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Original Research Article

A Study on Platelet Rich Plasma for the Treatment of Non Healing Leg Ulcers

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Abstract: Platelet-rich plasma (PRP) is an autologous concentration of human platelets contained in a small volume of plasma characterized by haemostatic and tissue repairing effects. Tissue repairing effects and being enriched by various kind of growth factors, has made them the focus of attention for different procedures. The efficacy of certain growth factors in healing of non-healing leg ulcers and the concentration of these growth factors found within PRP are the theoretical basis of healing of leg ulcers. To study the efficacy of PRP for the treatment of non-healing leg ulcers, we selected ten patients of non healing leg ulcers for the study. A total of 10 patients with 14 ulcers were treated with PRP. Specialized investigations including X-ray of leg/foot, color doppler study were done where ever required. The nonhealing ulcers were first debrided to remove the infected and necrotic tissues. Size of the ulcers was noted prior to the start of the treatment. The duration of ulcer ranged from 1 month to 6 months with a mean of 15.4 weeks. The mean baseline platelet count of the patients was 2.4 lac per cubic mm and mean platelet count of the patients after PRP was 5.2 lac per cubic mm. The mean duration of the healing of the ulcers was in 7.2 weeks (SD 3.1). The baseline mean area of the ulcer was 12.24 cm^2. The final area of the ulcer at the end of 8 weeks was 2.01 cm^2. The mean percentage improvement in the area of the ulcer was 83.57%. As the use of PRP increases, additional studies may establish PRP as an efficacious treatment modality.
Keywords: Platelet-rich plasma (PRP); leg ulcers; autologous; centrifuge; non healing; growth factors.

INTRODUCTION

Chronic ulceration of the lower leg is a frequent condition whose incidence is rising as a result of the ageing population and increased risk factors for atherosclerotic occlusion such as smoking, obesity and diabetes [1-4]. The current use of health care resources for the treatment of vascular ulcers is very high, both because complete closure of the wound is very difficult to achieve and because the ulcers tend to recur. Ulcers can be defined as wounds with a full thickness depth and a slow healing tendency [5]. In general, the slow healing tendency is not simply explained by depth and size, but caused by an underlying pathogenetic factor that needs to be removed to induce healing. The main causes are venous valve insufficiency, lower extremity arterial disease and diabetes [6-9]. Less frequent conditions are infection, vasculitis, skin malignancies and ulcerating skin diseases such as pyoderma gangrenosum. But even rarer conditions exist, such as the recently discovered combination of vasculitis and hypercoagulability. For a proper treatment of patients with leg ulcers, it is important to be aware of the large differential diagnosis of leg ulceration. Although most leg ulcers are caused by venous insufficiency (approximately 45–60%), arterial insufficiency (10–20%), diabetes (15–25%) or combinations of these well-known aetiological factors (10–15%), 4,5 rare underlying disorders may exist [10-13]. For a rational approach towards patients with leg ulcers, it is important to have detailed knowledge of the clinical picture, pathogenesis, diagnostic possibilities [14-16].

Venous ulceration is caused by increased pressure in the venous system. The main cause of venous hypertension is insufficiency of the valves in the deep venous system and the lower perforating veins [17]. These veins and good functioning of their valves are necessary for the return of venous blood to the heart at each contraction of the calf muscles. Intact valves but absent muscle contraction (immobility, paresis) may also cause oedema and ulceration, a condition known as dependency syndrome [18, 19]. Valve insufficiency may be acquired as in post-thrombotic syndrome or caused by congenital weakness of valves or vessels. The exact pathogenetic cascade leading from valve insufficiency to ulceration is still not full elucidated. The clinical symptoms of venous insufficiency are oedema, ipodermatosclerosis, hyperpigmentation, hyperkeratosis, and atrophie blanche preceding ulceration.

Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline [20-22]. PRP also has been referred to as platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel, and platelet releasate. Platelet releasates have been used to treat wounds since 1985. PRP serves as a growth factor agonist and has both mitogenic and chemotactic properties. It contains a high level of platelets and a full complement of clotting and growth factor PRP functions as a tissue sealant and drug delivery system with the platelets initiating wound repair by releasing locally acting growth factors via α-granules degranulation [23-25]. The secretory proteins contained in the α-granules of platelets include platelet-derived growth factor, transforming growth factor-β, platelet factor 4, interleukin-1, platelet-derived angiogenesis factor, vascular endothelial growth factor, epidermal growth factor, platelet-derived endothelial growth factor, epithelial cell growth factor, insulin-like growth factor , osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin , and thrombospondin-1. These growth factors aid healing by attracting un-differentiated cells in the newly formed matrix and triggering cell division. PRP may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration, promote new capillary growth, and accelerate epithelialization in chronic wounds [26, 27].

For the preparation of PRP, whole blood from the patient is first centrifuged to separate the plasma from packed red blood cells and then further centrifuged to separate PRP from platelet-poor plasma. This concentrate is then activated with the addition of thrombin or calcium, resulting in a gelatinous platelet gel. Clinically valuable PRP contains at least one million platelets per microliter. Lesser concentrations cannot be relied on to enhance wound.

AIMS

To study the efficacy of PRP for the treatment of non-healing leg ulcers.

MATERIAL AND METHODS

We selected ten patients of non healing leg ulcers for the study. It was a randomised controlled study. The patients were selected from the dermatology OPD and surgical OPD. Written informed consent was taken from all the patients before the start of the study. Prior approval of hospital ethical committee was also taken before the start of the study. Detailed history including name, age, sex, address, contact number, marital status, occupation, history of medication will be noted. Patients were thoroughly examined and ulcer size (length, breadth & width). All routine investigations of the patients were done before the start of the treatment including complete haemogram, bleeding time & clotting time, baseline platelet count and fasting blood sugar. Specialized investigations including x-ray of leg/foot, color doppler study were done where ever required. The non-healing ulcers were first debrided to remove the infected and necrotic tissues. Size of the ulcers was noted prior to the start of the treatment.

The Following Patients Were Included In Our Study:

- Ulcers of Less Than 6 Weeks Duration
- Patients Willing For the Procedure
- Patients above The Age Of 18 Years

The Following Patients Were excluded from our study:

- Patients with heart disease
- Patients with hypercholestraemia
- Patients with unrealistic expectations
- Un cooperative patients

Under aseptic precautions 20 ml of venous blood was drawn and added to a test tube containing acid citrate dextrose in a ratio of 9:1 (blood: Acid citrate dextrose), centrifuged at 5000 rpm for 15 min to separate the red blood cells from the platelets and plasma. Then the supernatant and the buffy coat composed of platelets and plasma was collected and centrifuged again at 2000 rpm for 5-10 min. The bottom layer about 1.5 ml was taken and 10% calcium chloride was added (0.3 ml for 1 ml of PRP). The activated PRP was applied onto the wound after proper surgical debridement and was dressed with a non-absorbent dressing (paraffin gauze). After the application of PRP, repeat platelet count of the plasma was done to see whether the platelet count has increased and how many times it has increased. After the procedure, the patients are instructed to refrain from any physical exercise or sunlight for 2-3 days. After 1 Week The Dressing Was Removed With Normal Saline And Assessed For The Improvement. The Platelet Rich Plasma Was Reapplied And Dressing Was Done. This Process Is Repeated Weekly For 8 Weeks. At every week the ulcer area and volume was calculated and photographs were taken. Wound area was calculated using the formula for an ellipse: Length × width × 0.7854 (an ellipse is closer to a wound shape than a square or rectangle that would be described by simple length × width). The wounds were covered with moist saline dressing. Daily dressing change without additional treatment was performed. At the end of 8th week the wound area was calculated by length×width×0.7854.photographs were taken before each application and at the end of 8 weeks (fig 1 and fig 1a). Data obtained was analyzed statistically using ‘z’ test.

RESULTS

The data was collected and tabulated and the results were analysed statistically.

Table-I: Showing Age Distribution of Patients

SR NO	AGE DISTRIBUTION	NUMBER	PERCENTAGE
1	0 - 10	-	-
2	11 - 20	-	-
3	21 - 30	2	20%
4	31 - 40	3	30%
5	41 - 50	4	40%
6	>50	1	50%

Table-II: Showing Sex Distribution of Patients

SR NO	SEX DISTRIBUTION	NUMBER	PERCENTAGE
1	MALES	9	90%
2	FEMALES	1	10%

Table-III: SHOWING AETIOLOGY OF ULCERS

SR NO	AETIOLOGY OF ULCERS	NUMBER	PERCENTAGE
1	DIABETIC ULCER	2	20%
2	VENOUS ULCER	1	10%
3	ARTERIAL ULCER	1	10%
4	IDIOPATHIC ULCER	5	50%

Table-IV: TABLE SHOWING PERCENTAGE IMPROVEMENT IN ULCERS

SR NO	NO OF WEEKS	MEAN PERCENTAGE IMPROVEMENT OF ULCERS
1	2	22%
2	4	43%
3	6	64%
4	8	88%

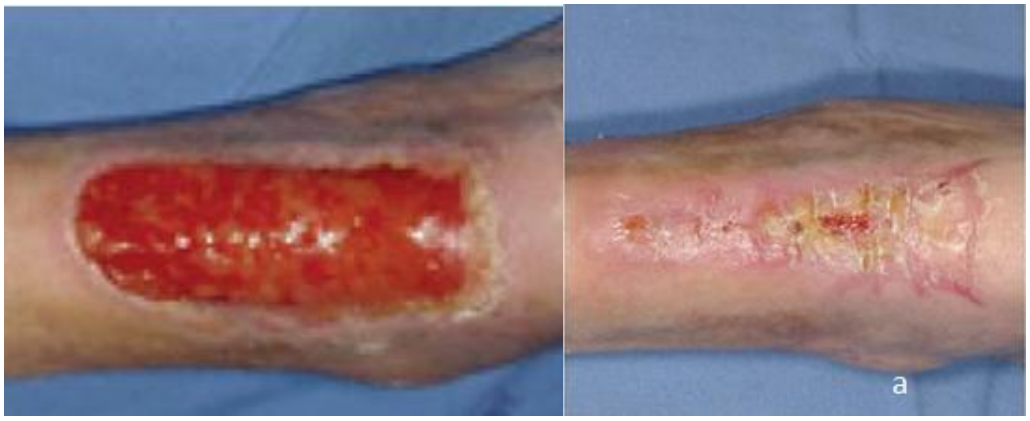

Fig-1&1a: Leg ulcers in a 42 years old patient before and after treatment with PRP

DISCUSSION

Regarding age distribution of patients, 20% patients were between 21- 30 years of age, 30% patients were between 31- 30 years of age, 40% patients were between 40- 50 years of age and 10% patients were more than 50 years of age. There were 9 males and 1female for the study. Male: female was 9:1.Regarding the aetiology of ulcers, diabetic ulcers were present in 20% patients, venous ulcers in 10% patients, arterial ulcers in 10% patients and idiopathic ulcers were present in 50% patients. A total of 10 patients with 14 ulcers were treated with PRP. The duration of ulcer ranged from 1 month to 6 months with a mean of 15.4 weeks. The mean baseline platelet count of the patients was 2.4 lac per cubic mm and mean platelet count of the patients after PRP was 5.2 lac per cubic mm. The mean duration of the healing of the ulcers was in 7.2 weeks (SD 3.1). The baseline mean area of the ulcer was 12.24 cm^2. The final area of the ulcer at the end of 8 weeks was 2.01 $cm^{2.}$ The mean percentage improvement in the area of the ulcer was 83.57%.Fibrinogen presents in the PRP polymerized into a fibrin gel, leading to the formation of platelet gel that adhered to the wound bed. No treatment associated adverse reactions were observed during the study.

Platelet rich plasma is a blood plasma that has been enriched with platelets. It is a concentrated source of autologous platelets. PRP contains several growth factors which help in the healing of leg ulcers [28]. Platelets collected in PRP are activated by the addition of thrombin and calcium chloride which induce the

release of these factors from the alpha granules contained in platelets. Chronic non healing ulcers are often difficult to heal because they lack the necessary growth factors to maintain the healing process. Conventional therapies such as dressings, surgical debridement and even skin grafting cannot provide satisfactory healing since these treatments are not able to provide necessary growth factors that can modulate healing processes. As an autologous preparation, PRP is safer to use than allogenic or homologous preparations and is free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever, and Creutzfeldt-Jakob disease. PRP requires no special considerations regarding antibody formation, effectively preventing the risk of graft vs. host disease and leading to better acceptance by patients. Chronic vascular ulcers are associated with a high use of resources. Conventional treatment consists of wound cleansing, necrotic tissue debridement, prevention, diagnosis, and, if necessary, treatment of infection and dressing application; although conventional treatment has limited effectiveness with wound healing (around 15-30%). Chronic nonhealing diabetic ulcers of lower extremity develop as a result of peripheral neuropathy, ischemia, and trauma. The goal of treatment is to obtain expeditious wound closure. The benefits of PRP in the treatment of severe and large ulcers have not been evaluated in randomized clinical trials [29, 30].

In a study on leg ulcers, 12 patients with 17 venous ulcers & 9 patients with 13 non venous leg ulcers all of them had failed to respond to atleast 4 weeks of conventional therapies [31]. Complete healing was achieved in 66.7% of the patients with venous ulcers in 7.1 weeks following an average of 2 applications of platelet rich fibrin matrix per patient.

In another study, it was seen that the use of autologous PRP was successful in healing a chronic lower extremity wound in a case study of a 57-year-old man with type 2 diabetes and a wound of six months duration [32]. Although this study is limited as a case study involving a single patient, it suggested that PRP can be successful in healing wounds that have failed to heal by other treatment techniques. In another study by Driver et al, a prospective, randomized, controlled multicenter trial in the United States was done regarding the use of autologous PRP for the treatment of diabetic foot ulcers [33]. In this study, investigators compared the effectiveness of autologous PRP gel to that of normal saline gel for 12 weeks.

Crovetti *et al*. published a prospective non-blinded study regarding the efficacy of platelet gel (PG) in healing cutaneous chronic wounds [34]. The wounds of the 24 patients enrolled in this study varied in origin, and etiologies included diabetes-related, vascular insufficiency, infectious disease, post-traumatic, neuropathic, and vasculitis-related.

CONCLUSIONS

As the use of PRP increases, additional studies may establish PRP as an efficacious treatment modality. It is important to motivate both patients and clinicians to attempt these more advanced treatment modalities, as treatment with growth factors may result in faster healing times. There are pros and cons in the use of autologous versus allogeneic blood materials. In the absence of pathogen inactivation treatment, a major advantage of using autologous platelet gel is avoiding the ethical and legal concerns of exposing the patient to the viral risks of allogeneic products, especially in countries with high infectious rates and limited donor screening and donation testing. Using autologous blood leads to better acceptance of the surgical procedure by some patients. Drawbacks of autologous products include potential larger individual variability in the quality of PRP compared to allogeneic products prepared from healthy blood following standardized working procedures of blood establishments. Another limitation relates to the difficulty of preparing autologous cryoprecipitate as a source for fibrin glue. Fibrin glue may be beneficial to stabilize the graft as it comes into direct contact with the wound and to avoid the use of staples or sutures. Finally, preparing autologous thrombin from the patient's plasma avoids relying on bovine thrombin that may carry immunological and infectious risks, most particularly transmissible spongiform encephalopathy agent responsible for Creutzfeldt-Jakob disease.

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