THE HISTOPATHOLOGICAL EVIDENCE OF IMPROVED SPLIT THICKNESS SKIN GRAFT OUTCOMES ON USING THE AUTOLOGOUS PLATELET-RICH PLASMA: A PROSPECTIVE CONTROLLED CLINICAL STUDY

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ABSTRACT

Background: The Platelet-rich plasma (PRP) used widely in several clinical settings. It is well recognized for its adhesive, hemostatic, and healing properties. These properties of PRP are owing to the several growth factors elaborated from the platelets into the wound environment. However, its useful outcome on the split-thickness skin graft (STSGs) is quite debatable.

Aim: The aim of this work is to assess the process of wound healing histopathologically in STSG after injection of PRP to the recipient bed compared to the traditional method of graft fixation to determine if PRP could improve STSG outcomes.

Patients and Methods: the study incorporated 60 patients had a large skin defect in the lower limb, with age ranging from 19-61 years old. Of these patients, 32 males and 28 females. The cause of these raw areas was trauma in 25 patients, tumor excision in 13 Patients, burn in 11 patients and chronic ulcer in 11 patients. Patients were allocated sequentially into one of the two groups. In the 1st group, the traditional methods of graft fixation were performed, while in the 2nd group, an autologous PRP was applied to wound beds prior to graft fixation. Two weeks after surgery, 2 rectangular punches were biopsied from the graft in both groups and sent for histopathology examination. The collected data were statistically analyzed.

Results: Application of PRP to the recipient bed prior to graft fixation improve the STSG outcomes as we found all the histopathological criteria of wound healing process (epidermal thickness, migration of keratinocytes, bridging cells, keratinization, melanin pigmentation, collagen fiber deposition and newly formed vessels in the dermis with inflammatory cells) were superior and statistically significant in the PRP group compared to the traditional group.

Conclusion: in the present study, we introduced the histological evidence that confirms the application of PRP may become an optimal choice to improve STSG outcomes.
INTRODUCTION

Skin grafts are the ideal approach for large dermatological defects in plastic and reconstructive surgery. Split-thickness skin grafts STSGs are standard therapy for surgical wounds, postoperative defects, chronic wounds, large traumatic wound, burns and for cosmetic purposes (Juan, et al., 2016; Schiestl, et al., 2011), mostly when it is not possible to close with primary intention (Läuchli, et al., 2013). Commonly STSGs are more appropriate than full-thickness surgical flaps or closure by secondary intention (Barrit and Brick-Sorensen, 2014; Harrison and MacNeil, 2008). Skin grafting may fail for numerous reasons. The majority of STSG failure cases are due to hematoma/seroma accumulation beneath the graft that precludes graft adherence to the underlying wound bed and impedes the graft from getting the required nourishment. The reason for graft failure may be attributed to the movement of the graft with disruption of the fragile attachment between the graft and the underlying raw area. The surface contamination, as well as poor blood supply, undoubtedly has a negative effect on graft survival (Jeffrey, et al., 2010).

Autologous PRP came to be popular as a treatment option in many surgical fields (Marx, 2004), especially in challenging wounds, (Margolis, et al., 2001) defects in maxillofacial bone, (Marx, et al., 1989) the cosmetic surgery (Bhanot and Alex, 2002) in addition to the spinal surgery (Hee, et al., 2003; Kakudo, et al., 2008). The PRP is defined as a concentration of platelet at least 10, 000,000 platelets/μL in 5 ml of plasma. It has a 3-5 fold rise in growth factor concentration (Foster, et al., 2009). Recent studies have shown that platelets contain over 800 proteins based bio-active factors (Boswell, et al., 2012). In addition, PRP contains a high percentage of platelets that has α-granules which contain molecules such as transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) that motivate tissue regeneration through activation of cell differentiation and proliferation. PRP has been documented to encourage epithelialization in STSG donor sites (Marx, 2004). However, its effectiveness still controversial. Moreover, the previous studies did not comment on the histological condition of the STSGs managed with PRP. Our present study aiming to evaluate the role of PRP in improving the take and quality of STSGs by studying the histopathological changes that happen in STSGs.

PATIENTS & METHODS

The present study was completed in the Plastic and Reconstructive Unit, and the department of pathology at Benha University Hospital in Egypt and King Saud Hospital in Saudi Arabia from September 2014 to September 2016. After approval of the study protocol by the Ethical Committee and obtaining a written fully informed consent. A separate consent was signed by all patients before surgery. The 60 Patients in our study were divided into two groups; the first group (the control group) for patients who were treated through the traditional method of grafting and included 30 cases, while the second group (the PRP group) for patients who were managed through an intra-operative injection of the recipient area of STSG with PRP before putting the graft, this group included 30 patients. The Inclusion criteria
in our study were: patients with a raw area requiring STSG for various clinical reasons with Clean and healthy granulating bed, patients’ age 18-60 years, and the patient’s ability to sign consent. Exclusion criteria were: Presence of one or more co-morbidities other than diabetes mellitus, active auto-immune or immune diseases, patient involvement in another study, and patients who have a prominent psychiatric disease.

**PRP preparation:**

In the laboratory, 35ml of autologous venous blood is withdrawn by venous puncture of one of the antecubital veins. Blood is collected in a sterile tube containing 3 ml of citrate phosphate dextrose (CPD) for anticoagulation to avoid platelet activation and degranulation.

**Centrifugation of the extracted blood 2 times:**

The first centrifugal, called “soft spin” (3600 rpm) in which blood separated into three layers, RBCs, PRP, and platelet poor plasma (PPP). Without anticoagulant, the PRP, PPP, and some RBCs were shifted to another tube. (Figure 1)

The second centrifugal, called “hard spin” (2400 rpm) in which the PRP was settled at the bottom of the tube with a few RBCs. (Figure 2)

This PRP was collected in a sterile tube to be ready for use in the operation.

The platelets and TGF-β1 have assessed in the pre-centrifuging blood samples as well as PRP samples.

**Before surgery:**

Assessment of the general condition of patients with control of diabetes in diabetic patients.

Preparation of the wound through a daily dressing.

Control of any infection by the parenteral antibiotic, according to the results of the culture.

Swapping of the wound for culture until it becomes free from bacteria.

**Operation:**

All operations underwent in the operation theatre under complete aseptic condition.

The recipient site was prepared well.

The STSGs were harvested from the thigh of the opposite side by a Hamby’s knife, then the donor site of STSGs was covered with wound dressing materials to moisten the environment of the wound.

In patients of the control group, the graft was fixed to the recipient bed in an ordinary manner.

In patients of the PRP group, the prepared PRP was mixed with calcium chloride 10% (0.1 ml) just before the time of application, then it was injected to the recipient bed through multiple injections (Figure 3), then the graft was fixed (Figure 4).

**After surgery**

Two weeks post-surgery, 2 punch biopsies were taken from grafts in both groups and sent for histopathological assessment.
Histopathology Handling Of The Specimen: the rectangular punch biopsy was received in formalin and bisected and totally submitted in to one cassette which is processed and stained with the routine hematoxylin and eosin stain and examined to compare the following histopathological criteria in both groups: the thickness of epidermis, surface keratinization, keratinocytes differentiation (cell migration, bridging, and keratohyaline granule formation) melanin deposition, formation of an early and mature collagen fibres, and neovascularization in the dermis in addition to the presence of inflammatory cells infiltrate.

Statistical analysis

Data presented as mean ± SD, ranges, numbers, and ratios. Results analyzed using Student's T-test and chi-square test. Statistical significance was considered when the p-value is <0.05. Statistical analysis was done using the SPSS (version 16 for Windows; SPSS Inc., Chicago, IL, USA) statistical package.

Figure (1): The blood sample after 1st centrifuge (PRP in the middle).

Figure (2): The sample after 2nd centrifuge (PRP in the bottom).
RESULTS

The present study included 60 patients who have a raw area (defect) in the skin of the lower extremity; the cause of the defects was trauma in 26 patients, tumor excision in 13 cases, burn in 10 cases, and chronic ulcer in 11 cases. No significant differences between both groups regarding the demographic data (Table 1).

We found that the mean platelet counts ±SD was increased about three folds in the PRP group in comparison with the pre-centrifuging blood sample (756.9 ±140.3 and 235.0 ±50.1 respectively). Also, there was a statistically significant difference between TGF-β1 concentration in the PRP and the patient’s blood sample (P-value = 0.004) (Table 2).

In comparison with the control group, the PRP group showed a statistically significant (P-value<0.001) mean epithelization of the surface area (Table 3). In the PRP group, the PO histological monitoring of the biopsy taken from the STSGs two weeks after surgery revealed a statistically significant correlation in all the parameters that were mentioned in table 4 in comparison with the control group (Table 4).
Table (1): Patients’ demographic data

<table>
<thead>
<tr>
<th>Findings</th>
<th>Strata</th>
<th>Control group</th>
<th>PRP group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td></td>
<td>39±2.2</td>
<td>37±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>18</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>Cause of the defect</td>
<td>Trauma</td>
<td>13</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Tumor excision</td>
<td>5</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Burn</td>
<td>6</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Chronic ulcer</td>
<td>6</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Associated co-morbidity</td>
<td>Diabetics</td>
<td>11</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>7</td>
<td>6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table (2): The mean platelet counts and the mean TGF-β1 concentration compared between the blood specimen and the PRP specimen.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Blood specimen</th>
<th>PRP specimen</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (x10³ cells/mm³)</td>
<td>309.7±84.1</td>
<td>764.1±115.7</td>
<td>0.000</td>
</tr>
<tr>
<td>TGFβ1 (ng/mL)</td>
<td>48.09±1.35</td>
<td>120.7±1.53</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 3: The mean epithelialization surface area 1 week after surgery.

<table>
<thead>
<tr>
<th>Finding</th>
<th>PRP group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The mean epithelialization surface area (mm)</td>
<td>43.1±4.33</td>
<td>21.0±4.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table (4): The postoperative histological monitoring in the PRP and control groups.

<table>
<thead>
<tr>
<th>Keratin formation</th>
<th>Total number in Strata (N)</th>
<th>PRP group N (%)</th>
<th>Control group N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin formation</td>
<td>Mild (19)</td>
<td>4(21.1%)</td>
<td>15(78.9%)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Moderate (21)</td>
<td>12(57.1%)</td>
<td>9(42.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked (20)</td>
<td>14(70%)</td>
<td>6(30%)</td>
<td></td>
</tr>
<tr>
<td>Keratinocytes differentiation</td>
<td>Differentiated (34)</td>
<td>24(70.6%)</td>
<td>10(29.4%)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Undifferentiated (26)</td>
<td>6(23.1%)</td>
<td>20(76.9%)</td>
<td></td>
</tr>
<tr>
<td>Neovascularization</td>
<td>Mild (15)</td>
<td>4(26.7%)</td>
<td>11(73.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate (22)</td>
<td>11(50%)</td>
<td>11(50%)</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Marked (23)</td>
<td>15(65.2%)</td>
<td>8(34%)</td>
<td></td>
</tr>
<tr>
<td>Collagen deposition</td>
<td>Mild (23)</td>
<td>6(26.1%)</td>
<td>17(73.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate (22)</td>
<td>12(54.5%)</td>
<td>10(45.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Marked (15)</td>
<td>12(80%)</td>
<td>3(20%)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory cells in the dermis</td>
<td>Mild (21)</td>
<td>17(81%)</td>
<td>4(1.9%)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Moderate (25)</td>
<td>15(60%)</td>
<td>10(40%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked (14)</td>
<td>11(78.6%)</td>
<td>3(21.4%)</td>
<td></td>
</tr>
<tr>
<td>Melanin deposition</td>
<td>Mild (24)</td>
<td>6(25%)</td>
<td>18(75%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Moderate (22)</td>
<td>13(59.1%)</td>
<td>9(40.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked (14)</td>
<td>11(78.6%)</td>
<td>3(21.4%)</td>
<td></td>
</tr>
</tbody>
</table>
Figure (5): The full-thickness skin biopsy stained sections of the PRP group; (A) the epidermis showed renewed intact epithelium with fully differentiated epidermal keratinocytes with overlying keratin layer (H&E ×100), (B) high power of the previous image inset showed differentiated polyhedral cells in stratum spongiosum with the cells in stratum granulosum are fully differentiated and display keratohyalin granules (green arrow) and basal melanin pigment deposition (yellow arrow) (H&E ×400), (C) dermis showed collagen deposition with mild perivascular inflammatory infiltrate with Moderate to marked increase neovascularization (H&E × 100), and (D) high power of the previous image inset showed moderate early and mature collagen deposition with mild perivascular inflammatory infiltrate with a decrease in the melanin (H&E x200).
DISCUSSION

Autologous PRP has been applied recently as an effective therapeutic option in several medical as well as surgical fields (Redaelli, et al., 2010). The PRP is a prepared autologous separated platelet in concentrated plasma (Redaelli, et al., 2010). The PRP includes a mixture of plasma derivatives and bioactive platelets (Anitua, et al., 2009). Several growth factors are released from α-granules in the platelets activated by aggregation inducers. The α-granules had more than 30 bioactive substances (Kim, et al., 2011; Cayırlı, et al., 2014). Tissue regeneration requires cell proliferation, new vessel formation, and cell migration. Fibroblasts showed many surface receptors that could simultaneously stimulate several molecules which elicit behavioural responses (Anitua, et al., 2009). Numerous cytokines and growth factors that help in accumulation of extracellular matrix and increase proliferation and differentiation of cells are triggered after PRP injection into the suggested tissue (Banihashemi and Nakhaeizadeh, 2014).

On studying the ECM remodelling, which necessitates activated dermal fibroblast? Kim et al showed that PRP induced increased human skin fibroblasts expression of collagen type I. PRP stimulates the fibroblasts to synthesize new collagen (Kim, et al., 2011). Kakudo et al found that the multiplying of human adipose-derived stem cells and human skin fibroblast were significantly promoted after adding “activated platelet-rich or PPP” to the cell culture. They found that PRP can augment the number of multiplying human skin fibroblast and adipose-derived stem cells. (Kakudo, et al., 2008) Cho et al revealed that PRP motivates the human dermal fibroblasts to increase the expression of collagen type I, MMP-1 and MMP-2 (Cho, et al., 2012). In a controlled study of Kakudo et al who evaluated the PRP effects on the STSG donor site, reporting the time needed for epithelialization and disappearance of pain during changing the gauze. They found that PRP encourages the epithelialization and angiogenesis of STSG donor sites (Kakudo, et al., 2011). Moreover, some experimental and in vitro work advocated that PRP increases wound healing through promoting fibroblast proliferation, synthesis of collagen, neoangiogenesis, and epithelialization. Shin et al. (2012) controlled study revealed that PRP application with a fractional laser, significantly increased dermal elasticity, collagen formation, fibroblasts number, and patient satisfaction.

Yuksel et al. (2014) reported the improvement of human facial skin on the application of PRP intradermal injections clinically, but they did not examine the skin histologically. In Abuaf, et al. (2016) study, they compared the collagen after intradermal injection of PRP with the patient baseline collagen levels as well as with the control side. They found that in the PRP side after four weeks, it is significantly improved compared to the baseline findings. They conclude that PRP was effective, and significantly increasing dermal collagen. Our findings were compatible with the previous studies. The importance of these findings because of that our study is a controlled clinical study of STSG. To the best of our knowledge, histological examination of the STSG recipient site after PRP application was not evaluated in the preceding studies, in vivo.
Our study findings confirmed that the intraoperative autologous PRP application to the recipient site before fixation of STSG is a harmless, effective, inexpensive and an easy procedure to enhance the healing activity. Using our technique is economically valuable and cost-effective. The PRP is mean to deliver several GFs required to stimulate the healing process that has been extensively confirmed. Economically, the expense of one disposable kit is cheaper compared to other methods. Both Histological and clinical findings of the current work establish solid proofs of efficacy, as well as safety. So that we confirm the role of PRP as the treatment modality of SG that improve the healing of STSG and quality of life in patients having raw areas of several etiologies and might decrease the possible graft complications.

CONCLUSION

We recommend the PRP application to the recipient site of STSG as it improves the output and accelerates wound healing. Furthermore, we encourage our colleagues to implement more studies based on the application of PRP in the different medical and surgical fields as we believe that it could provide the growth factors and scaffolds that could increase the regeneration power of the different tissues.

REFERENCES


الادلة النسيجية على تحسن نتائج الرقعة الجلدية باستخدام الصفائح الدموية الغنية:
دراسة سريرية مستقبليّة محكمه
محمّد توفيق يونس - أشرف محمد عبدالقادر - شيماء ضوه
قسم الجراحة العامّة - كلية الطب - جامعة بنها
قسم الباثولوجي* - كلية الطب - جامعة بنها

تستخدم الصفائح الدموية الغنية على نطاق واسع في العديد من الإعدادات السريريّة حيث أن معروف عنها خصائصها الاصطناعيّة والانتعاشية وترجع هذه الخصائص إلى وجود العديد من عوامل النمو التي تفرز في بيئة الجرح، على الرغم من ذلك فإن نتائجها المفيدة على الرقعة الجلدية لا زالت محل نقاش.

الهدف من البحث: يهدف البحث إلى دراسة النسيجية لعملية التنامي الرقعة الجلدية وذلك ب…”

تتراوح أعمارهم بين 19-67 سنة، منهم 38 رجل و 28 سيدة. هذه الجروح ناتجة عن صدمة إجمالية في 25 مريض، استتامول ورم في 13 مريض، حرق في 11 مريض وكذلك جرح مزمن في 11 مريض.

تم تقسيم المرضى إلى مجموعتين متساويتين في المجموعة الأولى تم تثبيت الرقعة بالطريقة التقليدية بينما في المجموعة الثانية تم حقن البلازما الغنية بالصفائح الدموية في الجرح قبل وضع الرقعة الجلدية.

بعد أسبوعين تم أخذ عينتين من الرقعة الجلدية في كلتا المجموعتين ليتم فحصها في معامل الأنسجة ثم تحليل البيانات التي تم جمعها احصائياً.

النتائج: استخدم البلازما الغنية بالصفائح الدموية على الجرح المستقبل الرقعة الجلدية أدى إلى تحسن جميع المعايير التشريحيّة النسيجية للرقعة (سماكة البشرة) 3.1. المجلة المصرية للعلوم الطبية 37 (2) 2013-10-5-2016.