INFLAMMATORY MARKERS IN ANDROGENETIC ALOPECIA IN WOMEN

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Summary

Introduction: *Androgenetic alopecia (AGA) in women is a common pathology but with a low success rate of drug therapy. The local inflammation has been shown to play an important role in the pathogenesis of the disease, this fact providing us with the opportunity to develop new therapies and to customize the existing ones. The systemic inflammation in AGA has not been extensively studied, but it raises the possibility of identifying new cardiovascular risk factors among female patients with AGA.*

Objective: *The aim of this study was to identify the association between the presence of androgenetic alopecia in women and the increase of certain serum markers of systemic inflammation (ESR, fibrinogen, CRP, TNF-α, IL-6).*

Material and Method: *The study was of observational, analytical, transversal, prospective, case-control type. It included a group of 30 female patients with AGA (age range of 30 to 60 years) and a control group of 30 patients without injuries of skin appendages, selected from the patients that addressed to the Dermatovenerology ambulatory and unit of the County Emergency Clinical Hospital of Oradea, between January 1st and December 31st, 2016. In order to evaluate the systemic inflammation (CRP, fibrinogen, VSH, TNF-α and IL-6), venous blood samples were collected between 8 and 9 a.m., 12 hours after the last meal, the consumption of alcohol being prohibited for 24 hours prior to collecting specimens.*

Results: *Statistically, CRP levels were significantly higher in the alopecia group compared to the control group ($p = 0.05$). No statistically significant differences were observed for the other analyzed parameters ($p > 0.05$). The Spearman's rho coefficient revealed a strong direct correlation between ESR and CRP ($p < 0.001$). IL-6 and TNF-α were not correlated with any of the other inflammatory markers.*

Conclusion: *Our study has revealed the presence of a certain degree of systemic inflammation through high ESR and CRP values in the androgenetic alopecia group compared to the control group. A correlation between IL 6, the serum TNF-α and AGA in women could not be demonstrated.*

Key words: *TNF alpha, Interleukin 6, androgenetic alopecia, inflammatory markers, cardiovascular risk.*

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Introduction

Androgenetic alopecia in women (AGA) or the female pattern hair loss (FPHL) is a topic of general social interest, as it represents 95% of all cases of alopecia and the pathophysiology of the disease is still unclear[1]. The fact that the success rate of drug treatment, even of the anti-androgenic therapy, hardly exceeds 30% in androgenetic alopecia shows us the importance of deepening the study of the pathophysiological mechanisms and of identifying new therapeutical weapons[2].

The specialty literature mentions, in the pathogenesis of the disease, the microvascular insufficiency and inflammatory abnormalities, besides the hormonal sexual and genetic factors. [3-4]. So far, by undergoing scalp biopsies and proving the presence of inflammatory cytokines and perifollicular fibrosis, several studies have

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reported the association of AGA in men with the increase of local inflammation. [5-7] These studies have even reported a decrease in the therapeutic effect of Minoxidil in patients with AGA suffering from local inflammation and have delivered the theory of the positive impact of combining topical anti-inflammatory drugs in the treatment of this pathology, a theory which has been validated through further studies[8]. Nevertheless, there is a lack of data regarding large population studies which would elucidate whether there is an association of serum inflammatory markers with androgenetic alopecia, especially in women.

The patients with androgenetic alopecia have, in theory, a prevalence of carotid atheromatosis with higher levels of inflammatory markers, both of C-reactive protein, fibrinogen and D-dimers types and of inflammatory cytokines type[9]. The chronic systemic inflammation is also a risk factor for the progressive development of atheromatosis and of other vascular and microvascular alterations. There is the concept of potentially protective (anti-inflammatory) mediators and pro-inflammatory mediators (pro-atherogens). The first category consists of leptin and adiponectin, while the second comprises the S100 protein, TNF-α, IL6, IL8, IL12, IL15, IL17, IL18, IL20, IL23, IFNγ, MCP1, MMPs (MMP9), CRP, PAI-1, TSP-1, MIF, M-CSF, sPLA2-IIA[10].

Most studies have not determined the serum inflammatory markers in patients with AGA [9,11,12], although there are two studies in which some of these parameters have had positive results. As the presence of inflammatory markers is associated with increased cardiovascular risk, patients with AGA have a higher cardiovascular risk which is not attributed to insulin resistance[13].

The purpose of our study was to identify associations between certain serum markers of systemic inflammation (ESR, fibrinogen, CRP, TNF-α, IL-6) and the presence of androgenetic alopecia in women in order to determine new cardiovascular risk factors in this population group.

Material and Method

The study was of observational, analytical, transversal, prospective, case-control type. It included a group of 30 female patients with androgenetic alopecia, aged between 30 and 60 years and a control group of 30 patients without injuries of skin appendages or other conditions. The patients were selected from the cases that addressed to the ambulatory care unit or to Dermatovenerology department of the County Emergency Clinical Hospital of Oradea between January 1 and December 31, 2016. The diagnosis of AGA was established on the basis of local dermatological exam and of family background: the onset at a young age, diffuse pattern of hair on the front and parietal scalp with maintenance of the front hair insertion line and the diversity of the hair diameters (seen through dermatoscopy).

The inclusion criteria were: voluntary participation in the study, normal liver and kidney functions, normal results in thyroid and adrenal glands evaluation, normal blood count and standard urine analysis.

The exclusion criteria comprised: the presence of congenital adrenal hyperplasia, thyroid disease including subclinical hypothyroidism (TSH>5 mUI/l), diabetes, smoking, Cushing's disease, antecedent viral hepatitis, cirrhosis or hepatic insufficiency, androgen or antiandrogen treatment, insulin medication, glucocorticoids, autoimmune diseases.

In order to evaluate the systemic inflammation (CRP, fibrinogen, ESR, as well as TNF-α and IL-6 cytokines), venous blood samples were collected between 8 and 9 a.m., 12 hours after the last meal, the consumption of alcohol being prohibited for 24 hours prior to blood collecting. ESR and fibrinogen were analyzed on DIASYS (German Diagnostic System GmbH) commercial kits, on CI4100 Architect Biochemistry Machine. TNF alpha, hs CRP, IL6 were processed from collected blood á jeun on non-anticoagulant tubes, centrifuged and frozen at -80 °C on commercial IVD kits, by the chemiluminescence technique, with kits that are compatible with the IMMULINE 1000 automatic analyzer.

The statistical analysis was performed with the MedCalc Statistical Software version 17.5.5 (MedCalc Software bvba, Ostend, Belgium, http://www.medcalc.org, 2017). The data were verified for normal distribution by the

Table 1. Differences in inflammatory parameters between the AGA group and the control group

Variable	Alopecia group (n=31)	Control group (n=9)	p
IL 6 (pg/mL)	2 (2; 4)	3.4 (2; 17.3)	0.2
TNF-α (pg/mL)	0.8 (0.3; 2.4)	1.1 (0.2; 2.1)	0.8
ESR 1h (mm/h)	2 (1; 6)	1 (1; 4)	0.1
ESR 2h (mm/h)	4 (2; 10)	2 (2; 6)	0.1
CRP (mg/L)	0.5 (0.4; 0.7)	0.4 (0.3; 0.5)	0.05

Table 2. Correlations between the inflammatory markers

		IL-6 pg/mL	TNF-α pg/Ml	ESR1	ESR2	CRP
IL-6 pg/mL	r	-	-0.011	0.177	0.166	0.210
	p	-	0.9	0.2	0.3	0.1
TNF-α pg/Ml	r	-0.011	-	-0.255	-0.226	-0.064
	p	0.9	-	0.1	0.1	0.6
ESR1h	r	0.177	-0.255	-	0.994	0.736
	p	0.2	0.1	-	<0.001	<0.001
ESR2h	r	0.166	-0.226	0.994	-	0.744
	p	0.3	0.1	<0.001	-	<0.001
CRP	r	0.210	-0.064	0.736	0.744	-
	p	0.1	0.6	<0.001	<0.001	-

Kolmogorov-Smirnov test. The variables were expressed using the medians and percentiles 25 and 75. The difference between the groups was assessed with the Mann-Whitney test. A $p < 0.05$ value was considered statistically significant.

Results

The comparison of variables between the group of women with androgenetic alopecia and the control group can be seen in Table 1.

Statistically, CRP levels were significantly higher in the alopecia group compared to the control group ($p = 0.05$).

No statistically significant differences were observed for the other analyzed parameters ($p > 0.05$). Table 1 shows that the normal value (NV) for IL-6 is <3.8 pg/mL[4], the minimum is of 2 pg/mL[4], and the maximum of 4 pg/mL[4], the average of the results being respectively normal. The other cytokine, TNF-α, was also proved to be within normal limits (NV=<8.1pg/ml[2]); the maximum value of 2.4 pg/ml[2] and the minimum value of 0.3 pg/ml[2].

In order to determine the existence of some correlations between the inflammatory markers, the Spearman's rho coefficient (the correlation between the inflammatory markers in the same patient) was calculated. (Table 2). This revealed a strong direct correlation between ESR and CRP ($p < 0.001$). IL-6 and TNF-α were not correlated with any of the other inflammatory markers.

Discussions

Undoubtedly, the androgenic hormones are the main modulators of hair loss in AGA. However, the association of androgenic alopecia with the local inflammation of the scalp, also called micro-inflammation, has been demonstrated in multiple studies over the past few years, this leading to miniaturization and involution of the hair follicle and to dermal fibroplasia around it(Mahe 2000)[2]. Jaworski et al., Messenger et al., Mahe et al. and Kramer et al. performed biopsies from the scalp areas of AGA patients by which they proved the presence of rich inflammatory infiltration in areas affected by alopecia, especially the perifollicular area around the infundibulum [2-3,6-7]. Philpott et al demonstrated the inhibitory effect of inflammatory cytokine TNF-α, also synthesized by epidermal keratinocytes, on hair follicle growth at a concentration of 1.0 ng / ml[14].

Given the fact that there are few research works in the literature regarding the presence of a systemic inflammation degree among AGA patients, we have evaluated the levels of serum

inflammatory markers in these patients, comparing them with non-alopecia patients, also aiming at identifying whether there is or not a high cardiovascular risk due to the presence of these markers in women. The results of the present study show that the values of fibrinogen, ESR, as well as the inflammatory cytokines IL-6 and TNF-α, are not pathologically modified among female AGA patients. However, the CRP level was statistically significantly high ($p = 0.05$) in the AGA group compared to the control group, a result that validated the data published by Vaya et al in 2016[11], data that were obtained on male population and by Arias-Santiago et al in 2010, on the female population[9].

As far as the cardiovascular system is concerned, the serum inflammatory factors favour the migration and accumulation of mononuclear cells in the intima vasculature, as well as in the platelet aggregation. Moreover, it has been demonstrated that CRP inhibits the synthesis of endothelial nitric oxide which affects vasoreactivity [15]. Both fibrinogen levels and CRP [have been independently associated with the incidence of coronary events, even after the adjustment of traditional cardiovascular risk factors [16].

Although more studies are required to establish the exact relationship between AGA and cardiovascular disease in both genders, the results of our study show that for women suffering from AGA the degree of systemic inflammation is lower than that of male patients suffering from the same disease. Therefore, considering the multifactorial theories which accept inflammation as one of the predisposing factors and which maintain AGA as one of the essential agents for initiating and triggering cardiovascular events, the presence of serum inflammatory markers has demonstrated, in the present study, the connection between the two pathologies, a closer connection among males and a less close connection in female patients [17-18].

Conclusions

Among the inflammatory markers representative for cardiovascular risk, C-reactive protein had slightly high values in patients with alopecia. Considering that studies conducted on male patients with AGA have determined a more intense expression of inflammation in them, we can estimate a lower level of inflammation in women than in men. It was not possible to demonstrate a correlation between IL 6 and serum TNF alpha and AGA in women.

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Bibliography

1. Olsen EA. Female pattern hair loss and its relationship to permanent/cicatricial alopecia: a new perspective. J Investig Dermatolol Symp Proc 2005; 10(3):217-21.
2. Mahé YF, Michelet JF, Billoni N et al. Androgenetic alopecia and microinflammation. Int J Dermatol 2000; 39(8):576-84.
3. Jaworsky C, Kligman AM, Murphy GF. Characterization of inflammatory infiltrates in male pattern alopecia: implications for pathogenesis.Br J Dermatol 1992; 127(3):239-46.
4. Ioannides D, Lazaridou E. Female pattern hair loss. Curr Probl Dermatol 2015; 47:45-54.
5. Rashid RM, Thomas V. Androgenic pattern presentation of scarring and inflammatory alopecia. J Eur Acad Dermatol Venereol 2010; 24(8):979-80.
6. Kramer J, Fahey M, Santos R et al. Alopecia in Rhesus macaques correlates with immunophenotypic alterations in dermal inflammatory infiltrate consistent with hypersensitivity etiology. J Med Primatol 2010; 39(2):112-22.
7. Messenger AG, Sinclair R. Follicular miniaturization in female pattern hair loss: clinicopathological correlations. Br J Dermatol 2006; 155(5):926-30.
8. Sakr FM, Gado AM, Mohammed HR et al. Preparation and evaluation of a multimodal minoxidil microemulsion versus minoxidil alone in the treatment of androgenic alopecia of mixed etiology: a pilot study. Drug Des Devel Ther 2013; 7:413-23.

9. Arias-Santiago S, Gutierrez-Salmeron MT, Castellote-Caballero L et al. Androgenetic alopecia and cardiovascular risk factors in men and women : a comparative study. J Am Acad Dermatol 2010; 63(3)420-9.
10. Fisman EZ, Motro M, Tenembaum A. Cardiovascular diabetology in the core of a novel interleukins classification: the bad, the good and the aloof. CardiovascDiabetol 2003 Sep 12;2:11.
11. Vayá A, Sarnago A, Ricart JM et al. Inflammatory markers and Lp(a) levels as cardiovascular risk factors in androgenetic alopecia. ClinHemorheolMicrocirc 2015; 61(3):471-7.
12. P. Hirsso, M. Laakso, V. Matilainen et al. Association of insulin resistance linked diseases and hair loss in elderly men. Finnish population-based study, Cent Eur J Public Health 197 14 (2006), 78–81.
13. P. Libby, P.M. Ridker, Novel inflammatory markers of coronary risk.Circulation 1999,1148–1150.
14. Philpott MP, Sanders DA, Bowen J et al. Effects of interleukins, colony-stimulating factor and tumour necrosis factor on human hairfollicle growth in vitro: a possible role for interleukin-1 and tumour necrosis factor-alpha in alopecia areata. Br J Dermatol 1996; 135(6):942-8.
15. O. Yousuf, B.D.Mohanty, S.S.Martin et al. High-sensitivityC-reactive protein and cardiovascular disease: A resolute belief or an elusive link?.J Am Coll Cardiol 2013, 397–408.
16. E. Corrado, M. Rizzo, G. Coppola et al. An update on the role of markers of inflammation in atherosclerosis.J Atheroscler Thromb 2010, 1–11.
17. Fedele S. Common oral mucosal diseases, systemic inflammation and cardiovascular diseases. Am. Heart J 2011; 161:344-350.
18. Ludwig E. Classifications of the types of androgenetic alopecia (common baldness) occuring in the female sex. Br J Dermatol Sept. 1977; 97(3) 247-54.

Conflict of interest
NONE DECLARED

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