthy controls. Methods: Twenty healthy volunteers of same age and gender were compared to sixty male AGA patients in this cross-sectional research. The following procedures were carried out: clinical evaluations, laboratory investigations, and ZAG level measurements. Using standard methods, we examined ZAG, lipid profile, fasting blood sugar, insulin, and fasting insulin resistance. Insulin resistance was determined using the HOMA-IR formula. The predictive impact of ZAG in AGA was assessed using multivariate logistic regression analysis. The fasting glucose levels of AGA patients were considerably higher (84±12 mg/dl) than those of controls (78±6 mg/dl), with a p-value of 0.003. In comparison to controls (95.9±10.1 mg/dl, P = 0.012), AGA patients had substantially higher levels of LDL (106.2±25.6 mg/dl). The median ZAG level in AGA patients was 12.1µg/ml, which is substantially higher than the control group's median level of 7.6µg/ml (P = 0.023). With a sensitivity of 80% and a specificity of 60%, ZAG showed an area under the curve of 0.671 (95 percent CI: 0.522-0.820, P = 0.023) in ROC analysis. The study's conclusion is that ZAG serum levels were greater in AGA patients compared to controls, suggesting that ZAG may contribute to the disease's pathophysiology. Particularly in cases of severe AGA, insulin resistance and metabolic syndrome are more prevalent in individuals with AGA.

Topics covered include male pattern baldness, insulin resistance, zinc alpha 2 glycoprotein, and androgenetic alopecia.

Keywords: Androgenetic Alopecia, Zinc Alpha 2 Glycoprotein, Insulin Resistance, Male Pattern Baldness.

Introduction

Androgenetic Alopecia areata, the most prevalent kind of gradual shrinkage of the hair follicles that does not leave scars, and it follows a certain pattern of distribution (1). Hair loss that begins at the vertex and works its way inward is the most common symptom of male pattern alopecia (thinning of the hair around the crown) in men. Androgens and their receptors are thought to be involved in the pathogenesis of AGA, however this is far from confirmed (2).

Numerous disorders have been linked to male pattern baldness, including obesity, insulin resistance, aberrant blood lipid profiles, and coronary artery disease (3).

Energy expenditure, insulin sensitivity, inflammation, and cardiovascular activity are just a few of the metabolic processes in which human adipokines are engaged. As a result, they may have therapeutic use as an indicator of fat tissue function and elevated metabolic risk (4).

The adipokine zinc alpha 2 glycoprotein (ZAG) is produced by adipocytes of both brown and white fat. In people, it's encoded by the AZGP1 gene. Due to a decrease in ZAG gene expression in adipose tissue associated with increased adiposity and blood insulin levels, the obesity gene is associated with insulin resistance in the obese. It has been shown that ZAG levels are lower in the fat of individuals who are obese (5).

Because of its importance in lipid mobilisation and insulin resistance, ZAG may have a function in diabetes and dyslipidemia, two of the leading causes of cardiovascular disease (6).

The researchers in this study set out to compare the insulin resistance and ZAG serum levels found in male patients with AGA to those of healthy controls.

Participants and procedures

Patients

In this case-control research, sixty individuals diagnosed with androgenetic alopecia (AGA) were compared to twenty healthy volunteers who were age- and gender-matched. Patients seen by the dermatologists, venereologists, and andrologists at Benha University Hospitals' outpatient clinics from June 2020 through August 2021 were the subjects of this study.
In compliance with the principles outlined in the Declaration of Helsinki, this research was approved by the Research Ethical Committee at Benha Faculty of Medicine. We made sure to get everyone's informed permission before we took their blood. At Benha University's Faculty of Medicine, the local Ethics Committee gave its stamp of approval to the whole research concept. The research upheld the principles of confidentiality and personal privacy. There were no repercussions for patients who opted out of the trial, and researchers did not utilise the data they acquired in any other way.

Only patients older than 18 years old with AGA were eligible to participate.

The following conditions were considered ineligible for inclusion in the study: a current or previous diagnosis of cancer or immunosuppressive treatment, a history of systemic diseases including diabetes mellitus, chronic liver or renal disease, autoimmune disease, alcoholism, malabsorption disorders, a history of taking immune suppressant drugs, a history of topical treatment for AGA for more than one month prior to the study, and a history of any other dermatological diseases.

Two groups were formed from all of the participants: Division A: Comprising sixty individuals diagnosed with AGA, Group B: A control group consisting of twenty healthy volunteers.

Methods

The following evaluations were conducted on all patients:

1. Comprehensive patient history: My background: Please include your full name, date of birth, employment, place of residence, and details about any health-related behaviours, such as smoking. Modern AGA: When it began, how it progressed, and how long it lasted. Family history of AGA. Background of autoimmune disorders. Background information about prior AGA therapies (type, dose, duration).

2. Comprehensive Health Screening: In order to rule out any evidence of systemic disorders, all subjects had a clinical examination. The body mass index was determined by measuring the subjects' height and weight (BMI).

3. Local Examination: In order to validate the diagnosis of AGA, a scalp examination was performed on all patients. There are seven phases to Hamilton-scale Norwood's of male balding that measure the pattern and degree of hair loss; these stages were used to evaluate the severity of AGA.

4. Laboratory Investigations: Insulin, blood sugar, triglycerides, cholesterol, HDL, LDL, zinc alpha 2 glycoprotein, and fasting blood sugar were tested for in all individuals (ZAG).

5. Sampling: Following an overnight fast, five millilitres of each participant's venous peripheral blood was taken in aseptic circumstances using plain tubes. Prior to centrifugation at 2000g for 10 minutes, the blood samples were left to clot at room temperature for 20-30 minutes. We kept the serum samples at -20°C until we could analyse them. A colorimetric enzymatic assay was used to determine fasting glucose levels.

As the sixth measurement, insulin resistance (HOMA-IR) was ascertained by use of the homeostasis model assessment. Those over 2.5 on the HOMA-IR indicated insulin resistance, but results below this threshold were deemed normal.

7. Laboratory Measurement of Serum Insulin Levels: Specific kits were used to conduct enzyme-linked immunosorbent assay (ELISA) tests on serum insulin levels.

8. Serum ZAG Measurement: The serum ZAG level was ascertained by using the ELISA method in conjunction with designated kits.

9. Serum Cholesterol Measurement: The ELISA method was used to test the serum cholesterol levels.

ELISA was used to assess the amounts of serum triglycerides, which is the tenth measurement.

Eleventh, high-density lipoprotein (HDL) levels in the blood were determined by precipitation analysis.

12. Serum low-density lipoprotein (LDL) measurement: Total cholesterol concentration was subtracted from the supernatant cholesterol concentration to get serum LDL values.

For each individual test, these procedures were carried out according to the manufacturer's guidelines.

Code for approval:

Data analysis using statistical software

The statistical analysis and data management were carried out using SPSS version 28. (IBM, Armonk, New York, United States). The Kolmogorov-Smirnov test, the Shapiro-Wilk test, and direct data visualisation approaches were used to examine the normality of the quantitative data. After then, medians and ranges, or standard deviations and means, were used to summarise the numerical data. Numbers and percentages were used to summarise the
categorical data. For numerical variables that did not follow a normal distribution, researchers used the Mann-Whitney U test, whereas for properly distributed variables, they used the independent t-test. ZAG underwent ROC analysis for the purpose of AGA diagnosis. The diagnostic indices, optimal cutoff point, and area under the curve were computed with a 95% confidence interval. The Spearman’s correlation was used to conduct the correlation analyses. The AGA was predicted using multivariate logistic regression analysis. We computed the odds ratios and the 95% confidence intervals. There were no one-sided statistical tests. Significance was determined by p-values lower than 0.05.

Results

There In terms of age (P = 0.086) and body mass index (BMI) (P = 0.203), there were no statistically significant differences between the AGA group and the control group. Table 1 shows that AGA lasts an average of eleven years, with a range of one to thirty-six years. A good family history was found in all of the cases. Type II AGAs accounted for 21.7% of all cases, followed by type V at 16.7%, types III and IV at 15% each, type VII at 13.3%, type I at 10%, and type VI at the very bottom (8.3 percent). See Figure 1 for a comparison of patient and control fasting glucose levels (mean ± SD: 84± 12 mg/dl and 78±6 mg/dl, respectively; P = 0.003). In terms of fasting insulin (P = 0.571) and HOMA-IR (P = 0.726), there were no statistically significant differences between the patient group and the control group. Compared to controls, AGA patients had substantially higher levels of LDL (mean ± SD: 106.2 ± 25.6 mg/dl vs. 95.9 ± 10.1 mg/dl; P = 0.012) in Table 2. There were no notable variations in total cholesterol (P = 0.875), triglycerides (median: 143.2 mg/dl, 106.7 mg/dl respectively; P = 0.091), or HDL (mean ± SD: 36.3 ± 8 mg/dl, 36.7 ± 8.3 mg/dl respectively; P = 0.821) between the patients and the controls. Table 3 shows that the zinc alpha 2 glycoprotein level (ZAG) was noticeably greater in AGA patients compared to controls, with a median of 12.1µg/ml and a range of 7.6 µg/ml, respectively, with a p-value of 0.023. The results in Figure 2 demonstrate that ZAG was positively correlated with both the duration of the disease (P = 0.002) and the severity of the disease (P < 0.001) in the patients’ group, but it was not significantly correlated with the age of the patients (P = 0.825), BMI (P = 0.397), HOMA-IR (P = 0.059), fasting insulin (P = 0.121), and fasting glucose (P = 0.05). ZAG also shown non-significant relationships with respect to total cholesterol (P = 0.293), triglycerides (P = 0.688), HDL (P = 0.854), and LDL (P = 0.213). In order to determine its function in distinguishing between patients and controls, the ROC analysis of ZAG level was performed (Figure 3). A 95% confidence range of 0.522 to 0.820 was shown for ZAG, and the area under the curve was 0.671 (P = 0.023). At >7.99 ng/ml, sensitivity was 80% and specificity was 60%, making it the optimal cutoff point. In order to assess ZAG’s ability to forecast AGA, multivariate logistic regression analysis was used (Figure 4). A significant independent predictor for AGA was found to be ZAG (OR = 1.137, 95 percent CI = 1.02 - 1.267), even after adjusting for patients’ age and BMI. Discussion from Table 4

Table (1) General characteristics of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 60)</th>
<th>Controls (n = 20)</th>
<th>Test P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42 ±13</td>
<td>36 ±12</td>
<td>1.739</td>
</tr>
<tr>
<td>BMI Mean ± SD</td>
<td>30.5 ±5.9</td>
<td>29.1 ±3.6</td>
<td>1.290</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD, median (min-max), or number (percentage).

Table (2) HOMA-IR, fasting insulin, and fasting glucose of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 60)</th>
<th>Controls (n = 20)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>84 ±12</td>
<td>78 ±6</td>
<td>3.031</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12.6 (1.2-60.8)</td>
<td>13.9 (1.2-64.2)</td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td>Fasting insulin (µIU/mL)</td>
<td>2.5 (0.3-13.8)</td>
<td>3 (0.2-11.4)</td>
<td>0.350</td>
<td>0.72</td>
</tr>
</tbody>
</table>
* P < 0.05 is significant t: independent t-test Z: Mann Whitney U test.

**Table (3) Lipid profile of the studied groups**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 60)</th>
<th>Controls (n = 20)</th>
<th>Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>171.7 ±38.1</td>
<td>170.3 ±25.1</td>
<td>t = -</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>143.2 (20.4-238.6)</td>
<td>106.7 (24.8-235.4)</td>
<td>Z= -</td>
<td>0.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>36.3 ± 8</td>
<td>36.7 ±8.3</td>
<td>t= -</td>
<td>0.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>106.2 ± 25.6</td>
<td>95.9 ±10.1</td>
<td>t=</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* P: < 0.05 is significant, t: independent t-test, Z: Mann Whitney U test.

**Table (4) Multivariate logistic regression analysis for prediction of AGA**

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI) *</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZAG</td>
<td>1.137 (1.02-1.267)</td>
<td>0.021*</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>1.1 (0.989-1.224)</td>
<td>0.08</td>
</tr>
<tr>
<td>LDL</td>
<td>1.025 (0.995-1.055)</td>
<td>0.105</td>
</tr>
</tbody>
</table>

OR: Odds ratio, 95%CI: 95% confidence interval, * Adjusted for, age and BMI, **P <0.05 is significant.

![Severity types](image)

**Fig. (1) Androgenic alopecia types of the studied patients**
Fig. (2) Zinc alpha 2 glycoprotein (µg/ml) of the studied groups

![Boxplot showing median ZAG levels for Patients and Controls with a p-value of 0.023.](image_url)

**Fig. (3)** A) Correlation between ZAG and disease duration and B) correlation between ZAG and disease grades

![Scatter plots showing correlation between ZAG and disease duration and grades.](image_url)

**Fig. (4)** ROC analysis of ZAG in diagnosing AGA

![ROC curve for ZAG with AUC of 0.671 (95% CI: 0.522 - 0.826).](image_url)
The shrinkage of hair follicles, most often in the fronto-temporal and vertex regions of the scalp in males, is the hallmark of androgenetic alopecia (AGA), a prevalent form of non-scarring hair loss caused by androgens. Cardiovascular disease is one of the many complications that may arise from metabolic syndrome, which is increased by AGA. Other risk factors include high blood pressure, insulin resistance, hyperinsulinemia, dyslipidemia, and hypertension (7). The secreted protein zinc alpha2 glycoprotein (ZAG) is classified as a new adipokine because of its function in lipid mobilisation, which stimulates lipolysis in fat cells. ZAG has been found in several organs. In addition to its involvement in glucose metabolism and insulin resistance, ZAG regulates the excess free fatty acids generated during increased lipolysis (8).

The current research shows that AGA patients had an average age of 42 ± 13 years. A research that found an average age of 47.1 ± 8.4 years is in agreement with this result (9). The average age of AGA patients was found to be 43.74 ± 16.07 years in a similar research (10). The average age of AGA patients was shown to be 38.33 ± 8.85 years in a different research (11). Furthermore, research showed that the average age was 36.36 ± 9.62 years (7). On the other hand, when looking at their patient group, researchers found that the average age was somewhat higher at 51.32 ± 16.31 years (12).

Participants with AGA had an average body mass index (BMI) of 30.5 ± 5.9 kg/m² in this research. A research found that the average body mass index (BMI) was 27.87 ± 4.3 (9), whereas another study found that the average BMI was 23.13 ± 3.43 kg/m² (11). Another research found that the average body mass index was 26.89 ± 2.79 kg/m², which led to these conclusions (7). An interesting finding from the research was a mean body mass index (BMI) of 24.13 ± 4.94 kg/m², which is countereintuitive (12). Possible explanations for the discrepancy between the present study's findings and those of the aforementioned investigations include variations in patients' dietary habits and social cultures.

The current research found that AGA may last anywhere from one year to thirty-six years, with eleven years being the median. In a study where the average illness duration was 11.27 ± 5.41 years, ranging from 4 to 25 years, this congruence with earlier data is clearly visible (7).

There was a favourable correlation between AGA and family history in this research. This lines up with the results of a research that found that 78.28% of people have AGA in their family tree (10). In addition, 52% of patients had an AGA family history, which was much greater than the control group's 30% (P = 0.025). (13). A similar research found that whereas 34.8% of individuals did not have a positive family history of AGA, 48.4% had first-degree relatives with the disease and 16.8% had second-degree relatives with the disease (11).

In this research, type II AGA was shown to be the most common, accounting for 21.7% of cases, with type V type AGA coming in a close second (16.7 percent ). Type VII made up 13.3% of the total, whereas types III and IV were equally common at 15% each. Fifteen percent of the cases were type I, and eight and a third percent were type VI. A research found slightly contradictory results, with 36 people having grade II, 24 having grade III, 20 having grade IV, 15 having grade V, and 5 having grade VI (14).

The findings of another research were different; it found that 19 patients (38 percent) had AGA grade III, 16 patients (32 percent) had grade IV, 13 patients (26 percent) had grade V, and 2 patients (4%) (13).

Statistical analysis revealed that the sick group in this research had significantly higher fasting glucose levels than the control group. When comparing the patient and control groups on HOMA-IR and fasting insulin levels, no statistically significant changes were found.

According to the research, patients had substantially higher fasting blood glucose and insulin levels compared to controls (P = 0.043 and P = 0.020, respectively), which is consistent with the current result. Statistical analysis revealed no significant difference (P = 0.23) in HOMA-IR scores across the categories. Research showing that those with AGA had significantly higher fasting blood glucose levels compared to the control group also came to similar conclusions (P = 0.004). They also found that HOMA-IR was considerably higher in AGA patients than in controls (P = 0.006) and that insulin levels were significantly higher in AGA patients than in controls (P = 0.020). (15).

The present lipid profile analysis is consistent with previous research showing that AGA is associated with substantially elevated levels of triglycerides, low-density lipoprotein (LDL) cholesterol, and total cholesterol in males. There were no statistically significant variations in HDL cholesterol levels between the AGA and control groups (9).
Patients with AGA had significantly higher mean levels of triglycerides, very low density lipoprotein, LDL, and total cholesterol compared to controls (P < 0.001), according to two scientists. The control group had a significantly higher mean HDL value than the AGA group (P < 0.001).

A research found a similar trend, with AGA patients having considerably higher LDL and significantly lower HDL compared to the control group (P = 0.0052 and P = 0.0052, respectively) (14).

Swaroop et al. found that triglycerides and HDL cholesterol were considerably higher in patients compared to controls, which contradicts the present findings (16). Cholesterol, low-density lipoprotein, and triglyceride levels were all considerably higher in AGA patients (P = 0.003), whereas HDL values were significantly lower (P = 0.003). (15).

Both the length of the illness (P = 0.002) and the severity of the disease (P < 0.001) showed significant positive relationships with ZAG in the current investigation. The research also found that there were no statistically significant relationships between patients' ages and ZAG. The findings of a research that showed favourable relationships between blood ZAG levels and age contradict this conclusion (17). Serum ZAG levels did not correlate significantly with age, according to the opposite research (18).

The current investigation did not find a statistically significant correlation between ZAG and patients' body mass index. A negative correlation (r = -0.170) between serum ZAG levels and BMI was also found in a research (18).

Correlations between ZAG and patients' fasting insulin, fasting glucose, and HOMA-IR were not statistically significant in this investigation.

Researchers found that ZAG and HOMA-IR correlated negatively (19). A previous research found no statistically significant relationships between serum ZAG levels and HOMA-IR, which is in line with the current investigation (18).

When it came to triglycerides, the present investigation found no statistically significant connections involving ZAG.

Researchers found that ZAG and triglycerides correlated negatively (19). An analogous finding was reported in a research that found a negative link (r=-0.140) between blood ZAG levels and triglycerides (18).

Additionally, there were no statistically significant relationships between ZAG and total HDL in this investigation. A correlation value of 0.186 between serum ZAG levels and HDL indicates a favourable link, according to a research (18).

There were no statistically significant correlations between ZAG and LDL in this investigation. Researchers found no statistically significant correlation between serum ZAG levels and LDL cholesterol (18).

A related research used multivariate logistic regression analysis to show that polycystic ovarian syndrome was still strongly correlated with circulating ZAG levels after controlling for anthropometric characteristics, blood pressure, lipid profile, and hormone levels, among other things. A lower frequency of metabolic syndrome and lower HOMA-IR were seen in women with poly cystic ovarian syndrome who had greater ZAG levels, which is an interesting finding (19).

Recognizing the study's shortcomings, such as its cross-sectional design and limited sample size, is crucial. Although there are certain limitations, this research adds to the increasing amount of information about the possible importance of ZAG in different medical disorders. To determine the therapeutic significance of ZAG in AGA and associated disorders, as well as the causative linkages, more multi-center and longitudinal investigations are necessary.

**Conclusion**

There may be a function for ZAG serum levels in the etiology of AGA, as they were greater in individuals with the illness compared to controls. Particularly in cases of severe AGA, insulin resistance and metabolic syndrome are more prevalent in individuals with AGA. Long-term follow-up with AGA patients should be meticulous. It is possible that AGA patients would be better managed if insulin resistance and metabolic syndrome were detected earlier.

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**Contribution of the author**

All writers had equal roles in writing the research.

**Problems with potential bias**

There are no potential biases.
References


