Evaluation of the clinical and histopathological effect of Platelet rich plasma on chronic wound healing

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Abstract

To evaluate the benefits of platelet-rich plasma (PRP) and its clinical and histopathological effect in chronic wound healing. The study included 20 patients with chronic wounds of different causes. The sample consisted of 13 males and 7 females with an average age of 22.1 yrs. The largest group wound diagnosis was deep burn ulcer of different causes (n =10) and 10 patients with chronic ulcer (venous and diabetic and post traumatic) (n =5and4and1 respectively).The patients were divided into two groups: Study group (n=10) in which PRP was used and control group (n=10). On day 7, ulcer size had reduced by an average 31.4 %( range from 15%-70%) in 8 of 10 patients of the study group compared to control group which reduced by 15.3 % (range from 10%-35%) in 6 of 10 patients. After 28 days, 2 wounds in study group had healed completely and 7 wounds had decreased in size to an average 55.5% (range from 30.6%-85%) of their original size. In control group, about 6 wounds decreased in size by an average of 21.5% (average from 7%-30%). Histopathologically, there was evidence of faster tissue regeneration and epithelialization in study group compared to control group. The use of autologous PRP should be reserved for treatment of recalcitrant wounds where there is a lack of improvement despite treatment of underlying causes and good local wound care.

Keywords: Platelet-rich plasma, Chronic wound, Tissue regeneration

INTRODUCTION

Platelet Rich Plasma (PRP) is defined as plasma containing above baseline concentrations of platelets which is from 140,000 – 400,000/µl. PRP is isolated through the centrifugation of whole blood and the resultant density based separation of its contents.

Simply put, its actions are based on the infusion of elevated platelets, thereby theoretically enhancing the biological healing capacity and tissue generation in the wound bed. Enzyme-linked immunosorbent assay studies of PRP have quantified the presence of 7, 10, and 30 fold increases in such growth factors as transforming growth factor B(TGF), sepidermal growth factor(EGF) and platelet derived growth factor(PDGF)( Ernesto C, 1992).

In physiological conditions, through activation of platelets, these cytokines and GFs are transformed into their bioactive status and actively secreted within 10 min after clotting, with >95% of the presynthesised GFs released within 1 h1 This process can be reproduced in clinical settings through activation of PRP by using an activator, for example, thrombin, resulting in the formation of platelet gel (PG). This gel acts as a drugdelivery system since it comprises a high concentration of platelets and their active cytokines and GFs, which stimulate physiological processes (Kazakos et al., 2009).

The process of wound healing is orchestrated by the complex interaction of a variety of biochemical mediators. Platelets play a prominent role by their secretion of numerous biologically active proteins (Rothe and Falanga, 1989). Platelet activation results in release of intracellular α-granules, releasing growth factors and cytokines include PDGF, TGFβ, vascular endothelial growth factor, platelet activating factor (PAF), EGF, and insulin like growth factor (ILGF) (Knighton et al., 1986; Crovetti et al., 2004; McAleer et al., 2006; Steenvorde et al., 2008; Eppley et al., 2004 and Mast and Schultz, 1996). These bioactive proteins stimulate a host of...
activities such as osteoblasts, fibroblasts and epidermal cell differentiation, proliferation, collagen synthesis and angiogenesis (Gürgen, 2007 and Bertone, 1989).

PRP has been used to augment wound healing in various clinical situations (Fitch and Swaim, 1995). Studies of soft tissue repair have examined. Disorders such as rotator cuff injury, lateral epicondylitis, burn and chronic wounds (Dugrillon and Kluter, 2002; Robson, 1997 and Marx, 2004) these studies describe varying effects from equivalence with placebo saline or anesthetic injections to decrease postoperative pain and faster rates of healing (Harrison and Cramer, 1996).

Naves et al. 2008, examined the benefits of PRP in the wound healing process following fractional carbon dioxide laser resurfacing in 25 patients. They noted a significant decrease in recovery time in the treatment of inner arm soft tissue with PRP compared to controls treated with normal saline, demonstrated by less trans epidermal water loss, erythema and inflammatory pigmentation (Dyson, 1997)

In this study, we evaluated the clinical effect of the application of PRP in different types of chronic wounds and also the histopathological effect as epithelial proliferation, differentiation and new angiogenesis.

**MATERIALS AND METHODS**

The current study was done between February 2012 and December 2013. The study included 20 patients with chronic wounds of different causes were included in this study. The sample consisted of 13 males and 7 females with an average age of 22.1 yrs. (18-50 yrs.). The largest group wound diagnosis was deep burn ulcer of different causes (n =10) and 10 patients with chronic ulcer (venous, diabetic and post traumatic) (n=5,4 and 1) respectively. The patients were then divided into two groups. Study group (n=10) in which PRP was used and control group (n=10) in which ordinary methods of dressing were used.

The inclusion criteria were chronic ulcer post burns, venous, diabetic and posttraumatic with duration about 4 weeks without clinical signs of infection.

Exclusion criteria were any chronic diseases such as cardiovascular, respiratory, renal or gastrointestinal. Also any infected wound was excluded. All patients gave written informed consent before enrollment into the study.

**Preparation of platelet rich plasma**

PRP was prepared by using desktop centrifugation system (gravitational platelet system) (GPS 2, Biomet biologics comp.) and reaction chamber to produce autologous thrombin. Making PRP starts by drawing a volume of 55 ml blood from the patient and mixing it with 5 ml citrate. The blood is then transferred to the G.P.S. disposable which is placed in a centrifuge. It is spun at 3200 rpm for 15 minutes.

During centrifugation, blood is separated into three different fractions: Platelet poor plasma, platelets and white blood cells and red blood cells (fig.1). The platelets poor plasma and concentrated platelets and white blood cells are drawn from the tube using separate ports.

Thrombin is produced by mixing platelet poor plasma with an ethanol-calcium chloride reagent in the TPD (temperature programmed desorption) reaction chamber.
Table 1. Study group characteristics

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>Wound diagnosis</th>
<th>Duration of wound</th>
<th>Wound size (cm)</th>
<th>N. of treatments</th>
<th>% change in area at day 7</th>
<th>% change at day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>F,53</td>
<td>Post burn ulcer</td>
<td>2 months</td>
<td>21.5 cm</td>
<td>3</td>
<td>22.5</td>
<td>28</td>
</tr>
<tr>
<td>M,72</td>
<td>Ch. Venous ulcer</td>
<td>5 yrs.</td>
<td>15.7</td>
<td>4</td>
<td>23.3</td>
<td>49.7</td>
</tr>
<tr>
<td>M,35</td>
<td>Post burn</td>
<td>3 months</td>
<td>22</td>
<td>3</td>
<td>77.7</td>
<td>100</td>
</tr>
<tr>
<td>F,20</td>
<td>Post burn raw area</td>
<td>3 wks.</td>
<td>25</td>
<td>3</td>
<td>2.1</td>
<td>15</td>
</tr>
<tr>
<td>M,42</td>
<td>Post burn</td>
<td>45 days</td>
<td>20</td>
<td>4</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>M,17</td>
<td>Post traumatic</td>
<td>2 months</td>
<td>35</td>
<td>4</td>
<td>31.8</td>
<td>69.3</td>
</tr>
<tr>
<td>F,60</td>
<td>Diabetic foot</td>
<td>6 months</td>
<td>32</td>
<td>3</td>
<td>10.5</td>
<td>35</td>
</tr>
<tr>
<td>F,52</td>
<td>Diabetic wound</td>
<td>1 year</td>
<td>23</td>
<td>4</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>M,65</td>
<td>Ch. Venous ulcer</td>
<td>3 yrs.</td>
<td>34</td>
<td>4</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>M,70</td>
<td>Ch. Venous ulcer</td>
<td>6 yrs.</td>
<td>22.5</td>
<td>3</td>
<td>00</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Table 2. Control group characteristics

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>Wound diagnosis</th>
<th>Duration</th>
<th>Size wound (cm)</th>
<th>% change in area at 7 days</th>
<th>% change in area at 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>F,35</td>
<td>Post burn ulcer</td>
<td>1 month</td>
<td>16 cm</td>
<td>6.5</td>
<td>10</td>
</tr>
<tr>
<td>M,46</td>
<td>Post burn ulcer</td>
<td>2 months</td>
<td>25 cm</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>M,67</td>
<td>Diabetic wound</td>
<td>1.5 year</td>
<td>32 cm</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>M,55</td>
<td>Post burn ulcer</td>
<td>3 months</td>
<td>27 cm</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>M,40</td>
<td>Post burn raw area</td>
<td>4 months</td>
<td>30 cm</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>F,62</td>
<td>Diabetic wound</td>
<td>2 yrs.</td>
<td>22 cm</td>
<td>00</td>
<td>10</td>
</tr>
<tr>
<td>F,50</td>
<td>Post burn</td>
<td>3 months</td>
<td>35 cm</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>M,72</td>
<td>Ch. Venous ulcer</td>
<td>4 yrs.</td>
<td>15 cm</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>M,75</td>
<td>Ch. Venous ulcer</td>
<td>3 yrs.</td>
<td>33 cm</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>M,45</td>
<td>Post burn</td>
<td>3 months</td>
<td>23 cm</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

Platelet concentrate and thrombin were then drawn into separate syringes. The platelet-thrombin ratio was at 10-1. When thrombin was mixed with platelets, it activates it. The result is platelet gel that sticks to the surface of the wound when applied, then the platelets release growth factors which help in wound healing.

The P.R.P was applied to the wounds three times weekly. Degradable dressing and 2ry absorbent layer were used to cover the wounds. Biopsies taken from the control group and study group were collected and fixed in 10% buffered formalin. After 24 hours of fixation, specimens were routinely processed for histopathology, sectioned at 4 microns, and stained with hematoxylin and eosin (H and the E) and Masson Trichrome (Sigma-Alorich). Immunohistochemical expression for cytokeratin AE1/AE3 and p63 (Dako, Denmark) were determined using streptavidin-biotin peroxidase method on paraffin sections using microwave antigen retrieval method. In order to evaluate the antibody specificity, known positive and negative controls were used. The sections were incubated with primary antibody at 4°C overnight. Light microscopic examination of the stained sections was performed by histopathologist who was unaware of which group the slides belonged to.

Evaluation: evaluation was done clinically and histopathologically. Clinical assessment of the wounds was done accurately using digital planimetry (visitrak, smithandnephew, hull, uk) to measure wound sizes. Digital measurements of the ulcers size were routinely taken on day 7 and 28.

The primary endpoint was time to heal, and the secondary endpoint was reduction in ulcer size if wounds had not healed. Skin biopsies from the edges of the wounds were taken at day 7 and 28.

RESULTS

Clinical results

Wounds were measured at day 0 with digital planimetry and showed an average wounds size 16.7 cm (range from 22.4-35.5cm).

On day 7, ulcer size had reduced by an average 31.4 % (range from 15%-70%) in 8 of 10 patients of the study group compared to control group which reduced by 15.3 % (range from 10%-35%) in 6 of 10 patients (table1 and 2).

In 2 wounds of the study group, they were unchanged in size compared to 4 wounds in control group. The wounds in study group were clinically assessed as improved.
Figure 2. Ulcer size at day 0 (left). Ulcer size at 28 days treated with P.R.P.

Figure 3. Light microscopy examination at day (7)
A). Partial re-epithelization of the epidermis in the control group with region of angiogenesis (arrow head).
B). Complete re-epithelization of the epidermis in the study group.
C). Cytokeratin AE1/AE3 immunohistochemical stain for the epidermis in the control group, and
D). Study group
Figure 4. Light microscopy examination at day (28).

A) Mild deposition of randomly oriented collagen fibers (green color) in the dermis in the control group.

B) Prominent deposition of green-staining collagen bundles in the study group in which the collagen fibers are well-orientated parallel to the overlying epidermis.

C) Nuclear expression of p63 immunostaining in the control group involving mainly the basal and suprabasal layers in reepithelialized epidermis.

D) p63 overexpression involving entire basal, suprabasal and spinous layers of the epidermis in the study group.

On day 28, 2 wounds in study group had healed completely and 7 wounds had decreased in size to an average 55.5% (range from 30.6%-85%) of their original size. All of those were clinically assessed as improved. One wound remained unchanged in both size and condition and there was infection in it. Culture sensitivity was done and pseudomonas organism was isolated and suitable antibiotic given (Fig.2).

On day 28, 6 wounds in control group decreased in size by an average of 21.5% (average from 7%-30%). 3 wounds got infection and culture sensitivity was done and antibiotic was given.

**DISCUSSION**

Wound healing is a complex process that is regulated by interactions between a large number of cell types, extracellular matrix proteins and mediators such as cytokines and growth factors. Lack of balance between these interactions may result in a chronic wound. One possible cause of imbalance in the wound healing process is the lack of balance between the repair and remodeling phases.
process is high bacterial counts leading to a prolonged inflammatory response with high levels of cytokines. This leads to increased production of matrix metalloproteases. High matrix metalloprotease activity results in uncontrolled breakdown of extracellular matrix and growth factors (Bennett and Schultz, 1993). If measures are not taken to re-establish the balance between the factors involved, a chronic wound fails to heal. Despite the fact that concentrated platelets have been used to treat chronic wounds for more than 20 years, there is a lack of high-quality studies describing their use in the literature. Literature findings do not allow drawing a clear conclusion on the use of platelet concentrates. Senet et al., used frozen autologous platelets that had no significant adjuvant effect on healing of chronic venous leg ulcers (Kevy and Jacobson, 2004). Crovetti et al. 2004, McAleer et al. 2006, and Steenvorde et al. 2008, reported wound closure rates in recalcitrant ulcers between 37.5% and 66%. The results of this small sample of patients show that 50% of all wounds healed. Treatment with platelet-rich plasma was reserved for patients with wounds that had not healed despite the use of other treatment strategies. Use of platelet-rich plasma not only leads to wound closures, but also to improve the healing time. The average healing time for the wounds that closed was less than 6 months. 42.8% of 7 venous ulcers in this study healed within 6 months after treatment with platelet-rich plasma and two layer compression bandage. Nelson, et al. showed 67% healing rates of venous ulcers within 24 weeks when using four-layer compression therapy and 49% healing rates when using single-layer compression therapy (Assoian at al., 1983). However, it might be difficult to compare outcomes because of the low number of patients included in the present study. No pattern in the speed of wound healing could be discerned. Some wounds showed rapid progression of healing after application of concentrated platelet gel, but later slowed down, and in some cases the wounds began deteriorating again. Other wounds did not seem to demonstrate any early healing after treatment, but showed significant progress by the end. There is no explanation for this. To date, there has been no determination of a standard treatment frequency or duration. The treatment program of Crovetti et al., 2004 recommends weekly application of platelet gel. Complete healing was achieved in 9 wounds with an average of 10 applications (Ashcroft at al., 1999). In this study, wound closure occurred in 7 wounds with an average of 1.7 platelet gel applications. Crovetti et al., 2004 used a different system to prepare platelet gel, which may have resulted in different concentrations of platelets and growth factors that were applied to the wounds. Many studies reported a beneficial influence of PRP on wound healing, with the main contributors being the improved proliferation of endothelial cells and vascularisation and the stimulatory effects on formation of granulation tissue. These advantageous effects are most likely to occur when PRP is repeatedly applied to the wound bed. Not only did more wounds heal when PRP was used, but also the time to healing was notably shorter (Saad et al., 2011).

In our study, there was evidence of beneficial effect of PRP in enhancing and accelerating wound healing process as evidenced by clinical and histopathological results.

CONCLUSION

The use of platelet-rich plasma can be an option when treating recalcitrant wounds of differing etiologies. It should be reserved to wounds that do not show any progress after 6 months with treatment of wound etiology and standard wound care. Further research in the field is required. Controlled studies with sufficient sample sizes are needed to prove the efficacy of platelet-rich plasma to treat wounds. Such studies should focus on outcomes as well as for variations in platelet counts, growth factor concentrations, and applicability and cost-effectiveness.

REFERENCES

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