ORIGINAL ARTICLE

Dermatology

Morphological Substantiation of Clinical Efficacy of Platelet Rich Plasma in the Treatment of Androgenetic Alopecia

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Abstract

The aim of the study was morphological substantiation of clinical efficacy of platelet rich plasma (PRP) in the treatment of patients with androgenetic alopecia (AGA).

Materials and Methods: The study included 25 men aged from 20 to 43 years. AGA degree II-IV on the Hamilton-Norwood scale was diagnosed in 23 (92%) patients and degree I in 2 patients. All patients received intradermal injections of PRP. The course of treatment consisted of 4 procedures with an interval of 4 weeks between each procedure. The clinical efficacy of the therapy was evaluated by the dynamics of morphometric indicators of hair growth. The morphological analysis included an assessment of indicators in horizontal sections and was carried out at 4 levels: at the level of subcutaneous adipose tissue, sweat glands, sebaceous glands, and mouths of hair follicle (HF). Sections were stained with H&E. Histological examination was performed before and after treatment. Against the background of treatment with PRP, hair density increased by 12% (P=0.000), average hair diameter by 12% (P=0.002), and the share of vellus and telogen hair decreased by 17% (P=0.002) and 16% (P=0.028), respectively. At the same time, the amount of anagen hair in relation to telogen hair increased by 18% (P=0.016). Histologically, the increase in hair density was accompanied by a significant increase in the number of HF at the mouth level (Δ+96%, P=0.004) and at the level of sweat glands (Δ+54%, P=0.037), as well as a tendency for their number to increase at the level of the sebaceous glands. These increases were combined with a significant decrease in the proportion of telogen hair (Δ-43%, P=0.023) and vellus hair (Δ-29%, P=0.037).

Conclusion: The positive clinical effect of PRP therapy is due to significant morphofunctional changes in hair follicles. (**International Journal of Biomedicine. 2018;8(4):317-320.**)

Key Words: androgenetic alopecia • hair follicle • platelet rich plasma • treatment • morphological substantiation

Introduction

Platelet rich plasma (PRP) is a very promising method for the treatment of androgenetic alopecia (AGA).[1] Z.J. Li et al.[2] indicated that PRP prolongs the anagen phase, promotes cell proliferation and lifespan of the dermal papilla during the hair growth cycle, and increases the cell survival time by inhibiting apoptosis. Clinically, this is manifested in a decrease in the intensity of hair loss (telogen fraction). It is believed that the growth factors contained in platelets bind to receptors sensitive to them and activate the proliferative phase of the hair.[3] Gkini Maria-Angeliki et al.[4] conducted a systematic review of 14 published studies on the use of PRP in the treatment of AGA, among which the main clinical results were an increase in density and a decrease in hair loss. The accumulated world experience in the use of PRP-therapy in the treatment of AGA is mainly clinical. Only scarce data are available on the analysis of the dynamics of hair growth indicators in accordance with the standards of morphological diagnosis on the background of PRP-therapy.

The aim of the study was morphological substantiation of clinical efficacy of PRP in the treatment of patients with AGA.

Materials and Methods

The study included 25 men aged from 20 to 43 years (mean age, 30.0±2.5 years). AGA degree II-IV on the Hamilton-Norwood scale[4] was diagnosed in 23 (92%) patients and degree I in 2 patients. The average disease duration was 3.4±0.97 years. To obtain PRP, blood samples (18 ml) were collected from each

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patient by venipuncture into 2 tubes with an anticoagulant. As an anticoagulant, 3.8% sodium citrate was used in a ratio of 1:9. Centrifugation was carried out twice. The first centrifugation was carried out at 1800 rpm for 5 min, after which the erythrocyte mass was separated. Plasma containing leukocyte and platelet layers was subjected to a second centrifugation at 2500 rpm for 10 min. As PRP, 2 ml of the lower part of the supernatant from each tube was used. The plasma was separated manually. An official calcium chloride solution was used as an activator in a ratio of 1:20. The surface of the scalp was treated with a chlorhexidine solution; local anesthesia was not applied. The resulting PRP was injected intradermally into the scalp by microinjection, at approximately 0.15 ml per injection. The course of treatment consisted of 4 procedures with an interval of 4 weeks between each procedure. Written informed consent was obtained from each patient.

The clinical efficacy of the therapy was evaluated by the dynamics of morphometric indicators of hair growth. A trichological study was performed using a digital video camera (Aram Huvis Co., Ltd; Korea) and the TrichoSciencePro v1.3 software. The density of hair was determined on 1 cm^2—the share of vellus and telogen hair - in %, the average diameter of all hair - in microns, and the ratio of hair in the telogen/anagen stage, as the amount of anagen hair per 1 telogen hair. The control points were marked with a tattoo mark and were in the parietal zone, approximately 2 cm closer to the center from the border of the thinning hair.

Skin biopsies for histological examination were obtained from 12 patients before and after treatment. Skin biopsies were performed under local anesthesia with a 1% lidocaine solution with epinephrine. The material was sampled by a punch for biopsy with a diameter of 4 mm.[5,6] Skin pieces were fixed in 10% neutral buffered formalin, dehydrated in alcohols of increasing concentration and embedded in paraffin according to the standard procedure. Sections were stained with H&E.

The morphological analysis included an assessment of indicators in horizontal sections and was carried out at 4 levels: at the level of subcutaneous adipose tissue, sweat glands, sebaceous glands, and mouths of hair follicle (HF). Hair density was evaluated at all levels. The diameter of the hair shafts and the number of vellus and miniaturized hair were calculated at the level of the sebaceous glands of HF. Hair was considered to be vellus if the core diameter was equal to or less than the thickness of the inner shell of the follicle and was ≤0.03 mm. Hair in the anagen, catagen and telogen stages was also counted at the level of the sebaceous glands. The telogenous hair was classified as hair without an inner shell or at the late telogen stage, as well as with irregular accumulation of basaloid star-shaped cells in the form of islands with nuclei located on the periphery. Hair characterized by the presence of the inner sheath of the hair root and the absence of cell necrosis was considered to be in the anagen phase. Histological examination was performed using the 3DHistech Pannoramic scanning system and the Pannoramic Viewer 1.15.4 v. software (3DHISTECH Ltd., Hungary).

Statistical processing and visualization of the results was carried out using Microsoft Office Excel (version 15.0.50.31.1000, 2013) and standard R programming language

tools (version 3.4.37). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of *P*<0.05 was considered statistically significant.

Results

Against the background of treatment with PRP, all hair growth indicators underwent significant changes. Hair density increased by 12% (*P*=0.000), average hair diameter by 12% (*P*=0.002), and the share of vellus and telogen hair decreased by 17% (*P*=0.002) and 16% (*P*=0.028), respectively. At the same time, the amount of anagen hair in relation to telogen hair increased by 18% (*P*=0.016) (Table 1, Figure 1).

Table 1.

Dynamics of morphometric indicators of hair growth

Variable	Before treatment	After treatment	Dynamics of change		*P*-value
			Abs.	Δ (%)	
Hair density on 1cm^2	381.5±45.4	426.1±50.1	44.6	12%	0.000
Share of vellus hair, %	49.6±7.3	41.0±7.7	-8.6	-17%	0.002
Average diameter of all hair, μm	39.8±3.5	44.4±4.5	4.6	12%	0.002
Share of telogen hair, %	42.0±6.4	35.3±7.0	-6.8	-16%	0.028
Ratio of hair in the telogen/ anagen stage	1/2.28±1/1.51	1/2.68±1/0.88	1/0.40	18%	0.016

(a)   (b)
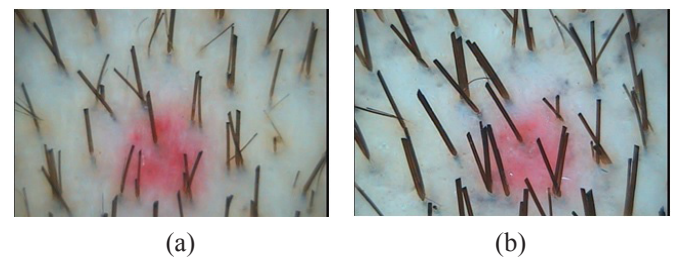
Fig.1. The patient who received PRP injections: (a) - before treatment; (b) - after treatment. Phototrichogram, magnification ×60.

The increase in hair density was accompanied by a significant increase in the number of HF at the mouth level (Δ+96%, *P*=0.004) and at the level of sweat glands (Δ+54%, *P*=0.037), as well as a tendency for their number to increase at the level of the sebaceous glands. These increases were combined with a significant decrease in the proportion of telogen hair (Δ-43%, *P*=0.023) and vellus hair (Δ-29%, *P*=0.037) (Table 2, Figure 2).

Table 2.

The morphological analysis of indicators in horizontal sections

Variable	Before treatment	After treatment	Dynamics of change Abs.	Δ (%)	*P*-value
The level of subcutaneous adipose tissue					
HF number	29.7±23.0	57.0±9.5	27.3	92%	0.087
The level of sweat glands					
HF number	44.0±16.9	68.0±14.9	24.0	54%	0.037
The level of the sebaceous glands					
HF number	67.2±21.9	92.0±32.0	24.8	37%	0.057
Share of vellus hair, %	38.2±8.2	26.8±7.6	-11.4	-29%	0.037
Average diameter of all hair, μm	38.7±4.8	45.0±12.1	6.3	16%	0.202
Share of telogen hair, %	25.4±5.3	14.6±8.4	-10.8	-43%	0.023
The mouth level					
HF number	77.1±10,3	151.0±68.0	73.9	96%	0.004
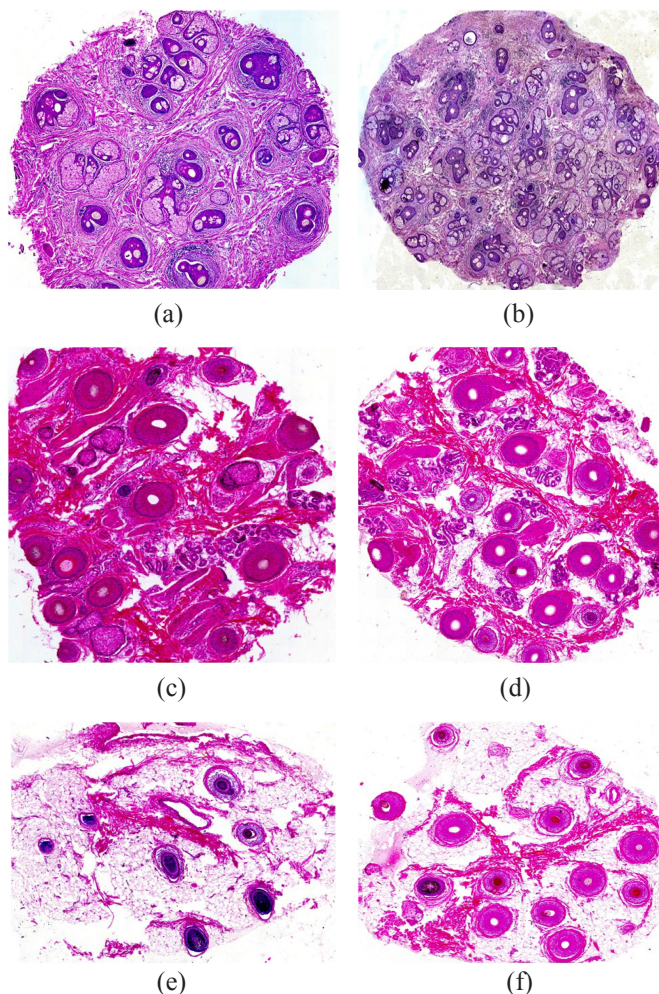
Fig. 2. Histological examination: horizontal sections, H&E staining. The number of HF at the mouth level (×60) before (a) and after treatment (b), at the level of sweat glands (×100) before (c) and after treatment (d), and at the level of the sebaceous glands (×100) before (e) and after treatments (f).

Discussion

Hair loss has a significant effect on the psycho-physiological state and is associated with low self-esteem and depression of patients. The treatment of AGA is limited and includes topical administration of minoxidil, oral finasteride (FDA approved), both as monotherapy and as a combination.

The regenerative potential of PRP has attracted the attention of doctors of many specialties, including trichology. It is known that growth factors activate the proliferative phase and transdifferentiation of hair and stem cells, and the proliferation of dermal papilla cells of HF, and thus play a key role in the growth of the hair shaft. In 2018, PRP-therapy was given attention in the European guidelines for the treatment of AGA, while it was recognized that clinical data are still insufficient for a good evidence base of this treatment method. The availability of a support protocol regarding the preparation of PRP, the frequency of use and the amount of plasma injected remain topical issues.

We prepared PRP by the double spinning method with "soft spin," in which the layers of blood cells were separated manually. We also applied platelet activation through coagulation, which causes the secretion of various growth factors and potentiates mitogenic effects in different cell types. Activated PRP promotes the proliferation of dermal papillary cells and prevents their apoptosis.

The course of our study demonstrated a significant increase in the density and average diameter of hair and a decrease in the share of vellus and telogen hair. This was accompanied by a significant increase in the number of HF at the level of mouths and sweat glands, as well as a tendency to increase them at the level of the sebaceous glands.

The decrease in the proportion of telogen and vellus hair was significant, both in clinical and histological data. The increase in the diameter of the rods, according to the phototrichogram, was significant, but it was not significant histologically. It is possible that some difference in indicators is related to different levels of counting: the phototrichogram shows the condition of the hair on the surface of the scalp, which, when morphologically examined, corresponds to the level of the HF mouth. Morphological analysis was carried out a little deeper, at the level of the sebaceous glands.

The results of our study are consistent with data published in the world literature on the effectiveness of PRP. A comparative analysis of the phototrichological and morphological results of treatment confirmed positive unidirectional dynamics.

Our study has some limitations. During the trichoscopic evaluation, objective results were obtained, but the sample size for the morphological analysis was small. Average patient follow-up time was also too short to show the long-term effectiveness of the treatment. Thus, further studies with longer observation and with a large number of samples are needed.

Conclusion

PRP injections are a simple, effective treatment for hair loss, and can be considered a valuable treatment for AGA.

The positive clinical effect of PRP therapy is due to significant morphofunctional changes in hair follicles. These effects of PRP on HF are possible under the condition that pathological processes are reversible and are not specific for AGA, which makes further research relevant to the possible effect of PRP on the expression of specific proteins that trigger pathological signaling pathways.

Conflict of interest

The authors declare that they have no competing interests.

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