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Original Article

Platelet-Rich Plasma Efficacy in Alopecia Areata Patients with Normal and Elevated Levels of Antibodies against Thyroglobulin and Thyroid Peroxidase

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Abstract

Aim: To evaluate and compare the efficacy of platelet-rich plasma (PRP) therapy in alopecia areata (AA) patients with normal and with elevated levels of anti-thyroglobulin antibodies and/or anti-thyroid peroxidase antibodies.

Materials and methods: The study included 30 AA patients divided into two groups: AA patients with normal (Group 1) and with elevated antithyroid antibodies (Group 2). PRP was applied in three consecutive monthly sessions. Treatment results were evaluated by clinical photography, assessment of scalp hair, trichoscopy, and assessment of patients' quality of life.

Results: The mean age of patients was 42.10 years, 23 (76.6%) patients were with normal and seven (23.3%) were with elevated antithyroid antibodies. Eleven (50%) patients from Group 1 had significant hair growth, of which ten with AA partialis (45.5%), one with AA reticularis (4.6%). Two patients with alopecia reticularis and two with alopecia totalis had insignificant hair growth. Worsening was found in four patients with alopecia reticularis and alopecia totalis. In Group 2 one patient with alopecia partialis (14.3%) had a significant hair growth, five patients with alopecia reticularis and one with alopecia totalis had insignificant hair growth, and none of the patients worsened.

Conclusions: PRP therapy was effective in patients with mild to moderate form of AA and most had normal antithyroid antibodies. Patients with severe AA, AA ophiasis and alopecia totalis, with normal and elevated antibodies, did not benefit from PRP therapy.

Keywords

alopecia areata, anti-thyroglobulin antibodies, anti-thyroid peroxidase antibodies, platelet-rich plasma

INTRODUCTION

Alopecia areata (AA) is an autoimmune disorder characterized by non-scarring hair loss of the scalp or body. It has an incidence rate of 0.1%–0.2% in the general population.[1] AA manifests as a single or multiple well-defined oval bald patches of different sizes. It may present as hair loss of the entire scalp (alopecia totalis, AT), entire body (alopecia universalis, AU), or bodily parts.[2] The course of the disease is unpredictable and spontaneous remissions are reported to occur in 34%–50% of patients within a year.[3,4] The loss of the growing hair shafts is connected

with the CD4+ and CD8+ T-lymphocytes, which violate the immune privilege of anagen hair follicles.[5,6] There are also environmental factors including viral infections, trauma and psychological stress.[7-9]

Data differ regarding the incidence of thyroid diseases in patients with AA.[10,11] AA is associated with the existence of antithyroid antibodies, including antibodies against thyroglobulin (anti-TG-Ab) and antibodies against thyroid peroxidase (anti-TPO-Ab).[12] Routine thyroid screening for asymptomatic patients with AA remains controversial.[13]

Diagnosis and activity of AA are based on clinical manifestation and trichoscopy.[14]

Application of platelet-rich plasma (PRP) is a therapeutic option in AA. Platelet-rich plasma includes concentrated platelets, 4-7 times above baseline values, that secrete growth factors (GF) including: platelet derived (PDGF), vascular endothelial (VEGF), transforming (TGF), fibroblast (FGF), connective tissue (CTGF), epidermal (EGF), and insulin-like growth factor-1 (IGF-1). They stimulate hair follicle proliferation and differentiation, anagen phase, neoangiogenesis and development of adnexal structures.[15-17]

AIM

The aim of this study was to evaluate and compare the efficacy of the PRP therapy in AA patients with normal and with elevated levels of anti-TG-Ab and/or anti-TPO-Ab.

MATERIALS AND METHODS

The study was conducted from September 2020 to September 2022.

Patients

This study enrolled 30 patients with AA. The inclusion criterion was patients of both sexes with AA above 18 years of age. Exclusion criteria were: presence of acute or chronic infection of the scalp, anticoagulants and antiaggregants intake, coagulation disorders (decreased number of platelets, platelet dysfunction, hypofibrinogenemia), haemodynamic instability (collapse), chronic liver diseases, keloid formation, pregnancy or lactation. At first visit, a detailed medical history was taken, and physical examination was done. The following data were collected: patient demographics, age at onset, number of relapses, total duration of disease, duration of last relapse. Dermatological examination determined the pattern of hair loss, size, and localization of bald patches. Trichoscopy was performed, and quality of life was measured.

Patients were divided into two groups: Group 1, AA patients with normal levels of anti-TG Ab and anti-TPO Ab, and Group 2 were AA patients with elevated levels of anti-TG-Ab and/or anti-TPO-Ab. The effect of the therapy was evaluated and compared between both groups.

Serum for the evaluation of anti-TG-Ab of IgG class and anti-TPO-Ab of IgG class was taken before the treatment. The tests were performed with anti-thyroglobulin ELISA (IgG) test kit and anti-TPO ELISA (IgG) test kit, Euroimmun, respectively, according to the manufacturer's instructions. Sera from AA patients were collected and stored at −80°C until assayed. Patients' sera were diluted 1:201 in sample buffer. The upper limit of the normal range (cut off) for anti-TG-Ab was a value <100 UI/ml, and a value equal or above 100 UI/ml was deemed to be elevated; for anti-TPO-Ab the normal range was <50 UI/ml and a value equal or exceeding 50 UI/ml was elevated (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany).

Treatment protocol

Therapy included application of autologous PRP in three consecutive monthly sessions. For the preparation of PRP, at each visit, 8-10 ml of the patient's venous blood was drawn by venipuncture and collected in a Pure PRP (REGEN Ltd). After centrifugation for 10 minutes at 3500 rpm, 5–6 ml of PRP were obtained and aspirated into two 3-ml syringes. The skin of the scalp was cleaned with alcohol pads prior injections. About 0.1 ml of PRP was injected in the scalp in the subfollicular plane through multiple intradermal injections, 1 cm apart with a 30 G needle.

Assessment and evaluation criteria

Treatment evaluation was done by hair loss assessment, global photography, trichoscopy and quality of life at baseline (BL), before initiation of therapy, and at follow-up (FU) one month after completion of therapy. Severity of Alopecia Tool (SALT) Score was used to assess hair loss severity. Results at BL were denoted as SALT BL, those at FU (one month after last procedure) as SALT FU. Final results were denoted as SALT, representing hair regrowth as the percentage of change from BL. The percentage change from baseline is calculated as follows:

Percentage change from baseline =
(SALTbaseline − SALTfollow-up)/SALT baseline × 100%

Patients' improvement after PRP therapy was considered a significant improvement for SALT ≥50%, insignificant for SALT <50, no change for SALT=0, and worsening for SALT<0.[18,19]

Trichoscopy was performed using a Dino-Lite Edge Digital Microscope AM7915MZT(R7), magnification ×70. Quality of life was assessed with the Dermatology Life Quality Index (DLQI) questionnaire.[20] Verbal Numerical Rating Scale (VNRS) was used to measure the level of pain during manipulation at the first PRP application. VNRS ranged from 0 (no pain) to 10 (most severe pain).

Each patient provided written informed consent before participation. The study was performed according to the

Folia Medica

Declaration of Helsinki. The Ethics Committee at the Medical University of Plovdiv approved the study.

Statistical analysis

Data were collected, computerized, and statistically analyzed using SPSS v. 23.0., (SPSS, Inc., Chicago, IL, USA). Continuous variables are given as means and standard deviations (±SD), and category variables in percentages. The Kolmogorov-Smirnov test was applied to establish the distribution of data about SALT change and disease duration. Bivariate Pearson correlation was used to test the relationship between the variables disease duration and SALT change. Statistical significance was considered significant at $p<0.05$.

RESULTS

AA patients ranged in age from 20 to 67, with a mean age of 42.10 (±11.684) years. Of these, 25 (83.3%) were women and five (16.7%) were men. The mean disease duration was 14.1657 (±13.57472) years, ranging from 0.08 to 41, and the mean duration of the last relapse was 2.5067 (±4.98603) years, ranging from 0.08 to 19. First episode of AA was reported by nine patients (30.0%), while 21 (70.0%) had recurrent episodes. Eleven patients (36.7%) had AA reticularis (AAR), 11 (36.7%) had AA partialis (AAP), six patients (20.0%) alopecia totalis (AT), one (3.3%) AA ophiasis (AAO), and one (3.3%) AA barbae (AAB). Sixteen patients (53.3%) reported involvement of other bodily parts. Normal levels of antithyroid antibodies were found in 23 (76.7%) and elevated levels in seven (23.3%) AA patients. In 12 (41.4%) of all patients, SALT was more than 50% and 91.7% were with normal antithyroid antibody levels. In eight (27.6%) patients, hair growth was less than 50%. No improvement was observed in five (17.2%) patients and worsening of AA in four (13.8%). In five patients (16.7%) SALT was 100% **(Table 1)**. The types of AA and SALT change are presented in **Table 2**. The duration of the disease and SALT change showed a negative, insignificant correlation ($r=-0.277$, $p>0.05$). Trichoscopy findings in patients with significant and insignificant improvement were upright regrowing hairs, pigtail hairs and vellus hairs; in patients with worsening: black dots, exclamation mark hairs, yellow dots, broken hairs and tapered hairs **(Fig. 1)**. DLQI measured at BL and FU is presented in **Table 3**. Thirteen patients (43.3%) reported the procedure as moderately painful. The mean value of patients' pain was 4.83±2.451, which counts as an 'average level of pain'. No side effects were observed in this study.

DISCUSSION

Twelve patients showed significant hair growth, 11 with AAP, one with AAR, and most had normal antithyroid antibody levels. Insignificant treatment outcomes were found

Table 1. SALT score and SALT change in AA patients treated with PRP

SALT	AA patients n=29 (%)	Group 1 n=22 (%)	Group 2 n=7(%)
SALT BL			
S0 = no hair loss	0 (0)	0 (0)	0 (0)
S1 <25% hair loss	13 (44.8)	12 (54.6)	1 (14.3)
S2 = 25%–49% hair loss	6 (20.7)	4 (18.2)	2 (28.6)
S3 = 50%–74% hair loss	2 (6.9)	0 (0)	2 (28.6)
S4 = 75%–99% hair loss	5 (17.2)	4 (18.2)	1 (14.3)
S5 = 100% hair loss	3 (10.3)	2 (9.1)	1 (14.3)
SALT FU			
S0 = no hair loss	5 (17.2)	4 (18.2)	1 (14.3)
S1 <25% hair loss	10 (34.5)	9 (40.2)	1 (14.3)
S2 = 25%–49% hair loss	3 (10.3)	1 (4.6)	2 (28.6)
S3 = 50%–74% hair loss	5 (17.2)	3 (13.7)	2 (28.6)
S4 = 75%–99% hair loss	1 (3.5)	1 (4.6)	0
S5 = 100% hair loss	5 (17.2)	4 (18.2)	1 (14.3)
SALT change			
SALT ≥50%	12 (41.4)	11 (50.0)	1 (14.3)
0< SALT <50	8 (27.6)	3 (13.6)	5 (71.4)
SALT = 0 (no change)	5 (17.2)	4 (18.2)	1 (14.3)
SALT <0 (worsening)	4 (13.8)	4 (18.2)	0 (0)

Table 2. Types of AA and change in SALT score

Type of AA	n=29(%)	Group 1 n=22 (%)	Group 2 n=7(%)
AA partialis	11 (37.9)	10 (45.5)	1 (14.3)
SALT ≥50	11 (37.9)	10 (45.5)	1 (14.3)
0 <SALT <50	0 (0)	0 (0)	0 (0)
SALT = 0 (no change)	0 (0)	0 (0)	0 (0)
SALT <0 (worsening)	0 (0)	0 (0)	0 (0)
AA reticularis	11 (37.9)	6 (27.3)	5 (71.4)
SALT ≥50	1 (3.5)	1 (4.6)	0 (0)
0 <SALT <50	8 (27.6)	3 (13.6)	5 (71.4)
SALT = 0 (no change)	0 (0)	0 (0)	0 (0)
SALT <0 (worsening)	2 (6.9)	2 (9.1)	0 (0)
AA totalis	6 (20.7)	5 (22.7)	1 (14.3)
SALT ≥50	0 (0)	0 (0)	0 (0)
0<SALT <50	0 (0)	0 (0)	0 (0)
SALT = 0 (no change)	4 (13.8)	3 (13.6)	1 (14.3)
SALT < 0 (worsening)	2 (6.9)	2 (9.1)	0 (0)
AA ophiasis	1 (3.5)	1 (4.5)	0 (0)
SALT = 0 (no change)	1(3.5)	1 (4.5)	0 (0)

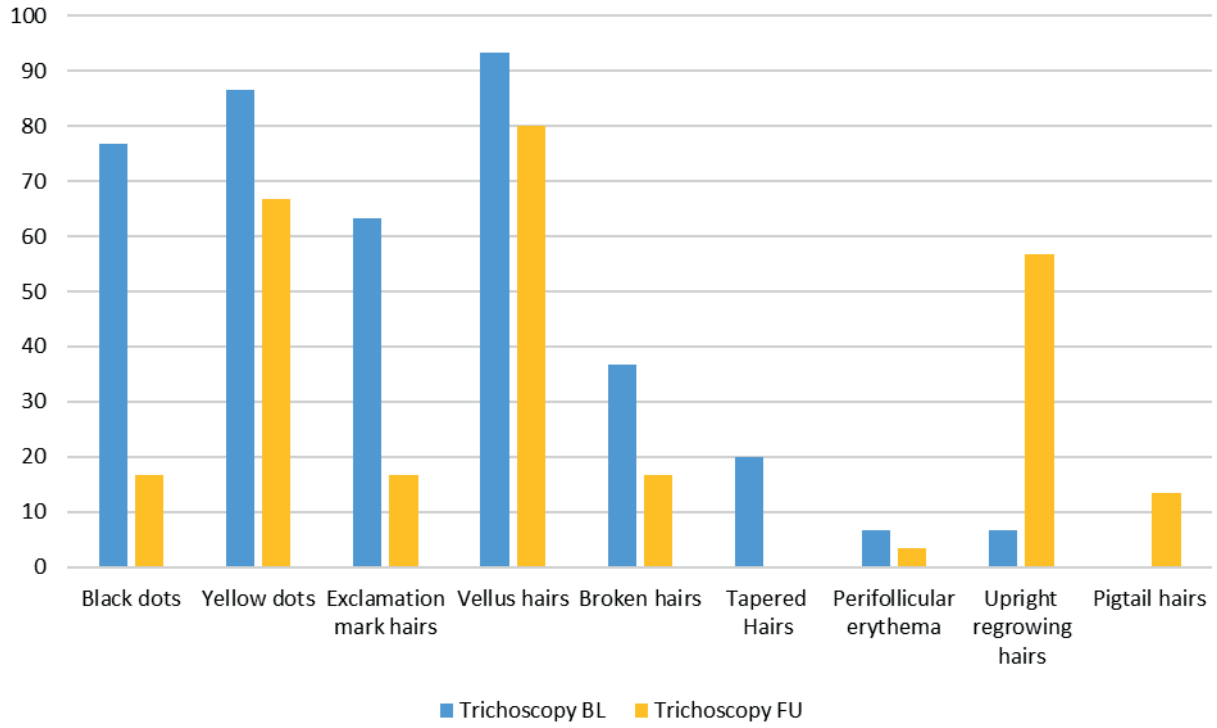

Figure 1. Comparison between trichoscopic markers at baseline (BL) and at follow-up (FU).

Table 3. DLQI of AA patients at base line (BL), and at follow-up (FU)

DLQI Score	DLQI BL n=30 (%)	Group 1 DLQI BL n=23 (%)	Group 2 DLQI BL n=7 (%)	DLQI FU n=30 (%)	Group 1 DLQI FU n=23 (%)	Group 2 DLQI FU n=7 (%)
0–1	4 (13.33)	4 (17.39%)	0 (0%)	13 (43.33)	11 (47.83%)	2 (28.57%)
2–5	10 (33.33)	9 (39,13%)	1 (14.29%)	11 (36.67)	7 (30.43%)	4 (57.14%)
6–10	8 (26.67)	3 (13.04%)	5 (71.43%)	4 (13.33)	3 (13.04%)	1 (14.29%)
11–20	8 (26.67)	7 (30.43%)	1 (14.29%)	2 (6.67)	2 (8.70%)	0 (0%)
21-30	0 (0)	0 (0%)	0 (0%)	0 (0)	0 (0%)	0 (0%)

in eight patients (27.6%) with AAR, three with normal and five with elevated antibodies. Five patients remained unchanged, four of whom had AT and one had AAO, four with normal and one with elevated antibody levels. Worsening was established in four patients, two with AAT and two with AAR, and all had normal antithyroid antibody levels.

The present study showed that PRP application resulted in significant improvement in hair restoration in patients with mild to moderate forms of AA. Trichoscopy results at FU showed a decrease in dystrophic hairs and an increase in growing hairs in comparison to BL. These findings are consistent with the results of other PRP studies. A nonrandomized, placebo-controlled study has established that PRP therapy is effective in mild cases of AA.[21] A placebo-controlled, randomized study of 90 AA patients compared the results of application of topical minoxidil 5%, PRP injections, and placebo. Both PRP and minoxidil showed a significant increase in hair growth. PRP treatment led to significant hair regrowth in AA partialis (70%) and AU (30%), but not in AT. Patients treated with PRP had better and earlier response in terms of fully pigmented hair regrowth, reduction of short vellus hair, yellow dots, and dystrophic hair.[22] A placebo-controlled, randomized trial that included 45 participants assigned to receive PRP, triamcinolone acetonide (TrA) or placebo found that both PRP and TrA led to significant hair growth in AA lesions compared to the placebo and baseline. PRP was found to be more effective than TrA based on dermoscopic findings.[23] A non-randomized trial compared the effect of intralesional TrA and PRP application among 74 AA patients with hair loss involvement less than 25%. The study found complete hair regrowth of all patients in both groups. Patients treated with PRP had an earlier response than patients treated with TrA with statistically insignificant difference.[24] A prospective study to assess the effectiveness of PRP applied in six sessions at 4-week intervals included 20 patients with chronic AA followed up at one year. The majority of the participants showed good hair regrowth, except for

Folia Medica

one patient who experienced minimal hair regrowth and relapse.[25] A case report of a successful PRP treatment of AA barbae aligns with the results of the patient with AA barbae in this study.[26] In a case series of nine patients suffering from chronic AA with more than half of the scalp affected, PRP treatment had no substantial effect at one-year follow-up.[27] In a case report, AA ophiasis was treated successfully with PRP.[28]

To the best of our knowledge, this is the first study to estimate the efficacy of PRP therapy in AA patients with normal or elevated levels of thyroid antibodies. The mechanism of relationship between AA and thyroid autoimmunity is not well established. Genetic and racial factors probably play a role in this relationship. A potential risk of autoimmune thyroid disease particularly in severe and refractory AA was suspected.[12]

The study also established improved quality of life due to hair growth stimulation.

Limitations of the study are the insufficient number of cases, lack of a control group, and a short follow-up period. A long-term follow-up may not be very informative because of the high rate of spontaneous remissions and relapses in AA.

CONCLUSIONS

Autologous PRP is a safe, relatively effective, minimally invasive treatment method. PRP therapy was effective in patients with mild to moderate form of AA and most had normal antithyroid antibodies. Patients with severe AA, AA ophiasis and alopecia totalis, with normal and elevated antibodies, did not benefit from the PRP therapy. Controlled trials with more subjects are needed to validate the reported results.

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Competing interests

The author has declared that no competing interests exist.

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Эффективность обогащённой тромбоцитами плазмы у пациентов с очаговой алопецией с нормальным и повышенным уровнем антител к тиреоглобулину и тироидной пероксидазе

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Резюме

Цель: Оценить и сравнить эффективность терапии обогащённой тромбоцитами плазмой (PRP) у пациентов с очаговой алопецией (ОА) с нормальным и повышенным уровнем антител к тиреоглобулину и/или антител к тироидной пероксидазе.

Материалы и методы: В исследование включены 30 больных ОА, разделённые на две группы: больные ОА с нормой (1-я группа) и с повышенными антитиреоидными антителами (2-я группа). PRP применялась тремя последовательными ежемесячными сеансами. Результаты лечения оценивали с помощью клинической фотографии, оценки состояния волос на голове, трихоскопии и оценки качества жизни пациентов.

Результаты: Средний возраст пациентов составил 42.10 года, у 23 (76.6 %) пациентов были нормальные и у семи (23.3 %) повышенные антитиреоидные антитела. У одиннадцати (50 %) пациентов из 1-й группы наблюдался значительный рост волос, из них у десяти – с частичной ОА (45.5 %), у одного – с сетчатой ОА (4.6 %). У двух пациентов с сетчатой алопецией и у двоих с тотальной алопецией рост волос был незначительным. Ухудшение было обнаружено у четырёх пациентов с сетчатой и тотальной алопецией. Во 2-й группе у одного пациента с частичной алопецией (14.3 %) наблюдался значительный рост волос, у пяти пациентов с сетчатой алопецией и у одного с тотальной алопецией рост волос был незначительным, и ни у одного из пациентов не ухудшилось состояние волос.

Заключение: PRP-терапия была эффективна у пациентов с лёгкой и умеренной формой ОА, большинство из которых имели нормальные антитиреоидные антитела. Пациенты с тяжёлой ОА, ОА офиазом и тотальной алопецией, с нормальными и повышенными антителами, не получали пользы от терапии PRP.

Ключевые слова

очаговая алопеция, антитела к тиреоглобулину, антитела к тироидной пероксидазе, обогащённая тромбоцитами плазма