www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i4.38

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Role of "Platelet Rich Plasma" in Treatment of Diabetic Foot Ulcers

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ABSTRACT
Non healing diabetic foot ulcers are a major complication in diabetics, being notoriously difficult to heal. Application of Platelet rich plasma gel (PRP), produces multiple growth factors that speed up healing.
Aims and Objectives - *To evaluate the effectiveness and rate of healing of PRP gel in treatment of diabetic foot ulcers.*
Materials and Methods - *Thirty patients were enrolled into the study and divided into two groups - Group A (PRP group) received once weekly application of PRP gel and Group B (Control group) received saline dressings.*
Observations and Results -*The % reduction in ulcer surface area (80.33%) and average rate and duration of healing in PRP group was much higher than control group. The average number of dressings in the PRP group was 4.6 while the same in the control group was 64.5. Complete wound healing was seen in 14 out of 15 patients in PRP group.*
Conclusion: *PRP is highly effective in treatment of diabetic foot ulcers. PRP stimulates the growth of wound healing thus speeding up the duration of hospital stay. PRP is also a safe product without any allergic reactions/ infections.*
Key Words: *diabetic, foot, ulcer, Platelet, rich, plasma, healing.*

Introduction

India has emerged as a global capital of diabetes mellitus. At present, the estimated diabetics in India are 67 million and 1.5 million new cases of diabetes are diagnosed every year. [1] Diabetes is associated with many complications like atherosclerosis and nephropathy, and eventually the loss of a limb as a consequence of non-healing diabetic foot ulcer. Up to 85% of lower limb amputations are preceded by foot ulcers that fail to heal and more than50% of patients aren't even aware that they are diabetic. [1]

Diabetes causes healing failure by decreasing blood flow and creating a wound environment rich in cytokines, serine proteases, matrix metalloproteases (MMPs) and tissue inhibitors of MMPs, which destroy the ECM components and degradation of growth factors .[2]

Platelet rich plasma (PRP) is a plasma fraction of autologous blood that concentrates platelets in a small fraction of plasma. While the normal platelet count in blood may vary from 1.5 to 3 lakhs the platelet count in PRP is 2 million per micro liter. [3]

PRP was initially developed in the 1970s and was first used in 1987 in an open heart surgery.[4] Today, PRP is widely used in the treatment of dengue, burns, cosmetic surgery, maxillofacial surgery and orthopedics.

This PRP, after a little activation with calcium chloride and/or thrombin, can be converted into a gel efficacious in wound healing due to its tissue sealant property. [5] Also, activation of PRP causes release of several growth factors contained in the alpha granules of platelets [6] like Platelet derived growth factor (PDGF),Transforming growth factor- beta (TGF-beta), Platelet factor 4 (PF4), Vascular endothelial growth factor (VEGF) and Epidermal growth factor (EGF). [7, 8]

PRP also suppresses cytokine release, promotes new capillary growth, epithelialization, promotes collagen synthesis and decreases dermal scarring. [9] Advantages of PRP:

- Safe and biocompatible.
- No chances of any immune mediated counter response.
- Has antimicrobial properties.[10]
- Concerns of transmissible diseases are absent.
- More cost effective and ensures economical use of resources.[11]

Aims and Objectives

1. To evaluate the effectiveness and rate of healing of autologous PRP gel in treatment of diabetic foot ulcers.
2. Compare the effectiveness of PRP gel with standard treatment (normal saline dressings).

Materials and Methods

A prospective study of 30 cases was done in a large tertiary care hospital.

Selection Criteria

- Patients with type 1 and type 2 diabetes mellitus.
- Ulcer with minimum surface area of 4 sq cm (length X width)
- Hb>10 g%

Exclusion Criteria

- Patients with any bleeding disorders/ malignancy
- Screening serum albumin < 2.5g/dl
- Screening hemoglobin< 10gm%
- Screening platelet count <100x10^9/l
- Presence of osteomyelitis
- Deformities like Charcot's foot

Protocol

A consistent protocol for patient management was followed.

1. Maintaining a tight blood sugar control [by oral anti diabetics /insulin]
2. Deep tissue culture and Systemic antibiotics according to culture sensitivity given before starting the PRP therapy.

Plan of Study

The patients were divided into 2 treatment groups.

(1) Group A patients: [PRP Gel group]- Foot ulcers cleaned with normal saline, and PRP was evenly distributed over the ulcer. Dressings were done once every five days/ twice a week depending on the wound exudate.

(2) Group B patients: [Control group]- The ulcers were first cleaned and then normal saline dressing was done. Dressings were done daily.

Method

A thorough evaluation of patient was done including detailed history, clinical examination and all relevant investigations including diabetic status.

(1) Group a Patients [receiving PRP gel therapy]

<15 ml blood drawn from each patient by venipuncture and collected in sterile glass tubes containing (CPD-Adenine) was sent for centrifugation [in REMI's centrifuge machine]. Three layers were obtained in the test tube out of which the middle layer is the Platelet rich plasma.

Along with PRP, Platelet Poor Plasma (PPP) was also pipetted out, to which 10% Calcium chloride solution was added (in a ratio of 33ul/ml) in a petri dish until it coagulated. On coagulation, the clot was discarded and the remaining solution is autologous thrombin which was then mixed with the previously obtained PRP (in a ratio of 0.16ml/ml of PRP), just before application and a sticky gel obtained.(Preparation time 30-45 minutes).

(2) For Group B Patients [control group]: Normal saline dressings were done.

Wound Measurement: (in both test and control groups)

- Using a sterile transparent A4 size sheet and a fine-tipped permanent black ink marker, the two largest perpendicular diameters of the ulcer were measured and multiplied to obtain the area in mmsq.
- Ulcer size was measured once a week till complete healing/up to maximum 8 weeks.

Observation and Results

The study included 30 patients (15 in test group versus 15 in control group). The baseline profile, effectiveness of the treatment protocols and rate and duration of healing was evaluated in terms of percentage healing and change in ulcer surface area upto 8 weeks.

1. **HbA1C Levels**

Mean HbA1C levels in Group A and Group B were almost equal as P value (>0.05).

2. **Ulcer Grade (Wagner's Classification)**

Most common ulcer grades observed in the study groups are I(9), II(16) and III(5). The ulcer grades in Group A and Group B were almost equal as p value (>0.05).

3. **Ulcer Surface Area**

- The mean ulcer surface area at onset was 1829.80+/- 1275.39 mm^2 in Group A and 1799.87+/- 631.52 mm^2 in group B. This difference was not statistically significant as P value is >0.05.
- After 1 week of PRP therapy, an increased amount of granulation tissue formation was seen in Group A. The wound surface area also reduced to 1373.47+/-1128.74 mm^2 in group A and 1684.53+/- 646.32 mm^2in group B which was still not significant as p value >0.05.
- At 3 weeks, a significant change in wound surface area was observed in Group A as 727.57+/-878.722 while that of group B was 1445.67+/-637.36 and p value was highly significant. Moreover, in the PRP group, in 1 patient wound had healed completely and was discharged.
- From week 4 - week 8, reduction in ulcer surface area in Group A continued to be more significant than Group B.
- The mean percentage (%) reduction in surface area after 1 week of therapy was 24.94% in Group A while the percentage reduction in control group was only 6.41%.
- We observed a significant increase in percentage reduction in Group A (63%), as compared with group B where it was 26.45%. at the end of 4 weeks.
- At week 8, (i.e., at the end of the study), PRP group showed an 80.33% reduction in ulcer surface area while the control group showed only 51.06% reduction.

4. **Duration of Healing**

The wounds in the PRP group healed after a mean of 4.87+/-1.64 weeks compared to 8.67+/-0.90 weeks in group B and the difference was highly significant as p value <0.0001.

5. **Rate of wound healing**

At 8 weeks, the average wound closure rate per week was 333.90+/-173.50 mm^2/wks in PRP group versus 114.866667+/-26.02 mm^2/wk. for the control group. This difference in rate of healing in both groups was highly significant.

6. **Number of dressings required in each group**

The average number of dressings in the PRP group was 4.6 (range: 2-8) and inthe control group was 64.5 (range: 60-72).Thus, 12 times more dressings were required in control group than PRP group.

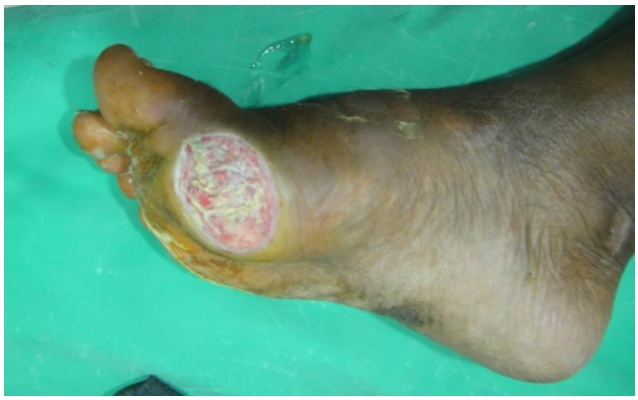

Figure 1: Diabetic foot ulcer before starting PRP dressings

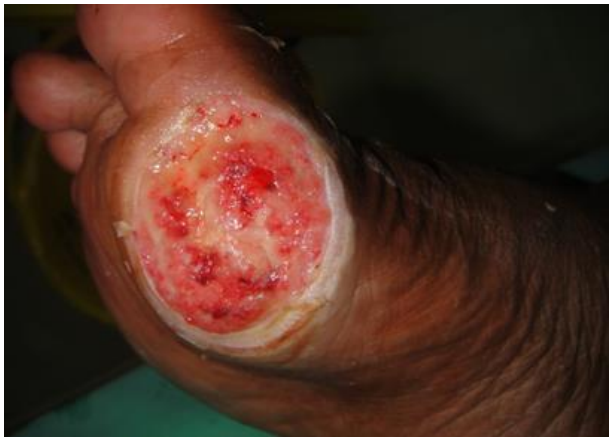

Figure 2: After 1 week of starting PRP dressings

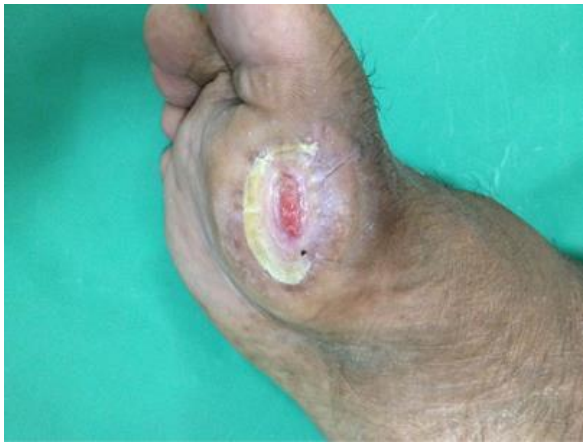

Figure 3: At the end of 4 weeks of therapy

Discussion

Diabetic foot ulcer, defined as a chronic, non – healing full thickness wound below the ankle in a person with diabetes, is notoriously difficult to heal.

One major cause of imbalance in the wound healing process, is high bacterial counts leading to a prolonged inflammatory response with high levels of cytokines, further worsened by diabetic neuropathy and ischemia. This leads to increased production of matrix metalloproteases which result in uncontrolled breakdown of ECM and growth factors.

The use of autologous platelet-rich plasma (PRP), which is rich in multiple growth factors, may bear some similarities to the natural wound healing process.

Margolis et al stated that the favorable results of PRP could be because PRP exhibited two important roles in healing. Firstly, gel fibrin formed a barrier to prevent the bacterial contamination into the wound bed. Secondly, the growth factors from platelets triggered wound healing and balanced the wound. [12]

Our study included 30 patients (15 in test group versus 15 in control group), the maximum number being in the age group 45-60 years. They were compared for the following variables:-HbA1C levels, Ulcer Grade, Ulcer surface area up to 8 weeks, % Reduction in ulcer surface area, Rate of healing, Duration of healing, and Number of dressings.

Glycosylated haemoglobin levels

There was no significant difference between the HbA1C levels of test and control groups. A similar finding was reported by Driver et al [13].

Ulcer Grade

No significant difference was observed between the Test and control group with respect to ulcer grade. While most of the ulcer grades in our study were Wagners Grades I, II, III, studies by Villela et al [14], Akingboye et al [15] and Croveti et al [16] included Grades IV and V as well, which were excluded from our study.

Ulcer Surface Area

Initially the ulcer surface areas in both PRP and control groups were comparable and not significant. This was similarly reported in studies by Driver et al, [13] Villela et al [14] and Saad Setta et al.[17]

The earliest evidence of granulation tissue formation was seen at week 1 to week 2, which was maximum in patients treated with PRP.

At week 3, a highly significant difference in ulcer surface area was observed in between the study groups and in the PRP group. One patient's wound had completely healed and was discharged.

A study by Marcus et al reported similar findings, however the changes were observed after week 4.[18]

% Reduction in Ulcer Surface Area

Macleer et al [19] and Steenvorde et al [20], reported % reduction in recalcitrant ulcers between 37.5% and 66%.Akingboye et al observed that 86.3% of wounds, showed a 47.5% area reduction.[15]

In our study, It was observed that at week 1 the mean % reduction in group A was 4 times greater than group B. The % reduction in the PRP group was 80% at the end of 8 weeks which was not only highly significant compared to the control group, but was also much more than the values reported in the previous studies.

Results of a Meta analyses of healing outcomes in the control group of other diabetic foot prospective studies, suggested that only 24% healing can be expected in the control group even after promoting good care. [12]

In our study, though patients of PRP group were more likely to heal than the control group, the percentage reduction in ulcer surface area in control group was higher than those reported in other studies. [12].

Rate of Wound Healing

The rate of wound healing in the PRP group was 3 times greater than the control group. Some wounds showed rapid progression of healing after one application of PRP gel, while others had a comparatively slower response. Thus, variation in patient characteristics also play an important role in the outcome of the therapy.

Duration of healing

There was a highly significant difference in the duration of healing in the test and control groups. Similar results were also observed in a study by Saad Setta et al.[17]

Number of dressings

Average number of dressings in control group was 12 times more than the PRP group. Crovetti et al, who had a different system for gel preparation and application, recommended a once weekly application of PRP gel similar to the protocol followed in our study. However in the study by Crovetti et al complete healing was achieved in 9 patients with an average of 10 PRP dressings.[16]

In our study, complete healing was achieved in 14 with an average of 4.6 PRP dressings.

PRP gel was obtained by activation with autologous thrombin and $CaCl_2$ and for application an externally created clot was preferred. This was done to avoid the loss of platelet concentrate that occurs while spraying the PRP onto the wound.

Another study by Marcus et al incorporated autologous thrombin for activation of PRP. In this study the average number of dressings required in the treatment group was 2.1. [18]

Safety of PRP

No allergic reactions or infections was observed in any patients treated with PRP. Similar results were observed in study by Driver et al.[13]

The only concern was about the effects of frequent though small amounts of blood collection (i.e., 10 ml per application) on health of the patients. Hence, the patients were constantly monitored for hemoglobin levels and these frequent blood draws did not reduce the hemoglobin, hematocrit, or platelet count. Thus showing that, PRP is not only an effective but also a safe treatment method.

Conclusion

PRP is highly effective in treatment of diabetic foot ulcers. PRP stimulates the wound healing and

reducing granulation tissue, thus speeding up the hospital stay.

Apart from being efficacious in wound healing, PRP is also a safe product. No allergic reactions/ infections were associated with PRP and the once a week drawing of 10 ml of blood has no effect on the haemoglobin, haematocrit, or platelet count of the patient.PRP was more efficacious than control group with respect to the number of dressings and duration of healing.

Implications for future include implementation of PRP gel in treatment of diabetic foot ulcers with challenging presentations (i.e., mild to moderate vascular disease/ exposed tendon).

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