THE EFFECT OF AUTOLOGOUS PLATELET RICH PLASMA (PRP) ON PATIENTS WITH ALOPECIA AREATA

By

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ABSTRACT

Background: Alopecia areata (AA) is a non-scarring, autoimmune hair loss condition. Despite available therapeutic options, searching for new and more effective treatment is continuous.

Objective: Evaluation of the efficacy of autologous platelet-rich plasma (PRP) for the treatment of localized AA.

Patients and methods: This was a prospective study that conducted at Al-Azhar University Hospital in which 30 patients who attended the outpatient clinic were enrolled. All the patients had age range from 14 to 50 years with localized AA "patchy AA", normal laboratory parameters for CBC, and thyroid functions. None of them had hematological disorders, thyroid dysfunction, low pain threshold, malnutrition, cicatricial alopecia, alopecia total is, alopecia universal is or other dermatological disorders contributing to hair loss.

Results: Eighteen patients (60.0%) were responders and twelve patients (40.0%) were non-responders. From 18 responders, 16 (88.9%) were good responders, and 2 (11.1%) were partial responders.

Conclusion: PRP has emerged as a new treatment modality in AA, and showed effective results and safety in treatment of alopecia areata.

Key words: Platelet Rich Plasma (PRP), Alopecia areata (AA).

INTRODUCTION

Alopecia areata (AA) is a common disease whose clinical manifestations range from mild lesions (plaques) to total hair loss (universal). Most cases can be recognized by well-defined patches of hair loss with exclamation mark hairs at the periphery *(Spano and Donovan, 2015).* Of unknown etiology, AA is autoimmune and genetic-related, affecting people of all ages. The lifetime prevalence of AA is about 2% *(Wu et al., 2013).* The loss of hair is related to alterations in the normal cycle of hair growth. A hereditary component has been identified in patients with AA, and according to current information it is most likely a polygenic disease. Substantial progress in basic and clinical immunology research suggests that AA is a CD8+ cell, Th1-type autoimmune reaction against anagen stage hair follicles *(McElwee et al., 2013 and Watanabe et al., 2015).* Many other cell types, including keratinocytes, fibroblasts, mast cells and dendritic cells also contribute to AA pathogenesis *(Bertolini et al., 2014, Kurashima et al., 2014 and Xing et al., 2014).* There is no evidence that treatment changes the individual

patient's final outcome. However, before counseling patients, one must take into account individual psychological states as hair loss can have a profound effect, and many patients will elect to undergo therapy *(Messenger et al., 2012)*. Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. Although the optimal PRP platelet concentration is unclear, the current methods by which PRP is prepared report 300-700% enrichment, with platelet concentrations consequently increasing to more than 1,000,000 platelets/L *(Li et al., 2012 and Moon et al., 2013).* PRP has attracted attention in several medical fields because of its ability to promote wound healing . Activation of alpha granules of platelets releases numerous proteins including platelet-derived growth factor (PDGF) and interleukin (IL)-1 *(Takikawa et al., 2011).* The mechanism of PRP in the treatment of AA remains unknown, but it is hypothesised that growth factors released from platelets may act on stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neova-scularisation *(Chaudhari et al., 2012).*

PATIENTS AND METHODS

This was a prospective study that conducted at Al-Azhar University Hospitals from March 2015 to November 2015 in which 30 patients who attended the outpatient clinic were enrolled. All the patients had age range from 14 to 50 years with localized AA "patchy AA", normal laboratory parameters for CBC and normal thyroid functions tests (TSH, T3, T4). None of them had hematological disorders, thyroid dysfunction, low pain threshold, malnutrition, cicatricial

alopecia, alopecia totalis, alopecia universalis or other dermatological disorders contributing to hair loss.

Before being admitted to the clinical study, the patients gave consents to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them. The patients were evaluated clinically and digitally photographe in every session.

PRP was prepared by collecting 10-20 cc of fresh blood in sodium citrate containing vaccutainers in minor operation theatre under proper aseptic precaution. The tubes were rotated in a centrifugation machine at 1500 revolutions per minute for 6 minutes. The first centrifugation is called "soft spin", which allowed blood separation into three layers, namely bottom RBC layer (55% of total volume), topmost acellular plasma layer called platelet poor plasma (PPP, 40% of total volume) and an intermediate PRP layer (5% of total volume) called the "buffy coat". Separated buffy coat with PPP was collected with the help of Finn pipette in another test tube. This tube was undergone a second centrifugation, which was longer and faster than the first, called "hard spin", comprising at 2500 revolutions per minute for 15 minutes. This allowed the platelets (PRP) to settle at the bottom of the tube. The upper layer containing PPP was discarded, and the lower layer of PRP was loaded in an insulin syringe containing calcium chloride (1 part calcium chloride and 9 parts of PRP) as an activator. One hour prior to administration of PRP, anesthetic cream was applied over the bald area. Area of the scalp to be treated was cleaned

with alcohol. With the help of insulin syringe, PRP was intradermally injected over affected through multiple small injections under proper aseptic precaution in minor operation theatre. A total volume of 1-2 cc was injected. The treatment was repeated every 2 weeks for 4 sessions. Clinical assessment of the degree of AA was determined according to the percentage of hair loss as regard to base line in affected sites by the Severity of Alopecia Tool score, i.e. SALT score (Figure 1 - *Olsen, 2011).* We evaluated all the patients clinically and digital photography at each visit and at the end of 12 weeks.
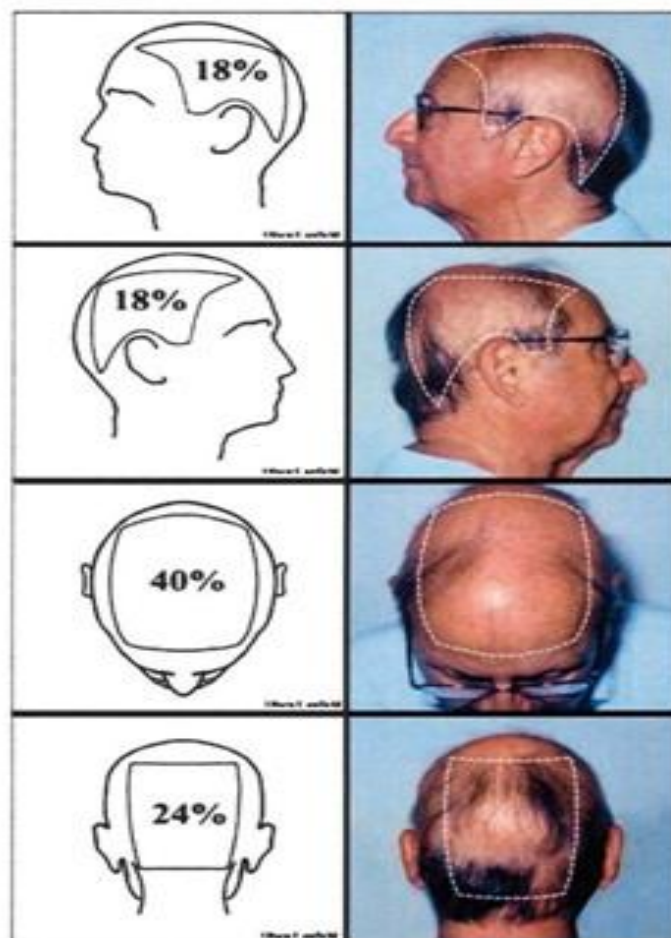

Figure (1): SALT score: The percentage of hair loss in any one of the four views (areas) of the scalp = the percentage hair loss X percent surface area of the scalp in that area. The percentage equals the sum of the scalp hair loss in each area. (a) Top (left side view) = 95% X .18 = 17.1(b) Second (right side view) = 90% X.18 = 16.2c) Third (top of scalp) = 95% X .40 = 38 (realizing that most of hair loss is probably male pattern hair loss) (d) Bottom (back of scalp) = 55% X.24 = 13.2+b+c+d = 17.1 + 38 + 16.2 + 13.2 = 84.5% hair loss *(Olsen, 2011).*

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Statistical analysis of data: The collected data were organized, tabulated and statistically analyzed using statistical package for social sciences (SPSS) version 19 (SPSS Inc, Chicago, USA), running on IBM compatible computer. For qualitative data, frequency and percent distributions were calculated and for comparison between numeric groups, (t) test was used. For comparison between categorical groups, the Chi square (X^2) test was used. For quantitative data, mean, standard deviation (SD), minimum and maximum were calculated, Paired t-test was used to assess the statistical significance of the difference between two means measured twice for the same study group, Student's t-test was used to assess the statistical significance of the difference between two study group means. Mann Whitney test (U test) was used to assess the statistical significance of the difference of a nonparametric variable between two study groups. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count was less than 5 in more than 20% of cells, Correlation analysis, by Pearson's method, was used to assess the strength of association between two quantitative variables. The correlation coefficient, denoted symbolically "r", defined the strength and direction of the linear relationship between two variables. For all tests, p value <0.05 was considered significant.

RESULTS

In the present work, 30 patients with patchy AA with age ranged from 15 to 50 years, the mean age was 30.50±11.05. The majority of patients were males (86.7%), and females represented 13.3%. Seventy percent of studied populations had a work, and 30.0% had no work. In addition, 63.3% of studied patients were married, and 36.7% were single. Smoking was reported in 76.7% of patients (Table 1).

Table (1): Characteristics of AA studied populations.

Characteristics		Statistics
Age (mean ± SD; minimum – maximum)		30.50±11.05; 15.0- 50
Sex (n,%)	Male	26(86.7%)
	Female	4 (13.3%)
Work (n,%)	Positive	21(70.0%)
	Negative	9(30.0%)
Marital state (n,%)	Single	11(36.7%)
	Married	19(63.3%)
Smoking	Positive	23(76.7%)
	Negative	7(23.3%)

90.0% of patients had negative past history of medical drugs, while 6.7% received anti-diabetic drugs, and 3.3% received anti-hypertensive drugs. In addition, 11 patients (36.7%) had previous treatment for alopecia areata, and 63.3% had no previous drugs for alopecia areata (Table 2).

Table (2): Past history of drug's treatment in studied populations.

Parameters		Statistics
Past history of Medical drugs	Negative	27(90.0%)
	Anti-Diabetic	2 (6.7%)
	Anti-hypertensive	1 (3.3%)
Past history of drugs Treatment for alopecia A.	Positive	11(36.7%)
	Negative	19(63.3%)

The family history was positive in 16.7%. The number of patches was one patch in 73.3%, two patches in 23.3% and three patches in 3.3%. The site of lesion was scalp in 73.3%, and other sites occurred in 26.7%. Finally, the nail was normal in 83.3%, while nail pitting was reported in 16.7% (Table 3).

Table (3): Data related to AA disease populations

Parameters		Statistics
Family history	Positive	5(16.7%)
	Negative	25(83.3%)
Number of Patches	One	22(73.3%)
	Two	7(23.4%)
	Three	1(3.3%)
Site	Scalp	22(73.3%)
	Beard	8(26.7%)
Nail condition	Normal	25(83.3%)
	Nail pitting	5(16.7%)

Patients were subdivided according to regrowth of hair to responders (Figure 2), partial (Figure 3), and non-responders.
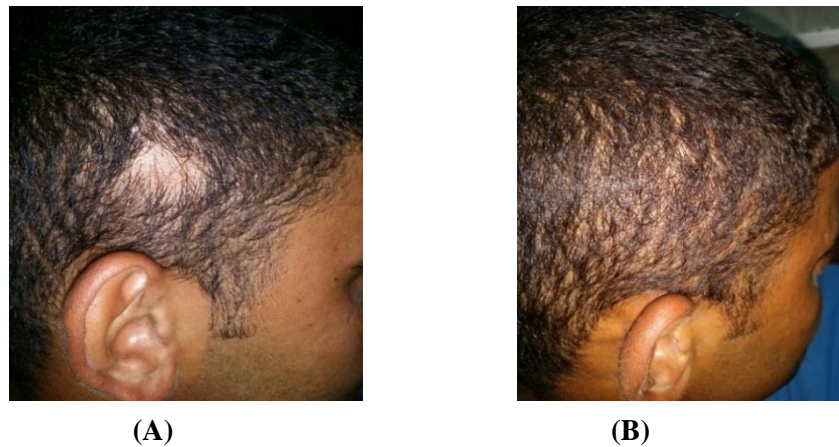
(A) **(B)**

Figure (2): AA patch in the scalp: A. before and B. after PRP injection showing complete hair regrowth at the end of the study.
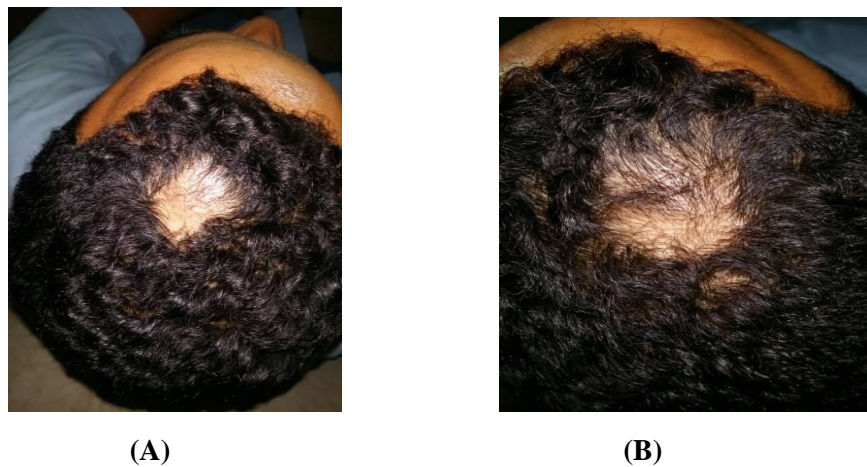

(A) **(B)**

Figure (3): AA patch in the scalp: A. before and B. after PRP injection showing partial hair regrowth at the end of the study.

Administration of autologous PRP has led to an observable hair regrowth in AA lesions. There was hair regrowth evaluated by clinical and digital photography at the end of the study. Responding patients 18 (60%) showed regrowth at the end of the study. As regard to the severity of disease before treatment, the mean SALT score was 3.46 ± 1.8. After treatment, the mean was 1.56 ± 2.14. There was a statistically significant decrease of severity after treatment when compared to corresponding values before treatment (Table 4).

Table (4): Severity percentage of alopecia in studied patients before and after treatment.

	% Before treatment	% After treatment	P
mean±SD	3.46±1.8	1.56±2.14	<0.001

As regard to response, 18 patients (60.0%) were responders and 12 patients (40.0%) were non-responders. From 18 responders, 16 (88.9%) were good responders, and 2 (11.1%) were partial responders (Table 5).

Table (5): Response in studied populations.

Parameters		n	%
Response	Responder	18	60.0
	Non-responder	12	40.0
Type of response	Good	16	88.9
	Partial	2	11.1

There was no significant difference between responders and non-responders as regard to age. The mean age in responders was 30.44±10.27 compared to 30.58 ± 12.60 in non-responders (Table 6).

Table (6): Comparison between ages of responders and non-responders.

Age / Response	Mean (years)	S D	Minimum	Maximum	P
Responder	30.44	10.27	15.00	50.00	0.97
Non-responder	30.58	12.60	15.00	49.00	

There was no association between response and previous treatment for alopecia areata, where only 33.3% of responder received previous treatment compared to 41.7% of non-responders (Table 7).

Table (7): Relation between response and previous treatment for alopecia areata.

Response / Drugs for AA	Responder		Non-responder		P
	N	%	N	%	
Positive	6	33.3%	5	41.7%	0.64
Negative	12	66.7%	7	58.3%	

In the present study, there was a statistically significant moderate positive (proportional) correlation between change in response and age. On the other hand, no significant difference was found between duration of disease (Table 8).

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Table (8): Correlation between improvement change and each patient age and duration of disease.

Change / Parameters	r	P
Age	0.525	0.025
Duration	0.446	0.064

DISCUSSION

Alopecia areata (AA) is an autoimmune, reversible, initially patchy hair loss most commonly involving the scalp, although other regions of the body may be affected *(Garcia and Blume, 2008)*.

Therapies are mostly immunosuppressive. Nevertheless, treatment is still a challenge in AA, and no treatment is either completely curative or preventive. Finding new therapies for this condition, and improving effectiveness of existing ones, are therefore of utmost important *(Alkhalifah, 2011)*.

Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma *(Choi et al., 2012)*. The role of PRP in promoting hair growth has also been investigated. It has been shown to promote hair survival and growth *(Trink et al., 2013)*.

The aim of the present study was to evaluate the efficacy of autologous PRP injection in treating AA. The study included 30 male and female patients with localized AA that ranged from 5 to 6 months duration, with normal blood parameters and normal thyroid functions tests.

At the end of the study, about 53.3% of patients had a complete regrowth of the hair, 6.6% of patients had partial hair regrowth, and 40% of patients showed no regrowth of hair. Our results showed statistically significant improvement with an increased hair densities in patches which treated by PRP for 8 weeks duration. As we rely on the percentage of hair loss as regard to base line to assess the severity, there was also a significant difference in the mean score after treatment. There was a statistically significant moderate positive (proportional) correlation between change in response and age. On the other hand, no significant difference was found between duration of disease.

Clark (2008) has studied AA in patients that were subjected to PRP therapy after traumatizing the scalp using micro needling roller, then PRP was injected in a retrograde fashion (from deep to superficial). His results had shown that patients grew hair one month after injection and hairs continued to grow over the next 10 months. He interpreted that the effect of PRP worked through initiation of signal transducer and activator of transduction (STAT) dependent keratinocyte migration toward the anagen progression and wound healing.

Our findings were in agreement with the study of *Rinaldi et al. (2012)* who examined 45 male and female AA patients with a chronic recurring disease with at

least 2 years duration. They have shown that PRP administration lead to major difference in AA lesions, with 60% of patients achieving complete remission.

There was a significantly higher response with autologous PRP than TrA administration, which is classically considered as treatment of choice for patch-stage AA and concluded that PRP is a safe simple and effective treatment in AA *(Shumez et al., 2015).*

This regenerative effect of PRP might be powered by the action of many GFs in PRP on dermal papillae and may indirectly act on hair follicles, thus improving involution of the vascular plexus around each hair follicle *(Nakamura et al., 2009).*

PRP is a potential useful therapeutic tool for alopecias, without major adverse effects *(Trink et al., 2013).*

CONCLUSION

PRP has emerged as a new treatment modality in A.A., and showed effective results and safety in treatment of alopecia areata.

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تأثير البلازما الغنية بالصفائح الدموية في علاج مرضى الثعلبة

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خلفية البحث: تعتبر الثعلبة من الأمراض الجلدية الشائعة التي تؤدي إلي سقوط الشعر الغير مصحوب بندابات. والثعلبة أحد أمراض المناعة الذاتية. وعلى الرغم من وجود العديد من العلاجات المتاحة إلا أن البحث عن علاج جديد وفعال مازال مستمرا.

الهدف من البحث: هو تقييم فاعلية البلازما الغنية بالصفائح الدموية في علاج الثعلبة .

المرضى وطرق البحث: أجريت الدراسة علي ثلاثين مريضا من المترددين علي العيادة الخارجية وقد تراوحت أعمارهم ما بين ٥٠-١٥ عاما. و قد تم حقن البلازما الغنية بالصفائح الدموية مرة كل أسبوعين لمدة أربعة جلسات لكل المرضي الذين شملتهم الدراسة وتم تصوير المريض قبل وبعد العلاج .

النتائج: نتج عن الدراسة مجموعتين:

١ـ المجموعة المستجيبة للعلاج (١٨ مريضا) : لوحظ وجود زيادة في نمو الشعر و كثافته وتقليص مساحة المنطقة المصابة وتمثل ٦٠% من المرضى الذين شملتهم الدراسة منهم ١٦ مريضا كانت إستجابتهم للعلاج جيدة ومريضين كانت إستجابتهم جزئية.

٢ـ المجموعة الغير مستجيبة للعلاج (١٢ مريضا) : لوحظ عدم وجود زيادة في نمو الشعر أو كثافته وتمثل ٤٠ % من المرضى الذين شملتهم الدراسة.

الاستنتاج: يمكن أن تستخدم البلازما الغنية بالصفائح الدموية كعلاج جديد وفعال للثعلبة ، ويمكن أن تستخدم بأمان دون وجود أثار جانبية تذكر.