

# **Study of Intralesional Triamcinolone Alone and in Combination with Pressure Garments for the Treatment of Hypertrophic Scars and Correlation of Outcome with Expression of Transforming Growth Factor Beta**

Jyoti Gupta<sup>1</sup>, Vaibhav Jain<sup>2</sup>, Santosh Singh<sup>3</sup>, Neeraj Gupta<sup>4</sup>, Pradeep Jain<sup>5</sup>

Research Scholar, Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India<sup>1</sup>

Senior Resident, Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India<sup>2</sup>

Junior Research Officer, Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India<sup>3</sup>

Assistant Professor, Institute of Bioscience and Technology, Sri Ramswaroop Memorial University, Lucknow Deva Road, Barabanki, UP, India<sup>4</sup>

Professor, Department of Plastic Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India<sup>5</sup>

**ABSTRACT:** Delayed process of wound healing leads to hypertrophic scarring especially in burn victims which prevents normal function of the affected body part. Various efforts made by the researchers to understand the mechanism of the abnormal healing and to find the best method to treat and control formation of hypertrophic scars have still not resulted in arriving at uniformly accepted method. Therefore, the present study has been designed to find out the efficacy of Triamcinolone alone and in combination with pressure garments therapy and correlate its outcome with expression of TGF beta gene which is supposed to be profibrotic. Eighty cases with hypertrophic scars were divided into two groups each containing forty cases. The total RNA was isolated from the cases before the treatment and at six months after the therapy using Trizol. The cDNA was synthesized for the quantitative gene expression analysis of TGF beta gene (1, 2 & 3) by Real time PCR. The mRNA expression of TGF  $\beta$ -2 gene was highest i.e. 3.9 fold increase when compared with controls. At the 6 months of follow-up, the mRNA expression of TGF beta-1 & 2 was significantly downregulated ( $p < 0.05$ ) in both Groups, when evaluated with pre-therapy cases. Complete improvement was observed in 22.5 % of cases after the combined treatment with PG+TAC. This study concluded that combination treatment of pressure garments and Triamcinolone was more efficacious and acceptable to patients with hypertrophic scars and produced better results as compared to triamcinolone injection alone.

**KEYWORDS:** Hypertrophic scar, Transforming Growth Factor- $\beta$ , Gene expression, Triamcinolone, Pressure Garments.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 2, February 2017

## I. INTRODUCTION

Skin is the largest organ of the body and it plays important roles in the wound healing process. Wound repair occurs with an initial inflammatory response, re-epithelialization, and the formation of a normal scar. Hypertrophic scarring results from aberrant behavior of the normal process of wound healing after burn injury. The hypertrophic scars not only have thick, coarse and itchy skin but also may at times prevent normal function of the affected body part. In burn victims, the wound healing process may lead to excessive deposition of collagen in the dermis and production of overabundant extracellular matrix (ECM), resulting in a raised hypertrophic scar. They are easily identified by color mismatch, stiffness and rough texture. Hypertrophic scars affect 1.5% to 4.5% of the general population. However, the exact prevalence of hypertrophic scarring in burn victims is really unknown. The incidence of hypertrophic scar occurrence following injuries has been reported in a range varying from 32-94%; 40 to 94% following surgery and 32 to 91% following burns [1-3]. The prevalence of hypertrophic scar is more in developing countries like India due to delayed surgical intervention, poor economy and less literacy [4]. Many factors that influence the severity of hypertrophic scar are genetic predisposition, race, anatomical location of the burn, age and depth of the burn [5]. Cytokines play important role primarily as mediators of inflammatory and immunomodulatory reactions. Transforming Growth factor beta is important for re-epithelialization, inflammation, angiogenesis, and granulation tissue formation during wound healing. TGF- $\beta$  iso-forms have different biological activities in wound healing and also play an important role in the pathogenesis of hypertrophic scarring via distinct molecular mechanisms [6-8]. Both TGF  $\beta$ -1 and TGF  $\beta$  -2 promote fibrosis and scar formation, whereas TGF  $\beta$  -3 is neither scar inducing nor reducing [9-12]. Numerous methods have been described for the treatment of hypertrophic scars, but it is yet to find the one with consensus agreement. The objective of the present study was to investigate the efficacy of a polytherapy regimen vs. monotherapy in hypertrophic scar cases such as Intralesional injection of Triamcinolone alone or in combination with Pressure garments and finds its correlation with expression of TGF beta gene in hypertrophic scars.

## II. RELATED WORK

Niessen et al [6] hypothesized that impaired production of keratinocyte-derived growth factors, such as IL-1 $\alpha$ , led to a decrease in the catabolism of the dermal matrix, whereas augmented epidermal PDGF production led to increased formation of the dermal matrix in hypertrophic scars. These observations supported the possible role of the epidermis in preventing the formation of hypertrophic scars. Babu et al [7] concluded that TGF-beta 1 stimulation of fibronectin production in keloid cells was likely to involve a transcriptional mechanism and keloid overproduction of extracellular matrix components might be due to an inherent modification of the TGF-beta regulatory program. Lee et al [9] characterized expression of TGF-beta isoforms in keloids at the protein level using Western blot analysis and found that TGF-beta1 and -beta2 proteins were at higher levels in keloid fibroblast cultures compared with normal human dermal fibroblast cultures. However, they found comparable expression of TGF-beta3 protein in both the normal and keloid cell lines.

## III. MATERIAL AND METHODS

### Collection of Samples

We studied 80 cases of post-burn hypertrophic scar (median age 37.35 years, range 10 – 70 years). The control group consisted 30 unrelated healthy individuals. All the patients with hypertrophic scar  $\geq 18$  months old in duration were included in the study. Patients were further randomized into two study groups each containing 40 cases. In group A, Intralesional injection of Triamcinolone (TAC) 40 mg was given to all patients every 4 weeks for 6 months. In group B, Triamcinolone injection in the same dose was given in combination with Pressure Garments therapy exerting 25 mm Hg pressure for 23 hours a day (TAC+PG). All the cases were followed up for 6 months. Tissue biopsies were collected before starting the treatment and then after 6 months in each patient in both the groups. Samples were collected from the department of Plastic surgery of a tertiary care teaching institution after prior approval from the Institute's ethical committee. Informed written consent was obtained from the attendants/patients in each case.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 2, February 2017

## RNA isolation and Quantitative Real-time PCR

Total RNA from tissue biopsies was isolated using Trizol reagent (Sigma, USA), and 2µg of total RNA was reverse-transcribed using standard reagents (Invitrogen, USA). Real-time PCR analysis was performed in a fluorescence temperature thermal cycler (Minicycler, Biored, USA) according to the manufacturer's instructions. The cDNA served as a template in a 10µl reaction containing 0.5 mM of each primer of TGF beta gene (1, 2 & 3), and 2x sybergreen mix (Biored, USA). Samples were incubated for an initial denaturing at 95°C for 10 min, followed by 45 cycles, each cycle consisting of 95°C for 10 s, 60°C for 10 s and 72°C for 10 s. Cycle-to-cycle fluorescence emission readings were monitored at 72°C at the end of each cycle. Melting curves were generated after each run to confirm amplification of specific gene (s). All quantifications were normalized to the housekeeping gene β-actin.

## Patient's self-assessment

The patients were observed every four weeks for 6 months. The self assessment of the patients' response was rated as 10 points for complete cure, 4-7 for moderate improvement, 3 points for minor changes and 0 point for no response to the treatment.

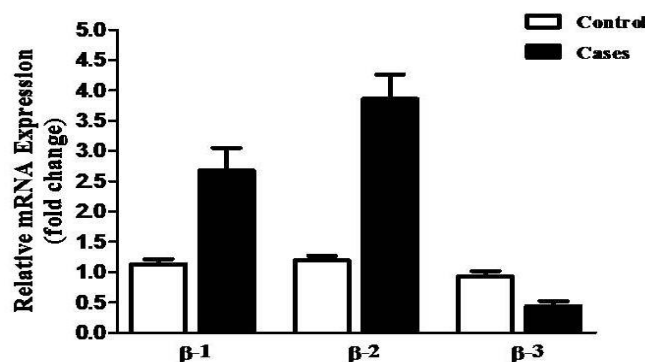
## Statistical analysis

The gene expression data were reported as means ± SD. The statistical analysis was done using ANOVA to evaluate significant differences in mean among the groups. Post hoc multiple comparison test was also done to analyze the significant difference between the pair of means in pre treated and post treated cases. All statistical analyses were performed using SPSS. Values were considered significant at P < 0.05.

## IV. RESULTS

### Expression of TGF-beta gene in hypertrophic scars

TGF β -1, 2 and 3 mRNA expression was investigated in hypertrophic scars and controls by real-time PCR (Fig. 1). The study observed that the mRNA expression of TGF β-1 and TGF β-2 was increased up to 2.6 and 3.9 fold respectively, whereas we observed the lower expression of TGF β-3 gene (0.43) in the hypertrophic scars when compared with controls.



**Fig. 1.** Real-time PCR was performed to detect the mRNA expression of TGF beta genes in cases (black bars) and in controls (white bars). The mRNA expression of TGF β-2 gene was highest while β-3 gene showed lower expression. Data were normalized to β-actin endogenous control. Data are represented as the means ± SD.

### Expression of TGF-beta gene after the treatment

After 6 months of treatment, the mRNA expression of TGF β -1, 2 and 3 was once again evaluated and compared with pre-therapy values (Fig. 2). Our study observed that the mRNA expression of TGF β -1 & 2 was significantly (P<0.01) down regulated after the treatment with Triamcinolone alone (group A) and in combination with Pressure Garments

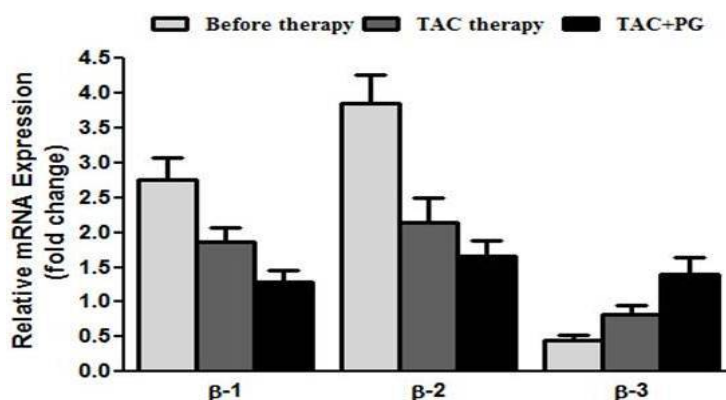
## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 2, February 2017

(group B) when compare with pre-therapy hypertrophic scar cases. Whereas, the mRNA expression of TGF  $\beta$  -3 increased in-significantly ( $P>0.05$ ).



**Fig. 2.** After the treatment the mRNA expression of TGF beta-1 & 2 decreases in hypertrophic scars cases in both the treatment groups while  $\beta$ -3 expression increases. Data are represented as the means  $\pm$  SD.

The statistical analysis was done using ANOVA to evaluate significant differences in mean among the groups: pre-treated cases, TAC (group A) and TAC+PG (group B). The mRNA expression of TGF  $\beta$  -1 ( $F(2, 117) = 382, r^2 = 0.86$  and  $p=0.003$ ) &  $\beta$ -2 ( $F(2, 117) = 454, r^2 = 0.88$  and  $p=0.001$ ) was down regulated significantly. Post hoc comparison test was also done to analyze the significant difference between the pair of means as described in Table 1. The post hoc test result showed highly significant ( $p=0.001$ ) mean difference between the pre-treated cases and TAC+PG (group B). Whereas, mRNA expression of TGF  $\beta$ -3 gene showed non-significant mean difference ( $p>0.05$ ) between pretreated cases and Triamcinolone (group A) therapy.

**Table 1.** Descriptive Statistical data analysis of ANOVA after post hoc comparison test in hypertrophic scars between pre and post treatment.

Variables	Mean diff.	SD( $\pm$ )	95% CI	p value
<b><math>\beta</math>-1 gene</b>				
Pre treated cases vs. A	0.88	0.32	0.75-1.0	0.05
Pre treated cases vs. B	1.47	0.30	1.37-1.56	0.01*
<b><math>\beta</math>-2 gene</b>				
Pre treated cases vs. A	1.71	0.62	1.52-1.92	0.05
Pre treated cases vs. B	2.20	0.38	2.0-2.3	0.001**
<b><math>\beta</math>-3 gene</b>				
Pre treated cases vs. A	0.38	0.15	-0.42- 0.32	ns
Pre treated cases vs. B	0.96	0.22	-1.0-0.88	0.05

Group A=Triamcinolone therapy, Group B=Triamcinolone+Pressure Garments therapy

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

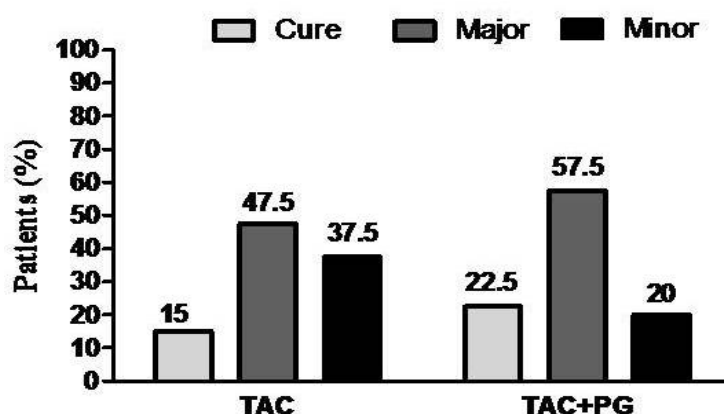
Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 2, February 2017

P<0.01\*, P<0.001\*\*, P>0.05=non-significant (ns).

### Patient self-assessment response

To evaluate the results of our treatment, we used patients' satisfaction scale. After 6 months of treatment 47.5% of patients in the group A and 57.5% of patients in group B expressed major improvement (Fig. 3). Similarly greater (22.5%) number of patients cured completely in combination treatment group B, compare to treatment with Triamcinolone alone (15 %). However, none of the patient showed failure of the treatment.



**Fig. 3.** Patients' self assessment and their percentage of rate of response. After the treatment with TAC+PG (group B) 22.5% of cases was cure and 57.5% of cases showed major improvement from hypertrophic scar.

## V. DISCUSSION

In the modern era, the patients with hypertrophic scars are much concerned for their disfigurement and therapeutic management and prevention of these types of complications is a challenge for the clinician. Understanding of the molecular and biological mechanisms involved in hypertrophic scar formation increased in recent years and it also allowed the development of more specific therapeutic options for these lesions. The present study demonstrates the expression of TGF  $\beta$  gene family in the hypertrophic scars and compares it with controls and with some of the prevalent treatment options. Our investigations showed that the mRNA expression of TGF  $\beta$ - 1 & 2 was higher, while TGF  $\beta$ -3 gene expression was lower in hypertrophic scar cases in comparison to controls and our findings are consistent with those of Lu et al. [13]. Numerous methods have been described for the treatment of hypertrophic scars and no single method of treatment can be taken as the best. With pressure garments therapy, pressure significantly reduces fibroblast growth, secretion of transforming growth factor beta, inhibits fibroblast activity and also decreases collagen deposition [14]. Layton et al. [15] reported that Intralesional injection of a Triamcinolone is helpful for the treatment of hypertrophic scar. A number of authors reported that Intralesional Triamcinolone injection could be more effective in combination therapies as compared to monotherapy for hypertrophic scar treatment [16, 17]. Therefore, the study was further extended to investigate the efficacy of a polytherapy regimen vs. monotherapy in hypertrophic scar cases.

Our study observed that the TGF beta 1 & 2 mRNA expression was significantly downregulated in Triamcinolone treatment group (p=0.05) and only 15% of cases were healed. Based on the previous reports, the study used the combination of Triamcinolone and Pressure Garments (TAC+PG) to reduce hypertrophy of scars and to achieve better results. The mRNA expression of  $\beta$ -1 & 2 was more significantly downregulated in TAC+PG group (p<0.01). After the treatment 22.5% of patient's revealed complete healing and 57.5% of cases responded major improvement from these abnormal scars in combination group as compared to much lower response in the Injection only group. The clinical

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 2, February 2017

improvement observed in the hypertrophic scars was amply supported by the altered expression of TGF beta at the molecular level.

The results of our study indicated that combined therapeutic approach gave better results than monotherapy. However, pressure garments are difficult to comply with in tropical climate and the patients may not find it easy to bear it almost half of the time in a year. Further, standardization of pressure garments in relation to the pressure exerted and achieved with continuous use is yet to be achieved. They need to be changed every 2-3 months depending on its quality. Similarly, Intralesional triamcinolone injection may at times cause systemic effect due to inadvertent entry into systemic circulation during the process of injection and causes lot of pain at least for first few injections. Thus patients' cooperation and compliance are important in achieving good outcome.

## VI. CONCLUSION

To best of our knowledge, this is the first randomized clinical study of these two modalities of treatment for hypertrophic scars in relation to growth factors expression. Both the treatment groups in our study had an acceptable improvement and satisfactory results. This study concluded that combination treatment of pressure garments and Intralesional Triamcinolone injection was more efficacious and acceptable to patients and produced better results than the injection alone. Our study suggests that the combination therapy of TAC + PG may prove to be an effective tool in the management of a wide variety of hypertrophic scars.

## VII. ACKNOWLEDGEMENT

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

## REFERENCES

- [1] Bombaro, K.M., Engrav, L.H., Carrougner, G.J., Wiechman, S.A., Faucher, L, Costa, B.A., Heimbach, D.M., Rivara, F.F., "What is the prevalence of hypertrophic scarring following burns", *Burns*, Vol. 29, No. 4, pp. 299-302, 2003.
- [2] Li-Tsang, C.W., Lau, J.C., Chan, C.C., "Prevalence of hypertrophic scar formation and its characteristics among the Chinese population", *Burns*, Vol. 31, pp. 610-616, 2005.
- [3] Leventhal, D., Furr, M., Reiter, D., "Treatment of keloids and hypertrophic scars: A meta- analysis and review of the literature", *Arch. Facial. Plast. Surg.* Vol. 8, pp.362-368, 2006.
- [4] Gupta, J., Patel, A., Jain, P., "Alteration in Transforming growth factor-  $\beta$ 1 gene expression in hypertrophic scar", *Ind J Biotech.* Vol.13, pp. 314- 317, 2014.
- [5] Davies, D.M., "Plastic and reconstructive surgery. Scars, hypertrophic scars and keloids", *Br Med J.* Vol. 290, No.6, pp.1056 – 8, 1985.
- [6] Niessen, F.B., Andriessen, M.P., Schalkwijk, J., Visser, L., Timens, W., "Keratinocyte- derived growth factors play a role in the formation of hypertrophic scars", *J Pathol.* Vol.194, 207–16, 2001.
- [7] Babu, M., Diegelmann, R., Oliver, N., "Keloid fibroblasts exhibit an altered response to TGF-beta", *J. Invest. Dermatol.* Vol. 99, No.5, pp. 650-5, 1992.[8] Moulin, V., "Growth factors in skin wound healing", *Eur. J. Cell. Biol.* Vol. 68, No.1, pp. 1-7, 1995.
- [9] Lee, T.Y., Chin, G.S., Kim, W.J., Chau D, Gittes GK, Longaker MT., "Expression of transforming growth factor beta 1, 2, and 3 proteins in keloids", *Ann Plast Surg.* Vol.43, No. 2, pp 179—84, 1999.
- [10] Roberts, A.B., Sporn, M.B., Roche, N.S., Wakefield, L.M., "Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro", *Proc. Natl. Acad. Sci. USA.* Vol.83, No.12, pp.4167-4171, 1986.
- [11] Yang, E., Moses, H.L., "Transforming growth factor- $\beta$  induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioal-lantoic membrane", *J. Cell Biol.* Vol.11, No.2, pp. 731-741, 1990.
- [12] Phillips, G.D., Whitehead, R.A., Stone, A.M., Ruebel, M.W., Goodkin, M.L., Knighton, D.R., "Transforming growth factor- $\beta$  stimulation of angiogen.: an electron microscopic Study", *J. Submicrosc. Cytol. Pathol.* Vol. 25, pp. 149-155, 1993.
- [13] Lu, L., Chen, Y.L., Zhang, Q.G., "Distribution and expression of transforming growth factor beta and receptors in hypertrophic scar", *Zhonghua Shao. Shang Za. Zhi.* Vol.20, No.1, pp. 30-33, 2004.
- [14] Chang, L.W., Deng, W.P., Yeong, E.K., "Pressure effects on the growth of human scar fibroblasts", *J. Burn Care Res.* Vol.29, No.5, pp. 835-41, 2008.
- [15] Layton, A.M., Yip, J., Cunliffe, W.J., "A comparison of intralesional triamcinolone and cryosurgery in the treatment of acne keloids", *Br. J. Dermatol.* Vol.130, No.4, pp. 498 – 501, 1994.
- [16] Yosipovitch, G., Widijanti, S.M., Goon, A.A., "Comparison of the combined effect of cryotherapy and corticosteroid injections versus corticosteroids and cryotherapy on keloids: a controlled study", *J. Dermatol Treat.* Vol.12, pp. 87 – 90, 2001.
- [17] Fotini, B.K., Amalia, T., Eleni, A., and Olga, M. "Hypertrophic and keloidal scars: an approach to polytherapy", *Interna, J. Dermatol.* Vol.44, pp. 324-327, 2005.