Interleukin 17 Expression in Psoriatic Skin Lesions before and after Treatment with Platelet Rich Plasma (PrP)

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Abstract

Background: Psoriasis is a chronic immune-mediated inflammatory disorder with epidermal hyperplasia. There is some evidence that the Interleukin (IL) 17 has a role in the pathogenesis of psoriasis. Platelet Rich Plasma (PrP) is autologous concentration of platelets may improve the condition because of its anti-inflammatory nature.

Aim of Work: This study has been conducted in an attempt to assess the effect of PrP as a new modality for treatment of chronic localized plaque psoriasis on IL17 expression in psoriatic skin lesions.

Patients and Methods: This study is a randomized controlled clinical trial study in which 24 psoriasis patients attending Dermatology and Andrology Outpatient Clinics in Suez Canal University Hospitals between January 2016 to January 2017 were enrolled. Two symmetrical plaques were injected weekly intradermally with PrP and saline as placebo and skin biopsy was taken from each plaque before and after treatment. Skin biopsies were assessed for IL17 expression.

Results: All the clinical parameters used for assessment of clinical response (size, erythema, thickness and scaling) significantly decreased after PrP injection by (27.39 ±29.33, 28.47±51.89, 25.35±66.23, and 24.31±44.36%) respectively, while plaques injected with saline showed significant decrease of scaling only. Moreover 6 plaques out of the 9 plaques that were positive for IL17 converted to negative after treatment with PrP, while all them remained positive for IL17 expression after injection with saline as placebo.

Conclusions: Although psoriatic plaques treated with PrP showed variable clinical and immunological improvement compared to lesions injected with saline as placebo, PrP treatment alone can't be considered as a therapeutic modality for treatment of localized plaque psoriasis.

Key Words: Psoriasis – Interleukin 17 – Platelet rich plasma.

Introduction

PSORIASIS is known to be a prevalent noncontagious autoimmune disease characterized by recurrent episodes of sharply demarcated red and scaly skin plaques affecting approximately 2% of world's population [1].

The precise etiology of psoriasis remains unclear, but psoriasis may result from a complex interaction between genetic, environmental, skin barrier disruption and immune dysfunction [2], that cause epidermal hyperplasia which occurs as a result of activation of immune system in a focal skin region which in turn is mediated by CD4+ and CD8+ T-lymphocytes that accumulate in the diseased skin. Psoriasis is now considered to be the most prevalent T-cell mediated inflammatory disease [3].

Interleukin 17 (IL 17) is a prototype member of the IL 17 family of cytokines, which contains six structurally related isoforms: IL-17A, IL-17B, IL-17C, IL-17E 9IL-25), and IL17F that share 20-50% homology to IL-17 [4]. The polypeptide of IL-17 consists of a 19-amino-acid signal sequence followed by 136-amino-acid-mature fragment. It contains one N-linked glycosylation site at least [5].

Interleukin 17 (IL 17) appeared to be one of the most essential players in the current model of psoriasis pathogenesis. It was thought to be produced mainly by T helper (Th)-17 cells, a subset of CD4 + T helper cells that is distinct from the Th1 and Th2 lineages [6,7]. IL17 expression was not detected in normal skin, but was found in lesional skin of psoriasis [8].

The need for new topical therapies to treat localized plaque psoriasis is getting increased because treatment options for psoriasis are limited and the success of treatment options for psoriasis...
are limited and the success of treatment is poor with up to 50% of patients are not satisfied with the current standard therapies including emollients, tar, topical steroids, vitamin D analogues, retinoids and calcineurin inhibitors [9,10].

Platelet Rich Plasma (PrP) which can be defined as a volume of autologous plasma with platelet concentration above baseline [11]. In the past PrP has been used to prevent infection and speed up the wound healing process by reducing bleeding and swelling after plastic-, general-, neuro-, orthopedic surgeries [12], now it has also been found to be beneficial therapies such as PrP with carbon dioxide fractional lasers for acne scars [13].

Although PrP has proliferation including effect, it is also a potent anti-inflammatory agent, which can suppress cytokine release and therapy limit local tissue inflammation [14]. Since psoriasis is characterized by extensive inflammatory infiltrate responsible for secretion of a variety of inflammatory cytokines, it is probable that the anti-inflammatory effect of PrP may be of benefit in this condition.

This study was conducted to assess the effect of intradermal injection of PrP as a new modality for treatment of localized plaque psoriasis on the expression of IL17 in psoriatic skin lesions compared to placebo.

Aim of the work:

This study has been conducted in an attempt to assess the effect of PrP as a new modality for treatment of chronic localized plaque psoriasis on IL17 expression in psoriatic skin lesions.

Patients and Methods

The study is a randomized controlled clinical trial study. It was conducted at Dermatology and Venereology Outpatient Clinics and Pathology Lab in Suez Canal University Hospitals in Ismailia, Egypt from January 2016 – January 2017. Patients clinically diagnosed to have chronic plaque psoriasis with two almost symmetrical plaques having similar clinical criteria and who consented after having received full data about the study was enrolled in this study.

Inclusion criteria:

Patients having chronic plaque psoriasis with almost symmetrical plaques similar clinical criteria aged 18 years or more.

Exclusion criteria:

- Pregnant and lactating females.
- Patients who have been on any modality for psoriasis for proceeding two months.
- Patients having comorbid illness as cardiovascular or hematological diseases as well as patients with hepatitis B and C viruses.
- Patients on anticoagulants or receiving blood products.

This study included 24 patients, and 48 skin biopsies taken from all as follows:

- In each patient two almost symmetrical plaques were selected to be studied, the two plaques had similar severity parameters.
- One plaque was injected intradermally with PrP and the other one was injected with normal saline as placebo.

All patients were subjected to the following:

1- Full medical history, include:
   - Demographic data; age, gender, occupation, residence, marital status.
   - Medical history (chronic illness, surgical interventions).
   - Any history of previous medication for psoriasis/duration of psoriasis.

2- Clinical assessment of severity of psoriatic plaque according to:
   - Plaque size.
   - Severity parameters: Thickness of plaque, degree of erythema and degree of scaling. severity parameters are measured on a scale 0 (no symptoms), 1 (mild), 2 (moderate), 3 (marked) and 4 (very marked).

3- Skin biopsy; five mm skin punch biopsies were taken from each patient before and after PrP or saline injection and were stained for H & E and for IL 17 by routine IHC protocol. For standard histology, the specimens were fixed in 10% formalin and processed for hematoxylin and eosin staining.

4- Clinical follow-up of patients once a week by clinical assessment of clinical severity parameters.

5- PrP preparation:
   - Obtain WB by venipuncture in Acid Citrate Dextrose (ACD) tubes.
- Do not chill the blood at any time before or during platelet separation.
- Centrifuge the blood using a ‘soft’ spin at 1200rpm for 4 minutes at 22°C.
- Transfer the supernatant plasma containing platelets into another sterile tube (without anticoagulant).
- Centrifuge tube at a higher speed (a hard spin) to obtain a platelet concentrate at 3500 rpm for 10 minutes at 22°C.
- The lower 1/3rd is PRP and upper 2/3rd is Platelet-poor Plasma (PPP). At the bottom of the tube, platelet pellets are formed.
- Remove PPP and suspend the platelet pellets in a minimum quantity of plasma (2-4mL) by gently shaking the tube.

6- PrP Injection: According to the size of the psoriatic plaque 1-4ml of PrP was injected intradermally at the plaque site using insulin U100 syringes. Patients were injected once per week for 10 weeks.

7- IHC staining for IL17 of psoriatic plaques: For immunohistochemical staining, 4-µm sections were cut and placed on slides coated with poly-l-lysine. Immunohistochemical staining was performed by the streptavidin-biotin complex method according to the manufacturer’s instructions. Rabbit polyclonal antibodies against mouse IL-17 (Abcam, USA, ab18568) at a 1:200 dilution were used as primary antibodies. The sections were washed and stained using the streptavidin-biotin complex. After the sections were rehydrated, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min at room temperature. Then the sections were first incubated in 5% bovine serum albumin for 20min and then in the primary antibody for 24h at 4°C, and incubated with the streptavidin-biotin complex for 20min. At last, the sections were developed with 3, 3-Diaminobenzidine (DAB) and observed under a light microscope. Instead of primary antibody, non-immune goat serum was taken as a control. Formalin-fixed and paraffin-embedded normal placenta samples were used as the positive control. In addition, formalin-fixed and paraffin-embedded breast samples were used as the negative control.

Statistical analysis:
Data were fed to the computer and analyzed using IBM SPSS software package Version 20.0 (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The Wilcoxon signed ranks test was used to compare. p-value was considered statistically significant when it was less than 0.5.

Ethical considerations:
An informed consent was taken from all studied patients after informed them about all details of the study and agree to involved in our study.

Results
This study is a comparative clinical trial study to evaluate the effect of PrP on psoriatic plaques compared to placebo (saline). The study included 24 patients whom ages ranged between 18 and 60 years, whom attended the Dermatology and Venereology outpatient Clinics in Suez Canal University Hospital, Ismailia, Egypt.

Demographic features:
The mean age of the patients was (40.29 ± 12.37) years and the mean duration since they have been diagnosed as chronic plaque psoriasis was (7.40 ± 7.37) years. The mean duration of the plaques treated with PrP was (6.69 ± 4.86) months and the mean duration of their symmetrical lesions on the other side that were treated with saline (6.58 ± 4.11) months (Table 1).

Clinical results:
Regarding the effect of treatment modules on the clinical parameters (size, erythema, thickness and scaling), no statistically significant difference between these parameters in psoriatic plaques treated with PrP versus saline at the star of the study. By the end of the study a significant reduction of all these clinical parameters in psoriatic plaques treated with PrP while plaques treated with saline showed reduction on scaling only (Table 2 & Figs. 1, 2).

Histopathological parameters results:
As regards to histopathological parameters changes of psoriasis (Parakeratosis, acanthosis, downward elongation of rete ridges, thin/no granular cell layer, suprapapillary thinning, Munro microabscesses, increased mitotic figures above basal layer, Prominent dermal capillaries, mixed dermal infiltrate of lymphocytes, macrophages and neutrophils) with different treatment modules (PrP versus saline), a statistically significant decrease of dermal perivascular inflammatory infiltrate in psoriatic lesion treated with PrP than treated with
IL17 expression results:

Positive IHC expression of IL17 were detected as brownish nuclear stain mainly in basal cells of epidermis and dermal perivascular inflammatory cells. Regarding the effect of treatment module (PrP versus Saline) on IL17 expression, pretreatment biopsies showed 19 out of 24 studied biopsies were positive for IL17. On correlating the duration of psoriatic plaque diagnosis and IL17 positivity, recent plaques showed more IL17 expression than old plaques with no statistically significant difference. No changes in IL17 expression in post-treatment biopsies taken from saline treatment while in post treatment biopsies with PrP reduce in IL17 expression occurs with cases showed moderate to marked improvement (as reduce dermal inflammatory cells infiltrate) (10 out of 24 biopsies were positive, and 9 cases were negative), this difference was statistically significant ($p=0.0171$) Figs. (6,7).

Table (1): Demographic data of the studied patients (n=24).

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Pre-</th>
<th>Post-</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs):</td>
<td>Min-max</td>
<td>18.0-60.0</td>
<td>40.29±12.73</td>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>25.83±29.02</td>
<td>7.40±7.37</td>
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<td></td>
<td>Median</td>
<td>16.50</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>↓8.45±15.99</td>
<td>&lt;0.001*</td>
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<tr>
<td>Gender:</td>
<td>Male</td>
<td>16</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Duration of disease (since diagnosis) (years):</td>
<td>Min-max</td>
<td>0.50-25.0</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>7.40±7.37</td>
<td>4.0</td>
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<tr>
<td></td>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
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<tr>
<td></td>
<td>Change</td>
<td>↓0.88±1.30</td>
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<tr>
<td>Duration of plaques treated with PrP (months):</td>
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<td>0.50-15.0</td>
<td>6.50</td>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>6.69±4.86</td>
<td>6.0</td>
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<tr>
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<td>Median</td>
<td>3.0</td>
<td>2.0</td>
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<tr>
<td></td>
<td>Change</td>
<td>↓0.63±1.10</td>
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</tr>
<tr>
<td>Duration of plaques treated with saline (months):</td>
<td>Min-max</td>
<td>0.50-13.0</td>
<td>6.0</td>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>6.58±4.11</td>
<td>7.0</td>
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<td>Median</td>
<td>3.0</td>
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<td></td>
<td>Change</td>
<td>↓0.63±1.10</td>
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| Wilcoxon signed ranks test showed a statistically significant reduction of clinical parameters of psoriatic plaques treated with PrP more than those treated with saline. $p_1$: $p$-value for comparing between pre and post-treatment. $p_2$: $p$-value for comparing between PrP and saline.
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Fig. (1): (A) Before Saline: Psoriatic plaque showed moderate erythema and scaling. (B) After Saline: Psoriatic plaque showed mild erythema and scaling. (C) Before PrP: Psoriatic plaque showed marked erythema and scaling. (D) After PrP: Psoriatic plaque showed mild erythema and scaling (more improvement than saline treatment).

Fig. (2): (A) Before Saline: Psoriatic plaque showed moderate erythema and scaling. (B) After Saline: Psoriatic plaque showed mild erythema and scaling. (C) Before PrP: Psoriatic plaque showed marked erythema and scaling. (D) After PrP: Psoriatic plaque showed mild erythema and scaling (more improvement than saline treatment).
Fig. (3): (A) Pre-treatment psoriasis, H & E X200: Showed parakeratosis (white line), Munro microabscesses (blue arrow), suprapapillary thinning (white star), downward elongation of rete ridges (white head of arrow), marked dermal perivascular inflammatory cells infiltrate (white arrow). (B) Post-PrP-treatment psoriasis, H & E X200: Showed no improvement, same histopathological parameters of pre-treatment.

Fig. (4): (A) Pre-treatment psoriasis, H & E X200: Showed parakeratosis (white line), suprapapillary thinning (white star), downward elongation of rete ridges (white head of arrow), marked dermal perivascular inflammatory cells infiltrate (white arrow). (B) Post-PrP-treatment psoriasis, H & E X200: Showed mild improvement as reduce in parakeratosis (white line), downward elongation of rete ridges (white head of arrow), while same marked dermal perivascular inflammatory cells infiltrate (white arrow) still present.

Fig. (5): (A) Pre-treatment psoriasis, H & E X200: Showed parakeratosis (white line), suprapapillary thinning (white star), downward elongation of rete ridges (white head of arrow), marked dermal perivascular inflammatory cells infiltrate (white arrow). (B) Post-PrP-treatment psoriasis, H & E X200: Showed marked improvement as reduce in parakeratosis (white line), downward elongation of rete ridges (white head of arrow), marked reduce in dermal perivascular inflammatory cells infiltrate (white arrow).
Fig. (6): IL17 IHC expression: (A) Pre-treatment; showed marked nuclear brownish positive stain of IL17 mainly in dermal perivascular inflammatory cells (black arrow) and scattered basal cells in epidermis. (B) Post PrP treatment; showed no changes in IL17 positive expression as in pre-treatment state and no improvement in histopathological/clinical parameters. (C) Post saline treatment; showed no changes in IL17 positive expression as in pre-treatment state and no improvement in histopathological/clinical parameters.

Fig. (7): IL17 IHC expression: (A) Pre-treatment; showed marked nuclear brownish positive stain of IL17 mainly in dermal perivascular inflammatory cells (black arrow) and scattered basal cells in epidermis. (B) Post PrP treatment; showed reduced in IL17 positive expression compared to pre-treatment state and improvement in histopathological/clinical parameters (as reduced in perivascular inflammatory cells in dermis) (red arrow). (C) Post saline treatment; showed no changes in IL17 positive expression as in pre-treatment state and no improvement in histopathological/clinical parameters.
Psoriasis is a chronic, immune mediated dermatological disease with an estimated prevalence ranging between 1-9% of the world population, varying according to age and geographic regions [15].

Due to the immediate visibility of the disease, psoriasis can lead to considerable psychological stress and psychosocial disability, with acute feelings of stigmatization [16]. There is an urgent need for new treatment strategy, as up to 50% of patients are not content with current therapies [17].

Regenerative medicine is the newest branch of medicine that provides revolutionary technology for developing therapies for previously untreatable and immune mediated diseases as psoriasis. Also, regenerative therapy may allow an absolute response and diminish potential toxicity that has been associated with conventional therapy [18].

Platelet rich Plasma (PrP) is one of the important regenerative therapies today, that accelerate the wound healing mainly. Several clinical studies using PrP as a trial treatment for several chronic dermatology illness with promising results. Platelets release cocktail of growth factors, which are believed to mimic physiological healing processes through NF-κb and other cytokines suppression [19]. Zhang et al., showed that PrP exerts anti-inflammatory effect which could be mediated by the presence of HGF among other factors [20]. Bendinelli et al., delineated that PrP also reduces chemotaxis by inhibiting chemokine transactivation and CXCR4-receptor expression, thus also control local inflammation [21].

Our study trying to evaluate the results of the application of PrP solely for the treatment of psoriasis to detect the efficacy and the safety of this potential method for the treatment of psoriasis. A total 24 adult patients with psoriasis attending to Dermatology Outpatient Clinics, Suez Canal University Hospitals were enrolled for a prospective randomized controlled clinical trial of 10 weeks duration aiming to assess the effect of intradermal injection of Prp on clinical/histopathological parameters and IL17 expression in psoriatic lesional skin as compared to saline (placebo).

By the end of the study, we found that although using intradermal injection of PrP for treatment of psoriatic plaques has significantly improved the clinical/histopathological parameters and reduce in IL17 expression more than saline treatment, but the percentage of change of clinical parameters wasn't enough to consider intradermal injection of PrP as a sole new modality for treatment of chronic localized plaque psoriasis.

This study agreed with that done by Chakravdhania and his colleagues; that evaluate the impact of PrP plus Methotrexate (MTX) treatment of chronic localized plaque psoriasis. They found that a significant improvement of PrP plus Methotrexate (MTX) treatment compared with Methotrexate (MTX) treatment only, also proved that this combined treatment was well tolerated by all patients without any serious side effects [22].

Concerning IL17 expression using IHC, pre-treatment biopsies showed 19 out of 24 studied biopsies were positive for IL17. On correlating the duration of psoriatic plaque diagnosis and IL17 positivity, recent plaques showed more IL17 expression than old plaques with no statistically significant difference. No changes in IL17 expression in post-treatment biopsies taken from saline treatment while in post-treatment biopsies with PrP reduce in IL17 expression occurs with cases showed moderate to marked improvement (as reduce dermal inflammatory cells infiltrate) (10 out of 24 biopsies were positive, and 9 cases were negative), this difference was statistically significant. So we can concluded that usage of PrP have an anti-inflammatory effect depend on causing a statistically significant reduction in dermal peri inflammatory cells infiltrate and IL17 expression.

Johansen et al., studied the levels of IL17 isoforms and receptors in psoriatic skin lesions by quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique in patients with moderate to severe psoriasis and found that the expression of IL17A, IL17C and IL17F mRNA were significantly higher in lesional skin than non-lesional and normal skin [22].

Yilmaz et al., evaluated the serum and tissue levels of IL17 in different subtypes of psoriasis have reported a positive correlation between IL17 levels and disease severity and they recommended measurement of IL17 levels as an objective parameter to determine disease severity [23].

Michalak-Stoma and Colleagues found no statistically significant differences in the serum IL17 concentrations between the psoriatic patients and control group, but they stated that elevated IL22 level without increase of IL 17 level may suggest that IL22 role may be more significant in inflammatory process of psoriasis. This supports our finding that IL17 is not necessarily high in all psoriatic patients [24].
Clinical improvement detected in our cases with PrP treatment compare to saline treatment can be attributed to some extent to blockage of the IL 17 (in form of reduce IL 17 expression). All confirm the anti-inflammatory effect of PrP treatment in psoriasis. Di Cesare et al., found that IL 17A and IL 17 F can mobilize, recruit and activate neutrophils so blocking IL17 pathway may improve clinical outcome [25].

Moreover, not all patients who showed clinically improvement of the treated plaques and were positive for IL17 expression before treatment turned out to negative expression after treatment, and this may point to different mechanisms by which PrP can improve clinical outcome in psoriasis and not only IL17 expression.

The precise mechanisms through which PrP alleviates inflammatory process in psoriasis is yet to be delineated. Further studies are recommended to identify specific inflammatory pathway PrP targets.

**Conclusion:**

Although psoriatic plaques treated with PrP showed statistically significant clinical, histopathological and IL17 IHC expression compared to lesions with saline as placebo. This improvement was mild (slow). So, PrP cannot be considered as a sole therapeutic modality for treatment of chronic localized plaque psoriasis.

**References**

التعبير عن الإنترلوكين 17 في الجلد المصابة بالصدفية

نستعرض أداء الإنترلوكين 17 كنصير من العناصر المسببة لمرض الصدفية، فيما أيضًا يتم الحديث عن استخدام الحقن بالبلازما الفنية بالصفائح الدموية كعلاج فعال في الكثير من الأمراض ومنها الجلدية. وقد تم تصميم هذه الدراسة لتحديد فعالية حقن البلازما الفنية بالصفائح الدموية داخل الأدوية كطريقة جديدة لعلاج الصدفية وتأثيرها على معدل التعبير عن الإنترلوكين 17 كنصير مسبب للصدفية في عينات نسيجية من الجلد قبل وبعد العلاج. تم إجراء الدراسة بالعوامل الخارجية للأمراض الجلدية وعمل تحليل البئولوجيا بالمستشفيات الجامعية لقنوات السويس لإنتاجية.

واستمرت على 24 مريض الصدفية اللوحية الموضوعية الممتنعة في الفترة ما بين يناير 2016 إلى يناير 2017 ويتراوح أعمارهم بين 18-60 عامًا. تم أخذ عينة من الجلد في المنطقة المصابة قبل الحقن وبعد وقوع حقن كل مريض مرة واحدة أسبوعيًا لمدة 10 أسابيع. وتمت تحليل النتائج من حيث التعبير عن وجود الإنترلوكين 17 قبل وبعد العلاج إلى إمكانية استخدام حقن البلازما الفنية بالصفائح الدموية مقتنيًا مع طرق علاجية أخرى للمرضى الصدافيين الموضوعية الممتنعة.