Alteration in transforming growth factor-β1 gene expression in hypertrophic scar

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In human, development of hypertrophic scar is a complex interaction of growth factors, cytokines and abnormal processes of wound healing. The exact role of transforming growth factor beta (TGF-β) in pathogenesis of scar has not been well looked into Indian setup. Hence, the present study becomes imperative to understand its pathogenesis with the aim to evaluate the role of cytokine, such as, TGF-β1, before and after 6 months of application of a herbal cream. This study is intended to find out the differential expression of TGF-β1 gene after the herbal cream treatment and improvement in the hypertrophic scar. The total RNA was isolated from the samples using Trizol and cDNA was prepared for RT-PCR analysis. The expression of TGF-β1 was high in 72.5% cases of hypertrophic scar, while 27.5% cases showed lower expression before treatment. After 6 months of application of the herbal cream, the expression of TGF-β1 gene was significantly down regulated (77.5%), while no changes were observed in 22.5% of hypertrophic scar cases. The variation in the gene expression of TGF-β1 could be due to several factors, such as, different age group, depth of injury and delay in healing of wounds. However, the efforts have been made to cure such patients as they suffer from psychological problems, especially with the scars on exposed parts of the body including face.

Keywords: Cytokine, hypertrophic scar, transforming growth factor beta (TGF-β), wound healing

Introduction

Hypertrophic scarring is an uncharacteristic feature of the normal processes of wound healing. It is very important to understand the normal wound healing process for deciding the proper treatment of hypertrophic scar. Cytokines play important role primarily as mediators of inflammatory and immunomodulatory reactions. Annually, over 1 million people require treatment for burns in the United States and 4 million patients in the developed world acquire scars each year as a result of burns. The incidence of hypertrophic scar is more in developing countries like India due to delayed surgical intervention, poor economy and less literacy. Previous studies reported diverging incidence of hypertrophic scarring that varied from 40 to 94% following surgery and from 30 to 91% following burns14. Transforming growth factor-beta (TGF-β) family is potent regulator of multiple cellular functions, including cell proliferation and differentiation. TGF-β has surfaced from abundant research as a key signal in orchestrating wound repair. The healing process at the injured site, which normally results in the formation of a scar, is an extremely complex process involving numerous cell types, cytokines and ECM (extracellular matrix) components5. Excessive blood loss from injured blood vessels is prevented by the formation of a blood clot, which further acts as a provisional wound matrix that attracts and guides inflammatory cells, endothelial cells, fibroblasts, and keratinocytes. Together they form new blood vessels, produce ECM and create a new layer that covers the surface of the wound. The surface of burn wounds differs from other type of wounds, as they are characterized by coagulation of superficial blood vessels and usually do not tend to bleed excessively5. In some individuals, and predominantly in burn sufferers, the wound healing process may lead to excessive production of ECM, resulting in a raised hypertrophic scar6,8. They are easily identified by colour mismatch, stiffness and rough texture. Many fibroblasts transform into myofibroblasts, which are considered to initiate collagen deposition and wound contraction9.

In the process of wound healing, keratinocytes play an important role as a key regulator of skin inflammation and remodeling. Keratinocytes have the capacity to produce growth factors, while cytokines promote chemotaxis, activation of macrophages and inflammatory cells, and proliferation of fibroblasts10-12. The hypertrophy of the scar has been associated with inflammatory cell adhesion through stimulation of fibroblast proliferation and excessive matrix

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production. Hence, the study of cytokine gene regulation becomes imperative in such clinical cases.

Material and Methods

Collection of Samples
We studied 40 hypertrophic scar cases of burn of all age group. The control group consisted of 10 unrelated healthy individuals. All the patients with hypertrophic scar ≥ 18-month-old in duration were included in the study. All the cases were followed up for 6 month with the herbal cream application. Samples were collected before starting the herbal cream application and then after 6 month of continuous application. Blood (1.0 mL) and tissue biopsies were collected from the department of Plastic surgery, Institute of Medical Sciences, BHU, Varanasi. The Institute’s ethical committee approved the protocol and informed written consent was obtained from the attendants/patients. Clinical assessment was carried out at the same time by using Vancouver Scar Scale.

Reverse Transcription PCR
The total RNA was extracted from samples using Trizol (Sigma, USA) and quantified on spectrophotometer. They were qualitatively analyzed on 1% agarose gel, followed by gel doc system. RT-PCR was performed according to an established protocol. The cDNA synthesis was carried out in 20 µL volume containing 5 µg total RNA, 4 µL RT-buffer, oligo dT 0.5 µg/µL, dNTP (10 mM) and 200 U of reverse transcriptase enzyme (Fermentas, USA). RT-PCR reaction was carried out by using forward and reverse oligonucleotide primers of TGF-β1 gene in total reaction volume (25 µL) containing 10× Tris (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 2.5 mM dNTP, 10 pM primers, 50-100 ng cDNA, and 3 U Taq DNA polymerase. PCR thermal cycling condition was 4 min of initial denaturation at 95°C, followed by 35 cycles at 95°C for 30 sec, at 60°C for 30 sec, at 72°C for 30 sec, and a final extension of 7 min at 72°C. PCR amplified products were visualized on 1.5% agarose gel stained with ethidium bromide and followed by gel documentation system.

Results
The present study evaluated the hypertrophic scars by two parameters, one by Vancouver Scar Scale (VSS) score and second by TGF-β1 gene expression. The difference in hypertrophic scar before and after its improvement with the herbal cream application was significant. It was reddish-pink, thick and hard before the application of herbal cream, while it became softer, pale and flattened six months after its continuous application.

VSS Score Assessment
VSS for scar evaluation was more practical and validated tool of our study for clinical assessment of hypertrophic scar. Different parameters were used for the assessment of hypertrophic scar before and after treatment, i.e., pliability, vascularity, pigmentation and thickness (Table 1).

VSS score was calculated individually for each patient at the beginning and after 6 months at the end of treatment (Table 2). The mean VSS score was 9.54±2.44 and, after 6 months of herbal cream application, it came down to 5.32±2.87, thus showing significant improvement.

The response to the treatment was rated on VSS as excellent, good, mild and no response based on the following guidelines. An excellent response was given when the scar scale changed 8 or more points after treatment, a good response for an improvement of between 4 to 7 points, mild response for an improvement of between 1 and 3 points, and no response was assigned to those patients who had no change in the VSS (Table 3).
6 month’s follow up. We also included 10 skins and blood samples of normal individuals as control. The expression of TGF-β1 was high in 72.5% (n=29) of cases of hypertrophic scar, while 27.5% showed low expression (but higher than that in controls) before the treatment of herbal cream. After the 6 months of cream application in the follow up cases, the expression of TGF-β1 gene was significantly down-regulated (n=31, 77.5%), while no changes were observed in remaining cases. The expression of TGF-β1 gene was also significantly lower in control group.

Statistical analysis was carried out using student’s t test. A significant difference was observed (p<0.05) between expression of TGF-β1 in tissue before and after herbal cream application. We also observed statistically significant difference ((p<0.001) in VSS scores and found improved clinical outcome in hypertrophic scars after 6 month of the treatment. However we could not obtain any correlation between gene expressions in blood and clinical outcome.

Discussion

Scarring, a consequence of dermal injury whether due to surgery or trauma including burns, has a wide spectrum of clinical manifestations. Post-burn hypertrophic scar is a serious clinical problem for plastic surgery and rehabilitative medicine. It frequently causes severe cosmetic and functional impairment in burn patients. Hypertrophic scar has been reported to have variable incidence rate from 40 to 94% following surgery and from 30 to 91% following burns. The functional significance of cytokines in dermal-epidermal interplay to regulate tissue repair and homeostasis is poorly understood. These cytokines have been primarily interpreted as mediators of inflammatory and/or immunomodulatory reactions. TGF-β1 is a multifunctional cytokine produced by platelets, macrophages, lymphocytes, fibroblasts and many other types of cells. TGF-β is considered to be the most potent and ubiquitous profibrogenic mediator out of all cytokines and plays an important role in almost every aspect of plastic surgery.

Among the various parameters used for clinical assessment, thickness and pliability improved before the improvement could be observed in pigmentation and vasularity. The present study is the preliminary work of TGF-β1 gene expression in hypertrophic scar cases and their treatment with herbal cream for the management of such type of scars. The present study showed increased expression of TGF-β1 gene in hypertrophic scar cases. These results are consistent with the findings of Lu et al where they showed an increased expression of TGF-β1 in hypertrophic scars. In our country while no study had been reported earlier about the treatment of herbal cream and the examination of TGF-β1 gene expression in patients with hypertrophic scars, a good number of cases outside showed lower expression of TGF-β1 gene expression after the treatment with herbal cream. The different results might be attributed to the different methods used or to differences in age and biopsy site of skin specimens. This makes us conclude that our herbal cream had some anti TGF-β1 property, which lowered the concentration of cytokine bringing it to the level of that preset in normal skin. From this study, it appears that the herbal cream might have suppressed the TGF-β1 gene at molecular level.
This study is being extended further to know the expression of other isoforms of TGF-β and also to evaluate other cytokines to see the impact of herbal cream and also other treatment modalities, such as, pressure garments, Triamcinolone injections and silicone gel sheet. It would also be worth trying to use the herbal cream as a primary topical therapy for burn wounds and look for a possible scar less healing.

References