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Efficacy of Platelet Rich Plasma after Skin Needling In the Treatment of Vitiligo

Rofaida Mahmoud Abdelaty*

Department of Appl. Math & Information Sciences, Zewail City of Science and Technology, Egypt

***Corresponding author:** Abdelaty M R, Department of Appl. Math & Information Sciences, Zewail City of Science and Technology, Egypt, Tel: 01064703209; Email: Einstein.123456@outlook.sa

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Abstract

Vitiligo is an idiopathic disorder in which the selective destruction of skin melanocytes results in the development of depigmented patches. It affects 0.5-1 % of the general population without any racial, sexual or regional differences in prevalence. The etiology of vitiligo has not yet been fully established; therefore, different factors are said to be potentially involved, including autoimmune, neural, genetic and biochemical hypotheses.

Platelet rich plasma (PRP) is the autologous preparation of platelets in condensed plasma. Various growth factors, including a platelet-derived growth factor, a transforming growth factor, a vascular endothelial growth factor, and an insulin-like growth factor, are excreted from a-granules of condensed platelets triggered by aggregation inducers.

These factors are known to control many processes, including cell migration, attachment, proliferation, differentiation, and the promotion of extra cellular matrix aggregation through binding to various cell surface receptors.

Micro needling is an office technique that produces thousands of microchannels across the epidermis in the papillary dermis. This tiny wounds in the papillary dermis produce a confluent surface bleeding region that is an effective catalyst to initiate the natural process of wound healing with the release of multiple growth factors. These growth factors stimulate the migration and proliferation of fibroblasts that promote collagen deposition.

This research was conducted to assess the role of autologous PRP with skin needling in the treatment of vitiligo in a group of (26) Vitiligo patients who were exposed to the use of freshly prepared autologous Platelet Rich Plasma (PRP) double spinning technique every 2 weeks for 8 sessions. Results were evaluated quantitatively using the vitiligo area severity index (VASI) score and qualitatively by the percentage of repigmentation as excellent (> 75%), good (50-75%), moderate (25-50%), and mild (<25%).

There was a statistically meaningful drop in the VASI score in post-treatment patients relative to pre-treatment with a mean reduction of 8.21 %. No lasting side effects have been found in all patients. Transient ecchymosis has been identified in only one perioral area patient with injection.

Introduction

Vitiligo is an inherited pigmentation condition characterized by depigmented spots due to the absence of functional melanocytes from the epidermis. This disease, which is cosmetically disfiguring especially in dark-skinned people, makes the infected skin more susceptible to sunburn and affects 0.1-2% of the world's population, irrespective of gender and race [1].

Vitiligo etiology is still unclear, but genetic factors, autoimmunity, environmental factors, or lack of melanocyte growth factors can lead to disease precipitation in susceptible individuals [2].

Establishing the correct diagnosis for hypomelanotic skin disorders requires a good history, a detailed physical examination, the use of special lighting techniques, such as Wood's light, and sometimes a biopsy of the abnormally pigmented skin and the normally pigmented skin [3].

Vitiligo can be treated successfully with medical, physical and surgical techniques. These techniques include topical corticosteroids, PUVA, depigmentation, broadband and narrowband UVB, recent topical treatments like (tacrolimus & pimicrolimus ointment, vit.D derivatives, PGE2 and antioxidants), Surgical therapies (the transfer of melanocytes, melanocytes and keratinocytes or full-thickness skin from normally pigmented areas to hypomelanotic patches) and treatment with tissue-engineered skin [4].

Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. Various growth factors, including platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, and insulin-like growth factor, are secreted from α-granules of concentrated platelets activated by aggregation inducers [5]. These factors are known to regulate many processes including cell migration, attachment, proliferation, differentiation, and promoting extra cellular matrix accumulation by binding to specific cell-surface receptors [6].

Platelet Rich Plasma has attracted attention in the field of dermatology specifically in the esthetic field for skin rejuvenation, acne scars, and hair loss, it is considered an

excellent safety profile with relatively low cost. Based on the collection of indirect evidence, it could be postulated that PRP therapy in vitiligo lead to repigmentation through these possible mechanisms, stimulation of proliferation, and interaction of both keratinocyte and fibroblast with melanocytes, attraction and stimulation of undifferentiated stem cell and anti-inflammatory effect of PRP which limits the release of cytokines and subsequently limit apoptosis of melanocytes [7].

Skin needling is a technique predominantly used to improve the appearance of cutaneous scarring and photodamage. Fine needles puncture the skin, resulting in increased dermal elastin and collagen, collagen remodeling, and thickening of the epidermis and dermis. Additionally, skin needling creates small channels, which increase the absorption of topically applied preparations a property which has been used in various dermatological treatments [8].

Subjects and Methods

This study was carried out at the outpatient clinics of Dermatology and Venereology Department, Faculty of Medicine, Zagazig University Hospitals in the period from January 2019 to September 2020. Twenty-six patients (9males and 17females) aged 8 - 65 years with stable vitiligo were randomly chosen and enrolled in this clinical trial. The study had the approval of The Institutional Review Board (IRB) at Zagazig University under the number 2724-22-3-2016. All participants signed a written informed consent.

Full history was taken from each case including:

- Personal history: including name, age, sex, occupation, and residence.
- History of present dermatological disease: onset, course, duration, site, and history of previous treatment for the disease.
- History of associated other dermatological diseases.
- History of systemic diseases.
- History of drug intake.
- Family history.

All patients were subjected to:

- General examination of body systems was performed to discover associated medical conditions.
- Dermatological examination to assess the skin type, vitiligo lesions site, size and number.

Inclusion criteria for treatment included:

- Patients diagnosed clinically with stable vitiligo [absence of new areas of depigmentation or enlargement of the preexisting lesions for 12 months and absence of Koebner phenomena during the same period].
- Willing to sign informed consent.

Exclusion criteria for treatment included:

- Pregnant & lactating female.
- Patients who were known to have a good or rapid response to conventional modalities.

Before treatment, an informed consent was taken from all the patients. We asked the patient about his expectations about the treatment to avoid unrealistic expectations. We emphasized to the patient the unpredictability of vitiligo treatment and there is no quick, easy and permanent fix to the problem. Possible side effects of the procedure as erythema, edema and pain were explained.

Procedure

Local anesthetic cream (eutectic mixture of lidocaine and prilocaine, EMLA cream, APP pharmaceuticals, Fresenius Kabi, USA) was applied to the lesion for approximately 30 minutes before the procedure.

After sterilization with alcohol, every patient undergone micro needling of the lesions to be treated by micro needling pen as a first pass, PRP is introduced into the lesion and spread by gentle massage, lastly a second pass by micro needling pen was done to ensure maximum benefit of PRP. This procedure was repeated every 2 weeks for maximum 4 months (8 sessions).

Platelet Rich Plasma preparation

It was obtained by double spin method. The patient was first sent to a clinical pathological laboratory where 10 ml of autologous whole blood was collected under complete aseptic condition into tubes containing sodium citrate (10:1) as an anticoagulant. The collected blood was firstly centrifuged at 3000 rpm for 7 min to separate the plasma and platelets from the red and white cells (soft spin). Then the upper plasma was pipetted to undergo another centrifugation at 4000 rpm for 5 min (hard spin) leading to separation of the plasma into 2 portions: - platelet rich plasma in the bottom of the tube (with a platelet count ≥4 times more concentrated than the whole blood), and a platelet-poor plasma (PPP) in the upper part which was removed. PRP was activated by adding calcium chloride at ratio of 10:1 (0.1 cc of $CaCl_2$ to each 1 cm of PRP) immediately before the session [7].

Post procedure care

Topical antibiotic was prescribed for three days after treatment but application of sunscreen was not required.

Clinical assessment

Digital color photographs were taken using a Canon digital camera (IXUS 160, China) for all patients before (at baseline) and after the end of treatment.

The repigmentation response was expressed qualitatively as: excellent: >75 to 100%, good: >50% to 75 %, moderate: >25% to 50% and mild: <25%. Quantitative evaluation of the response was also performed in a numerical percentage for precise statistical evaluation using VASI scoring system (Figure 1).

Evaluation of treated patients had been done at each visit for any side effects. Follow up of responders had been done monthly for 3 months after the end of therapy for detection of any recurrence.

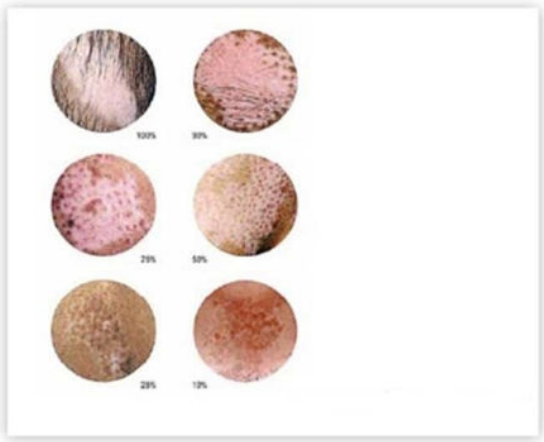

Figure1: VASI score for Vitiligo

At 100% depigmentation, no pigment is present; at 90%, specks of pigment are present; at 75%, the depigmented area exceeds the pigmented area; at 50%, the depigmented and pigmented areas are equal; at 25%, the pigmented area exceeds the depigmented area; at 10%, only specks of depigmentation are present.

VASI= ∑ All Body Sites [Hand Units] × [Residual Depigmentation]

Results

The study included 26 stable vitiligo cases. The age of the studied cases ranged from 8 to 65 years with mean 32 years. The percentages of males (9 cases) and females (17 cases) were 34.6% and 65.4%, respectively Regarding skin type among cases 15 were skin type III with a percentage of 57.7, and 11 cases were skin type IV with a percentage of 42,3 as shown in (Table 1). On studying the duration of the disease among the studied group, it ranged from 1 to 20 years with mean 5.42 years. Regarding lesion distribution most frequent were acral & extremities (53.8% & 34.6% respectively), Regarding no. of sessions among the studied group, it ranged from 3 to 8 with mean 6.92 and 73.1% of them completed 8 sessions. Regarding complications of the treatment 61.5% of the studied cases had complications, 1 case had erythema, 2 cases had burning sensation and 13 cases had pain during sessions (Table 2). There was statistically significant decrease in VASI score among the studied cases post treatment compared to pre with mean % of reduction 8.21% (Table 3). Regarding response to treatment, 61.5% of the studied cases showed no response to treatment and 38.5% showed mild response (Table 4).There was no statistically significant difference between cases had no response and cases had mild response in sex distribution. But there was statistical significance decrease in mean age among cases had mild response compared to cases had no response (Table5). There was no statistically significant difference between cases had no response and cases had mild response regarding skin type There was no statistically significant difference between cases had no response and cases had mild response in disease duration or distribution as shown in (Table 6). There was no statistically significant difference between cases had no response and cases had mild response in no. of sessions or frequency of complication as shown in (Table 7). There was no statistically significant difference between cases had no response and cases had mild response in VASI score before treatment as shown in (Table 8).

Table1: Age & sex and Skin type of the studied cases

Variable	**(n=26)**	
Age: (year)		
Mean ± SD	32 ± 16.90	
Median	30	
Range	Aug-65	
Variable	N	%
Sex:		
Male	9	34.6
Female	17	65.4
	(n=26)	
Variable	No	%
Skin type:		
III	15	57.7
IV	11	42.3

Table2: Duration of disease, distribution of lesion, number of sessions and Complication of treatment and among the studied cases

Variable	**(n=26)**	
Duration: (year)		
Mean ± SD	5.42 ± 4.62	
Median	4.5	
Range	20-Jan	
	No	%
Distribution:		
Acral	14	53.8
Face	1	3.8
Extremities	9	34.6
Trunk	2	7.7
	(n=26)	
Variable	No	%
No. of sessions:		
3	2	7.7
4	4	15.4
6	1	3.8
8	19	73.1
Mean ± SD	6.92 ± 1.87	

Complication:		
No	10	38.5
Yes	16	61.5
Pain during session	13	50
Erythema	1	3.8
Burning sensation	2	7.7

Table3: Comparison of VASI score pre and post treatment among the studied group

Variable	Pre	Post	Paired	p	% of change
	(n=26)	(n=26)	W		(n=26)
Mayo score: (%)					
Mean ± SD	1.39 ± 1.14	1.31 ± 1.13	2.83	0.005	-8.21 ± 12.11
Median	1	0.8		**	0
Range	0.5 – 4.5	0.3 – 4.5			-40

SD: Standard deviation Paired W: Paired Wilcoxon test **: highly significant (P<0.01)

Table4: Response to treatment among the studied cases

	(n=26)	
Variable	**No**	**%**
Response to treatment:		
No response	16	61.5
Mild	10	38.5
Moderate	0	0
Good	0	0
Excellent	0	0

Table5: Relation between response and Age & sex of the studied cases

Variable	No response		Mild response		MW	P
	(n=16)		(n=10)			
Age: (year)						
Mean ± SD	36.25 ± 16.08		25.2 ± 14.31		2.48	0.04*
Median	36		18			
Range	Aug-65		13 - 55			
Variable	N	%	N	%	χ2	P
Sex:						
Male	6	37.5	3	30	0.15	0.7
Female	10	62.5	7	70		NS

Table6: Relation between response, Skin type, and Duration of disease and distribution of lesion among the studied cases

Variable	No response (n=16)		Mild response (n=10)		χ2	P
	No	%	No	%		
Skin type: III IV	10 6	62.5 37.5	5 5	50 50	0.39	0.59 NS
Variable	No response (*n*=16)			Mild response (n=10)	MW	t
Duration: (year) Mean ± SD Median Range	6.56 ± 5.56 5 1 – 20			3.6 ± 1.35 3 2 - 6	1.5	0.13 NS
	No	%	No	%	χ2	P
Distribution: Acral Face Extremities Trunk	9 0 6 1	56.2 0 37.5 6.2	5 1 3 1	50 10 30 10	1.86	0.60 NS

SD: Standard deviation MW: Mann Whitney test χ2: Chi square test NS: Non signi icant (P>0.05)

χ2: Chi square test NS: Non signi icant (P>0.05)

Table7: Relation between response a nd N o. o f s essions and Complication of treatment among the studied cases

Variable	No response		Mild response		MW	T
	(n=16)		(n=10)			
No of sessions: (year)						
Mean ± SD	6.63 ± 4.62		7.4 ± 1.35		0.29	0.77
Median	8		8			NS
Range	8-Mar		8-Apr			
	No	%	No	%	χ2	P

Complication:						
No	9	56.3	1	10		
Yes, Pain during session	5	31.3	8	80	7.09	0.07
Erythema	1	6.2	0	0		NS
Burning sensation	1	6.2	1	10		

Table8: Relation between response and VASI score pretreatment among the studied group

Variable	No response	Mild response	MW	p
	(n=16)	(n=10)		
Mayo score: (%)				
Mean ± SD	1.49 ± 1.27	1.24 ± 0.96	0.85	0.4
Median	1	1		
Range	0.5 – 4.5	0.3 – 3.6		

SD: Standard deviation; MW: Mann Whitney test; NS: Non significant (P>0.05)

Figure2: Illustrative cases

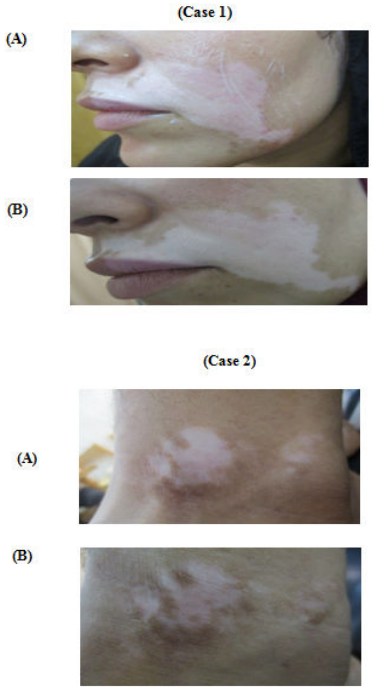

Figure3: Facial lesion (A) before treatment.

(B) after 8 sessions, pigmentation starts at periphery

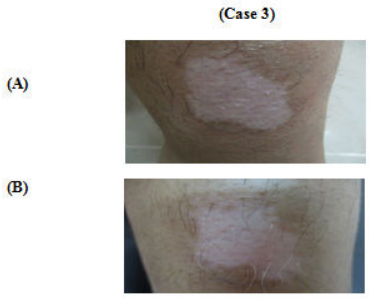

Figure4: Lesion at medial aspect of right leg,

(A)before treatment.

(B) after 8 sessions, notice hyperpigmented margin (Trichrome vitiligo).

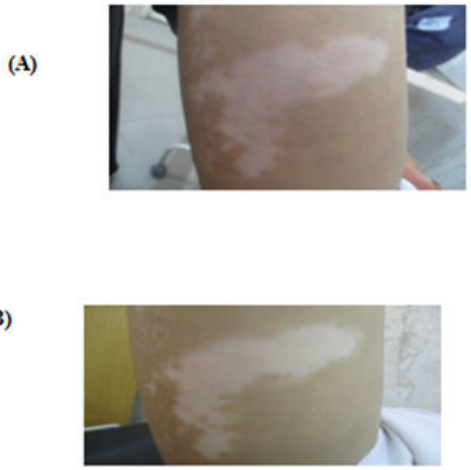

Figure5: Lesion at left leg (A) before treatment, notice convex border

(B) after 4 sessions, notice concave border

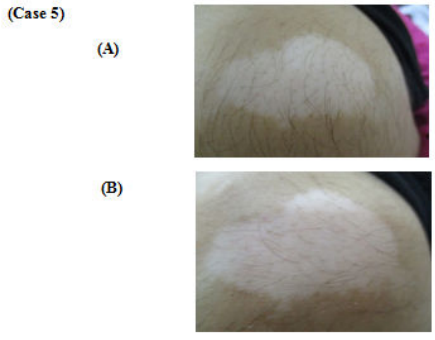

Figure6: Lesion at right arm,

(A) before treatment.

(B) after 8 sessions with visible repigmentation at periphery

Discussion

Vitiligo is a generalized autoimmune disorder that manifests as acquired white patches due to melanocyte failure. It is the most common pigment disease with a prevalence rate of 0.1–2 % demonstrating multifactorial etiology and polygenic inheritance [9].

The causes of vitiligo are still unclear but tend to rely on the combination of immunological, genetic and neurological influences. While all these theories are founded on certain facts, none of them can completely describe the disease on its own. Cellular and humoral immunities have been involved in the production of vitiligo [10] It has been shown that 84% of vitiligo patients have anti-melanocyte antibodies in circulation. In the other hand, several experiments have indicated the T-cell mediated melanocyte death in vitiligo [11]. Traditional repigmentation surgery, including topical and phototherapy, is the mainstay of modern care. Topical drugs include corticosteroids, calcineurin inhibitors and vitamin D analogs. Phototherapy treatments include narrowband UVB (NB-UVB) or psoralen and UVA (PUVA).

However, the battle for new and quicker response therapy in Vitiligo, either alone or in tandem with other modalities, is still ongoing. Platelet rich plasma is a modern advance in soft-tissue recovery. It comprises platelets at a concentration of 7 times the average blood level (about 1 million) from which many growth factors are made accessible to the tissue where they are used.

PRP is an important accumulation of various fundamental growth factors (GFs) on the basis of platelets alone (stored as-granules in platelets) and plasma proteins, including fibrin, fibronectin and vitronectin. These GFs include a platelet-derived growth factor, a transformative growth factor, a vascular endothelial growth factor, and an insulin-like growth factor that is essential to tissue repair and regeneration regulation.

The beneficial effect of platelet rich plasma in vitiligo may be inferred by these growth factors, which promote the spread of keratinocytes and fibroblasts, with the resulting enhancement of their association with melanocytes contributing to the stabilization of melanocytes.

Platelet rich plasma therapy has been shown to induce accelerated proliferation and migration of fibroblasts through up-regulation of cyclin E and cyclin-dependent kinase 4, which is essential for cell migration and proliferation [7].

This research was performed to determine the outcome of intralesional autologous platelet rich plasma after skin needling in chronic vitiligo patches and to study the protection profile of PRP therapy in vitiligo.

The present research involved 26 healthy cases of vitiligo of both sexes and ages ranging from 8 to 65 years of age. The study found no statistically significant difference between cases with no response and cases with mild response in sex distribution, but there was a statistically significant decrease in mean age of cases with mild response relative to cases with no response , So younger patients appear to respond better to PRP therapy.

Out of 26 patients, only 19 completed the 8 sessions of treatment with a percentage of 73.1% . And it's because of insufficient enforcement and a firm conviction that Vitiligo is a debilitating condition that has no definitive solution.

The first research on the role of PRP in Vitiligo was carried out Twenty Vitiligo patients were treated with intradermal PRP injections monthly for 10 weeks which concluded that PRP was not successful in the treatment of Vitiligo. However, in our study, 10 out of 26 cases (38.5%) showed a moderate reaction in the form of clear repigmentation.

Another research carried out here in Egypt (7) 'the effect of platelet-rich plasma on short-term narrowband–ultraviolet B phototherapy in the treatment of vitiligo' resulted in 55% of patients in the PRP community achieving excellent repigmentation and 20 % achieving strong repigmentation over a 4-month period compared to the NB-UVB side. In our sample, 38.5 % (10 cases) demonstrated visible repigmentation (mild reaction) and there was a statistically significant decrease in VASI score among the post-treatment cases tested relative to pretreatment with a mean 8.21 % reduction but there was no moderate, strong or outstanding response to treatment.

A recent research conducted in India by found that out of 40 patients, 15 patients had a positive response, 12 patients had an average response and 13 patients showed no response to treatment with 67.5 % of patients having clear repigmentation. The increase in these cases was shown as a reduction in the size of the lesions with pigmentation beginning from the periphery and eventually spreading to the middle of the lesion, rather than the perifollicular pigmentation usually seen with other topical treatments compatible with our research.

The research performed by [7] was analyzed with respect to the body site and it was observed that vitiligo of the ears, trunk and extremities showed the best results. The study conducted by found that the facial lesions displayed a stronger response than the truncated lesions and extremities showed a weaker response. However, in our research, there was no statistically meaningful difference between the non-response cases and the moderate response in disease distribution.

The observed side effects in the [7] sample were mild and were well tolerated by all patients. Pain resulted in 50 % of patients that was normal and tolerable. Ecchymosis at the injection site occurred at 15%. Side effects in the sample were also small, with a total of 4 patients having side effects, accounting for 10% of cases of full recovery within 24 hours.

In our study, the adverse effects were also small, including pain at the injection site (13 cases), erythema (1 case) and burning sensation (2 cases) . The only patient with perioral injection registered intense burning discomfort and upper lip ecchymosis within one hour of injection, possibly due to unintended deep dermal needling, however, she recovered within one day with cold compresses. A total of 16 patients had

side effects which account for 61.5% of cases, which however recovered in 24 hours.

Conclusion

Vitiligo has a profound effect on quality of life of a patient; hence newer efficacious treatments are always sought by patients and dermatologists. PRP offers a simple, minimally invasive, inexpensive treatment for vitiligo. It may be combined with topical therapies, surgical modalities and phototherapy. PRP may be considered as an additional therapy in patients not responding adequately to traditional therapies. It was also felt that patients might require more than 8 sittings for complete repigmentation.

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