THE STUDY OF EFFECTIVENESS OF PLATELET-RICH PLASMA (PRP) TREATMENT FOR LICHEN PLANUS PIGMENTOSUS (PILOT STUDY)

BY

PITCHARAT PITUVONG

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Abstract

This is the first pilot study utilizing intradermal platelet-rich plasma (PRP) in the treatment of lichen planus pigmentosus (LPP). Five participants with a diagnosis was LPP by clinical and histologic features enrolled in these studies. The participants received three sessions of intradermal PRP 2 weeks apart, with a follow up after 2 weeks, 4 weeks, 8 weeks. The melanin index (MI), clinical improvement of pigmentation, patient satisfaction score, and monitored adverse effects were measured.

The mean MI score of the subjects was 450.33±69.91 and declined to 392.60±73.88 at the end of study (week 12). All patients demonstrated a significant reduction of the MI score since the first PRP injection (week 2). Clinical improvement as evidenced by escalation of mean QGS grading by 2 dermatologists was initially observed at week 6. The mean patient self-assessment score at week 12 was reported to be thr level of satisfaction, “very satisfied”. Adverse effects were minimal including swelling at injection sites and bruising, which spontaneously resolved in a few days. In summary, intradermal injection of PRP could be an effective alternative or complementary treatment option for treatment-resistant lichen planus pigmentosus. The limitation of our study was the small sample size and short follow-up period.

(Total 32 pages)

Keywords: Lichen planus pigmentosus, Platelet-rich-plasma, Macrophages, Melanin, Pigment

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Abbreviations and Symbols

Symbol	Meaning
ADMH	Acquired dermal macular hyperpigmentation
RM	Riehl melanosis
PCD	pigmented contact dermatitis
EPS	Erythema dyschromicum perstans
DIF	Direct immunofluorescence
PRP	Platelet-rich plasma
MI	Melanin index
QGS	Quartile grading scale
PDGF	Platelet-derived growth factor
TGF	Transforming growth factor
FGF	Fibroblast growth factor
EGF	Epidermal growth factor
VEGF	Vascular endothelial growth factor
MITF	Microphthalmia-associated transcription factor
PAX	Paired-box homeotic gene
TRP	Tyrosine releasing protein

Chapter 1

Introduction

1.1 Background and significance of the problem

Acquired dermal macular hyperpigmentation (ADMH) is a collective term for hyperpigmentation groups such as lichen planus pigmentosus (LPP), Riehl melanosis (RM), pigmented contact dermatitis (PCD), and erythema dyschromicum perstans (EPS) (Kumarasinghe et al., 2019). There is considerable overlap between the clinical and histopathologic features of these diseases (Kumarasinghe et al., 2019).

LPP belongs to the group of ADMH. LPP presents as poorly defined, slate gray to brownish-black macules in sun-exposed areas, predominantly on the face and neck, in the flexural fold (Bhutani, Bedi, Pandhi, & Nayak, 1974).

In 1974, LPP was first described by Bhutani et al. (1974) in 40 Indian patients with acquired macular pigmentation, where the term LPP was coined. LPP is widespread and occurs in young to middle-aged female adults with skin phototype 3 and 4 especially in patients of Indian, Latin American, or Middle Eastern origin. Clinically, it presents as irregularly shaped or oval brown to gray-brown blemishes and patches in sun-exposed areas (especially forehead, temples, and neck) or intertriginous areas with asymptomatic to mild pruritus or a burning sensation. The etiology of LPP is still unknown. Photodistribution in some patients suggests that UV light may play a pathogenic role, and topical application of mustard oil, which contains allyl isothiocyanate and amla oil have been suggested as a possible trigger (Rieder, Kaplan, Kamino, Sanchez, & Pomeranz, 2013). For certain diagnosis, we must refer to the clinical and histopathological findings: vacuolar degeneration of the basal cell layer, perivascular mononuclear cell infiltrate in the upper dermis, increased epidermal melanin, dermal melanophages, lichenoid reaction, and colloid bodies (Bhat, Mathanda,

Jayaprakash, & Dandakeri, 2017). Histological staining displays the deposition of IgM or C3 linear patterns at the basement membrane zone in direct immunofluorescence (DIF). The patch test showes a negative result in most cases (Sehgal, Srivastava, Sharma, Sehgal, & Verma, 2011).

Previous studies have used topical treatments such as bleaching agent (azelaic acid cream, 2-4% hydroquinone), calcineurin inhibitor (0.1% tacrolimus), corticosteroid (fluticasone/mometasone, 1% mometasone, clobetasol), oral treatment of vitamin A analog (isotretinoin, acitretin), oral corticosteroid (dexamethasone, prednisolone), colchicine, tranexamic acid, chemical peeling, narrow band UVB, and laser light-based therapy (Q-switched Nd-YAG laser). The previous treatment studies have found that none of them is universally effective (Syder, Sicco, & Gutierrez, 2022).

The lesions significantly impact the appearance of patients. The treatment of lichen planus pigmentosus is limited due to its efficacy. According to the American College of Physicians grading system, the highest evidence amongst all available studies was grade 2. Al-Mutairi and El-Khalawany (2010) conducted an open-label, nonrandomized, prospective study of 13 patients treated with topical 2% tacrolimus ointment. The result was that seven patients showed improvement in pigmentation. Muthu et al. (2016) showed a low dose of isotretinoin (20mg /day) for 6 months in treating 27 patients, with 15 patients experiencing moderate improvement. Few studies used light-based treatment. In the study by Bhari, Sharma, Singh, Parihar, and Arava (2020) nine patients were treated with a Q-switched Nd YAG laser. After six laser sessions, an average clinical improvement of 25.7% in lesions was noted by physician assessment. Several of the studies had a small sample size and a low level of evidence. Furthermore, none of these were universally effective, making LPP a complex cosmetic problem to treat.

Platelet-rich plasma (PRP) is autologous blood plasma with a concentration of platelets above baseline and reportedly releases high growth factors that may be valuable for numerous applications. PRP is recently used as an adjunctive therapy for

many medical conditions because of the numerous growth factors and very few adverse effects (Ehrenfest et al., 2014).

PRP is recently used as adjunctive therapy for several conditions (Wang & Avila, 2007), such as orthopedic indications, and various purposes in dermatologic treatments, such as wound healing, facial skin rejuvenation, scar revision, and hair restoration (Alam et al., 2018). The numerous growth factors that are actively secreted by platelets initiate many processes in the cell, such as platelet-derived growth factor (PDGF), which stimulates tissue and collagen healing and transforming growth factor-(TGF-), which decreases melanogenesis and signal protein in melanogenesis (Xian, Chowdhury, Saim, & Idrus, 2015). In addition, fibroblast growth factor (FGF) promotes fibroblasts in angiogenesis. The epidermal growth factor (EGF) promotes mesenchymal and epithelial cell development. Keratinocyte growth factor promotes epithelial cell stimulation, while vascular endothelial growth factor (VEGF) stimulates collagen production (Kim et al., 2011).

PRP has been used to treat pigmentary lesions such as periorbital hyperpigmentation (Al-Shami, 2014) and dermal melasma (Amini, Ramasamy, & Yew, 2015; Çayırlı, Calışkan, Açıkgöz, Erbil, Ertürk, 2014). Dermal melasma shared some histological features as in LPP lesions: melanin incontinence, dermal melanophage. We hypothesized that PRP injection could have therapeutic effects to LPP as in melasma lesion.

1.2 Research objectives

1.2.1 To investigate the effectiveness of platelet-rich plasma (PRP) in treating lichen planus pigmentosus

1.2.2 To study the safety of intradermal platelet-rich-plasma (PRP)

Chapter 2

Literature Review

According to the classification proposed by Ehrenfest et al. (2014), four main families of preparations can be defined, depending on their cell content and fibrin architecture.

Type	Preparation method	Final component
Leucocyte poor platelet rich plasma (LP-PPP)	Anticoagulated whle blood is centrifuged, PPP and portion of the buffy coat are collected. Then, high force centrifugation is preferred	Fibrin-rich plasma with concentrated platelets
Leucocyte rich platelets rich plasma (LR-PRP)	Aniticoagulated whole blood is centrifuged. Then , PPP and buffy coat are collected	Fibrin rich plasma with concentrated platelets, leucocytes ,and red blood cell
Leucocyte poor platelets rich fibrin (LP-PRF)	Anticoagulated whole blood is centrifuged. PPP and buffy coat are ollected with separate gel	Fibrin polymerized clot that is rich in platelets and leucocytes
Leucocyte rich platelet rich fibrin (LR-PRF)	Venous blood is collect in glass tube and centrifuged without anticoagulant	Fibrin polymerized clot that is rich in platelets and leucocyte

Figure 2.1 Types of PRP

Source: Ehrenfest et al., 2014

There are wide variations in the reported protocols for standardization and preparation of PRP. There are many commercially available PRP kits in the market. Our study used pure PRP for treatment, which consisted of low levels of white blood cell, which is proinflammatory cell, to avoid the inflammation process which could aggravate lesions in patients.

From the study of Hofny, Abdel-Motaleb, Ghazally, Ahmed, and Hussein (2019), platelet-rich plasma increases transforming growth factor-beta (TGF-β) in patients with skin melasma and nearby skin, which decreases melanogenesis and

signaling protein in melanogenesis. This treatment effectively reduces melanin in the lesions to a significant extent, as evidenced by a study by Sirithanabodeekul, Dannarongchai, and Suwanchinda (2020), who studied melasma patients of mixed type by administering PRP intradermally on one side of the face compared to normal saline on the other side. After four sessions two weeks apart, there was a decrease in mMASI score and 3D-measured melanin on the side treated with intradermal PRP.

The therapeutic effect of PRP to pigment could be due to:

2.1 PRP induces phagocytosis in cells

Macrophages are skin resident phagocytes in the epidermis and dermis. They help in maintaining the immunotolerant environment of the skin. Specialized macrophages in the skin called melanophages can engulf melanocyte fragments and melanin (Mizushima, 2018). These melanocyte fragments and melanin are processed by autophagy. The UK1 and Beclin1 are autophagy mediators to macrophage in skin (Sil, Wong, & Martinez, 2018).

Cell type	Autophagy mediator	Processes that require the autophagy machinery
Macrophages	UK1, Beclin1, ATG14, ATG16L1	Pigmentation, removal of damaged protein/organelles

Figure 2.2 Autophagy mediator to immune cell in skin

Source: Adapt from Sil et al., 2018

The macromolecular activators of phagocytosis from platelets (MAPP: l-MAPP and s-MAPP) in platelet-rich plasma induces phagocytosis in immune cells (Sakamoto et al., 2011; Ogawa et al., 2000) as shown in Figure 2.2. From the study of Czakai et al. (2017), PRP can promote phagocytosis activity of macrophages.

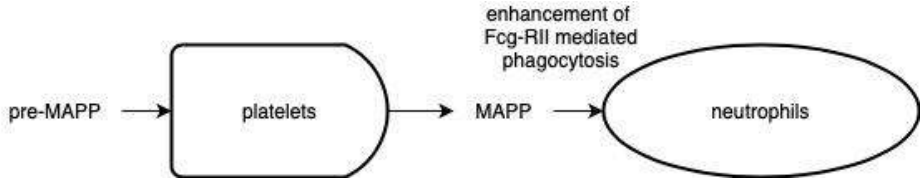


Figure 2.3 Macromolecular activators of phagocytosis from platelets (MAPP) in PRP to stimulate phagocytosis

Source: Adapt from Ogawa et al., 2000

2.2 PRP inhibits melanogenesis

TGF-β is released as an inactive form from the platelet alpha-granules of PRP and then become activated by the proteolytic cleavage. In the skin, TGF-β family regulated several cell functions including cell proliferation, differentiation, and melanogenesis. In the spontaneously immortalized mouse melanocytes cell line, TGF-β significantly suppressed melanin synthesis through several mechanisms such as delayed extracellular signal-regulated kinase activation. TGF-β can reduce the activity of tyrosinase, tyrosinase related protein and the promotor of the microphthalmia transcription factor (MITF) (Yasumoto, Yokoyama, Takahashi, Tomita, & Shibahara, 1997). TGF-β can delay the activation of extracellular signal-regulate kinase. TGF-β can repress the expression of paired-box homeotic gene (PAX), which regulate UV-induced melanogenesis (Tachibana, 2000).

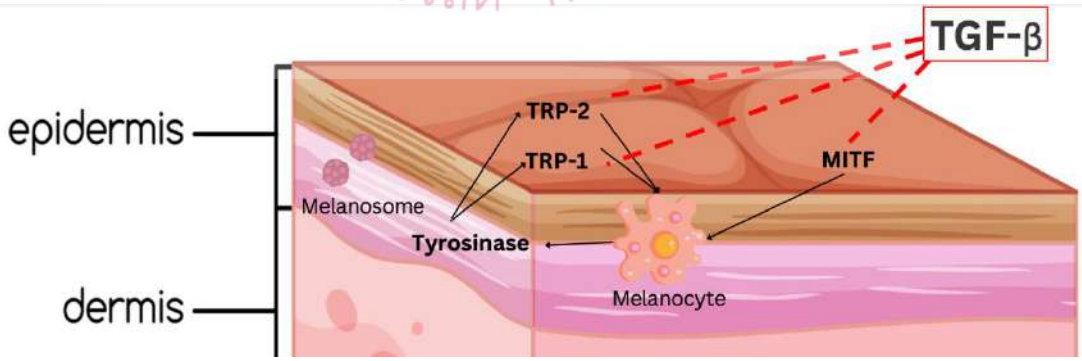


Figure 2.4 Process of TGF-β inhibition in melanogenesis

Source: Adapt from Tachibana, 2000

TGF-β in PRP can increase chemotaxis, stimulate collagen matrix deposition, and decrease signaling response in melanogenesis through microphthalmia-associated transcription factor (MITF) in melanocytes (Chiaverini et al., 2008). This effect may decrease the activity of tyrosine and tyrosine-releasing protein (TRP) in melanogenesis, which slows down signaling in the activation of the extracellular signal-regulated kinase (Lambert, Vancoillie, & Naeyaert, 1999). In the recent decade, many researchers studied about microphthalmia-associated transcription factor gene (MITF). Indeed, MITF appears to have an important role in regulating the network of transcription factors and signaling pathways that control the survival, proliferation, and differentiation of melanoblasts and melanocytes (Vance & Goding, 2004). The melanocyte development and pigmentation via its transcriptional regulatory effect on tyrosinase, tyrosine releasing protein 1 (TRP-1) and tyrosine releasing protein 2 (TRP-2). Therefore, MITF plays a central role in melanin synthesis, as well as melanosome biogenesis and transport (Murakami, Matsuzaki, & Funaba, 2009).

Based on these hypotheses, the pigmentary lightening effect of PRP may be reasoned to several factors including inhibition of melanogenesis, stimulation of phagocytosis activity to dermal melanocyte, and melanin granule that could be seen in lichen planus pigmentosus lesions. Therefore, we hypothesize that PRP could be the promising alternative treatment for lichen planus pigmentosus.

Chapter 3

Research Methodology

This was the first pilot clinical trial to noninvasively and objectively measure the effectiveness of intradermal platelet-rich plasma (PRP) in the treatment of lichen planus pigmentosus and to study safety in using PRP in LPP.

3.1 Population and samples

This research was an experimental pilot study. This study was reviewed and approved by the Ethics Committee (EC) at the Institute of Dermatology, Bangkok. After the study was submitted to the Ethics Committee, we looked for five volunteers from the outpatient department who fulfilled the criteria:

3.1.1 Inclusion criteria:

3.1.1.1 Male or female, 18-60 years old

3.1.1.2 A patient diagnosed with lichen planus pigmentosus, with an onset of six months or longer

3.1.1.3 There are lesions on the face

3.1.1.4 The biopsy showed relevant results for histological findings: perivascular infiltration, lichenoid reaction and melanin, melanophages in epidermis and dermis

3.1.1.5 The patch test was negative to rule out pigmented contact dermatitis

3.1.2 Exclusion criteria:

3.1.2.1 Pregnancy or breastfeeding

3.1.2.2 Individuals with a history of hypertrophic scar and/or keloid formation

3.1.2.3 Active infection at the location that is yet to be treated

3.1.2.4 Having a history of bleeding disorders

3.1.2.5 The use of anticoagulants

3.1.2.6 Having other hyperpigmentation conditions such as pigmented contact dermatitis, fixed drug eruption, lichenoid drug reaction, ochronosis, melasma, PIH, or endocrinopathies

Written informed consent was obtained from all participants. At the first meeting, all volunteers were informed about the the study, the steps of the study, and to make an appointment when required. Next, six appointment dates are comprised of three treatments, two weeks apart, with a follow-up at two weeks, one month, and two months. If a volunteer cannot participate on a previously stated appointment date, they can come the day after. If they cannot participate more than one day after the scheduled appointment with reasons that are not relevant to the study, the researcher will not collect their data. However, if there are reasons presented that are relevant to adverse events from the study, the researcher will record the date, adverse events, and treat the complications accordingly. The study took place at the bioengineering unit at Institute of Dermatology, Bangkok.

All volunteers were advised:

1) Not to take NSAID at least 14 days before the treatment

2) Not directly have exposure to sunlight for 24 hours before the treatment

3) Not to apply any makeup 24 hours after the treatment

4) Not to use any topical bleaching agents, such as vitamin A analog, while in the study

5) Can only apply moisturizer and sunscreen that was given by the researcher

All volunteers were advised to clean make up with soap before the study.

The lesions were marked by non-permanent marker.

All of the steps were performed under a sterile environment.

PRP preparation:

1) Prepared 2 cc. of anticoagulant agent (ACD-A) in a 20 cc. syringe

2) Collect 10 cc. of venous blood from the forearm with 20 cc. with a 20 Gauge disposable needle.

3) Draw the blood in to e+PRP Kit (Minos®, Gibthais.co.,LTD) and put in the centrifuge device (L500 Tabletop Low Speed Centrifuge). The setting for the centrifugation was: 1 round at 2000G/5minutes.

4) Draw the 2-3cc. of PRP or buffy coat layer from the e+PRP Kit to a 1 cc. syringe

After having prepared the PRP of each individual, 0.05 cc. of PRP was injected intradermally with a 30 Gauge needle at the marked lesion, 1 cm. apart and 1-2 mm. depth in anterograde direction.

3.2 Research instruments

We measured melanin index, a photograph at baseline was taken. We recorded the patient satisfaction score and side effects were noted after 2, 4, 8 week after the last sessions.

Figure 3.1 Evaluation for participants

3.2.1 Melanin index

To objectively measure the pigment outcome, the mexameter® (MX18, Courage & Khazaka (C&K) Electronic GmbH, Germany) was used to measure the melanin content in terms of melanin index (MI).

The investigators took 3 digital photographs of each participant's face including front, right lateral, and left lateral views using Visia® device to evaluate the hyperpigmented lesion in every visit. The melanin content of the 3 sites of lesions (frontal area, left and right side) of participants, The MI score was measured using the Mexameter® at the same point, marked by transparent paper. These measurements were repeated for each participant in every visit.

Figure 3.2 Mexameter® (MX18, Courage & Khazaka (C&K) Electronic GmbH, Germany)

Source: Courage & Khazaka Electronic, 2023

3.2.2 Clinical improvement

Clinical photographs were taken by using A Canfield Visia-CR System®. Using the transparent paper to mark the lesion, we chose both sites of the face on three points which have the most prominent pigmentation. Thus, we were able to evaluate the improvements between the baseline picture and after each treatment by two physicians.

To judge the final result, 2 independent blinded physicians rated the improvement based on baseline photo (week0) and 8 weeks after last session of PRP (week12) photograph using the quartile grading scale (QGS) : 0= no improvement, 1 = mild improvement (0-24%), 2 = moderate improvement (25-49%), 3= good improvement (50–74%), 4= excellent improvement (75-100%), and an average score was calculated.

Figure 3.3 A Canfield Visia-CR System®

Source: Dermatology Associates of Plymouth Meeting, 2023

3.2.3 Patient satisfaction Score

Figure 3.4 Visual analog scale

Overall satisfaction levels were scored with a visual analog scale (ranging from 0 = extremely unsatisfied, to 10= extremely satisfied). A ruler was used to provide measurement in centimeters.

3.2.4 Adverse effects

Any adverse effects were recorded (erythema, edema, bleeding, bruising, post inflammatory hyperpigmentation, and others).

3.3 Data collection

All data was recorded in Microsoft Excel program.

3.4 Data analysis

Data is expressed as a mean ± standard deviation. The Wilcoxon signed-rank test was used to analyze differences in outcomes between each visit. The SPSS software version 23 was used for all analyses. The P value <0.05 was considered statistically significant.

Chapter 4

Research results

Four female and one male patient with lesions on the face were enrolled in this study. All completed the treatment protocol. Their average age was 47.6 (range,35-56 years). Among these 5 patients, 3 (60%) had skin phototype IV and 2 (40%) had skin phototype V. The duration of illness ranges from 1 years – 6 years. All the patients showed negative results in the patch test and the direct fluorescence test. Most of the biopsy results showed lichenoid reaction. Their past 6month treatment was 4% hydroquinone and tacrolimus cream (Table 4.1).

Table 4.1 The demographic data and clinical featured of participating patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Female	Male	Female	Female	Female
Age	56	39	52	35	56
Skin phototype	V	IV	IV	IV	V
Location	Face, extremities	Face, neck, trunk	Face, neck, trunk	Face	Face, neck
Duration of illness	2 years	3 years	2 years	5 years	2 years
Patch test	Neg	Neg	Neg	Neg	Neg
Biopsy Result	Lichenoid reaction	Infiltration of some melanophages in papillary dermis, lichenoid reaction	Infiltration of some melanophages in papillary dermis, lichenoid reaction	Lichenoid reaction	Infiltration of some melanophages in papillary dermis, lichenoid reaction
Treatments	Hydroquinone	Hydroquinone	Hydroquinone	Hydroquinone	Hydroquinone, tacrolimus cream

4.1 Decrease of mean melanin index following PRP treatment

Mean melanin index values as measured by the Mexameter® fell from the score of 450.33±69.91 to 392.60±73.88 at week 8 after the third PRP treatment, which was significant (P<0.05) (Table 4.2).

Table 4.2 The mean melanin index of each participant and the percentage of decrease in melanin index

Visit		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean ±SD	P-value
Before PRP (week 0)	Melanin index	420.33	437.67	402.00	418.33	573.33	450.33 ±69.91	ref
After 1st PRP (week 2)	Melanin index	384.67	412.00	383.33	389.67	552.33	424.4 ±72.44	0.043
	Decrease (%)	35.66 (8.48)	25.67 (5.87)	18.67 (4.64)	28.66 (6.85)	21 (3.66)	25.93 (5.76)	
After 2nd PRP (week 4)	Melanin index	359.33	418.00	368.00	378.33	538.00	412.33 ±73.75	0.043
	Decrease (%)	61 (14.51)	19.67 (4.49)	34 (8.46)	40 (9.56)	35.33 (6.16)	38 (8.44)	
After 3rd PRP (week 6)	Melanin index	378.00	411.67	357.00	371.67	529.67	409.6 ±70.04	0.043
	Decrease (%)	42.33 (10.07)	26 (5.94)	45 (11.19)	46.66 (11.15)	43.66 (7.62)	40.73 (9.04)	
After 3rd PRP (week 8)	Melanin index	356.33	403.00	350.00	366.33	522.67	399.67 ±71.76	0.042
	Decrease (%)	64 (15.23)	34.67 (7.92)	52 (12.94)	52 (12.43)	50.66 (8.84)	50.67 (11.25)	
After 3rd PRP (week 12)	Melanin index	352.00	394.67	340.67	356.00	519.67	392.6 ±73.88	0.043
	Decrease (%)	68.33 (16.26)	43 (9.82)	61.33 (15.26)	62.33 (14.9)	53.66 (9.36)	57.73 (12.82)	

There was a trend towards gradual reduction of mean melanin index after every visit. (Figure 4.1) In each participant, the mean melanin index in each visit is shown in Figure 4.2.

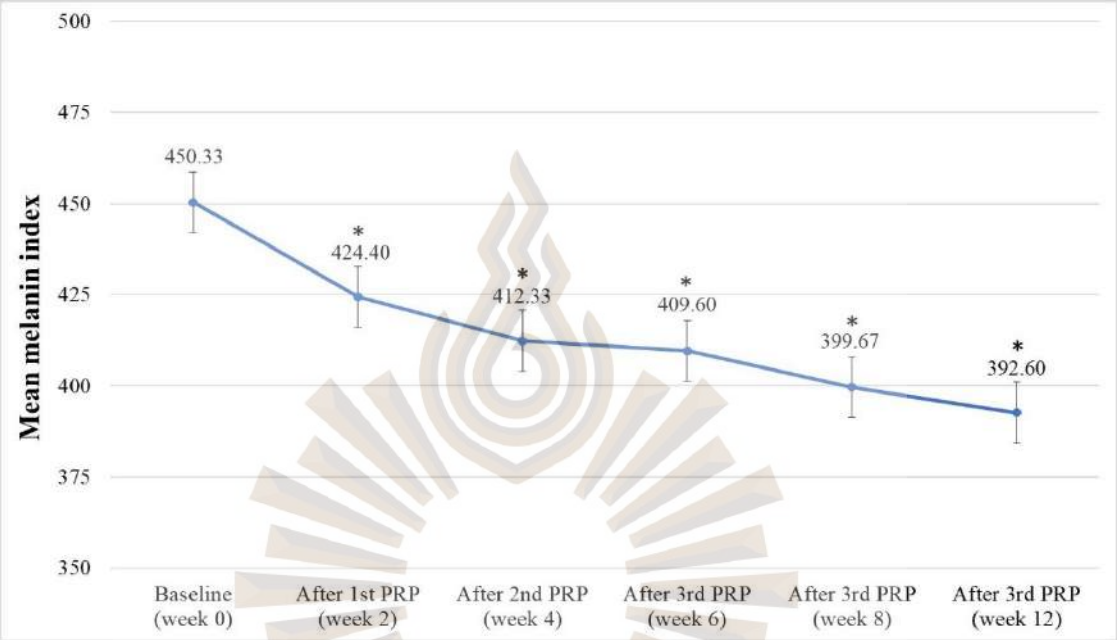


Figure 4.1 Decrease of mean MI noted in each visit. The line diagram demonstrates a trend of reduction of the mean MI at different treatment sessions and follow-up visits. *P<0.05

Figure 4.2 Mean melanin index in each patient at week 0, 2, 4, 6, 8, 12

The relative melanin index difference compared to baseline also showed significant differences with PRP after 2 weeks of 1st PRP treatment ($p<0.05$)

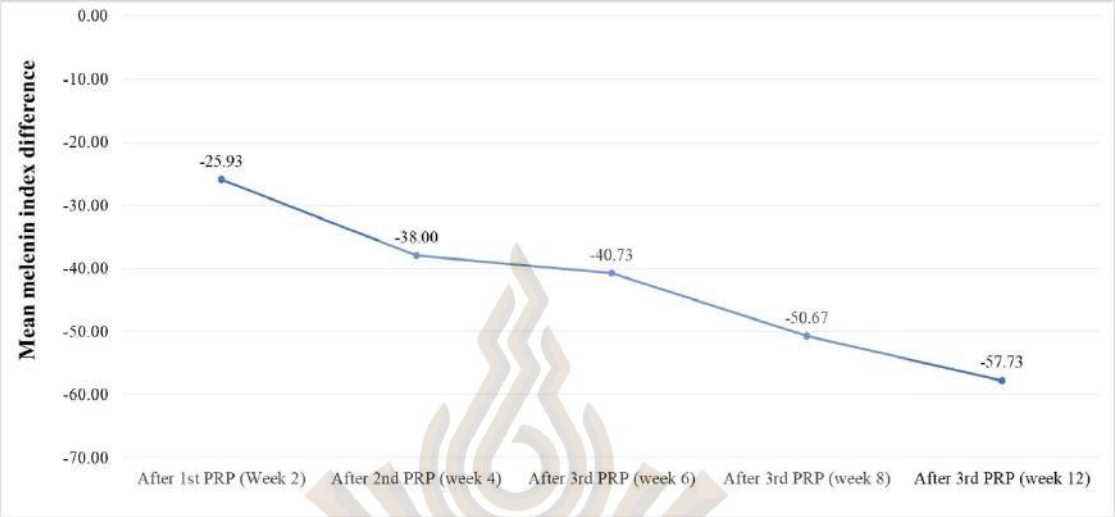


Figure 4.3 Mean melanin index reduction from baseline in each visit

4.2 Clinical improvement of lesions by two physicians

To judge the final result, two independent blinded physicians rated the improvements based on the before and after photographs using the quartile grading scale (QGS), where 0 = no improvement (less than 1%),1 = mild improvement (1-25%), 2 = moderate improvement (26-50%), 3 = good improvement (51–75%), 4 = excellent improvement (>75%). Their results were shown in Figure 4.4, and an average was calculated.

After the first PRP and second PRP, the mean improvement was 1.4 and 1.9 respectively, which is mild to moderate improvement. The mean improvement was moderate at week 6 and 8. There was good improvement after 8 weeks after the third PRP.

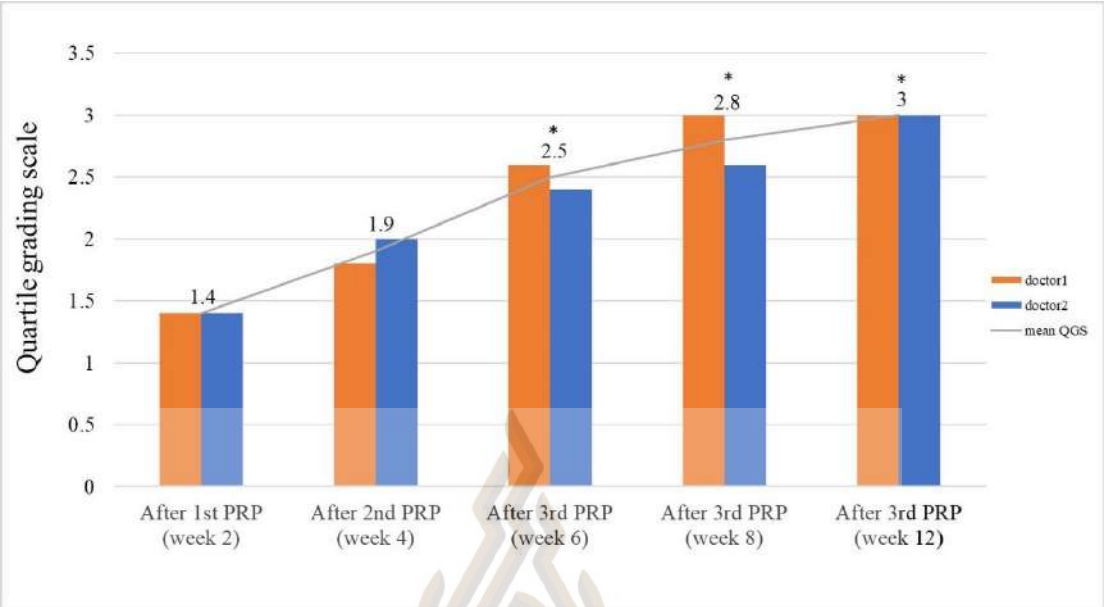


Figure 4.4 Improvement of the mean quartile grading scale from week 2 to week 12.

*P<0.05

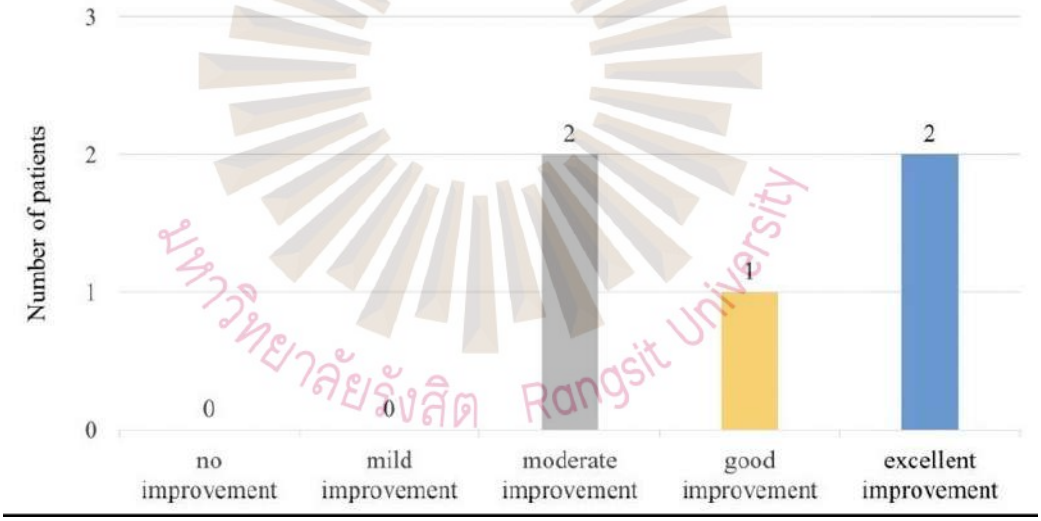


Figure 4.5 Number of patients with clinical improvement at 8 weeks after 3rd PRP (week 12)

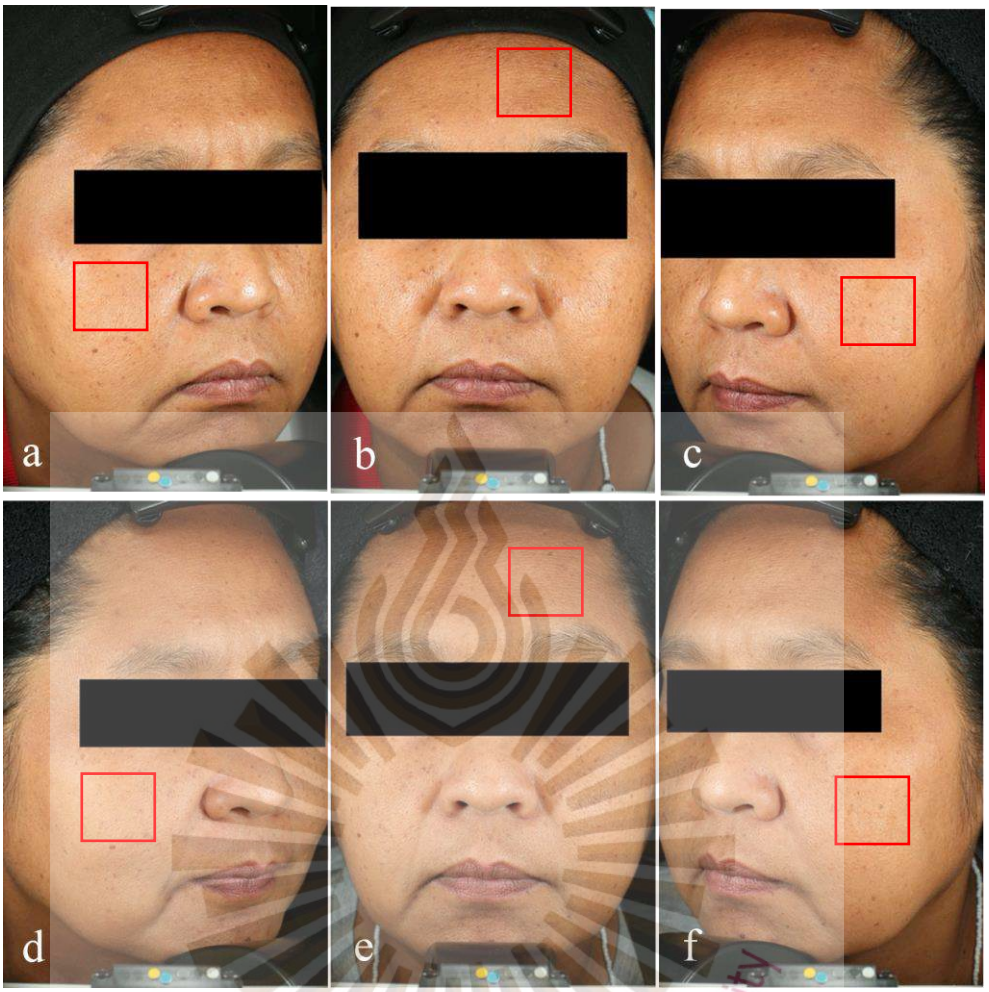


Figure 4.6 Clinical photograph of a patient at baseline (a-c) and after 3 sessions of PRP treatment at week 12 (d-f). The red rectangles demonstrate the area that were treated with intradermal injection of PRP.

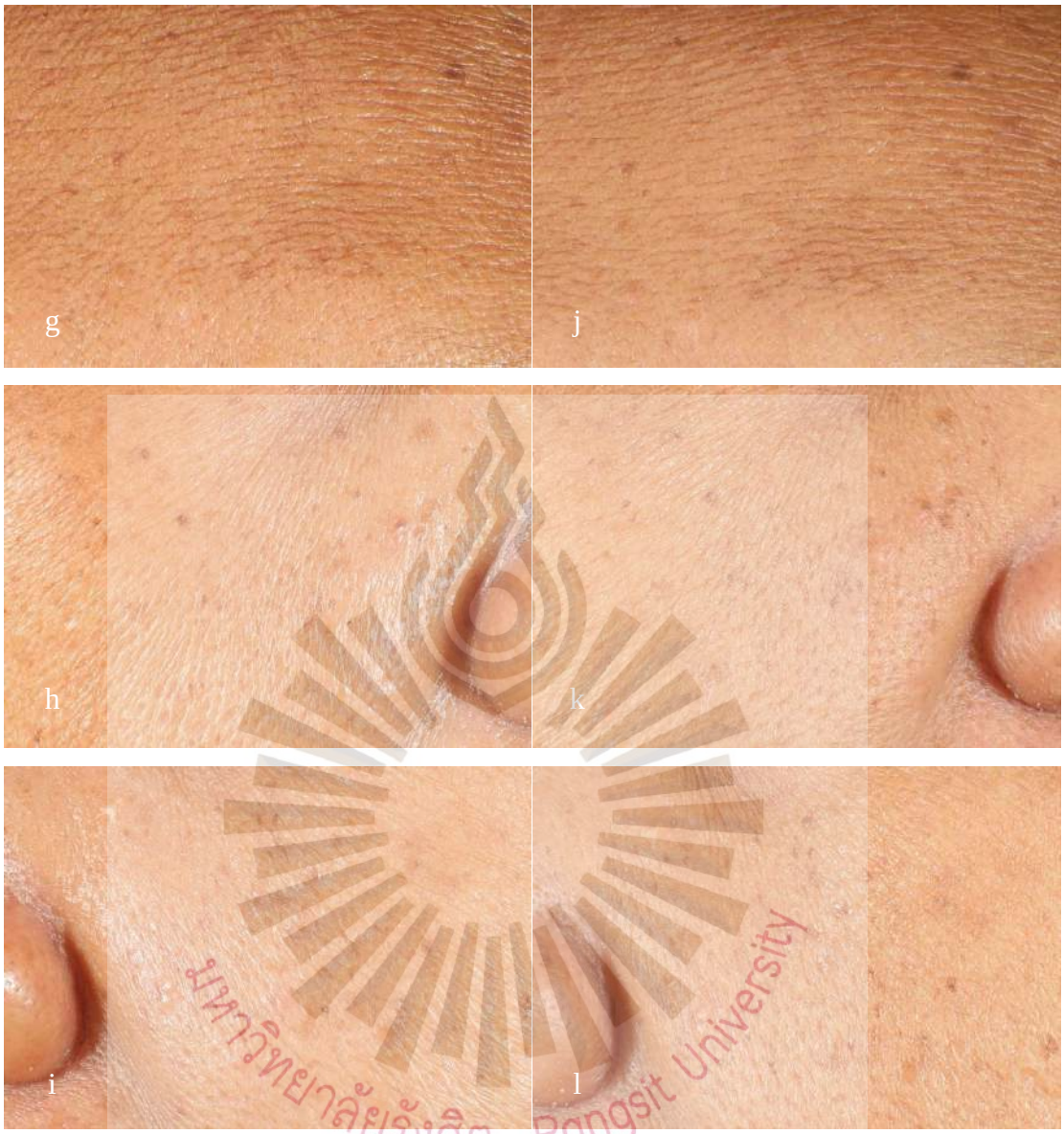


Figure 4.7 Lightening of hyperpigmentation after PRP treatment. (g-i) Baseline clinical photographs of representative areas of LPP patients at week 0. (j-l) Post treatment clinical photographs at the end of the study (week 12).

4.3 Participants' overall satisfaction assessment

Participants were asked to rate their overall satisfaction score after treatment every time on a 0-10 scale: 0-1 = not satisfied, 2-4 = slightly satisfied, 5=neutral, 6-8= very satisfied, 9-10 = extremely satisfied.

In terms of the patient's satisfaction, after the first PRP treatment, the mean of the overall satisfaction score was slightly satisfied but since after second PRP of, the mean of the overall satisfaction score was increased to very satisfied (Figure 4.8).

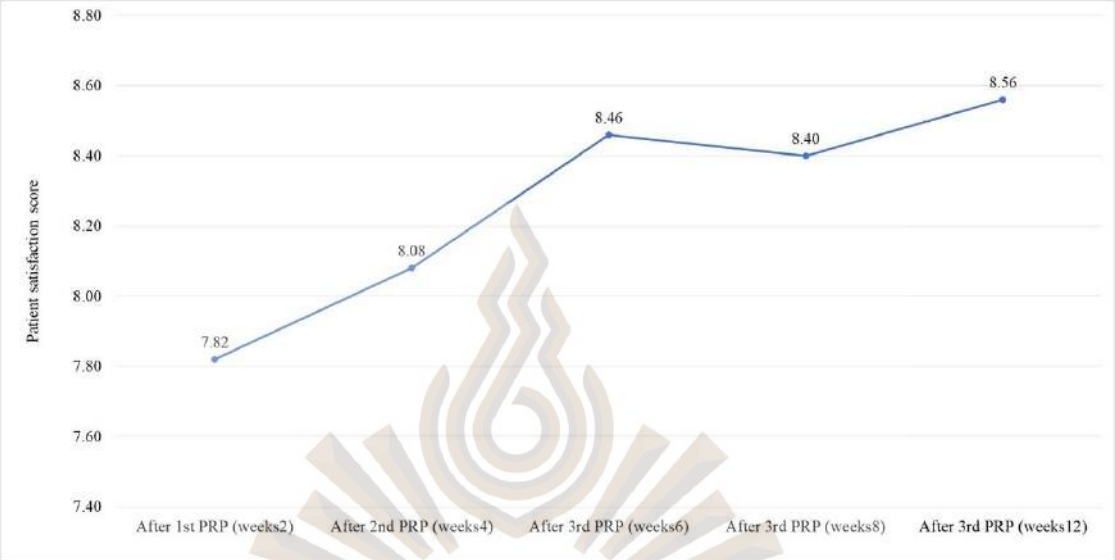


Figure 4.8 Mean patient satisfaction score from patients in each visit

4.4 Adverse effects

Most patients experienced swelling on the sites of injection ranging from 1-3 hours after treatment, and it spontaneously resolved without any treatment. One of the participants experienced bruising for 2 days and it was resolved in day 3. None of the participants reported infection or post-inflammatory hyperpigmentation.

Chapter 5

Conclusion and recommendations

5.1 Discussion

LPP is a chronic, acquired pigmentary disorder characterized histologically by the presence of pigment and pigment laden macrophage in the papillary dermis (Rieder et al., 2013; Bhat et al., 2017). The treatment of LPP remains controversial. The available treatment modalities for LPP include topical hydroquinone, corticosteroids, tranexamic acid, tacrolimus, oral isotretinoin and light-based therapy ex. Q-switched Nd:YAG laser, Narrowband UVB (Syder et al., 2022).

From the past researchs that were prospective studies, Al-Mutairi and El-Khalawany (2010). studied 13 patients and treated them with 0.03% topical tacrolimus for 6-12 weeks, with 58.8% showing lightening of lesion after 12 weeks. Pruritus was recorded in 9 patients. Muthu et al. (2006) demonstrated low dose of isotretinoin for 6 months in 27 patients, with 55.7% of patients experiencing moderate improvement. The adverse effects were menorrhagia, cheilitis, xerosis, and transaminitis in a few patients. Bhari et al. (2020) studied 13 patients that were treated with 1064 Q-switched Nd-YAG laser for 5-6 sessions every 4-8 weeks. 34.8% of patients demonstrated significant improvement assessed by 2 independent dermatologists by visual inspection and comparing serial photographs. One patient experienced depigmentation on forehead and other patients showed some scarring in the periorbital area. There is very limited data regarding effectiveness treatments for LPP, and most of the available data was collected from case reports and series.

PRP injections have been extensively used in dermatology (Alam et al., 2018). A number of studies have declared that PRP could ameliorate skin hyperpigmentation,

including periorbital hyperpigmentation and melasma. Al-Shami (2014) reported significant improvements in infraorbital color homogeneity after PRP injection. Moreover, numerous studies have revealed beneficial effects of PRP in treating melasma (Amini et al., 2015; Çayırlı et al., 2014; Hofny et al., 2019; Sirithanabadeekul, 2020). Improvement of hyperpigmentation after PRP treatment is likely due to growth factors in alpha granules such as transforming growth factor- (TGF- and epidermal growth factor (EGF), which inhibit melanogenesis via several signal transduction pathways. In addition, macromolecular activators of phagocytosis from platelets (MAPP) in PRP have been shown to enhance macrophage phagocytosis activity and subsequently reduce melanocyte fragments and melanin granule (Sit et al., 2018). Based on these knowledge, we speculated that PRP might utilized these mechanisms to improve LPP and had a few minor adverse effects. Ultimately, we hereby propose PRP as a noval therapy for LPP which could be a promising alternative in treatment-resistant cases.

In our study, after completing 3 sessions of PRP injection at the sites of hyperpigmentation lesion in five patients, the mean melanin index values measured by Mexameter® was decreased, and this change was significant. ($P < 0.05$). There was a trend towards a gradual decrease in the mean melanin index at each visit. The difference in the relative melanin index compared to the baseline also showed a significant difference with PRP after 2 weeks of the first PRP treatment ($p < 0.05$). According to the global assessment of two physicians, the mean improvement increased from mild to moderate after the first PRP (week 2) and second PRP (week 4) to moderate improvement after the third PRP (week 6) and showed an excellent improvement after 8 weeks of the third PRP (week 12). For the patient's satisfaction, after the first PRP treatment, the mean of overall satisfaction was slightly satisfied After the second PRP, the overall satisfaction was very satisfied. Finally, side effects of the treatment includes edema and bruising, but these were mild and resolved spontaneously within a few days.

5.2 Conclusion

In conclusion, this is the first PRP trial to treat hyperpigmentation in lichen planus pigmentosus. We found that the PRP intradermal injection significantly

improved lesions within 3 treatments without causing serious adverse effects. However, to the best of our knowledge, we still have limited information on the action mechanism of PRP.

5.3 Recommendations

Further well-designed studies with larger sample size are necessary to confirm this preliminary observation. to the best of our knowledge, we still have limited information on the action mechanism of PRP.

References

Al-Mutairi, N., & El-Khalawany, M. (2010). Clinicopathological characteristics of lichen planus pigmentosus and its response to tacrolimus ointment: an open label, non-randomized, prospective study. *Journal of the European Academy of Dermatology and Venereology: JEADV, 24*(5), 535–540. doi:10.1111/j.1468-3083.2009.03460.x

Al-Shami, S. H. (2014). Treatment of periorbital hyperpigmentation using platelet-rich plasma injections. *American Journal of Dermatology and Venereology, 3*(5), 87-94. doi:10.5923/j.ajdv.20140305.03

Alam, M., Hughart, R., Champlain, A., Geisler, A., Paghdal, K., Whiting, D., … Poon, E. (2018). Effect of Platelet-Rich Plasma Injection for Rejuvenation of Photoaged Facial Skin: A Randomized Clinical Trial. *JAMA dermatology, 154*(12), 1447–1452. doi:10.1001/jamadermatol.2018.3977

Amini, F., Ramasamy, T. S., & Yew, C. H. (2015). Response to intradermal autologous platelet-rich plasma injection in refractory dermal melasma: Report of two cases. *Journal of Health and Translational Medicine, 18*(2), 1-6. doi:10.22452/jummec.vol18no2.1

Bhari, N., Sharma, V. K., Singh, S., Parihar, A., & Arava, S. (2020). Effect of Q-switched Nd-YAG laser on the clinical, pigmentary, and immunological markers in patients with lichen planus pigmentosus: A pilot study. *Dermatologic Therapy, 33*(2), e13208. doi:10.1111/dth.13208

Bhat, R. M., Mathanda, T. R., Jayaprakash, C. S., & Dandakeri, S. (2017). Clinical, Histopathological Characteristics and Immunohistochemical Findings in Lichen Planus Pigmentosus. *Indian Journal of Dermatology, 62*(6), 612–617. doi:10.4103/ijd.IJD_148_17

Bhutani, L. K., Bedi, T. R., Pandhi, R. K., & Nayak, N. C. (1974). Lichen planus pigmentosus. *Dermatologica, 149*(1), 43–50. doi:10.1159/000251470

Çayırlı, M., Calışkan, E., Açıkgöz, G., Erbil, A. H., & Ertürk, G. (2014). Regression of melasma with platelet-rich plasma treatment. *Annals of dermatology, 26*(3), 401-402. doi:10.5021/ad.2014.26.3.401

References (Cont.)

Courage & Khazaka Electronic. (2023). *Mexameter® MA 18*. Retrieved April 23, 2024. From https://www.courage-Khazaka.de/en/scientific-products/occupational-health/occupational-health/169-mexameter-e

Chiaverini, C., Beuret, L., Flori, E., Busca, R., Abbe, P., Bille, K., … Ballotti, R. (2008). Microphthalmia-associated transcription factor regulates RAB27A gene expression and controls melanosome transport. *The Journal of Biological Chemistry*, *283*(18), 12635–12642. doi:10.1074/jbc.M800130200

Czakai, K., Dittrich, M., Kaltdorf, M., Müller, T., Krappmann, S., Schedler, A., … Löffler, J. (2017). Influence of Platelet-rich Plasma on the immune response of human monocyte-derived dendritic cells and macrophages stimulated with Aspergillus fumigatus. *International Journal of Medical Microbiology*, *307*(2), 95–107. doi:10.1016/j.ijmm.2016.11.010

Dermatology Associates of Plymouth Meeting. (2023). *Canfield VISAI Camera System*. Retrieved April 23, 2024. From https://playmouthmeetingdermatology.com/canfield-visai-camera-system/

Ehrenfest, D. M. D., Andia, I., Zumstein, M. A., Zhang, C., Pinto, N. R., & Bielecki, T. (2014). Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscles, Ligaments and Tendons Journal*, *41*, 3-9.

Hofny, E. R. M., Abdel-Motaleb, A. A., Ghazally, A., Ahmed, A. M., & Hussein, M. R. A. (2019). Platelet-rich plasma is a useful therapeutic option in melasma. *The Journal of dermatological treatment*, *30*(4), 396–401. doi:10.1080/09546634.2018.1524821

Kim, D. H., Je, Y. J., Kim, C. D., Lee, Y. H., Seo, Y. J., Lee, J. H., & Lee, Y. (2011). Can Platelet-rich Plasma Be Used for Skin Rejuvenation? Evaluation of Effects of Platelet-rich Plasma on Human Dermal Fibroblast. *Annals of Dermatology*, *23*(4), 424–431. doi:10.5021/ad.2011.23.4.424

References (Cont.)

Kumarasinghe, S. P. W., Pandya, A., Chandran, V., Rodrigues, M., Dlova, N. C., Kang, H. Y., … Parsad, D. (2019). A global consensus statement on ashy dermatosis, erythema dyschromicum perstans, lichen planus pigmentosus, idiopathic eruptive macular pigmentation, and Riehl's melanosis. *International Journal of Dermatology*, *58*(3), 263–272. doi:10.1111/ijd.14189

Lambert, J., Vancoillie, G., & Naeyaert, J. M. (1999). Molecular motors and their role in pigmentation. *Cellular and Molecular Biology (Noisy-le-Grand, France)*, *45*(7), 905–918.

Mizushima, N. A. (2018). Brief history of autophagy from cell biology to physiology and disease. *Nature Cell Biology*, *20*, 521–527. doi:10.1038/s41556-018-0092-5

Murakami, M., Matsuzaki, F., & Funaba, M. (2009). Regulation of melanin synthesis by the TGF-beta family in B16 melanoma cells. *Molecular Biology Reports*, *36*(6), 1247–1250. doi:10.1007/s11033-008-9304-6

Muthu, S. K., Narang, T., Saikia, U. N., Kanwar, A. J., Parsad, D., & Dogra, S. (2016). Low-dose oral isotretinoin therapy in lichen planus pigmentosus: An open-label non-randomized prospective pilot study. *International Journal of Dermatology*, *55*(9), 1048–1054. doi:10.1111/ijd.13293

Ogawa, Y., Sakamoto, H., Oryu, M., Shinnou, M., Sakamoto, N., Yanghong, W., … Nishioka, M. (2000). Production of macromolecular activators of phagocytosis by lysed platelets. *Thrombosis Research*, *97*(5), 297–306. doi:10.1016/s0049-3848(99)00178-4

Rieder, E., Kaplan, J., Kamino, H., Sanchez, M., & Pomeranz, M. K. (2013). Lichen planus pigmentosus. *Dermatology Online Journal*, *19*(12), 20713.

Sakamoto, H., Wu, B., Nagai, Y., Tanaka, S., Onodera, M., Ogawa, T., & Ueno, M. (2011). Platelet high-density lipoprotein activates transferrin-derived phagocytosis activators, MAPPs, following thrombin digestion. *Platelets*, *22*(5), 371–379. doi:10.3109/09537104.2010.533797

References (Cont.)

Sehgal, V. N., Srivastava, G., Sharma, S., Sehgal, S., & Verma, P. (2011). Lichenoid tissue reaction/interface dermatitis: recognition, classification, etiology, and clinicopathological overtones. *Indian Journal of Dermatology, Venereology and Leprology*, *77*(4), 418–430. doi:10.4103/0378-6323.82389

Sil, P., Wong, S. W., & Martinez, J. (2018). More Than Skin Deep: Autophagy Is Vital for Skin Barrier Function. *Frontiers in immunology*, *9*, 1376. doi:10.3389/fimmu.2018.01376

Sirithanabadeekul, P., Dannarongchai, A., & Suwanchinda, A. (2020). Platelet-rich plasma treatment for melasma: A pilot study. *Journal of Cosmetic Dermatology*, *19*(6), 1321–1327. doi:10.1111/jocd.13157

Syder, N., Sicco, K., & Gutierrez, D. (2022). Updates In Therapeutics for Lichen Planus Pigmentosus. *Journal of Drugs in Dermatology*, *21*(3), 324–330. doi:10.36849/JDD.6454

Tachibana, M. (2000). MITF: a stream flowing for pigment cells. *Pigment Cell Research*, *13*(4), 230–240. doi:10.1034/j.1600-0749.2000.130404.x

Vance, K. W., & Goding, C. R. (2004). The transcription network regulating melanocyte development and melanoma. *Pigment Cell Research*, *17*(4), 318–325. doi:10.1111/j.1600-0749.2004.00164.x

Wang, H. L., & Avila, G. (2007). Platelet rich plasma: myth or reality?. *European Journal of Dentistry*, *1*(4), 192–194.

Xian, L. J., Chowdhury, S. R., Saim, A. B., & Idrus, R. B. (2015). Concentration-dependent effect of platelet-rich plasma on keratinocyte and fibroblast wound healing. *Cytotherapy*, *17*(3), 293–300. doi:10.1016/j.jcyt.2014.10.005

Yasumoto, K., Yokoyama, K., Takahashi, K., Tomita, Y., & Shibahara, S. (1997). Functional analysis of microphthalmia-associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. *The Journal of Biological Chemistry*, *272*(1), 503–509. doi:10.1074/jbc.272.1.503

Appendix

Case record form

Visit .....

Date .....

Case record form

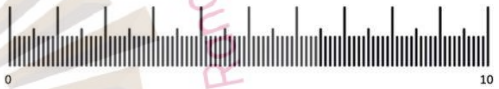
Case no.

Melanin index 1..... 2..... 3.....

Side effects

- Erythema
- Edema
- Oozing
- Bruising
- PIH
- Others.....

Patient satisfaction score



Score ____

Record by .....

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