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# NEDD4 Transcript Variant 3 and IGF-1 as Molecular Markers in the Development and Prognosis of Keloids

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## ABSTRACT

**Background:** Keloids are benign fibrous growths resulting from abnormal wound healing, commonly affecting individuals with darker skin tones. Genetic factors, particularly the Neural Precursor Cell Expressed Developmentally Down-Regulated Protein 4 (NEDD4) gene transcript variant 3 (NEDD4-TV3) and growth factors like insulin-like growth factor-1 (IGF-1), are implicated in keloid formation.

**Objective:** This study aimed to assess the expression levels of NEDD4-TV3 and IGF-1 in keloid tissue and their potential role in keloid pathogenesis.

**Patients and methods:** This case-control study was conducted involving 30 keloid patients and 20 individuals of matched age, sex and BMI as a control group. Comprehensive history, examination, and laboratory investigations were performed, including PCR for NEDD4-TV3 and IGF-1 gene expression.

**Results:** NEDD4-TV3 and IGF-1 gene expressions were significantly higher in keloid patients compared to controls ($P \leq 0.001$). NEDD4-TV3 ≥75852 predicted keloid formation with 95% sensitivity, 95% specificity, 97.4% PPV, 90.5% NPV, and 95% accuracy (AUC = 0.983, 95% CI: 0.95-1.0). IGF ≥8490 had 77.5% sensitivity, 70% specificity, 97.4% PPV and 90.5% NPV. NEDD4-TV3 significantly correlated positively with pigmentation score, vascularity score, height score, total Vancouver scale ($P \leq 0.001$) for all, and IGF-1 expressions ($P = 0.014$). IGF-1 significantly correlated with pigmentation score, vascularity score, and total Vancouver scale ($P = 0.03, 0.016, 0.005$) respectively.

**Conclusions:** Significantly elevated NEDD4-TV3 and IGF-1 gene expressions were associated with increased susceptibility to keloid formation, suggesting their potential role in keloid etiopathogenesis and their predictive roles.

**Keywords:** Keloids, NEDD4-TV3, IGF-1, Gene expression, Biomarkers, PCR analysis.

## INTRODUCTION

Keloids are benign fibrous growths that arise from an abnormal wound healing process, often following dermal injuries, burns, tattoos, or acne [1]. They are prevalent in individuals with darker skin tones, such as non-albino African-Americans, Hispanics, and Asians, and those with a family history, affecting 30-90% of patients depending on region and race [2]. Keloids can be tender, painful, pruritic, or cause a burning sensation, with cosmetic concerns being a primary reason for seeking treatment [3].

Genetic predisposition plays a significant role in keloid formation, with the E3 ubiquitin ligase Neural Precursor Cell Expressed Developmentally Down-Regulated Protein 4 (NEDD4) gene, specifically its transcript variant 3 (TV3), implicated in regulating NF-κB/STAT3-mediated inflammation, thereby promoting keloid development.[4, 5] NEDD4-TV3 is more abundantly expressed in keloid tissues compared to normal skin, suggesting its significant role in keloid pathogenesis.[5]

Additionally, growth factors such as insulin-like growth factor-1 (IGF-1) are linked to keloid formation by activating fibroblasts and inducing extracellular matrix deposition and collagen production [6, 7]. Understanding the roles of NEDD4-TV3 and IGF-1 in keloids could lead to better therapeutic strategies. Hence, the purpose of this study was to assess NEDD4 TV3 and IGF-1 expressions in patients with active keloid ≤ 6 months duration.

## PATIENTS AND METHODS

This case-control study included 40 patients of keloids and 20 subjects of matched age, sex and BMI as a control group. Patients were recruited from the Outpatient Clinic of Dermatology and Andrology Department, Benha University Hospital through the period from May 2019 to January 2020.

**Inclusion criteria:** Patients of both genders aged 18-45 years old with clinically confirmed diagnosis of keloid with duration ≤ 6 months.

**Exclusion criteria:** Pregnant and lactating patients, patients with past history of systemic disease (diabetes mellitus, hypertension and cardiac and renal patient), psychological illness that may result in non-compliance with the procedure and the required follow up, cancer patients and who were receiving immune depressive therapy.

**All the studied patients were subjected to:**

A. **Comprehensive history and examination:** This included personal details (age, sex and occupation), duration and course of the condition, family history, and history of other skin diseases or drug intake. A thorough general examination, focusing on the abdomen, chest, and neurological systems, and a detailed dermatological examination using the Vancouver Scar Scale to assess keloid sites.
B. **Laboratory investigation:** Four mm punch biopsies were taken from each subject, stored at -80°C, and



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tested for NEDD4 and IGF-1 gene expression in keloid tissue via PCR. A complete blood picture was analyzed using a Hematology Autoanalyzer Symex XS-1000i (Japan), nucleated RBCs were manually counted with a hemocytometer, and HB electrophoresis was performed using Sebia mincap SN 2447 (IVD, France). Relative quantitation of NEDD4 and IGF-1 gene expression was done by real-time PCR.

**1. Sample preparation**

A fresh tissue sample (20-50 mg) was homogenized with 300 µl Lysis Buffer (with 2-ME), followed by an additional 200 µl Lysis Buffer and vortexing for 15-30 seconds. After centrifugation at 100,000 g for 10 minutes, if debris remained, 500 µl chloroform was added, vortexed for 15-30 seconds, and centrifuged again. The supernatant or upper aqueous phase was then transferred to a new tube.

**2. Total RNA Extraction**

For total RNA extraction, optional column activation involved adding 100 µl activation buffer to a spin column, centrifuging at 100,000 g for 30 seconds, and discarding the flow-through. For column loading, 300 µl isopropanol was added to the lysate, vortexed and transferred to the spin column, and centrifuged at 100,000 g for 30 seconds, discarding the flow-through. The column was washed twice: First with 700 µl washing buffer and second with 700 µl washing buffer, each followed by centrifugation at 100,000 g for 30 seconds and discarding the flow-through. For RNA elution, 60-50 µl Elution Buffer was added, incubated for 1 minute, and centrifuged at 100,000 g for 1 minute. RNA was stored at -20 °C or -80 °C.

**C. Ultraviolet Spectrophotometric quantification of RNA by nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA):**

For significance, a 260 readings should exceed 0.15. An absorbance of 1 unit at 260 nm equals 40 µg RNA/ml. Pure RNA has an A260/A280 ratio of 1.9-2.3 [8].

1. **Relative quantitation of mRNA of NEDD4 and IGF gene by quantitative real-time PCR (qRT-PCR):** Relative quantitation (RQ) using comparative cycle threshold (CT) measures the change in target gene expression relative to a calibrator [9]. In this study, human NEDD4 and IGF were the target genes, with beta actin as the reference and healthy control as the calibrator. Gene expression was assessed by qRT-PCR using a two-step RT-PCR. RNA was converted to cDNA with a VeritiTM Thermal Cycler and High-Capacity cDNA Reverse Transcription kit. Template RNA and distilled water were added to Maxime RT PreMix tubes.
2. **Dissolve the clear pellet by pipetting:** The cDNA synthesis involved standing the mixture at room temperature for 1-2 minutes for pellet dissolution, followed by the reaction using a PCR machine. Optionally, the reactant was diluted with 20-50 µl sterile water. PCR cycles were performed as specified. In the second step, NEDD4 and IGF gene expression were quantified using a StepOne real-time PCR system and the SensiFAST Sybr Hi-Rox Kit, with beta actin as the housekeeping gene. Melting curve analysis confirmed assay specificity.

**Ethical considerations: The study was done after being accepted by The Research Ethics Committee of Faculty of Medicine, Benha University. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

***Statistical analysis***

Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL). Categorical data were presented as numbers and percentages and analyzed using the Chi-square test. Quantitative data were tested for normality using the Shapiro-Wilks test. Normally distributed variables were expressed as mean ± SD and analyzed by Student's t-test for two independent groups, while non-parametric variables were presented as median and IQR and analyzed using the Mann-Whitney U test for two groups and Kruskall-Wallis test for three groups. Spearman's correlation coefficient (rho) assessed non-parametric correlation, and Pearson's coefficient (r) assessed parametric correlation. ROC curve analysis determined the cutoff value of markers for predicting keloid formation, with $P \leq 0.05$ considered significant.

**RESULTS**

The age and sex were matched between the cases and control groups with no significant difference (***p*** = 0.66, 0.84 respectively) (Table 1).

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**Table (1):** Demographic criteria of the studied groups

| Variable | | Keloid patients (N=40) | | Controls (N=20) | | Test of significance | *P* |
|---|---|---|---|---|---|---|---|
| **Age (ys)** | **Mean ± SD** | 25.0 ± 7.2 | | 25.8 ± 7.1 | | **St."t"=** | **0.66** |
| | **Range** | 15-36 | | 16-36 | | **0.43** | **(NS)** |
| | | **No.** | **%** | **No.** | **%** | | |
| **Sex** | **Male** | 29 | 72.5 | 14 | 70.0 | **$\chi$2=** | **0.84** |
| | **Females** | 11 | 27.5 | 6 | 30.0 | **0.04** | **(NS)** |

*P* < 0.05 is significant, N: Number, SD: Standard Deviation

All cases received either topical or systemic treatment with 32.5% of cases reported good response to treatment. Regarding onset of keloid, the studied patients reported 40% one month after trauma, 37.5% 2 months after trauma and 22.5 % 2 weeks after trauma. All cases reported progressive course. Head was the most common affected sites in the studied patients (35%). The mean length was 5.25 cm, while mean width was 2.37 cm (**Table 2)**.

**Table (2):** History and clinical findings in the patients

| Variable | | | N (n=40) | (%) |
|---|---|---|---|---|
| **Marital status** | **Single** | | 16 | 40.0 |
| | **Married** | | 24 | 60.0 |
| **Smoking** | **No** | | 20 | 50.0 |
| | **Yes** | | 20 | 50.0 |
| **Family history** | **Negative** | | 14 | 35.0 |
| | **Positive** | | 26 | 65.0 |
| **Cause of keloid** | **Injection site** | | 6 | 15.0 |
| | **Post burn** | | 11 | 27.5 |
| | **Incision of surgery** | | 10 | 25.0 |
| | **Piercing** | | 4 | 10.0 |
| | **After stitch** | | 5 | 12.5 |
| | **Insect bite** | | 4 | 10.0 |
| **Onset** | **2 weeks after trauma** | | 9 | 22.5 |
| | **1 month after trauma** | | 16 | 40.0 |
| | **2 months after trauma** | | 15 | 37.5 |
| **Course** | **Progressive** | | 40 | 100.0 |
| **Duration** | **Weeks** | | 8 | 20.0 |
| | **Months** | | 32 | 80.0 |
| **Site** | **Head** | | 14 | 35.0 |
| | **Trunk** | | 11 | 27.5 |
| | **UL** | | 12 | 30.0 |
| | **LL** | | 3 | 7.5 |
| **Size (cm)** | **Length** | **Mean ± SD** | 5.25 ± 1.9 | |
| | | **Median (range)** | 5.0 (2-8) | |
| | **Width** | **Mean ± SD** | 2.37 ± 1.7 | |
| | | **Median (range)** | 2.0 (0.5-5.0) | |

N: Number, SD: Standard Deviation

Among the studied group the mean of pigmentation was 1.62, vascularity 1.65, pliability 1.75 and height 1.45. The mean of total Vancouver scale was 6.5 ± 1.69 (Table 3).

**Table (3):** Descriptive statistics of Vancouver scale among the studied patients

| | | Pigmentation | Vascularity | Pliability | Height |
|---|---|---|---|---|---|
| **N** | | 40 | 40 | 40 | 40 |
| **Mean** | | 1.62 | 1.65 | 1.75 | 1.45 |
| **SD** | | 1.030 | 1.00 | .98 | .96 |
| **Minimum** | | .00 | .0 | .0 | .00 |
| **Maximum** | | 3.0 | 3.0 | 3.0 | 3.0 |
| **Percentiles** | **25** | 1.0 | 1.0 | 1.0 | 1.0 |
| | **50** | 2.0 | 2.0 | 2.0 | 1.0 |
| | **75** | 2.75 | 2.0 | 3.0 | 2.0 |

N: Number, SD: Standard deviation

There was a highly significant difference between the 2 groups regarding NEDD4TV-3 and IGF gene expressions (***P***≤0.001) (Table 4).

**Table 4:** NEDD4TV-3 and IGF gene expressions among the studied groups

| Variable | Patients (n=40) | | Controls (n=20) | | $Z_{MWU}$ test | *P* |
|---|---|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD | | |
| **NEDD4TV-3** | 105875.4 | 22160.3 | 57611.2 | 12937.9 | 6.06 | ≤0.001 (HS) |
| **IGF** | 11115.3 | 3260.7 | 7324.5 | 2027.7 | 4.75 | ≤0.001 (HS) |

ZMWU test: Z value of Mann Whitney U test, N: Number, SD: Standard Deviation, IGF: insulin like growth factor.

NEDD4TV-3 ≥75852 significantly predict keloid formation with 95% sensitivity, 95% specificity, 97.4% PPV, 90.5% NPV and 95% accuracy. AUC (95%CI) = 0.983 (0.95-1.0). IGF ≥ 8490 significantly predict keloid formation with 77.5% sensitivity and 70% specificity. NEDD4 was significantly more accurate than IGF-1 (Figure 1).

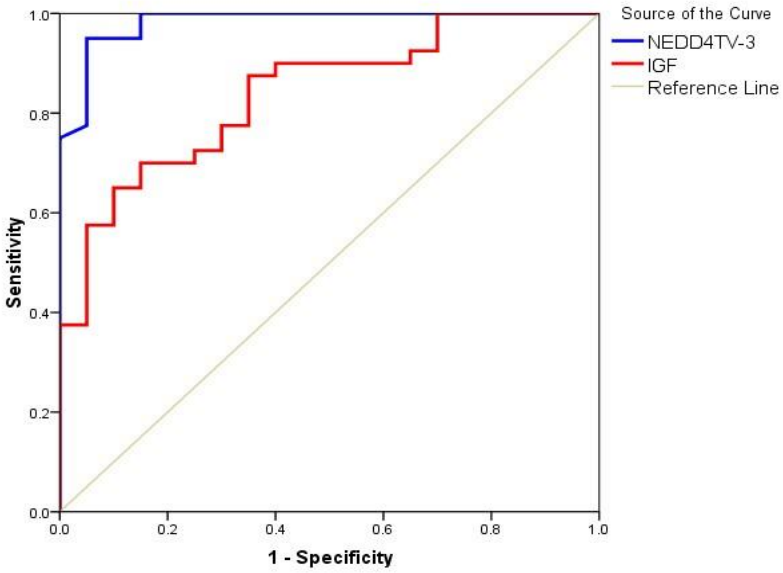
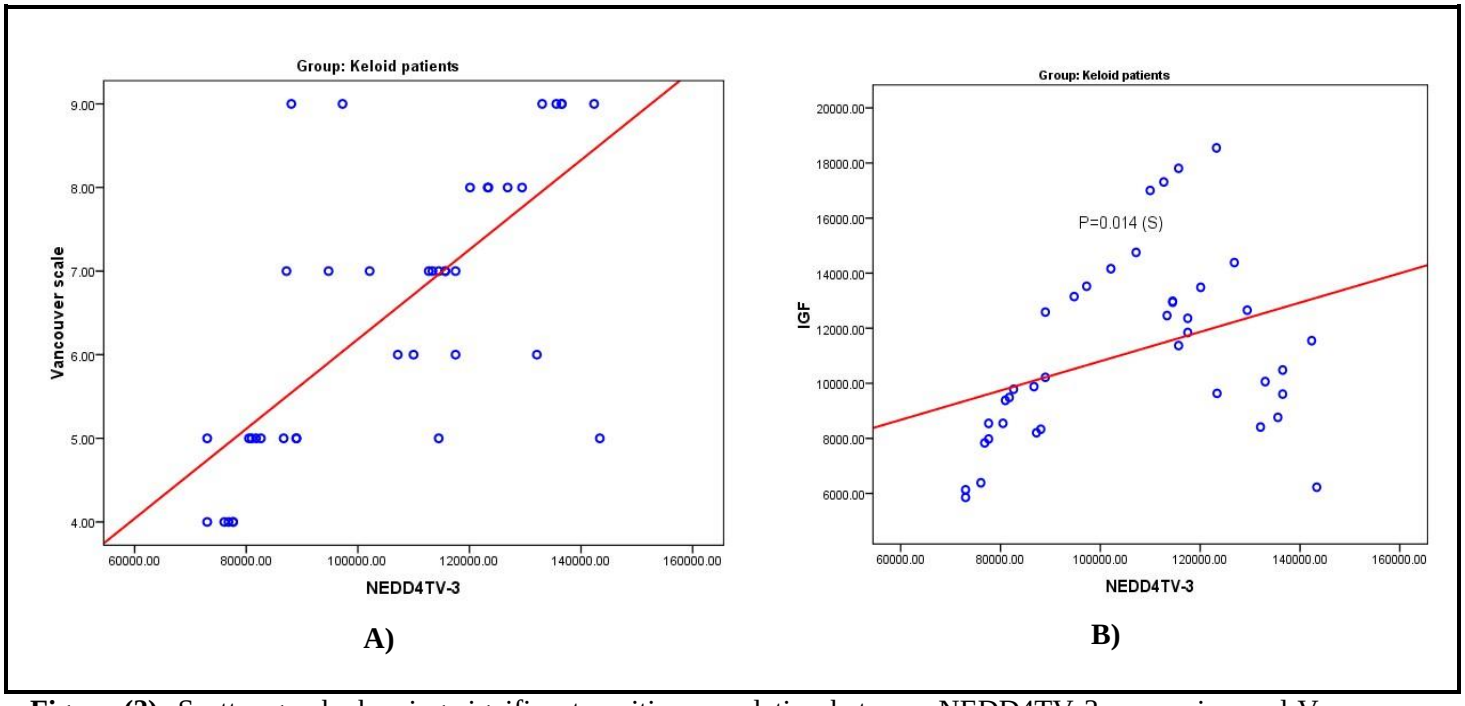



**Figure (1):** ROC curve for the performance of the studied markers in prediction of Keloid formation.

There was significant positive correlation between NEDD4TV-3 and pigmentation score, vascularity score, height score and total Vancouver scale (***P***≤0.001). Also, there was significant positive correlation between NEDD4TV-3 and IGF expressions (***P***≤0.014) (Figure 2).




**A)** **B)**

**Figure (2):** Scatter graph showing significant positive correlation between NEDD4TV-3 expression and Vancouver scale (A) and IGF (B).

There was significant positive correlation between IGF expression and pigmentation score, vascularity score and total Vancouver scale (P values were 0.03, 0.016 and 0.005, respectively) (Figure 3).

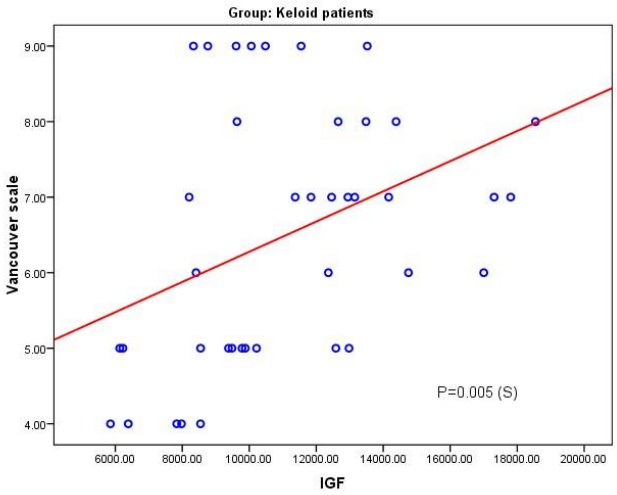



**Figure (3):** Scatter graph showing significant positive correlation between NEDD4TV-3 expression and Vancouver scale

NEDD4TV-3 level was found not to be affected significantly with the sex, marital status, smoking, family history, cause, onset, duration and site of keloids (Table 5).

**Table (5):** NEDD4TV-3 level according to socio-demographic variables and characters of keloid among patients

| Variable | | N | NEDD4TV-3 Median | Min. | Max. | test | *P* |
|---|---|---|---|---|---|---|---|
| **Sex** | **Male** | 29 | 107156.0 | 72997.0 | 143356.0 | $Z_{MWU}$ =1.19 | 0.23 (NS) |
| | **Female** | 11 | 114490.0 | 80494.0 | 142344.0 | | |
| **Marital status** | **Single** | 16 | 87636.5 | 72997.0 | 136529.0 | $Z_{MWU}$ =1.73 | 0.084 (NS) |
| | **Married** | 24 | 113925.5 | 72997.0 | 143356.0 | | |
| **Smoking** | **Yes** | 20 | 113598.0 | 77624.0 | 142344.0 | $Z_{MWU}$ =0.66 | 0.51 (NS) |
| | **No** | 20 | 100954.5 | 72997.0 | 143356.0 | | |
| **Family history** | **Negative** | 14 | 110822.0 | 72997.0 | 143356.0 | $Z_{MWU}$ =1.0 | 0.31 (NS) |
| | **Positive** | 26 | 111342.0 | 72997.0 | 142344.0 | | |
| **Cause** | **Injection site** | 6 | 115995.5 | 88982.0 | 136529.0 | **KW test** =2.67 | 0.75 (NS) |
| | **Post burn** | 11 | 88982.0 | 76071.0 | 136529.0 | | |
| | **Incision of surgery** | 10 | 113599.0 | 72997.0 | 129431.0 | | |
| | **Piercing** | 4 | 110257.0 | 82612.0 | 142344.0 | | |
| | **After stitch** | 5 | 107156.0 | 87203.0 | 143356.0 | | |
| | **Insect bite** | 4 | 98338.5 | 72997.0 | 123378.0 | | |
| **Onset** | **2 weeks after trauma** | 9 | 88982.0 | 80960.0 | 135566.0 | **KW test** =2.27 | 0.44 (NS) |
| | **1 month after trauma** | 16 | 117503.0 | 76071.0 | 143356.0 | | |
| | **2 months after trauma** | 15 | 107156.0 | 72997.0 | 142344.0 | | |
| **Duration** | **Weeks** | 8 | 88526.0 | 72997.0 | 132090.0 | **KW test** =1.33 | 0.18 (NS) |
| | **Months** | 32 | 113924.5 | 72997.0 | 143356.0 | | |
| **Site** | **Head** | 14 | 99678.0 | 72997.0 | 135566.0 | **KW test** =2.96 | 0.39 (NS) |
| | **Trunk** | 11 | 115674.0 | 80494.0 | 143356.0 | | |
| | **UL** | 12 | 113924.5 | 77624.0 | 142344.0 | | |
| | **LL** | 3 | 107156.0 | 81764.0 | 123267.0 | | |

ZMWU test: Z value of Mann Whitney U test.

IGF level was found to be significantly higher in the married (P value=0.005) and smoker patients (P value=0.017). While there was no significant correlation between IGF level and the cause, onset, duration and site of keloids (Table 6).

**Table (6):** IGF-1 level according to socio-demographic variables and characters of keloid among patients

| Variable | | N. | IGF Median | IGF Min. | IGF Max. | test | *P* |
|---|---|---|---|---|---|---|---|
| **Sex** | **Male** | 29 | 10481.0 | 5855.0 | 17311.0 | $Z_{MWU=}$ 0.51 | 0.62 (NS) |
| | **Female** | 11 | 10061.0 | 6227.0 | 18548.0 | | |
| **Marital status** | **Single** | 16 | 9432.5 | 5855.0 | 14384.0 | $Z_{MWU=}$2.79 | 0.005 (S) |
| | **Married** | 24 | 12522.5 | 6132.0 | 18548.0 | | |
| **Smoking** | **Yes** | 20 | 11954.5 | 8544.00 | 18548.00 | $Z_{MWU=}$2.38 | 0.017 (S) |
| | **No** | 20 | 9322.0 | 5855.00 | 14753.00 | | |
| **Family history** | **Negative** | 14 | 10349.5 | 5855.0 | 18548.0 | $Z_{MWU=}$0.14 | 0.88 (NS) |
| | **Positive** | 26 | 10715.5 | 6132.0 | 17806.0 | | |
| **Cause** | **Injection site** | 6 | 12151.5 | 10218.0 | 13485.0 | **KW**=9.41 | 0.094 (NS) |
| | **Post burn** | 11 | 8763.0 | 6387.0 | 13148.0 | | |
| | **Incision of surgery** | 10 | 12816.0 | 6132.0 | 17806.0 | | |
| | **Piercing** | 4 | 12535.5 | 9782.0 | 18548.0 | | |
| | **After stitch** | 5 | 8333.0 | 6227.0 | 14753.0 | | |
| | **Insect bite** | 4 | 9757.0 | 5855.0 | 17002.0 | | |
| **Onset** | **2 weeks after trauma** | 9 | 10218.0 | 8763.0 | 17806.0 | **KW**=0.45 | 0.8 (NS) |
| | **1 month after trauma** | 16 | 11162.5 | 6227.0 | 14384.0 | | |
| | **2 months after trauma** | 15 | 9881.0 | 5855.0 | 18548.0 | | |
| **Duration** | **Weeks** | 8 | 8896.0 | 5855.0 | 13523.0 | **KW**=1.72 | 0.085 (NS) |
| | **Months** | 32 | 11459.0 | 6132.0 | 18548.0 | | |
| **Site** | **Head** | 14 | 10862.5 | 5855.0 | 17002.0 | **KW**=4.05 | 0.25 (NS) |
| | **Trunk** | 11 | 9607.0 | 6227.0 | 13148.0 | | |
| | **UL** | 12 | 11954.5 | 7977.0 | 17806.0 | | |
| | **LL** | 3 | 14753.0 | 9485.0 | 18548.0 | | |

ZMWU test: Z value of Mann Whitney U test.

## DISCUSSION

Other studies have highlighted the potential roles of genetic factors, particularly NEDD4-TV3, and growth factors like IGF-1 in promoting aberrant wound healing.[10, 11] So, we included 40 patients of keloids and 20 subjects of matched age, sex and BMI as a control group to assess the expression levels of NEDD4-TV3 and IGF-1 in keloid tissue and their potential role in keloid pathogenesis.

In the current study, the age and sex were matched between the cases and control groups. The mean age in patients of keloid in the current study was 25.0 ± 7.2 years with male predominance (72.5%). Keloids can occur at any age, but they are most likely to occur between the ages of 11 and 30 years. A slight female predominance is also noted, but this could be related to the higher rate of earlobe piercing in females [12]. **Marneros *et al.*** [4] showed equal incidence of keloids in male and female subjects.[4]

In the current study, there was a non-significant correlation between patients with positive family history and negative family history regarding NEDD4TV-3 gene expression. These results are in agreement with those of **Zhao *et al.*** [13]. On the other hand, there was a non-significant difference between patients with positive family history and negative family history regarding IGF gene expression. These results are in agreement with those of **Demendi *et al.*** [14].

In the current study, the sex was found to have no significant impact on the NEDD4 nor IGF-1 gene expression. This is an agreement with results of the study conducted by **Farag *et al.*** [15], who reported that there was no statistically significant difference regarding sex.

Neural Precursor Cell Expressed Developmentally Down-Regulated Protein 4 expression was found to be significantly higher in the patient group compared to the control group. This was in disagreement with the results of the study conducted by **Fujita *et al.*** [5] who reported that there was no statistically significant difference in the mRNA levels of total NEDD4 amount between normal and keloid keratinocytes. But they also found that NEDD4 TV3 was significantly more abundant in keloid skin compared to normal skin. The discrepancy between results may be explained by the geographically different population studied, the difference in sample size, difference in the study design and difference in study population ethnicity.

The current study showed that there was a high statistically significant difference between patients and control groups regarding IGF-1 gene expression. IGF gene expression has been reported to be associated with

a number of other diseases in which fibro-genetic and inflammatory factors play a role in its pathogenesis including acne hypertrophic scar formation [16] idiopathic pulmonary fibrosis [17], Alzheimer's disease [18] and follicular dermal papillae in androgenic alopecia [19].

In the present work, ROC curve analysis showed that NEDD4TV-3 significantly predicted Keloid formation with 95% accuracy. In addition to IGF, which showed association with keloid formation with 75% accuracy. So, patients with NEDD4TV-3 or IGF gene expression have high chance for development of keloid at site of any skin injury. Therefore NEDD4TV-3 and IGF gene expression can be used as biomarker of keloid development.

The obtained results showed that there was significant correlation between NEDD4 (TV-3) expression and total Vancouver scale. Regarding Vancouver score, the obtained results showed that there were positive correlations between NEDD4 TV-3 expression and pigmentation score, vascularity score and height in the patients with keloid formation. Therefore, patients of keloid with high expression of NEDD4TV-3 gene showed high score of Vancouver scale.

The present study detected significant positive correlation between NEDD4TV-3 expression and IGF. The results obtained showed that there was significant correlation between IGF expression and total Vancouver scale. Regarding Vancouver score, the obtained results showed that there were positive correlations between IGF expression and pigmentation score and vascularity score among patients with keloid formation. Therefore, patients of keloid with high expression of IGF gene showed high score of Vancouver scale.

The obtained results demonstrated that there was significant correlation between IGF expression and marital status and smoking among the patients with keloid. Smoking can be considered a risk factor of keloid development. **Noishiki *et al.*** [20] found that endothelial dysfunction could cause keloid formation and/or aggravation and keloids tend to be worse in smoker patients. Endothelial dysfunction is associated with hypertension, smoking, dyslipidemia, diabetes mellitus, obesity and aging. As during normal wound healing, the capillary vessels and peripheral nerve fibers multiply and fibroblasts increase their production of collagen fibers. Keloids, which are a cutaneous fibroproliferative disorder, are the result of impaired wound healing that leads to persistent inflammation in the wound and the continuous deposition of collagen fibers.

## LIMITATIONS

Our study had some limitations including a small sample size and a single-center design. Additionally, the study did not explore the detailed mechanisms by which NEDD4-TV3 and IGF-1 contribute to keloid pathogenesis and did not assess other potential gene expressions or emerging treatments. Further research with larger multicenter studies is needed to address these aspects.

## CONCLUSION

NEDD4 TV3 and IGF were associated with scarring susceptibility among Egyptian patients with keloid. NEDD4 TV3 and IGF can be used as a potential diagnostic marker and therapeutic target for keloid.

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## REFERENCES

1. **Shaheen A (2017)**: Comprehensive review of keloid formation. Clin Res Dermatol Open Access, 4: 1-18.
2. **Swenson A, Paulus J, Jung Y *et al.* (2024)**: Natural History of Keloids: A Sociodemographic Analysis Using Structured and Unstructured Data. Dermatol Ther (Heidelb), 14: 131-49.
3. **Berman B, Maderal A, Raphael B (2017)**: Keloids and Hypertrophic Scars: Pathophysiology, Classification, and Treatment. Dermatol Surg., 43 (1): S3-s18.
4. **Marneros A, Norris J, Olsen B *et al.* (2001)**: Clinical genetics of familial keloids. Arch Dermatol., 137: 1429-34.
5. **Fujita M, Yamamoto Y, Jiang J *et al.* (2019)**: NEDD4 Is Involved in Inflammation Development during Keloid Formation. J Invest Dermatol., 139: 333-41.
6. **Kang S, Hur J, Kim D (2019)**: Advances in diagnostic methods for keloids and biomarker-targeted fluorescent probes. Analyst, 144: 1866-75.
7. **Lee C, Tsai C, Chen C *et al.* (2023)**: An updated review of the immunological mechanisms of keloid scars. Front Immunol., 14: 1117630.
8. **Wilfinger W, Mackey K, Chomczynski P (1997)**: Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques, 22: 474-6.
9. **Livak K, Schmittgen T (2001)**: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25: 402-8.
10. **Cao X, Lill N, Boase N *et al.* (2008)**: Nedd4 controls animal growth by regulating IGF-1 signaling. Sci Signal, 1: ra5.
11. **Jayaprakash S, Hegde M, BharathwajChetty B *et al.* (2022)**: Unraveling the Potential Role of NEDD4-like E3 Ligases in Cancer. Int J Mol Sci., 23(20):12380.
12. **Chike-Obi C, Cole P, Brissett A (2009)**: Keloids: pathogenesis, clinical features, and management. Semin Plast Surg., 23: 178-84.
13. **Zhao Y, Liu S, Xie J *et al.* (2016)**: NEDD4 single nucleotide polymorphism rs2271289 is associated with keloids in Chinese Han population. Am J Transl Res., 8:544-55.
14. **Demendi C, Börzsönyi B, Nagy Z *et al.* (2012)**: Gene expression patterns of insulin-like growth factor 1, 2 (IGF-1, IGF-2) and insulin-like growth factor binding protein 3 (IGFBP-3) in human placenta from preterm deliveries: influence of additional factors. Eur J Obstet Gynecol Reprod Biol., 160: 40-4.
15. **Farag A, Khaled H, Hammam M *et al.* (2020)**: Neuronal Precursor Cell Expressed Developmentally

Down Regulated 4 (NEDD4) Gene Polymorphism Contributes to Keloid Development in Egyptian Population. Clin Cosmet Investig Dermatol., 13: 649-56.

16. **Yang J, Yoon J, Moon J *et al.* (2018)**: Expression of inflammatory and fibrogenetic markers in acne hypertrophic scar formation: focusing on role of TGF-β and IGF-1R. Arch Dermatol Res., 310: 665-73.
17. **Ruan W, Ying K (2010)**: Abnormal expression of IGF-binding proteins, an initiating event in idiopathic pulmonary fibrosis? Pathol Res Pract., 206: 537-43.
18. **Rivera E, Goldin A, Fulmer N *et al.* (2005)**: Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. J Alzheimers Dis., 8: 247-68.
19. **Tang L, Bernardo O, Bolduc C *et al.* (2003)**: The expression of insulin-like growth factor 1 in follicular dermal papillae correlates with therapeutic efficacy of finasteride in androgenetic alopecia. J Am Acad Dermatol., 49: 229-33.
20. **Noishiki C, Takagi G, Kubota Y *et al.* (2017)**: Endothelial dysfunction may promote keloid growth. Wound Repair Regen., 25: 976-83.