

Tissue Expression of Survivin in Hypertrophic Scars before and after Intralesional Bleomycin

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Abstract

This 16.5 kDa protein is a member of the IAP gene family, which controls cell division, proliferation, and survival by inhibiting the apoptotic process. No detectable or only extremely low levels of survivin expression are present in normal tissues; on the other hand it is present at comparatively larger levels in certain malignant tissues, embryonic and foetus tissues, as well as in only few adults and some of their normal tissues, such as their skin. Antitumour, antibiotic, and antiviral properties are all present in the cytotoxic substance, bleomycin. pyrimidine and purine bases are eliminated by binding to DNA and producing strand scission. Purpose of Study: To discover how survivin plays a role in hypertrophic scar (HTS) aetiology as well as to measure tissue levels of survivin before and after intralesional bleomycin therapy. In this study, the intralesional bleomycin was administered to 20 patients with HTS, while 20 healthy persons of same age and sex served as a control group (Group B). Real-time polymerase chain reaction was used to measure the serum levels of survivin in all of the participants in the study (RT-PCR). Survivin gene expression was considerably elevated in the HTS group prior to treatment compared to healthy control gene expressions. The HTS group, on the other hand, demonstrated a substantial decrease in survivin gene expression after treatment as compared to the control group and before to treatment. Substantial positive link with scar size and total baseline VSS score was found for survivin gene expression at baseline; however, significant negative correlations were found for improvement. There was a substantial positive link between total VSS score after therapy and survivin, whereas a significant negative correlation with improvement. Survivin did not have any significant associations with any of the other HTS parameters. Using age, gender, smoking, and baseline survivin gene expression as confounders, logistic regression analysis was used to predict the development of HTS. Survivin was believed to be a risk factor for HTS. Age, gender, smoking, duration, previous treatment, and baseline survivin gene expression were all taken into account as potential confounders in a linear regression model used to estimate the severity of HTS (higher VSS score). Survivin gene expression was shown to be an independent risk factor for the severity of HTS ($B=6.586$, $p=0.045$) at the beginning of the study. Survivin gene expression may have a role in HTS pathogenesis, according to our results. In addition, its level may be used as a predictor of HTS vulnerability and severity on its own.

Key words: Bleomycin, Hypertrophic scar, Survivin.

1. Introduction

Burns, severe trauma, or surgical operations may all cause hypertrophic scarring, a form of cutaneous fibroproliferative disease [1]. Hypertrophic scars (HTS) are often red, inflamed, itchy, elevated, stiff, and sometimes painful. They're a result of the body's natural healing process [2]. Between 4.5 and 16 percent of the general population suffer from HTSs, and around 35 percent of surgical skin wounds develop HTSs after one year [3].

HTS creation is complicated, and the process behind it is yet not entirely understood. Specifically, a number of variables, including mechanical stress, local inflammation, and fibroblast activation, have been shown to have a significant influence in human HTS development [4].

Streptomyces verticillus is the source of Bleomycin, a water-soluble glycopeptidic antibiotic. Antibacterial, antiviral, and anticarcinogenic properties are all attributed to it. Treatment for hemangiomas, neurofibromas and hard-to-treat warts may all benefit from the use of this drug in combination with other treatments [5].

Due to the inhibition of enzyme lysyl-oxidase and transforming growth factor-beta (TGF-beta), it inhibits

collagen formation. Bleomycin is used to treat keloids and HTS because of this mode of action [6].

When it comes to cell division and apoptosis inhibition, Survivin is one of the most crucial members of the inhibitor of apoptosis protein family. Breast, lung, and pancreatic malignancies all have high levels of this gene's expression [7].

Certain liver disorders and stimulation of hepatic stellate cells have previously been linked to an increase in survivin levels. Survivin may play a role in the fibrogenesis process by regulating apoptosis, as this study shows to a large extent [8].

These findings will provide light on the function of survivin in the development of HTS, as well as how survivin is expressed in tissues before and after bleomycin therapy.

2. Subjects and method

The study population:

This study was conducted as a prospective case-control study. All patients were selected from the outpatient clinic of Dermatology, Venereology and Andrology Department of Benha University Hospitals from October 2019 to September 2020 after the approval by Research Committee at Faculty of

Medicine, Benha University. This study was conducted on 20 patients with keloids and HTS of varying sizes and durations treated by intralesional bleomycin (Group A) and 20 apparently healthy individuals of matched age and sex as a control group (Group B). Every subject was informed about the aim of the study and an informed consent was obtained from each individual before being enrolled in the study. Pregnant women or if they want to be pregnant, lactating women, any subject was suffering from hepatic dysfunction and renal dysfunction and anyone having active infectious disease and complaining from blood dyscrasia, were excluded from this study. Patients were subjected to full history-taking including: onset, course, duration of HTS, previous treatment, as well as history of other skin diseases, and History of medications. Complete dermatological examination to evaluate the site and extent of scars.

The included 20 patients were injected intralesionally with bleomycin at a concentration of 1.5 IU/ml. Initially, local anesthetic (Mepivacaine HCl3%) was administered at the lesion site. Then, multiple intralesional injections of bleomycin at a dose of 0.5–1 ml/cm² with a maximum dose of 4 ml per session using insulin syringe were administered with one week interval [9].

Real time PCR of survivin:

Hypertrophic scars and normal tissue were carefully excised then 4-mm punch biopsies were taken from every sample and stored at 80°C for further investigation. All studied subjects were tested for evaluation of serum level of survivin by RT-PCR.

Statistical Analysis:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Student T Test was used to assess the statistical significance of the difference between two study group means. For the comparison of the three groups' means, one way analysis of variance (ANOVA) was used. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test: was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Paired sample t

test was used to assess changes in parameters over time. Correlation analysis: To assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables. The ROC Curve (receiver operating characteristic) provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. The optimum cut off point was defined as that which maximized the AUC value. The area under the ROC curve (AUC) results were considered excellent for AUC values between 0.9-1, good for AUC values between 0.8-0.9, fair for AUC values between 0.7-0.8, poor for AUC values between 0.6-0.7 and failed for AUC values between 0.5-0.6 [10]. Regression analysis: Logistic and linear regression analyses were used for prediction of risk factors, using generalized linear models. Odds ratio and 95% confidence interval were calculated. Beta regression coefficient describes the relationship between a predictor variable and the response. The sign of beta coefficient indicates the direction of the relationship. The coefficient value represents the mean change in the response given a one unit change in the predictor. If the p-value for a variable is <0.05, data provide enough evidence of correlation, and changes in the independent variable are associated with changes in the response. N.B: *p* is significant if <0.05 at confidence interval 95%.

3. Results

The HTS group before treatment showed significantly upregulated survivin gene expression when compared healthy control gene expressions ($p < 0.001$). While, HTS group after treatment showed significantly downregulation gene expression of survivin when compared before treatment gene expressions as well as when compared to control group ($p < 0.001$ each) Table (1).

Baseline survivin gene expression showed significant positive correlation with scar size, total baseline VSS score, significant negative correlation with improvement. Moreover, survivin after treatment showed significant positive correlation with total VSS score after treatment, significant negative correlation with improvement. Otherwise, no significant correlations of survivin were found with other parameters in HTS group Table (1).

Table (1) Comparison of survivin gene expression between all studied groups before and after treatment.

| Variable | | Control N=20 | | HTS N=20 | | P ¹ | P ² | P ³ |
|---|---------|-----------------|-------|--------------|-------|----------------|----------------|----------------|
| | | | | Before | After | | | |
| Survivin gene expression | Mean±SD | 8927.5±1894.9 | | 13611±2999.3 | | <0.001 | <0.001 | <0.001 |
| | Min-max | 6325 | 12365 | 9057 | 20402 | | | |
| Survivin gene expression (log ₁₀) | Mean±SD | 3.9±0.1 | | 4.1±0.1 | | <0.001 | <0.001 | <0.001 |
| | Min-max | 3.8 | 4.1 | 4.0 | 4.3 | | | |

Table (3) Regression analysis for prediction of HTS susceptibility.

| Variable | P | OR | 95% CI | |
|-------------------|--------------|-------|--------|-------|
| Age | 0.905 | 1.003 | 0.961 | 1.045 |
| Gender | 0.490 | 0.741 | 0.316 | 1.738 |
| Smoking | 0.214 | 1.974 | 0.675 | 5.777 |
| Baseline survivin | 0.001 | 2.315 | 1.879 | 4.371 |

Linear regression analysis was conducted for prediction of HTS severity (higher VSS score) using age, gender, smoking, duration, previous treatment and baseline survivin gene expression as confounders. Higher baseline survivin gene expression was suggested to be independent risk predictor for HTS severity (B=6.586, p=0.045) Table (2).

4. Discussion

Hypertrophic scars are defined as visible and elevated scars that do not spread into surrounding tissues and that often regress spontaneously [11]. After a wound has been infected or the incision has been closed with severe strain, necrosis may ensue [12]. HTS fibroblasts show lower apoptosis and higher proliferation, even if the precise processes are yet unknown [13].

As an anti-cancer agent, bleomycin is a glycopeptide antibiotic known as In addition to treating cancers including Hodgkin's lymphoma, squamous cell carcinoma, and testicular cancer, this medication is also used to cure plantar warts [14].

Cell proliferation and angiogenesis are both enhanced by Survivin, a member of the apoptosis-inhibiting protein family [15]. It is found in the cytoplasm of a few basal epidermal cells, where it is expressed in healthy human skin [16].

Certain liver disorders and stimulation of hepatic stellate cells have previously been linked to an increase in survivin levels. Survivin may play a part in the fibrogenesis process by regulating apoptosis, as this study shows to a considerable degree [17].

Survivin gene expression was significantly upregulated in the HTS group before treatment compared to healthy control gene expressions (p0.001), while survivin gene expression was significantly downregulated in the HTS group after treatment compared to before treatment gene expressions (p0.001 for each).

HTS patients have not yet had survivin levels measured, to our knowledge. The purpose of this work was thus to investigate the involvement of survivin in the pathophysiology of HTS and to evaluate the gene expression of survivin before and after intralesional bleomycin therapy.

A strong association between scar size, total baseline VSS score, and improvement in survivin gene expression was found in our study findings. There was a substantial positive link between total VSS score after therapy and survivin, whereas a significant negative correlation with improvement.

We observed that age, gender, smoking, and the expression level of the survivin gene at baseline were all confounding factors in our logistic regression model for predicting HTS development. Survivin was believed to be a risk factor for HTS. Using age, gender, smoking, duration, prior treatment, and baseline survivin gene expression, linear regression was used to predict HTS severity (a higher VSS score). Survivin gene expression was shown to be an independent risk factor for the severity of HTS (B=6.586, p=0.045) at the beginning of the study.

Numerous research have been conducted on the Survivin molecule because its effects on apoptosis and the immune system suggest that it may be involved in the pathophysiology of autoimmune/autoinflammatory illnesses such as rheumatoid arthritis, MS, SLE, and psoriasis. Autoimmune/autoinflammatory illnesses are hypothesised to develop when apoptosis is disrupted, leading to the development of autoreactive cells [18].

In hyperproliferative skin illnesses, such as cancer and psoriasis, Survivin is implicated [19]. Psoriasis is a chronic inflammatory disease of the immune system that affects those who are predisposed to it according to their genetic makeup (20). Additionally, keratinocyte stem cells may be identified and isolated using Survivin as an effective marker [21].

At the cellular level, the survivin molecule has been shown to be elevated in psoriasis patients [22]. Survivin, which has been linked to a number of psoriasis-related alterations, may potentially play a role [23].

When exposed to UV radiation, cisplatin, c-rays, or H2O2, aged human skin fibroblasts display higher levels of apoptosis resistance than younger counterparts, which has been linked to increased production of the survival protein survivin. Flavopiridol or particular shRNAs boosted the apoptotic response of aged fibroblasts to those genotoxic chemicals by inhibiting/down-regulating Survivin [24]. New insights into the complicated link between apoptosis, survivin, and the human skin fibroblasts have been provided by these findings, which support the importance of survivin in apoptosis resistance [25].

There is also a systemic reaction to damage that affects the healing process, with increased IL-4, IL-10, and TGF- circulating in the lymphocytes of patients with fibrosis [26].

Survivin promotes the formation of a polarised Th2 phenotype by increasing IL-4 expression [27]. Survivin expression was shown to be elevated in response to IL-10 in an immunoblotting experiment. Through the activation of survivin, the IL-10 boosted cell cycle

progression and prevented apoptosis in part [28]. Cell cycle progression is induced by TGF- β , which regulates survivin activity [29].

In the study of keloid, Wang et al. [30] found that SMAD3 and its connection to TGF- β are critical. Procollagen gene expression and ECM deposition by keloid fibroblasts were reduced considerably when SMAD3 expression was downregulated. TGF- β induces apoptosis by down-regulating survivin expression via SMAD3 in the antiproliferative TGF- β pathway [31].

4. Conclusion

We infer that an increased expression of survivin may play a role in the pathophysiology of HTS. In addition, its level may be used as a predictor of HTS vulnerability and severity on its own.

5. Recommendations

Because of the study's limited sample size, the findings should be regarded with caution. Expanding and validating present results need more research. The specific mechanisms by which survivin contributes to the pathophysiology of HTS remain to be investigated further. Survivin overexpression, genetic and hereditary variables and anatomical aspects must be studied further to understand how HTS develops. In order to better understand how survivin overexpression and genetic variables interact to affect the cellular environment, it is necessary to investigate survivin gene polymorphism. Survivin inhibition as a therapy for HTS should be investigated.

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