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Our Experience with Cell-Based Therapy in Chronic Burn Wounds

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Abstract
Wounds are very common in a surgery practice. They lead to major morbidity and mortality in low socioeconomic countries due to inability to manage them. Management of wounds using Human Umbilical Cord Blood (HUCB) seems a feasible option. 40 patients with chronic wounds were treated in a tertiary care hospital using HUCB after proper informed consent and the results were compared to the use of ionic silver which is one of the most common substances used for wound dressings. At the completion of study, HUCB group showed improvement not only in clinical criteria but also histologically when compared to the control. The results are encouraging but large community trials are needed to further use HUCB at large scale.

INTRODUCTION

Burn injuries are among the most devastating of all injuries and a major challenge for all the health care professionals involved in its management. Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls and interpersonal violence[1,2]. More than 90 percent of burns and over 96% of burn related deaths occur in low to middle-income countries lacking the necessary infrastructure to reduce the incidence and manage severe burns[3,4]. Although the early tangential excision and split thickness skin grafting is the gold standard for managing burn wounds, some patients suffer from graft loss and many others either refuse surgical intervention or remain deprived because of extensive burns or due to lack of infrastructure. In such instances, burn wound modulation by means of extraneous sources such as cord blood is an encouraging and viable option. We present here our experience of using the Human Umbilical Cord Blood in managing chronic burn wounds of more than 6 weeks duration.

MATERIALS AND METHODS

This prospective interventional study was carried out at a tertiary care teaching hospital in 40 patients with chronic burn wounds who signed informed consent for their participation. The study was also approved by the Institute Ethical Committee. All the cord blood donors also signed informed consent for freely willing to donate cord blood and did not opt for its banking.

All patients with full thickness chronic burn wound (>6 weeks old) received human umbilical cord blood (HUCB) as topical application on = 200 cm^2 area with regular dressing and another wound preferably of same size, depth and location in the same patient was dressed with Ionic Silver and taken as control. The patients with Diabetes and grossly infected wounds were excluded. Serial monitoring of the wounds was done with recording of various parameters as per wound assessment chart on a weekly basis.

The Wound surface area was measured with the help of a formalin-sterilized transparent tracing paper placed on the wound surface at day 0 after removing dead tissue if any or after scraping the wound bed only once with gauze. Subsequently, the wound was measured at weekly interval without disturbing the wound bed. This tracing paper was photographed and the area within the marked area was calculated. The nature and the quantity of the exudate and the wound bed-tissue type: i.e. healthy granulation (pink), unhealthy granulation (fluffy red and easy to bleed on touch), over-granulation, or slough (Yellow) was also noted. Wound swab was taken for culture and sensitivity and biopsy from the center of the wound for histopathological examination at the beginning and at end point of the study (4 weeks) in both case and control groups.

Human umbilical cord blood (HUCB) was obtained from the Department of Obstetrics and Gynaecology after obtaining a written informed consent from a HIV, HBs and HCV Ag negative mother. One minute after the delivery, the cord was clamped and transected. Blood was collected from placental end of the cord in a sterile heparinised syringe. Usually 10 ml was collected each time. It was stored at 4-8 °C till application (as early as possible but within 24 hours of collection).

HUCB was applied as whole blood topically on weekly basis on wounds with needle-less syringe drop by drop taking care not to spill from wound margin and after 5 minutes, Paraffin gauze or tulle was applied followed by secondary dressing with sterile gauze and cotton pad. Subsequently, alternate day saline dressing was done till the next application a week later. The control wound was managed with alternate day dressing with Ionic Silver.

Wound assessment, measurements and photographic documentation were done weekly in both the groups (HUCB and controls) at the time of HUCB application.

End Point of Study:

- 4 week duration since first application of cord blood.
- Healed wound.
- Any deterioration of wound.

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RESULTS AND DISCUSSIONS

At the beginning of the study, the mean wound surface area was 71.39 cm^2 in the HUCB group and 62.59 cm^2 in ionic silver group (p = 0.235). Although initially there was no significant difference in the wound area between the cases and controls, it had significant mean reduction in wound surface area of 52.70 cm^2 in HUCB and only 33.18 cm^2 in control groups at the end of the study at 4 weeks (p = 0.001, Table1., Fig. 1).

Wound Bed Quality: During weekly analysis of amount of exudate from wounds, there was no significant difference found between both the groups (p = 0.970) initially, but with the progression of the study significant difference in amount and quality of exudate

was observed at 3 weeks (p = 0.001). In HUCB cases all wounds either healed (36, 90%) with soft and supple scar (Fig.2) or were sterile with healthy granulation tissue and minimum or no exudate and significantly reduced in size (4, 10%., Fig.3). These four wounds also healed subsequently in another 4 weeks with supple scar without any further application of cord blood or surgical intervention. However, in the control group there remained 11 patients (27.50%) who had unhealthy granulation with exudate and positive culture (p = <0.001., Fig.4) and required surgical intervention. By the time of preparing the manuscript, all the patients had more than 3 years of follow-up without any untoward happening like hypertrophic scar or contracture in HUCB group as compared to 65% of the patients developing hypertrophic scar among controls.

Histology: The pre-therapy chronic wounds showed plenty of inflammatory infiltrate and moderate to profound granulation tissue (Fig. 5 A). As the wounds healed, inflammatory infiltrate disappeared mostly and granulation tissue also vanished or remained scanty (Fig. 5 B). The collagen fibers were vertically oriented to begin with and became horizontal in healed wounds. The remarkable observation was the finding of rete pegs (Fig. 6A) and hair follicles (Fig. 6 B) in healed wounds where HUCB was applied.

The wishful goal of treating cutaneous burns for any treating Burn Surgeon is eventually to get the damaged skin replaced with the skin structurally and functionally similar to the one lost in thermal damage. Recent research on stem cells and their clinical application has certainly shown a ray of hope to achieve this goal.

The mean reduction in wound surface area in our patients was significantly greater in wounds treated with HUCB. The unhealed wounds at the end of 4 weeks in HUCB group(4) were significantly less than those in Ionic silver group (11) and had healthy granulation, with minimal exudate and free of bacterial load which was significantly inferior in the ionic silver group. The unique outcome of this study was that the full thickness wounds in HUCB group healed with soft, supple, and durable skin with well-organized collagen lattice and even with regeneration of sweat glands and hair follicles, which was entirely wanting in the ionic silver group. Such regeneration of cutaneous adnexa using stem cell therapy has also been reported by others but by using special approach[5,6]. We believe that application of stem cells to a wound bed not only helps in healing but also prepares the wound with improved neovascularization and healthy granulation with augmented local growth factor production, which altogether achieve rapid closure of the wound with

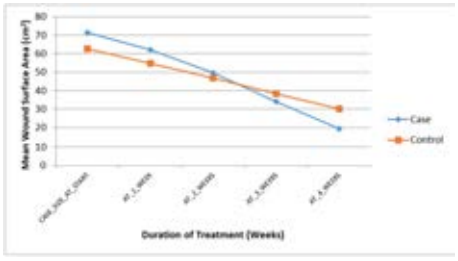

Fig. 1: Graphical representation of comparison of burn wound healing

Fig. 2: Post Electric Burn wound x 9 months
2a. At presentation 2b. After application
2c. Week 1 2d. Week 2
2e. Week 3(post application) 2f. Week 6

Fig. 3: Post burn wound for 14 months
3a. At presentation 3b. After application
3c. Week 1 3d. Week 2
3e. Week 3 (post application) 3f. Week 6

Fig. 4: Post Thermal Burn wound with failure of skin graft x 6 months
4a. At presentation 4b. Week 1
4c. Week 2 4d. Week 4
4e. Week 6

Table 1: Comparison of wound healing: mean reduction in wound surface area

Time of Evaluation	Mean Case Area (cm^2) HUCB Applied	Mean Control Area (cm^2) Ionic Silver dressing	Inter group Comparison Student's t-test
Week 0	71.39±34.15	62.59±21.00	t = 1.201 p = 0.235
1 Week	61.99±30.21	54.63±19.00	t = 1.130 p = 0.263
2 Weeks	49.55±25.16	46.80±16.62	t = 0.497 p = 0.621
3 Weeks	33.95±19.42	38.43±14.07	t = -1.017 p = 0.314
4 Weeks	19.33±12.63	30.01±11.84	t = -3.350 p = 0.001
Total Reduction Achieved	52.70±26.20	33.18±12.31	t = 3.691 p = 0.000

supple scar. We also presume to have better graft take in large and extensive wounds applied with cord blood because of cellular alteration in the wound bed and this view is also supported by Huang and Burd[7].

We found significant reduction in pain suffered by the patients and also in the bacterial load and amount of exudate even before the wounds healed completely, making the quality of life much better in these patients. This also increased the confidence of the patients to continue with the therapy, which they had never heard of. We stopped applying cord blood after four applications in incompletely healed wounds at 4 week stage because the wounds had reduced dramatically in size by the end of the study period and all these wounds healed in another 4 weeks suggesting that the changes once initiated at the cellular level by the stem cells continue for some time.

In an adult, generally the wounded tissue is replaced by scar tissue, often hypertrophic or keloidal in nature and is characterized by disorganized collagen structure, loss of hair follicles and irregular vascular structure. However, the foetal wound heals by replacement with normal tissue through the activity of stem cells[8]. Therefore, one may expect to modify adult wound healing by placing/recruiting stem cells into the wound environment and achieve scarless healing with restoration of skin adnexa.

Stem cells are thought to mediate repair via five primary mechanisms: 1) providing an anti -inflammatory effect, 2) homing to damaged tissues and recruiting other cells, such as endothelial progenitor cells, that are necessary for tissue growth, 3) supporting tissue remodelling over scar formation, 4) inhibiting apoptosis and 5) differentiating into bone, cartilage, tendon and ligament tissue[9,10]. They also cause formation and/or recruitment of new blood cells to the damaged region. The anti-inflammatory effect of stem cells also has the potential for attenuating the systemic inflammatory response following severe burn injuries thereby limiting infectious complications and achieve better outcomes[11,12].

Autologous stem cells intended for regenerative therapy are generally isolated either from the patient's bone marrow (Hematopoietic and Mesenchymal Stem Cells) or from adipose tissue (Mesenchymal Stem Cells). The number of stem cells transplanted into damaged tissue may alter efficacy of treatment. Stem cells may be cultured in specialized laboratories for expansion to millions of cells[13]. Although adipose -derived tissue also requires processing prior to use, the culturing methodology for adipose-derived stem cells is not as expensive and extensive as that for bone marrow-derived cells. The less challenging collection techniques and the multi-cellular micro environment already present in adipose-derived stem cell (ADSC) fractions make it the preferred source for autologous transplantation[14].

Mesenchymal Stem Cells have also been found in cord blood which is in abundance due to large number of deliveries world over. Besides the banking of cord blood which is done in few instances only and not as a routine in many places yet, the remaining amount goes waste. This precious cord blood therefore can be used as an abundant source of multipotent stem cells with certain precautions like screening for common viral markers and HLA typing particularly if cord blood need to be injected. Initial studies have shown that cord blood has 8-18 stem cells/mL[18].

MSCs are the most common and comprehensively studied stem cells for their role in tissue repair and regeneration. These cells are capable to differentiate into cells with the mesodermal, ectodermal and endodermal characteristics[15]. They participate in wound repair and regeneration through direct differentiation or transdifferentiation into tissue-specific cells to regenerate the tissue and are also known to release paracrine factors to stimulate the survival and functional recovery of the resident cells in the wound. However these cells have low immunogenicity[16]. MSCs also enhance angiogenesis and suppress immune response, which make them a potentially therapeutic modality for managing burns and wounds[17].

The important feature of our study was the simplest, easiest, safest and the most cost effective way to use the HUCB as whole blood that was applied

on the wound surface drop by drop with no component separation or addition of any support or delivery system. As the cord blood was used from the booked patients who had undergone screening for all the common viral markers such as HBSAg, HIV 1 and 2 and HCV twice, one at the time of first antenatal visit and another during onset of labour pains, the risk of viral transmission was remote.

On the contrary, others have injected the cord blood in the wound bed or at its margins at multiple places or even transfused it[10]. This is important as our technique of topical application had no chance to allow the host and donor cells to come in contact and so did not require any testing for HLA or ABO incompatibility in spite of the fact that the stem cells present in the cord blood are considered to have low immunogenic potential as mentioned above. The only precaution required to apply it on the wounds is to maintain aseptic condition and therefore it can be carried out even in remote areas. This is of utmost importance in the places with lack of adequate facilities and where the patients can't afford costly treatment or in the patients with extensive burns with lack of skin donor site.

Bone marrow aspirate was also used by many authors for healing of chronic wounds[18]. but we refrained from using it for burn patients as it is technically more demanding and at risk particularly in patients with extensive burns. Moreover, some have found exhaustion of bone marrow and repeatedly negative aspirate in patients with extensive burns[19]. However, Huang and Burd applied autologous bone marrow aspirates to chronic unhealed burn wounds and also on slow-healing donor site wounds and observed a dramatic healing response.

The cord blood is not only a rich source of hematopoietic stem cells with naïve immune status for clinical application[20], non-hematopoietic stem cells are also found in umbilical cord blood and have been demonstrated to be able to differentiate into epithelial cells under both in vitro and in vivo condition[21-23].

Although we are convinced with the results of topical application of HUCB on chronic burn wounds, some questions still remain unanswered. One has no clue as to what should be the amount of cord blood per unit wound surface area or the stem cell density and how frequently it should be applied. The range of transplanted stem cells recommended is between 1 × 106 and 3 × 106 cells[22,24]. but others have used it in much higher concentration. For instance Bliley *et al*. transplanted 6.8 × 106 Adipose tissue derived stem cells (ADSCs) after twenty-four hours of the burn[25] and Franck *et al.* injected 3.2x106 ADSCs twice, once immediately after the burn and again after 4 day[26] Cord blood may also be used as component therapy in form of plasma and cells separately or by expanding the stem cells through culture. Cord blood cells may also be combined with platelet rich plasma as combination therapy. To retain the transplanted stem cells in the wound, delivery vehicles have also been used[27,28]. Although adoption of all these techniques may improve the outcome, the results found with our simple technique without all these costly maneuvers needs attention.

We agree that one has to wait for the long-term follow-up before the doubt about their potential of unending division and their consequences is clear. Lots of questions need to be answered such as how frequently and how to apply (the route), in what concentration and dose, and whether was there any risk of developing tumor in long run. Encouraged by our initial results we have also used HUCBs in moderately large wounds with amazing results and we hope the same advantages may be extrapolated to wounds of any size and any nature, may be with alteration in the amount, composition, frequency and technique of application. It might also be possible to use multiple sources of HUCB at the same time either separately or in pooled form while using our technique of simple topical application.

CONCLUSIONS

We found very encouraging results in our study. The outcome of our study may have a dramatic impact on management of burns if others also validate the real potential of such an easy approach. Although ours is a pilot study with good results, it needs approval from competent authorities to make it widely acceptable and useful for patients at a large scale. From social point of view particularly in countries like India where the placenta and the cord are considered a waste, its acceptability as a therapeutic modality would need some years. It still remains to be included as the tested and accepted treatment for chronic burn wounds by the health professionals and academicians.

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