A Prospective Study of Autogenous Platelet- Rich Plasma (Prp) in The Treatment of Second Degree Deep Burns.

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Abstract:

Background: wound healing is a complex, well-coordinated process whereby an injured tissue is removed and replace by normal tissue this process involves an interplays of inflammatory cells, mediators, cytokines and growth factors. Platelets plays a pivotal role in growth factors, Materials and Methods: A 2 years hospital based prospective study was carried out in the burn unit of department of plastic surgery, RIMS, Imphal to evaluate the effectiveness of autogenous platelet-rich plasma (PRP) in the treatment of second degree burns. A total of 15 cases age 14 years and above presenting within 48 hours of sustaining deep second degree burns due to flames, scalds or electric burns were included in the study; wounds of similar dimensions were chosen in the same patient as cases and controls, autogenous platelet rich plasma was prepared and injected at different levels in the selected case sites. Results; on histopathological anagysis of the tissue sent by incisional biopsies from both case and control site, the PRP treated areas were observed to epithelialise at a faster rate and healing occurred without hypertrophic scarring,

Keywords: Platelets, Plasma, Deep dermal burn.

I. Introduction

Wound healing is a tissue remodelling process in which, an injured tissue is removed and replaced by normal tissue (1) this process involved a complex interplay of cells, mediators, growth factor and cytokines. The cascade of events begin with blood clotting, aggregation of inflammatory cells and then proceeds to a highly proliferative state, where in fibroblasts synthesize collagen, keratinocytes form a new epithelial layer and angiogenesis leads to neovascularisation. (2) wound repair in most mammals follows a similar orderly sequence of events. (3)

Platelets are small discoid blood cells made in bone marrow with a life span of 7-10 days, and contain intracellular structures containing glycogen and lysosomes. Several angiogenesis regulating proteins carried by platelet within the alpha granules of their cytoplast play a major role in wound healing.

Platelet rich plasma (PRP) is defined as a volume of the plasma fraction of autologous blood having a platelet concentration above the baseline value normal platelet concentration is 2 lacs/UL, studies have shown that clinical efficacy of wound healing can be expected to improved with a minimum increase of platelets four time above the baseline value (1 million/UL) platelets contain many growth factor such as PDGF (platelet derived growth factor), TGF-beta (transforming growth factor –beta) and VEGF (vascular endothelial growth factor). PRP has been reported to enhance wound healing when used in cardiac surgery oral-maxillofacial surgery and orthopaedics.

Platelets rich plasma (PRP) is obtained by centrifugation of anti-coagulated blood, after centrifugation the separated buffy coat layer, consisting of platelets and WBCs, form the PRP remain stable for approximately 8 hours, as in normal platelet deposition, degramulation of alpha, granules in platelets releases pre-packaged growth factor. The cascade of blood clotting, and begin within 10 minute after platelets aggregation, in PRP activation of platelet derived factors is triggered by the Addition of calcium chloride and thrombin. Collagen in soft tissue triggers the induction of release of mediators from platelets hence PRP can be injured directly into soft tissue, in the initial burst with the first hour, about 95% of pre synthesize growth factor are released, during the remaining 7 days of their viability, platelets synthesize and secrete by chemotaxis to the zone of injury also get stimulated by platelets, here platelets continue to regulate wound healing by secreting additional growth factor such as tumor necrosis factor (TNF-) and basic fibroblast growth factor(6 FGF).

PRP has been reported to facilitate wound healing in burns, radiotherapy burns, cosmetic surgery, e plastic surgery, dental surgery, orthopaedic surgery, and cardiothoracic surgery. To evaluate the use of PRP in burns therapy, two effect of PRP must be considered; an immediate hemostatic and tissue adherent effect and a long term regulation of wound healing.

II. Materials and methods

This was a prospective study conducted in Department of plastic surgery, Regional Institute of Medical science, Imphal, Manipur over a period of two years from November 2012 to November 2014, patients who were 14 years above presenting within 48 hours with 2nd degree deep normal burns due to scalds, flame on electric burn ay multiple sites and who were willing to participate in the study were included. Therefore those patients age below 14 years, and who late i.e,48 hours were excluded, likewise, patients suffering from malignancy, systemic disease, immunocompromised states were also excluded, altogether, 15 cases presenting with deep dermal burns were taken up for the study.

Procedure And Data Collection:

A detailed case history, physical examination and routine laboratory investigation were conducted, for all the case, information written consent was oftained prior to the stud, similar size deep burn area in the same patient were chosen as study and control wound.

Dimensions of the wound in terns of length and breadth were measured, only 5% of the total burn area was utilised for the study, status of the wound was recorded and photographic documentation done, saline dressing with topical antibiotic was done for both sides. Sample for culture and sensitivity was sent after 48 hours of burn.

Platelets Rich Plasma (PRP) Preparation:

10ml of venous blood was aspiration using a 10ml disposable syringe and equally distribution in two vacutainers each containing 1.5ml of anti coagulant acid citrate dextrose (ACD).the sample was antrifuged at the rate of 3000 rpm for 10 minute resulting in separation of plasma above and RBCs below. Plasma is further composed of platelet poor plasma (PPP) above and platelet rich plasma (PRP) below, by gentle aspiration using a 23 G spinal needle, 40-50% of the supernatant platelet poor plasma is removed and discarded.

The remaining plasma is gentle drawn and pumped back against the separation surface which is repeated 2-3 times to mix as much platelets as possible from the surface. By gentle vortexing, plasma with high concentration of platelets is pepare and kept ready for injection.

Injection of PR: the wound were cleaned with normal saline. PRP was injected at six different sites in the wound in equal amounts, four intra dermal and two sub dermal. Dressing was done with paraffin gauze for both the study and control sites. the wound were photographed and the dimension measured first on admission and subsequently at 2nd and 3rd weeks, by the end of the 2nd week tissue for histopathological examination was send by taking an incisional biopsy from both the study and control sites, the result were compared and analysed.

III. Result

During the study period, 15 patients suffering from second degree deep burn were treated by injected of platelets rich plasma (PRP) majority of patients were in 15-25 years age group (four patients), followed by two patients each in the 26-35 years, 36-45 years and 56- 65 years, two patients were treated in the 46-55 years age category.

Out Of 15 Patients,8 Were Males And 7 Females (Table 2).

Among 15 patients, 13 were Hindus and the remaining Christians. (table 3)

Occupation, seven patients were house wives, three were businessmen, three were manual labourers and two were unemployed, (table 4).

Six out of the 15 patients sustained burns at home accidentally, four patients attempted suicide by burning themselves, four patients caught fire at their place of work, Yet one person sustained electric flash burn in the office (table 5).

On estimating the total body surface area burnt at the time of admission, majority (ten out of fifteen patients) had burn between 10-20%. there patients sustained burns between 20-30% burn (table 6).

Wound swab for culture and antibiotic sensitivity was send after 48 hours of admission, in eight patients swab was sterile. Escherichia Coli, Klebsiella and Staphylococcus aureus were isolated in two patients each only one patients showed Proteus (table 7).

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Table 1: Age group

Age in year	No. Of case
15-25	4
26-35	3
36-45	3
46-55	2
56-65	3

Table 2: sex distribution

Sex	Total No.
Male	8
Female	7
Total	15

Table 3: Religion

Religion	Number of cases
Hindus	13
Christians	2
Muslim	0

Table 4: Occupation

occupation	Number of cases
House wives	7
business	3
Manual labourer	3
Unemployed	2

Table 5: Mode/ circumstance of injury.

Mode of injury	Number of cases
Accidentally at home	6
Suicidal	4
Accidentally at work place	4
Electric burn	1

Table 6: Distribution according to percentage of burns

Percentage of burn	Number of cases
10-20%	10
20-30%	3
30-40%	2

Table 7: Distribution according to microorganism isolated

Microorganism	No. Of cases
none	8
Escherichia coli	2
Klebsiella	2
proteus	1

IV. Discussion

In the prospective study of the years 15 patients who sustains deep second degree burns and admitted to the burn unit were enrolled into the study. Majority of the patients belonged to 15 to 25 years age group of the 15 patients 8 were males and 7 were females.

13 patients were Hindu and the remaining Christians.

All the 7 females patients were house wives, four of them sustained burns in a suicidal attempt and the rest accidentally burnt. Four of the male patients sustained burns at their place of work, while one suffer from electric burn. In five patients, the total body surface area burnt ranged from 20-40% and they were resuscitated within the initial 48 hours. In the remainder of patients the TBSA burnt was below 20% and were hemodynamically stable on admission, Therefore they were allowed ton have oral diet.

Wound swab for culture and sensitivity was reportedly sterile for 8 patients, nevertheless, broad spectrum antibiotics was administered for five days in all patients, none of the patients have any co-mordibities like diabetes mellitus, asthma, tuberculosis etc, pain was the commonest complaint similar to the study by Crane and Evert.

In our study, analgesic or local anaesthetic were use to reduce pain of intradermal or subdermal injections, NSAIDS were not given within 48 hours of PRP injection, as they may inhibit inflammation, prior

explanation about pain during injection was done similar thing was followed in the study done by Bieleckie et al.Blood was collected in vacutainers containing acid citrate dextrose, as an anticoagulant would prevent activation prior to use. The procedure of blood collection to PRP injection took 45-60 minutes in every patients, this was well within the platelets survival time, as shown in the study by Max RE et al, that concentrated platelets remain viable for upto 8 hours, PRP was neit5her activated with thrombin nor calcium chloride, as previous studies had proved that collagen in soft tissue trigger the release of inflammatory mediators.

PRP was injected into 4 intradermal and 2 subdermal location at each study site, Similar injections and was administered as platelet rich fibrin matrix by Schafani et al.

By the end of 2^{nd} week post burn, there was a 50-75% reduction in the dimension of burn wound in five patient; while in 3 cases, 25-50% reduction was noted, only 1 case had complete epithelialisation at the end of 2 week. Thus 43% of the cases shown full healing by 2^{nd} week whereas among the control comparable to those shown by Hao et al,

At the end of 3rd week, 78% of t5he cases and 65% of the controls reported complete healing, these figures agree with the study done by Hao etal where in it was 88% and 73% respectively. P value by paired t-test was (0.000) which was statistically significant. By the end of 3rd week, 11 cases out of 15 manifested complete healing. On the other hand, only 5 out of 15 controls shown complete healing.

By the end of 2nd week, tissue biopsy to monitor epithelialisation, was obtained from both cases and control sites, 11 out of 15 cases manifested epithelialisation with dense fibroblastic proliferation, whereas only 4 out 15 controls epithelialisation, Tissue biopsy taken on the 13th day to look for epithelial thicken and amount of collagen in the study by Kakudo N et al shown similar results.

2nd degree deep dermal burns due to flame on contact are unlikely to heal within 3 week. Therefore, early excision and skin grafting is done to avoid hypertrophic scarring secondary to delayed healing however this procedure is found with the danger of blood ion. Platelet rich plasma may be use in such case. Hao T et al [2010] showed in their study of platelet rich plasma gel following tangential excision of 2nd degree deep burns to produce faster wound healing as compared to control areas. Our study gives faster healing in the PRP injected areas too, with subsequently lesser chances of developing hypertrophic scarring.

V. Conclusion

Wound healing is a complex process of biochemical, cellular and environmental regulation between serum enzyme cascades, growth factors platelets, monocytes, tissue macrophages, fibroblasts etc. Platelets work by releasing potent locally acting growth factors. This study was conducted in the department of plastic surgery, Regional Institute of Medical science, Imphal over a period of 2 years (November 2012 to November 2014) in 15 patients admitted with deep second degree burns to find out the efficacy of platelet rich plasma injection synthesized from their own blood. 43% of PRP injected burns sites healed satisfactorily by the of second week, as compared to 29.5% of the control sites furthermore 78% of PRP injected sites healed completely by the end of third week in contrast to 65% in the control sites. That finding was statistically significant (p value 0.000)

Hence in our study, autologous platelet rich plasma helped the deep 2nd degree burn wounds achieve faster epithelialisation and healing without hypertrophic scarring. It also reduced the requirement of split skin grafting in such burn wounds.

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