ASSESSING EFFICIENCY OF THE AUTOLOGOUS PLATELET-RICH PLASMA (PRP) THERAPY IN THE TREATMENT OF CHRONIC ULCERS

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ABSTRACT

The study is conducted on 26 patients in order to evaluate efficiency of the autologous PRP therapy in the treatment of chronic ulcers. Patients with the chronic ulcers at stage III and stage IV (76.8% of the patients caused by decubitus ulcer) have the average age of 42.5 (ranged from 18 to 80 years old), in which, there are 20 male and 6 female patients. All patients have combination chronic diseases; average amount of chronic ulcers per patient is 1.5 ulcers (ranged from 1 to 6 wounds); average area of the chronic ulcers is 32.5± 22.3 cm2 (ranged from 5 to 100 cm2); average existence time of the chronic ulcers is 9.3 weeks (ranged from 4 to 50 week long). Implementing fractionation and injection of autologous PRP (about 4 - 5ml of fractionated solution) in compliance with the procedure of treatment for chronic ulcers in two times (duration between two times is 1 week). The results show that PRP therapy has the impact on reducing inflammation, stimulating regeneration and epithelization, reducing infection at the ulcers, preparing the ulcers foundation to successfully ensure surgery of flap transfer and skins graft and reducing treatment time. The proportion of patients who are totally healed is 100% with the average treatment time of 33.3±10.7 days. The autologous PRP therapy used in the treatment of chronic ulcers is safe for patients (both local treatment and whole body treatment).

Keywords: Platelet rich plasma, chronic wound, ulcer.

PROBLEM STATEMENT

The chronic wound has a common connection with varicose veins, inflammation, trauma, neuropathy, and immunodeficiency….., and this is more and more increasing. In the United States, the chronic wound affects about 3 to 6 million people, in which, a proportion of over-65-year-old people accounts for 85%. The annual cost of chronic wound treatment is estimated at $ 3 billion (Mathieu D. et al. 2006 [1], Menke N.B. et al. 2007 [2]). By 2014, the care cost of pressure ulcer is estimated at $ 11 billion per year, and the average cost for an ulcer is from $ 500 to$ 70,000 [3]. In the UK, the annual care cost of the chronic wound is estimated at $ 2,165 billion in 2014 (Dowsett C., 2014). The chronic wound is still considered a medical challenge due to the association with chronic diseases of the whole body, immunodeficiency, hematopoiectic disorders, infections ... and especially disorder of regeneration and functional recovery. Many therapies have been researched and applied to accelerate wound healing as therapies of topical negative pressure, hyperbaric oxygen, immunity, anti-inflammatory; using the modern material for wound dressing; cell culture such as fibroblasts, etc.

Platelet-rich plasma (PRP) has more concentrated platelets than normal plasma [5, 6]. Activated platelets lead to α-granule secretion within the platelets, thereby releasing proteins of anti-inflammatory cytokines and chemokines and dozens of growth factors such as PDGF, TGFβ, IGF, PD-ECGF, EGF, FGF, VEGF ... playing an important role in wound healing.

Growth factors affect damaged progenitor cells to stimulate proliferation and improve wound healing through autocrine and paracrine mechanisms. This process will create cell proliferation, collagen synthesis and basic ingredients, etc involving in regeneration and repair of damages of cartilage, bone, joint and soft tissue. Platelets also release large amounts of hemostatic agents, such as serotonin, catecholamines, fibrinogen, fibronectin, factor V, factor VIII, thromboxane A2 and calcium [7, 8]. As a result, blood clots are formed. Fibrin matrix stimulates monocyte stimulation, fibroblasts and other cells playing an important role in wound healing. At present, there are many published publications on the use of PRP in various fields such as orthopedics, medicine, sports, dentistry, ENT, neurosurgery, ophthalmology, urology, wound regeneration, aesthetics, and cosmetics ... due to high efficiency and safety, fast and easy production, relatively low cost and minimal invasiveness [6,8,9].

In Vietnam, PRP therapy is initially used to treat osteoarthritis, facial or aesthetic areas for good results. In the field of the wound, especially chronic wound, there have not had much research yet, these are small initial researches concentrated on the group of diabetic patients. Therefore, the study is conducted on:"Assessing the efficiency of the autologous platelet-rich plasma (PRP) therapy in the treatment of chronic ulcers" in order to: 1) evaluate the efficiency of the PRP therapy in the treatment of chronic ulcers. 2) Evaluate the safety of PRP therapy.

OBJECTS AND METHODOLOGY

Research objects

There are 26 patients with the chronic ulcer, treated at the Department of Wound healing - Le Huu Trac National Institute of Burns from December 2015 to August 2016.

- Criteria of studied patient selection: they are 18 years old and over and has chronic ulceration with stage III and stage IV (according to classification of US NPUAP and European EPUAP in 2009 [10]); existence time of ulcer over 4 weeks [9, 11,12,13,14] and agree to participate in the study and do not contraindicate in the PRP.
- Exclusion criteria [9]: Absolute contraindications: Platelet deficiency syndrome; severe thrombocytopenia (<50×10G/L); hemodynamic disorders; sepsis; blood fibrinogen decrease; systemic infection, acute inflammation at the injection site; the patients agree to participate in the study. Relative contraindications: fever; cancer (especially in liver and bones); Hb <100g / L, platelets <150 G / L, pregnant women, cardiovascular disorders, blood glucose control disorders.
- Moral criteria in the study: Patients who met the study criteria are fully explained for treatment therapy. The study is only conducted when having the voluntary consent of the patients. Procedures are conducted in accordance with the Helsinki Declaration, which maintains the safety and confidentiality of the patients. The study is approved by the ethics board in research of the Le Huu Trac National Institute of Burns.

Research material

New PRP Pro kit of Geneworld brand is licensed for circulation in Vietnam and its accompanying machines such as the centrifuge, etc.

Methodology:

- Prospective study, clinical trials, comparison of results before and after treatment process.

Procedure

PRP collection process

Based on the area of injury, the study chooses the use kit suitably. According to Vladimir N. Obolenskiy, Darya A. Ermolova [15], the amount of blood collected for PRP fractionation is equivalent to about half of the lesion area. In this study, the area of the lesion is mainly from 40 to 60 cm^2. Therefore, the average amount of blood is taken about 24 - 26mL.

PRP collection: Application of the PRP fractionation procedure (according to the supplier's instructions, ensuring the sterile principle) includes the basic steps: Venipuncture/Vacuum blood collection with specialized instruments on three blood *collection* tubes (about 25mL). The next steps are carried out in the secondary biosafety box. Carrying out the first centrifugation with the speed of 2000 round/min during 10 minutes to separate plasma - leukocyte - red blood (Blood after centrifugation will be separated into three layers: the yellow top layer is plasma mainly including leucocyte, the red below layer is erythrocyte, and the platelet is the thin layer between plasma and erythrocyte). After that, collecting plasma and deleting leucocyte and red erythrocyte. Conducting the second centrifugation with the speed of 3500 rounds/min during 5 minutes to fractionate PRP. The collected PRP is activated by calcium chloride within 15 minutes. Removing platelet-poor plasma. The final product contains 4 - 5 ml of hyaline yellow solution and ensures sterile. The all activated PRP solution is absorbed in the sterile cylinder to inject into the wound. The time from blood collection to the collection of activated PRP is about 30 minutes.

Conducting a PRP injection on the wound site

Preparation at the chronic wound site: conducting PRP injection when the ulcer is stable, there is no acute inflammation with the denotation of swelling, heat, redness, pain and no necrotic tissue at the wound site [16].

Patients are changed the bandage on a wound immediately before injecting PRP according to the standard procedure, ensuring sterile principle. Cleaning the wound with Betadine 3% solution.

The injection technique is conducted at the dressing room, immediately after collecting the activated PRP (within about 15 first minutes after collecting the activated PRP).

The PRP product is directly injected into the peripheral skin area which is about 1 cm from the wound edge at positions corresponding to 3h, 6h, 9h, and 12h. Each site is injected with an amount of 1 ml and is 4 mm deep. The injection technique is similar to local anesthesia injection(Mehrannia M. [17]). After injection, covering with Betadine 3% bandage, sterile dry bandage and bandaging closely. Changing the bandage according to the daily standard with Betadine 3%.

Conducting the second injection and duration between two times is 1 week. The injection technique is similar to the first injection. After injection, the patients continue to be changed the bandage until the wound is healed or are used combination therapy such as skin graft surgery or flap transfer surgery (1 week after the second injection).

Whole body treatment: During the course of treatment, the patient may still be able to use general systemic medication via taking a medicine, body injection, supporting the body

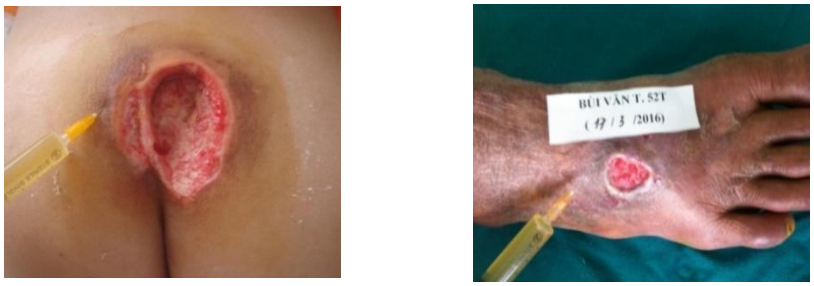


Image 2.1. Illustration images of PRP injection into the ulcer

Research criteria

Some criteria of the patient's whole body

Age, gender, foundation pathology of patients; location, number, existence time and depth of ulcer.

Systemic manifestation: pulse, blood pressure, temperature before and after PRP injection, daily temperature during treatment and number of treatment days;

Paraclinical tests: hematology test, routine serum biochemistry test

Some research criteria at the ulcer site

- Clinical features at the wound site: Area of the ulcers (cm^2). The features of the ulcer edge: Palate, segmentaire, epithelialization. The features of the ulcer foundation: caseation, directly exposing tendon, muscle and bone, pseudomembranes and granulation tissue. Unwanted effects after injection. Taking a photo of the wound.

- Bacterial test of the wound: Taking succus of the wound exudate and determining the type of bacteria according to routine techniques of microbiology laboratory .

Evaluation time: before injection (T_0), after 7 days (T_1), after 14 days (T_2).

- On-site treatment method combined after PRP injection*:* change the bandage, flap transfer surgery, skin graft surgery and result of surgery

- Data processing: processed by Statistical Software SPSS 20.0. The difference has significant meaning when $p < 0.05$.

RESEARCH RESULTS

Some characteristics of the patients and ulcers

Table 3.1. Distribution of patients by age and gender

Criteria		Amount of patient	Rate (%)
Age:	20 – 60	18	69.2
	61 – 80	8	30.8
Average age		42.5 (20 – 80)	
Sex:	Male	20	76.9
	Female	6	23.1

Table 3.2. Combination pathology of patients

Combination pathology	Amount of patients	Rate (%)
Traumatic spinal column and spinal cord injuries (causing the Paralysis of the lower part of the body**)**	11	42.3
Cerebral traumatic brain injury, cerebral stroke, and cerebrospinal meningitis	6	23.1
Hypertension	2	7.7
Peripheral vascular disease	2	7.7
Gout	1	3.8
Pyoderma gangrenosum	1	3.8
Heel spur	1	3.8
Femoral *neck fracture*	1	3.8
Tetanus	1	3.8
Total	**26**	**100%**

Comment: 100% of patients have combination pathology. In which, patients with traumatic spinal column and spinal cord injuries, cerebral traumatic brain injury, cerebral stroke, and cerebrospinal meningitis account for high rate.

Table 3.3. Some clinical characteristics of ulcer

Criteria		Amount of patient	Rate (%)
Amount of ulcer: 1 ulcer 2 - 3 ulcers 4 - 6 ulcers		 20 4 2	 76.9 15.4 7.7
Average amount of ulcer per patient:		1.5 ± 1,1 (1- 6)	
Stage of wound*:*	stage II, III stage III, IV	2 24	15.4 84.6
Wound area: 40 - 60 cm^2 60 - 100cm^2		 24 2	 92.3 7.7
Average area of wound		32.5 ± 22.3 cm^2	
Existence time of ulcer From 4 to 6 weeks From 6 to 50 weeks		 15 11	 57.7 42.3
Average existence time of ulcer		9.3 weeks (from 6 to 50 weeks)	
Ulcer site:	Sacrum, Coccyx Trochanter Foot Heel Femoral	20 2 2 1 1	76.8 7.7 7.7 3.8 3.8

***Comment*:** Patients with one ulcer occupy a high rate. The study is mainly conducted in patients with stage-IV ulcer: 24/26 patients (in which, there are 2 patients with stage III combining with stage IV). The ulcers in the sacrum and coccyx area occupy the highest rate: 20/26 patients.

Manifestation at the ulcer site after injection of the autologous PRP
Clinical manifestation
- Manifestation at the injection site
The patients are well-adapt with the therapy and do not have bleeding, infection, or complications associated with the therapy, including swelling, heat, redness at the injection site immediately after PRP injection and during the treatment process.

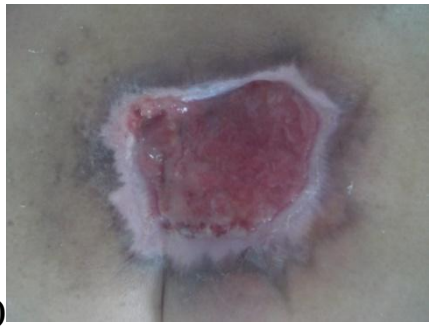
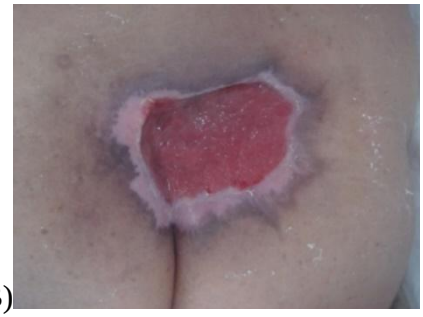
Table 3.4. Clinical manifestation at the ulcer site

Features of the wound	**Time**			p
	T0	**T1**	**T2**	
Directly exposing tendon, muscle and bone	16 wounds	5 wounds	3 wounds	p ($T_{1,2-0}$)<0.05
Pseudomembranes	17 wounds	10 wounds	10 wounds	p($T_{1,2-0}$)<0.05
Epithelialization at the wound edge	7 wounds	21 wounds	24 wounds	p($T_{1,2-0}$)<0.05
Feature of granulation tissue: - There is no granulation tissue - Unhealthy granulation tissue - Not healthy granulation tissue yet - Healthy granulation tissue	11 (42.3%) 9 *(34.6%)* 6 *(23.1%)* 0	0 9 *(34.6%)* 10 *(38.5%)* 7 *(26.9%)*	2 *(7.7%)* 11 *(42.3%)* 13 *(50%)*	$p_{(T2/T0)}$<0.05
Level of discharge: - Many - Medium - Little	13 (50%) 13 (50%) 0 (0%)	*0* *17(65.4%)* *9 (34.6%)*	*0* *5 (19.2%)* *21 (80.8%)*	$p_{(T2/T0)}$<0.05
Allergy	No	No	No	
Inflammation, edema and congestion of healthy skin around the wound	No	No	No	
Necrosis	No	No	No	
Secondary necrosis	No	No	No	
Wound area (cm^2)	31.1 ± 22.5	25.3 ± 21.7	20.8 ± 19.4	$p_{(T2/T0)}$<0,05

Comment:
The wound status with the pseudomembranes and directly exposing tendon, muscles and bone significantly reduces after 1 week (p<0.05).

Before the injection, the wound foundation does not have healthy granulation tissue and after the injection, the wound has granulation tissue covering tendon, muscle and bone. After 2 weeks: 100% of the wounds have granulation tissue, number of the wounds having healthy granulation tissue increases significantly (p <0.05).

After 1 week, number of the wounds having epithelialization at the wound edge increases significantly and after 2 weeks, 24/26 wounds have this phenomenon (p<0.05).
The wound area after 2 weeks is significantly narrowed, p <0.05.

(A)  (B) 

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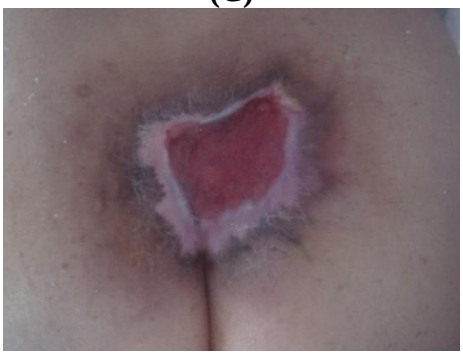
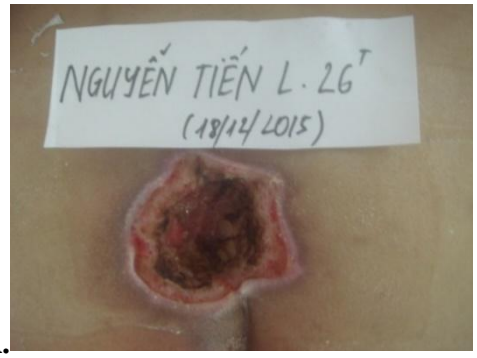
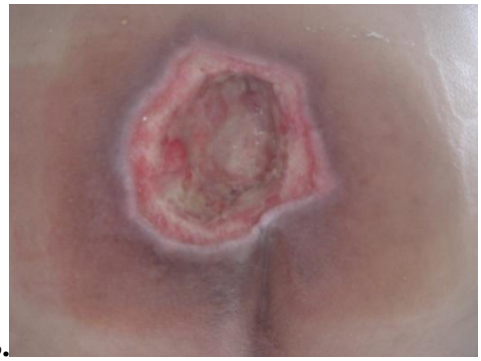
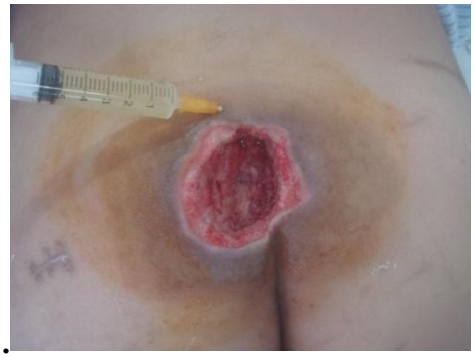


Image 3.1. Image of stage-IV ulcer (The patient of Vu Thi X, 72 years old, case-record number: 9160). Case A: the ulcer at time of T0: a large amount of secreted fluid, blue pus, having pseudomembranes. B: the ulcer at time of T1. C: the ulcer at time of T2: The wound is clean and does not have pseudomembranes, the wound area is narrowed, the granulation tissue of the wound is redder and more flat.

A. 

B. 

C. 

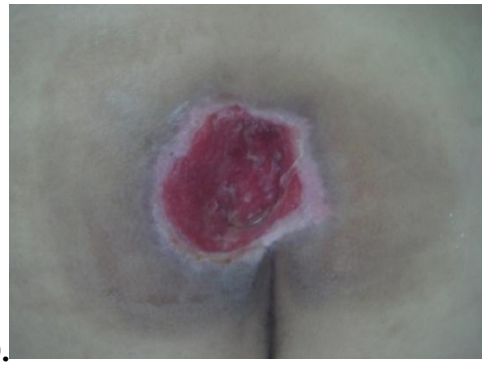
D. 

Image 3.2. Image of stage-IV ulcer (The patient of Nguyen Tien Lanh, 26 years old, case-record number: 9582). Case A: the ulcer when the patient is hospitalized: there is necrosis. B: the ulcer in the period of 1 week after treatment does not have necrosis and still has the pseudomembranes. C: the image of PRP injection, the ulcer at time of T0 occurs the

granulation tissue. D: the ulcer at time of T2: the granulation tissue is red, healthy and the edge of the epithelium is clear.

Bacterial manifestation at the wound surface

Table 3.5. Type of bacteria at the wound surface

Time / Type	T0 (n=26)	T1 (n=26)	T2 (n=26)	Total (%)
P.aerugenosa	6	3	4	13 (16,7%)
S.aureus	3	2	2	7 (8,9%)
K.pneumoniae	2	1	1	4 (5,1%)
Aci. baumannii	1	1	1	3 (3,8%)
S.epidermidis	2	0	0	2 (2,6%)
E.coli	2	0	0	2 (2,6%)
S.hemolyticus	1	0	0	1 (1,3%)
E.aerogenes	1	0	0	1 (1,3%)
Ent.faecalis	1	0	0	1 (1,3%)
Total number of bacteria occurrence samples	19	7	8	34 (43,6%)
Total number of samples which do not have bacteria occurrence	7	19	18	44 (56,4%)

Comment: Types of bacteria at the wound site are various, but mainly are P.aeruginosa, S.aureus. In the 2nd and 3rd injections, the number of the wounds which do not have bacteria occurrence increase significantly (p>0.05).

Evaluating safety of the PRP therapy

Table 3.6. Signs of existence before and after PRP injection (average value)

Injection time	Temperature (^{0}C)		p	Pulse (cycle /1 min)		p	Blood pressure (mmHg)		p
	before	after		before	after		before	after	
1st	36.9	37	*p>0,05*	85	86	*p>0.05*	116/69	117/71	*p>0.05*
2nd	36.9	37.1	*p>0,05*	85	86	*p>0.05*	121/71	121/72	*p>0.05*

Comment: Indicators of pulse, temperature and blood pressure of patients do not significantly change in the period of 24 hours before or after the injection. There are not the patients who occur systemic allergic reactions.

Table 3.7. Change of some hematological criterias

Criteria	Time			p
	T0	T1	T2	
Erythrocyte (T/L)	3,9 (2,9 ÷ 4,9)	3,9 (2,9 ÷ 5,3)	4,1 (3,0 ÷ 5,4)	$p_{T1,2/T0}>0,05$
HST (g/L)	112 (90 ÷ 139)	114 (90 ÷ 148)	115 (94 ÷ 146)	$p_{T1,2/T0}>0,05$
Hematocrit (L/L)	0,3 (0,2 ÷ 0,4)	0,3 (0,2 ÷ 0,4)	0,3 (0,2 ÷ 0,4)	$p_{T1,2/T0}>0,05$
Leucocyte (G/L)	7,8 (3,3 ÷ 13,9)	6,9 (3,4 ÷ 11,3)	8,3 (4,0 ÷ 16,8)	$p_{T1,2/T0}>0,05$
Platelet (G/L)	303 (70 ÷ 609)	283 (97 ÷ 498)	276 (173 ÷ 396)	$p_{T1,2/T0}>0,05$

Comment: Indicators of erythrocyte, Hb, hematocrit, leucocyte, and platelet are different and do not have significant statistics meaning (p> 0.05).

Table 3.8. Change of some biochemical criterias

Criteria	Time			p
	T0	T1	T2	
Protein (g/L)	65 (55 ÷ 77)	65 (54 ÷ 77)	65 (58 ÷ 74)	$p_{T2,T3/T1}>0,05$
Albumin (g/L)	34 (26÷ 44)	35 (25÷ 46)	37 (26÷ 48)	$p_{T3/T1} = 0,011$
Glucose (mmol/L)	6,1 (4,4 ÷ 9,5)	6,0 (3,2 ÷ 12,2)	5,1 (2,5 ÷ 7,8)	$p_{T3/T1} = 0,001$
Ure (mmol/L)	4,3 (3,2 ÷ 6,1)	4,3 (1,8 ÷ 6,4)	4,5 (1,2 ÷ 7,6)	$p_{T2,T3/T1}>0,05$
Creatinin (µmol/L)	78 (43 ÷ 144)	79 (48 ÷ 129)	72 (31 ÷ 105)	$p_{T2,T3/T1}>0,05$
SGOT (U/l)	30 (17,3 ÷ 63,8)	29 (16,7 ÷ 58)	29 (18 ÷ 67)	$p_{T2,T3/T1}>0,05$
SGPT (U/l)	30 (8,8 ÷ 72)	32 (8,7 ÷ 79)	31 (13,1 ÷ 132)	$p_{T2,T3/T1}>0,05$

Comment: Albumin content increases and glucose content decreases with p <0.05, but they remain within normal limit. Other indicators are within normal limits and do not have the difference between the periods.

Treatment result

Table 3.9. Treatment method after PRP injection

Patient	Treatment method			
	Change the brandage	Flap transfer		Skin graft
		Healing in the first phase	Healing in the second phase	
Amount of patient	3	18	2	3
Rate %	11.5	69.2	7.7	11.5

Comment: 100% of the wounds are completely healed.

There are three patients (in which, two ulcers are caused by peripheral vascular disease *and* one ulcer is caused by pyoderma gangrenosum) with a small area, so after PRP treatment, they are continued to change the bandage until healed.

23/26 ulcers are used the surgery method (20 patients are transferred flap and three patients are grafted skin), the patients who are completely healed in the first phase occupy the high rate with the number of 21/23 patients. Two patients who are completely healed in the second phase have pressure ulcer in the sacrum and coccyx areas on the *cervical spine injury*. After the surgery, the patients are not immobilized in a favorable position for the flap leading to the tense flap, the malnutrition of a flap part. One patient has to take extra care and sutures the wound, one patient must be conducted the second surgery.
The mean number of days of treatment is 33.3 ± 10.7 (from 21 to 65 days).

DISCUSSION

Some characteristics of chronic wound relating to PRP

Study has shown that 100% of patients having a combined pathology, mainly traumatic spinal column and spinal cord injuries, cerebral traumatic brain injury, cerebral stroke, and cerebrospinal meningitis. This is the group of patients lossing, reducing the capacity of movement, feeling in a part of the body, so this group has a higest risk of pressure ulcers. This is also consistent with records of most chronic wounds that are ulcerated relating to ischemia, venous stasis, or pressure ulcers [1, 2, 14, 18]. In America, 3–5% of all hospitalized patients have spinal cord injuries which suffer from ulcers. National cost for ulcer pressure is $1.3 billion per year. (Diegelmann and Evans, 2004 [18]).

A chronic wound is a wound whose regenerative process is disordered. Duration for a wound to become a chronic wound is determined from 4 weeks to more than 3 months [11, 12, 13, 14].

Patients in the study mainly suffer from pressure ulcers, among which there are a number of patients who have not only 1 but also 2, 3 or even 6 ulcers. In particular, ulcer at the same sacrum accounted for the highest rate with 20/26 patients (76.9%). All studied ulcers are ulcers at level III, IV, with prolonged existence, 9.3 weeks in average (the longest duration is 50 weeks - over 1 year). Patients have experienced many treatment places, applying many treatment methods in clinics at different levels but they still do not recover. These characteristics require that patients should be applied many methods, especially new ones in the hope of wound healing.

The effect of PRP on chronic ulcer

Reducing inflammation

Chronic wound is a wound that does not heal in a physiological process and prolonged time of the wound. Depending on the area, depth, body's resistance, the wound basically develops in the following stages (continuous, overlapping, influencing each other): inflammation; proliferation, maturation. The inflammatory response stage and the transition from inflammatory response to granulation tissue rebuilding play an important role in the ulcer healing. If the inflammation stage is missed or prolonged, wound healing process will be stagnated. As prolonged inflammation produces more inflammatory cells, slowing down the response of GF, thin layers of fibrin are formed limiting development of granulation tissue. (Hart, 2002, [19]). Studied patients have prolonged chronic wounds with following signs and symptoms: the edge of the wound has multiple sclerosis, the wound has multiple

pseudomembrances, a large amount of fluid oozes from the wound multiple discharge. After applying PRP, within 1 - 2 weeks, symptom of inflammation is markedly reduced. The amount of oozed fluid decreases, the amount of wound with less oozed fluid increases significantly, there are no more wounds with large amount of oozed fluid. The amount of pseudomembranous wound also decreases ($p < 0.05$).

Effects of anti-inflammation and pain reliever relate to growth factors in PRP such as HGF, PDGF, IGF, TGF - β, EGF, FGF và [5, 20, 21]; soluble factor produced from SVF or ADSCs such as HGF, VEGF, NGF, EGF, FGF, and TGF- β [22, 23, 24].

PRP can also limit inflammation by suppressing cytokine release in the wound and improve the regeneration process by promoting blood vessel formation and tissue regeneration thanks to the presence of an amount of white blood cell [17, 25]. According to Manish Suthar et al (2017, [26]), effects of anti-inflammation and pain reliever in the wound was noted within 1 week of treatment and this may result from the anti-inflammatory effect of PRP, including white blood cell.

Effect of stimulating epithelialization - replicate and healing the wound of PRP

Chronic wounds have basic characteristics as disorders of reproductive regeneration process The degenerated recurrent vascular becomes older and decrease, and endothelial cells decrease in cell division. Epithelial cells around the wound decrease or stop cell dividing process, not growing into the center of the wound The intercellular tissue components produced by fibroblasts are laminin, decorin, and fibronectin, which are decreased that the epithelial cells can not adhere and slide on them to migrate to the wound, carrying out the epithelialization to cover the injury Injured intercellular tissue structure is unhealable, wound becomes fibrous. The fibroblasts are weak or inefficient to perform function fully so the epithelial cells can not divide or migrate into wound [27]. Chronic wounds is often difficult to cure, has a substantial cost and creates a burden for patients and society in general [1, 2] The main objective of any treatment is to heal the wound quickly. Common treatments include the removal of necrotic tissue, infection control, re-circulation of anemic tissues, and avoidance of excessive pressure on wound [25].

Our research indicates that the wound before using PRP is in the absence of granulation tissue, multiple pseudomembranes, exposing the tendon muscles. The margin of wound before the study also has no epithelial symptom, boundary between edge and skin is clear. After applying the PRP treatment, the wound is gradually healed with more healthy granulation tissue, 100% of the wound has granulation tissue After 2 weeks, the number of healthy granulation tissue increased remarkably. Granulation tissue is formed, gradually covering the tendons, muscles (the number of wound which reveal muscle and tendon increased remarkably compared to before the treatment). Epithelialization significantly from the edge, which contributed to significant reduction in area ($p < 0.05$). Histopathology also show relevant results with clinical: NBS concentration and proliferation, neovascular focus in wound significantly increases.

PRP with high concentrations of GFs is capable of repairing tissue. In 1986, the first clinical study by Knighton et. al. recorded the usage of auto platelet factors that accelerated epithelial growth, which led to the healing of chronic skin ulcers. PRP contains a variety of cytokines and GFs (especially PDGFs, VEGFs, TGF-β, IGFs, FGFs that attract progenitor cells to stimulate proliferative and differentiation activities, stimulate to heal wound via autocrine and paracrine mechanism [6, 15]. PDGF, TGF - β, VEGF, EGF and IGF are GFs that play an

important roles in all stages of healing the wound. They stimulate the cells to migrate, develop and form new neovascular. PDGF increases the evacuation of macrophages, monocytes and fibroblasts; stimulating to increase fibroblasts that helps promote collagen synthesis and proteoglycan. TGFα - Create mitogenic and Positive chemotaxis for kinatinocytes and fibroblasts. TGFβ1 and TGFβ2 stimulate angiogenesis, increase collagen production, inhibit degradation, stimulate positive chemotaxis and activation of inflammatory monocytes cells, macrophages, fibroblasts leading to extracellular matrix and collagen formation. . VEGF - stimulates angiogenesis in hypoxia. FGF - stimulates angiogenesis, granulation and epithelialization via endothelial cells, fibroblast and keratinocyte migration. IGF-1 has two important functions: Positive chemotaxis of endothelial cell of vascular into wound to proliferation, differentiation of several cell lines including chondroblasts, osteoblasts, myoblasts, and hematopoietic cells [5, 6, 20, 26, 29, 30].

The GFs released from PRP are important in the modification of dependant cell selection, in proliferation and extracellular matrix synthesis to heal the wound [31]. PDAF is a polypeptide which is capable of stimulating new capillary development by stimulating the movement of endothelial cells. PDECGF is responsible for neutrophil leukocyte concentration in the wound, stimulating multiple cell division, including epithelial and fibroblast cells. More recently, platelet factors affecting angiogenesis and cardiovascular reproducing processes, which have stimulated granulation. [32]

The activated platelets, in addition to the GF, also secrete a large number of coagulation factors, such as fibrinogen, serotonin, fibronectin, factor V, factor VIII, and calcium (factor IV). This produces antiplatelet, which results in stable platelet by fibrin and glycoprotein cross-linking. Fibrin promotes the penetration of monocytes white blood cells, fibroblasts and other progenitor cells that play an important role in ulceration [7, 8, 15].

PRP is used in a variety of forms, such as liquid, gels, or by injection into wound for various causes (diabetic ulcer, pressure or venous ulcer, postoperative wound or trauma). In 2010, authors Conde-Montero E, de la Cueva Dobao P, Martínez González JM [30] reviewed the study using PRP as adjuvant therapy for chronic wounds recently: 10 uncontrolled studies (seven for prospective, one for clinical trial), 54 retrospective studies and a series of observations using a multi-center registry database. Sample sizes range from 11 to 285 wounds. Both the time and the initial area of injury are uneven (1-17 months). Weekly treatment is the most frequent. The number of sessions varies greatly from 1 week to 14 months. The most recorded result is the wound being healed immediately. PRP therapy combined with pain relief showed a significant reduction in the number of intravenous sedation medications. The ineffective cases related to the poorly prepared of wound base (necrosis, infection ...), related to the high concentration of protease in wound exudate. No adverse symptoms associated with PRP. Narrowing of the lesion area averaged 50% in most studies at week 4. The median duration varies from 4 to 10 weeks. PRP has shown promising results when combined with other therapeutic tests, such as fat-derived stem cells. PRP represents a viable alternative to chronic ulcers, of which effectiveness has been demonstrated both in blood collection tubes and in the body.

The effect of reducing infection at the wound

Infections are a common condition in chronic wounds, which decreasing the healing process of wound. Inflammation is a normal part of the wound healing process and is very important for the removal of infectious microorganisms. However, without effective decontamination, inflammation may be long lasting. Both bacteria and endotoxin can lead to prolonged

inflammatory cytokines such as IL-1 and TNF-α, resulting in prolonged inflammation. If continued, wound is easily become chronic and cannot be healed. This prolonged inflammation also increases MMPs (a protease family that can cause ECM depletion) but causes a reduction in protease inhibitors. This change may cause rapid decomposition of GFs that develop in chronic wound [2, 33, 34]. Clinical manifestations of infectious wound infection are inflammation of the wound and the egde of the normal skin, exudate and pus. According to Edwards, Harding (2004, [33]; Davis (2008, [35]): S. aureus, P. aeruginosa, and β-hemolytic Streptococci are clinically most common bacteria in chronic wounds. S. aureus, P. aeruginosa plays an important role in wound infections. Many chronic ulcers may be healed by the presence of biofilm containing P. aeruginosa, which shields the bacteria from the effects of polynucleotides (PMNs) or antibiotics [36].

In the study, bacteriological results prior to treatment with PRP at the wounds indicated the degree of bacterial abundance at chronic wounds. Bacteria is mainly *P. aeruginosa, S. aureus, K. pneumoniae.*

Treatment of wound infection in the wound area is a determining factor for successful wound healing. In the study, the wounds prior to the PRP treatment showed multiple exudate, multiple pseudomembranes. After the PRP treatment, the degree of secretion of the ulcers tends to decrease. This result also corresponds to bacterial evolution at the wound. After 1 to 2 weeks of PRP treatment, the number of bacterial species was significantly reduced. Some rare bacteria in transplant 1 such as *Ent.faecalis, S.hemolyticus, E.coli, S.hemolyticus* did not appear in the second or third transplant.

Drago acknowledged PRP's ability to inhibit the growth of *Enterococcus faecalis, Candida albicans, Streptococcus agalactiae, and Streptococcus oralis* [37]. PRP is resistant to *S. aureus* and *E. coli* [38, 39].

PRP has antimicrobial ability because it contains many leukocytes, neutrophils, monocytes and lymphocytes. Neutrophils, monocytes are rich in granules containing myeloperoxidase, catalyzing the oxidation of chloride to produce hypochlorous acid and other toxic oxygen derivatives for microorganisms and fungi.

Treatment results

In our study, 100% of ulcers completely recovered with the average number of days of treatment from 33.3 ± 10.7 days (4.7 weeks). As such, PRP therapy has contributed to stimulating wound healing, facilitating the success of surgical treatment of the wound. This is a remarkable achievement of PRP therapy.

The results from our series are consistent with previous studies on the duration of treatment.

Sarvajnamurthy [41] studied 17 patients with venous ulcers of the lower limb who received successful PRP treatment with an average duration of 5.1 weeks. In another clinical study by Pham Van Phuc, six diabetic foot ulcers patients treated with PRP, resulting in completely recovery after an average of seven weeks [24].

According to Tsvetan Sokolov [42], PRP is a convenient, inexpensive treatment for pressure ulcers, which is the alternative to traditional methods (especially when traditional therapy is not effective enough and surgery is not possible). It reduces the time, treatment cost and time to stay hospital. Obolenskiy [15] evaluated the effects of PRP on 44 patients with chronic

ulcers (37 patients who were treated) who reported that PRP usage is safe, clinically effective, and cost-effective.

Manish Suthar [26] studied on 24 patients with an average age of 62.5 ± 13.53 years, with 1 patient with wound /ulcer due to different causes treated with a PRP injection around the wound The duration of chronic wounds ranged from 9 to 24 weeks with an average of 16 weeks. All patients' wounds were healed and the average duration of treatment was approximately 8.2 weeks ± 1.9.

PRP itself directly promotes the healing of lower limb ulcers due to diabetes, indications in intravenous and arterial ulcers, pressure ulcers, burn lesions, at the skin area, scars [43]. Driver (2006 - Multi-center trial report in the United States [44]), Marcus Gurgen (2010) [45] found that PRP had analgesic effect, shortened treatment time and lowered ulcer healing. Chronic failure from 4 weeks to 8 years, has failed with conventional methods (due to diabetes, vascular events, infections, traumas, nerves and vasculitis). Rebecca L. Knox used PRP for the treatment of ulcer over the past year, despite the use of a variety of therapies such as HBO, laser ... The authors found that, after treatment, the area and depth of the ulcer decreased, Clean wound, facilitating skin grafting. [46]

PRP safety in the treatment of chronic ulcers

PRP was first used in 1987 by Ferrari and has been studied extensively in various fields during the last two decades. Animal and human studies have demonstrated the safety and effectiveness of PRP for 20 years.

In the study, we conducted PRP self-extraction in accordance with the procedure, according to the sterile principle (duration of about 25-30 minutes). Subsequently, PRP injections were administered at the same time, at the changing room According to the Marx studies [8], Obolenskiy [15], after activation, platelets release about 70% of the growth factor retained within the first 10 minutes. Release of GF from platelets completed within 1 hour. Many growth factors have short half-life. Therefore, platelets should be activated immediately before using PRP.

With the procedure appied for over 26 patients, we found PRP safe Patients did not change systemic parameters such as temperature, pulse and blood pressure immediately before and after treatment with PRP ($p < 0.05$). PRP is safe in the treatment of patients with chronic ulcers due to self-blood sampling, low amount of blood and sterile separation procedures so that there is no risk of blood transfusions, do not affect all body status, allergic reactions or at the injection site.

During the study, no cases of allergic reactions, no inflammation of the skin, skin healing wound or secondary necrosis at the wound. We did not see any patients with bleeding, infection, or side-effects related to the procedure. In addition, there were no adverse effects on the day of treatment and during follow-up time of patient. The PRP procedure is relatively simple, does not require expensive equipment, then injected back to the patient, thus eliminating the risk of infection contagious, has created peace of mind for patients.

The hematological and biochemical indexes across the study periods are within normal limits. The albumin index increased significantly as statistics ($p < 0.05$) and blood glucose decreased ($p < 0.05$). This is a signal that the patient is progressing well, stable blood glucose after treatment. .

Manish Suthar et al (2017, [26]), Javier Ramos-Torrecillas [29] reported a PRP injection for safe chronic wound healing, considered it as a safe and effective treatment for those who did not have chronic ulcer.

Lacci [47], Mehta [48] noted that PRP as a self-medication should be a safe treatment compared to allogenic preparations and hasvno concern for infectious disease, has no risk of graft versus transplantation and lead to better acceptance of the patient. Singh [31] did not find systemic and on-site side effects after PRP on 49 patients with chronic wound. Tsvetan Sokolov [38], Steven Sampson [49] noted that PRP itself reduces the risk of blood-borne diseases. No studies have shown PRP to stimulate hyperplasia, carcinogenicity or cancer. Development factors affect the cell membrane rather than the cell nucleus and only activate the normal gene [30, 50].

CONCLUSION

PRP is effective in treating chronic wound healing at skin site, reducing exudate, reducing inflammation, antibacterial, stimulating regeneration-modulating and narrowing the wound. PRP therapy is safe in treating patients with chronic ulcer.

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