|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online atwww.ijmbs.info
PubMed (National Library of Medicine ID: 101738825)
Index Copernicus Value 2018: 75.71
Original Research Article
Volume 3, Issue 7; July: 2019; Page No. 149-155

ALTERATION IN GENE EXPRESSION OF TRANSFORMING GROWTH FACTOR- B FOLLOWING TREATMENT OF HYPERTROPHIC BURN SCARS WITH THREE DIFFERENT THERAPEUTIC MODALITIES

Vaibhav Jain[1], Jyoti Gupta[1], Neeraj Gupta[2], Pradeep Jain[1]
[1]Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi
[2]Institute of Bioscience and Technology, Sri Ram Swaroop Memorial University, Deva Road, Lucknow

Article Info: Received 23 June 2019; Accepted 21 July. 2019
DOI: https://doi.org/10.32553/ijmbs.v3i7.403
Address for Correspondence: Prof. Pradeep Jain. Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India
Conflict of interest: Nil

Abstract

Background: Hypertrophic scar (HTS) is a dermal form of fibro-proliferative disorder often caused by thermal injury to the deep dermis. Transforming growth factor β1 & 2 are well known pro-fibrotic cytokines promoting ECM production and tissue fibrosis. The present study was designed to evaluate the different therapeutic modalities for management of hypertrophic scar and correlate it with altered expression of TGF beta gene at the molecular level.

Materials and Methods: One hundred and twenty patients with hypertrophic post burn scar were randomly distributed into three different treatment groups of Pressure Garments, Silicone Gel Sheet and Triamcinolone Injection. Total RNA was isolated from the scar tissue in cases before and 6 months after the therapy and from normal skin in controls to evaluate the expression of TGF beta (1, 2 & 3) by real time PCR.

Results: Following treatment, the expression of TGF β-1 & 2 was down regulated while that of β-3 up regulated. The overall positive response (combining all the groups) was 94% out of which, 16.6% were cured, 47.5% showed major improvement and minor changes were observed in 30.8% of patients.

Discussion: All the three modalities of treatment were effective in bringing down the level of TGF β-1&2 and in up-regulating antifibrotic β3 and this correlated well with the clinical improvement in the scar thickness, pliability etc.

Conclusion: Out of all, intralesional Triamcinolone Injection achieved the best result.

Keywords: Hypertrophic Scar, Transforming Growth Factor β, Pressure Garments, Silicone Gel Sheet, Triamcinolone Injection

Introduction

Abnormal wound healing represents a tremendous clinical challenge and causes significant burden on patients and healthcare professionals. Delayed and prolonged process of wound healing leads to abnormal scar formation like hypertrophic scar that does not extend beyond the initial zone of injury. These scars may cause abnormal sensations including pain, itchiness, severe functional impairment, and psychological morbidity.[1,2] The incidence of hypertrophic scar ranges from 40% to 94% following surgery and from 30% to 91 % following burns.[3-5] Transforming growth factor beta (TGF- β) is a cytokine that plays a central role in growth, differentiation, development and immune response and is involved in all stages of wound healing.[6] It is also important for migration of Keratinocytes and regulation of wound re-epithelialization.[7]

TGF-β1 & 2 are one of the most important stimulators of collagen and proteoglycan synthesis and affect the extra cellular matrix (ECM) not only by stimulating collagen synthesis but also by preventing its breakdown .[8,9] In contrast, TGF-β3, which is predominantly induced in the later stages of wound healing, has been found to reduce connective tissue deposition. The increased expression of TGF-β1& 2 leads to an excessive deposition of collagen by fibroblasts and less collagenase activity which may play an important role in formation of hypertrophic scar.[10,11]

The aim of this study was to evaluate three different treatment modalities in different groups of cases and to find the best therapy in reference to

altered expression of Transforming Growth factor beta.

Material and Methods

Collection of samples: We studied 120 patients who had received no treatment for hypertrophic burn scars in the past six months. All the patients had hypertrophic scar ≥18 months post burn. There were 68 females with mean age of 35 years and 52 male patients with mean age of 38 years (range 12-65 years). The control group consisted of 30 unrelated healthy individuals of similar age and sex who were operated for some other plastic surgical procedure. Full thickness skin pieces (5 mm) were collected at the time of biopsy or surgery before starting the treatment and then after 6 months of therapy. The Institute's Ethical Committee approved the protocol and informed consent was obtained from each patient.

Treatment of the Hypertrophic Scar cases: All the patients were randomly distributed into three treatment groups, A, B & C each containing forty cases and their treatment was continued for 6 months. The patients in Group A were treated with custom made pressure garment exerting 25 mm Hg pressure for 23 hours a day. In group B, Silicone gel sheet, (SGS; Nagor, USA) was applied over the hypertrophic scar continuously for 23 hours a day with ½ an hour break twice in the day. Intralesional injection of Triamcinolone (TAC) 40 mg/dose at every 4weeks was used in the third group of hypertrophic scar cases, Group C.

Real-time PCR : Real-time qRT-PCR was performed on BioRad iCycler (Bio-Rad, California, USA) using the BioRad iQ™ SYBR Green Supermix. Total RNA from tissue biopsies was isolated using Trizol reagent (Sigma, USA), and 2µg of total RNA was reverse-transcribed to cDNA that served as a template using standard reagents (MBI Fermentas, Ontario, Canada). The amplification mixtures contained 1.0 µl of 1:5-diluted cDNA template, 6.25 µl SYBR Green PCR Master Mix Buffer (2X), and 10 pmol of forward and reverse primers of TGF beta gene (1, 2 & 3) in a total volume of 12.5 µl. The cycle was initiated at 95°C for 10 minutes and then each of the 40 amplification cycles consisted of a 10 second denaturation step at 95°C followed by a 30 second annealing at 60°C and extension at 72˚C for 30 seconds. All PCR reactions were studied in duplicate and housekeeping gene β-actin were used to allow relative quantification, along with one non template control (NTC) sample. Melting curves were generated after each run to confirm amplification of specific genes. The threshold cycle (Ct) value was calculated at the cycle where the fluorescence of the sample exceeded a threshold level. Relative quantification of gene expression was done with the normalization of data with endogenous control β-actin.

Vancouver Scar Scale Assessment: Clinical assessment was carried out using Vancouver Scar Scale (VSS), a validated subjective scale to document a change in appearance of the scar. [12] The VSS is composed of four parameters-pigmentation, vascularity, pliability, and height as described in the Table 1. The results were recorded from 0-13 points where 0 reflected normal skin. [13] Scar assessment was performed at the beginning of the treatment, and at the end of the sixth month, Table 2.

Statistical analysis: All data of hypertrophic scar cases and controls were presented as mean ± SD (standard deviation). Student t-test was used to compare between two means of hypertrophic scar cases before and after treatment, and to find out the significant difference from each other. ANOVA was applied to evaluate significant differences in mean among the groups. Post hoc multiple comparison tests were also done to analyze the significant difference between the pair of means in pre treated and post treated cases. The mean and standard deviation of VSS score was also determined. Changes in VSS components – pigmentation, vascularity, pliability and height, before and after treatment differences were analyzed by using student t-test. SPSS version 21.0 (IBM Corp., Armonk, NY, USA) was used for all of the statistical analysis. Probability levels $P < 0.05$ were considered statistically significant.

Results

The gene expression of transforming growth factor beta (1, 2 & 3) was investigated in 120 cases of hypertrophic scars before and after 6 months of therapy by real-time quantitative PCR. The study observed the higher mRNA expression of TGF β-1 (2.6 ±0.37 fold) & TGF β-2 gene (3.8 ± 0.40) in pre-treated hypertrophic scar cases when compared

with controls. Whereas, TGF β-3 gene (0.44 fold) expression was lower.

After using Pressure garments for 6 months, the mRNA expression of only TGF β-1 was significantly downregulated (t=12.61, df=39, r=0.34* & p=0.01) as shown in Fig. 1.

After the application of Silicone gel sheet, the mRNA expression of TGF β -2 gene was significantly downregulated (p=0.01) as in Fig. 2. However, TGF β -3 gene showed in-significant upregulation of the mRNA expression.

Treatment with Intralesional Triamcinolone resulted into significant down-regulation of the mean mRNA expression of TGF β-1(1.86±0.19) and TGF β-2 (2.08±0.33) gene but only insignificant increase in mRNA expression of TGF β-3 gene when compared with pre therapy levels as represented in Fig. 3.

Vancouver Scar Scale assessment and rate of response to the treatment: The data of Vancouver scar scale in regards to four components and their mean values was calculated. The decreasing mean value of the score indicates clinical improvement in the scar. The difference between before and after treatment scores for each of the three groups was statistically significant ($p<0.05$). The study observed that the most affected component of the Vancouver scar scale was height, and then vascularity and pigmentation for all these treatment methods. The mean score with all three different therapies has been represented in Table 2. The rate of response was best with Triamcinolone and least with Pressure Garments.

Table 1: The Vancouver Scar Scale parameters and their score.

Scar Characteristics	**Score**
Vascularity	
Normal	0
Pink	1
Red	2
Purple	3
Pigmentation	
Normal	0
Hypopigmentation	1
Hyperpigmentation	2
Pliability	
Normal	0
Supple	1
Yielding	2
Firm	3
Ropes	4
Contracture	5
Height (mm)	
Flat	0
<2	1
2-5	2
>5	3
Total Score	13

Table 2: Paired t-test for comparison between pre and post treatment mean values of Vancouver Scar Assessment Scale of the three different treatment groups.

VSS parameters	Before therapy Mean±SD	After therapy Mean±SD	Mean difference	t	p value
Pigmentation					
Group A	2.15±0.62	1.225±0.69	0.92	5.7	0.05
Group B	2.20±0.75	0.65±0.48	1.55	12.5	0.04
Group C	2.30±0.64	0.67±0.47	1.62	15.4	0.01
Vascularity					
Group A	2. 52±0.50	1.32±0.97	1.20	6.0	0.01
Group B	2.53±0.65	0.95±0.71	1.57	9.8	0.01
Group C	2.47±0.55	0.87±0.75	1.60	8.6	0.0001
Pliability					
Group A	2. 97±0.80	1.05±0.67	1.92	11.6	0.05
Group B	2.82±0.84	0.82±0.44	2.0	15.4	0.02
Group C	2.8±0.79	0.95±0.59	1.85	13.1	0.01
Height					
Group A	2. 25±0.49	0.92±0.57	1.32	9.7	0.01
Group B	2.02±0.57	0.65±0.53	1.37	13.3	0.01
Group C	1.80±0.51	0.57±0.50	1.22	12.5	0.01

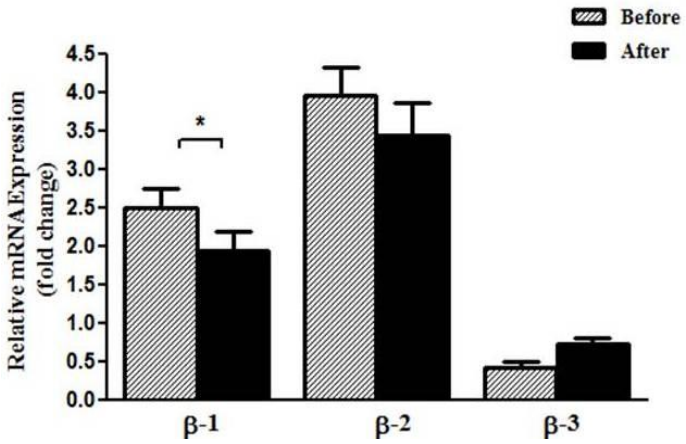

Figure 1: TGF- β 1, 2 & 3 gene expression in cases before and after pressure garments therapy. The expression of β-1 and β-2 decreases, while up-regulation of β-3 gene was observed.
Figure 1

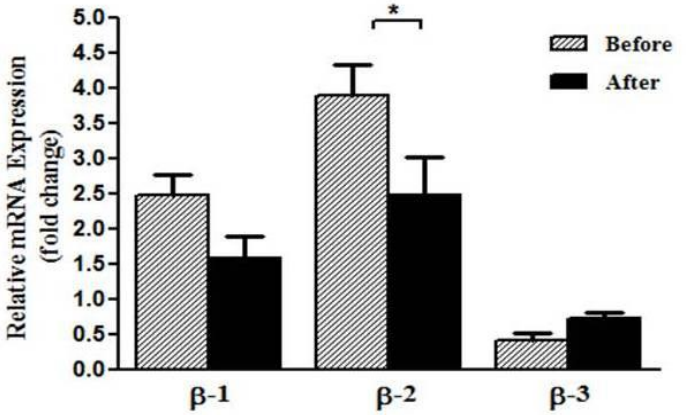

Figure 2: The expression of β-2 gene after SGS treatment significantly decreases. While, β-3 gene expression was insignificantly up regulated.

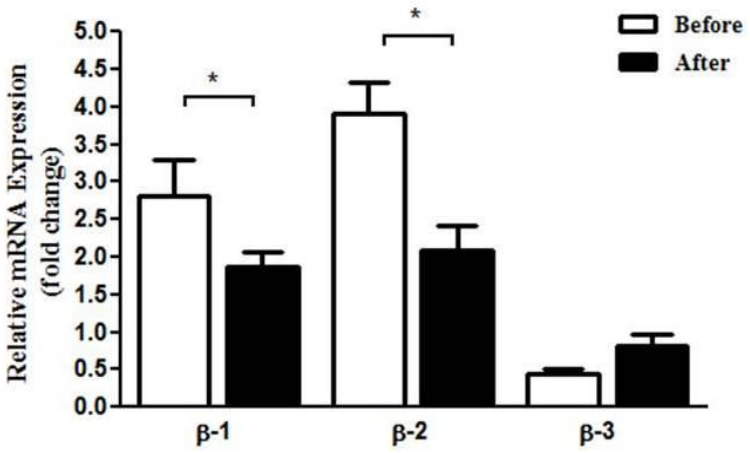

Figure 3: Altered expression of TGF beta gene after Triamcinolone injection therapy. The mRNA expression of TGF β-1and β-2 genes showed significant ($p<0.05$) down regulation.

Discussion

Since the hypertrophic scars have been found to be difficult to treat, different therapeutic modalities were used in the study to evaluate the better option for effective treatment of such type of scars. Transforming growth factor beta (TGF-β) pathway plays a very well known role in collagen synthesis and fibrosis. The present study demonstrates the alteration of TGF β (1, 2 & 3) gene expression in relation to the formation of hypertrophic scars. Our investigations showed that in hypertrophic scars the gene expression of TGF β- 1 & 2 was higher while TGF β-3 gene expression was lower in comparison to controls. Similar findings were also observed in a study of Lu et al. [14.]

Three different therapeutic modalities were used in the study to investigate the altered expression of TGF beta gene in post-treated hypertrophic scar cases and compare it with pre-treated levels. After the pressure garments therapy, TGF β -1 gene expression was decreased significantly in hypertrophic scar cases. Atiyeh et al also observed that the Pressure significantly reduced TGF-β1 secretion, similar to our finding.[15] The study observed 90 % of cases confirmed the response to the treatment in which 12.5 % of cases claimed to be cured. The other patients who did not respond to the therapy might be due to, below desired level of pressure (25 mm Hg) exerted by pressure garments which is difficult to monitor consistently.

After the application of Silicone Gel sheet significant down-regulation of TGF β -2 gene was observed as also reported by Kuhn et.al.[16] The hypertrophic scars, before the therapy, were reddish-pink, thick and hard and became pale, flattened and softer 6 months later in 92% patients in which 47.5% cases showed major improvement and 17.5% were completely cured. Berman and Flores have also reported the reduction of redness, itchiness and tenderness. [17] It is hypothesized that there is decreased capillary activity which down regulates fibroblasts' activity, reduces collagen deposition and scar thickness. [18,19] Other studies reveal that silicone gel sheeting prevents recurrence of abnormal scarring in 79–100% of patients.[20,21] Various authors suggested that silicone gel sheeting has an important role in scar management. It is a safe and effective management option for hypertrophic scars.[22] However, the cost is a prohibitive factor for some. It also needs to be properly secured and cleaned repeatedly to enhance its life and effectiveness.

Intralesional injection of Triamcinolone (TAC) was also used as emerging treatment strategy in hypertrophic scar cases which showed that the TGF β-1 & 2 both gene expression was reduced significantly in comparison to pre-treated cases. Insignificant enhancement of TGF β-3 expression with all the three common therapeutic modalities described so far leads one to presume that antifibrotic TGF β-3 takes longer time to reflect any change in its level. A number of studies reported the rate of response of TAC vary from 50–100% with a recurrence rate of 9–50% .[23] Our study had the 100% rate of response with Triamcinolone from

the patients in which 20% of cases were cured and 52.5% of cases responded with major improvement. Down-regulation of TGF β-1 & 2 brought relief to the patients, with decreased height, pigmentation, and vascularity. Even pliability of the scars, which was found by many others to be more resistant to change, improved to patients' satisfaction following injection.

In our study there was no drop out and all the parameters were studied. A great number of patients rated their better response to the different therapies. The study also calculated the overall effectiveness of the all three therapies on the basis of rate of response. The study observed major improvement in 47.5% of cases, minor changes in 30.8%, and cure in 15.8%. When the rate of response was studied with all the three therapies and correlated with the treatment modality used, expression level of TGF beta gene was found to have maximum reduction with the intralesional Triamcinolone therapy as compared to other two treatment modalities. This better understanding will facilitate further research into this promising field and may help to promote the development of pharmacological interventions that could ultimately prevent, reduce, or even reverse scar formation or progression.

Acknowledgment

Authors are thankful to all the patients and their attendants for their participation in the study.

References

1. Gangemi EN, Gregori D, Berchialla P, Zingarelli E, Cairo M, Bollero D, Ganem J, Capocelli R., Cuccuru F, Cassano P, Risso D, Stella M. Epidemiology and risk factors for pathologic scarring after burn wounds. Arch Facial Plast Surg 2008; 10: 93-102.
2. Schneider JC, Holvanahalli R, Helm P, Goldstein R, Kowalske K. Contractures in burn injury: defining the problem. J Burn Care Res, 2006; 27: 508-14.
3. Lewis WHP, Sun KKY. Hypertrophic scars: a genetic hypothesis. Burns 1990;16:176-8.
4. Bombaro KM, Engrav LH, Carrougher GJ, What is the prevalence of hypertrophic scarring following burns. Burns 2003; 29: 299-302.
5. Gupta J, Patel A, Jain P. Alteration in Transforming growth factor- β1gene expression in hypertrophic scar. Ind J Biotech 2014; 13: 314-7.
6. O'Kane S, Ferguson MW. Transforming growth factor beta s and wound healing. Int J Biochem Cell Biol 1997; 29: 63-78.
7. Ramirez H, Patel SB, Pastar I. The Role of TGF b Signaling in Wound Epithelialization. Advances In Wound Care 2014; 3: 482-91
8. Szulgit G. Alterations in fibroblast alpha1 beta1 integrin collagen receptor expression in keloids and hypertrophic scars. J Invest Dermatol 2002; 118: 409-15.
9. Kose O, Waseem A. Keloids and hypertrophic scars: are they two different sides of the same coin?. Dermatol Surg 2008; 34: 336-46.
10. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J Cell Sci 1995;108: 985-1002
11. Tredget EE, Shankowsky HA, Pannu R., Nedelec B, Iwashina T, Ghahary A. Transforming growth factor-beta in thermally injured patients with hypertrophic scars: effects of interferon alpha-2b. Plast Reconstr Surg 1998; 102:513-7.
12. Roques C, Teot L. A critical analysis of measurements used to assess and manage Scars. Int J Low Extrem Wounds 2007; 6:249-53.
13. Baryza, MJ, Baryza, GA. The Vancouver Scar Scale: an administration tool and its Inter-rater reliability. J Burn Care Rehabil 1995;16: 535-8.
14. Lu L, Chen YL, Zhang QG. Distribution and expression of transforming growth factor beta and receptors in hypertrophic scar. Zhonghua Shao Shang Za Zhi 2004; 20: 30-3.
15. Atiyeh BS, Al-Amm CA, El-Musa K. Improved scar quality following primary and secondary healing of cutaneous wounds. Aesth Plast Surg 2003; 27: 411-7.
16. Kuhn MA, Moffit MR, Smith PD, Lyle WG, Ko F, Meltzer DD. Silicone sheeting decreases fibroblast activity and downregulates TGFbeta2 in hypertrophic scar model. Int J Surg Investig 2001; 2: 467-74.
17. Berman B, Flores F. Comparison of a silicone gel-filled cushion and silicone gel sheeting for

the treatment of hypertrophic or keloid scars. Dermatol Surg 1999;25: 484-6.

18. Quinn KJ, Evans JH, Courtney JM, Gaylor JDS. Non-pressure treatment of hypertrophic scars. Burns 1985; 12: 102-8.

19. Suetake T, Sasai S, Y-X Zhen, Tagami H. Effects of silicone gel sheet on the stratum corneum hydration. Brit J Plast Surg 2000; 53: 503-7.

20. Dockery G.L., Nilson RZ. Treatment of hypertrophic and keloid scars with Silastic Gel Sheeting. J Foot Ankle Surgery 1994; 33(2): 110-9.

21. Katz B. Silastic gel sheeting is found to be effective in scar therapy. Cosmetic Dermatology 1992; 6: 1-3.

22. Poston. J. The use of silicone gel sheeting in the management of hypertrophic and keloid scars. J Wound Care 2000; 9: 10-6.

23. Niessen FB, Spauwen PH, Schalkwijk J, Kon M. On the nature of hypertrophic scars and keloids: a review. Plast Reconstr Surg 1999; 104: 1435-58.