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Defective Expression of the Gap Junction Protein Pannexin-1 Channel Contributes to the Formation of PCOS-Realted Androgenetic Alopecia

Sule Gencoglu[1*], Fatma Tanilir Cagiran[2], Zercan Kali[3]

[1]Dermatology, Private Gözde Akademi Hospital, Malatya, Turkiye
[2]Private Clinic Obstetrics and Gynecology, Diyarbakır, Turkey
[3]Gozde Academy Hospital Obstetrics and Gynecology, Malatya, Turkey
Email: *sulegencoglu2309@gmail.com

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Abstract

Objective: To determine serum pannexin-1 channel levels and their association with hair loss in women with PCOS diagnosed with female androgenetic alopecia (FAGA). **Materials and Methods:** Thirty-five women with PCOS who presented with diffuse and treatment-resistant progressive hair loss and were diagnosed with FAGA were included in the study. 25 patients who were diagnosed with female androgenetic alopecia but did not have PCOS were considered as the control group. PCOS and control groups were matched by age. Follicular miniaturization, displacement of terminal hairs with vellus hairs, and a diffuse decrease in hair density were accepted as FAGA in the trcihoscopy examination of the vertex and bitempoaral area. On the third day of the menstrual cycle serum FSH, LH, testosterone, PRL and insulin levels were measured. Insulin resistance was calculated with HOMA-IR. Serum pannexin-1 channel levels of each group were mesured with ELISA. **Results:** Serum pannexin 1 channels levels of FAGA group due to PCOS were found to be significantly higher than FAGA patients in the control group (2.72 ± 1.09 ng/mL vs 1.65 ± 0.97 ng/mL, $p < 0.01$). Serum LH, insulin and testosterone levels of PCOS group were significantly higher than controls. HOMA-IR values were significantly higher and >2.5 in the PCOS group compared to the controls. PRL values were similar except for one patient with elevated PRL. Serum FSH values were the same in both groups. A positive and significant correlation was found between pannexin 1 channels levels and HOMA-IR and serum testosterone levels ($r = 0.650$, $p < 0.02$, $r = 0.544$, $p < 0.03$ respectively). **Conclusions:** In addition to hyperandrogenemia, increased pannexin

1 channel levels may play a role in the etiology of PCOS associated FAGA, as it impairs the communication between the skin and hair follicle.

Keywords

Androgenetic Alopecia, PCOS, Androgen, Pannexin-1 Channel, Inflammation

1. Introduction

In the last two decades, despite the rapid developments in medical research, female androgenetic alopecia (FAGA), which started to appear from the adolescence age, continues to increase both its physical and psychological effects [1]. Although the pathological mechanisms of FAGA are not fully understood, the increase in systemic and local inflammation together with genetic predisposition causes damage to hair follicles, leading to diffuse and progressive thinning of the hair, and subsequently to hair loss. Local or systemic inflammation is an adaptive immune response to exogenous or endogenous pathological stimuli [2]. If the adaptive immune response is adequate and regular, it limits the pathological event, while the uncontrolled and long-term inflammatory response triggers a process leading to hair follicle damage followed by the death of the follicles and hair loss. Innate immunity triggered by androgens or inflammatory molecules leads to amelioration of the inflammatory response or perpetuation of the detrimental effect in the hair follicles. However, while FAGA causes hair loss in the vertex and bitemporal regions, the preservation of the frontal hairline suggests the presence of a limited inflammatory response to a specific area rather than a systemic effect. If areas of hair loss specific to FAGA are genetically determined, adaptive immune response may occur in these areas. The autosomol dominant nature of FAGA supports a genetic predisposition [1].

Maintaining homeostasis in the skin and its appendages requires intercellular communication [3]. Pannexins, which are gap junction proteins, play a role in intercellular communication, transport of ions and metabolites [4]. Pannexins (Panxs), which are two main gap junction proteins, are expressed in three different ways as Panx1, 2 and 3 [5]. Pannexin-1 (Panx1) channels are inflammation regulators involved in skin formation and hair follicle regeneration [6]. Exogenous or endogenous activation of Panx1 channels initiates the inflammatory process by providing leukocyte chemotaxis and accumulation [7] [8]. PCOS is the most common endocrine pathology in which FAGA is seen. High androgen, low-grade inflammation and insulin resistance can trigger FAGA formation [9] in PCOS. However, since only 30% of FAGA patients have hyperandrogenemia, it is thought that molecules other than androgens may also play a role in the ethology. The role of novel channel molecules responsible for inflammation, such as Pannexin, in the etiology of PCOS-related FAGA is unknown. This study was planned to determine serum pannexin-1 channels levels and their associa-

tion with hair loss in PCOS patients diagnosed with FAGA.

2. Materials and Methods

Thirty-five women with PCOS who presented with diffuse and treatment-resistant progressive hair loss and were diagnosed with androgenetic alopecia were included in the study. 25 patients who were diagnosed with female androgenetic alopecia but did not have PCOS were considered as the control group. PCOS and control groups were matched by age. The medical histories of the patients in both groups were taken in detail. When the hair loss started, family history and the drugs he used were questioned. Adrenal pathologies causing hair loss, ovarian tumors and other endocrine pathologies associated with elevated prolactin levels were excluded. Drug use, chemical exposure and occupations that can cause hair loss were questioned. In order to diagnose PCOS, presence of at least two of the criteria of hypernadrogenemia, ovulation dysfunction and polycystic ovarian morphology was required. Blood samples were taken to determine the basal hormonal values of both groups. On the third day of the menstrual cycle, blood was drawn in the morning following a night fast. Serum FSH, LH, testosterone, PRL and insulin levels were measured. Insulin resistance was calculated with HOMA-IR. Serum pannexin-1 channels levels of each group were calculated in the same blood samples.

Patients with alopecia areata, adrenal or ovarian tumors, and adrenal hyperplasia were excluded from the study. Those who were exposed to drugs or chemicals causing hair loss and those with cicatricial alopecia were also excluded from the study. Those with high PRL and those who had PRP and hair transplantation were not included in the study because of their possible effects on pannexin-1 channels proteins. Follicular miniaturization, displacement of terminal hairs with vellus hairs, and a significant and diffuse decrease in hair density were accepted as FAGA in the trcihoscopy examination of the vertex and bitempoaral area [10]. Preservation of the frontal hairline strengthened our diagnosis. Except for two patients, routine scalp biopsy was not required.

2.1. Measurement of Serum Pannexin-1 Channels with ELISA

Pannexin-1 channels levels in serum samples were measured using the Human Pannexin-1 (PANX1) ELISA kit (Sunred Biotechnology Company. Catalog no: 201-12-4490, Shanghai, CHINA) with the principle of quantitative sandwich enzyme immunoassay. The absorbances of the samples studied in accordance with the kit procedure were measured in a Bio-Tek ELx800 (BioTek Instruments, USA) device at a wavelength of 450 nanometers. The concentrations corresponding to all absorbances were calculated with the formula obtained with the help of the standard curve graph. Test results were expressed as ng/mL. The measuring range of the PANX1 kit was 0.05 - 15 ng/mL, and the minimum measurable level was 0.045 ng/mL. The kit's Intra-Assay CV value was <10%, while the Inter-Assay CV value was <12%.

2.2. Statistical Analysis

The collected data of FAGA with PCOS and control FAGA group were analyzed with the Statistical Package for Social Sciences software for Windows. The Shapiro-Wilk test was used to determine whether the data showed normal distribution or not. Differences in demographic, clinical and laboratory findings of the both groups were evaluated with Mann-Whitney U test. Correlations between pannexin 1 channels levels and demographic and laboratory parameters were analyzed with Pearson's correlation. Results were presented as mean ± SD, number of cases, or percentage. All comparisons with $p < 0.05$ were considered significant.

3. Results

Age and BMI values of the participants in both groups were found to be similar. Two patients required scalp biopsy for the diagnosis of FAGA. A patient with elevated PRL was included in the study after medical treatment was started. In both groups, there was no patient with a history of chronic disease and using drugs that cause hair loss. Three patients wore wigs for psychological reasons. These patients were included in the study and psychiatric consultation was recommended. Dermatological findings of the syndrome were observed in some patients in the PCOS group. Cases with hirsutismus remained in the study. Blood samples were taken before ovarian stimulation in two patients who were planned to be treated with assisted reproductive techniques.

Demographic and clinical data of the patients are shown in Table 1. Serum pannexin 1 channels levels of FAGA group due to PCOS were found to be significantly higher than FAGA patients in the control group (2.72 ± 1.09 ng/mL vs 1.65 ± 0.97 ng/mL, $p < 0.01$). This elevation was accepted as evidence of the presence of low-grade inflammation in PCOS. Serum LH, insulin and testosterone levels of PCOS group were significantly higher than controls. HOMA-IR

Table 1. Demographic and laboratory parameters of PCOS and FAG groups.

	PCOS plus severe hair loss	Female Androgenetic Alopecia	P values
N	35	25	P < 0.05
Age (y)	27.1 ± 3.78	26.7 ± 5.11	0.65
BMI (Kg/m^2)	24.7 ± 2.32	23.8 ± 4.87	0.23
Pannexin 1 (ng/mL)	2.72 ± 1.09	1.65 ± 0.97	<0.001
LH (mIU/mL)	8.59 ± 3.09	5.65 ± 3.22	<0.01
FSH (mIU/mL)	5.03 ± 1.21	5.13 ± 1.05	0.43
Testosterone (ng/dL)	41.9 ± 4.30	29.3 ± 3.09	<0.01
Insulin resistance	2.54 ± 1.09	1.89 ± 0.66	<0.05

Data are presented Mean ± SD.

values were significantly higher and >2.5 in the PCOS group compared to the controls. HOMA-IR values in the control group were recorded as <2.5. PRL values were similar except for one patient with elevated PRL. Serum FSH values were the same in both groups. A positive and significant correlation was found between Pannexin 1 channels levels and HOMA-IR and serum testosterone levels ($r = 0.650$, $p < 0.02$, $r = 0.544$, $p < 0.03$ respectively). There was no significant correlation between other parameters and pannexin 1 channels levels.

4. Discussion

Characterized by follicle miniaturization, androgenetic alopecia affects the dermal papilla and its sheath, leading to shrinkage of follicles and reduction of hair in the anagen phase [11]. Alopecia is often irreversible. Although miniaturization sometimes returns spontaneously or with medical treatments, complete recovery is often not possible [12]. Although the exact etiology of the disease is not known, it has been shown in twin studies that the genetic potential is high [13]. Although the most accused mechanism in the etiology of FAGA is the presence of high androgens, only one of the three FAGA cases has hyperandrogenemia. While androgens shorten the anagen phase by disrupting the hair cyle, they increase the telogen phase and cause a progressive hair loss [1] [14]. The occurrence of FAGA in both men and women supports the role of androgens in the etiology. PCOS is the most common endocrine pathology with hyperandrogenemia and FAGA. Hirsutism, acne vulgaris and acanthosis nigricans are the most common dermatological manifestations of PCOS. FAGA often occurs with virilization findings in PCOS patients. Depending on the increased androgens, muscle hypertrophy, deepening of the voice, reduction in breast size and FAGA may occur. However, although androgens are responsible for most of the dermatological findings related to PCOS, there is no clear correlation between androgens and FAGA [15].

The lack of a clear link between androgen elevation and FAGA has brought forward that different aetiological mechanisms may be responsible for FAGA. The presence of metabolic syndrome components in most FAGA patients and the close relationship between high BMI and FAGA suggest that chronic low-grade inflammation and insulin resistance play a role in the etiology [14]. In our study, all patients with FAGA had hyperandrogenemia and insulin resistance. Serum pannexin 1 channels levels, which we consider as an inflammation marker, were significantly higher in the PCOS group. Increased serum pannexin 1 channels levels are critical evidence of chronic low grade inflammation. Increased pannexin 1 channels levels in the FAGA group due to PCOS initiate leukocyte chemotaxis in hair follicles and trigger inflammation [7] [8]. Panx1 provides skin formation and hair follicle regeneration under normal conditions [5]. In the presence of PCOS, the increase in Panx1 levels stimulates the release of inflammatory cytokines in the dermal papilla, leading to local inflammatory damage and loss of hair in the anagen phase. Since the inflammatory follicular microen-

vironment causes increased oxidative stress and disrupts the nutrition of the dermal sheath and hair follicles, progressive hair loss may occur.

The skin is the largest organ in the organism, and with its immunological and thermoregulatory functions, it provides both a barrier function and a suitable environment for hair follicles to live [5]. Intercellular communication between the skin and its appendages is critical for the survival of many resident cells [6]. Hemostasis between the skin and its appendages is achieved through channels called gap junctions. Panx1 is an important gap junction protein [6] [16]. The significant increase in Panx1 levels in FAGA patients with PCOS prevents these channels from performing their functions due to inflammation. As the occlusion of the Panx1 channels will prevent the passage of ions, metabolites and seconde messengers, the growth of hair follicles both in nutrition and anagen phase will be prevented, leading to progressive follicle loss and hair loss. In addition to the increased androgens in PCOS, increased panx1 levels may play a role in the etiology of FAGA, as it impairs the communication between the skin and hair follicle. Panx1 immunohistochemistry studies on the skin and appendages of FAG patients will help us reach a clearer conclusion. If panx1 pathology is confirmed by other studies, a new treatment approach can be put forward through gap junctions in the treatment of FAGA.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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