A Study on Molecular Biology and Pathology of Keloid

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Abstract

Introduction: Keloids are dermal fibro proliferative disorder characterized by excessive deposition of collagen in dermis and subcutaneous tissue after tissue injury or spontaneously. Angiogenesis inhibition has been shown to be effective in treatment of malignancy in both animal models and human beings. Recently, it has been reported that vascular endothelial growth factor (VEGF), a potent angiogenic factor, is over expressed in keloid tissue and may have a potential role in its evolution.

Materials and Methods: 40 cases of keloid were studied. Histopathological features were studied in Haematoxylin and eosin stained sections from paraffin block. Expression of VEGFR-2 was studied by Immunohistochemistry in 40 cases of keloid.

Results: The most common site observed was Ear lobe followed by sternum. 52.5% cases had previous history of surgery or trauma. Family history of keloid was present only in 17.5% cases. 62.5% cases presented to the clinic asymptomatically for cosmetic reasons. 87.5% of Keloid case studied shows Normal thickness of epidermis with Rete pegs. Basal cell arrangement was regular in 97.5% of keloid. Basal cell Vacoulation was present in 87.5% of keloid. In 95% of Keloid collagen deposition was found in both papillary and reticular dermis. Haphazard arrangement of collagen fibres was found in 95% of keloid. Broad acellular glassy collagen was found in 57.5% of keloid. Moderate amount of Inflammation was found in 67.5% of keloid. In all cases of keloid, blood vessels was found aggregating below the epidermis. VEGFR-2 expression was increased involving all layers of epidermis, fibroblast and endothelial cells in 57.5% of keloid. Intense staining was noted in 32.5% of keloid whereas moderate staining in 25% of keloid.

Conclusion: Our findings demonstrates that overlying epidermis is the major source of VEGF, which suggests novel therapies for keloids by inhibiting the production and effect of keratinocyte-derived VEGF or by inhibiting keratinocyte-derived VEGF activity on its endothelial receptors.

Keywords: Keloid, VEGFR-2, Wound, Healing, Histopathology.
histopathology of keloid which in future can be applied in the treatment of keloid by blocking that growth factor.

Objective
1. To study the expression of growth factor receptor (VEGFR -2) in keloid by immunohistochemistry.
2. To study the histopathological features of keloid.

Materials and Methods
Study Design: Descriptive study
Study Setting: Dept of pathology, Govt. Medical College, Trivandrum. Rajiv Gandhi Centre for Biotechnology, Trivandrum.
Study Duration: 2 YEARS (2014-2016)
Study Population: All keloid specimens received in the department of Pathology, Trivandrum during the study period.
Methodology: All the resected specimens were fixed in 10% neutral buffered formalin. Bits were taken from the Keloid. The tissue was processed, paraffin embedded sections were stained with Hematoxylin and Eosin .The following parameters were studied in detail under light microscope.
- Epidermis (normal finding or flattened or hyperplastic)
- Epidermal features associated (hyper parakeratosis or hypergranulosis or spongiosis)
- Basal cell organization (regular palliate or disarray)
- Basal cell vacuolar change (present or absent)
- Papillary dermis (normal or scarring)
- Collagen site (papillary or reticular dermis)
- Collagen arrangement (haphazard, nodules or parallel to the skin surface)
- Collagen quality (large, broad hyalinized or fibrillar, regular or wavy)
- Collagen cellularity (Myofibroblasts: numerous, scant or a cellular)
- Orientation of blood vessels (horizontal, vertical or aggregating)
- Inflammatory infiltrate (mild or moderate and its location)

Immunohistochemistry
4 μm thick sections were taken from paraffin block in APES coated slides and incubated in 600C for one hour followed by overnight incubation in 370C. Then sections were deparaffinised in xylene, rehydrated through descending grades of alcohol and subjected to immunohistochemistry using monoclonal rabbit anti-human VEGFR-2 antibody. Heat induced antigen retrieval was performed for 10 minutes in boiling citrate buffer (0.01M) following endogenous peroxidase block by dipping the slides in 1.5% hydrogen peroxide in methanol for 20 minutes. The unspecific antibody binding sites were blocked by incubating the slides with 3% BSA for 1 hour and boundaries were marked using DPX mountant. The tissues were then incubated over night at 40 C with the primary antibodies diluted as 1:500 for VEGFR-2. Then incubated with C-linker containing serum blocker for 30 minutes. An ABC detection system was utilized for signal development and positive reaction was visualized with 3,3’-diaminobenzidine (DAB) chromogen substrate solution. Negative controls were processed by incubating slides with 3% BSA in PBS and omitting the primary antibodies. Age and Sex matched five controls taken from surgical samples done for cosmetic reasons (Gynaecomastia).

Materials and Methods
Expression of VEGFR-2 was assessed based on the Intensity of brownish deposit in fibroblast and endothelial cells and epidermal layers and graded as (Ahmed salem et al)
0 - Negative staining.
1 - Mild staining involving fibroblast, endothelial cells and basal layer of epidermis only.
2- Moderate staining involving whole layer of epidermis except stratum corneum, fibroblast and endothelial cells.
3- Strong/Intense staining involving whole layer of epidermis except stratum corneum, fibroblast and endothelial cells.

Inflammation in Keloid was graded according to the number of lymphocytes seen per high power field as follows:
- Mild: 0-50/HPF.
- Moderate: 50-100/HPF.
- Intense: >100/HPF.

Statistical Analysis
All Quantitative variables are expressed in mean (SD) and Categorical Variables as Proportions. Bi-variate Analysis was done using Chi-square test to see any significant association between the exposure and outcome Variables.

Observation
Total number of patients taken for this study were 40. Majority of patients in this study were Females (72.5%). Majority of the population in this study group belongs to 10-30 years. Youngest of this group was 10 years. Oldest of this group was 64 years.

The most common site of keloid was found to be ear (52.5%) followed by sternum (37.5%). Only 17.5% of population studied had family history of keloid. 52.5% of keloid cases studied had previous history of surgery mainly in the form of ear lobe piercing which accounts for ear lobe as the most common site. Majority of keloid patients presented to clinic for cosmetic reasons. Only 37.5% presented with complaints of pain and itching. Majority of keloid shows normal thickness of epidermis with rete pegs, Basal cell vacoulation and regular arrangement of basal cells. Majority of keloid shows haphazard arrangement of collagen and collagen deposition was found to be in both papillary and reticular dermis. Broad a cellular glassy collagen was found in 57.5% of cases. 67.5% of population studied shows moderate inflammatory cell infiltration. All keloid cases studied shows blood vessels aggregating below the epidermis. VEGFR-2 expression was found to be increased in 57.5% of cases. Out of which 32.5% of cases shows intense staining whereas 25% of case showed moderate staining in all layer of epidermis, vessels and Fibroblast. 42.5% of cases show Mild staining in basal layer of epidermis and vessels and fibroblast.
Discussion

The majority of the patient in the present study belongs to 10-30 yrs age range which was 85%. Schierle HP et al found that hormones found to have influence on Keloid formation, the incidence of Keloid was increased during Pregnancy and Puberty. This may hold true since our study also demonstrated most common population belongs to younger age group. The majority of patients studied in my population is female which accounted for 72%. Kelly et al studied that higher rate of ear lobe piercing was responsible for slight female predominance over males. The most common site of keloid in the present study was ear lobe which accounted for 52.5%. Ear lobe was found to be the common site due to presence of minor trauma in the form of ear piercing. In our study, family predisposition was found only in 17.5% of keloid. Majority of population in this study shows normal thickness of epidermis with rete ridges accounted for 87.5%. But this was in contrast to the studies by Lee JY et al, David W et al, Bennet RG et al, Ehrlich HP et al which showed hyperplastic epidermis. This can be explained partly, by the phenotypic variations in the study groups. Majority of keloid cases in our study show moderate amount of inflammatory cell infiltration (63%) around perivascular location composed mainly of lymphocytes which was similar to findings studied by Borgognoni et al, Blackburn et al and Boyce DE et al. No significant association was found between VEGFR-2 expression and amount of Inflammatory cell infiltration in keloid in our study. 87.5% of keloid scar in our study demonstrated the presence of large, broad, glassy, eosinophilic focally fragmented and haphazardly arranged collagen complexes referred to as “keloid collagen.” 100% of haphazard arrangements are reported in other studies done by Moshref et al, Lee JY et al and Ehrlich HP et al.

Expression of VEGFR-2 and collagen cellularity was found to have statistical significance in our study. VEGFR-2 expression was confined only to basal layer of epidermis in normal skin which was reported by various studies, whereas in our study majority (57.5%) of keloid, showed increased expression of VEGFR-2 involving both basal and upper layer of epidermis except stratum corneum. Among them 32.5% shows intense expression of VEGFR-2 and 25% shows moderate staining involving all layers of epidermis except stratum corneum. VEGFR-2 expression was also found to be increased in vascular endothelial cells and fibroblasts. Localisation of VEGFR-2 receptors was found to be in both cytoplasm and membrane. VEGFR-2 expression in normal skin taken as control, was found to be staining mildly involving only basal layer of epidermis, vessels. Our result demonstrated abundant expression of VEGFR-2 in the overlying epidermis which is the major source of Keloid Angiogenesis. Similar finding was observed by Man XY et al.

Conclusion

- The frequent age group observed was 10-30 years and Females predominated.
- The most common site observed was Ear lobe followed by sternum.
- 52.5% cases had previous history of surgery or trauma.
- Family history of keloid was present only in 17.5% cases.
- 62.5% cases presented to the clinic asymptatically for cosmetic reasons.
87.5% of Keloid case studied shows Normal thickness of epidermis with Rete pegs.

Basal cell arrangement was regular in 97.5% of keloid.

Basal cell Vacoulation was present in 87.5% of keloid.

In 95% of Keloid collagen deposition was found in both papillary and reticular dermis.

Haphazard arrangement of collagen fibres was found in 95% of keloid.

Broad acellular glassy collagen was found in 57.5% of keloid.

Moderate amount of Inflammation was found in 67.5% of keloid.

In all cases of keloid, blood vessels was found aggregating below the epidermis.

VEGFR-2 expression was increased involving all layers of epidermis, fibroblast and endothelial cells in 57.5% of keloid. Intense staining was noted in 32.5% of keloid whereas moderate staining in 25% of keloid.

Bivariate Analysis was done using Chisquare test to see the association of VEGFR-2 expression and collagen cellularity with any of exposure Factors.

Expression Of VEGFR-2 and clinical presentation of patient was found to have a statistical significance with a p value of 0.024. The odds ratio (95% CI) was found to be 5.143 (1.289-20.518).

Expression of VEGFR-2 and collagen Cellularity was found to be statistically Significant.

References
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