THE EFFECTIVENESS OF INTRADERMAL PLATELET-RICH PLASMA FOR THE TREATMENT OF ACQUIRED BILATERAL NEVUS OF OTA-LIKE MACULES (HORI'S NEVUS), IN INSTITUTE OF DERMATOLOGY, THAILAND, PILOT STUDY

BY

PASINEE DEEUDOMWONGSA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
IN DERMATOLOGY AND DERMATOSURGERY
FACULTY OF MEDICINE

GRADUATE SCHOOL, RANGSIT UNIVERSITY
ACADEMIC YEAR 2023

Thesis entitled

THE EFFECTIVENESS OF INTRADERMAL PLATELET-RICH PLASMA FOR THE TREATMENT OF ACQUIRED BILATERAL NEVUS OF OTA-LIKE MACULES (HORI'S NEVUS), IN INSTITUTE OF DERMATOLOGY, THAILAND, PILOT STUDY

by

PASINEE DEEUDOMWONGSA

was submitted in partial fulfillment of the requirements

for the degree of Master of Science in Dermatology and Dermatosurgery

Rangsit University

Academic Year 2023

Asst.Prof.Dr. Direkrit Chiewchengchol, M.D., Ph.D.	Dr. Wanida Limpongsanurak, M.D.
Examination Committee Chairperson	Member
Dr. Praneet Sajjachareonpong, M.D.	Dr. Chanisa Kiatsurayanon, M.D., Ph.D.
Member and Adviser	Member and Co-Advisor

Approved by Graduate School

(Asst.Prof.Plt.Off. Vannee Sooksatra, D.Eng.)

Dean of Graduate School

July 3, 2023

Acknowledgements

This thesis would not have been possible without the assistance and support of several mentors, especially my academic advisor, Dr. Praneet Sajjacharoenpong, M.D. and Dr. Chanisa Kiatsurayanon, M.D., Ph.D. They have provided me with unwavering support and guidance throughout my study. Their insightful comments and encouragement have been invaluable to my research and personal development.

In addition, I would like to thank my co-advisor, Dr.Chumsaeng Chusaengsri, for his contributions to my research. His knowledge of the topic has significantly influenced my research methodology and analysis.

Moreover, I would like to thank my thesis committee members for their time and suggestions. Their expertise has helped me improve my work, for which I am appreciative. To those who contribute to the accomplishment of the study, gratitude and appreciation are expressed.

Throughout this process, I would like to thank my family and friends for their unwavering encouragement and support. Their love and encouragement have been the driving force behind my achievements.

Pasinee Deeudomwongsa

Researcher

6406190	:	Pasinee Deeudomwongsa
Thesis Title	:	The Effectiveness of Intradermal Platelet-rich Plasma for The Treatment of Acquired Bilateral Nevus of Ota-like Macules (Hori's Nevus), in Institute of Dermatology, Thailand, Pilot study
Program	:	Master of Science in Dermatology and Dermatosurgery
Thesis Advisor	:	Dr. Praneet Sajjachareonpong, M.D

Abstract

Hori's nevus is a pigmentation condition that affects many Asian women. Today's treatments aren't all effective. Recent studies have proposed platelet-rich plasma (PRP) for skin pigmentation issues. This study assessed intradermal PRP therapy for Hori's nevus's effectiveness and safety. Ten female patients received bilateral intradermal PRP every two weeks for four time. The modified dermal pigmentation and severity index (mDPASI), mean melanin index, brightening score, and patient self-assessment were evaluated at two-, four-, eight-, and twelve-weeks post-treatment. The safety of treatment was evaluated by monitoring adverse events.

At twelve weeks post-treatment, mDPASI improved 38.86% from 0.929 ± 0.617 to 0.568 ± 0.415 ($p = 0.003$). The mean melanin index fell 12.75% from 208.650 ± 26.319 to 182.052 ± 17.028 ($p < 0.0001$). In addition, mean brightness score between two experts is 1.4, indicating moderate lightening (>25-50% improvement). At week 12, 20% of the 10 patients reported a significant improvement (25-50% improvement), 50% a marked improvement, and 30% near-normal skin. Side effects included pain, mild edema, and bruising, which spontaneously resolved within day 3. This study reveals PRP may be an effective alternative to standard Hori's nevus treatments. However, larger samples and longer follow-ups are needed to corroborate these results.

(Total 81 pages)

Keywords: Platelet-rich plasma, Hori's Nevus, Acquired Bilateral Nevus of Ota-like Macules, ABNOM, Nevus, Hyperpigmented

Student's Signature …..... Thesis Advisor's Signature .....

Table of Contents

Table of Contents (continued)

List of Tables

List of Figures

Abbreviations and Symbols

Symbol	Meaning
ABNOM	Acquired bilateral nevus of Ota-like macules
ADMH	Acquired dermal macular hyperpigmentation
ADP	Adenosine triphosphate
ATP	Adenosine diphosphate
DPASI	Dermal hyperpigmentation score and severity index
EDP	Erythema dyschromicum perstans
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
HGF	Hepatocyte growth factor
IGF	insulin-like growth factor
KGF	keratinocyte growth factor
LPP	Lichen planus pigmentosus
MAPK	Mitogen activated protein kinase pathway
mDPASI	Modified dermal hyperpigmentation score and severity index
MITF	Micropthalmia associated transcription factor
PCD	Pigmented contact dermatitis
PDGF	Platelet-derived growth factor
PRP	Platelet-rich plasma
PSAL	Picosecond alexandrite laser
QSNYL	Q-switched (QS) Nd:YAG laser
QSAL	Q-switched alexandrite laser
QSRL	Q-switched ruby laser
RM	Riehl's melanosis
SCF	Stem cell factor
TGF-β	Transforming growth factor beta
TRP	Tyrosinase-related protein
VEGF	vascular endothelial growth factor

Chapter 1

Introduction

1.1 Background and Significance of the Problem

Acquired bilateral nevus of Ota-like macules (ABNOM) or Hori's nevus is a hyperpigmentation disorder in the dermis mostly found in Asian women. The clinical characteristic is bilateral brown-to-grey macules mainly on both cheeks. The most common risk factors of Hori's nevus include genetic factor, UV light exposure, dermal inflammation, hormonal change and aging process. (Park, Tsao, & Tsao, 2009)

There are several reports of effective treatments of Hori's nevus. For example, energy-based devices and dermabrasion. Melanin-targeting lasers such as Picosecond alexandrite laser (PSAL), Q-switched (QS) Nd:YAG laser (QSNYL), QS alexandrite laser (QSAL), and QS ruby laser (QSRL) have proven effective in the treatment of Hori's nevus. (Kaur et al., 2020) However, multiple sequential treatments are required to achieve the treatment goal. Following several treatments of LASER therapies, there are common complications such as post-inflammatory hyper- and hypopigmentation, irritation and erythema. Although dermabrasion has shown the desired improvement of Hori's nevus, the common problems encountered are the uncontrollable depth of ablation, downtime and infections. (Kaur et al., 2020; Kunachak, Kunachakr, Sirikulchayanonta, & Leelaudomniti, 1996; Manuskiatti, Sivayathorn, Leelaudomlipi, & Fitzpatrick, 2003)

Recently, platelet-rich plasma (PRP) has been used in many dermatologic conditions such as acne scar, skin rejuvenation, alopecia and melasma. Growth factors contained in the platelet alpha granules act through several signal transduction pathways to inhibit melanogenesis.

Hence, we are interested in intradermal PRP injection as an alternative treatment of Hori's nevus due to its efficacy on reduce melanin production, safety and less problem. In addition, there is no research on PRP injection for the treatment of Hori's nevus.

1.2 Research Objectives

To study the effectiveness of intradermal platelet-rich plasma for the treatment of Hori's nevus in Institute of dermatology, Thailand

1.3 Research Questions/ Assumptions

Our research question is whether the intradermal platelet-rich plasma injection could be an effective treatment for reduction of hyperpigmentation of Hori's nevus in Institute of dermatology, Thailand

1.4 Research Framework

Patient in Institute of dermatology, Thailand who was diagnosed as Hori's nevus

↓

Treatment with intralesional intradermal platelet-rich plasma

↓

Growth factors in platelet alpha granule help reduce in melanin production via transduction signaling pathway

↓

Also, platelet-rich plasma induced melanin phagocytosis which result in reducing persistent dermal melanin

↓

Improvement in hyperpigmented lesion of Hori's nevus

1.5 Definition of Terms

Melanocyte "Cells that reside predominatly in the epidermis and synthesize melanin whose primary function is to absorb and block the sun's damaging ultraviolet light (UV)"

Melanin "Skin pigment within melanocytes which protect the skin from UV"

Melanogenesis "The process of melanin synthesis"

Dermal fibroblast "The central cells of the dermis which secrete collagen"

Dermal mast cell "The cells which play an important role in allergic tissue inflammation and secrete histamine"

Macrophage "The cells which are involved in the elimination of invading microbes"

Melanophage "Specialized macrophages which canengulf melanocyte fragments and melanin"

Phagocytosis "Ingestion and killing of cells"

Tyrosinase "Enzyme that regulate the production of melanin"

Chapter 2

Literature Review

2.1 What is Hori's nevus?

Acquired bilateral nevus of Ota-like macules or Hori's nevus is an acquired pigmentary disorder commonly found in middle-aged Asian women. Hori et al. were the first to describe it in 1984 (Hori, Kawashima, Oohara, & Kukita, 1984). Clinically, Hori's nevus is characterized by bilateral multiple blue-brown and/or slate-gray macules on the malar regions (68%) or less commonly forehead (1.9%), upper eyelids (1.2%), temple (3.2%) and root and alar of nose (3.7%). Discrete brown macules were the most common presentation in early stage, whereas confluent slate-gray macules present in late stage (Ee, Wong, Goh, & Ang, 2006; Park et al., 2009). Hori's nevus is clinically similar to nevus of Ota. On the other hand, Hori's nevus is not observed in the conjunctiva and mucosa (Manuskiatti et al., 2003). Most patients were Fitzpatrick skin type IV (89%) and Fitzpatrick skin type III (17%) (Ee et al., 2006). Histologically, actively melanin-synthesizing dermal melanocytes are found in the papillary and middle layers of the dermis (Manuskiatti et al., 2003).

2.1.1 Pathogenesis of Hori's nevus

Apparently, pathogenesis of Hori's nevus is still unclear but there were several studies and assumptions mentioned about it.

2.1.1.1 Drooping off of epidermal melanocytes

Falling off of epidermal melanocytes from the basal layer of epidermis into the dermis (Hori et al., 1984; Kaur et al., 2020).

2.1.1.2 Migration of hair bulb melanocytes

Relocation of follicular bulb melanocytes into the dermis (Hori et al., 1984; Kaur et al., 2020)

2.1.1.3 Reactivation of preexisting dermal melanocytes

The manifestation of latent dermal melanocytosis as a result of several triggering factors including dermal inflammation, atrophy of dermis and epidermis, aging, ultraviolet exposure, hormonal change, pregnancy and genetic factors (Hori et al., 1984; Kaur et al., 2020).

2.1.1.4 Increase of melanogenic cytokines in dermis from dermal fibroblast

Dermal fibroblast was stimulated by dermal inflammation, aging and sun exposure. Then, releasing melanogenic cytokines which are dermal stem cell factor, c-kit receptor, hepatocyte growth factor and dermal mast cells (Lee et al., 2011).

1) Dermal stem cell factor (SCF)/c-kit: SCF is one of dermal melanogenic paracrine networks and c-kit is SCF's receptor. When dermal fibroblast was activated from dermal inflammation, aging and UV irradiation, expression of dermal SCF/c-kit are increased. In Lee J.Y.et al study, dermal SCF and c-kit expression were significantly increased in the histologic dermal lesions of patients with Hori's nevus. The SCF/c-kit pathway is the paracrine linkages between dermal fibroblasts and dermal melanocytes. SCF/c-kit will enhance activity of mitogen activated protein kinase pathway (MAPK) which result in increased expression of micropthalmia associated transcription factor (MITF). This sequentially increase tyrosinase-related protein 1 and 2 (TRP-1,-2), and tyrosinase activity causing more melanin production (Fu et al., 2020)

2) Hepatocyte growth factor (HGF): HGF is also secreted by dermal fibroblast. It stimulated melanocyte proliferation in vitro and in vivo. In immunohistochemistry, HGF expression is also increased in lesional dermis. Increase

in HGF expression is a pathogenesis of Hori's nevus due to HGF signaling stimulate migration of melanocytes from epidermis to dermis (Lee et al., 2011).

3) Dermal mast cells: Due to SCF is a growth factor for mast cells, increasing in SCF/c-kit expression also increase dermal mast cell. In Lee J.Y.et al study, dermal mast cell expression was also significantly increased in the histologic dermal lesions of patients with Hori's nevus. Dermal mast cell released histamine which is a substance that induce the proliferation and migration of melanocyte (Hofny, Hussein, Ghazally, Ahmed, & Abdel-Motaleb, 2019; Lee et al., 2011).

2.1.2 Triggering factors of Hori's nevus

Triggering factors of Hori's nevus include hormonal medication (6.2%), pregnancy (19.3%), chronic sun exposure (27.3%), stress (0.6%) and trauma (0.6%). Some patients didn't have any risk factors (37.3%). Chronic sun exposure and hormonal fluctuation during pregnancy have been reported to cause the reactivation of latent melanocytes in the dermis. In addition, UV irradiation is believed to induce melanogenesis via increase expression of tyrosinase activity by melanocyte-stimulating hormone (Ee et al., 2006).

2.1.3 Measurement of Hori's nevus severity

Hori's nevus has no reliable measurement scale comparable to the MASI score for melasma. Vinay, K., Dabas, G., Parsad, D., & Kumaran, M. S. have developed the validate quantitative scale for acquired dermal macular hyperpigmentation (ADMH) which includes lichen planus pigmentosus (LPP), Riehl's melanosis (RM), pigmented contact dermatitis (PCD), and erythema dyschromicum perstans (EDP). The scale was called dermal hyperpigmentation score and severity index (DPASI) which was calculated by 2 x (percentage of forehead x grade) + 2 x (percentage of right cheek involvement x grade) + 2 x (percentage of left cheek involvement x grade) + 1 x (percentage of central face involvement x grade) + 1.5 x (percentage of right neck involvement x grade) + 1.5 x (percentage of left neck

involvement x grade). The severity of disease is graded by grade 0: no change in color/normal pattern in dermoscopy; grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy; grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy; grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy; grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy (Vinay, Dabas, Parsad, & Kumaran, 2018)

In 2019, Kumaran, M. S., Dabas, G., Vinay, K., & Parsad, D. aimed to validate the proposed ADMH severity scale (DPASI) by evaluating its reliability, validity, and usability. They concluded the DPASI is a reliable measure of ADMH severity comparative to physician global assessment score (Kumaran, Dabas, Vinay, & Parsad, 2019).

Hori's nevus, on the one hand, is also an acquired dermal hyperpigmentation. In contrast, Hori's nevus is less common on forehead and neck. Hence, in this study we modified the DPASI scale into modified dermal hyperpigmentation score and severity index (mDPASI) by adjust the multiplication factor in DPASI score (Figure 2.1).

mDPASI = 4 x (percentage of right cheek involvement x grade) + 4 x (percentage of left cheek involvement x grade) + 2 x (percentage of central face involvement x grade. The score ranges from 0 to 40.

The severity of disease is graded by grade 0: no change in color/normal pattern in dermoscopy; grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy; grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy; grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy; grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy.

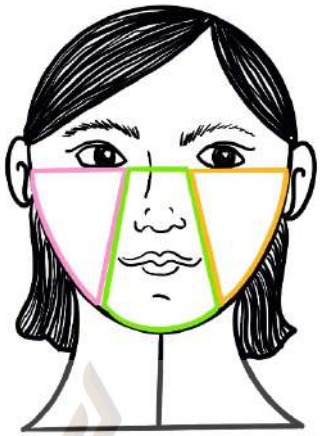

Figure 2.1 Area division of face based on mDPASI score. Area which commonly have Hori's nevus is divided in to three segments – right cheek, left cheek all of which constituting 40% area each and central face which constitute 20% of area.
Source: Vinay et al., 2018

2.2 Current treatment of Hori's nevus

2.2.1 Laser and light therapy

2.2.1.1 Picosecond alexandrite laser (PSAL)

Yu, W., Zhu, J., Yu, W., Lyu, D., Lin, X., & Zhang, Z. assessed the effectiveness of PSAL and QSAL in a randomized, split-face, single-blind study (n=30). Each patient receives PSAL (750 ps, 2-2,5mm, 4.07-6.37 J/cm^2, 2.5 Hz, single pass without overlapping) on one side of the face; and QSAL (70 ns, 3mm, 6.0-8.0 J/cm^2, 2 Hz, single pass without overlapping) on another side of the face at intervals of six months, for three sessions. According to the study, PSAL is more effective and secure than QSAL. Quartile improvement scale score were 3.73 ± 0.521 for the PSAL group and 2.4 ± 0.894 for the QSAL group. The PSAL treated side had lower PIH rates and a greater patient satisfaction rating with average discomfort during treatment (Yu, Zhu, Yu, Lyu, Lin, & Zhang, 2018).

2.2.1.2 Q-switched Nd:YAG laser (QSNL)

QSNL 1064 nm with higher fluence 8-10 J/cm^2 was applied on the lesion (pinpoint bleeding was taken as the end-point), 97% of patients showed 100% clearance of the lesions with mean 2.8 sessions of the treatment. The side effect was transient hyperpigmentation (Kunachak & Leelaudomlipi, 2000).

Another study of QSNL 1064 nm with lower fluence 2.2-2.6 J/cm^2 was done 2-3 passes on the lesion of Hori's nevus, followed by 2-3 passes or until fine petechiae appeared at fluence 4-6 J/cm^2 for 3-8 sessions at 1-2 weeks interval. Overall patient satisfaction was satisfied. There was no evidence of post-laser hypo or hyperpigmentation (Cho, Park, Kim, & Bu, 2009).

2.2.1.3 Q-switched alexandrite laser (QSAL)

Effectiveness of QSAL in therapy of Hori's nevus was evaluated in a retrospective study by Lam, A. Y., Wong, D. S., Lam, L. K., Ho, W. S., & Chan, H. H. in 2001. Each patient (n=32) received QSAL (755 nm, 3 mm spot size, 100ns pulse duration, 8 J/cm^{2}fluence and 7 mean treatment sessions, with 33 days treatment interval). Eleven patients showed complete resolution of Hori's nevus. However, sixteen patients developed hypopigmentation, four patients developed post-laser hyperpigmentation, and thirteen patients developed transient erythema. The degree of clearance varied according to the number of treatment sessions (Lam, Wong, Lam, Ho, & Chan, 2001).

QSAL is effective in the treatment of Hori's nevus, but the most common side effects are erythema and post-laser hypo or hyperpigmentation (Kaur et al., 2020).

2.2.1.4 Q-switched ruby laser (QSRL)

QSRL (694 nm, 25 ns, 3-4 mm, 4.5-6 J/cm^2, 10 sessions with 3-4 weeks interval) was treated on the lesion of Hori's nevus. The lesions were significant improved after sixth month follow-up (Lee, Nam, Cha, Park, & Kim, 2018).

The side effect was skin color change, stinging sensation. Bleb formation and transient hyperpigmentation. Some cases developed prolong and persistent hyperpigmentation (Kunachak, Leelaudomlipi, & Sirikulchayanonta, 1999).

2.2.2 Dermabrasion

Kunachak et al. investigated dermabrasion as a treatment option for Hori's nevus. 97 percent of patients received a full recovery. No resurrence was seen at 1-7 years follow-up (Kunachak et al., 1996).

2.3 Platelet-rich plasma (PRP)

2.3.1 What is platelet-rich plasma?

PRP is an autologous solution of plasma containing 4-7 times of the baseline concentration of human platelets (Leo, Kumar, Kirit, Konathan, & Sivamani, 2015). PRP was first originate in the 80s, when Helena Matras explained the use of fibrin glue which helped repair tissue in oral and maxillofacial surgical operations (Matras, 1982). Then the PRP has become popular in many clinical fields such as sport medicine, dental specialties, orthopedics, plastic surgery, otorhinolaryngology, neuroscience and etc. Recently, PRP has been used in various dermatologic conditions such as acne, alopecia, melasma, and skin ulcers (Merchan, Gomez, Chasoy, Alfonso-Rodriguez, & Munoz, 2019).

2.3.2 Component of platelet-rich plasma

Platelets are cytoplasmic fragments of megakaryocytes. They contain α-granules, dense granules, lysosomes and mitochondria (Kahr, 2009). In α-granules,

there are so many growth factors which are essential for tissue repair (Merchan et al., 2019). In addition, these various growth factors containing in α-granules stimulate cell such as osteoblasts, adult mesenchymal stem cells, endothelial cells, fibroblasts, and epidermal cells. Hence, they induce cellular proliferation, osteoid production, matrix formation, and collagen synthesis (Tuknayat, Bhalla, & Thami, 2021). The growth factors containing in α-granules include transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), and insulin-like growth factor (IGF) (Merchan et al., 2019; Tuknayat et al., 2021). While dense granules contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), calcium, histamine, serotonin and magnesium (Tuknayat et al., 2021). Furthermore, ATP and ADP in dense granules of PRP help stimulate macrophage phagocytosis, which is the main pathogenesis of dermal pigment reduction in Hori's nevus (Sakamoto & Firkin, 1984).

Table 2.1 Growth factors in α-granule and its function

Growth factors	Function
TGF-β	• Inhibits MITF, TRP1&2, tyrosinase • Promotes collagen synthesis
PDGF	• Angiogenic • Macrophage activator • Mitogen for mesenchymal and neuronal cells • Favours the formation of type 1 collagen • Synthesis hyaluronic acid

Table 2.1 Growth factors in α-granule and its function (Cont.)

Growth factors	Function
EGF	• Inhibits tyrosinase & PGE2
FGF	• Removes photodamaged ECM collagen and formation of new collagen
IGF-1	• Stimulates the synthesis of type 1 collagen, alkaline phosphatase and osteocalcin

Source: Merchan et al., 2019

After the whole blood was centrifuged, the blood components are arranged by density in the following order, from the bottom to the top of the tube: the red blood cell, white blood cell, platelet-rich plasma (PRP), platelet-poor plasma (PPP) (Figure 2.2). When considering the effectiveness of PRP, the first thing to be checked is determining the platelet concentration necessary to enhance tissue healing. Normally, in the whole blood platelet counts range from 150,000/μL to 350,000/μL. Most commercially platelet-concentrating machines can be divided into lower platelet concentrating machines (>1x-3x baseline) and higher platelet concentrating machines (>4x-9x baseline). Report recommended that platelet concentration of 2.5x-3x baseline were ideal. For the contamination of RBC, PRP systems producing low platelet concentrations generally contain minimal or no RBCs, whereas highly concentrating systems are allowed to have higher RBC residual (5-15% hematocrit). For the contamination of WBC, it depends on the PRP classification the researcher demand (Mautner et al., 2015).

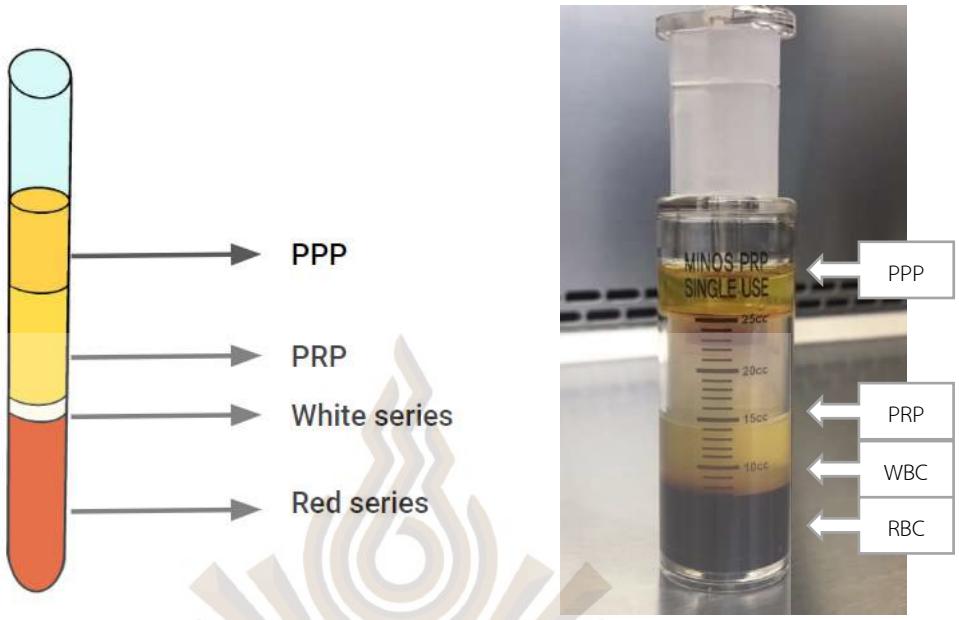

Figure 2.2 Blood component after configured by MINOS PRP tube

Source: Merchan et al., 2019

2.3.3 Platelet-rich plasma preparation

The 18 ml of patient's whole blood is collected in a 20-ml syringe using a butterfly cannula. In the 20-ml syringe, there is the anticoagulant citrate dextrose A (ACD-A) 2 ml. The syringe was then shaken slowly to allow the blood to mix with ACD-A. Then gently put the whole blood with ACD-A in the commercial kit and follow each of the manufacturer guidelines. After centrifugation, the whole blood will give 3-5 ml of PRP depended on the centrifugation device, the technique and the baseline platelet count of an individual.

In this study we choose MINOS PRP tube because it is easy to separate PRP with only kit, separation can be done with only one time of centrifugation, this tube is a close system and it gives us a high concentration of platelets (16x baseline) with less contamination of RBC (9.2%) and WBC (0.1%). The advantage of close system is that PRP is not exposed to the environment during the process of PRP preparation. After

we put the whole blood with ACD-A in MINOS PRP tube, then centrifuge it with Ugaiya Cence L500 table top low speed centrifuge with speed 3500 rpm for 5 minutes. Another advantage of MINOS PRP tube is that we can get maximum PRP with less PPP contamination.

In addition, this study chose acid citrate dextrose as an anticoagulant for the preparation of PRP. The common anticoagulants used for blood preparation include ACD-A, trisodium citrate (TSC), heparin and EDTA. ACD-A has a lower pH and lower extracellular calcium ion concentrate than TSC which is good for fibroblast, growth factor (TGF-β) proliferation and prevention of platelet aggregation (Dashore, Chouhan, Nanda, & Sharma, 2021).

2.3.4 Classification of platelet-rich plasma

Dohan Ehrenfest, D. M., Rasmusson, L., & Albrektsson, T. established a classification of PRP in 2009 based on two basic parameters: the presence or absence of leucocytes and fibrin architecture. This will cause 4 categories of PRP (Ehrenfest, Rasmusson, & Albrektsson, 2009).

2.3.4.1 Pure PRP (P-PRP) or leucocyte- poor PRP: preparation without or low WBC and without fibrin network.

2.3.4.2 Pure platelet-rich fibrin (P-PRF) or leucocyte-poor platelet-rich fibrin: preparation without or low WBC and with high-density fibrin network. When P-PRP is mixed with activator (CaCl2) and allowed to incubate for some time, a stable PRF clot can be collected.

2.3.4.3 Leucocyte-rich PRP (L-PRP): preparation with elevated WBC value and without fibrin network. L-PRP shows similar platelet and growth factors concentrations but higher leucocyte, providing antimicrobial effects, and pro-inflammatory cytokine concentrations compared with P-PRP.

2.3.4.4 Leucocyte-rich and platelet-rich fibrin: preparation with elevated WBC value and with high-density fibrin network. The method is to collect blood without anticoagulant and immediately centrifuged.

Table 2.2 Classification of PRP

	Pure platelet-rich plasma (P-PRP)	Pure platelet-rich fibrin (P-PRF)	Leucocyte-rich PRP (L-PRP)	Leucocyte platelet-rich fibrin (L-PRF)
With WBC	-	-	✓	✓
High density fibrin network	-	✓	-	✓

Source: Dohan Ehrenfest et al., 2009

2.3.5 Platelet-rich plasma in clinical practice

2.3.5.1 PRP with acne and acne scar

PRP can inhibit the growth of *Cutibacterium acnes* in vitro (Intravia et al., 2014). Gómez, L., Romero, V., & Merchan, W. applied PRP every month for three months to the patients who had acne. The results showed decrease in both inflammation and scar formation by acne (Gómez, Romero, & Merchan, 2017). Asif, M., Kanodia, S., & Singh, K. used PRP with microneedling in patient with atrophic scar from acne. The results show that the PRP combination with the microneedle is more effective than only microneedling (Asif, Kanodia, & Singh, 2016).

2.3.5.2 PRP with alopecia

Shetty, V. H., & Goel, S. injected PRP every 3 week for 3 months in patients who have androgenetic alopecia. The results were evaluated by using dermoscopy at baseline and at 3 months. Patients' hair counts, hair diversity improved

significantly after treatment. Also, the patient's hair growth assessment score improved by 50-70 percent (Shetty & Goel, 2019)

PRP is rich in growth factors, which promote dermal papilla cell differentiation and growth through various signaling pathways. PDGF, TGF, VEGF, and IGF are the main growth factors involved in androgenetic alopecia (Gkini, M. A., Kouskoukis, A. E., Tripsianis, G., Rigopoulos, D., & Kouskoukis, K., 2014). These growth factors promote the transdifferentiation of hair and stem cells, which results in the formation of new follicular units (Shetty & Goel, 2019).

2.3.5.3 PRP in skin rejuvenation

There is a systematic review of the safety and effectiveness of PRP for skin aging and rejuvenation. Injection PRP alone has been shown to temporarily improve facial skin appearance, texture, and lines. Fine lines and pigmentation around the eyes may also benefit. This paper summarized that PRP injections are safe and may have a minor benefit for aging skin (Maisel-Campbell et al., 2020).

2.3.5.4 PRP in periorbital hyperpigmentation

One study assessed the efficiency of prp in the treatment of periorbital hyperpigmentation, with significant changes obtained in all 50 patients after three sessions (Al-Shami, 2014). Another study was done by Mehryan, P., Zartab, H., Rajabi, A., Pazhoohi, N., & Firooz, A., using PRP intradermal injections on ten patients, and the results show an improvement in infraorbital color homogeneity from the first session (Mehryan, Zartab, Rajabi, Pazhoohi, & Firooz, 2014).

2.3.5.5 PRP in melasma

Cayirli, M., Caliskan, E., Acikgoz, G., Erbil, A. H., & Erturk, G. had injected PRP in both melasma lesion in patient for 3 times every 2 weeks. The results

showed that more than 80% reduction in hyperpigmentation (Cayirli, Caliskan, Acikgoz, Erbil, & Erturk, 2014).

Sirithanabadeekul conducted a randomized. Controlled, split-face trial in patients with melasma. In this study, they administered intradermal PRP injection on one-sided face and another-sided face was injected with normal saline. The injections were done every 2 weeks for 4 sessions. After one-month follow-up the PRP-injected-side showed an improvement in mMASI score and melanin level (Sirithanabadeekul, Dannarongchai, & Suwanchinda, 2020).

Tuknayat et al. treated melasma with PRP intradermally injection monthly for 3 sessions. At the third month follow-up, the mMASI score reduced 54.5%, patients' satisfaction score was up to 90%, and there was no recurrence during the period of the study. (Tuknayat et al., 2021)

There was a systematic review on efficacy and safety of PRP in melasma done by Zhao L et al. It proved that PRP can significantly reduce mMASI in melasma and could be a promising therapy for melasma with less serious side effect (Zhao, Hu, Xiao, Zhou, Li, Xiong, & Li, 2021).

2.3.5 Platelet-rich plasma in Hori's nevus

Firstly, Kim et al. had done research on TGF-β1's role in melanogenesis by using a preserved mouse melanocyte cell line, Mel-Ab. The results show that TGF-β1 significantly inhibit melanogenesis in a dose-dependent way, subsequentially, reduce tyrosinase activity via down regulation of micropthalmia associated transcription factor (MITF) pathway. To summarized, TGF-β1 reduce MITF activity, tyrosinase, tyrosinase-related protein-1 and 2 productions. Moreover, TGF-β1 also delay activation of extracellular signal-regulated kinase (ERK) which contribute to MITF down-regulation to decrease melanin production (Kim, Park, & Park, 2004).

Additionally, Tuknayat et al. evaluated the effectiveness of PRP in treating melasma and discovered that TGF- β1 in PRP can significantly inhibit MITF and the paired-box homeo-c gene (PAX3), hence reducing tyrosinase activity and reducing melanogenesis (Tuknayat et al., 2021).

Secondly, according to Yun et al., EGF reduces melanogenesis via inhibiting prostaglandin 2 (PGE2) which is also activate tyrosinase through cAMP signaling pathway and phospholipase C (PLC) (Fu et al., 2020). Additionally, this research demonstrated that normal human melanocytes contain EGF receptors and responded to EGF via ERK signaling. Recently, EGF has been utilized as a whitening agent in dermatologic practice to alleviate PIH caused by laser or UV light (Yun et al., 2013).

Thirdly, fibroblast growth factor (FGF), a component of PRP, also aids in the removal of photodamaged ECM collagen and promotes the production of new collagen. Furthermore, platelet-derived growth factor (PDGF) stimulated the production of new collagen and hyaluronic acid. The hyperpigmented lesion appears more radiant as the skin's volume grows (Tuknayat et al., 2021).

Fourthly, a study on the components of PRP discovered that macromolecular activators of phagocytosis from platelets (MAPP), which stimulates Fc receptors and induces macrophage, melanophage, dermal dendritic cells, and neutrophil phagocytosis activity (Czakai et al., 2017; Ogawa et al., 2000; Sakamoto et al., 2011) can also reduce latent dermal melanocyte fragment and melanin granules. (Sil, Wong, & Martinez, 2018) Additionally, ATP and ADP in dense granules of PRP contribute to accelerated macrophage phagocytosis, which is the main pathogenesis for reducing dermal pigmentation in Hori's nevus (Sakamoto & Firkin, 1984). As a result, PRP can help to minimize dermal hyperpigmentation.

Finally, phagocytosis of macrophage can also reduce cell debris from epidermal and dermal degeneration from aging process (Sil et al., 2018). This dermal degradation caused dermal inflammation and dermal inflammation via SCF/c-kit and mast cell degranulation (Hofny, Hussein, Ghazally, Ahmed, & Abdel-Motaleb, 2019;

Lee et al., 2011) which is the pathogenesis of Hori's nevus (Hori et al., 1984). It is a reasonable assumption that minimizing dermal degradation can also reduce dermal inflammation, SCF/c-kit, and mast cell degranulation, which will limit melanogenesis, melanocyte proliferation and migration and dermal hyperpigmentation.

To summary, PRP is believed to be a new promising therapy among these theories which can be compared with LASER and dermabrasion as showns in table 2.3.

Table 2.3 Advantages and disadvantages of the current treatments of Hori's nevus and platelet-rich plasma

Treatment	Advantage	Disadvantage
LASER	Several supported studies	Post-inflammatory hyper-, hypopigmentation Downtime Several sessions required Expensive cost
Dermabrasion	Studies showed fully recovery with no recurrence	Post-inflammatory hyper-, hypopigmentation Downtime Infection
PRP	Less side effect included post-inflammatory hyper-, hypopigmentation Cheaper cost No downtime Safe	Several sessions required

2.3.5 Side effect of platelet-rich plasma

PRP can cause temporary adverse effects such discomfort where it is injected, edema, transient erythema, and bruises, all of which are self-limiting and resolved in a few days (Tuknayat et al., 2021).

Chapter 3

Research Methodology

3.1 Population and Samples

3.1.1 Population

Patient who was diagnosed as Hori's nevus in Institute of dermatology, Thailand.

3.1.2 Sample size

Due to the lack of research on the effectiveness and safety of intradermal platelet-rich plasma injection for the treatment of Hori's nevus on a national and worldwide scale, this study was done as a pilot study with a sample size of 10.

3.1.3 Inclusion criteria

Patients between the ages of 25 and 65 with bilateral Hori's nevus.

3.1.4 Exclusion criteria

1) Pregnancy and breastfeeding

2) History of PRP allergy

3) History of bleeding disorder such as thrombocytopenia (<100,000 platelets/μL) or thalassemia

4) History of blood-borne disease include hepatitis B, hepatitis C or HIV

5) Patient who currently on anti-oxidant supplement such as vitamin C, vitamin E within three months prior to the study

6) Patient who got chemotherapy and anti-coagulant within three months prior to the study

7) Patient who had done LASER or IPL within three months prior to the study

8) Patient who concomitant use of whitening agents/chemical peeling within three months prior to the study

9) Patient who significantly had co-existing hyperpigmented lesion except Hori's nevus

10) Patient who has any active skin disease on the PRP injected area such as infection, skin cancer, dermatitis, and etc.

3.2 Research Instruments

3.2.1 Platelet-rich plasma

3.2.1.1 PRP extraction tube

We used MINOS PRP tube from neogenesis, South Korea which was distributed in Thailand by Gibthai company. This tube is designed for PRP separation specifically. It is a closed system which can reduce contamination from environment. By doing a single centrifugation, it is able to isolate the buffy coat layer, concentrated platelets, and the necessary amount of plasma. In addition, Minos tube offers 16–20 times the concentration ratio of comparable PRP kits (4-6 times)

3.2.1.2 Centrifuge

Ugaiya Cence L500 table top low speed centrifuge.

3.2.1.3 Anti-coagulant

Acid citrate dextrose A (ACD-A)

3.2.1.4 Instrument for PRP preparation

1) 20-ml syringe
2) 10-ml syringe
3) 3-ml syringe
4) 23g x ¾” scalp vein
5) 30g x 13 mm needle
6) Tourniquet
7) 70% alcohol

3.2.1.5 Instrument for patient’s preparation

1) Topical lidocaine
2) Cleansing

3.2.2 Mexameter®

In this study we use Narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electronic GmbH) to assess melanin index on the lesional area.

3.3.3 Canfield Visia-CR System®

We used Canfield Visia-CR System® to record the picture of the patient before and after treatment.

3.3 Data Collection

3.3.1 Research design

This is a pilot study, prospective trial to evaluate the effectiveness of intradermal PRP injection in the treatment of Hori’s nevus. Ten patients with bilateral

Hori's nevus were enrolled in the trial. All of the patients were injected PRP every 2 weeks for 4 sessions to both lesional face (week 0, 2, 4, 6 of the study). After each injection, the researcher will call each patient to ask about the side effect occurred on the injected side on day 1 and day 3 post-treatment. Then we follow-up on week 8, 10, 14, 18 of the study.

3.3.2 Method

3.3.2.1 After the study was approved by the ethics committee, the researcher will recruit the volunteers who were between 25 – 65-year-old and diagnosed with Hori's nevus in Institute of dermatology, Thailand

3.3.2.2 The researcher uses inclusion and exclusion criteria to include and reject volunteers.

3.3.2.3 The researcher with give two expert dermatologists, who are not involved in the study, pictures of 20 different Hori's nevus cases to determine inter-rater reliability of the experts on outcome measurement (mDPASI score and brightening score) before starting the trial. Then, we calculated an intraclass correlation coefficient (ICC) which should be more than 0.75. The researcher will arrange a meeting with both experts to review the criteria and reevaluate if the ICC is less than 0.75.

3.3.2.4 Before the trial begins, the researcher will arrange a meeting with the volunteers to obtain their informed consent.

3.3.2.5 On the day of the appointment, the researcher will gather the general data relevant to the study, including gender, underlying diseases, Fitzpatrick skin type, and risk factors for Hori's nevus such as history of hormonal used, pregnancy, and unprotected sun exposure.

3.3.2.6 Before beginning the treatment, all of the volunteers will visit with both experts to determine baseline mDPASI. On the same day, the volunteers will

also analyze the melanin index using a narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electrical GmbH), and will use the Canfield Visia-CR System to capture a picture of the whole face (week 0 of the study). Before beginning any of these procedures, the volunteers will be instructed to remove all of their makeup and wash their faces with water and facial cleansers.

3.3.2.7 PRP was injected into both lesional facial areas of each patient four times, at intervals of every two weeks (week 0, 2, 4, 6 of the study).

3.3.2.8 Each session includes the following steps.

The participants will be advised to remove all makeup and wash their faces with water and facial cleansers. They will next apply topical anesthesia cream, occlude the lesion on both sides for 45 minutes, and then wash it off to get completely dry skin.

The blood will be collected from the subjects using a 23g x 34" scalp vein for 18 milliliters and placed in a syringe with ACD-A for 2 milliliters, for a total of 20 milliliters. The blood is then transferred to the MINOS PRP kit and centrifuged for 5 minutes at 3500 rpm to extract the PRP. The mixture is then separated into four layers, from bottom to top, which are RBC, WBC, PRP, and PPP. After that, the research will extract PRP from the kit.

With a 30g needle, PRP was intradermally injected at the Hori nevus on both sides of the face. Each injection points are 1 cm apart and 0.5-1 cm in diameter.

After finishing PRP injection, the volunteers will be cleaned their face with sterile pads and can be able to apply sunscreen after the treatment 12-24 hours. They will be advised to avoid sunlight, heat, and irritation to the face.

3.3.2.9 The researcher will contact the participants through Line video call following the injection to examine the side effects that occurred on day 1 and day 3.

3.3.2.10 The participants will receive sunscreen and moisturizer throughout the trial duration. They will be told to use sunscreen every morning and, if possible, every two hours while being outside. Additionally, use a certain moisturizer twice daily, in the morning and at night.

3.3.2.11 The participants will be assigned to evaluate the effectiveness of PRP four times following the last PRP session, at two weeks, four weeks, eight weeks, and twelve weeks (week 8, 10, 14, and 18 of the study). The following steps are included in each session. To determine the mDPASI and brightening score, two expert dermatologists must first be consulted. Second, utilizing the narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electrical GmbH) to measure the melanin index. Third, the Canfield Visia-CR System is then used to take a photograph of the entire face. Last, evaluate patient self-assessment score. All of the results will be documented in case record forms. The primary goal of this study is to compare the outcomes at baseline (week 0 of the study) and twelve weeks after therapy (week 18 of the study). The two-week, four-week, and eight-week post-treatment follow-ups (week 8, 10, and 14 of the study) were used to determine the progression of improvement.

Table 3.1 The participant's activity

Week / Activity	0	2	4	6	8	10	14	18
Collecting general information	✓							
Taking photos	✓				✓	✓	✓	✓
Measuring melanin index	✓				✓	✓	✓	✓
Meeting two experts to determine mDPASI	✓				✓	✓	✓	✓
Meeting two experts to determine Brightening score					✓	✓	✓	✓
Meeting the researcher for injecting PRP	✓	✓	✓	✓				
Evaluating Patient self-assessment					✓	✓	✓	✓
Evaluating side effect	✓*	✓*	✓*	✓*	✓	✓	✓	✓

Note: ✓* is day 1 and day3 following the injection, the researcher will contact the participants through Line video conversation to investigate the adverse effects that happened. Taking photos by the Canfield Visia-CR System. Measuring melanin index by using the narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electrical GmbH)

3.3.3 Outcome measurement

3.3.3.1 Primary outcome

The researcher used Modified Dermal Pigmentation Area and Severity Index (mDPASI) as a primary outcome to assess the effectiveness of PRP in the treatment of Hori's nevus. mDPASI is a scoring the researcher modified from Dermal Pigmentation Area and Severity Index (DPASI) to evaluate the severity of Hori's nevus which is calculated by this following. (Vinay et al., 2018)

mDPASI = 4 x (percentage of right cheek involvement x grade) + 4 x (percentage of left cheek involvement x grade) + 2 x (percentage of central face involvement x grade

The score ranges from 0 to 40.

The severity of disease is graded by grade 0: no change in color/normal pattern in dermoscopy; grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy; grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy; grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy; grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy (Vinay et al., 2018).

The mean mDPASI of two expert dermatologists will be compared between baseline (week 0 of the study) and the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study)

3.3.3.2 Secondary outcome

1) Mean melanin index

Melanin index was measured by the narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electrical GmbH) which is be compared between baseline (week 0 of the study) and the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study). The researcher will measure two representative points on right and left cheek marked with a permanent marker on a flexibly, plastic sheet. (Manuskiatti et al., 2003) Each point will measure three times and calculate the mean of both points.

2) Brightening score

The brightening score was evaluated by two expert dermatologist which was graded as 4 levels: 0-25%; no change to slight lightening, >25-50%; moderate lightening, >50-75%; marked lightening, >75-100%; near normal skin. (Hofny, Abdel-Motaleb, et al., 2019)

The mean brightening score of two expert dermatologists will be collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study)

3) Patient self-assessment score

The patient self-assessment was graded as 5 levels by using quartile grading system as: 0 = no improvement; 1= slight improvement (<25%); 2 = moderate improvement (25-50%); 3 = marked improvement (50-75%); 4 = near normal skin (>75%). Score will be collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups. (Manuskiatti, 2003)

The patient self-assessment score will be collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study)

4) Side effects

Side effect which the researcher interested were pain score grading by 0 to 10, edema score grading by: 0 = no edema; 1 = mild edema; 2 = moderate edema; 3 = severe edema, erythema (presence or absence), hyperpigmentation (presence or absence), infection (presence or absence; if presence determined whether it is from bacteria; virus; or fungus), transient ischemia (presence or absence), bruise (presence or absence), papules (presence or absence), pustules (presence or absence).

On each session, short-term side effect data will be collected on day 1 and day 3 post-treatment by Line video call. The long-term side effect will be collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study)

3.4 Data Analysis

All statistical analyses will be performed by using SPSS.

3.4.1 General information of patient

Quantitative data will be presented as mean, median, and interquartile range for continuous data and percentage for discrete data. Quantitative data will be presented as a percentage.

3.4.2 Primary outcome

Modified Dermal Pigmentation Area and Severity Index (mDPASI) will be reported as means between two expert dermatologists.

3.4.3 Secondary outcome

1) Mean melanin index will be reported as means

2) Brightening score will be reported as means between two expert dermatologists.

3) Patient self-assessment score will be reported as frequency and percentage.

4) Side effects will be reported as frequency and percentage.

3.4.4 Comparative of outcome between baseline and post-treatment

The researcher will use mean mDPASI between two expert dermatologists and mean melanin index to compared between baseline (week 0 of the study) and the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study).

If the data are in a normal distribution, a paired t test will be performed to determine the significance of the improvements of the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study). compared with the baseline (week 0 of the study). A P-value of 0.05 or less was regarded as statistically significant.

If the data are not in a normal distribution, Wilcoxon signed-rank test will be performed.

3.4.5 Comparative tendency of improvement between different timepoint of post-treatment-follow-up

Tendency of improvement between the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study) will be used Reapeated ANOVA to evaluate the data if the data are normal distribution. If the data are not in a normal distribution, Friedman test will be performed.

3.4.6 Inter rate reliability

Before the trial begins, the researcher will show two expert dermatologists who are not participating in the study images of 20 different examples of Hori's nevus to assess the inter rater reliability of the experts on outcome measurement (mDPASI score and brightening score). Following that, we determined an intraclass correlation coefficient (ICC), which should be more than 0.75. If the ICC is less than 0.75, the researcher will schedule a meeting with both experts to review the criteria and reevaluate.

Chapter 4

Research Results

4.1 Demographic data

This investigation enrolled ten female patients with acquired bilateral nevus of Ota-like macules.

Each participant completed the protocol. The average age was 32.8 years old with Fitzpatrick skin type III (%) and IV (%). Table 4.1 displayed the demographic information in further depth.

Table 4.1 Demographic data

		Count (%)	Mean ± SD
Sex	Female	10(100%)	
Age (year)			33 ± 5
Duration (year)			6.9 ± 1.5
Onset (year)			25.9 ± 1.4
Underlying disease	None	7 (70%)	
	Hypertension	1 (10%)	
	Dyslipidemia	1 (10%)	
	Migraine	1 (10%)	
	Allergic rhinitis	1 (10%)	
Fitzpatrick	III	4 (40%)	
	IV	6 (60%)	

Table 4.1 Demographic data (Cont.)

		Count(%)	Mean ± SD
Hormonal use	No	7 (70%)	
	OCP	3 (30%)	
History of pregnancy	No	8 (80%)	
	Yes	2 (20%)	
Area			
Forehead	No	10 (100%)	
Both cheek	Yes	10 (100%)	
Central face	No	6 (60%)	
	Yes	4 (40%)	
Severity	Mild	3 (30%)	
	Moderate	4 (40%)	
	Severe	3 (30%)	
	Very severe	0 (0%)	

4.2 Primary outcome

All enrolled patients had been evaluated modified dermal pigmentation area and severity index (mDPASI) by two-independent dermatologists to determine the improvement at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups (week 8, 10, 14 and 18 of the study)

Twelve weeks after treatment, the mean mDPASI between two expert dermatologists decreased substantially from 0.929 ± 0.617 at baseline to 0.568 ± 0.414 ($p = 0.001$). Although the maximum reduction in mDPASI is observed at four weeks post-treatment (p-value = 0.001), mDPASI levels at eight- and twelve-weeks post-treatment are still significantly reduced from baseline (p-value = 0.003), whereas the increase in mDPASI from four-weeks post-treatment is not statistically significant (p-value > 0.05).

Table 4.2 mDPASI results of each patient

	Baseline	2-wk post PRP (% decrease)	4-wk post PRP (% decrease)	8-wk post PRP (% decrease)	12-wk post PRP (% decrease)
Patient 1	1.22	0.56 (54.09)	0.56 (54.09)	0.54 (55.74)	0.63 (48.36)
Patient 2	0.24	0.17 (29.16)	0.15 (37.5)	0.14 (41.67)	0.14 (41.67)
Patient 3	1.7	1.2 (29.41)	1.08 (36.47)	1.2 (29.41)	1.2 (29.41)
Patient 4	0.96	0.64 (33.33)	0.64 (33.33)	0.84 (12.5)	0.88 (8.33)
Patient 5	0.86	0.48 (44.18)	0.46 (46.51)	0.58 (32.56)	0.7 (18.60)
Patient 6	1.98	1.28 (35.35)	1.24 (37.36)	1.04 (47.47)	1.12 (43.43)
Patient 7	0.82	0.3 (63.41)	0.23 (71.95)	0.2 (75.61)	0.3 (63.41)
Patient 8	1.14	0.6 (47.37)	0.54 (52.63)	0.54 (52.63)	0.54 (52.63)
Patient 9	0.15	0.09 (40)	0.07 (53.33)	0.07 (53.33)	0.05 (66.67)
Patient 10	0.22	0.17 (22.73)	0.12 (45.45)	0.16 (27.27)	0.12 (45.45)

Table 4.3 mDPASI results summary of all patients

	Mean (SD)	Mean difference* (SD)	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	*P value*
mDPASI baseline	0.929 (0.617)				
mDPASI 2-wk post PRP	0.549 (0.412)	0.380 (0.247)	0.203	0.557	0.001
mDPASI 4-wk post PRP	0.509 (0.399)	0.420 (0.258)	0.235	0.604	0.001
mDPASI 8-wk post PRP	0.531 (0.396)	0.398 (0.311)	0.176	0.620	0.003
mDPASI 12-wk post PRP	0.568 (0.414)	0.361 (0.284)	0.158	0.564	0.003

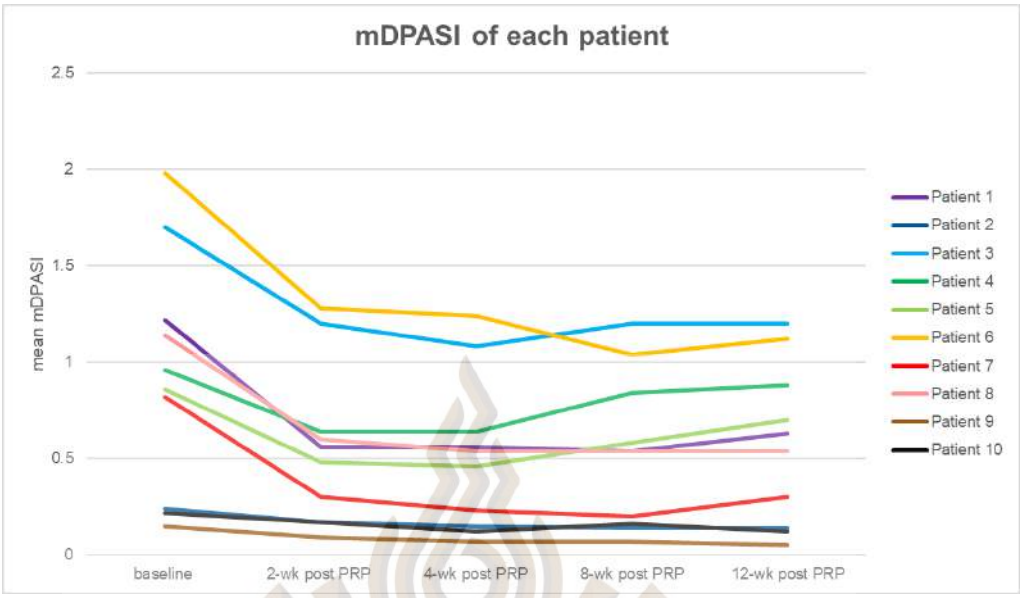

Figure 4.1 Mean mDPASI at baseline, two-week, four-week, eight-week, and twelve-week post PRP treatment of each patient

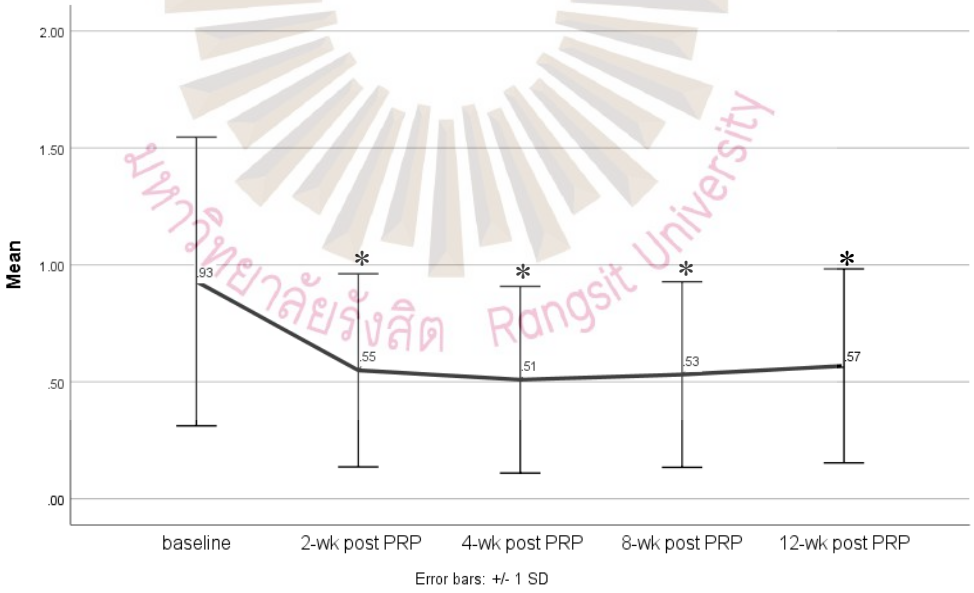

Figure 4.2 mean ± SD of mDPASI at baseline, two-week, four-week, eight-week, and twelve-week post PRP treatment *(* P <0.05)*

4.3 Secondary outcome

4.3.1 Mean melanin index

All enrolled participants were evaluated melanin index which was measured by the narrow-band reflectance spectrophotometer (Mexameter® MX18; Courage + Khazaka electrical GmbH)

It is statistically significant that the mean value of the melanin index decreased from 208.650 ± 26.319 at baseline to 182.052 ± 17.027 twelve-weeks after treatment. The maximum mean melanin index reduction is at eight-week posttreatment which is 181.199 ± 19.690. At twelve-week posttreatment seem to have an elevation of mean melanin index according to eight-week follow-up though it is not statistically significant (p-value > 0.05).

Table 4.4 Mean melanin index results of each patient

	Baseline	2-wk post PRP (% decrease)	4-wk post PRP (% decrease)	8-wk post PRP (% decrease)	12-wk post PRP (% decrease)
Patient 1	208.83	195.16 (6.55)	188.66 (9.66)	179.83 (13.88)	180.17 (13.72)
Patient 2	173.67	162.66 (6.34)	157.83 (9.12)	159.83 (7.97)	159.17 (8.35)
Patient 3	218.00	211.50 (2.98)	182.00 (16.51)	172.33 (17.96)	182.50 (16.28)
Patient 4	231.33	200.00 (13.54)	201.00 (13.11)	199.67 (13.68)	197.17 (14.77)
Patient 5	182.50	173.17 (5.11)	169.00 (7.40)	170.83 (6.39)	167.50 (8.22)

Table 4.4 Mean melanin index results of each patient (Cont.)

	Baseline	2-wk post PRP (% decrease)	4-wk post PRP (% decrease)	8-wk post PRP (% decrease)	12-wk post PRP (% decrease)
Patient 6	262.83	251.00 (4.50)	230.80 (12.19)	224.50 (14.58)	218.17 (16.99)
Patient 7	197.50	186.17 (5.74)	167.60 (15.14)	179.83 (8.95)	188.17 (4.73)
Patient 8	217.67	167.50 (23.05)	165.33 (24.04)	162.17 (25.50)	165.67 (23.89)
Patient 9	209.67	200.50 (4.37)	200.83 (4.22)	193.00 (7.95)	184.83 (11.85)
Patient 10	184.50	179.83 (2.53)	174.00 (5.70)	170.00 (7.86)	177.17 (3.97)

Table 4.5 Mean melanin index results of all patients

	Mean (SD)	95% Confidence Interval			
		Mean difference* (SD)	Lower Bound	Upper Bound	P value
MI baseline	208.650 (26.319)				
MI 2-wk post PRP	192.749 (25.819)	15.90 (14.07)	5.83	25.97	0.006
MI 4-wk post PRP	183.705 (22.147)	24.94 (13.67)	15.17	34.72	<0.0001
MI 8-wk post PRP	181.200 (19.690)	27.45 (15.17)	16.59	38.30	<0.0001
MI 12-wk post PRP	182.052 (17.027)	26.59 (15.15)	15.76	37.43	<0.0001

*Compare with baseline P≤0.05 was statistically significant

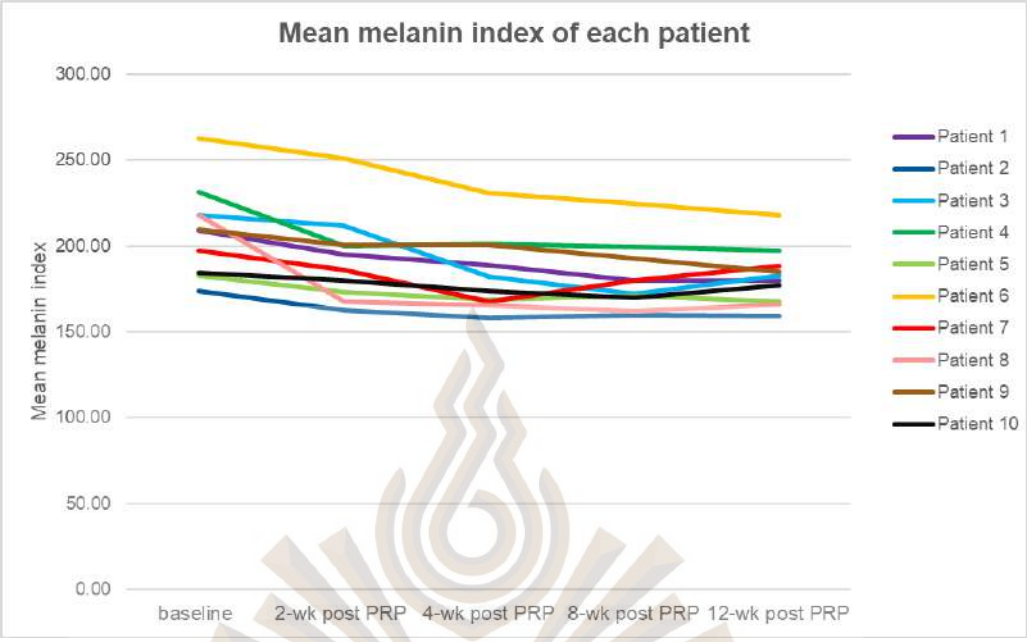

Figure 4.3 mean melanin index at baseline, two-week, four-week, eight-week, and twelve-week post PRP treatment of each patient

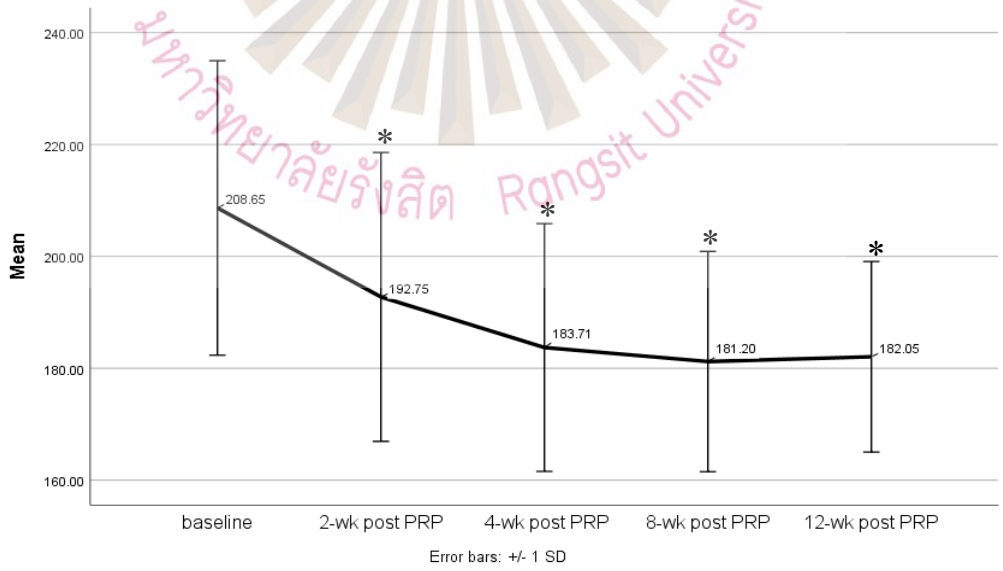

Figure 4.4 mean ± SD of melanin index at baseline, two-week, four-week, eight-week, and twelve-week post PRP treatment *(* P <0.05)*

4.3.2 Brightening score

The brightening score was evaluated by two expert dermatologist which was graded as 4 levels: 0-25%; no change to slight lightening, >25-50%; moderate lightening, >50-75%; marked lightening, >75-100%; near normal skin. The mean brightening score of two expert dermatologists were collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups.

Corresponding with mDPASI and mean melanin index, brightening score also showed the improvement toward time. At two-week, four-week, eight-week, and twelve-week post treatment the brightening score is 0.3, 0.8, 1.3 and 1.4. The mean brightening score of two expert dermatologist at twelve-week after treatment was 1.4 which was interpret as >25-50%; moderate lightening.

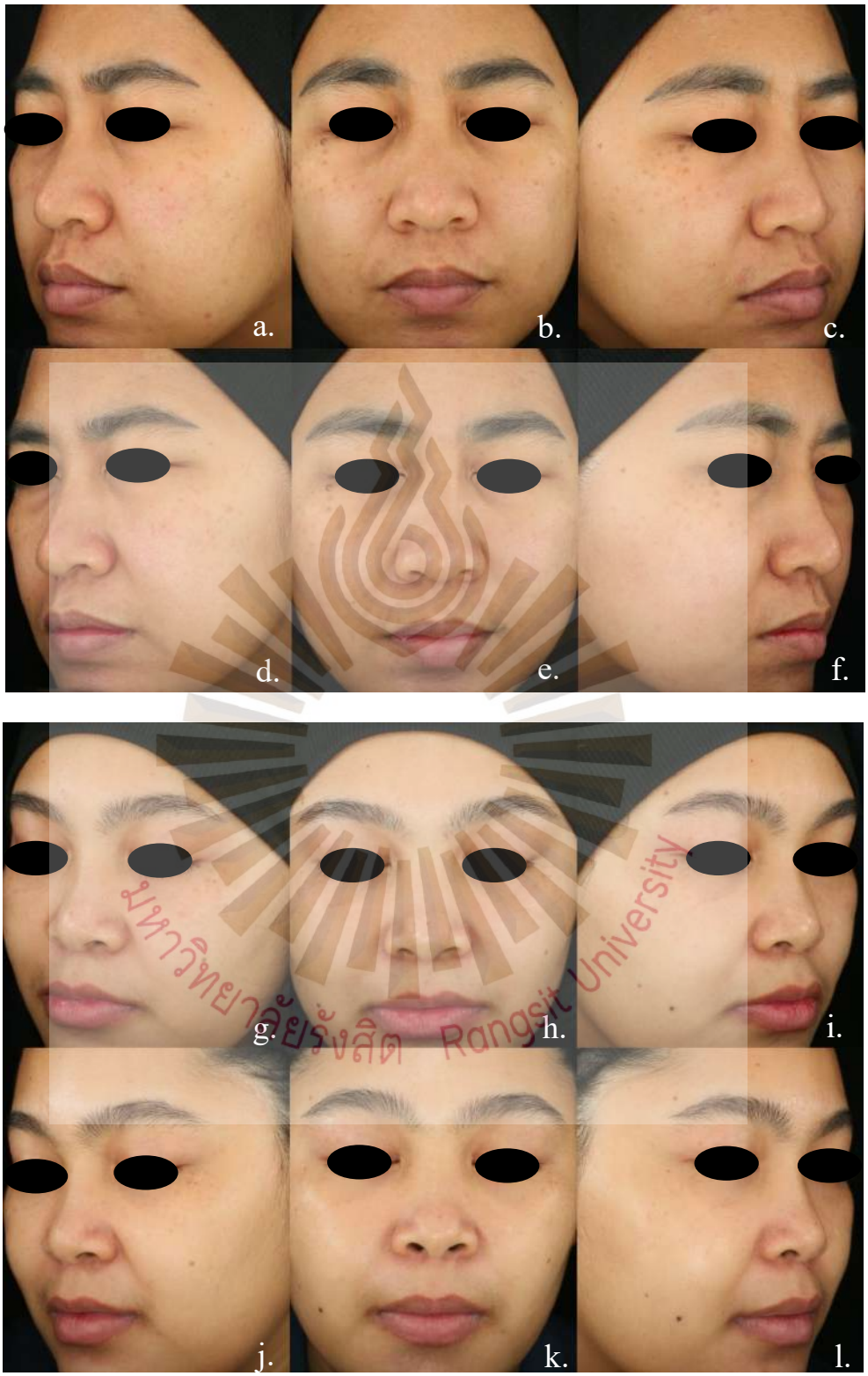

Figure 4.5 Clinical photograph of a patient at baseline (a,b,c,g,h,i) and after four sessions of PRP treatment at twelve-week post-treatment follow-ups (d,e,f,j,k,l)

4.3.3 Patient self-assessment score

The patient self-assessment was graded as 5 levels by using quartile grading system as: 0 = no improvement; 1= slight improvement (<25%); 2 = moderate improvement (25-50%); 3 = marked improvement (50-75%); 4 = near normal skin (>75%). Score was collected at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups.

Of the 10 patients evaluated at week 12, 20% graded the improvement as moderate (25–50% improvement), 50% reported the improvement as marked improvement, and the remaining 30% graded the improvement as near normal skin or >75% improvement, as shown in Figure 4.6.

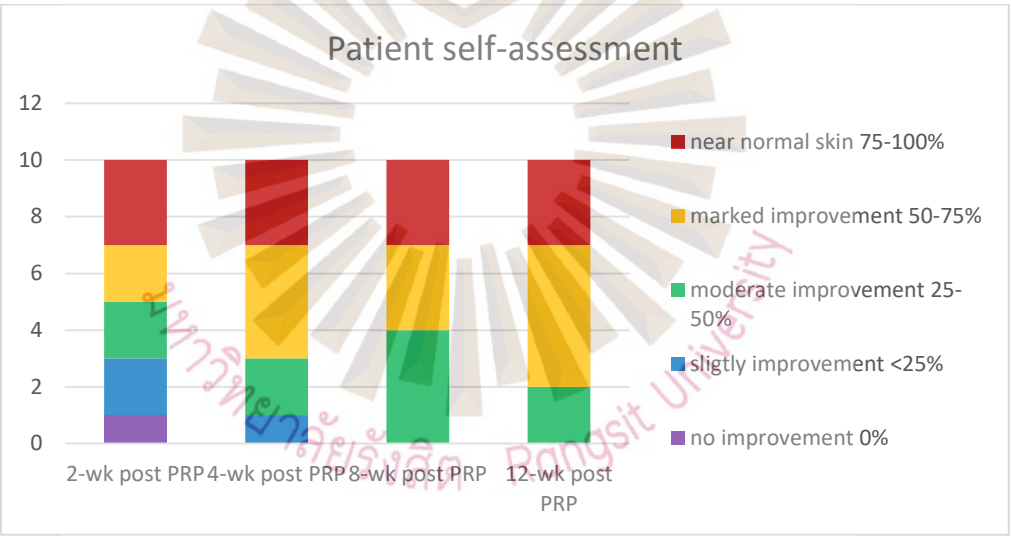

Figure 4.6 Patient self-assessment at the two-week, four-week, eight-week, and twelve-week post-treatment follow-ups

4.3.4 Side effect

Most patients experience minimal side effects such as pain, mild edema, and bruise on day 1 as shown in Figure 4.7, Figure 4.8, and Figure 4.9. Most of

them resolved in day 3. None of the participant report severe side effects such as transient ischemia, hyperpigmentation, and infection.

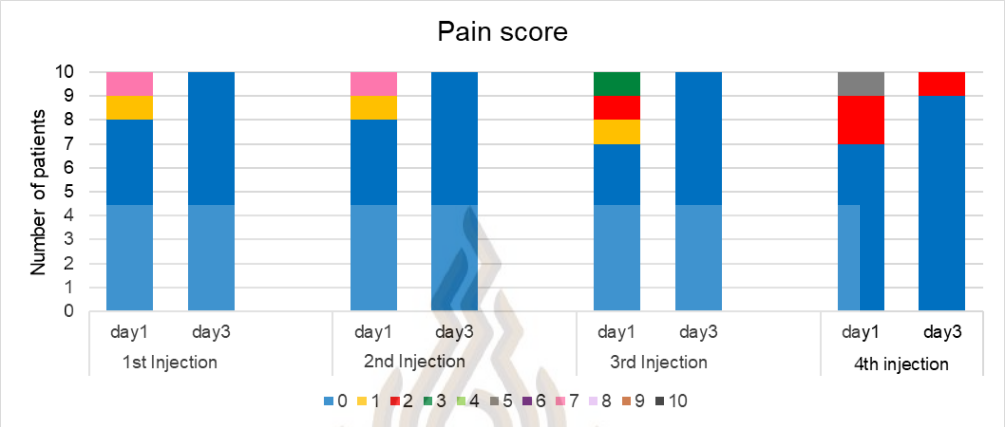

Figure 4.7 Pain score on day 1 and 3 post-PRP injection each session

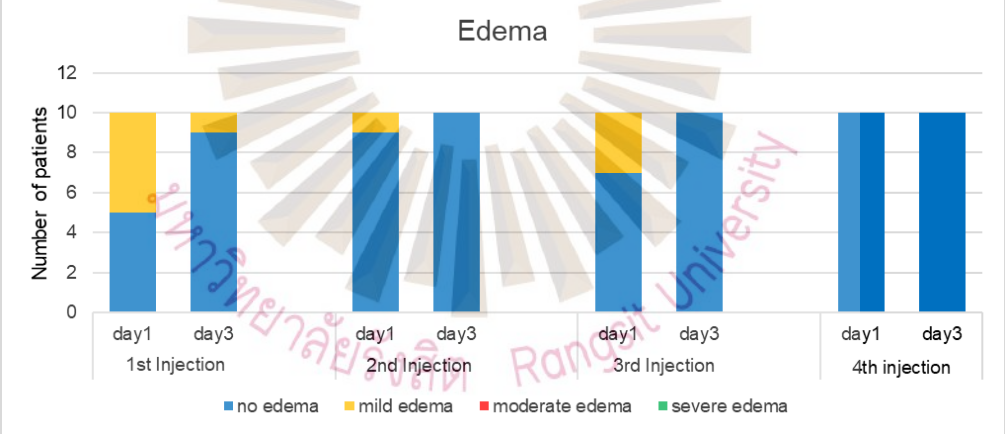

Figure 4.8 Edema score on day 1 and 3 post-PRP injection each session

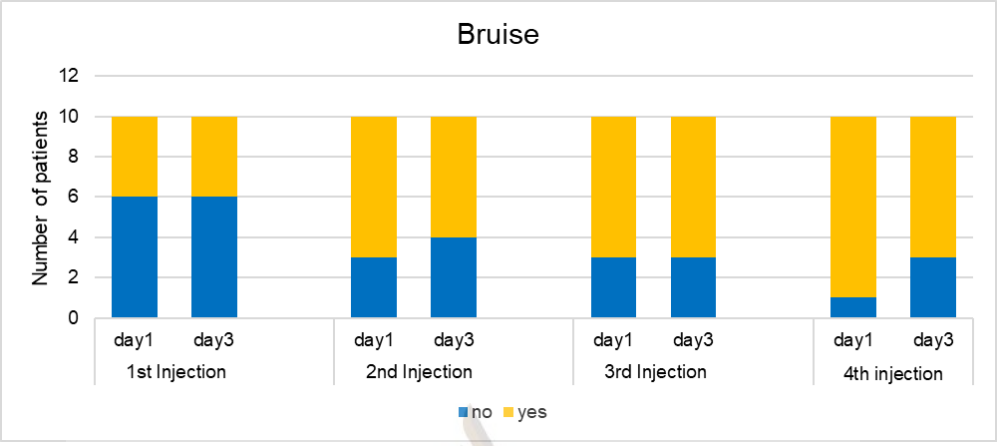

Figure 4.9 Appearance of bruise on day 1 and 3 post-PRP injection each session

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

The acquired bilateral nevus of Ota-like macule, also known as Hori's nevus, is a type of pigmentary lesion that is quite common yet challenging to treat. Currently available treatment options are laser and dermabrasion. In spite of multiple treatments, the lesions heal inconsistently and are accompanied by complications such as post-inflammatory hyperpigmentation. The majority of patients have recurrent lesions. (Kaur et al., 2020)

Uncertainty still surrounds the pathogenesis of Hori's nevus. Several assumptions exist regarding it. Hori et al. discovered in 1984 that Hori's nevus was caused by the drooping off of epidermal melanocytes, migration of hair bulb melanocytes, reactivation of preexisting dermal melanocytes by sun exposure, dermal inflammation, ageing, and atrophy or degeneration of the epidermis and dermis, which stimulated dermal melanogenesis. (Hori et al., 1984) Second, an increase in melanogenic cytokines in the dermis from dermal fibroblasts, such as dermal stem cell factor (SCF)/c-kit, which upregulate mitogen activated protein kinase pathway (MITF) and tyrosinase activity, hepatocyte growth factor (HGF), which induces melanocyte migration to the dermis, and dermal mast cells. Histamine secreted by mast cells in the dermis stimulated the proliferation and migration of melanocytes. (Lee et al., 2011) Third, Hori's nevus is triggered by chronic solar exposure (27.3%), hormonal changes and pregnancy (25.5%), stress (0.6%), and trauma (0.6%). 37.3% of patients did not have any risk factors. Similarly, it is believed that UV irradiation induces melanogenesis by increasing the expression of tyrosinase activity by melanocyte-stimulating hormone. (Ee et al., 2006).

Platelet-rich plasma, or PRP, has emerged as a new trend in the dermatology field in recent years. PRP is an autologous plasma solution with a high platelet concentration. Platelets contain numerous growth factors that are necessary for tissue repair, cell proliferation, and collagen synthesis. (Merchan et al., 2019)

α-granules contain a variety of growth factors, such as transforming growth factor beta (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), etc. Additionally, platelets contain macromolecular activators of phagocytosis from platelets (MAPP), which activate Fc receptors and induce macrophage, melanophage, dermal dendritic cell, and neutrophil phagocytotic activity. (Tuknayat et al., 2021)

In our study, we enrolled ten patients with bilateral Hori's nevus. They received intradermal platelet-rich plasma injections on both sides every two weeks for a total of four treatments (at weeks 0, 2, 4, and 6 of the trial). We then followed up with them four times, at two-week, four-week, eight-week, and twelve-week post treatment. During these follow-ups, we evaluated various factors including mDPASI, melanin index, brightening score, patient self-assessment score, and side effects. The most notable finding of our study was that mDPASI showed a significant reduction from 0.929 ± 0.617 to 0.568 ± 0.414, indicating a 38.86% reduction at twelve weeks post-treatment (p value = 0.003). Additionally, we observed a significant reduction in the mean melanin index, which decreased from 208.650 ± 26.319 to 182.052 ± 17.028, representing a 12.75% improvement at twelve weeks post-treatment (p value < 0.0001). The brightening score also showed improvement over time, consistent with the changes in mDPASI and mean melanin index. At two-week, four-week, eight-week, and twelve-week post-treatment, the brightening scores were 0.3, 0.8, 1.3, and 1.4, respectively. The mean brightening score assessed by two expert dermatologists at twelve weeks post-treatment was 1.4, indicating moderate lightening (>25-50% improvement).

Overall patient self-assessment at week 12, 20% graded the improvement as moderate (25–50% improvement), 50% reported the improvement as marked improvement, and the remaining 30% graded the improvement as near normal skin or >75% improvement. Most patients experienced minimal side effects such as pain, mild edema, and bruising on day 1, with the majority resolving by day 3. None of the participants reported severe side effects such as transient ischemia, hyperpigmentation, or infection.

The mechanism that PRP can improve Hori's nevus is to reduce dermal melanocyte. First, Kim et al. investigated the function of TGF-β1 in melanogenesis. TGF-β1 inhibits the production of MITF, tyrosinase, and tyrosinase-related proteins-1 and 2. In addition, TGF-β1 delays the activation of extracellular signal-regulated kinase (ERK), which contributes to the downregulation of MITF in order to reduce melanin production. (Kim et al., 2004). Second, Yun et al. found that EGF inhibits prostaglandin 2 (PGE2), which in turn activates tyrosinase via the cAMP signaling pathway and phospholipase C (PLC). This results in a decrease in melanogenesis, which leads to a lighter skin tone (Fu et al., 2020). Moreover, the results of this study indicated that normal human melanocytes contain EGF receptors and respond to EGF by means of the ERK signaling pathway. In recent years, EGF has been introduced to use in dermatological treatment as a whitening agent in order to reduce PIH brought on by laser or UV radiation. (Yun et al., 2013). Correlatedly with the significant reduction of mDPASI and mean melanin index and improvement of the brightening score in our study. TGF-β1 and EGF in PRP can reduce tyrosinase activity which is the result from increasing in SCF/c-kit and HGF in Hori's nevus. Third, the PRP component fibroblast growth factor (FGF) assists in the removal of photodamaged ECM collagen and stimulates the production of new collagen. PDGF also stimulated the synthesis of new collagen and hyaluronic acid. As the skin's volume increases, the hyperpigmented lesion becomes more radiant. (Tuknayat et al., 2021) Along with the improvement of brightening score in our study. Overall brightening score showed more than 25-50% improvement and moderate lightening which reflect the rejuvenation effect from FGF and PDGF in PRP.

Furthermore, a study of the components of PRP identified macromolecular phagocytosis activators from platelets (MAPP). MAPP enhances phagocytosis activity in macrophages, melanophages, dermal dendritic cells, and neutrophils through stimulating Fc receptors (Czakai et al., 2017; Ogawa et al., 2000; Sakamoto et al., 2011). Hence, this can also diminish dormant melanocyte fragments and melanin granules in the dermis (Sil et al., 2018). Besides, ATP and ADP found in dense granules of PRP contribute to increased macrophage phagocytosis, which is the principal pathophysiology responsible for the reduction in skin pigmentation seen in Hori's nevus. (Sakamoto & Firkin, 1984). As a consequence of this, PRP has the potential to assist in the reduction of dermal hyperpigmentation. Last but not least, the act of phagocytosis performed by macrophages can help minimize the amount of cell debris caused by the epidermal and dermal degeneration associated with the natural aging process. (Sil et al., 2018). This dermal deterioration resulted in dermal inflammation via SCF/c-kit and mast cell degranulation, which is the pathogenesis of Hori's nevus (Hofny, Hussein, et al., 2019; Hori et al., 1984; Lee et al., 2011). There is a plausible notion that limiting dermal degradation can also minimize dermal inflammation, as well as SCF/c-kit and mast cell degranulation. This will, in turn, restrict melanogenesis, as well as melanocyte proliferation and migration, as well as dermal hyperpigmentation. Corresponding with the significant reduction of mDPASI and mean melanin index in our study. To emphasized, MAPP in PRP can reduce dermal melanocyte and dermal inflammation from SCF/c-kit and mast cell degranulation in Hori's nevus via increasing phagocytosis activity.

The improvement of the mDPASI and melanin index gradually increases after completing four sessions of PRP. This may be because PRP has delayed effects on reducing melanogenesis and phagocytic activity, which in turn decrease melanocyte and melanin granule. Additionally, since PRP contains some RBC, there may be hemosiderin at the injection site, and over time, the hemosiderin disappears, making the lesion appear brighter.

There is some rebound in mDPASI at the 8-week follow-up, which may be due to a reduction in the number of associated growth factors over time. However, the

increases in mDPASI at the 8-week and 12-week follow-up from the 4-week follow-up are not statistically significant.

At the twelve-week follow-up, 20% of all patients graded the improvement as moderate improvement, 50% reported the improvement as marked improvement, and the remaining 30% graded the improvement as near normal skin. Although a few minimal side effects such as discomfort, mild swelling, and bruising were observed, they all resolved spontaneously within three days of injection. Despite the low incidence of side effects, it's important to remember that everyone reacts differently to PRP treatment, so it's important to closely monitor each patient's progress. Nonetheless, these findings suggest that PRP is a safe and effective treatment for Hori's nevus, with high patient satisfaction.

To sum up, this is the initial test examining the effectiveness of intradermal platelet-rich plasma for treating acquired bilateral nevus of Ota-like macule, commonly known as Hori's nevus. Our results demonstrate that PRP injection into the skin notably enhanced the lesions in both objective and subjective assessments after four sessions. We also witnessed a gradual decline in mDPASI and melanin index. As a result, we suggest that PRP is a promising new treatment option for Hori's nevus.

5.2 Recommendations and Limitations

This is the first trial of using PRP to treat Hori's nevus. The study has some drawbacks, including a small number of participants, a single location, and a brief observation period. To verify this early study, larger, randomized, placebo-controlled studies are necessary. To determine the duration of the treatment's impact and the likelihood of recurrence, it is advisable to conduct a long-term follow-up. Additional investigation into the role of other growth factors in melanogenesis and inflammation reduction could yield more information on improving hyperpigmentation.

References

Al-Shami, S. H. (2014). Treatment of Periorbital Hyperpigmentation Using Platelet-Rich Plasma Injections. *American Journal of Dermatology and Venereology*, *3*(5), 87-94. https://doi.org/10.5923/j.ajdv.20140305.03

Asif, M., Kanodia, S., & Singh, K. (2016). Combined autologous platelet-rich plasma with microneedling verses microneedling with distilled water in the treatment of atrophic acne scars: a concurrent split-face study. *J Cosmet Dermatol*, *15*(4), 434-443. https://doi.org/10.1111/jocd.12207

Cayirli, M., Caliskan, E., Acikgoz, G., Erbil, A. H., & Erturk, G. (2014). Regression of melasma with platelet-rich plasma treatment. *Ann Dermatol*, *26*(3), 401-402. https://doi.org/10.5021/ad.2014.26.3.401

Cho, S. B., Park, S. J., Kim, M. J., & Bu, T. S. (2009). Treatment of acquired bilateral nevus of Ota-like macules (Hori's nevus) using 1064-nm Q-switched Nd:YAG laser with low fluence. *Int J Dermatol*, *48*(12), 1308-1312. https://doi.org/10.1111/j.1365-4632.2008.04061.x

Czakai, K., Dittrich, M., Kaltdorf, M., Muller, T., Krappmann, S., Schedler, A., . . . Loffler, J. (2017). Influence of Platelet-rich Plasma on the immune response of human monocyte-derived dendritic cells and macrophages stimulated with Aspergillus fumigatus. *Int J Med Microbiol*, *307*(2), 95-107. https://doi.org/10.1016/j.ijmm.2016.11.010

Dashore, S., Chouhan, K., Nanda, S., & Sharma, A. (2021). Preparation of Platelet-Rich Plasma: National IADVL PRP Taskforce Recommendations. *Indian Dermatol Online J*, *12*(Suppl 1), S12-s23. https://doi.org/10.4103/idoj.idoj_269_21

Dohan Ehrenfest, D. M., Rasmusson, L., & Albrektsson, T. (2009). Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*, *27*(3), 158-167. https://doi.org/10.1016/j.tibtech.2008.11.009

Ee, H. L., Wong, H. C., Goh, C. L., & Ang, P. (2006). Characteristics of Hori naevus: a prospective analysis. *Br J Dermatol*, *154*(1), 50-53. https://doi.org/10.1111/j.1365-2133.2005.06689.x

References (Cont.)

Fu, C., Chen, J., Lu, J., Yi, L., Tong, X., Kang, L., . . . Zeng, Q. (2020). Roles of inflammation factors in melanogenesis (Review). *Mol Med Rep*, *21*(3), 1421-1430. https://doi.org/10.3892/mmr.2020.10950

Gkini, M. A., Kouskoukis, A. E., Tripsianis, G., Rigopoulos, D., & Kouskoukis, K. (2014). Study of platelet-rich plasma injections in the treatment of androgenetic alopecia through an one-year period. *J Cutan Aesthet Surg*, *7*(4), 213-219. https://doi.org/10.4103/0974-2077.150743

Gómez, L., Romero, V., & Merchan, W. (2017). The use of platelet-rich plasma in the treatment of acne and its scars: A pilot study. *Surgical & Cosmetic Dermatology*, *9*. https://doi.org/10.5935/scd1984-8773.201792932

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. *J Dermatolog Treat*, *30*(4), 396-401. https://doi.org/10.1080/09546634.2018.1524821

Hofny, E. R. M., Hussein, M. R. A., Ghazally, A., Ahmed, A. M., & Abdel-Motaleb, A. A. (2019). Increased expression of TGF-beta protein in the lesional skins of melasma patients following treatment with platelet-rich plasma. *J Cosmet Laser Ther*, *21*(7-8), 382-389. https://doi.org/10.1080/14764172.2019.1668016

Hori, Y., Kawashima, M., Oohara, K., & Kukita, A. (1984). Acquired, bilateral nevus of Ota-like macules. *J Am Acad Dermatol*, *10*(6), 961-964.

Intravia, J., Allen, D. A., Durant, T. J., McCarthy, M. B., Russell, R., Beitzel, K., . . . Mazzocca, A. D. (2014). In vitro evaluation of the anti-bacterial effect of two preparations of platelet rich plasma compared with cefazolin and whole blood. *Muscles Ligaments Tendons J*, *4*(1), 79-84.

Kahr, W. H. (2009). Granules and thrombus formation. *Blood*, *114*(5), 932-933. https://doi.org/10.1182/blood-2009-05-220665

Kaur, H., Sarma, P., Kaur, S., Kaur, H., Prajapat, M., Mahendiratta, S., . . . Medhi, B. (2020). Therapeutic options for management of Hori's nevus: A systematic review. *Dermatol Ther*, *33*(1), e13167. https://doi.org/10.1111/dth.13167

References (Cont.)

Kim, D. S., Park, S. H., & Park, K. C. (2004). Transforming growth factor-beta1 decreases melanin synthesis via delayed extracellular signal-regulated kinase activation. *Int J Biochem Cell Biol*, *36*(8), 1482-1491. https://doi.org/10.1016/j.biocel.2003.10.023

Kumaran, M. S., Dabas, G., Vinay, K., & Parsad, D. (2019). Reliability assessment and validation of the dermal pigmentation area and severity index: a new scoring method for acquired dermal macular hyperpigmentation. *J Eur Acad Dermatol Venereol*, *33*(7), 1386-1392. https://doi.org/10.1111/jdv.15516

Kunachak, S., Kunachakr, S., Sirikulchayanonta, V., & Leelaudomniti, P. (1996). Dermabrasion is an effective treatment for acquired bilateral nevus of Ota-like macules. *Dermatol Surg*, *22*(6), 559-562. https://doi.org/10.1111/j.1524-4725.1996.tb00374.x

Kunachak, S., & Leelaudomlipi, P. (2000). Q-switched Nd:YAG laser treatment for acquired bilateral nevus of ota-like maculae: a long-term follow-up. *Lasers Surg Med*, *26*(4), 376-379. https://doi.org/10.1002/(sici)1096-9101(2000)26:4<376::aid-lsm5>3.0.co;2-j

Kunachak, S., Leelaudomlipi, P., & Sirikulchayanonta, V. (1999). Q-Switched ruby laser therapy of acquired bilateral nevus of Ota-like macules. *Dermatol Surg*, *25*(12), 938-941. https://doi.org/10.1046/j.1524-4725.1999.99176.x

Lam, A. Y., Wong, D. S., Lam, L. K., Ho, W. S., & Chan, H. H. (2001). A retrospective study on the efficacy and complications of Q-switched alexandrite laser in the treatment of acquired bilateral nevus of Ota-like macules. *Dermatol Surg*, *27*(11), 937-941; discussion 941-932. https://doi.org/10.1046/j.1524-4725.2001.01094.x

Lee, J. Y., Kim, E. H., Kim, K. H., Kang, H. Y., Lee, E. S., & Kim, Y. C. (2011). Acquired bilateral naevus of Ota-like macules: an immunohistological analysis of dermal melanogenic paracrine cytokine networks. *Br J Dermatol*, *164*(3), 580-585. https://doi.org/10.1111/j.1365-2133.2010.10066.x

References (Cont.)

Lee, S. J., Nam, S. M., Cha, H. G., Park, E. S., & Kim, Y. B. (2018). The Efficacy of a Q-Switched 694-nm Ruby Fractional Laser for Treating Acquired Bilateral Nevus of Ota-Like Macules. *Arch Aesthetic Plast Surg*, *24*(1), 20-25. https://doi.org/10.14730/aaps.2018.24.1.20

Leo, M. S., Kumar, A. S., Kirit, R., Konathan, R., & Sivamani, R. K. (2015). Systematic review of the use of platelet-rich plasma in aesthetic dermatology. *J Cosmet Dermatol*, *14*(4), 315-323. https://doi.org/10.1111/jocd.12167

Maisel-Campbell, A. L., Ismail, A., Reynolds, K. A., Poon, E., Serrano, L., Grushchak, S., . . . Alam, M. (2020). A systematic review of the safety and effectiveness of platelet-rich plasma (PRP) for skin aging. *Arch Dermatol Res*, *312*(5), 301-315. https://doi.org/10.1007/s00403-019-01999-6

Manuskiatti, W., Sivayathorn, A., Leelaudomlipi, P., & Fitzpatrick, R. E. (2003). Treatment of acquired bilateral nevus of Ota-like macules (Hori's nevus) using a combination of scanned carbon dioxide laser followed by Q-switched ruby laser. *J Am Acad Dermatol*, *48*(4), 584-591. https://doi.org/10.1067/mjd.2003.193

Matras, H. (1982). The use of fibrin sealant in oral and maxillofacial surgery. *J Oral Maxillofac Surg*, *40*(10), 617-622. https://doi.org/10.1016/0278-2391(82)90108-2

Mautner, K., Malanga, G. A., Smith, J., Shiple, B., Ibrahim, V., Sampson, S., & Bowen, J. E. (2015). A call for a standard classification system for future biologic research: the rationale for new PRP nomenclature. *Pm r*, *7*(4 Suppl), S53-s59. https://doi.org/10.1016/j.pmrj.2015.02.005

Mehryan, P., Zartab, H., Rajabi, A., Pazhoohi, N., & Firooz, A. (2014). Assessment of efficacy of platelet-rich plasma (PRP) on infraorbital dark circles and crow's feet wrinkles. *J Cosmet Dermatol*, *13*(1), 72-78. https://doi.org/10.1111/jocd.12072

Merchan, W. H., Gomez, L. A., Chasoy, M. E., Alfonso-Rodriguez, C. A., & Munoz, A. L. (2019). Platelet-rich plasma, a powerful tool in dermatology. *J Tissue Eng Regen Med*, *13*(5), 892-901. https://doi.org/10.1002/term.2832

References (Cont.)

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

Park, J. M., Tsao, H., & Tsao, S. (2009). Acquired bilateral nevus of Ota-like macules (Hori nevus): etiologic and therapeutic considerations. *J Am Acad Dermatol*, *61*(1), 88-93. https://doi.org/10.1016/j.jaad.2008.10.054

Sakamoto, H., & Firkin, F. (1984). Characterization of leucocyte phagocytic stimulatory material released by activated human platelets. *British journal of haematology*, *57*(1), 49–60. https://doi.org/10.1111/j.1365-2141.1984.tb02864.x

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. https://doi.org/10.3109/09537104.2010.533797

Shetty, V. H., & Goel, S. (2019). Dermoscopic pre- and posttreatment evaluation in patients with androgenetic alopecia on platelet-rich plasma-A prospective study. *J Cosmet Dermatol*, *18*(5), 1380-1388. https://doi.org/10.1111/jocd.12845

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

Sirithanabadeekul, P., Dannarongchai, A., & Suwanchinda, A. (2020). Platelet-rich plasma treatment for melasma: A pilot study. *J Cosmet Dermatol*, *19*(6), 1321-1327. https://doi.org/10.1111/jocd.13157

Tuknayat, A., Bhalla, M., & Thami, G. P. (2021). Platelet-rich plasma is a promising therapy for melasma. *J Cosmet Dermatol*, *20*(8), 2431-2436. https://doi.org/10.1111/jocd.14229

References (Cont.)

Tuknayat, A., Thami, G. P., Bhalla, M., & Sandhu, J. K. (2021). Autologous intralesional platelet rich plasma improves melasma. *Dermatol Ther*, *34*(2), e14881. https://doi.org/10.1111/dth.14881

Vinay, K., Dabas, G., Parsad, D., & Kumaran, M. S. (2018). A novel scale for measurement of acquired dermal macular hyperpigmentation severity. *J Eur Acad Dermatol Venereol*, *32*(6), e251-e253. https://doi.org/10.1111/jdv.14772

Yu, W., Zhu, J., Yu, W., Lyu, D., Lin, X., & Zhang, Z. (2018). A split-face, single-blinded, randomized controlled comparison of alexandrite 755-nm picosecond laser versus alexandrite 755-nm nanosecond laser in the treatment of acquired bilateral nevus of Ota-like macules. *J Am Acad Dermatol*, *79*(3), 479-486. https://doi.org/10.1016/j.jaad.2017.12.053

Yun, W. J., Bang, S. H., Min, K. H., Kim, S. W., Lee, M. W., & Chang, S. E. (2013). Epidermal growth factor and epidermal growth factor signaling attenuate laser-induced melanogenesis. *Dermatol Surg*, *39*(12), 1903-1911. https://doi.org/10.1111/dsu.12348

Zhao, L., Hu, M., Xiao, Q., Zhou, R., Li, Y., Xiong, L., & Li, L. (2021). Efficacy and Safety of Platelet-Rich Plasma in Melasma: A Systematic Review and Meta-Analysis. *Dermatol Ther (Heidelb)*, *11*(5), 1587-1597. https://doi.org/10.1007/s13555-021-00575-z

Appendice

Appendix A
Case Record Form

CRF Week 0| ID_____

Case Record Form: Week 0 Date_____

○ General Information

Sex ☐ male ☐ female

Underlying Disease ☐ HTN ☐ DM ☐ DLP

☐ other _____

Fitzpatrick Skin Type ☐ I ☐ II ☐ III ☐ IV ☐ V

○ Risk Factor

Hormonal used ☐ No ☐ Yes

Pregnancy ☐ No ☐ Yes

Unprotected sun exposed ☐ No ☐ Yes _____ hr/day

○ Disease Characteristic

- Area
  - ☐ Forehead
  - ☐ Cheek
    - ☐ Rt.cheek
    - ☐ Lt.cheek
    - ☐ Both cheek
  - ☐ Central face

- Dermoscopy Severity grade:
  - ☐ Normal
  - ☐ Chinese letter/semi-arcuate pattern
  - ☐ Reticulate pattern
  - ☐ Diffuse pattern

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 0| ID_____

mDPASI by Dermatologist 2

mDPASI = 4 x (percentage of right cheek involvement x grade) + 4 x (percentage of left cheek involvement x grade) + 2 x (percentage of central face involvement x grade (score 0-40)

grade 0: no change in color/normal pattern in dermoscopy;

grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy;

grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy;

grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy;

grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy

Area	Multiplication factor	% of area involvement	grade	Total score
Rt. cheek	4			
Lt. cheek	4			
Central face	2			
Total score				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 0| ID_____

Melanin Index: Baseline

	Melanin Index		
Rt. Cheek			
Lt. Cheek			
Cheek (mean)			

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 0| ID_____

Case Record Form: Week 0

- ○ Side effect: DAY 1
- ○ Date _____

Pain Score (0-10)				
Edema Score	0: no	1: mild	2: mod	3: severe
	Yes		No	
Erythema				
Hyperpigmentation				
Infection	If yes: ☐ bacteria, ☐virus, ☐fungus			
Transient Ischemia				
Bruise				
Papules				
Pustules				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

5

CRF Week 0(ID_____

O Side effect: DAY 3

O Date _____

Pain Score (0-10)				
Edema Score	0: no	1: mild	2: mod	3: severe
	Yes		No	
Erythema				
Hyperpigmentation				
Infection	If yes: ☐ bacteria, ☐virus, ☐fungus			
Transient Ischemia				
Bruise				
Papules				
Pustules				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 8| ID_____

Case Record Form: Week 8 (Two-week post PRP) Date_____

mDPASI by Dermatologist 1

mDPASI = 4 x (percentage of right cheek involvement x grade) + 4 x (percentage of left cheek involvement x grade) + 2 x (percentage of central face involvement x grade (score 0-40)

grade 0: no change in color/normal pattern in dermoscopy;

grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy;

grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy;

grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy;

grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy

Area	Multiplication factor	% of area involvement	grade	Total score
Rt. cheek	4			
Lt. cheek	4			
Central face	2			
Total score				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 8| ID_____

mDPASI by Dermatologist 1

Brightening score

0	1	2	3
0-25%	>25-50%	>50-75%	>75-100%
No change or slightly lightening	Moderate lightening	Marked lightening	Near normal skin

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 8| ID_____

mDPASI by Dermatologist 2

mDPASI = 4 x (percentage of right cheek involvement x grade) + 4 x (percentage of left cheek involvement x grade) + 2 x (percentage of central face involvement x grade (score 0-40)

grade 0: no change in color/normal pattern in dermoscopy;

grade 1: mild disease/light brown color change and/or dotted pattern on dermoscopy;

grade 2 moderate disease/bluish or violaceous color and/or Chinese letter/semi-arcuate pattern on dermoscopy;

grade 3 severe disease/slate grey or brown color and/or reticulate pattern on dermoscopy;

grade 4 very severe disease/dark brown to black color and/or diffuse pattern on dermoscopy

Area	Multiplication factor	% of area involvement	grade	Total score
Rt. cheek	4			
Lt. cheek	4			
Central face	2			
Total score				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 8| ID_____

mDPASI by Dermatologist 2

Brightening score

0	1	2	3
0-25%	>25-50%	>50-75%	>75-100%
No change or slightly lightening	Moderate lightening	Marked lightening	Near normal skin

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

CRF Week 8| ID_____

Melanin Index

	Melanin Index		
Rt. Cheek			
Lt. Cheek			
Cheek (mean)			

Patient's satisfaction

0	1	2	3
ไม่พอใจ	พอใจเล็กน้อย	พอใจ	พอใจมาก

ผลข้างเคียง

Pain Score (0-10)				
Edema Score	0: no	1: mild	2: mod	3: severe
	Yes		No	
Erythema				
Hyperpigmentation				
Infection	If yes: ☐ bacteria, ☐ virus, ☐ fungus			
Transient Ischemia				
Bruise				
Papules				
Pustules				

ลงชื่อ.....ผู้บันทึกข้อมูล

วันที่_____/_____/_____

17

Appendix B
Ethical Approval Document

Study Protocol Approval

The Ethics Committee of the Institute of Dermatology, Bangkok, Thailand has approved the following to be carried out according to the protocol dated and/or amended as follows:

Study Title : The effectiveness of intradermal platelet-rich plasma for the treatment of acquired bilateral nevus of Ota-like macules (Hori's nevus), in Institute of dermatology, Thailand, pilot study

Study Code : IRB 002/2565

Center : Institute of Dermatology, Bangkok, Thailand

Principal Investigator : Chumsaeng Chumsaengsri, M.D.

Protocol Date : 2 June 2022

This is to certify that Institute of Dermatology Ethics Committee is in full Compliance with International Guidelines for Human Research Protection such as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and The International Conference on Harmonization in Good Clinical Practice (ICH – GCP)

Date of Approval : 24 June 2022

Vice - Chairperson of Ethics Committee:.....

(Signature)

Chavalit Supsrisunjai, M.D.

Director of Institute of Dermatology:.....

(Signature)

Mingkwan Wichaidit, M.D.

Clinical trial ethics committee / IRB APPROVAL FORM

TO: Chumsaeng Chumsaengsri, M.D.

NAME OF ETHICS COMMITTEE/IRB:
Ethics committee /IRB of the Institute of Dermatology, Bangkok, Thailand
ADDRESS: Institute of Dermatology, 420/7 Rajavithi Road, Bangkok 10400, Thailand.
Tel: (02) 354-5222 Fax: (02) 354-8042, (02) 354-8046

PROTOCOL IRB/IEB code no. 002/2565 The effectiveness of intradermal platelet-rich plasma for the treatment of acquired bilateral nevus of Ota-like macules (Hori's nevus), in Institute of dermatology, Thailand, pilot study	**PROTOCOL DATE:** 2 June 2022
PRINCIPAL INVESTIGATOR: **ADDRESS:** Institute of Dermatology, 420/7 Rajavithi Road, Bangkok 10400, Thailand.	**SPONSOR:** Institute of Dermatology

The following [√] have been Approve in connection with the above study to be conducted by the above investigator
[√] Protocol (Date 2 June 2022)
[√] Case Report Form (Date 2 June 2022)
[√] Patient information sheet (Date 2 June 2022)
[√] Informed Consent Form (Date 2 June 2022)
[ ] Questionnaire (Date .....)

Date of Meeting: 26 May 2022
Date of Approval: 24 June 2022

SIGNATURE .....

Chavalit Supsrisunjai, M.D.
NAME OF ETHICS COMMITTEE/IRB
CHAIRPERSON/DELEGATE

Appendix C
Inform Consent Form

เอกสารชี้แจงผู้เข้าร่วมการวิจัย

(Patient Information Sheet)

ในเอกสารนี้อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามหัวหน้าโครงการวิจัยหรือผู้แทนให้ช่วยอธิบายจนกว่าจะเข้าใจดี ท่านอาจจะขอเอกสารนี้กลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อน สนิท แพทย์ประจำตัวของท่าน หรือแพทย์ท่านอื่น เพื่อช่วยในการตัดสินใจเข้าร่วมการวิจัย

ชื่อโครงการวิจัย การศึกษานำร่องประสิทธิผลของการฉีดพลาสมาเกล็ดเลือดเข้มข้นเข้าชั้นหนังแท้ในการรักษากระลึกในประชากรผู้ป่วยที่มารับการรักษาที่สถาบันโรคผิวหนังของไทย

ชื่อหัวหน้าโครงการวิจัย นพ.ชุมแสง ชุมแสงศรี, พญ.ภาสินี คือคุณวงศา, ดร.พญ.ชนิศา เกียรติสุระยานนท์

สถานที่วิจัย สถาบันโรคผิวหนัง กรมการแพทย์: 420/7 ถนนราชวิถี แขวงทุ่งพญาไท เขตราชเทวี กรุงเทพฯ 10400

สถานที่ทำงานและหมายเลขโทรศัพท์ของหัวหน้าโครงการวิจัยที่ติดต่อได้ทั้งในและนอกเวลาราชการ
สถาบันโรคผิวหนัง กรมการแพทย์ 420/7 ถนนราชวิถี แขวงทุ่งพญาไท เขตราชเทวี กรุงเทพฯ 10400
โทรศัพท์ 095-207-2811, 095-207-2812

ผู้สนับสนุนทุนวิจัย สถาบันโรคผิวหนัง กรมการแพทย์

ระยะเวลาในการวิจัย ตุลาคม 2564 – กันยายน 2566

โครงการวิจัยนี้ทำขึ้นเพื่อ เพื่อศึกษาประสิทธิผลของการฉีดพลาสมาเกล็ดเลือดเข้มข้นเข้าชั้นหนังแท้ในการรักษากระลึก (Hori's nevus) ในประชากรผู้ป่วยที่มารับการรักษาที่สถาบันโรคผิวหนังของไทย

ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย แพทย์และบุคลากรทางการแพทย์สามารถนำความรู้จากการศึกษาครั้งนี้มาใช้เป็นทางเลือกในการรักษาผู้ป่วยกระลึกได้อย่างมีประสิทธิภาพ อีกทั้งเพื่อเป็นข้อมูลเชิงประจักษ์ที่แสดงให้เห็นถึง ทางเลือกใหม่ในการรักษากระลึกนอกเหนือจากที่มีอยู่ในปัจจุบันและสามารถนำไปพัฒนาต่อยอดได้

ท่านได้รับเชิญให้เข้าร่วมการวิจัยนี้เพราะ ท่านมีอายุระหว่าง 25-65 ปี มีกระลึกบริเวณใบหน้าทั้งสองข้าง ซึ่งได้รับการวินิจฉัยโดยแพทย์ผิวหนังและมีบันทึกการวินิจฉัยในเวชระเบียน

จะมีผู้เข้าร่วมการวิจัยนี้ทั้งสิ้น 10 คน

หากท่านตัดสินใจเข้าร่วมการวิจัยแล้ว จะมีขั้นตอนการวิจัยดังต่อไปนี้คือ

1. ผู้วิจัยจะมีการนัดประชุมเพื่อชี้แจงข้อมูลเกี่ยวกับด้านการวิจัยให้อาสาสมัครทุกท่านรับทราบพร้อมกันและจะมีการขอคำยินยอมเข้าร่วมวิจัยจากอาสาสมัครทุกท่านก่อนเริ่มดำเนินการวิจัย
2. ผู้วิจัยจะทำการเก็บข้อมูลส่วนบุคคลและข้อมูลทั่วไปที่เกี่ยวข้องกับการวิจัยในวันที่นัดประชุม ได้แก่ เพศ, โรคประจำตัว, ประเภทสีผิว, ประวัติความเสี่ยงในการเป็นโรคกระลึก ได้แก่ การใช้ยาฮอร์โมน การ

ตั้งครรภ์ และประวัติการโดนแสงแดด เป็นต้น โดยข้อมูลทั้งหมดจะถูกเก็บเป็นความลับและไม่มีการระบุตัวตนของอาสาสมัคร

3. อาสาสมัครทุกท่านจะได้การการประเมินความรุนแรงของรอยโรคกระลึกโดยแพทย์ผิวหนัง 2 ท่าน โดยจะมีการใช้กล้องส่องผิวหนังเพื่อประเมินรอยโรคด้วย, ตรวจวัดความแข็งของเม็ดสีบริเวณรอยโรคด้วยเครื่องวัดปริมาณเม็ดสีใต้ผิวหนังและถ่ายภาพรอยโรคด้วยเครื่องถ่ายภาพและวิเคราะห์สภาพผิวใบหน้าก่อนทำการรักษา (สัปดาห์ที่ 0)
4. การเตรียมตัวก่อนเข้ารับการรักษา
   4.1 หากมีการทานยาในกลุ่มแก้ปวด แก้อักเสบ ควรหยุดยากลุ่มนี้ก่อน 7-10 วัน เนื่องจากยากลุ่มนี้มีผลรบกวนไขกระดูกในการผลิตพลาสมาเกล็ดเลือดเข้มข้นที่มีคุณภาพ
   4.2 ท่านไม่ต้องงดน้ำงดอาหารก่อนทำหัตถการ หรือ เจาะเลือด
5 อาสาสมัครทุกท่านจะได้รับการรักษาด้วยการฉีดพลาสมาเกล็ดเลือดเข้มข้น (PRP) เข้าชั้นหนังแท้บริเวณรอยโรคบนใบหน้าทั้งสองข้าง ทุก 2 สัปดาห์ รวมทั้งสิ้น 4 ครั้ง (สัปดาห์ที่ 0, 2, 4 และ 6 หลังเริ่มการวิจัย)
5. โดยในแต่ละครั้งจะมีขั้นตอนดังต่อไปนี้ อาสาสมัครทำความสะอาดใบหน้าและล้างเครื่องสำอาง หลังจากนั้นทายาชาบริเวณรอยโรคที่จะทำการรักษาเป็นเวลา 45 นาทีก่อนทำหัตถการ หลังจากนั้นเช็ดออกให้สะอาด ผู้วิจัยทำการเจาะเลือดอาสาสมัครด้วยเข็มเบอร์ 21 ยาว 1 นิ้ว ประมาณ 18 มิลลิลิตร ใส่ใน Syringe ที่มีสารกันเลือดแข็งตัวของเลือด (ACD-A) อยู่ 2 มิลลิลิตร รวม 20 มิลลิลิตร จากนั้นถ่ายเทสารดังกล่าวลงสู่หลอดปั่นคัดแยก PRP แบบสำเร็จรูป (e-PRP Kit) จากนั้นนำมาปั่นด้วยเครื่องปั่นตกตะกอนชนิดตั้งโต๊ะ ด้วยความเร็ว 3500 รอบต่อนาที นาน 5 นาที จำนวน 1 รอบ เพื่อแยกพลาสมาเกล็ดเลือดเข้มข้นออกมา หลังจากได้พลาสมาเกล็ดเลือดเข้มข้นแล้ว ผู้วิจัยทำการฉีดพลาสมาเกล็ดเลือดเข้มข้นเข้าที่ชั้นหนังแท้ บริเวณรอยโรค ที่ใบหน้าทั้งสองข้าง โดยใช้เข็มเบอร์ 30 ยาว ½ นิ้ว โดยแต่ละจุดที่ฉีดมีขนาดเส้นผ่านศูนย์กลางประมาณ 0.5-1 เซนติเมตร และมีระยะห่างกันประมาณ 1 เซนติเมตร หลังการรักษาผู้วิจัยจะทำการเช็ดใบหน้าอาสาสมัครด้วยสำลีแห้งปลอดเชื้อ หลังฉีดเสร็จอาสาสมัครสามารถทาครีมกันแดดและครีมบำรุงได้ภายหลังการรักษา 12-24 ชั่วโมง
6. แนะนำให้ผู้ป่วยหลีกเลี่ยงแสงแดด ความร้อน หรือการเสียดสีบริเวณใบหน้าหลังได้รับการรักษา ตลอดระยะเวลาของการวิจัยอาสาสมัครจะได้รับครีมกันแดดและครีมให้ความชุ่มชื้นสำหรับผิว โดยให้ทาครีมให้ความชุ่มชื้นทั่วใบหน้าในตอนเช้าและก่อนนอน และทาครีมกันแดดทั่วใบหน้าทุกวันในตอนเช้าและหากออกแดดต่อเนื่องต้องทาครีมกันแดดทุกสองชั่วโมงตลอดการรักษาและหลังสิ้นสุดการรักษาอย่างน้อย 8 สัปดาห์
7. หลังการรักษาในแต่ละครั้ง อาสาสมัครจะได้รับการติดต่อทางโทรศัพท์ (วิดีโอคอล ผ่านทาง แอปพลิเคชันไลน์) จากแพทย์ผู้วิจัย เพื่อประเมินผลข้างเคียงระยะสั้นที่อาจเกิดขึ้นภายหลังการรักษาในวันที่ 1 และ 3 หลังการรักษา โดยจะมีการสอบถามอาการดังนี้ ปวด, บวม, รอยแดง, รอยดำ, การติดเชื้อ, ผิวหนังบริเวณที่ฉีดมีสีซีดลง, รอยช้ำ, เกิดตุ่มนูน หรือ มีเกิดตุ่มหนองขึ้นหรือไม่
8. อาสาสมัครจะได้รับการนัดหมายเพื่อมาประเมินผลการรักษาทั้งหมด 4 ครั้ง หลังจบการรักษา 2 สัปดาห์, 4 สัปดาห์, 8 สัปดาห์ และ 12 สัปดาห์ (สัปดาห์ที่ 8, 10, 14 และ 18 หลังเริ่มการวิจัย) โดยในแต่ละครั้ง

อาสาสมัครจะได้พบแพทย์ผิวหนัง 2 ท่านเพื่อประเมินผลการรักษา โดยจะมีการใช้กล้องส่องผิวหนังเพื่อประเมินรอยโรคด้วย, ตรวจวัดความเข้มของเม็ดสีบริเวณรอยโรค โดยเครื่องวัดปริมาณเม็ดสีใต้ผิวหนัง, ทำแบบประเมินความพึงพอใจต่อผลลัพธ์ของการรักษา และถ่ายภาพรอยโรค โดยเครื่องถ่ายภาพและวิเคราะห์สภาพผิวใบหน้าผลการประเมินทั้งหมดจะถูกบันทึกไว้ในแบบบันทึกข้อมูล โดยข้อมูลทั้งหมดจะถูกเก็บเป็นความลับและไม่มีการระบุตัวตนของอาสาสมัคร

โดยสรุปอาสาสมัครจะอยู่ในโครงการวิจัยเป็นระยะเวลาประมาณ 18 สัปดาห์ มีการนัดหมายทั้งหมด 8 ครั้ง

วิธีดำเนินการวิจัย คัดเลือกอาสาสมัครเข้าร่วมโครงการวิจัยที่ผ่านเกณฑ์การคัดเลือกที่กำหนด

ความเสี่ยงที่อาจจะเกิดขึ้นเมื่อเข้าร่วมการวิจัย

1. การเจาะเลือดอาจทำให้เกิดรอยช้ำหรือการติดเชื้อบริเวณที่เจาะได้ โดยสามารถลดอาการช้ำได้โดยการใช้สำลีกดบริเวณที่เจาะเลือดเป็นเวลา 5 นาที หรือใช้น้ำแข็งประคบ และหากมีการติดเชื้อสามารถรักษาได้ด้วยการให้ยาฆ่าเชื้อรับประทานต่อเนื่องติดต่อกัน 7-10 วัน
2. การใช้ยาชาบริเวณใบหน้า อาจมีอาการแพ้ยาชาได้ในบางราย เช่น ความดันโลหิตต่ำ หัวใจเต้นผิดจังหวะได้ โดยจะมีการสังเกตอาการและวัดสัญญาณชีพเป็นระยะหลังจากทายาชา ในบางรายอาจมีผื่นแพ้สัมผัสจากยาชาได้ โดยอาจแสดงลักษณะเป็นผื่นลมพิษ หรือ ผื่นปื้นแดง มีอาการแสบและคันบริเวณใบหน้า หากมีอาการแพ้ดังกล่าวเกิดขึ้น อาสาสมัครจะได้รับการรักษาโดยยาแก้แพ้ชนิดทาและชนิดรับประทานจนกว่าอาการดีขึ้น หากเกิดรอยดำขึ้นภายหลังการรักษาผื่นแพ้ยาชาหายแล้ว อาสาสมัครจะได้รับการรักษารอยดำ โดยจะได้รับยาทาลดรอยดำ และติดตามการรักษาจนกว่ารอยดำจะจางลง
3. ในระหว่างที่ทำการรักษาด้วยพลาสมาเกล็ดเลือดเข้มข้นอาจมีอาการเจ็บจากการฉีดยาเข้าชั้นผิวหนังได้
4. ภายหลังการรักษาด้วยพลาสมาเกล็ดเลือดเข้มข้นอาจจะพบอาการที่ไม่พึงประสงค์ตามมาได้ เช่น รอยช้ำบวม รอยดำ หรือเกิดรอยแดง เป็นต้น ซึ่งอาการเหล่านี้สามารถหายได้เองโดยใช้เวลาประมาณ 7-14 วัน

หากท่านไม่เข้าร่วมในโครงการวิจัยนี้ ท่านก็จะได้รับการรักษาโรคของท่านตามวิธีมาตรฐานคือ การใช้ยาทา, การใช้เลเซอร์สำหรับกำจัดเม็ดสี เป็นต้น

หากมีข้อข้องใจที่จะสอบถามเกี่ยวข้องกับการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัย ท่านสามารถติดต่อ พญ.ภาสินี ดีอุดมวงศา โทร. 062-236-2632 (ตลอด 24ชั่วโมง), นพ.ชุมแสง ชุมแสงศรี โทร. 081-477-2010 (ตลอด 24 ชั่วโมง) และ ตร.พญ.ชนิศา เกียรติสุระยานนท์ โทร. 091-718-2555 (ตลอด 24ชั่วโมง)

ท่านจะได้รับการช่วยเหลือหรือดูแลรักษาการบาดเจ็บ/เจ็บป่วยอันเนื่องจากการวิจัยตามมาตรฐานทางการแพทย์ โดยผู้รับผิดชอบค่าใช้จ่ายในการรักษาคือ สถาบันโรคผิวหนัง กรมการแพทย์ กระทรวงสาธารณสุข เป็นผู้รับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลในสถานพยาบาลของรัฐ เท่านั้น

ประโยชน์ที่คิดว่าจะได้รับจากการวิจัย การเข้าร่วมวิจัยนี้ ผู้เข้าร่วมวิจัยจะได้รับการรักษาภระสึกที่มีประสิทธิภาพ และมีผลข้างเคียงน้อย

ผู้เข้าร่วมการวิจัยจะได้รับเป็นค่าสนับสนุนการเดินทาง จำนวน 175 บาท/ครั้ง

หากมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ทราบโดยรวดเร็วไม่ปิดบัง

ข้อมูลส่วนตัวของผู้เข้าร่วมการวิจัย จะถูกเก็บรักษาโดยไม่เปิดเผยต่อสาธารณะเป็นรายบุคคล แต่จะรายงานผลการวิจัยเป็นข้อมูลส่วนรวมโดยไม่สามารถระบุข้อมูลรายบุคคลได้ ข้อมูลของผู้เข้าร่วมการวิจัยเป็นรายบุคคลอาจมีคณะบุคคลบางกลุ่มเข้ามาตรวจสอบได้ เช่น ผู้ให้ทุนวิจัย สถาบัน หรือองค์กรของรัฐที่มีหน้าที่ตรวจสอบ รวมถึงคณะกรรมการจริยธรรมการวิจัยในคน สถาบันโรคผิวหนัง เป็นต้น

ผู้เข้าร่วมการวิจัยมีสิทธิ์ถอนตัวออกจากโครงการเมื่อใดก็ได้ โดยไม่ต้องแจ้งให้ทราบล่วงหน้า และการไม่เข้าร่วมการวิจัยหรือถอนตัวออกจากโครงการวิจัยนี้ จะไม่มีผลกระทบต่อการบริการและการรักษาที่สมควรจะได้รับตามมาตรฐานแต่ประการใด

หากท่านได้รับการปฏิบัติที่ไม่ตรงตามที่ระบุไว้ในเอกสารชี้แจงนี้ ท่านสามารถแจ้งให้ประธานคณะกรรมการจริยธรรมการวิจัยในคน สถาบันโรคผิวหนัง กลุ่มงานวิจัยฯ ชั้น 6 ตึกสยามบรมราชกุมารี สถาบันสุขภาพเด็กแห่งชาติมหาราชินี โทร. 095-207-2870

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย / วันที่.....
(.....)

ลงชื่อ.....หัวหน้าโครงการวิจัย/ผู้ร่วมวิจัย วันที่.....
(.....)

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย

(Informed Consent Form)

วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า.....อายุ.....ปี

อาศัยอยู่บ้านเลขที่.....ถนน.....แขวง/ตำบล.....

เขต/อำเภอ.....จังหวัด.....รหัสไปรษณีย์.....

โทรศัพท์.....

ขอแสดงเจตนายินยอมเข้าร่วมโครงการวิจัยเรื่อง การศึกษาหน้าร่องประสิทธิผลของการฉีดพลาสมาเกล็ดเลือดเข้มข้นเข้าชั้นหนังแท้ในการรักษาตะลักในประชากรผู้ป่วยที่มารับการรักษาที่สถาบันโรคผิวหนังของไทย

โดยข้าพเจ้าได้รับทราบรายละเอียดเกี่ยวกับที่มาและจุดมุ่งหมายในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ประโยชน์ที่คาดว่าจะได้รับของการวิจัย และความเสี่ยงที่อาจจะเกิดขึ้นจากการเข้าร่วมการวิจัย รวมทั้งแนวทางป้องกันและแก้ไขหากเกิดอันตรายขึ้น โดยได้อ่านข้อความที่มีรายละเอียดอยู่ในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด อีกทั้งยังได้รับคำอธิบายและตอบข้อสงสัยจากหัวหน้าโครงการวิจัยเป็นที่เรียบร้อยแล้ว ข้าพเจ้าจึงสมัครใจเข้าร่วมโครงการวิจัยนี้

หากข้าพเจ้ามีข้อข้องใจเกี่ยวกับขั้นตอนของการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัยขึ้นกับข้าพเจ้า ข้าพเจ้าจะสามารถติดต่อกับ แพทย์หญิงภาสินี ดือตมวงศา โทรศัพท์ 062-236-2632, นายแพทย์ชุมแสง ชุมแสงศรี โทรศัพท์ 081-477-2010 และ ดร.พญ.ชนิศา เกียรติสุระยานนท์ โทร. 091-718-2555 (ตลอด 24ชั่วโมง)

หากข้าพเจ้าได้รับการปฏิบัติไม่ตรงตามที่ระบุไว้ในเอกสารชี้แจงเข้าร่วมการวิจัย ข้าพเจ้าสามารถติดต่อกับประธานคณะกรรมการจริยธรรมการวิจัยในคน สถาบันโรคผิวหนัง โดยที่ กลุ่มงานวิจัยและประเมินเทคโนโลยี ชั้น 6 ตึกสยามบรมราชกุมารี สถาบันสุขภาพเด็กแห่งชาติมหาราชินี โทร. 095-207-2870

ข้าพเจ้าได้ทราบถึงสิทธิที่ข้าพเจ้าได้รับข้อมูลเพิ่มเติมทั้งทางด้านประโยชน์และโทษจากการเข้าร่วมการวิจัย และสามารถถอนตัวหรือตัดสินใจเข้าร่วมการวิจัยได้ทุกเมื่อโดยไม่ต้องแจ้งล่วงหน้าหรือระบุเหตุผล โดยไม่มีผลกระทบต่อการบริการและการรักษาพยาบาลที่ข้าพเจ้าจะได้รับต่อไปในอนาคต และยินยอมให้ผู้วิจัยใช้ข้อมูลส่วนตัวของข้าพเจ้าที่ได้รับจากการวิจัย แต่จะไม่เผยแพร่ต่อสาธารณะเป็นรายบุคคล โดยจะนำเสนอเป็นข้อมูลโดยรวมจากการวิจัยเท่านั้น

ข้าพเจ้าได้เข้าใจข้อความในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และหนังสือแสดงเจตนายินยอมนี้โดยลงชื่อตลอดแล้ว จึงลงลายมือชื่อไว้

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย / วันที่.....
(.....)

ลงชื่อ..... หัวหน้าโครงการวิจัย / วันที่.....
(.....)

ในกรณีผู้เข้าร่วมการวิจัยอ่านหนังสือไม่ออก ผู้ที่อ่านข้อความทั้งหมดแทนผู้เข้าร่วมการวิจัยคือ .....จึงได้ลงลายมือชื่อไว้เป็นพยาน

ลงชื่อ.....พยาน / วันที่.....
(.....)

Appendix D
Additional Data

Additional data

Since the committee were agreed to continue follow up all of the patient up to 6 months, the researcher continued the study. Unfortunately, there were 5 patients cannot continue the trial. Some stopped applying sunscreen after the last follow up. Some had done laser after the last visit and some had move to rural area and had transport problem. The results of mDPASI and melanin index of 5 patients at 18-weeks after the last PRP session as shown in Table 1 and 2. At 18-week post follow-up, 40% of patients develop rebound of hyperpigmentation at Hori's nevus area which were assessed by mexameter. However, mDPASI of all patients still have an improvement but less than 12-week follow-up in all patients.

Table 1 mDPASI of 5 patients

	Baseline	2-wk post PRP (% decrease)	4-wk post PRP (% decrease)	8-wk post PRP (% decrease)	12-wk post PRP (% decrease)	18-wk post PRP (% decrease)
Patient 2	0.24	0.17 (29.16)	0.15 (37.5)	0.14 (41.67)	0.14 (41.67)	0.20 (16.67)
Patient 6	1.98	1.28 (35.35)	1.24 (37.36)	1.04 (47.47)	1.12 (43.43)	1.16 (41.41)
Patient 7	0.82	0.3 (63.41)	0.23 (71.95)	0.2 (75.61)	0.3 (63.41)	0.4 (51.22)
Patient 8	1.14	0.6 (47.37)	0.54 (52.63)	0.54 (52.63)	0.54 (52.63)	0.58 (49.12)
Patient 10	0.22	0.17 (22.73)	0.12 (45.45)	0.16 (27.27)	0.12 (45.45)	0.12 (45.45)

Table 2 mean melanin index of 5 patients

	Baseline	2-wk post PRP (% decrease)	4-wk post PRP (% decrease)	8-wk post PRP (% decrease)	12-wk post PRP (% decrease)	18-wk post PRP (% decrease)
Patient 2	173.67	162.66 (6.34)	157.83 (9.12)	159.83 (7.97)	159.17 (8.35)	170.67 (1.72)
Patient 6	262.83	251.00 (4.50)	230.80 (12.19)	224.50 (14.58)	218.17 (16.99)	240.17 (8.62)
Patient 7	197.50	186.17 (5.74)	167.60 (15.14)	179.83 (8.95)	188.17 (4.73)	198.33 (-0.42)
Patient 8	217.67	167.50 (23.05)	165.33 (24.04)	162.17 (25.50)	165.67 (23.89)	175.50 (19.37)
Patient 10	184.50	179.83 (2.53)	174.00 (5.70)	170.00 (7.86)	177.17 (3.97)	190.67 (-3.34)

Biography

Name	Pasinee Deeudomwongsa
Date of birth	24 May 1996
Place of birth	Bangkok, Thailand
Education background	Mahidol University Doctor of Medicine, 2020
Address	100/5 Ratchapruek, Meung, Nonthaburi 11000
Email Address	pasineedee.md@gmail.com