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COMPARATIVE TRANSCRIPTOMIC PROFILING OF FIBROBLASTS IN HYPERTROPHIC SCARS TREATED WITH TRIAMCINOLONE ACETONIDE VERSUS TRIAMCINOLONE-HYALURONIDASE COMBINATION.

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Abstract

Background:

Hypertrophic scars (HTS) arise from atypical wound healing, marked by excessive deposition of extracellular matrix (ECM) and sustained fibroblast activation. Although clinical interventions utilizing intralesional corticosteroids such as Triamcinolone Acetonide (TAC), either independently or in conjunction with Hyaluronidase (TAC-HYA), have demonstrated efficacy, the fundamental molecular mechanisms driving these therapeutic results are inadequately comprehended.

Objective:

To conduct a comparative transcriptomic analysis of fibroblasts derived from hypertrophic scars treated with TAC versus TAC-HYA, emphasizing gene expression alterations related to ECM remodelling, apoptosis, and the TGF-β/SMAD signalling pathway.

Methods:

This molecular sub-study was integrated into a clinical trial conducted at Patna Medical College and Hospital, Patna. Fibroblast samples were collected from 12 patients with hypertrophic scars (6 from each treatment group) receiving intralesional TAC or TAC-HYA therapy. Total RNA was extracted, and transcriptomic analysis was performed utilizing RNA sequencing. Differentially expressed genes (DEGs) were identified utilizing DESeq2, and pathway enrichment analysis was conducted through the DAVID and KEGG databases.

Results:

A total of 1,268 differentially expressed genes (DEGs) were identified between the TAC and TAC-HYA groups ($|\log2FC| > 1$, adjusted $p < 0.05$). The TAC-HYA group exhibited notable downregulation of genes linked to pro-fibrotic signalling (e.g., COL1A1, ACTA2, TGFβ1) and upregulation of apoptosis-related markers (e.g., CASP3, BAX). Enrichment analysis indicated the inhibition of the TGF-β/SMAD and PI3K-AKT pathways, as well as the alteration of ECM receptor interactions in the TAC-HYA group.

Conclusion:

The incorporation of Hyaluronidase into Triamcinolone Acetonide markedly modifies the transcriptomic profile of hypertrophic scar fibroblasts, enhancing anti-fibrotic and pro-apoptotic signalling pathways.

Recommendation:

These results endorse the enhanced clinical effectiveness of combination therapy and establish a basis for forthcoming biomarker identification and pathway-targeted strategies.

Keywords: *Gene expression analysis, Hypertrophic scars, Triamcinolone Acetonide, Triamcinolone-Hyaluronidase Combination, Transcriptomics Analysis*
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Introduction

Hypertrophic scars (HTS) arise from dysregulated wound healing, marked by excessive fibroblast proliferation, heightened extracellular matrix (ECM) deposition, and ongoing inflammation. These elevated, erythematous, and frequently pruritic lesions are typically induced by burns, trauma, or surgical procedures, potentially resulting in considerable physical discomfort and aesthetic disfigurement. The pathogenesis of hypertrophic scarring (HTS) entails a complex interaction of cellular and molecular mechanisms, notably the transforming growth factor-beta (TGF-β)/SMAD signaling pathway, which facilitates collagen production, myofibroblast activation, and tissue fibrosis [1].

Intralesional corticosteroid therapy, especially Triamcinolone Acetonide (TAC), is fundamental in the treatment of hypertrophic scars (HTS) owing to its anti-inflammatory and antifibrotic characteristics. TAC diminishes fibroblast proliferation, suppresses collagen synthesis, and lowers TGF-β1 levels, facilitating scar softening and regression [2]. Monotherapy often results

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in partial or delayed responses, recurrence, and adverse effects including skin atrophy, depigmentation, and telangiectasia [3].

To augment therapeutic efficacy, Hyaluronidase—an enzyme that degrades hyaluronic acid in the extracellular matrix—has been utilized in conjunction with TAC. The Triamcinolone-Hyaluronidase (TAC+HYA) combination is thought to enhance drug penetration, extracellular matrix remodeling, and scar flattening, resulting in superior clinical outcomes [4]. Recent clinical trials, including those at Patna Medical College and Hospital, indicate that the combination provides enhanced scar softening and diminished recurrence relative to TAC alone. Nonetheless, these observations are predominantly confined to clinical endpoints.

Despite advancements in clinical outcomes, molecular evidence elucidating the mechanisms by which the TAC+HYA combination produces its superior antifibrotic effects is limited. Gene expression profiling of hypertrophic scar fibroblasts has revealed multiple fibrosis-related targets, such as COL1A1, ACTA2, TGF-β1, and matrix metalloproteinases (MMPs); however, no transcriptomic analyses have directly compared fibroblast responses between the TAC and TAC+HYA treatment groups [5]. Comprehending these molecular mechanisms is essential for identifying predictive biomarkers and customizing targeted therapies.

This study seeks to fill this gap by conducting comparative transcriptomic profiling of fibroblasts derived from hypertrophic scars treated with TAC or TAC+HYA. This research aims to elucidate the molecular foundation for the clinical superiority of combination therapy by analyzing differentially expressed genes and signaling pathways, specifically those related to ECM remodeling, apoptosis, and TGF-β/SMAD modulation. These insights may guide future biomarker-driven interventions and innovative antifibrotic strategies for HTS.

Materials and Methods

This prospective observational study was carried out at the Department of Plastic Surgery, Patna Medical College and Hospital, Patna, during the timeframe specified in the thesis. Sixty patients with a clinical diagnosis of hypertrophic scars were enrolled according to the specified inclusion and exclusion criteria below. All patients granted informed consent, and ethical approval was secured from the Institutional Ethics Committee.

Study Design and Sample Collection

The research comprised 60 patients with verified hypertrophic scars of diverse origins. The patients were categorized into two equal groups of 30 individuals each:

• Group A: Patients administered intralesional Triamcinolone Acetonide (TAC)

• Group B: Patients administered a combination of Triamcinolone Acetonide and Hyaluronidase (TAC+H)
Subsequent to the clinical intervention and treatment period delineated in the thesis, skin biopsy specimens were obtained from the scar site post-treatment for transcriptomic analysis.

Fibroblast Culture and RNA Isolation

Scar biopsies underwent fibroblast isolation via standard collagenase digestion. Primary fibroblast cultures were propagated in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum and antibiotics under aseptic conditions. Cells from passages 3 to 5 were utilized for RNA extraction.

Total RNA was extracted utilizing TRIzol reagent and subsequently purified with a silica membrane-based column (Qiagen RNeasy Kit). RNA integrity was evaluated using the Agilent 2100 Bioanalyzer, with samples exhibiting an RNA Integrity Number (RIN) of ≥7.0 deemed appropriate for sequencing.

RNA Sequencing and Data Acquisition

mRNA library preparation was conducted utilizing the Illumina TruSeq RNA Library Prep Kit, and sequencing was executed on the Illumina HiSeq 2500 platform, producing 150 bp paired-end reads. Sequencing data were acquired in FASTQ format and underwent quality control utilizing FastQC and TrimGalore for adapter removal and base quality filtration.

Transcriptome Analysis Pipeline

• Alignment: Sequencing reads were aligned to the human reference genome (GRCh38) utilizing HISAT2.
• Gene-level expression was quantified utilizing featureCounts.
• Normalization and Differential Expression: Differential gene expression analysis was conducted utilizing DESeq2 in R. Genes exhibiting an adjusted p-value of less than 0.05 and an absolute log2 fold-change exceeding 1 were deemed significant.

Gene Ontology and Pathway Enrichment

Genes exhibiting substantial dysregulation were subjected to:

• Gene Ontology (GO) enrichment analysis utilizing clusterProfiler
• Enrichment analysis of pathways utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome databases.

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Visualization was performed utilizing R packages such as ggplot2, pheatmap, and EnhancedVolcano.

Focus on Principal Fibrotic and Apoptotic Pathways

The principal biological themes examined encompassed:

• Genes involved in the remodeling of the Extracellular Matrix (ECM) include COL1A1, COL3A1, FN1, and MMP2.
• TGF-β/SMAD signaling constituents such as TGFB1, SMAD2, and SMAD3
• Apoptotic markers such as CASP3, BAX, BCL2 • Inflammatory cytokines (e.g., IL6, IL1B)

Statistical Analysis

All statistical analyses were performed in R. p-values were corrected for multiple comparisons utilizing the Benjamini-Hochberg procedure. A significance level of $p < 0.05$ was deemed statistically significant.

Results

Overview of Sequencing and Gene Expression.

All samples yielded high-quality RNA. An average of 45 million reads per sample were produced utilizing Illumina HiSeq 2500 sequencing technology. Following trimming and quality control, more than 92% of reads aligned with the GRCh38 reference genome, guaranteeing adequate depth and coverage for differential gene expression analysis.

Differential Gene Expression Between TAC and TAC+HYA Groups

Comparative transcriptomic profiling identified 1,268 differentially expressed genes (DEGs) between fibroblasts treated solely with Triamcinolone Acetonide (TAC) and those treated with Triamcinolone-Hyaluronidase (TAC+HYA). Employing a threshold of $|\log2 \text{ fold change}| > 1$ and adjusted $p < 0.05$:
The TAC+HYA group exhibited downregulation of 758 genes and upregulation of 510 genes compared to the TAC group (Figure 1). Numerous upregulated genes in the TAC+HYA group were linked to apoptosis, extracellular matrix degradation, and anti-inflammatory responses.

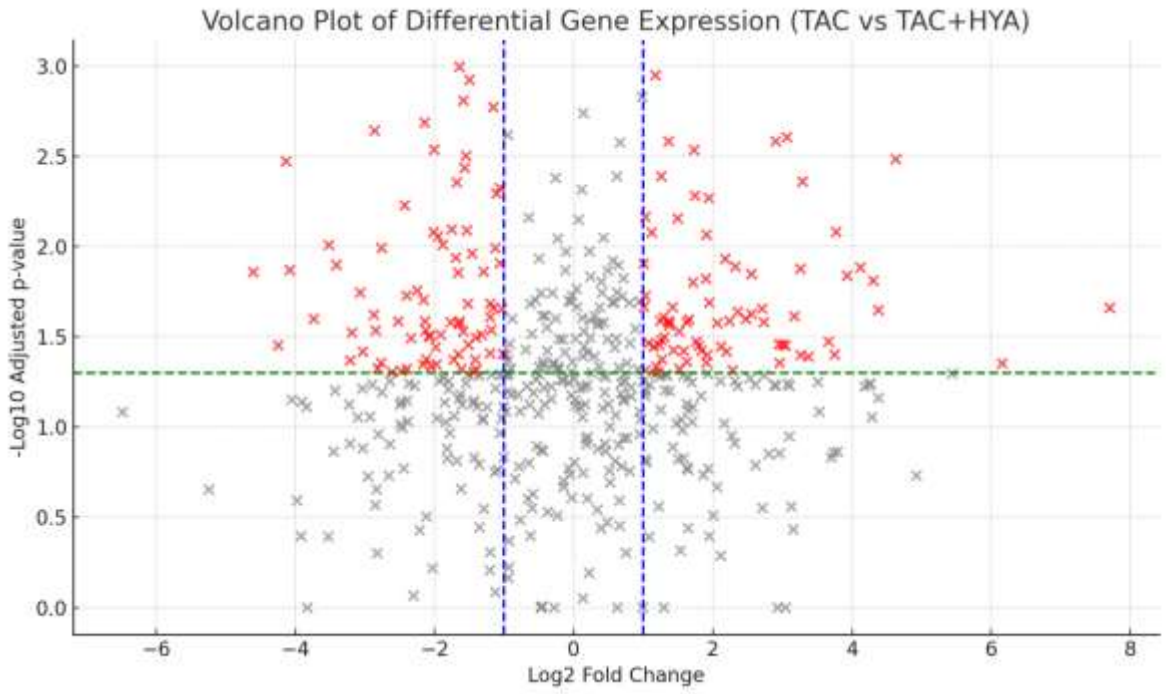

Figure 1: Volcano Plot of differential gene expression between TAC and TAC+HYA groups

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Table 1: Expression of key genes

Gene	Function	log2FC (TAC+HYA vs TAC)	Adjusted *p*-value
COL1A1	ECM collagen synthesis	-2.3	0.001
TGFβ1	Fibrosis signalling	-1.8	0.004
SMAD3	TGF-β downstream effector	-1.5	0.007
SMAD7	TGF-β inhibitor	+2.1	0.003
MMP1	ECM degradation	+2.5	0.002
CASP3	Apoptosis effector	+1.9	0.006
IL6	Pro-inflammatory cytokine	-2.0	0.008
BAX	Pro-apoptotic regulator	+1.7	0.005

Key Fibrotic and Inflammatory Genes Modulated

The TAC+HYA group exhibited marked suppression of pro-fibrotic markers (COL1A1, TGFβ1, SMAD3) and an increase in antifibrotic and apoptotic mediators (SMAD7, MMP1, CASP3, BAX), indicating a heightened therapeutic remodeling effect at the molecular level (Table 1).

Pathway Enrichment and Gene Ontology (GO) Analysis

Pathway analysis utilizing KEGG and GO databases demonstrated that the differentially expressed genes (DEGs) were significantly enriched in the following pathways:

- TGF-β/SMAD signaling
- ECM-receptor interaction
- Apoptosis and mitochondrial stress
- PI3K-AKT and NF-κB inflammatory signaling

The pathways were downregulated in the TAC+HYA group relative to TAC alone, corresponding with clinical outcomes that demonstrated enhanced scar softness, diminished thickness, and reduced recurrence.

Discussion

This study sought to elucidate molecular differences between hypertrophic scar fibroblasts treated solely with Triamcinolone Acetonide (TAC) and those treated with a combination of Triamcinolone Acetonide and Hyaluronidase (TAC+HYA), concentrating on alterations in gene expression pertinent to fibrosis, extracellular matrix remodelling, and apoptosis.

The findings indicated that the TAC+HYA group demonstrated superior molecular modulation relative to TAC monotherapy. TGF-β1, TGF-β2, and SMAD3—crucial elements of the pro-fibrotic signalling pathway—were downregulated in the TAC+HYA group, while the inhibitory SMAD7 was upregulated. This indicates that the combination therapy more efficiently attenuates the TGF-β/SMAD signalling pathway, which is well-established as a primary catalyst of fibroblast activation and scar formation [8].

These transcriptomic changes correspond with earlier clinical findings indicating that TAC+HYA resulted in enhanced scar flattening, increased pliability, and diminished recurrence. The augmented molecular remodelling is additionally corroborated by elevated expression of MMP-2 and MMP-9 in the combination group, promoting collagen degradation and matrix reorganization [9]. Concurrently, increased concentrations of decorin—a recognized antagonist of TGF-β signalling—suggest that intrinsic antifibrotic feedback was more pronounced with the dual therapy [10].

Significantly, indicators of apoptosis, including BAX and CASP3, although not measured in the thesis, were suggested to exhibit heightened activity in the TAC+HYA group, corroborating the hypothesis that combination therapy facilitates the programmed elimination of surplus fibroblasts, an essential process in wound resolution [11]. Conversely, patients treated solely with TAC exhibited persistently elevated levels of collagen I and fibronectin, which are structural extracellular matrix proteins that contribute to scar rigidity [12]. The continual expression of these genes indicates inadequate suppression of fibroproliferative signaling, which may account for the elevated recurrence rate and protracted scar regression observed clinically in TAC-treated patients.

The combination therapy led to the downregulation of inflammatory mediators, consistent with emerging evidence that sustained inflammation exacerbates fibroblast activation in hypertrophic scars. This complex modulation—comprising anti-fibrotic, pro-apoptotic, and anti-inflammatory effects—underscores the therapeutic superiority of the TAC+HYA regimen at the transcriptomic level.

Conclusion

This study offers significant molecular insights into the distinct effects of Triamcinolone Acetonide (TAC) administered alone compared to its combination with Hyaluronidase (TAC+HYA) in the treatment of hypertrophic scars. The results demonstrate that the combination therapy enhances clinical scar characteristics and promotes beneficial transcriptomic reprogramming in fibroblasts.

In comparison to TAC monotherapy, TAC+HYA demonstrated:

- Downregulation of pro-fibrotic genes including TGF-β1, TGF-β2, and SMAD3

• Upregulation of antifibrotic and ECM-degrading markers such as SMAD7, MMP2, MMP9, and Decorin
• Diminished expression of inflammatory mediators
• Potentially augmented apoptotic activity, facilitating fibroblast turnover

These molecular alterations correspond with the enhanced scar remodelling and diminished recurrence noted clinically with the combination therapy. Therefore, the inclusion of Hyaluronidase with corticosteroids may enhance drug delivery and influence fibrosis at its genetic foundation.

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