THE EFFICACY OF PLATELET-RICH PLASMA COMBINATION THERAPY FOR VITILIGO: A SYSTEMATIC REVIEW AND META-ANALYSIS

BY

MISS KYI MAR TUN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
THE MASTER OF SCIENCE IN DERMATOLOGY
CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE
THAMMASAT UNIVERSITY
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was approved as partial fulfillment of the requirements for
the degree of Master of Science (Dermatology)

on April 29, 2020

Chairman _____
(Assistant Professor Therdpong Tempark, M.D.)

Member _____
(Assistant Professor Suparuj Lueangarun, M.D., M.Sc.)

Member and advisor _____
(Assistant Professor Premjit Juntongjin, M.D.)

Director, Graduate Studies _____
(Professor Kesara Na-Bangchang, Ph.D.)

Dean _____
(Professor Adis Tasanarong, M.D., Ph.D.)

Thesis Title	THE EFFICACY OF PLATELET-RICH PLASMA COMBINATION THERAPY FOR VITILIGO: A SYSTEMATIC REVIEW AND META-ANALYSIS
Author	MISS KYI MAR TUN
Degree	Master of Science (Dermatology)
Major Field/Faculty/University	Clinical Dermatology Chulabhorn International College of Medicine Thammasat University
Thesis Advisor, Member	Assistant Professor Premjit Juntongjin, M.D.
Thesis Committee Members	Assistant Professor Therdpong Tempark, M.D. Assistant Professor Suparuj Lueangarun, M.D., M.Sc.
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ABSTRACT

Background: A recalcitrant, disfiguring, autoimmune disorder, vitiligo is resulting from the depletion of melanocyte in epidermis. Many therapeutic approaches have been used with various outcomes. Platelet-rich plasma (PRP) has recently been applied as an option in numerous skin disorders, including vitiligo.

Objective: To evaluate the efficacy of platelet-rich plasma modality as an adjunctive therapy for vitiligo.

Materials and methods: Articles search for clinical studies in human was conducted in the PubMed, Cochrane and Scopus databases with specific MeSH (Medical Section Heading) terms. This meta-analysis analyzed how the efficiency of PRP combination therapy in vitiligo by measuring clinical improvement with percentage of repigmentation. All data analyzing and grouping are performed with STATA version 14.0 (Stata Corp LP, College Station, TX).

Results: After matching with the study inclusion criteria, total 5 articles were involved in present meta-analysis. In vitiligo, PRP as an adjunctive therapy with other standard treatment modalities compared with PRP alone or other control group, there was clinically as well as statistically significant in ≥75% repigmentation, especially in energy based (laser and light-based) intervention. Risk ratio (RR) for ≥75% repigmentation: 2.95, 95% confidence interval (CI): 1.17– 7.43), p value was 0.02 which had no much bias and stronger evidence than in term of ≥50% repigmentation (RR: 2.54, 95% CI: 1.28 – 5.03). Generally, it is safe to use PRP as a combination therapy apart from minor side effects like pain, erythema immediately after procedure.

Limitations: Different treatment modalities, treatment regimes, sequence of PRP preparation and application protocols, comparative outcomes of each study made certain amount of variation in standard outcome data evaluation either in overall or subgroup analysis.

Conclusions: A novel autologous therapeutic PRP modality, high concentration of growth factors leads to skin repigmentation as well as adjuncts for standard vitiligo treatment. However, larger population with longer duration clinical trials is necessary to understand the exact mechanism of PRP on vitiliginous skin.

Keywords: Vitiligo, platelet-rich plasma, combination therapy, meta-analysis

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Miss Kyi Mar Tun

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
α	alpha
β	beta
γ-GT	γ-glutamyltranspeptidase
μg	Microgram(s)
μl	Microliter(s)
°C	Degree(s) Celsius
/	Per
CD4	Cluster of differentiation 4
CD5	Cluster of differentiation 5
CO2	Carbon dioxide
cm^2	Centimeter square
CXCL10	CXC chemokine ligand 10
CXCR3	CXC motif chemokine receptor 3
DLQI	Dermatology life quality index
Er: YAG	Erbium-doped yttrium aluminum garnet
EGF	Epithelial growth factor
FDA	Food and drug administration
GHQ	General health questionnaire
IGF	Insulin-like growth factor
IL	Interleukin
J	Joule(s)
LLLT	Low-level light therapy
L-PRP	Leukocyte and PRP
LXA4	Lipoxin A4
mJ	Milijoules(s)
mm	Millimeter(s)

MBEH	Monobenzyl ether of hydroquinone
MCP-1	Monocyte chemotactic protein -1
mRNA	Messenger ribonucleic acid
NB UVB	Narrowband ultraviolet B
NAFL	Non-Ablative fractional laser
Nd: YAG	Neodymium-doped yttrium aluminum garnet
Nm	Nanometer
NSV	Non-segmental vitiligo
PUVA	Psoralen and ultraviolet A
PRP	Platelet-rich Plasma
PRFM	Platelet rich fibrin matrix
PRF	Platelet-rich fibrin
RANTES	Regulated on Activation, Normal T Cell Expression and Secretion
TNF	Tumor necrosis factor
TGF	Transforming growth factor
UVB	Ultraviolet B
VAS	Visual analogue scale
VASI	Visual area scoring index
VEGF	Vascular endothelial growth factors
VETF	Vitiligo European task force
VETI	Vitiligo extent tensity index
VIDA	Vitiligo disease activity score
Wnt	Wingless-type MMTV integration sire family member

CHAPTER 1
INTRODUCTION

Vitiligo is a recalcitrant, disfiguring autoimmune disorder with loss of epidermal melanocyte and symptomatic depigmentation with unpredictable loss of melanin in epidermal layer with macules and patches. The epidemiology of vitiligo presents with 0.5% to 2% of prevalence according to geographic variation, regardless of all races and genders. Vitiligo was higher prevalence in the immediate relatives of patient's offspring that showed early evidence of its heritability and the inheritable risk of developing disease is six percent and twenty three percent in identical twin.[1]

A White depigmented patch of vitiligo affects patient's self-esteem and reduces dermatological life quality index so it should not regard as "simply cosmetic". Many misconceptions have been delivered by some historical and cultural beliefs especially in countries where the white patches/macules are regarded as contagious disease such as leprosy, which caused psychosocial burden in vitiligo patients. Regards to literature, more than thirty five percent of vitiligo patients are suffering depressive symptom with psychosocial stigmata. [2]

Therefore, introducing with a suitable modality as soon as possible to help these patients not only for their physical stigmas but also for psychological support. However, many therapeutic options for vitiligo including FDA approved and off-label modalities provide only modest efficacy and remain challenges, these might encourage to explore its safety and efficacy by well indulgent of disease pathogenesis and identifying different curative approaches. For instance, topical and systemic immunomodulators, phototherapy, lasers and surgical therapies are established but the prolong duration of therapy, social and financial reason are the main concern for patients' noncompliance.

Platelet-rich plasma (PRP) is derived from autologous venous blood having platelets in plasma concentrate including variety of growth factors such as platelet-derived growth factors (PDGFaa, PDGFbb and PDGFab), transforming growth factor beta (TGFβ1 and 2), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin like growth factor which regulate for cell migration, activation, differentiation and stimulation to accumulate the extra cellular matrix and specific surface receptors to bind the keratinocyte and melanocyte growth. PRP therapy has been innovated

to rejuvenate the skin, treatment of acne scars, alopecia, wound healing and vitiligo with high popularity because of its safety and cost effectiveness. [3]

Regards to many published clinical trial results about PRP combined with various modalities for vitiligo repigmentation, this study focused to evaluate the efficacy of PRP as combination therapy as well as to analyze the beneficial and cost-effective procedure in term of meta-analysis and systematic review.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Vitiligo

2.1.1 Introduction

Vitiligo is the most common acquired disfiguring depigmented disorder involving skin and hair, with progressive loss of melanocyte which results in amelanotic macules or patches with variable sizes. Initially, depigmentation may occur focally at face, acral regions then, other visible area of the whole body which impact physical and psychosocial devastation. Disease prognosis varies with individuals depends on their treatment compliance and recognition of depigmented patterns can be regarded as a clue for diagnosis and prognosis.[4] Various assumptions have been anticipated about the manner of causation of vitiligo but the exact theory is still uncertain. [5]

2.1.2 Epidemiology and Quality of life

About epidemiological data, the prevalence of vitiligo is 0.5% to 2% in global population regardless of races and sexes. Average onset is at younger age groups, 50% prevalence before age of 20, with or without progressive nature in their whole life presenting with various patterns[6]. Since vitiligo is not only the cosmetic condition, it apparently effects psychological impairment and negative effect on the dermatology life quality index (DLQI) similarly with psoriasis and atopic dermatitis[7, 8]. Therefore, clinicians should take seriously about the patients' psychosocial stress and mood disturbance, consider for regular assessment and refer to counseling service if required.

2.1.3 Clinical Classification

Widespread depigmented macules and patches are the main feature of vitiligo. It may spread symmetrically with increasing in numbers and area regardless of disease duration, starting over the face and extremities especially at sun exposed sites. There is multifactorial correlation with incidence of disease such as prolong sunburn, pregnancy, trauma and severe stressful conditions. Depigmentation may present with koebnerization at the areas of trauma. [9] Focal and mucosal vitiligo are termed as localized type. Isolated, small, depigmented patch 10 to 15 cm^2 unilateral involvement, lack of obvious clinical symptoms without progression lesion is called focal vitiligo and the similar presentation which occur in the oral mucosa and genital area is called mucosal vitiligo. If the lesion is strictly occurred at the head, upper or lower limbs and facial orifice, treatment outcome

can be challenging and termed as acrofacial vitiligo or lip-tip vitiligo. When the depigmentation area is completely reached to the whole body (≥ 80% of BSA), either may or may not involve the body hair, it is called universal vitiligo. [10]

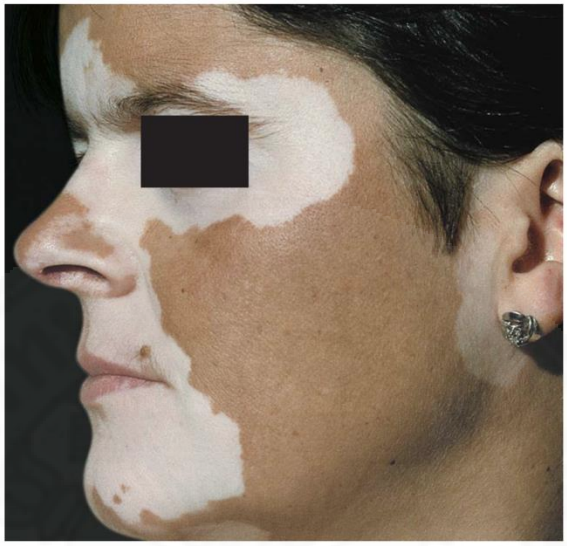

Figure 2.1 Vitiligo: face Extensive depigmentation of the central face. Involved vitiliginous skin has convex borders, extending into the normal pigmented skin. Note the chalk-white color and sharp margination. *Note also that the dermal melanocytic nevus on the upper lip has retained its pigmentation. [11]*

Depigmentation induced by exposure of certain chemical like phenol or monobenzyl ether of hydroquinone (MBHE) which accelerate the depigmentation initially start at the site of exposure progress to nearby skin, widespread with typical pattern is known as chemically induced vitiligo.[12] Progressive loss of melanocyte, with linear or block like pattern, presented by unilateral depigmentation in segmental vitiligo. Rarely, typical segmental pattern of vitiligo reaches beyond outside of primary lesion which is termed as mixed vitiligo.[13]

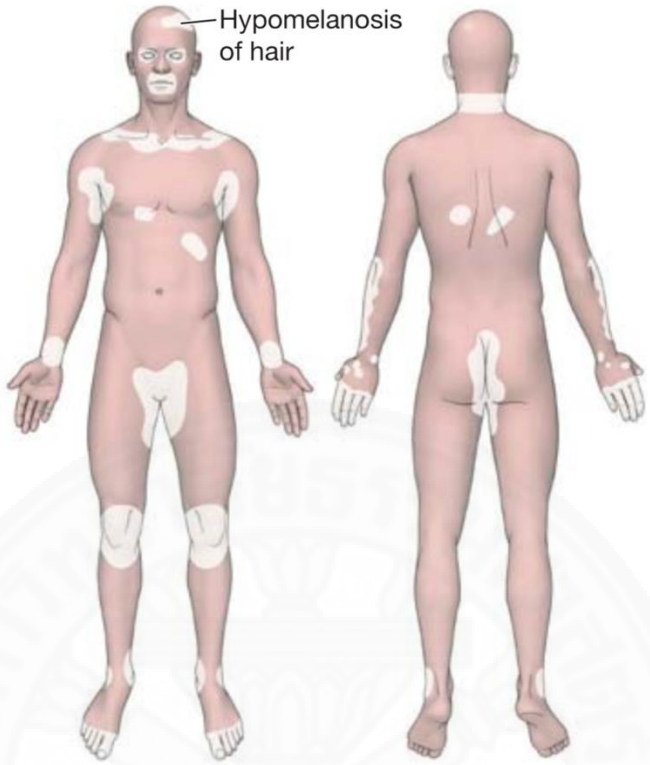

Figure 2.2 Vitiligo Predilection Sites [11]

2.1.4 Clinical markers of disease activity

Extensibility of depigmentation is characterized by koebnerization, trichrome lesion (mixed with depigmented lesion skin, normal skin color and zone of hypo pigmented skin), inflammation and confetti-like depigmentation. The greater BSA involvement, the poor response to the treatment combined with koebnerization. [14] Scaly erythematous and pruritus within or border of hypopigmented or depigmented lesion uncommonly present as inflammatory vitiligo. Although inflammatory response occurs in short duration, it can accelerate depigmentation. [15] While higher disease progression with more active and aggressive response described as confetti-like depigmentation, patients may exhibit higher vitiligo disease activity score together with Koebner phenomenon. [16]Severity assessment markers are essential to analyze the disease prognosis as well as plan for management.

2.1.5 Diagnosis

Generally, diagnosis of vitiligo is made by proper history taking and thorough physical examination. Wood's lamp is one the investigation tool to reveal the exact diagnosis resulting a chalky white enhancement of depigmented skin. Apparently

different result compared with hypopigmented skin, it is not enhanced with Wood's lamp. It is important to exclude other hypopigmented skin disorders which resulted only off-white under Wood's lamp examination. Histopathological investigation rarely requires for diagnosis. In early lesions, it demonstrates interface dermatitis with CD8+ cytotoxic T cells infiltration in epidermis and expanding edge of active lesions shows a perivascular and perifollicular lymphocyte infiltrate with absolutely depletion epidermal pigmentation and melanocyte at basal layer.[17] Visible depigmented skin shows sparse inflammation with absence of T cell infiltration, may appear after up to 48-hour of melanocytes apoptosis. [18]

2.1.6 Disease Association

Since vitiligo is acquired autoimmune disorder, high prevalence of autoimmunity in patients including their relatives and offspring. Most commonly correlated with autoimmune diseases [19, 20] autoimmune thyroid disease is most common (prevalence ~19%) so it is required to make annual thyroid stimulating hormone (TSH) testing and autoantibody (Ab) screening.[21]

Table 2.1 Differential diagnosis of segmental vitiligo. [7]

Disorder	Clinical presentation	Diagnosis
Nevus depigmentosus	At birth or first few years of life; grows in proportion to child; usually hypopigmented, has a jagged border, and lacks leukotrichia	Normal number of melanocytes histologically but decreased melanin
Nevus anaemicus	Presents at birth; mostly on the upper aspect of the chest; poorly demarcated white macule with surrounding erythema	Merges with surrounding skin with diascopy; no accentuation with Wood's lamp examination

Table 2.2 Differential Diagnosis of Vitiligo[7]

Disorder	Clinical presentation	Diagnosis
Congenital conditions		
Piebaldism	Midline depigmentation; present at birth; lesions contain islands of normal pigment	Dominantly inherited; other affected family members
Waardenburg syndrome	White forelock, some with depigmented patches	Other stigmata of the syndrome, including hearing loss
Multiple ash leaf macules of TS	Multiple, well-demarcated, hypopigmented macules	Other cutaneous signs of TS, epilepsy, and other organ involvement
Hypomelanosis of Ito	Blaschkoid hypopigmentation present at birth	May or may not have other stigmata
Inflammatory conditions		
Pityriasis alba	Poorly demarcated hypopigmented macules; scale, erythema may be seen; most commonly in children with skin of color	Does not fluoresce with Wood's lamp; evidence of eczema may be noted
Postinflammatory hypopigmentation	Poorly demarcated hypopigmentation in an area of previous inflammation; may see primary dermatosis (eg, seborrheic dermatitis, eczema)	Decreased number of melanocytes with or without other inflammatory patterns
Lichen sclerosus et atrophicus	Typically on genitals; atrophic skin with or without fissures; figure-of-8 pattern surrounding vaginal introitus and anus	Lichenoid inflammation; epidermal atrophy; sparing of melanocytes
Discoid lupus erythematosus	Head, face, and neck erythematous, scaly macules and plaques with scarring, dyspigmentation and alopecia	Interface dermatitis with sparing of melanocytes
Hypopigmented sarcoidosis	Hypopigmented macules or patches; may be other manifestations of sarcoidosis	Histopathology reveals noncaseating granulomas
Cutaneous malignancy		
Mycosis fungoides (hypochromic variant)	Especially seen in skin of color; bathing suit distribution; with or without scale and signs of inflammation	Epidermotropism; atypical lymphocytes
Infections		
Acquired progressive macular hypomelanosis	Young adults; trunk (especially lower back and axillae)	Wood's lamp may reveal *Propionibacterium acnes* (pink fluorescence)
Tinea versicolor	Hypopigmentation; trunk	Positive skin scraping with potassium hydroxide preparation; green fluorescence of untreated lesions
Leprosy (tuberculoid or indeterminate)	Hypopigmented, hypoaesthetic white patches	Skin smear and biopsy specimen reveal *Mycobacterium leprae*
Pinta (late-stage)	Depigmented lesions, typically on distal extremities or other exposed part of the body	Rapid plasma reagin—positive; spirochetes on dark-field microscopy or histopathology
Exogenous causes		
Idiopathic guttate hypomelanosis	Exogenous ultraviolet light exposure causing nonprogressive 1-5 mm hypomelanotic macules in older adults; chronically sun-exposed sites; no leukotrichia	
Trauma-induced hypo- or depigmentation	Geometric shapes and history of trauma or surgical intervention	

Any single, unilateral lesion of these diagnoses could also be included on the differential diagnosis of segmental variant of vitiligo.
TS, Tuberous sclerosis.

2.1.7 Severity Assessment

Since vitiligo impacts not only physical appearance but also psychosocial well-being, many experts generate specific tools to evaluate the severity of diseases which help the clinicians to offer specific treatment management and assessment for prognosis. Some tools in literature are in the list below:

1. Vitiligo Area Scoring Index (VASI)
2. Vitiligo Disease Severity Score (VIDA)
3. Vitiligo European Task Force Assessment (VTEFa)
4. Vitiligo Extent Tensity Index (VETI)
5. The Dermatology Life Quality Index (DQLI)
6. Vitiligo Impact Patient Scale (VIPs)
7. Repigmentation Score

1. Vitiligo Area Scoring Index (VASI)

Regards to PASI score (Psoriasis area and severity index), Hamzavei et al. generated VASI which is quantitative parametric score ranging from 0-100 in order to measure repigmentation of vitiligo by calculating in hand unit. Degree of extended depigmentation within one hand unit represents 1% of body surface area so it is estimated as 0%, 10%, 25%, 50%, 75%, 90% or 100% respectively. [22] VASI score equation is as below:

$$\text{VASI} = \sum_{\substack{\text{All Body} \\ \text{Sites}}} [\text{Hand Units}] \times [\text{Residual Depigmentation}]$$

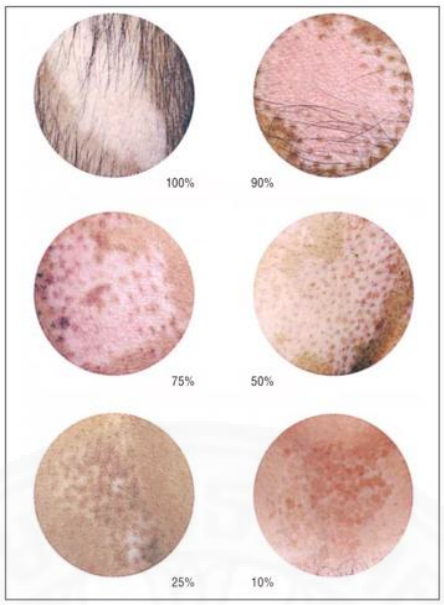

Figure 2.3 Standardized assessments for estimating the degree of pigmentation to derive the Vitiligo Area Scoring Index. At 100% depigmentation, no pigment is present; at 90%, specks of pigment are present; at 75%, the depigmented area exceeds the pigmented area; at 50%, the depigmented and pigmented areas are equal; at 25%, the pigmented area exceeds the depigmented area; and at 10%, only specks of depigmentation are present. [22]

2. Vitiligo Disease Severity Score (VIDA)

Based on patients' opinion, VIDA score is generated to evaluate the disease activity with 6 scales (+4 to -1). The lower the VIDA score, the lesser disease activity.

VIDA 4: Activity of 6 weeks or less period

VIDA 3: Activity of 6 weeks to 3 months

VIDA 2: Activity of 3 months to 6 months

VIDA 1: Activity of 6 months to 12 months

VIDA 0: Stable at least for 1 year

VIDA -1: Stable at least 1 year with spontaneous repigmentation

3. Vitiligo European Task Force Assessment (VETFa)

Taieb[23] proposed VETF scoring system which is used to assess treatment outcomes by analyzing the extent by rule of 9, diseases stages 0-3 and disease progression (spreading) by using Wood's lamp (+1 to -1).

Extent is measured with rule of 9. Approximately 1% of BSA represent the patient's palm involved with digits. Under 5 years of age, total BSA is 18% for head and neck and 13% of each leg.

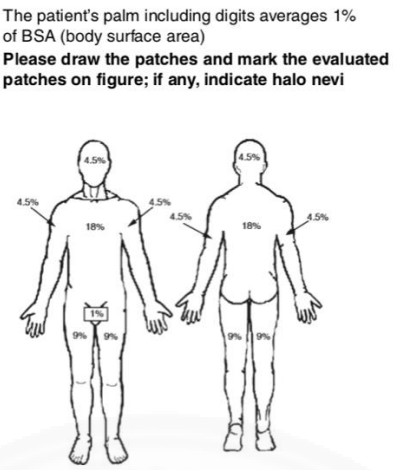

Figure 2.4 Recommendation for scoring extent. [23]

Staging focus to measure the hair and skin repigmentation by using Wood's Lamp

Score 1 - Stage 0: normal pigmentation (no depigmentation in area graded);

Score 2 - Stage 1: incomplete depigmentation (including spotty depigmentation, trichrome and homogeneous lighter pigmentation);

Score 3 - Stage 2: complete depigmentation (may include hair whitening in a minority of hairs, <30%);

Score 4 - Stage 3: complete depigmentation plus significant hair whitening (>30%).

Spreading is measured by using Wood's Lamp, score: (+1 is progressive, 0 is stable, -1 is regressive)

Table 2.3 Recommendation for stage and spread of Vitiligo: rule of 9 scoring table. [23]

Area	% Area	Staging* (0–4)	Spreading* (–1 +1)
Head and neck (0–9%)			
Trunk (0–36%)			
Arms (0–18%)			
Legs (0–36%)			
Hands and feet			
Totals (0–100%)		0–20	(–5 +5)

*largest patch in each area

4. Vitiligo Extent Tensity Index (VETI)

By using rule of 9,Feily A[24] studied VETI score to measure disease extent of vitiligo at 5 areas (head, upper limb, trunk, lower limb and genitalia) with 5 stages:

Stage 0: Normal skin

Stage 1: Hypopigmentation (including trichrome and homogenous lighter pigmentation)

Stage 2: Complete depigmentation with black hair and with perifollicular pigmentation

Stage 3: Complete depigmentation with black hair and without perifollicular pigmentation

Stage 4: Complete depigmentation with compound of white and black hair with/without perifollicular pigmentation

Stage 5: Complete depigmentation plus significant hair whitening

By using the formula below, VETI can be calculated from all body regions:

VETI score: (Percentage of head involvement × grade of tensity) + (Percentage of trunk involvement × grade of tensity) 4+ (Percentage of upper limbs involvement × grade of tensity) 2+ (Percentage of lower limbs involvement × grade of tensity) 4+ (Percentage of genitalia involvement × grade of tensity) 0.1

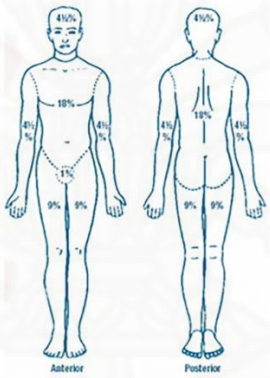

Figure 2.5 Rule of nine in burn assessment, (Copyright: ©2014 Feily.) [24]

5. The Dermatology Life Quality Index (DLQI)

To evaluate the impact of dermatological disorder to the quality of life, DLQI is assessed by using 10 validated questionnaires which is proven as valuable for vitiligo patients. These questionnaires structured to analyze the physical, social and functional aspects of life having four alternative responses as below:

- Score 0: not at all
- Score 1: a little
- Score 2: a lot
- Score 3: very much

After summarized total scores, the result will be maximum of 30, the worst quality of life and minimum of 0, the best quality of life which is related to dermatological

diseases. On the other hand, DQLI score is directly related with quality of life impairment. Generally, DLQI score can be interpreted as follow:

- 0-1: No effect at all on patient's life
- 2-5: Small effect on patient's life
- 6-10: Moderate effect on patient's life
- 11-20: Very large effect on patient's life
- 21-30: Extremely large effect on patient's life

6. Vitiligo Impact Patient Scale (VIPs)

Salzes [25] and team generated VIPs tool to calculate the incidence and assess burden caused by vitiligo according to skin phototypes by using vitiligo-specific burden questionnaire. Responses are using 6-point scale: "Never or Not applicable" (rated 0), "rarely" (1), "sometimes" (2), "often" (3), "very often" (4), "constantly" (5). Total score ranges from 0 to 110, for fair skinned phototypes, VIP- Fair skin (VIPs-FS) will be 0 – 110 and for dark skin phototypes, VIPs- dark skin (VIPs – DS), will be 0-130 respectively and final sore will be calculated as 100 then expressed as percentage.

Containing psychometric analysis, this tool aimed to evaluate the vitiligo burden in adults and also for individual burden before and after treatment so the value of VIPs score reflects directly to vitiligo burden.

7. Repigmentation Score

Repigmentation in vitiligo is one the main outcome for vitiligo intervention and main clinical studies are used to calculate the main outcome of the treatment. Based on literatures, more than 75% of repigmentation in vitiliginous skin is regarded as excellent outcome and rated the score as 4. If repigmentation is 51-75%, it is regarded as good outcomes with score 3 and 26-50% repigmentation as moderate improvement with score 2. The 1-25% repigmentation is graded as minimal improvement with score 1. If nothing changes or improvement, it is scored as 0 and the score will be -1 when worsen clinical outcomes. This scale is individualized approach to measure the efficacy of vitiligo treatment.

Table 2.4 Repigmentation Score

Grade	Repigmentation Condition
-1	Worsen
0	No Change
1	1-25% improvement (Minimal)
2	26-50% improvement (Moderate)
3	51-75% Improvement (Good)
4	76-100% improvement (Excellent)

2.1.8 Pathogenesis

Autoimmune disorder with loss of melanocytes may initiate disease through release of inflammatory signals by recruitment of natural killer (NK) cells and inflammatory dendritic cells. [26]Self-melanin production leads toxic to own melanocytes with autoreactive T cells. The effector arm of cytotoxic CD8+ T cell in melanogenic pathway acts autoimmunity in vitiligo such as gp100, MART1, tyrosinase and tyrosinase related protein 1 and 2. [27]IFN-ϒ plays important role in recruitment of specific melanocyte, induced chemokine CXCL10 and CXCR3 receptors, then autoreactive CD8+ T cell in order to recruit the T cell in the circulation.[28] For prevention and control of disease, CD4+ T regulatory cell (Tregs) takes place as critical role. Nevertheless, patients prone to suffer vitiligo who deprived of Tregs with immune polyendocrinopathy and associated with X linked syndrome. [29]

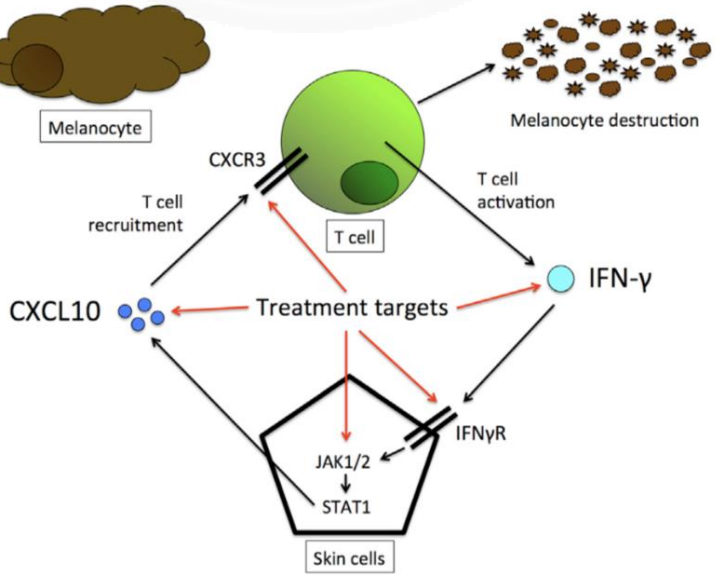

Figure 2.6 Schematic diagram of current understanding of vitiligo pathogenesis[7]

There are several mechanisms debated about the pathophysiology of non - segmental vitiligo in literature with several hypotheses such as autoimmune theory, melanocytorrhagy, oxidative stress and neural mechanism. Among these, autoimmune hypothesis tends to consider as main pathway in current update literature. In innate immunity, the initial trigger of depigmentation is Koebner phenomenon at the site of chronic friction or after trauma. It is frequently found in patients with active diseases. During active stage, researchers observed inflammatory responses as stress and destruction of epidermal melanocyte with danger associated molecular patterns (DAMP) and heat shock proteins (HSP).[30] These conditions may bridge the innate with adaptive immunity as IFN alpha induce lymphocyte attractive chemokines. At the same time, there is high D100B levels in active disease state therefore, it may spontaneously occur inflammatory responses. [31]

Koebner phenomenon might be one of the intrinsic adhesion defects of melanocytes which tended to associate with deficient E-cadherin expression. In vitiligo, alteration of E cadherin expression level before the development of depigmentation, deficiency of E cadherin levels may lose of melanocytes adhesion throughout oxidation or melanocyte stress. [32]In vitiligo, there are elevation of multiple oxidative stress markers and a collapse of antioxidative mechanism which leads to subsequent immune mediated melanocyte destruction. [33]

Segmental vitiligo seems to be melanocyte residing in a particular area following the dermatomal distribution compared with non-segmental vitiligo. Some literatures proposed about increasing neuropeptide is relating with effect of inflammation. [34] Moreover, unilateral distribution pattern of segmental vitiligo might be the most overlapping with segmental lentiginosis compared with other unilateral skin disorders. [35]

2.1.9 Risk Factors

Genetic factors are strongly influence the development of disease, approximately 2% in epidemiological prevalence, around 6% in family or sibling and 23% in identical twin. It is a form of polygenic pattern and multifactorial genetically related disease. Some key factors overwhelming the majority of immune gene in innate immunity are IFIH1, CASP7, NLRP1, I1CAM1 and others, on the other hand, for adaptive immunity

are CTLA4, CD80, HLA, GZMB, FOXP3, and so on. TYR, OCA2, and MC1R are risk alleles related with melanocytes so as to initiate the disease. [36, 37]

Furthermore, some external factors trigger the risk of vitiligo development such as exposing the phenolic compound, rhododendron, chemical involved in permanent hair dyes, detergents and monobenzyl ether of hydroquinone (MBEH) which promote the depigmentation in vitiligo. [38, 39] Nonspecific inflammation could induce localized lesions for koebnerization and cutaneous trauma lead to promote the autoreactive migration of melanocyte and T cell in vitiligo skin. [7, 40]

2.1.10 Therapeutic options in Vitiligo

Because of multifactorial and pathomechanical involvement in vitiligo, it causes challenging in management. However, based on current understanding pathogenesis, there are 3 distinct approaches for successful vitiligo treatment. They are: to reduce melanocyte stress, regulate the autoimmune response and stimulate melanocyte regeneration.[1]

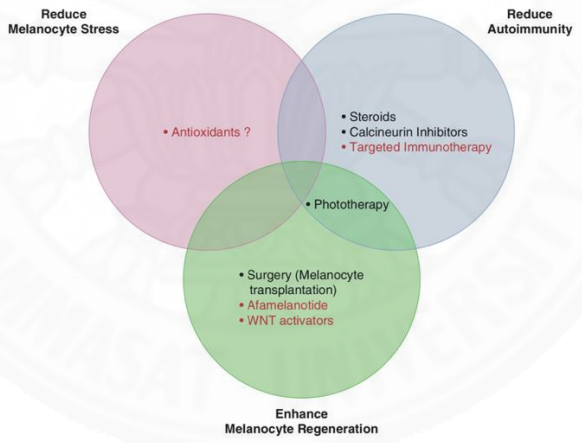

Figure 2.7 Current and emerging treatments address 3 major goals in vitiligo treatment are listed in black and emerging treatment in red [1]

Moreover, management has to initiate with individual personalized approach together with several multifactorial facts which are influencing the therapeutic choice such as disease duration, treatment impact and side effect, patients' demographic information, suffering site, psychosocial and cultural variation. Clinicians should mindful not only the patient's satisfaction in addition to expectation but also have to explain thoroughly about the treatment benefit and outcomes. [2]

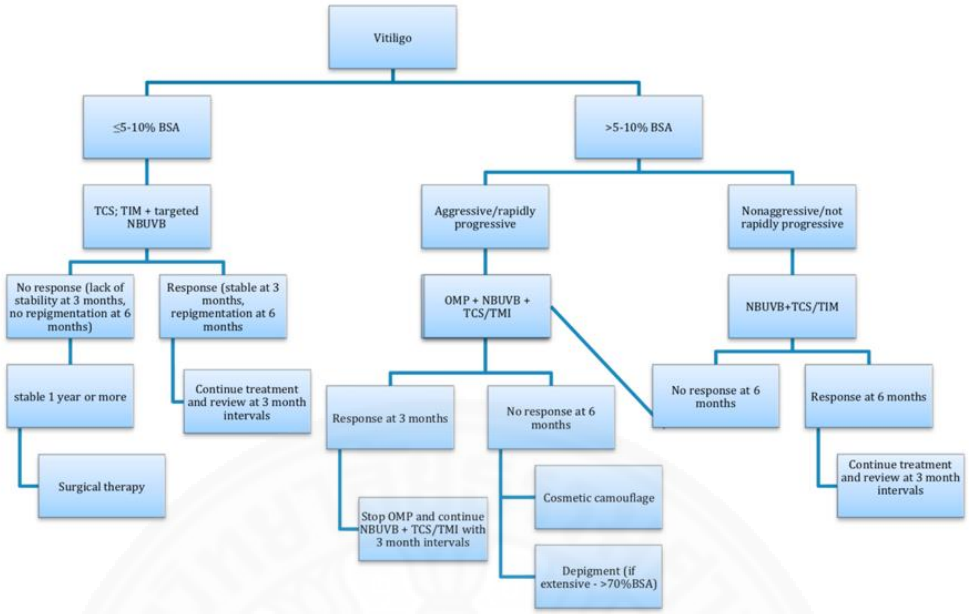

Figure 2.8 Treatment algorithm for Vitiligo [4]

1. General Measure

It is essential to avoid triggering all physical factors such as friction and trauma that tend to develop new depigmentation. Enough sun protection with broad spectrum sun screen or clothing might help to cover sun burn on depigmented area especially at summer. Sometime, spontaneous and treatment induced repigmentation can occur during summer times because of ultraviolent (UV) exposure. Therefore, moderate amount of UV exposure is involving as one strategy for repigmentation in vitiligo. [41, 42]

2. Topical Treatment

a. Topical Steroid

Topical steroid is considered as the first line since it has anti-inflammatory and autoimmune regulatory action that suppress the disease progression. There is similar repigmented efficacy between potent and ultra-potent corticosteroids. Treatment limitations may include skin atrophy, telangiectasia, striae and acneiform eruption. To monitor the treatment efficacy, potent corticosteroid should continue for at least 6months for better result. [41] Repigmentation more apparent at summer period especially on face, it can regard as successful treatment if no more disease progression. [43]

b. Topical Immunomodulator

Calcineurin inhibitors such as topical tacrolimus and pimecrolimus have effect on T cell activity which cause decreasing the production of proinflammatory cytokine. Some clinical trials proved that calcineurin inhibitors promote melanocyte migration and pigmentation [44, 45] with twice daily applications. Overall efficacy is similar with topical corticosteroid and repigmentation will start at the face rather than other sites of the body. The response rate of tacrolimus is slightly higher than pimecrolimus because of its higher potency and it has been proved in koebner induction study [40] Transient red-hot feeling during first 2 weeks of application is the most frequent complaints from the topical immunomodulatory therapy.

c. Topical Antioxidants

Natural herbal products and vitamin supplements become nominated as possible therapeutic option because of their antioxidants and anti-inflammatory properties. There are higher level of ROS and significant lower level of catalase enzyme in the epidermis of lesional skin. Antioxidants therapy or controlling reactive oxygen species might be beneficial. [46]Herbal extract Ginkgo biloba showed the efficacy of controlling the disease activity by limiting and slow spreading of vitiligo and also promoting repigmentation in vitiliginous area in two small groups human clinical trials. [47, 48]

3. Phototherapy

Phototherapy is generally known as competent option for vitiligo therapy. Among them, twice or thrice weekly NB-UVB is the best-choice phototherapy meanwhile it can stimulate repigmentation in most vitiligo patients, however, complete clinical recovery is found only at small group. [49] Phototherapy with tacrolimus two times per day application has been revealed as reducing chance of recurrence. [50]NB UVB show more efficacy of repigmentation and less side effect compared to photochemotherapy but 308nm excimer laser need shorter treatment duration for repigmentation compared with NB UVB. There is some dose dependent hyperpigmentation side effect with burning and risk of skin cancer increased with multiple phototherapy. [51, 52]

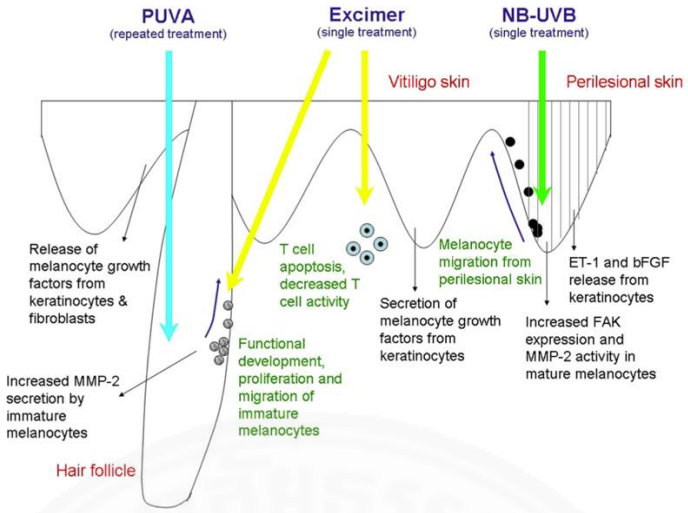

Figure 2.9 Schematic diagram showing the proposed mechanisms of different forms of phototherapy (PUVA, excimer laser/excimer light, NB-UVB) in inducing vitiligo repigmentation. [53]

In 1997, first clinical trial to compare between the clinical effectiveness of NB-UVB phototherapy and topical PUVA in vitiligo intervention which was done by Westerhof and team [54]. After two times per week for 4-month with NB UVB phototherapy, 67% of patients achieved significantly clinical recovery in skin color while, only 46% of patients got repigmentation in two time per week with PUVA therapy. Based on the several clinical trials about NB UVB, it can consider as the first line safety and efficacy for generalized vitiligo in all ages compared with PUVA. [55]

4. Systemic Agents

a. Oral corticosteroids

Partial pulse medication with moderate dose for oral corticosteroid 2.5 - 10mg dexamethasone, continuously two days in a week can halt the disease development in 88% of patients after taking 18.2-week treatment, rarely result with repigmentation. [56] A few adverse effects are unwanted fat deposition, acne, mood swings, sleep disturbance, unwanted hair growth, and irregular menstruation. Nevertheless, there are some rapid repigmentation with stop disease progression when combined with NB UVB together with long term side effect because of immunosuppression. [57]

b. Antioxidants

There are some clinical trials done with small sample size about combination therapy of systemic antioxidants (polypodium leucotomos, vitamin E and C) and NB UVB tend to increase recovery rate. However, large scale trials are required for

more accurate results. [58, 59]

5. Surgical intervention

Various surgical intervention technique has been available for selective type of vitiligo in order to perform pigment cell transplantation. There have 2 types: tissue grafting (including punching or split thickness grafting and epidermal blister grafting) and cellular grafting for melanocyte regeneration. Disease stability is quite important for successful surgical intervention and most effective for segmental vitiligo type. [60]

6. Depigmentation therapy

Treatment with depigmentation procedure is suitable only for the patients with depigmentation more than 50% to 60% of body surface area involvement (Generalized vitiligo or vitiligo Universalis). Treatment will be depigmented to the remaining pigmented area. Several bleaching agents (example monobenzone ether ester), laser therapy or cryotherapy have been reported in clinical trials. According to the previous clinical data, monobenzone ether ester treatment need longer duration of treatment around 5 to 12 months for satisfactory result. Laser and cryotherapy will also require several sessions for desired depigmentation. Strict sun protection during and after treatment is essential for successful outcomes. [61]

7. Cosmetic: Camouflage

Since appropriate cover-up of depigmented lesion can recover the dermatological life quality index (DLQI), camouflage is regarded as valuable option for vitiligo patients. Various methods have been launched such as tattooing, dihydroxyacetone, general cosmetics and numerous camouflage application such as microskin. Depend on the location of depigmentation, skin photo type and individual preference, clinicians have to consult wisely for available options with their advantages and disadvantages[62].

8. Combination Therapy

Even though multiple treatment modalities are established for repigmentation for vitiligo, variable outcome responses with unsatisfactory results are facing until these days which are influenced by multifactorial and polygenic nature of pathogenesis of vitiligo. In order to consider about these facts, clinicians are approaching to the combination therapies rather than treating with monotherapy to get better repigmentation and outcomes. [63] While NB UVB is keystone for vitiligo therapy, many clinical trials are being conducted for the combination therapies with medical or surgical

modalities to reduce the patient's battle against this intolerable disease.

In non-surgical treatment of vitiligo, a simple, safe, tolerable and cost-effective therapy has been studied which is combination therapy of intradermal 5-FU with NB-UVB. 5-FU reduces NB-UVB treatment period and resulted with superior outcomes and repigmentation except acral regions. In resistant localization of vitiligo, preceding laser dermabrasion combined with NB-UVB and potent topical steroid significantly improved the repigmentation rate however, the chance of repigmentation in such difficult areas is low and risk to have side effects and poor tolerance in current daily practice. [64]

Using oral psoralen plus ultraviolet A together is called PUVA, photo chemotherapy has been played the main role in generalized vitiligo meanwhile 1950s and has been substituted with NB-UVB. In current practice, it is rare to use NB-UVB as monotherapy and used to combined with topical corticosteroids and immunomodulatory medication which has been proved to accelerate clinical improvement.[65] Combination of two immunosuppressant reported as prone to cancer in literature, nevertheless safety and risks of each modalities are still unclear as well as long-term follow up data are still unavailable yet.[2]

2.2 Platelet-rich Plasma (PRP)

2.2.1 Introduction of platelet-rich plasma

Platelets mean thrombocytes, colorless cell fragments, which is mainly function as to stop bleeding. PRP contains relatively high concentration of platelets in plasma fraction compared with the whole blood. Platelets are concentrate in the plasma with various growth factors such as platelet derived growth factor (PDGF), transforming growth factor-b (TGF-b), epidermal growth factor (EGF), vascular endothelial growth factors (VEGF) and cytokine from alpha granules which regulate cell migration, local immune regulation, proliferation, attachment, variation and promotion extra cellular matrix accumulation, melanocyte regeneration and attachment to their specific receptors. [66-68]

Table 2.5 Key regenerative growth factors stored in platelet alpha granules and their functions[69]

Key regenerative growth factors stored in platelet alpha granules and their functions

Growth Factor	Function
PDGF	Stimulates cell proliferation, chemotaxis, and differentiation Stimulates angiogenesis
TGF-β	Stimulates production of collagen type I and type III, angiogenesis, re-epithelialization, and synthesis of protease inhibitors to inhibit collagen breakdown
VEGF	Stimulates angiogenesis by regulating endothelial cell proliferation and migration
EGF	Influences cell proliferation and cytoprotection Accelerates re-epithelialization Increases tensile strength in wounds Facilitates organization of granulation tissue
bFGF	Stimulates angiogenesis Promotes stem cell differentiation and cell proliferation Promotes collagen production and tissue repair
IGF-1	Regulates cell proliferation and differentiation Influences matrix secretion from osteoblasts and production of proteoglycan, collagen, and other noncollagen proteins

Since the effect of PRP on the inflammatory responses is still unclear, El-sharkawy studied about the PRP ‘s growth factor, the effect of PRP on monocyte, cytokine release and lipoxin A4 (LXA4) generation in 2007 by using peripheral blood from healthy donor, made isolating and culturing regardless the content of PRP. This study proved that PRP have several growth factors more than the whole blood and platelet-poor plasma. By the mechanism of chemoattractant for fibroblasts and macrophages, mitogen for firbroblasts and synthesis of extracellular matrix components, the growth factors of PRP can slow down the skin aging. It was dose dependent fashion, PRP stimulate monocyte chemotaxis and monocyte migration so it may reduce cytokine release, regulate infection and encourage tissue regeneration. [70] Furthermore, PRP can accelerate the healing, proliferation and regeneration of tissue so it has been studies in bone related application for past several decades.

2.2.2 Types of Platelet-rich Plasma

Autologous solution PRP contains 4-7 folds the mean of platelet concentration of whole blood achieved by centrifuging an autologous whole blood. In theory, there are 4 subsets of platelet-rich plasma: pure PRP or leukocyte-poor PRP (P-PRP), leukocyte-rich PRP (L-PRP), pure platelet-rich fibrin or leukocyte-poor PRF (P-PRF), leukocyte-rich fibrin and PRF (L-PRF) which control sustainable release of growth factors for a few months.[71] In PRP, two key important parameters include leucocyte content and density or architecture of fibrin.

Table 2.6: Types of PRP preparation[72]

	Leukocyte poor	Leukocyte rich
PRP	P-PRP (small volume, minimal fibrin polymerization)	L-PRP (small volume, minimal fibrin polymerization)
PRF	P-PRF (larger volume, dense fibrin polymerization)	L-PRF (larger volume, dense fibrin polymerization)

In P-PRP and L-PRP, immature fibrin network consists of fibrillae with a minute diameter because of simple fiber polymerization. It is quickly dissolved like a fibrin glue. P-PRP is lack of leukocytes but L-PRP contain leukocytes. Both activated P-PRP and L-PRP have a low-density fibrin network. Their main differences are types of platelet concentrates, amount leucocyte and effects of the leucocytes on propagation, diversity, immunity and infection.[72]

In P-PRF and L-PRF, multiple fiber assembly caused thick fibrin fibers and having a resistant matrix as fibrin biomaterials. P-PRF is lack of leukocytes, vice versa, in L-PRF has leukocytes, while both have a high- density fibrin networks. Most clinical trial conducted with L-PRP and pure PRP, little leukocyte and most platelet are condensed at buffy coat layer. [71] Several studies point out the key role of leukocyte in PRP especially for their micro bactericidal and immunomodulatory action.[70] On the other hand, the leucocyte content has supportive effects or theoretically advantageous effects of PRP especially in orthopedic treatment.[73]

Table 2.7 Difference in platelet-rich products[74, 75]

Platelet product	Anticoagulant	Activation	Fibrinogen polymerization	Fibrin architecture
Platelet-rich plasma	Added before centrifugation	In vivo	Low	None
Activated platelet-rich plasma (or gel)	Added before centrifugation	In vitro activation with Ca^{2+} or thrombin	Low-moderate	Weak
Platelet-rich fibrin	None	In vitro activation without additive	High	Strong

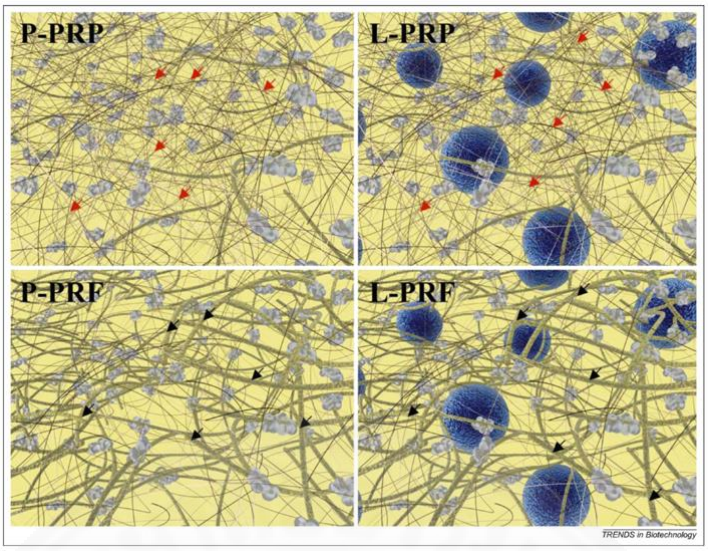

Figure 2.10 Schematic illustration of the matrix and cell architecture of the four categories of platelet concentrates.[71]

PRP is more platelet concentration than PPP, approximate 9.4-fold more than the whole blood. Average platelet count for whole blood, PRP and PPP were $1.8x10^5$, 1.7 x 10^6 and 3.1x 10^4 respectively. [76] There are variable reports about the significant of PRP variants, numbers of growth factors and standard dose response relationship in many clinical reports so Delong et al proposed that the PAW classification system based on 3 components: the absolute count of platelets, the manner platelet activation and the presence or absence of leukocytes. [77]

In order to create clinical guideline and make adjustment to accurate PRP dosage FIT PAW classification is generated and including 7 critical components: the force of centrifugation, the Iteration or sequence of centrifugation; the Time of centrifugation; Platelet concentration (baseline of patient's whole blood and final PRP product); Anticoagulant use; the utilization of an Activator including the type and amount; and the composition of White blood cells as shown in the figure below: [78]

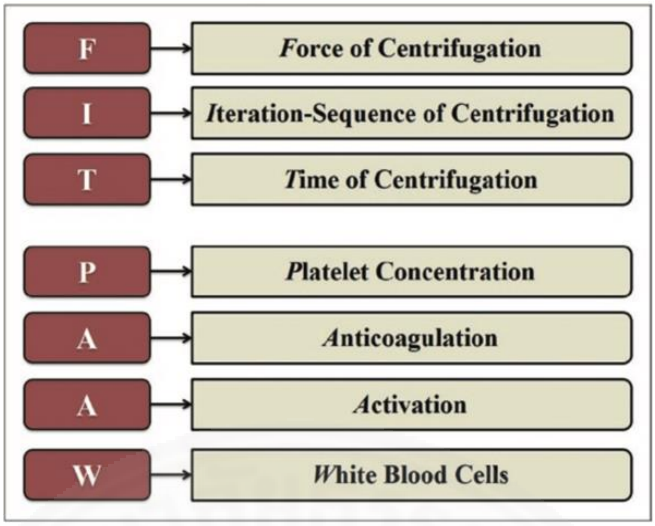

Figure 2.11 FIT PAAW classification for PRP[78]

2.2.3 Methods of preparations

There are several techniques to generate various subsets of PRP including blood collection, centrifugation, plasma aspiration, potential second centrifugation, selected supernatant removal, mixing/resuspension of platelets, activation and application.

10-60ml of whole blood is collected and mixed with anticoagulants (acid citrate dextrose or sodium citrate) in order to inhibit ex vivo coagulation and early secretion of growth factors (alpha granules). According to Stoke law, centrifuging of blood separate the cell types with different layers because of specific gravity.[69] Platelet-rich plasma derived at the inferior layer of plasma portion in the single-spin method. In order to get more platelet concentration and isolates plasma, second centrifugation is made. Then, buffy coat layer is obtainable at the top as in figure below.

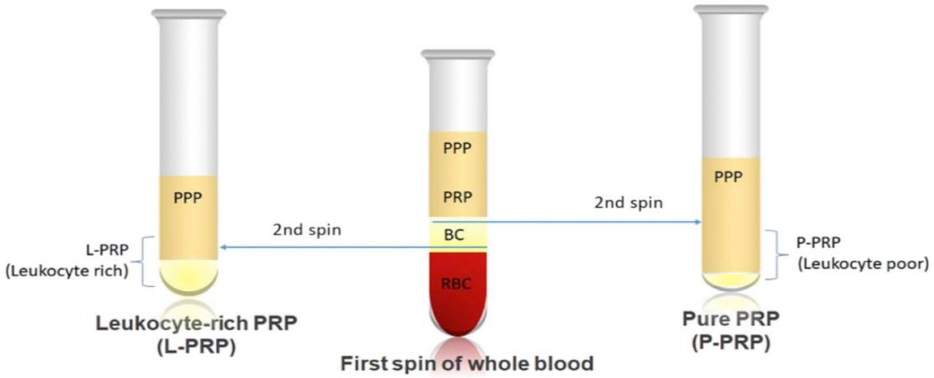

Figure 2.12 Isolation of P-PRP and L-PRP in a single-spin(softspin) technique or a 2-spin technique. (BC = Buffy coat; L-PRP = leukocyte- and platelet-rich plasma; PPP = platelet-poor plasma; P-PRP=pure platelet-rich plasma; PRP=platelet-rich plasma.) [74]

Dohan Ehrenfest et al studied the method to produce L-PRP and P-PRP by using single-spin (Soft-spin) and double-spin (2 spin) techniques. The whole buffy coat layer is filled with L-PRP, the top buffy coat layer is pure PRP (P-PRP) which is lower plasma portion. [71]

For activation of PRP, platelet activators, thrombin or calcium chloride (CaCl2) add to the whole blood and centrifuge to get loose fibrin matrix called PRFM, which has several growth factors concentrate and keep releasing them over a week which is applied mostly at fat grafting and soft tissue augmentation. [79] Leukocyte and PRFM (L-PRFM), as final subset, can be used without adding any anticoagulants and activators. Different processing methods affect configuration of PRP, growth factors proliferation and cellular concentrations in platelet result. [74]

2.2.4 Mechanism of action of platelet-rich plasma

Even though several clinical reports got several positive outcomes from PRP therapy, the detail contact of growth factors and biological mediation of PRP are controversial until now. Some literatures proposed that growth factors stimulated the fibroblast proliferation, extracellular matrix formation, promote the collagen and total protein synthesis. In cell regeneration, PRP also enhance antiphlogistic activity by lacking the macrophage activation and inhibition of Monocytes chemotactic protein-1 (MCP-1). By inhibiting the T cell regulation, it normalized the T cell expression, secretion (RANTES) level delivery and lack of lipoxin A4 (LXA4).

Leucocyte provides anti-inflammatory action and immune regulation, production of VEGF and crucially significant in augmenting angiogenesis especially in vascular endothelial lining integrity. RANTEs activates more adherence of monocytes to endothelial cells, potential for potent chemoattractants.

In alopecia, Li discovered that PRP encourages the propagation of dermal papilla (DP) cells and accelerates phosphorylated extracellular signal-regulated kinases (pERKs) and AKt expression in DP cells tends for cell proliferation, growing, helps survival and halts apoptosis in vitro. Moreover, PRP increase antiapoptosis protein BCL-2 which modifies the appearance of apoptotic molecules and cell existence. In 2019, Hesseler M studied a statistical meta-analysis about application of PRP for dermatology in clinical practice, analyzed different dermatology procedures with PRP therapy. It resulted that PRP significantly advantages in wound healing process as well as local microbicidal actions. In T cell mediated disease like vitiligo, platelet has a role in

controlling in vivo T cell immunity through TGF-beta, so activate autologous P-PRP suggested as superior outcomes with earlier result and can optimized in vitiligo repigmentation as level of evidence 2b especially in stable vitiligo. Moreover, PRP adjuvant therapy can minimize the used of systemic medication as well as their unwanted side effect profiles.[74]

2.2.5 Adverse reaction and contraindication of platelet-rich plasma

Recently years, many clinical trials proved about the beneficial effects of PRP in treatment of vitiligo but there was one case report of female patient who developed facial vitiligo after injection of platelet-rich plasma. Occurrence of vitiligo with Koebner phenomenon in one of the areas where PRP had been injected which was reported by Ejjiyar M in early 2019. [80]

Generally, PRP is regarded as harmless with minor side effects and few contraindications. One recent report in PRP injection of periorbital rejuvenation, there has documented about vascular compromised and irreversible blindness. [81]Moreover, there are warrant cautions for PRP therapy as medical contraindication may include blood dyscrasias, current infections being treated by antibiotics, use of antiplatelet agents, and use of systemic immunosuppressant, medications such as glucocorticoids. Others may include unable to withstand injection therapies or phobias for any invasive or noninvasive intervention. [69]

Regards to the past studies, absolute contraindication for PRP therapy are critical thrombocytopenia, platelet dysfunction, hemodynamic instability, sepsis, local infection at site of PRP administration, unwillingness of accepting risk and there are some relative contraindications: NSAID use within 48 hours, intradermal or intramuscular glucocorticoid administration at lesions within 4weeks, using intravenous glucocorticoid within 14days, smoking, recent illness, cancerous condition mainly at bone or hematolymphoid, anemia to haemoglobin < 10g/dl and thrombocytopenia to more than 105 platelet per microliter. [82]

2.2.6 Purposes of platelet-rich plasma

1. Evidence of Platelet-rich Plasma in non-dermatology

Since PRP is valuable in numerous dermatologic as well as non-dermatologic applications, many clinical studies measured its efficacy. Since 1980 and 1990s, the earliest clinical reports in the field of cardiac, dental and maxillofacial surgery and later gained popularity in regenerative medicine. Many researchers aim for future best

practice in various approaches of surgery and clinical procedures, including repairing of challenging wounds, maxillofacial bone defects, aesthetic operation and gastrointestinal surgeries.

a. Bone and musculoskeletal medicine

Grassi [83] and team conducted the meta-analysis and systematic review about the effect of PRP injection therapy in acute muscle injuries with 6 studies, 374 participants. All 6 studies were significantly shorter duration in patients treated with PRP compared with placebo injection or physical therapy. However, re-injuries and complications were also similar between 2 groups. Therefore, 4 articles were lack of patient blinding and evidence quality was inferior according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE). Conclusion in this study showed that using PRP in treatment of acute muscular injuries has no effect on pain management, functioning and relapse.

Numerous studies proved that effect of PRP enriched the bone grafts, promote bone augmentation and regeneration with superior effects. Marx et al studied that PRP enriched bone graft by randomized control trials. Max and colleagues conducted study with similar technique of PRP preparation and used in enhancement of bone graft procedure. This study resulted that PRP might influence not only high in bone density but also faster radiographic maturation. [84]

b. Wound healing

Many studies evaluated about the role of PRP in wound healing especially chronic ulcer which is the most prevalence in general population. De Leon et al[85] conducted a large observational clinical trial by applying the activated autologous PRP gel in 285 refractory chronic wounds, with multifactorial etiology, once or twice a week. 86.3% of the wound healing well with site 47.5% decrease especially in decubitus ulcer, diabetic and stasis ulcers achieved drastically rapid in recovery compared with other etiology. One prospective cohort studied with 44 patients evaluated with topical autologous L-PRF on small stasis ulcers, diabetic foot ulcers or complicated wounds. 3 months after post treatment resulted with full closure of venous ulcers after a mean of 12.6 treatments. [86]

To compare the activated autologous L-PRP gel and platelet poor plasma, Saad seta et al [87]evaluated 21 patients who had chronic diabetic ulcer >12 weeks in duration. Randomly two time per week for treatment showed that topical L-PRP gel had

shorter in average curative time than topical platelet poor plasma.

c. Dentistry

Since growth factors of PRP are capable to promote healing process, using PRP in surgery have been shown two to three times better healing than conventional surgery. [84] Using PRP become a natural booster and enrich the wound healing mechanism, many periodontal and oral surgery including bone grafting, implantation and maxillofacial related surgeries have been shown clinically effectiveness of PRP as a combination therapy. Nathan E reviewed several articles about PRP in wound healing mechanisms dealing with oral and maxillofacial surgery and periodontics fields. All reviewed articles expressed that platelet derived growth factors (PDGF) and transforming growth factor beta (TGF- beta) stimulated significant new granulation tissue in vivo therefore, these growth factors are important in healing of full thickness dermal wounds as well as accelerate post-surgical healing in both periodontal and oral surgical application. [88]

d. Pain control

59 patients with acute traumatic wound, which were not required flap surgery, studied with topical autologous, activated PRP gel compared with Vaseline petrolatum gauze weekly for 3 weeks. PRP formed the early improvement for first week, consequently reduced pain on second and third week.[89]

Johal and teams [90] studied total 78 randomized controlled trials with 5308 participants so as to analyze characteristic and effect of PRP on patient-reported musculoskeletal pain and treatment condition. Meta-analysis reported that PRP impact on pain in orthopedic surgery mainly in pain reduction but limited clinical efficacy.

e. Others

Moreover, not only PRP promotes healing ulcer but also might contributes to peripheral nerve regeneration. After weekly treated neuropathic ulcer with topical activated L-PRP for 6 times in 50 leprosy patients, almost 92% resulted with widespread re-epithelialization after treatment for six times .[91] There are a few studies claimed that variety of benefits with PRP therapy in regards to wound healing, reducing pain, pruritus and burning sensation in pressure ulcers[92] and venous ulcers[93].

2. Evidence of platelet-rich plasma in clinical and cosmetic dermatology

In the field of dermatology, recently PRP as adjuvant therapy is being attractive and got attention in treatment of androgenic alopecia (AGA), scar repaired therapy, acne scars revision, skin rejuvenation, dermal volumizing and striae distensae removal besides vitiligo.[76] Utilizing the various protocols of PRP preparation, different outcome measurements highlight the role of PRP in medical dermatology.

a. Fat Grafting

Autologous fat grafting is being choice of treatment for facial volumizing and repairing facial scars in current years mainly at cosmetic surgery. Some studies conducted about the combination of PRP with autologous fat grafting may have superior results with increased graft longevity. Since platelet-rich plasma promote angiogenesis, wound healing and proliferation of adipose-derived stem cell together with fat grafting. [94] Nevertheless, PRP has no advantages in combination with breast fat when compared with conventional method. Retrospective study done with 42 women showed that there was higher rate of fat necrosis and no effect on the need of further fat grafting. [95]

b. Alopecia

PRP therapy in androgenic alopecia (AGA) conducted with injection of PRP and PRP/dalteparin and protamine mircroparticles (PRP-DP) for 2- 3weeks interval for 3 months. PRP and PRP-DP groups had similar results with increasing number of hairs but PRP-DP group was considerably growth in hair breadth compared to the other groups. Action of improved in collagen fibers, fibroblasts and angiogenesis about hair follicles are increased by PRP and PRP-DP effects[96].Another study proved on non-cicatricial alopecia that 5 PRP injections within 2 months duration revealed increasing hair volume and quality clinically and microscopically. [97]

Alopecia areata (AA) is a common autoimmune inflammatory disorder that provoked hair loss but there has no efficient treatment until now. There was a pilot, split scalp study done with 45 patients suffering alopecia areata studied half scalp with intralesional PRP, triamcinolone acetonide (TA) or placebo and another half scalp was untreated as a control. Significant hair regrowth and reduction of burning, itching achieved at the side of PRP therapy compared with the control side. [98] Another study for AA with PRP therapy at 90 patients, divided into 3 subgroups, treating with topical minoxidil 5% solution, PRP injections, and the rest with placebo as control respectively. At PRP group,

hair regrowth occurred as the earliest response, short vellus hair and dystrophic hair reduction unlike the groups with topical minoxidil and control. Therefore, PRP has applied as safe, cost effectiveness, innovative treatment in hair loss because of these beneficial roles in hair regrowth.

c. Scar

Combination therapy with Er: YAG laser and topical PRP gel on facial acne scar revealed that more than 50% scar improvement in 68% and 91% of patients after the first and third treatment respectively. It was hard to evaluate efficacy of each modality since it did not conduct with split face study. [99] Furthermore, PRP therapy appears speedy recovery after cosmetic procedures with fractional ablative laser and rhytidectomy. [100] Applying L-PRP after fractional ablative laser markedly reduced the erythema and melanin index at the treated area compared with control, moreover PRP can reduce the transepidermal water loss and promote the collagen regeneration. [101]

d. Skin Rejuvenation

The advantages of possessing variety of growth factors in PRP proposed the improvement in skin smoothness and reduction of winkles in skin rejuvenation. After single injection of PRP to infraorbital dark circles and crow's feet wrinkles showed drastically improvement in skin color consistency, melanin, wrinkle volume assessed by physicians at post 3 months treatment period. [102] About facial rejuvenation and dermal grafting, there were two case reports pointed out using PRP combination with autologous fat grafting nevertheless, controlled studies are required for more scientific data. [103],[104]

e. Striae Distensae

There were some studies utilizing PRP as combination therapy in striae distensae which are dermal scar with epidermal atrophy because of continuous stretching. Intradermal PRP together with radiofrequency applied on the striae distensae once every 4-week interval, before and after comparison in 19 patients. 5.3% achieved excellent and 31.6% showed moderate improvement. Among 19 patients, 12 patients felt satisfactory or very satisfactory result on intradermal PRP with radiofrequency intervention. [105]

Even PRP is relatively new treatment modalities for aesthetic dermatology, there have a lot of evidence based proven beneficial effects on many cosmetic procedure and scar revision treatments. However, further clinical studies still required to analyze by comparing with control treatment as well as split sides treatment to

reveal the efficacy of PRP more apparently.

f. Vitiligo

Even though multiple treatment modalities are established for repigmentation for vitiligo, variable outcome responses with unsatisfactory results are facing until these days which are influenced by multifactorial and polygenic nature of pathogenesis of vitiligo. In order to consider about these facts, clinicians are approaching to the combination therapies rather than treating with monotherapy to get better repigmentation and outcomes. [63] Sardana studied scopes of medication and light- based intervention based on their pathogenesis and analyze the PRP function. Even though PRP monotherapy is not effective in repigmentation but superlative results when combined with another modality like NBUVB phototherapy. Another issue mentioned that PRP does not help repigmentation in some difficult areas like elbows, pressure bearing area or acrofacial area. This study pointed out that repeated injection of PRP twice weekly interval would not possible option and negatively painful option in real practice that would probably induced koebnerization [106] but large scale clinical measures are required since accurate mechanism of PRP on vitiligo is uncertain up till now.

Although NB UVB is safe and efficient therapy for vitiligo, patients' non-compliance results are relatively high because of longer treatment duration and adjusted dosage of phototherapy. Ibrahim conducted prospective study to explore the short-term NB UVB outcome combined with PRP injection in stable-vitiligo patients in early 2016. Total 60 stable vitiligo with symmetrical lesions are recruited to evaluate NB UVB alone as control and the other for combination NB UVB phototherapy with intradermal PRP injection for twice a month for 16 weeks. There was statistically improved in clinical result at combination therapy (NB UVB + PRP) compared with phototherapy alone. That study concluded about intradermal PRP injection combined with NB UVB was simple, tolerable and cheap technique, shorten the duration of NB UVB phototherapy and better patient's compliance. [107]

Fractional CO2 is one of the ablative laser which is widely used in many dermatological fields as well as in cosmetic procedure. In 2014, Helou J introduced using the 10600nm fractional CO2 laser as ablative treatment options for treatment of vitiligo followed by sun exposure got better improvement in non-segmental vitiligo (NSV).[108]

In 2018, Abdelghani conducted the study about combination treatment for vitiligo with fractional carbon dioxide (Fr: CO2) laser, intradermal PRP injection and NB

UVB for stale segmental vitiligo patients to consider about the repigmentation grade, patients' satisfaction and evaluate the side effect after trials. Participants are divided into 4 groups: each group received Fr: CO2 laser alone, PRP alone, combined Fr: CO2 and PRP, combined Fr: CO2 and NB UVB with 2-month duration of treatment. Among them, laser and PRP combination got excellent outcome: 60% of patients became repigmentation > 50% and 40% of patients got >75% of repigmentation, compared with other groups. Therefore, based on this trial combination fractional CO2 with PRP injection is the most effective treatment for repigmentation followed by combination fractional CO2 with NB UVB phototherapy, however, there is unsatisfactory results for monotherapy of fractional CO2 and PRP injection groups. [109]

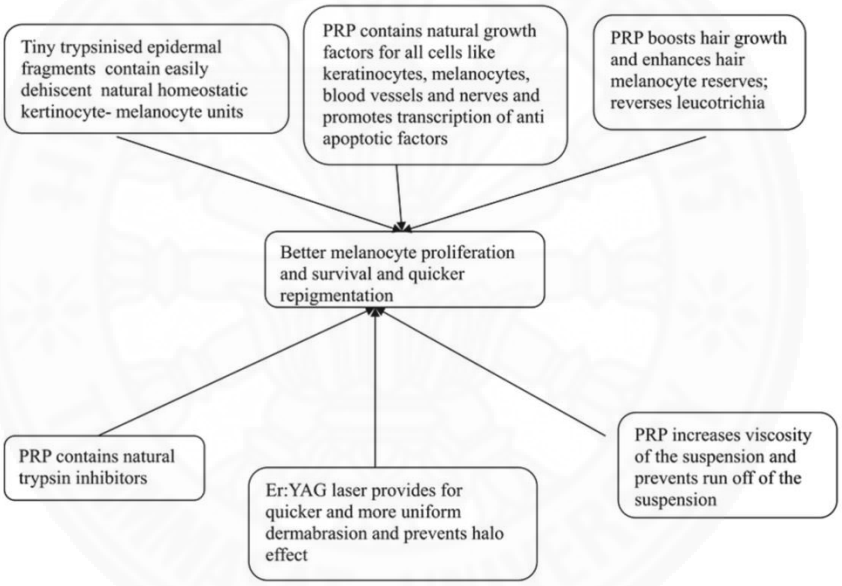

Figure 2.13 Summary of the advantages offered by LA-PEEST over the conventional NCES technique[110]

About the effective surgical modality for stable vitiligo in cellular graft, there are non-cultured epidermal melanocyte suspension graft and non-cultured follicular root sheath suspension graft. Non-cultured epidermal cell suspension (NCES) is transplantation of basal epidermal melanocyte into depigmented vitiliginous patch. [110]Parambath was using these beneficial facts of repigmentation in NCES transplantation and PRP to compare the efficacy of PRP and phosphate buffered saline (PBS). Total 21 stable vitiligo patients compared 20 lesions in the PRP arm by at least two sessions and another 20 lesions in non PRP arm as control (in PBS) by single session of treatment with 6 months follow up. Regards to this double blind split body randomized control trial that NCES suspended in PRP achieved significant repigmentation and more

patients' satisfaction compare to suspend in PBS, non PRP arm. Moreover, this study threw light into the capability of PRP in promoting repigmentation in vitiligo at low cost and effective treatment.[111]

Even considerable improvement of medical intervention in past decades, the chance of complete repigmentation is too hard to achieve in all patients. Therefore, several surgical modalities are introducing as alternative therapy for stable vitiligo since 1952. [112] The main concern for surgical intervention is to restore pigment uniformly, promote the melanin regeneration and replace the melanocyte in depigmented area.

CHAPTER 3

RESEARCH METHODOLOGY

This systematic review and meta-analysis is relied on Cochrane Handbook for Systematic Reviews of Interventions. Regards to Cochrane review, there are core methods for planning and determine the scope and questions, identify research evidence that fits pre-specified criteria and grouping for synthesis. Systematically searching and selecting studies to minimizing biases, collecting data, preparing for synthesis according to effect measures thus providing more reliable outcome results and guiding reviewers to report precisely.

The characteristics of a systematic review are; able to approach highly reliable quality, relevant, accessible and innovative data for healthcare personnel, to reduce bias through the use of pre-specified research questions and methods that are documented in protocols, and by basing their findings on reliable research, cconducted by domain expertise and methodological expertise in a team, who are free of potential conflicts of interest.People who might make – or be affected by – decisions around the use of interventions should be involved in main assessments about the review.It is critical for good data management, project management and quality assurance mechanisms. (Cochrane Handbook for Systematic Reviews of Interventions version 6.0, updated July 2019)[113]

Statistical grouping of two or more independent analyses is termed meta-analysis. Systematically numerical combination of all results to yield an overall statistic for summarizing the value of current experiment compared with comparators. Possible benefits are improving precise results particularly in comparison of categories of interventions and generating the new hypothesis by the exploration of differences between studies. [114]

Research Question

- How much the efficacy of platelet-rich plasma modality as adjunct therapy for vitiligo?

Population

Participants are human population who diagnosed vitiligo with any criteria, all age groups and both genders with all Fitzpatrick's skin types.

Intervention and Comparisons

The comparators which are using PRP in combination with standard vitiligo treatment. Comparative studies, before and after therapy, comparison between each modality in intervention domain, clinical trial, evaluation study, multi-center clinical trials, observational or pragmatic clinical trial, randomized control trial or twin study which involved patients with vitiligo were treated by platelet-rich plasma combination therapy.

Outcome Measurement

Clinical improvement of vitiligo defined as more than or equal 75% of repigmentation as primary outcome and secondary was more than or equal 50% repigmentation of depigmented skin.

Study Design

This study will be done with all comparative clinical studies involving both quasi experimental and randomized control trials (RCT).

3.1 Material and Method

3.1.1 Identification and searching data

About data searching, it was performed at the electronic database via PubMed, the Cochrane Central Register of controlled trials and Scopus and reference lists of articles by using related search terms for specific type of diseases and intervention up to 31st December, 2019. Articles and clinical trial references were searched by using with Medical subject headings (MeSH) terms followed by Cochrane Highly Sensitive Search strategy.

3.1.2 Selection of data

After analyzing the databases, the clinical data are extracted based on selected inclusion and exclusion criteria. All studies data are searched from PubMed, Cochrane and Scopus databases with MeSH (Medical Section Heading) terms: ((“vitiligo” OR “leukoderma”) AND (“platelets” OR “platelet-rich plasma” OR “platelet gel” OR “platelet-rich fibrin” OR “platelet-releasate” OR “PRP” OR “leukocyte platelet plasma” OR “LPRP” OR “L-PRP” OR “LPRP gel” OR “leukocyte and platelet-rich plasma gel” OR “pure platelet-rich plasma” OR “P-PRP” OR “PPRP” OR “advanced platelet-rich plasma” OR “advanced PRP” OR “A-PRP” OR “APRP” OR “autologous cells” OR “plasma rich in growth factors”) ; (“Clinical trial”; “Full text”; “Human”; “English”)). All data analyzing and grouping are done with Review Manager 5.3 (Rev Man) and STATA version 14.0 (Stata Corp LP, College Station, TX).

Studies which are in trials with animals, case reports, review articles, conference reports, clinical trials done with non-extractable data as well as clinical papers with limitation of full text assessment were excluded. After inputting all the published data from respective databases, duplicated paper and some irrelevant articles were excluded. Then, further screening was continued and detail extraction data of this analysis was mentioned in the figure 5.1.

Table 3.1 Inclusion and Exclusion Criteria

	Inclusion Criteria	Exclusion Criteria
Populations	Vitiligo patients diagnosed with any criteria All age groups All skin types Both male and female genders.	
Intervention	Use of PRP in combination with standard vitiligo treatment	Clinical trials without PRP therapy.
Comparison	Comparative studies Before and after treatment Comparison between each modality in intervention domain Clinical study Clinical trial Evaluation study Multi-center study Observational study Pragmatic clinical trial Randomized control trial or twin study	
Outcomes	Clinical improvement with ≥75% and ≥50% repigmentation	
Study Design	Clinical trial Language: English Full text	Duplicate publication Studies with non-extractable data Animal study Review articles Case reports & series Conference reports Non-clinical articles

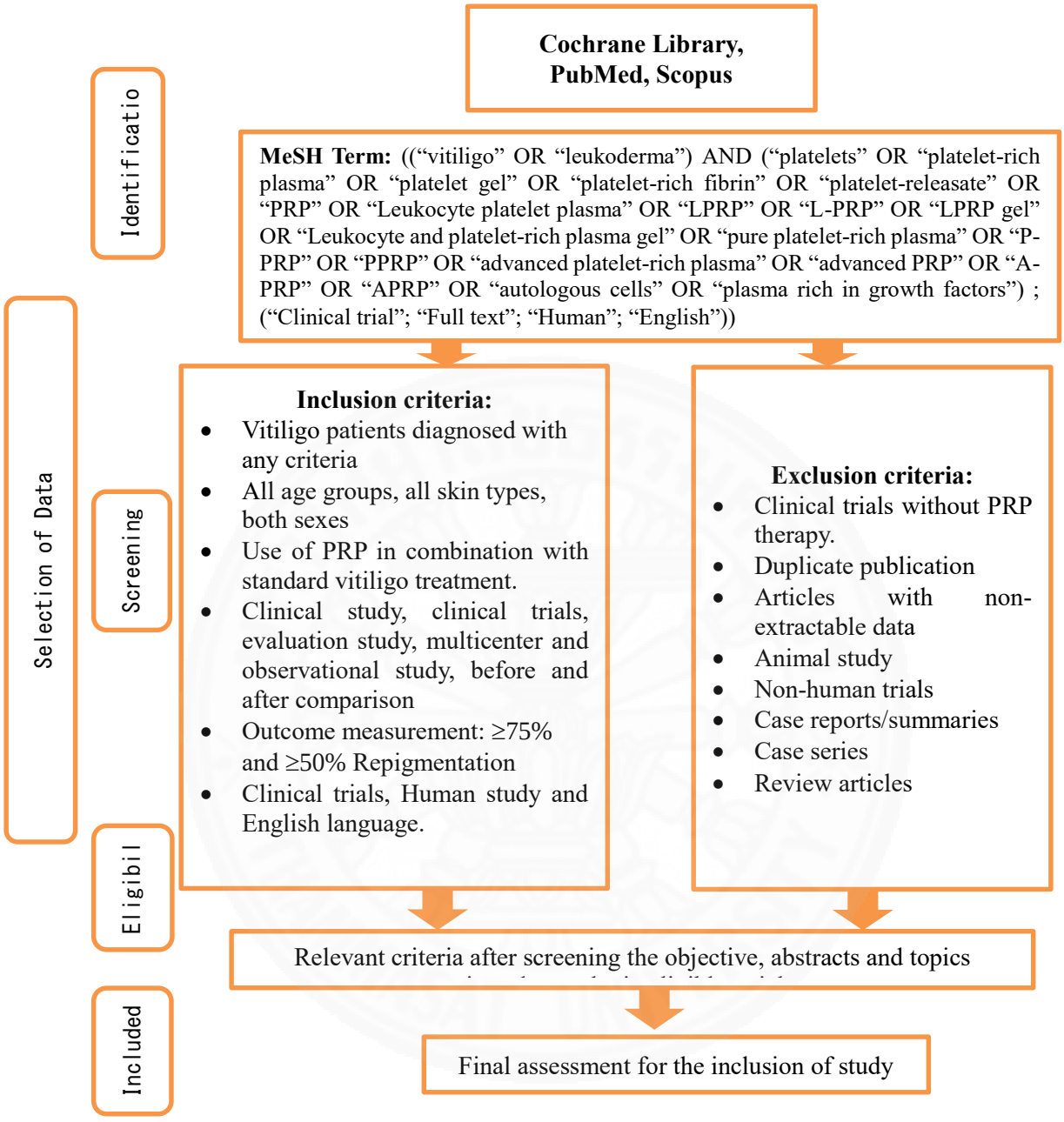

Figure 3.1 Diagram of data exploration and selection of eligible articles

3.1.3 Data Extraction

The data collection was done by 2 investigators (KYI and YUSUF) according to inclusion and exclusion criteria, selection from eligible articles by using data extraction table for each study to reveal detail characteristic, intervention and outcome measurement as table 5.2 by using Microsoft office excel 2017.

Table 3.2 Data extraction tables

Characteristic data of the articles

Study, Year	Study Design	Vitiligo Subtype	Participants			Mean disease duration±SD (range) (month)	Intervention with PRP and combination therapy	Outcome measurement
			No. of patient /patch	Mean age±SD (range) (year)	Skin type			

Characteristic table for method of Platelet-rich Plasma Preparation

Study, Year	Whole blood (cc)	Anticoagulant/ Activator	PRP preparation	Centrifugation		Protocol	Mean Follow up (Month)	Adverse Effect of PRP
				Soft	Hard			

Results of included studies using PRP and PRP combination therapy

Study, Year	Total Patients	Intervention – number of patients/patch	Outcome Measurement			
			VAS (Mean ± SD)	Repigmentation > 75% (Percentage, n)	Repigmentation >50% (Percentage, n)	Surface area reduction (Mean ± SD)

VAS = Visual Analogue Score

3.1.4 Risk of bias assessment

Final inclusion studies were analyzed independently by 2 investigators (KYI & YUSUF), counter checked by third party (A. PREMJIT) by judging the risk of bias with "Risk of bias assessment tool" from Cochrane Handbook for systematic reviews of intervention version 6, 2019 as table 5.3

Table 3.3: Criteria for judging risk of bias in the 'Risk of bias assessment tool'[114]

RANDOM SEQUENCE GENERATION **Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence.**	
Criteria for a judgement of 'Low risk' of bias.	The investigators describe a random component in the sequence generation process such as: • Referring to a random number table; • Using a computer random number generator; • Coin tossing; • Shuffling cards or envelopes; • Throwing dice; • Drawing of lots; • Minimization*. *Minimization may be implemented without a random element, and this is considered to be equivalent to being random.
Criteria for the judgement of 'High risk' of bias.	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: • Sequence generated by odd or even date of birth; • Sequence generated by some rule based on date (or day) of admission; • Sequence generated by some rule based on hospital or clinic record number. Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgement or some method of non-random categorization of participants, for example: • Allocation by judgement of the clinician; • Allocation by preference of the participant; • Allocation based on the results of a laboratory test or a series of tests; • Allocation by availability of the intervention.
Criteria for the judgement of 'Unclear risk' of bias.	Insufficient information about the sequence generation process to permit judgement of 'Low risk' or 'High risk'.

ALLOCATION CONCEALMENT **Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment.**	
Criteria for a judgement of 'Low risk' of bias.	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: • Central allocation (including telephone, web-based and pharmacy-controlled randomization); • Sequentially numbered drug containers of identical appearance; • Sequentially numbered, opaque, sealed envelopes.
Criteria for the judgement of 'High risk' of bias.	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers); • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); • Alternation or rotation; • Date of birth; • Case record number; • Any other explicitly unconcealed procedure.
Criteria for the judgement of 'Unclear risk' of bias.	Insufficient information to permit judgement of 'Low risk' or 'High risk'. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgement – for example if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.

BLINDING OF OUTCOME ASSESSMENT **Detection bias due to knowledge of the allocated interventions by outcome assessors.**	
Criteria for a judgement of 'Low risk' of bias.	Any one of the following: • No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; • Blinding of outcome assessment ensured, and unlikely that the blinding could have been broken.
Criteria for the judgement of 'High risk' of bias.	Any one of the following: • No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; • Blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding.
Criteria for the judgement of 'Unclear risk' of bias.	Any one of the following: • Insufficient information to permit judgement of 'Low risk' or 'High risk'; • The study did not address this outcome.

INCOMPLETE OUTCOME DATA **Attrition bias due to amount, nature or handling of incomplete outcome data.**	
Criteria for a judgement of 'Low risk' of bias.	Any one of the following: • No missing outcome data; • Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; • Missing data have been imputed using appropriate methods.
Criteria for the judgement of 'High risk' of bias.	Any one of the following: • Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; • 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization; • Potentially inappropriate application of simple imputation.
Criteria for the judgement of 'Unclear risk' of bias.	Any one of the following: • Insufficient reporting of attrition/exclusions to permit judgement of 'Low risk' or 'High risk' (e.g. number randomized not stated, no reasons for missing data provided); • The study did not address this outcome.

SELECTIVE REPORTING **Reporting bias due to selective outcome reporting.**	
Criteria for a judgement of 'Low risk' of bias.	Any of the following: • The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; • The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
Criteria for the judgement of 'High risk' of bias.	Any one of the following: • Not all of the study's pre-specified primary outcomes have been reported; • One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; • One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); • One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; • The study report fails to include results for a key outcome that would be expected to have been reported for such a study.
Criteria for the judgement of 'Unclear risk' of bias.	Insufficient information to permit judgement of 'Low risk' or 'High risk'. It is likely that the majority of studies will fall into this category.

OTHER BIAS **Bias due to problems not covered elsewhere in the table.**	
Criteria for a judgement of 'Low risk' of bias.	The study appears to be free of other sources of bias.
Criteria for the judgement of 'High risk' of bias.	There is at least one important risk of bias. For example, the study: • Had a potential source of bias related to the specific study design used; or • Has been claimed to have been fraudulent; or • Had some other problem.
Criteria for the judgement of 'Unclear risk' of bias.	There may be a risk of bias, but there is either: • Insufficient information to assess whether an important risk of bias exists; or • Insufficient rationale or evidence that an identified problem will introduce bias.

3.1.5 Statistical Analysis

All clinical data were estimated outcomes from all studies in term of ≥50% repigmentation, ≥ 75% repigmentation as clinical improvement that patients treated with PRP combination therapy compared other modalities and control group. Using risk ratio (RR), dichotomous outcome for the Mantel- Haenszel method was applied. The percentage of weight is depended on study size and variation of data (SD). Heterogeneity testing was performed by I^2 value. If I^2 is <50% or p-value was less than 0.1, fixed effect model will be used. Vice versa for I^2 is >50% or p-value from Cochrane Q test is ≥ 0.1, random effect model will be applied. All data were pooled and performed with Review Manager 5.3 (Rev Man) for statistical analysis.

This comprehensive meta-analysis was referenced from Cochrane Handbook for Systematic Reviews of Interventions, 2019. Risk of bias was graphed and summarized by using "Risk of bias assessment tool" from Cochrane Handbook for systematic reviews of intervention version 6,2019. Forest plots were used for overall and subgroups data analyzing. Funnel plots explored the possibility of publication bias and outcomes were tested using Egger's test accordingly.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Characteristic features of the studies

Total 263 studies were identified from PubMed, Cochrane and Scopus online databases after thoroughly screening with MeSH (Medical Section Heading) terms. Among them, 126 articles were remained after removing the duplicated papers and 22 records were found after screening detail for each abstract and title, therefore, 9 articles were left for further review with full paper access. Regards to the exclusion criteria, case reports/series, literatures and unidentified treatment articles were excluded so only 13 studies were eligible among the full text assessable articles. Although 7 papers can proceed for qualitative analysis, two studies were unappropriated for quantitative purpose. Therefore, final five articles were left for quantitative meta-analysis and were included in this study.

These articles included prospective, randomized trials comparative studies, two half sides intrapatient, studied population who were diagnosed with vitiligo for any criteria. Accessing the clinical improvement in term of repigmentation percentage as outcome measurement, number of patients who achieved repigmentation were compared with different treatment groups or with control.

Platelet-rich plasma (PRP) combination with standard laser and light-based treatment such as NB-UVB phototherapy, excimer laser or fractional ablative CO2 laser or/and PRP monotherapy compared with control groups as comparative studies or split study treatment for vitiligo were selected. Furthermore, treatment of vitiligo with PRP for surgical procedure as suspension agent and PRP utilized as systemic therapy, the primary outcome measurement was done with percentage of repigmentation in treatment area compared with control or other different treatment groups. Percentage of repigmentation and visual analogues scale were found to be the most frequent assessment tools in evaluation of treatment outcomes in vitiligo and were used in other meta-analysis of vitiligo studies done by King, 2018.[115] The detailed selection of articles was shown in the figure 6.1 as flow chart.

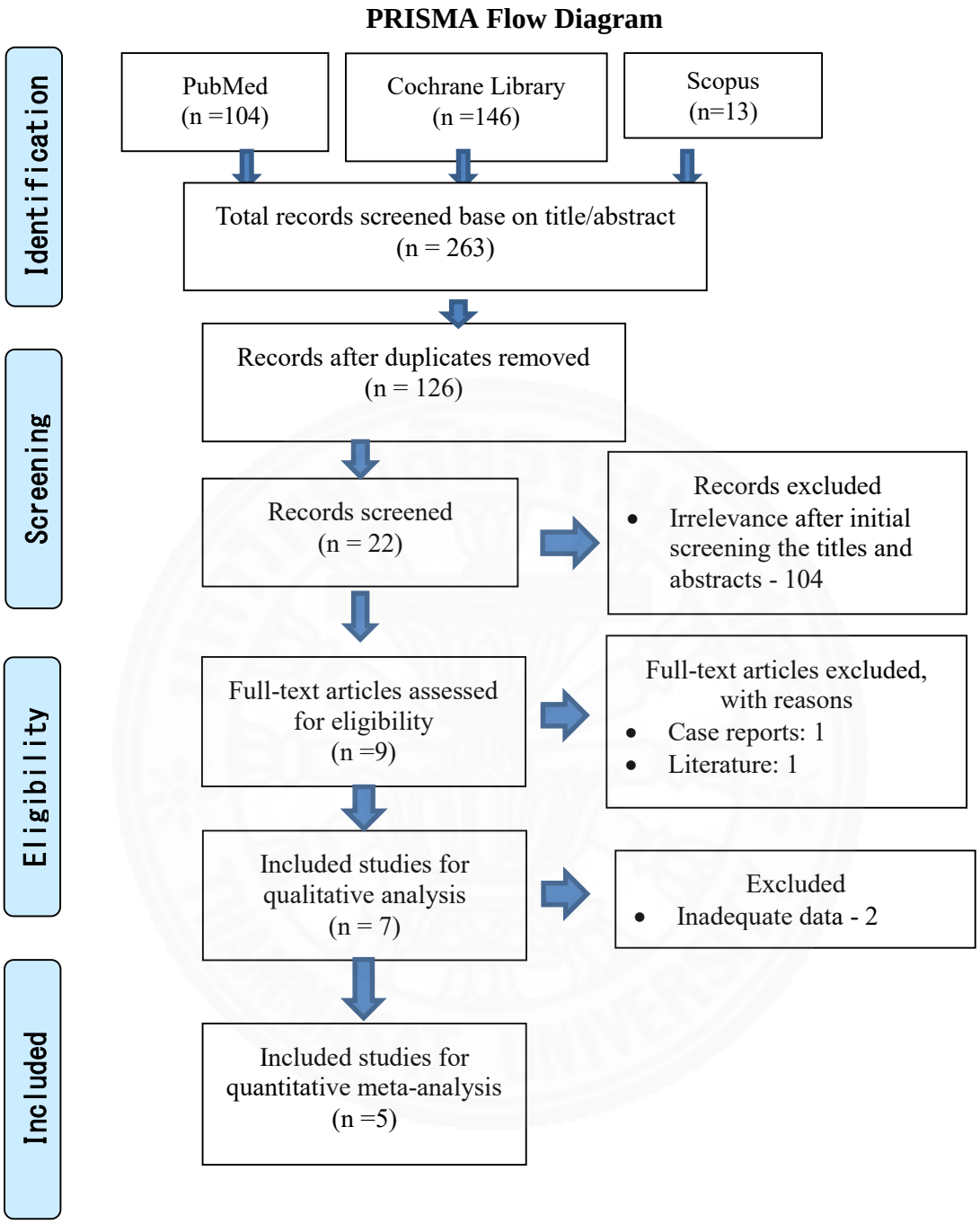

Figure 4.1 Study selection with PRISMA Flow Diagram for vitiligo platelet-rich plasma combination therapy

Table 4.1: Characteristic data of the articles

Study, Year	Study Design	Vitiligo Subtype	Participants			Mean disease duration±SD(range, month)	Intervention with PRP and combination therapy	Outcome measurement
			No. of patient /patch	Mean age±SD (range, year)	Skin type			
Energy based intervention (PRP with laser and light-based)								
Kadry M,2018[116]	RCT, NB, WP	Stable NSV	30 pt/ 120 pc	32.03±12.29 (18-59)	II, III, IV	22.53±23.75 (6-120)	PRP alone PRP+ FXCO2 FXCO2 alone Control	-Surface area reduction (VACAG) -Mean improvement score by physician (MISP) -IGA for % of repigmentation -VAS -Histopathology
AbdelghaniR, 2018[109]	RCT, parallel groups, NB	Stable NSV	80 pt	34.90±15.39 33.90±11.89 36.95±13.04 29.60±10.80 (29 – 37)	III, IV	NA	PRP alone PRP+ FXCO2 FXCO2 + NBVUB FXCO2 alone	-VAS - IGA for repigmentation grade
Ibrahim ,2016 [107]	WP, NB	NSV	60 pt	28± 5.56 (18-35)	III, IV	5.9±6.2 (1-10)	PRP + NBUVB NBUVB alone	-VAS -IGA for % of repigmentation
Khattab, 2019 [117]	RCT, single blinded	Stable NSV and segmental, symmetrical	52 pt	25.42±7.60 24.9±5.60 (18-40)	III-IV	NA	PRP + Excimer laser Excimer laser alone	-VAS -IGA for % of repigmentation -Histopathological assessment
Non- energy based intervention (PRP with local, systemic therapy and vitiligo surgery)								
Saify,2019[118]	RCT, NB	NA	120 pt	31.96 ±11.60 (NA)	NA	NA	IV PRP and local PRP IV CB+ IV PRP Local PRP IV CB only	-IGA for % of repigmentation
Garg S, 2019[110]	NB, WP	Stable vitiligo	10 pt / 20pc	NA (21-30)	IV	(0-6)	PRP suspension + Er: YAG	-VAS - IGA for % of repigmentation -Using digital image analysis system
Parambath,2019[111]	WP, double blinded	Stable NSV and segmental	20pt /40 pc	23.1± 7.6 (21-25)	NA	(54)	PRP Control	- VAS - IGA for % of repigmentation

CB= cord blood, FXCO2 = fractional CO2, IGA = investigator's global assessment, NSV = non-segmental vitiligo, NA = not available, NB=Non-blinded, RCT=Randomized Comparative Trial, VAS = visual analogue scale, VACAG = vitiligo analysis by computer-assisted grid, WP=within patient.

Table 4.2: Characteristic table for method of Platelet-rich Plasma Preparation and adverse effects

Study, Year	Whole blood (cc)	Anticoagulant/ Activator	PRP preparation	Centrifugation		Protocol	Mean Follow up (Month)	Adverse Effect of PRP
				Soft	Hard			
Energy based intervention (PRP with laser and light-based)								
Kadry M.2018[116]	8	Regen Lab Kit	Single	1500 rpm x 5 min		ID q 2 wk, *6Tx (0.1ml per injection,1cm apart)	3	Pain & PIH
Abdelghani R,2018[109]	10 - 20	Sodium citrate /Calcium chloride	Double	1500 rpm x 10 min	2000 rpm x 10 min	ID q 3 wk, *4Tx (0.1ml per injection,0.5cm apart)	3	NA
Ibrahim ,2016[107]	10-20	Sodium citrate /Calcium chloride (10:1)	Double	3000 rpm x 7min	4000 rpm x 5min	ID q 2 wk, *8Tx (0.1ml per injection, 2cm apart)	3	Pain
Khattab, 2019 [117]	25	Trisodium citrate	Double	1157 - 1336rpm x 10min	1500-2000 rpm x 15min	ID q 3 wk, *6Tx (0.1ml per injection, 1cm apart)	3	PIH
Non- energy based intervention (PRP with local, systemic therapy and vitiligo surgery)								
Saify,2019[118]	NA	FDA protocol of Government of India	NA	NA	NA	Local ID/topical or Systemic IV q 30 days, *6T	3	Erythema
Garg S, 2019[110]	10	Citrate dextrose, Y cell bio kit	Single	3200rpm x 4min		1.5ml of thick suspension of PRP with dermoepidermal fragments stored in 37°C incubator for 1 minute *1T	6	Pain
Parambath,2019[111]	NA	NA	Double	945 rpm x 7 min	2835 rpm x 12 min	Suspending NCES in PRP *1T	6	Pain

ID= intradermal, PIH = post inflammatory hyperpigmentation, NA = not available, rpm= revolution per minute, NCES = non-cultural epidermal suspension

All articles that correlate with the inclusion criteria: authors, published year, study design and nature of participants, platelet-rich plasma combination modalities and outcome measures for detailed screening and selecting the articles were done by two independent investigators and data collection were proceeded as the next steps with Microsoft Office Excel 2017 and summarized as shown in table 6.1 for PRP combination therapy. Each study had its own method of PRP preparation, amount of blood collected, sequence of centrifugation and different treatment protocols. In order to analyze and compare each characteristic of PRP preparation and adverse effects, characteristic table for PRP preparation methods are mentioned in table 6.2.

In most articles, most of the treatment's outcome measurement in vitiligo were stated with visual analogue scale (VAS) for mean with standard deviation and number of patients for over 75% and over 50% of repigmentation during ancd after treatment follow up for certain period. To analyze the efficacy of PRP, the different methods of vitiligo treatments such as laser and light-based therapy, local and systemic therapy and laser with vitiligo surgery were pooled in separated groups in the table 6.3.

Among treatment with laser and light-based therapy, Kadry [116] studied prospective randomized comparative study with 30 stable non-segmental vitiligo subjects for four different treatment groups. Using VAS as outcome measurement to analyze the effectiveness of treatment, mean and standard deviation (mean SD) is 6.87±2.65 for PRP with fractional CO2 laser treatment, mean SD 6.67±2.37 for PRP alone group, mean SD 4.87±2.19 for laser alone and mean SD 1.30±1.91 for control group respectively after 6 treatment sessions. This study demonstrated about the PRP combined with CO2 laser was the best for repigmentation, PRP monotherapy was ranked moderate effectiveness, though the fractional CO2 laser monotherapy was ranked the least efficacy.[116]

Another study was Abdelghani [109] who did randomize comparative trials at 80 stable NSV subjects with different treatment groups. Among them, PRP combined with fractional CO2 laser resulted visual analogue scale (VAS) for mean and standard deviation (mean SD) is 8.2±0.62, 8 subjects got >75% repigmentation and 20 subjects achieved >50% repigmentation which is the highest achievement among PRP alone, CO2 laser with NB-UVB and CO2 laser alone followed by total 4 treatments. Comparing NB-UVB monotherapy with PRP+NB-UVB combination therapy, Ibrahim

[107]studied prospective, intrapatient, comparative study at 60 vitiligo subjects for 8 treatment sessions. 33 subjects became >75% repigmentation and 12 subjects had >50% repigmentation compared with mono-phototherapy, which was statically significant result for combination therapy. Similar study was engaged by Khattab [117]with 52 subjects for PRP with excimer laser compared with laser monotherapy. Combination of PRP with laser resulted mean SD 10±0.41 for VAS, 9 patients in >75% repigmentation and another 9 subjects in >50% repigmentation compared with monotherapy after total 6 treatment sessions.

Regards to the combination therapy of PRP for local and systemic therapy, Saify [118]conducted open prospective study with 120 subjects in India. There were 4 different treatment groups for local and systemic therapy with PRP and umbilical cord blood transfusion for 6 sessions each group. Among them, intravenous umbilical cord blood transfusion with local PRP therapy achieved 30 subjects for >75% repigmentation and 15 patients for >50% repigmentation which was maximum response rate compared with other groups.

In vitiligo surgery, Garg [110]engaged the study with PRP as suspension agent after Er: YAG laser ablation in 10 vitiligo subjects. 8 subjects achieved >75% repigmentation after non-cultural epidermal suspension surgery with minimal complication. Parambath [111]evaluated the efficacy of PRP by using split side study for PRP with control in 21 vitiligo patients. Patient gratification at PRP combination side resulted 72±30% SD (p value = 0.001) and mean repigmentation by area calculating at 6 months follow up was 75.6±30% SD (p value 0.0036) compared with control side.

Table 4.3: Overall treatment response using PRP combination therapy in vitiligo

Study, Year	Total Patients	Intervention – number of patients/patch	Outcome Measurement				p value
			VAS (Mean ±SD)	Repigmentation ≥75% (percentage)	Repigmentation ≥50% (percentage)	Surface area reduction (Mean ± SD)	
Energy based intervention (PRP with laser and light-based)							
Kadry, 2018[116]	30	PRP alone – 30 pc PRP+FxCO2 – 30 pc FxCO2 alone – 30 pc Control – 30 pc	6.67±2.37 6.87±2.65 4.87±2.19 1.30±1.91	NA	NA	57.01±29.67 54.22±37.08 38.08±36.43 13.79±40.32	<0.001
Abdelghani, 2018[109]	80	PRP alone – 20 pt PRP+FxCO2 – 20 pt FxCO2+NB-UVB – 20 pt FxCO2 alone – 20 pt	3.85±3.68 8.2±0.62 5.56±3.42 4.50±2.76	20 40 5 10	20 100 30 10	NA	0.025 0.001 0.062 0.037
Ibrahim, 2016[107]	60	PRP + NB-UVB – right side (60pt) NB-UVB alone – left side (60 pt)	NA	75 0	90 6.7	NA	<0.001
Khattab, 2019[117]	52	PRP + Excimer – 26 pt Excimer alone – 26 pt	10±0.41 0	34.6 0	84.6 34.6	NA	0.000[a](HS)
Non- energy based intervention (PRP with local, systemic therapy and vitiligo surgery)							
Saify, 2019[118]	120	IV PRP+local PRP – 20 pt IV CB + local PRP – 50 pt Local PRP – 20 pt IV CB only – 30 pt	NA	30 60 50 40	80 90 80 80	NA	NA
Garg, 2019[110]	10	PRP suspension+ Er: YAG – 20 pc	NA	80	90	NA	NA
Parambath, 2019[111]	20	PRP arm – 20 pc Control arm– 20 pc	NA	80 55	80 55	NA	0.001

VAS = visual analogue scale, FXCO2 = fractional CO2, CB = cord blood, HS= highly significant, NSV = non-segmental vitiligo, NA = not available, pt = patient, pc= patch, a= statistical significant

4.1.2 Risk of bias and quality assessment

By using Cochrane Collaboration's tool for assessing risk of bias (Figure 6.2 and 6.3), all possible risk of bias was assessed with different levels for all studies in summary which included in this research. Level of heterogeneity related to the level of bias risk generally, high risk of bias could stand for high level of heterogeneity in that study. Due to insufficient information in included studies, most result showed unclear risk of bias for selection with randomization and allocation for participants for clinical trials. Five studies had low risk of bias in performance about blinding of participants information and personnel. Average unclear and low risk of detection bias in blinding outcome assessment and most included studies provided completed outcome data, therefore reduced risk in attrition bias. Nevertheless, three studies came with high risk of bias and one study showed unclear risk of bias in reporting of selective data and unclear risk of bias due to absence of illustration of outcome data completely after treatment and follow up in overall pooled studies.

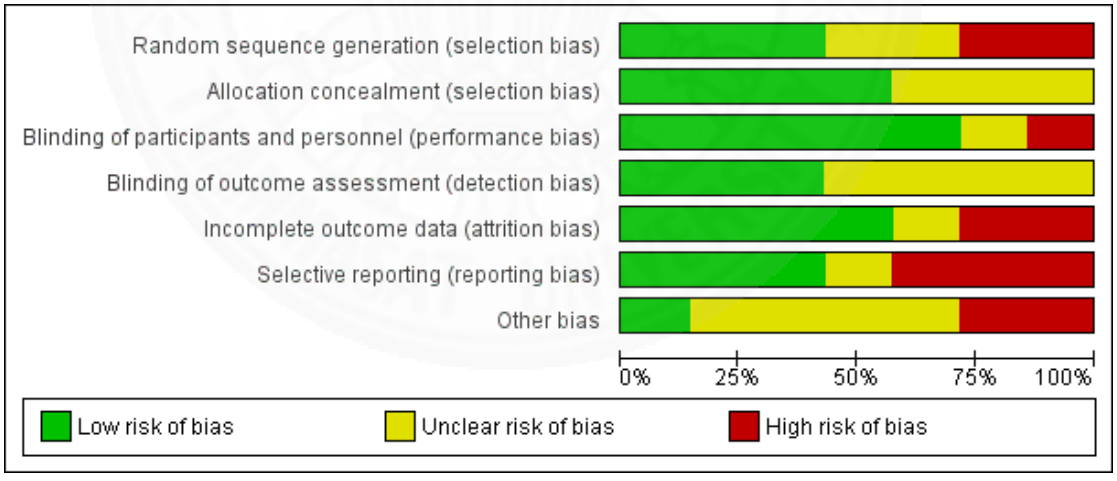

Figure 4.2 Graph showing risk of bias assessment

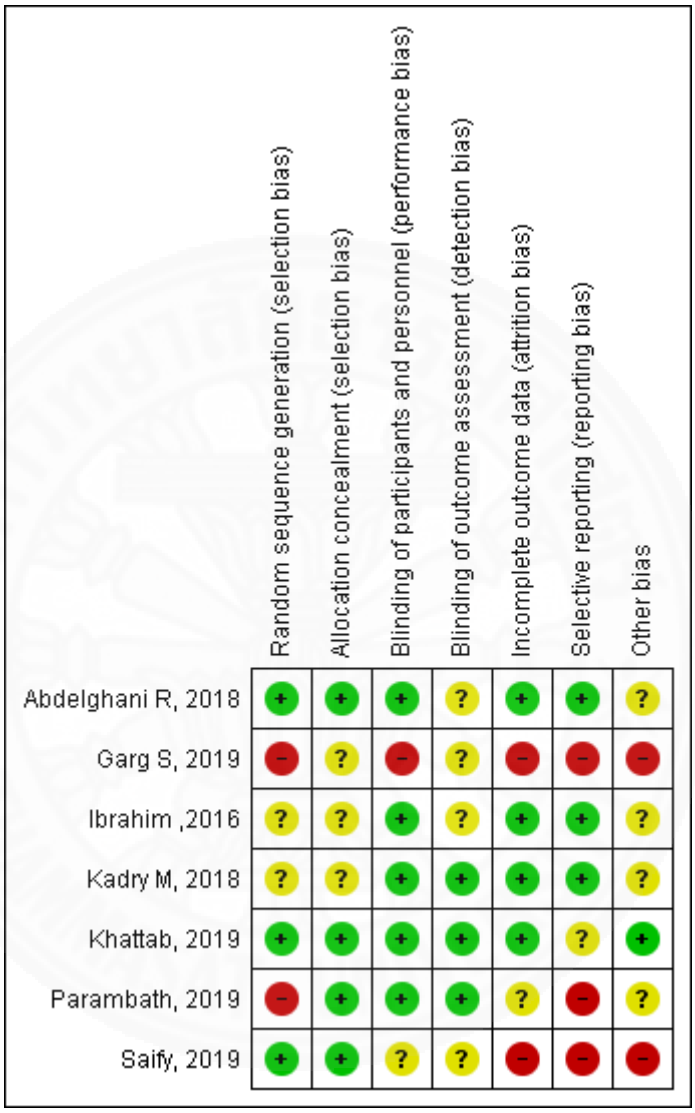

Figure 4.3 Summary of bias assessment for pooled studies

4.1.3 Statistical Analysis

Statistical analysis for the meta-analysis was pooled together with Review Manager 5.3(Rev Man) and STATA (Stata Corp LP, College Station, TX) according to the outcome measurement in order to estimate overall mean difference of treatment efficacy by using Comprehensive Meta-analysis program. Risk ratio with dichotomous data for number of subjects in repigmentation scale for ≥75% and ≥50% were respectively calculated for included studies.

Among seven identified studies, intervention protocol and methodology varied greatly. Therefore, five studies (Abdelghani R 2018[109], Ibrahim 2016[107], Khattab 2019[117], Parambath 2019[111] and Saify 2019[118]) using PRP combination management compared with control for vitiligo treatment in term of repigmentation score used as outcome measurement in this meta-analysis. Others studies were excluded because of unqualified data or inability for data extraction. Total 332 participants in five included studies which were adequate data for this study. The standard mean difference for the whole and individual study were interpreted by using forest plot, I-squared statistic was affected for heterogeneity. Heterogeneity of the pooled studies was regarded as significant if $P < 0.1$ and statistical difference were considered significant if $P < 0.05$. All seven studies will be evaluated qualitatively as systematic review.

4.1.3.1 Overall data treatment response based on the percentage of repigmentation

According to the forest plot, certain degree of variation of each study was shown in 95% confidence interval (CI) and horizontal line for favorable outcome of clinical trials and controls. Some studies applied the outcome measurement for percentage of repigmentation with different outcome achievements. Random effect model was applied and got favorable outcome for PRP combination with ≥75% repigmentation as figure 6.4. Out of total 216 clinical trials for PRP combination therapy, 122 events (56%) resulted favorable outcome in ≥75% repigmentation. As in another groups, total 206 clinical trials as control and only 38 events (18%) achieved favorable repigmentation outcome in vitiligo studies. The sample size of each clinical studies reflected the percentage of weight. Mean repigmentation with 95% CI was narrow with 2.95 (1.17 – 7.43) in which good response was observed and weighted mean difference applied for these studies using Mantel Haenszel statistical method. Outcome of ≥75% repigmentation was 2.95, increase as a result of risk ratio (RR) with high heterogeneity for 84%, p value was 0.02 resulted. Therefore, it was statistically significant for favorable PRP combination treatment compared with control group.

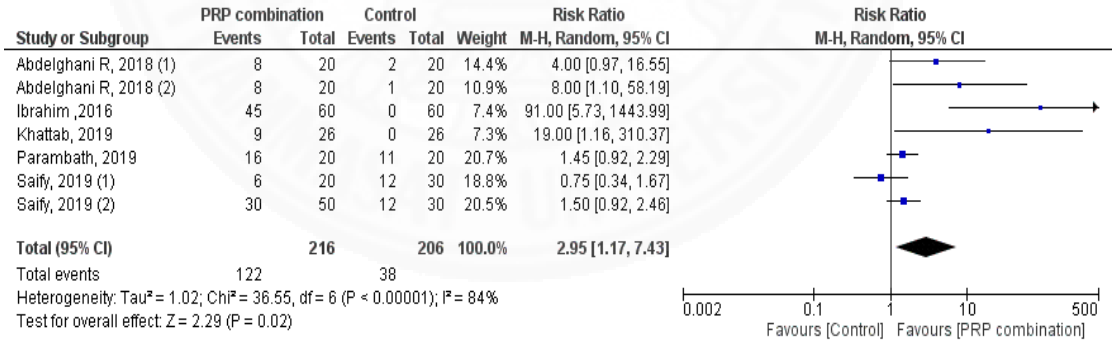

Figure 4.4 Forest plot overall treatment response regarding ≥75% repigmentation

Among total 216 events for PRP combination therapy, 89% (193 events) of the studies resulted favorable outcome for ≥50% repigmentation. In control groups for total 206 trials, only 39% (80 events) got ≥50% repigmentation outcome. Risk ratio of ≥50% repigmentation in forest plot (figure 6.5) was 2.54 which resulted increasing in uses of PRP with combination therapy compared to control groups. High

heterogeneity with I-squared value was 94%. Mean repigmentation with 95% CI was 2.54 (1.28 – 5.03) which showed narrow confidence interval in the forest plot for ≥50% repigmentation. Subsequently, favorable outcome for PRP combination therapy as outcome measurement for ≥50% repigmentation scale, p value was 0.008, statistically significant in term of repigmentation scale compared to control group.

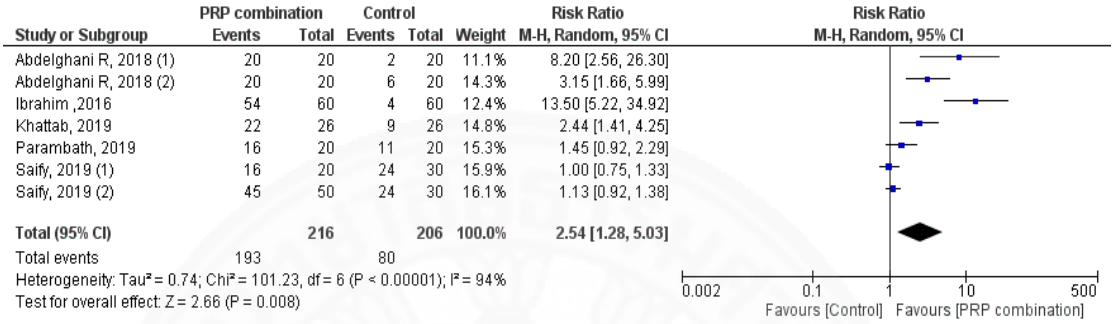

Figure 4.5 Forest plot for overall treatment response regarding ≥50% repigmentation

4.1.3.2 Subgroup analysis regarding to energy based and non-energy based therapies in percentage of repigmentation

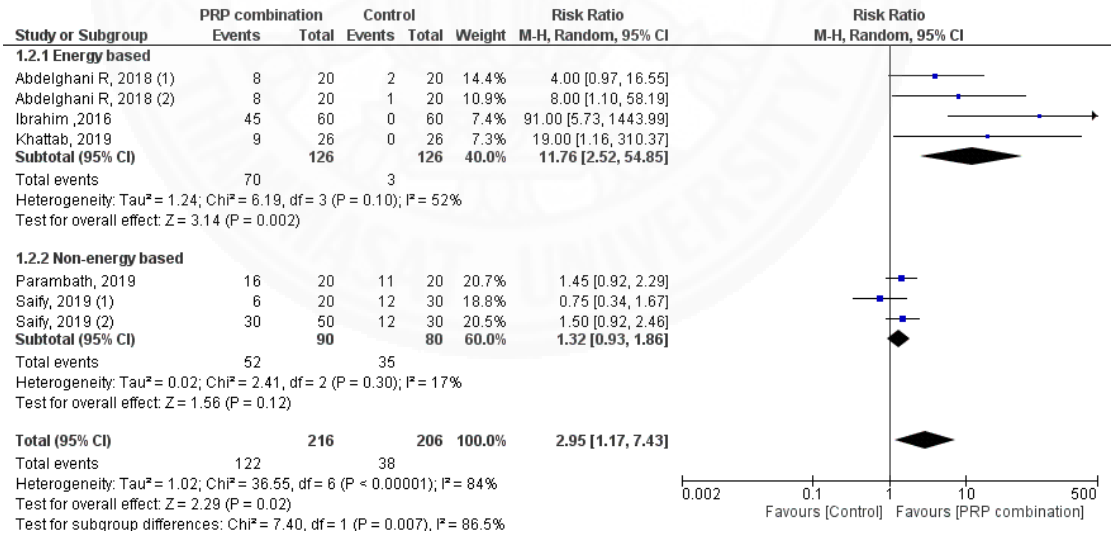

Figure 4.6 Forest plot comparing energy based and non-energy based therapies in ≥75% repigmentation

Regards to forest plot for subgroups analysis in ≥75% repigmentation, risk ratio for energy based therapy was 11.76 with 95% CI: (2.52 – 54.85), moderate heterogeneity for I-squared 52% showed statistically significant (p value = 0.002) in

PRP combination group compared with control. For non-energy based group, risk ratio for PRP combination therapy was 1.32 with narrow confidence interval, 95% CI: (0.93 – 1.86) and mild heterogeneity, I-squared was 17%. In non-energy based group, PRP combination therapy was statistically insignificant compared with control. Therefore, PRP combination with energy based therapies achieved more favorable outcome in repigmentation compared with non-energy based therapies, high risk of heterogeneity (I^2 = 86.5%) was resulted in subgroup analysis.

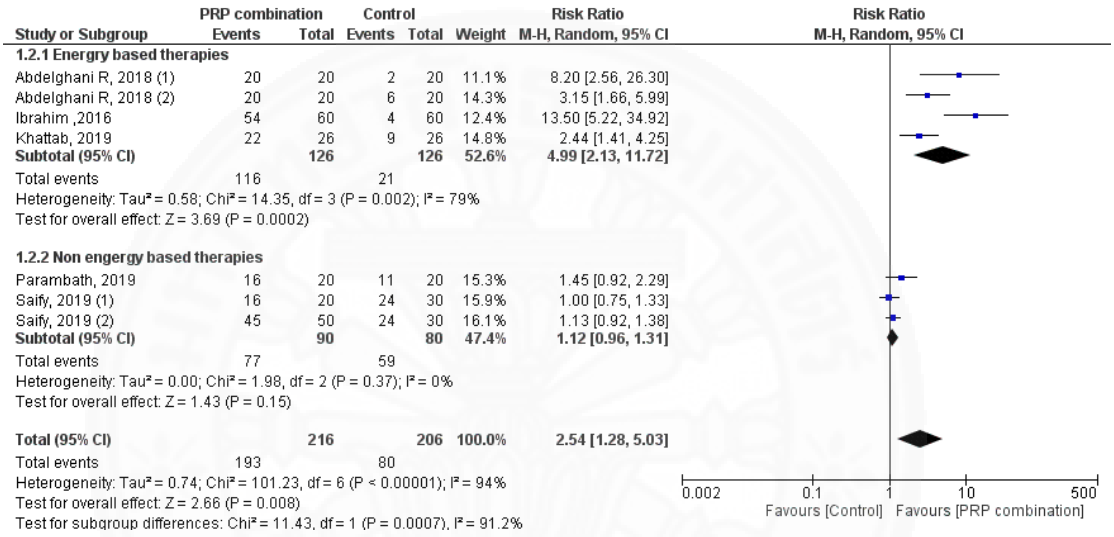

Figure 4.7 Forest plot comparing energy based and non-energy based therapies in ≥50% repigmentation

In ≥50% repigmentation, risk ratio for energy based therapies in PRP combination therapy was 4.99 time more favorable outcome with 95% CI: (2.13 – 11.72) in repigmentation than control. There was high heterogeneity with I^2 for 79% and p value was 0.0002 for statistical significant result. However, risk ratio for non-energy based therapy was 1.12, 95% CI: (0.96 - 1.31) with I-squared for 0%, mean repigmentation for PRP combination therapy was statistically insignificant (p value = 0.15) compared with control. In subgroup analysis for ≥50% repigmentation, energy based therapy resulted more favorable outcome in term of risk ratio compared with non-energy based therapies together with high risk of bias, I^2 for 91.2%.

4.1.3.3 Funnel plot for pooled studies

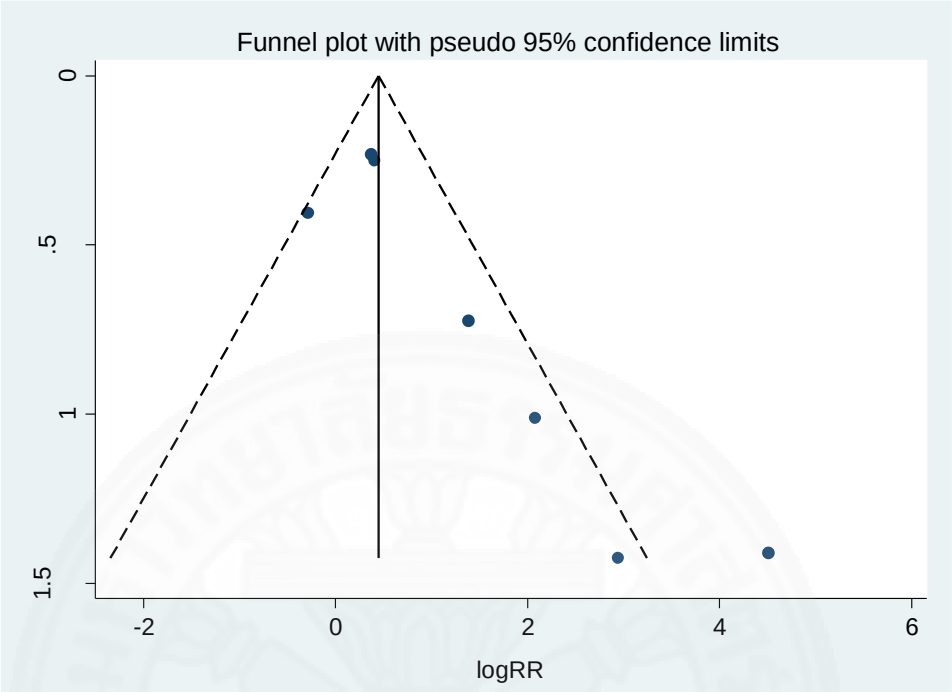

Figure 4.8 Funnel plot of pooled studies in ≥75% repigmentation

Funnel plot is an exploratory tool that used to assess the possibility of detecting bias or systematic heterogeneity, represented with scatter of dots for each study's treatment effect against a measurement of study size.[114] Standard error of the effect estimation mentioned at Y axis and mean difference for treatment effect measure of each study offered at X axis. In both funnel plots for ≥75% and ≥50% repigmentation, some of the studies were out of the lines of 95% confidence interval limit for heterogeneity bias.

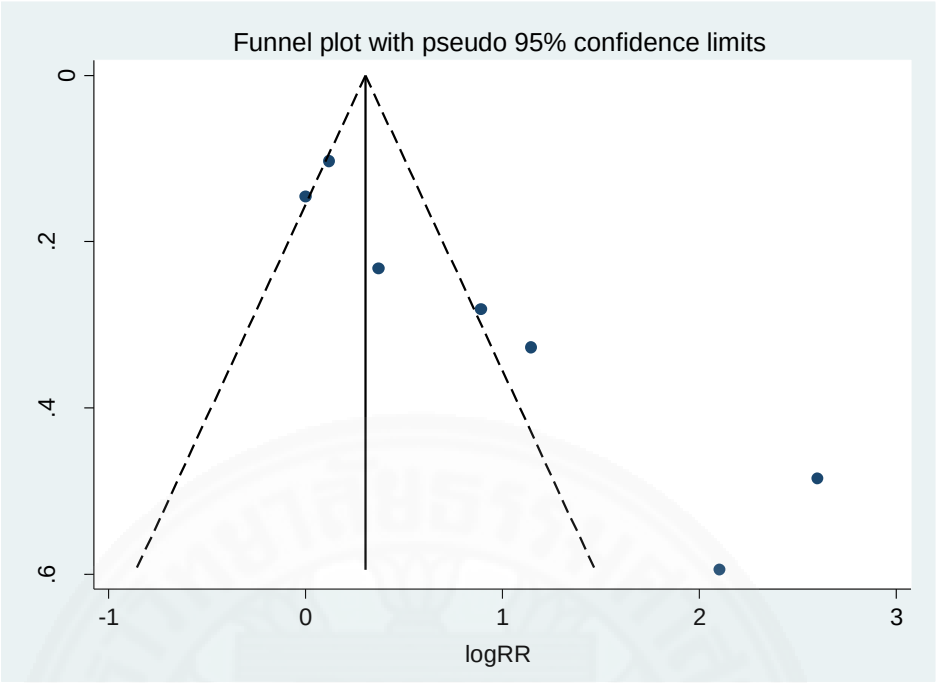

Figure 4.9 Funnel plot of pooled studies in ≥50% repigmentation

Asymmetrical plot resulted with P = 0.032 for statistical Egger's test in ≥75% repigmentation and P = 0.003 in ≥50% repigmentation which were <0.05 for P value, therefore, those asymmetrical funnel plots resulted significant bias. Publication bias for overall treatment response by repigmentation scale together with high heterogeneity which mentioned the relationship between treatment effect and study size. Furthermore, asymmetry suggested a systematic variation in study size. Other possible causes of asymmetry were due to by chance and high risk of reporting bias especially in discerning outcome reporting and selective analysis reporting together with weak methodology like study design, evaluation and fraud of these pooled studies.

4.1.4 Qualitative Description

Seven articles were included for qualitative analysis as systematic review. Two studies were not appropriated for meta-analysis because of lacking homogeneity. Regards to the pooled studies, two subgroups were divided according to different approaches in the clinical trial of PRP as combination therapy using energy based (laser or light-based treatment) and non-energy based intervention (local, systemic therapy and application of PRP in vitiligo surgery).

Different types of intervention were engaged to strengthen the repigmentation in each clinical trial of vitiligo treatment respectively. The type of combination with PRP therapy may vary for melanocyte proliferation and synthesis in vitiliginous skin in vitiligo repigmentation and percentage of repigmentation. Visual analogue scale and percentage of repigmentation were used for outcome measurement. Apparently, PRP combination therapy achieved superior repigmentation and satisfactory repigmentation result in VAS compared with control or monotherapy groups.

Comparative efficacy was observed in different PRP preparation protocols and methods of PRP application. Under the different subgroups, each article was using PRP as intradermal injection, topical application or systemic infusion into the depigmented skin. Khattab,2019 [117] study included 52 participants where clinical and statistical significant repigmentation by combined with excimer laser and intradermal PRP injection compared with control, laser monotherapy. Similarly, there was favorable outcome for Ibrahim,2016 [107] study, significant repigmentation in intradermal PRP combination with NB-UVB compared with phototherapy alone.

Thirty non-segmental vitiligo patients received 6 treatment sessions for different combination modality demonstrated that fraction CO2 laser and PRP combination had excellent in repigmentation, PRP monotherapy was ranked moderate effectiveness and laser alone was last with statistical significant difference in repigmentation grade, mean improvement score by physician and VAS, p value was <0.001. [116] Similar study was done by Abdelghani[109] with 80 NSV participants. 40% of patients achieved the best outcome for > 75% repigmentation and patients' satisfaction in laser and PRP groups, 5% of patients in laser and NBVUB groups, 10% in laser group and 20% in PRP alone group.

One open prospective study with 120 participants aimed to discern the actual application and benefit of PRP and umbilical cord blood (UCB) treatment in vitiligo patients. Saify 2019 [118] proved that ≥75% of repigmentation in 48.4% of patients ,the maximum response, by using local PRP and UCB transfusion especially in old, chronic and non-responding vitiligo. There were no adverse effects in UCB transfusion, local PRP application and micro needling procedure.

Most included studies suggested the positive impact for PRP combination therapy on vitiligo repigmentation, however, there was limited data from Garg,2019 [110]which was used Er: YAG laser with PRP suspension in vitiligo surgery in 10 participants, lacked of control groups. That study evaluated with percentage of repigmentation before and after trials, clinically significant results on PRP suspension with laser therapy were seen after 2 weeks post-therapy in 60% after the procedure. Parambath [111] proved that transplantation of non-cultured epidermal suspension in PRP is an applicable in surgery for stable vitiligo compared with that of suspended in phosphate buffered saline as control. In PRP arm, 75.6% repigmentation at 6 months with p value 0.0036 resulted which was better than control side.

Mostly trials averagely treated with four to six sessions, mean follow up for three months [107, 109, 116-118]and six months[110, 111]. Outcome results were visible on three months duration but some studies needed for longer duration for significant outcome. Overall treatment intervention was tolerable without severe complication. Some minor side effects were reported for pain, erythema, color mismatch and post inflammatory hypopigmentation or transient hyperpigmentation in some studies.

4.2 DISCUSSION

Vitiligo is one the autoimmune disorder, with unpredictable loss of functioning melanocyte, caused progressive depigmentation characterized by well demarcated, depigmented patches on the skin, hair and mucous membrane such as in the mouth and genitalia. There are significant hypopigmented patches in darker skinned individuals with a fine, dry surface, scales which can be mistaken with a superficial yeast infection: tinea versicolor. Regardless of age and sex, the prevalence of vitiligo varies according to the demographic region, certain genetic mutations and polymorphisms.[119]

Several articles evaluated about the vitiligo outcomes measurement index based on different clinician-reported trials, they are Visual Analogue Scale (VAS), Repigmentation score, Vitiligo European Task Force assessment (VETFa), Vitiligo area scoring index(VASI) and point counting. Furthermore, a few are based on patients' reported outcomes; Skindex-29, Skindex-16, Skindex-Teen, Dermatology Life Quality Index (DLQI), Patient Benefit index (PBI) and Pictorial Representation of Illness Measure (PRISM) and the left are based on observer-reported outcomes; Digital Image Analysis System (DIAS) and Image Analysis Technique (IAT).[120]

The vitiligo therapeutic management is recognized as challenging; therefore, better understanding of the disease pathogenesis may lead to superior outcome for recovery of disease. Therefore, scientists are doing research into the complex and multifactorial pathogenesis of vitiligo which is still unclear. For examples, the first treatment for vitiligo involves topical medications such as potent or super potent topical steroids: betamethasone valerate or clobetasol propionate, topical calcineurin inhibitors; pimecrolimus or tacrolimus, then phototherapy with NB-UVB, PUVA (Psoralen in combination with UVA) and excimer light. There is some supportive measure by using cosmetic camouflage and sunscreens. Treatment with phenol (Hyodroquinone monobenzyl ether) for depigmentation therapy used in extensive vitiligo patients. Mild or moderate adverse effects and recurrence are common in systemic therapy with oral dexamethasone, oral antibiotic minocycline, statins, methotrexate, azathioprine and some biologic drugs with or without combination with standard treatment protocol. After mean success rate of vitiligo surgery was high, it was

an attractive option as alternative treatment together with cognitive therapy and psychological support. [121]

The earliest combination therapy for vitiligo was microdermabrasion followed by a topical calcineurin inhibitor, some more advance techniques such as Er: YAG laser, CO2 laser also combined with light-based devices, NB-UVB, topical steroid, topical 5-FU and platelet-rich plasma for enhancing the repigmentation in vitiligo. [109, 122] By using this experience, Kadry did prospective, comparative study to compare the efficiency of PRP monotherapy versus combined fractional CO2 (Fr: CO2) laser with PRP in the treatment of NSV lesion at 2018. Total 30 NSV patients were allocated for 4 subgroups: first group treated with intradermal PRP alone, another is Fr: CO2 laser alone, the left is Fr: CO2 and PRP combination therapy and one group as control. The outcome measurement was done by vitiligo analysis by computer assisted grid (VACAG). Based on the analyzed data, combination therapy was the least side effect and maximum response at face and upper limbs compared to the other groups. Trunk showed higher response in PRP alone group and lower limbs showed improvement in laser alone group. According to the experiment data, Fr: CO2 and PRP combination therapy got superior repigmentation results than compared to intradermal PRP alone group and had safety and effective to treat as an alternative option for NSV. [116]

Several mechanisms proposed to enhance the efficacy of repigmentation by using different intervention methods as a supportive option with platelet-rich plasma. There were multiple growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-b), vascular endothelium growth factor (VEGF), a fundamental growth factor for fibroblasts (bFGF), and growth factors similar to insulin (IGF) in autologous PRP. These multiple types of growth factors lead to melanocyte migration, synthesis and melanocyte interaction lead to stabilization by enhancement of fibroblast from perilesional skin of vitiliginous skin. [123] In vivo study with narrowband ultraviolet B (NB-UVB) therapy, basic fibroblast growth factor (bFGF) from keratinocyte promoted human melanocyte proliferation and melanocyte migration via increased expression of phosphorylated focal adhesion kinase (p125(FAK)) on melanocytes.[124]

Furthermore, PRP suppressed the release of cytokine as interleukin-1, interferon- gamma and tumor necrosis factor which were offering anti-inflammatory effect and subsequently limited the melanocyte apoptosis. [70] However, one study from Lim. K, 2011 pointed out PRP monotherapy did not benefit in induction of repigmentation of vitiligo ,therefore, he recommended to consider direct and indirect effect of PRP on melanocyte in microenvironment with large clinical trial in future. [125] One synergistic result of PRP combination therapy has the stimulatory effect on melanocyte regeneration by UV radiation and ablative CO2 laser remove the skin barriers so it made better penetration of PRP into the depigmented skin for improving repigmentation. Similar synergistic mechanism was applied on the Abdelghani 2018[109], aimed to study the effect of combined therapy with fractional CO2 laser, intradermal PRP and NB-UVB phototherapy. Combination of laser and PRP group resulted the best outcome for repigmentation as well as patients' satisfaction, followed by laser combination phototherapy and PRP monotherapy was the last rank for clinical improvement. During inflammation and wound healing process, cytokines were released because of ablative CO2 laser and anti-inflammatory effects of PRP. These agonist effect of cytokines promoted melanocyte production and migration from the perilesional skin.

However, most interventions (especially energy based devices: laser and light-based modalities) have an anti-inflammatory/immunosuppressive capability to prevent the autoimmunity for vitiligo, some do not have direct or synergistic effect on melanocyte differentiation, migration and proliferation like growth factors from PRP. For clinical improvement of vitiligo, repigmentation requires the proliferation and movement of melanocyte stem cells typically from hair follicles bulge. Based on its polygenic nature of vitiligo, some facts pave the way to combine different treatments for vitiligo instead of monotherapy. [2]

Previously, King, 2018 evaluated the systematic review and meta-analysis for the efficacy of ablation-based combination therapy for vitiligo [115] resulted with ≥75% repigmentation for odd ratio (OR): 5.812, 95% confidence interval (CI): 2.194 – 15.393, I^2 =67.3%, p <0.001 and ≥50% repigmentation for OR: 10.490, 95% CI: 4.632- 23.757, I^2= 50%, p=0.00. Moreover, another study (Chiu, 2018) specified with the fractional carbon dioxide laser was a supportive treatment in non- segmental

vitiligo in a systematic review and meta-analysis[126] , outcome resulted were ≥75% repigmentation for risk ratio (RR): 2.8, (95% CI: 1.29 – 6.07, I^2=0%, p= 0.009) and ≥50% repigmentation for RR: 2.26, (95% CI: 1.23 – 5.9, I^2=33%, p=0.0002). It is a complex process to offer medication in treatment of vitiligo. Arora, 2020[127] studied the clinical efficacy and safety of tacrolimus monotherapy and adjunctive role in vitiligo. That meta-analysis revealed the tacrolimus and NB-UVB combination therapy was more superior than phototherapy alone in inducing more than 75% repigmentation (RR:1.34, 95%CI: 1.05-1.71, I^2=7%, p=0.02). Therefore, there has no meta-analysis study for platelet-rich plasma combination therapy for vitiligo. In our scope of knowledge, this study will be the foremost to emphasize the platelet-rich plasma as adjunct therapy in the treatment of vitiligo and outcome measurement analyzed with clinical improvement for repigmentation score results. Statistical analysis used with 95% confidence interval and risk ratio for continuous data analysis compared with PRP combination therapy and control groups by measuring the clinical improvement with ≥75 and ≥50% repigmentation.

Based on present meta-analysis with the forest plots in overall treatment response for ≥75% repigmentation scale, there was favorable outcome in clinically and statistically for repigmentation score by PRP combination compared with control groups, risk ratio (RR) was 2.95, (95% CI (1.17– 7.43), I^2: 84%, p = 0.02) on the other hand in term of ≥50% repigmentation, overall PRP combination therapy for RR was 2.54, (95% CI (1.28 – 5.03), I^2= 94%, p=0.008). There was significant benefit in PRP as an adjunctive therapy in term of ≥75% repigmentation which was less heterogeneity and more significant than in term of ≥50% repigmentation. Specifically, the main significant outcomes came RPR with energy based (laser and phototherapy) combination treatment for vitiligo which achieved 12 and 5 times better than control in term of ≥75% and ≥50% repigmentation respectively. However, PRP with non-energy based (topical, systemic or surgical procedure) combination therapy did not show statistically significant result compared with the control. Nevertheless, PRP as combination therapy showed higher chance of clinical improvement (in term of repigmentation) compared with Fr: CO2 laser or tacrolimus as an adjunctive role according to this meta-analysis results.[126, 127]

Generally, autologous platelet-rich plasma therapy is regarded as a safe with minimal side effects, no special consideration regarding antibodies formation and free from transmissible diseases. In this study, there were some minimal side effects associated with combination therapies including pain, erythema and temporary post inflammatory hypo or hyperpigmentation. The mentioned minor complications severity might vary according to their intervention protocol and parameters. In included studies, there was no reported case for Koebner phenomenon which is one of the most serious complication of vitiligo surgery or any intervention that causes trauma in vitiliginous skin. Repeated intradermal injection of PRP as monotherapy will be a painful option in a real practice and also induced risk of Koebnerization [80, 106]. Most participants in our included studies are stable vitiligo so this might be the reason for the absence of such complication in this study. PRP therapy was less chance for repigmentation in certain areas like pressure bearing areas or acrofacial sites and elbows.[106] Therefore, right candidates are important to choose carefully for successful intervention.

Additional treatment combination with large population clinical studies are recommended to get the proper guideline for using PRP in combination therapy for vitiligo are required for real clinical practice in order to increase treatment efficacy with minimized the risk of adverse effects and statistical heterogeneity. In spite of that, this study would be an option for vitiligo treatment with high efficient outcome for repigmentation in future.

4.3 LIMITATIONS

Variation of inclusion participants such as mean age, duration of disease, vitiligo subtypes, intervention protocols and comparative features of different types of treatments are unavoidable limitations of this meta-analysis. Furthermore, there were different features of comparison either combination or monotherapy in separated groups including laser, light- based therapy, vitiligo surgery and PRP as systemic therapy with comparative outcome measurements as clinical improvement adjusted in term of ≥75% repigmentation as well as ≥50% repigmentation in order to get homogenous outcome assessment. Numerous studies were being excluded based on the exclusion criteria such as non-extractable data for meta-analysis, insufficient outcome analysis and measurements. Systematically search for related articles and restricted data for homogenous comparative outcome measurement are selected and will be included in this study. Moreover, there was no split comparative before and after clinical trial for platelet-rich plasma (PRP) alone and other modality alone to analyze the efficacy of PRP. These limitations resulted the certain degree of bias which would lead to high heterogeneity for I-squared values in subgroups analysis.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSIONS

This is the first study for statistically calculating the efficacy of platelet-rich plasma as combination therapy for vitiligo in our scope of knowledge. Total five studies with 332 participants for present meta-analysis. Statistically significant resulted in ≥75% repigmentation scale for overall study analysis in risk ratio was 2.95 (1.17 – 7.43) with 95% confidence interval (CI) and p value was 0.02. Risk ratio in ≥50% repigmentation scale was 2.54 with 95% CI: (1.28 – 5.03) with p value, 0.008. Using PRP as an adjunctive therapy resulted drastically benefit in term of ≥75% repigmentation which had not much bias and stronger evidence than ≥50% repigmentation. Moreover, regarding to present statistical analysis adjunctive PRP significantly showed benefits, especially PRP in combination with energy based treatment (laser and light-based interventions) which provided around 12 and 5 times better than control for ≥75% and ≥50% repigmentation, respectively while, PRP with non-energy based modalities did not show benefit compared with control.

Based on the several studies clinical data analysis, there were only some minor complication: pain, erythema, post inflammatory hyper or hypopigmentation during and after completed treatment. Besides the standard treatment for vitiligo, platelet-rich plasma therapy can be considered as safe and effective treatment in repigmentation of vitiligo. Furthermore, standard protocol for platelet-rich plasma preparation such as collected blood, anticoagulant usage, numbers and time of centrifugation are essential for standardized therapy. Even though the mean follows up for each study was 6 months, large participants groups with longer duration of follow up measure are recommended to evaluate the efficacy of PRP as well as repigmentation and recurrence rate of vitiligo. In conclusion, the efficacy of platelet-rich plasma as combination therapy was found to be efficient in vitiligo intervention.

5.2 RECOMMENDATIONS

5.2.1 Recommendations for clinical practice

This study focused to evaluate for the efficacy of platelet-rich plasma as combination therapy in vitiligo. There were proven data about clinical improvement in PRP as combination therapy compared with control (in term of ≥75% repigmentation, RR: 2.95, (95% CI (1.17– 7.43), I^2: 84%, p = 0.02 and ≥50% repigmentation RR: 2.54, (95% CI (1.28 – 5.03), I^2= 94%, p=0.008) without serious complication so PRP can be considered as an option for future perspective for adjunctive modality besides the standard vitiligo therapy. Standard PRP preparation and protocol for vitiligo combination treatment with long follow up duration might be required for better clinical practice.

5.2.2 Recommendation for future research

In fact, there should be a large-scale population to analyze the efficacy of PRP as monotherapy beside another standard modality or control (placebo groups) in split study or double blinded randomized control trial for prospective study to minimize the heterogeneity and publication bias. Intervention and comparison for standard treatment regime with homogenous outcome measurement between each study are essential for future meta-analysis in order to get better understanding which treatment options are more repigmentation in vitiligo treatment. Additionally, more researches were required to understand the multi-pathogenesis nature of vitiligo related with platelet-rich plasma mechanism and the effect of growth factors on melanocyte activities since these facts were still hard to clarify. Regards to reduce the risk of bias, numbers of treatment sessions, cumulative does, longer follow up duration, extractable data for degree of complication and recurrences rate with standardized outcome measurement should be computed as well as clarifying the definite selective criteria in further studies.

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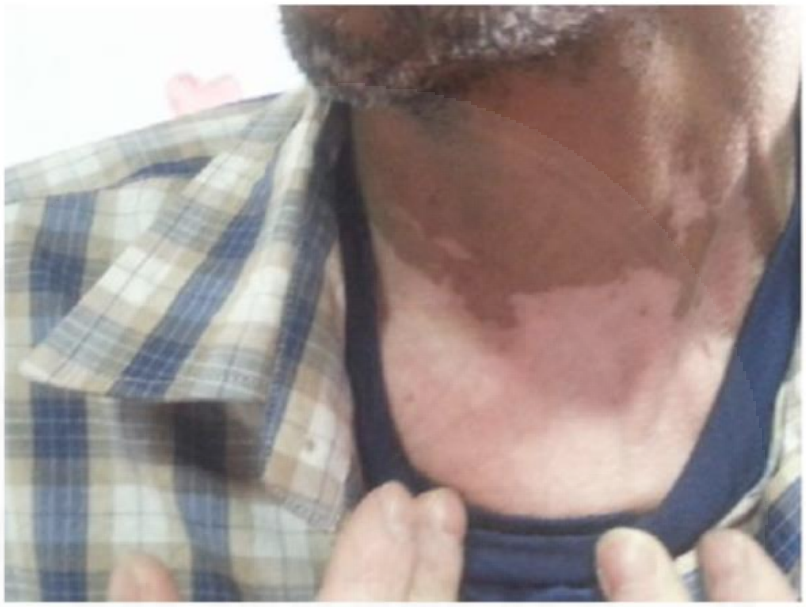
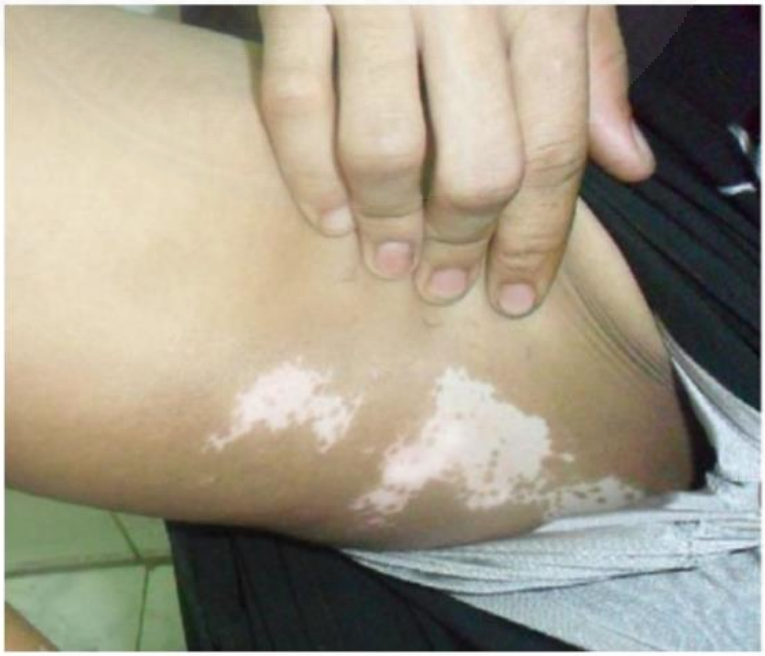
APPENDICES

APPENDIX A

PHOTOS OF VITILIGO

Vitiligo on the neck and inner thigh – fully demarcated vitiliginous macules and patches. [109]

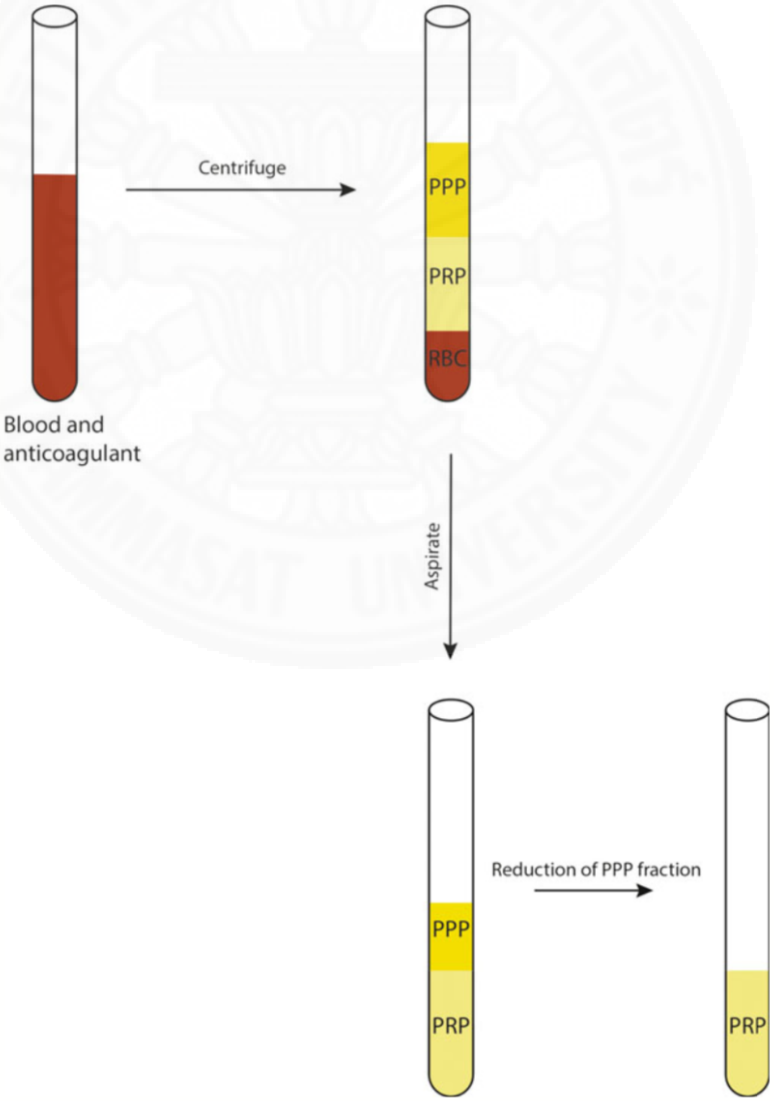




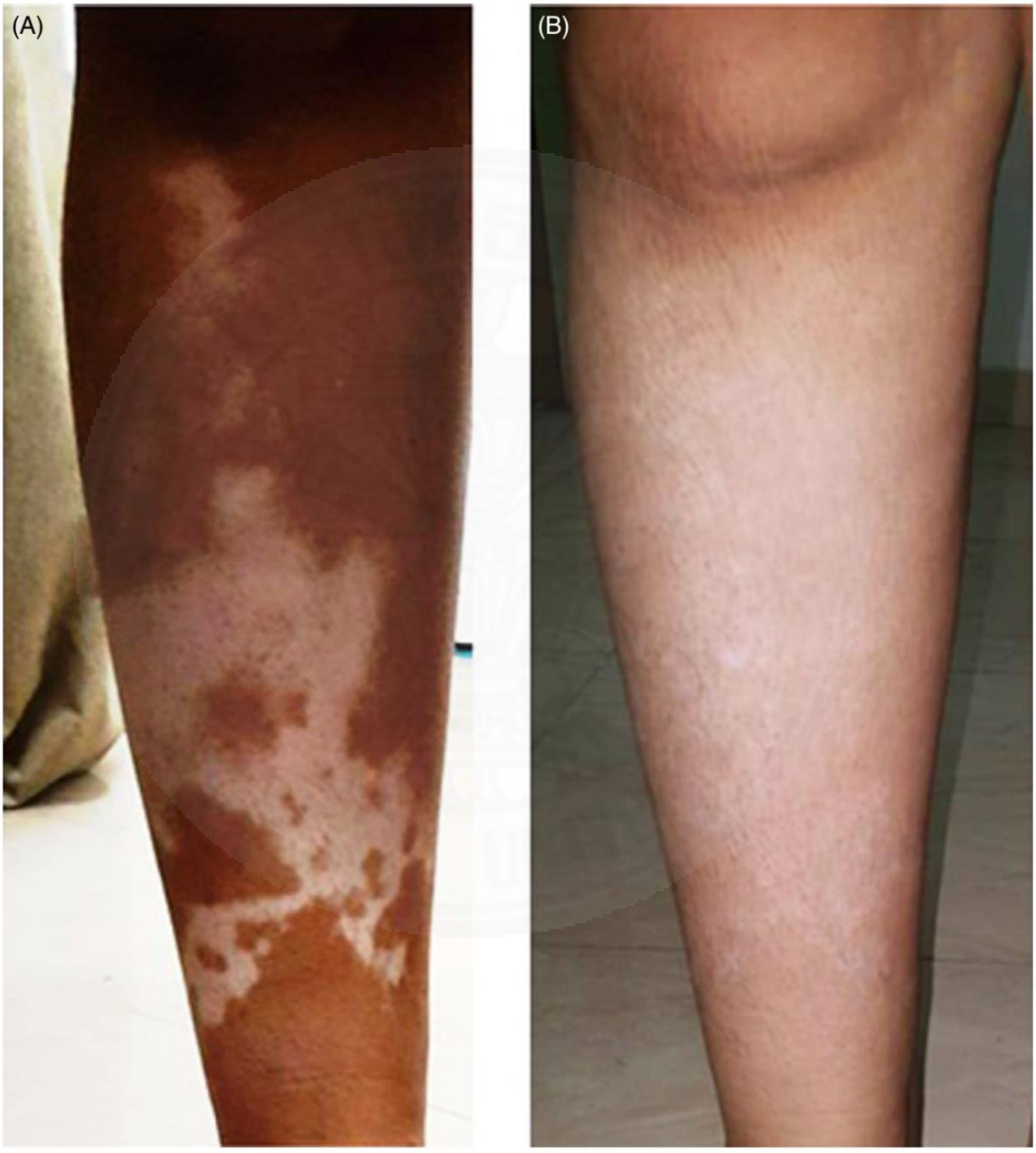
APPENDIX B

PROCESS OF PLATELET-RICH PLASMA PREPARATION

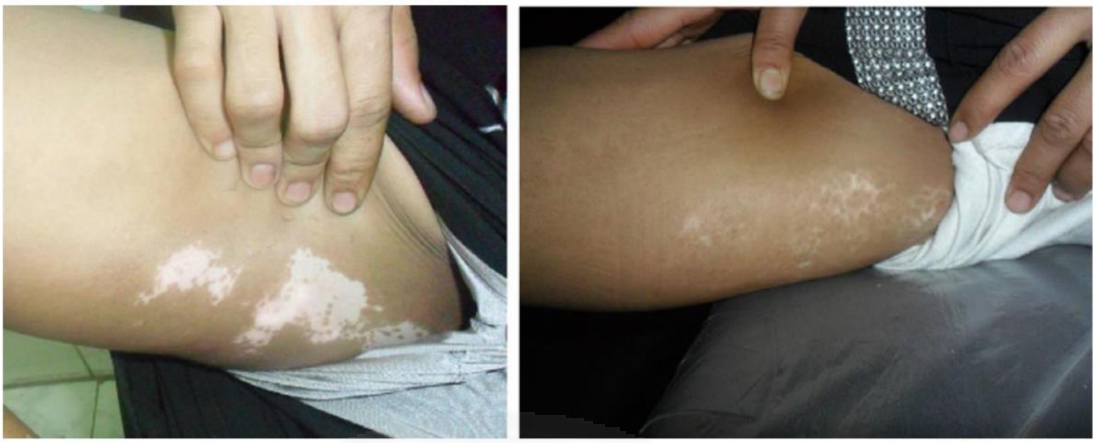
Platelet-rich plasma is prepared by withdrawn the whole blood and mixed with anticoagulant. After centrifuging, it separated into three layers: platelet poor plasma (PPP) at the top, platelet-rich plasma (PRP) in middle portion, and red blood cells (RBCs) at the bottom according to specific gravity. In order to get pure PRP, the RBCs layer is discarded and spin again. After finished 2-spins, the top layer of the PPP is discarded, and the final bottom layer contains mainly concentrate of PRP and mixed with a few percentage of PPP. At the last, the platelet is activated with thrombin or calcium chloride. [3]



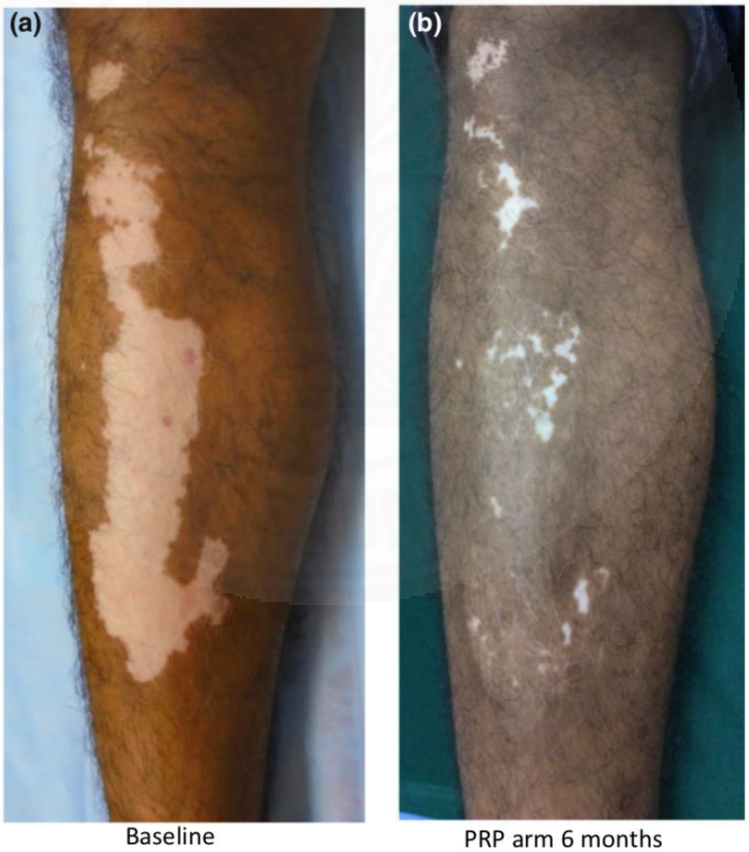
APPENDIX C
PHOTOS DEMONSTRATING BEFORE AND AFTER THE TREATMENT



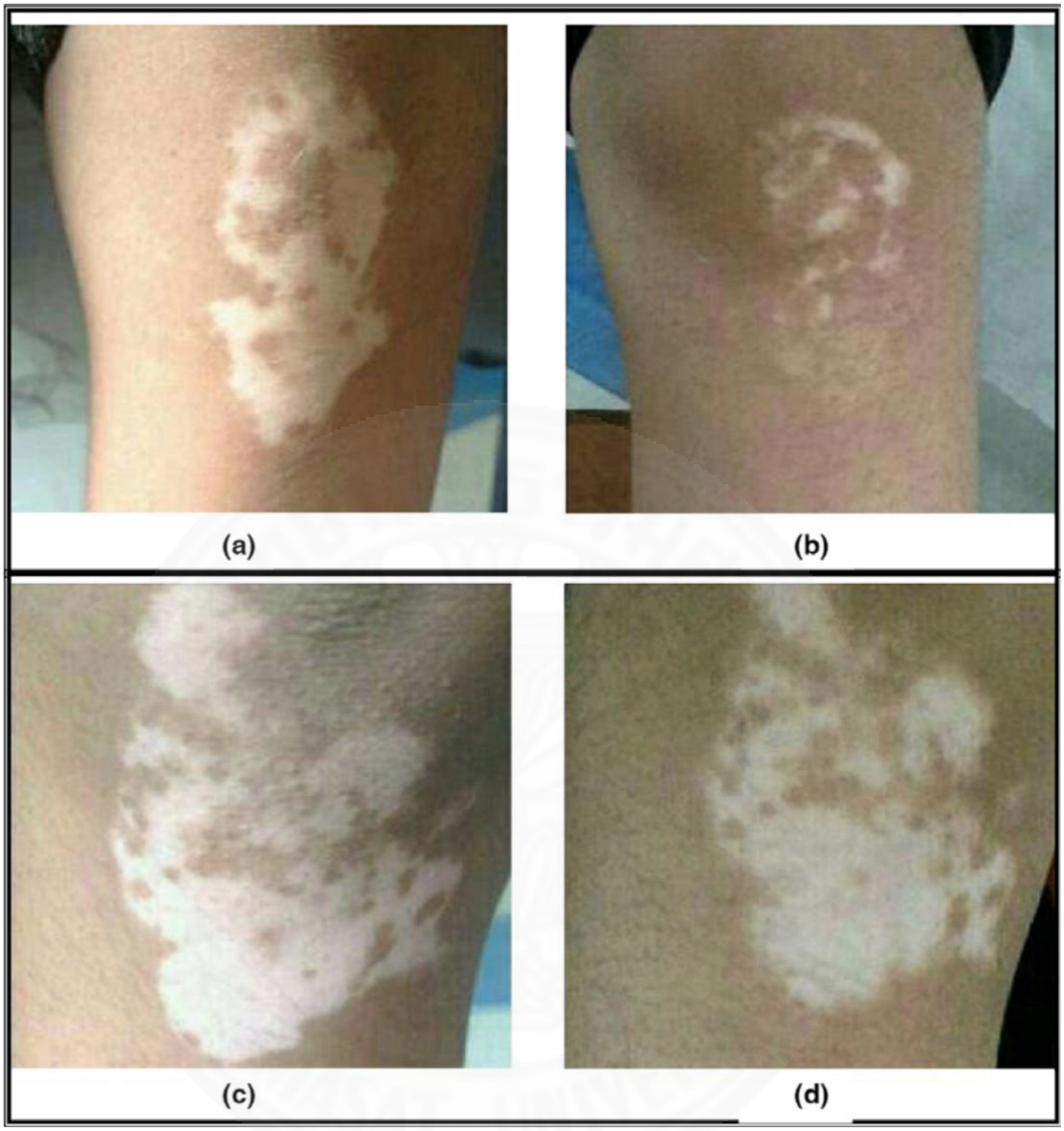
A female vitiligo patient, lesion at leg. A, Before treatment. B, 4 moths after PRP + excimer laser with excellent response [117]



Combination of fractional CO2 laser and PRP injection for lesions at inner side of the thigh; Right: before treatment, Left: 3 months after the treatment with repigmentation >75% [109]



Use of PRP to suspend noncultural epidermal cell suspension imporves repigmentation after autologous transplantation in stable vitiligo. Platelet-rich plasma (PRP) arm showing 92% improvement in 6 months with improvement in leukotrichia [111]



Female patient with vitiligo on: (a) right leg before treatment, (b) 4 months after PRP injection and NB-UVB phototherapy with excellent response, (c) left leg before treatment, (d) 4 months after NB-UVB phototherapy with mild response. [107]

APPENDIX D

ACCPETANCE LETTER FROM THE PROCEEDING

RSUSSH 2020
ACCEPTANCE & INVITATION LETTER
The 5th RSU National and International Research Conference on Science and Technology, Social Science, and Humanities 2020 (RSUSSH 2020)
Rangsit University, Pathum Thani, Thailand

April 21, 2020

Dear: Kyi Mar Tun, M.D, Premjit Juntongjin, M.D.

Thank you for submitting a paper to **The 5th RSU National and International Research Conference on Science and Technology, Social Science, and Humanities 2020 (RSUSSH 2020).** Herewith, the RSUSSH 2020 scientific committees are delighted to inform that, your paper entitled "Efficacy of platelet-rich plasma in combination therapy for vitiligo" has been accepted for **Poster (Full Paper)** in our conference. Your paper will be enclosed in our conference proceeding of RSUSSH 2020 with the detail described below;

Code:	IN20-298
Author(s):	Kyi Mar Tun, M.D, Premjit Juntongjin, M.D.
Title:	Efficacy of platelet-rich plasma in combination therapy for vitiligo
Session:	Medical, Dental and Health Sciences
Presentation:	Poster (Full Paper)

We cordially invite you to participate, share your experience and give your presentation in the RSUSSH 2020 conference held at the **Student Center building, Rangsit University**, Pathum Thani, Thailand, on **1st May 2020**. The Organizing Committee members would like to thank you in advance for your participation.

Sincerely yours,

Assoc. Prof. Dr. Kanda Wongwailikhit
Program Chair of RSUSSH 2020
Rangsit University, THAILAND.

APPENDIX E

CERTIFICATE FROM THE PROCEEDING

BIOGRAPHY

Name	KYI MAR TUN
Date of Birth	APRIL 03, 1989
Educational Attainment	2018 – present Master of Science (Dermatology) Chulabhorn International College of Medicine, Thammasat University, Thailand. 2013 – 2015 Master of Business Administration (MBA) (International Business Management) International College, University of The Thai Chamber of Commerce, Thailand. 2005 – 2013 Bachelor of Medicine and Bachelor of Surgery (M.B., B.S) University of Medicine 1, Yangon, Myanmar.
Work Position	General Practitioner at Everest Polyclinic, Yangon, Myanmar.
Work Experience	2012 – 2013 House Surgeon, Internship. Yangon General Hospital, Myanmar. Yangon East General Hospital, Myanmar. Yangon West General Hospital, Myanmar. Yangon Children Hospital, Myanmar. 2013 – 2018 General Practitioner