

Cutaneous Squamous Cell Carcinoma, an Immunohistological Analysis

Gull-e-Atif*, A H Nagi** and Nadeem Afraz***

*Department of Pathology, Islamabad Medical and Dental College., ** Department of Pathology, University of Health Sciences, Lahore. ***Department of Forensic Medicine, Islamabad Medical and Dental College, Islamabad

Abstract

Background: To detect the presence of keratin in apparently non-keratinizing squamous cell carcinomas by immunoperoxidase staining. This is important because keratinizing tumours are less radiosensitive and non-keratinizing are more radio responsive.

Methods: This prospective study was conducted at King Edward Medical College and Post Graduate Medical Institute, Lahore over six months, A total of 45 patients suffering from squamous cell carcinomas of skin were included in the study. Both H and E and immunoperoxidase stainings were performed. Positive and negative controls were set up. The results of both types of staining were compared for each case.

Results: Four groups were identified. Nineteen cases showed obvious keratinization on both H and E and immunoperoxidase staining. Eight cases had doubtful keratinization on H and E but showed more obvious keratinization on immunoperoxidase staining. Seven cases were non-keratinizing on H and E staining but revealed keratin on immunoperoxidase analysis. Eleven cases were non-keratinizing on H and E as well as on immunoperoxidase analysis.

Conclusion: Immunohistological technique can help us in revising and modifying our H and E impression of a squamous cell carcinoma. It can help us in better diagnosis of squamous cell cancers on basis of keratinization.

Key words: Squamous Cell Carcinoma, Keratinization, Immunoperoxidase study.

Introduction

Squamous cell carcinoma is defined as a tumour in which cells may resemble any or all of the layers of stratified squamous epithelium^{1,2}. Keratinization is an important feature of squamous cell carcinoma. Stratified squamous epithelium is traditionally associated with keratin³. During neoplastic transformation there are no major changes in the synthesis of the intermediate filament proteins by the malignant cells. Thus a well differentiated squamous cell carcinoma shows abundant keratinization⁴. On the other hand keratinization is

almost completely absent in poorly differentiated tumours⁵.

Immunohistochemistry can be used to localize cytokeratin in difficult cases. Immunoperoxidase staining with anti-keratin antibodies shows that : a) Well differentiated squamous cell carcinomas stain intensely with anti-keratin antibodies. b) Moderately differentiated tumors show intermediate staining intensity. c) Poorly differentiated tumors reveal weak staining^{6,7}. Squamous cell carcinomas are radiosensitive^{8,9}.

Previously it was thought that there are 19 distinct types of cytokeratins but latest research shows that there are much more cytokeratin types¹⁰. Keratin was isolated from human callus using the salt extraction method of sun and green. Stratum corneum obtained from the sole of the human foot was purified.

In 1975, Kohler and Milstein¹¹ developed monoclonal antibodies. In 1982 a monoclonal antikeratin antibody AE1 was developed. Spagnolo¹² showed that all carcinomas with the exception of hepatocellular, adrenocortical and basal cell carcinomas and occasional renal cell and pulmonary anaplastic carcinomas reacted with AE1 antibody irrespective of differentiation. Later Klein-Szanto¹³ showed that AE1 keratin antibody reacts with the acidic keratin species of 40K, 48K, 50K, 54K and 56.5K. They also described a monoclonal antibody AE3. Immunohistological staining for keratin is important as keratinizing tumors are less radiosensitive and non keratinizing are more radio responsive¹⁴. Immunostaining for localization of keratin would help the oncologist in selection of a correct therapeutic regime and prognostic evaluation of the patients.

The present study was undertaken to compare the results of hematoxylin eosin staining and immunohistochemical staining for detection of keratin in squamous cell carcinomas of skin.

Patients and Methods

A total of 45 patients suffering from squamous cell carcinoma of skin were included in the

study. They were collected from the Radiotherapeutic units of Mayo Hospital and Services Hospital Lahore.

The patients included in the study belonged to both sexes, and all age groups. A detailed history was taken and clinical examination carried out on all patients. Clinical proforma was filled with special reference to age, sex and presenting complaints. In most cases biopsy was taken before the initiation of radiotherapeutic treatment. Tissue specimen was taken by punch biopsy in majority of patients.

Haematoxylin and Eosin Staining and Immunoperoxidase Staining for Cytokeratin were done. Biotin-streptAvidin Amplified system was used. Cytokertain Polyclonal Antibody commercially prepared by Biogenex Laboratories, USA was used.

After blocking endogenous enzyme and non specific protein binding sites in the tissue, a 3 step labelling method was used. The sections were sequentially treated with the following antibodies:

i) **Primary Antibody:** Antibody type, Polyclonal. Raised in, Rabbit. Immunogen, Cytokeratin. ii) **Linking Antibody** iii) **Labelling Antibody** The antibody was in the ready to use form.

Positive controls comprised normal human skin and sections from well-differentiated keratinizing squamous cell carcinoma, skin. Sections cut from lymphomas and fibrosarcomas were used as negative controls. With each staining run a slide cut from a well differentiated squamous cell carcinoma was used.

The relative intensity of cytokeratin staining was arbitrarily graded as follows: Negative 0, Weakly Positive +1, Moderately Positive ++2, Strongly Positive +++3, Very Strongly Positive ++++4.

Positive staining was seen as reddish brown keratin antikeratin deposits.

Results

Of the total 45 cases, 26 (57.8%) were male and 19(42.2%) were female. Analysis of tumour size showed that the majority of the patients, 30 (66.7%) had large tumour size that is 2-5cm.

Majority of patients fell in the range of 41 to 50 years, that is 16(35.5%). Second commonest age group was 51 to 60 comprising 11(24.4%) patients. Eight (17.8%) were aged between 31 to 40 , six (13.3%) between 61 to 70 and two each (4.4%) in the 21 to 30 and 71 to 80 year age group

Maximum number of patients belonged to the well differentiated group. Next commonest was poorly differentiated and least common was moderately differentiated squamous cell carcinoma. (Fig 1).

1. Cases which showed obvious keratinization on both H&E and immunoperoxidase staining were 19 (42.2%).
2. Cases which showed doubtful keratinization on H&E staining but revealed more obvious keratinization on immunoperoxidase staining were 8 (17.8%).
3. Cases which were non-keratinizing on H&E staining but showed keratinization on immunoperoxidase analysis were 7(15.5%).

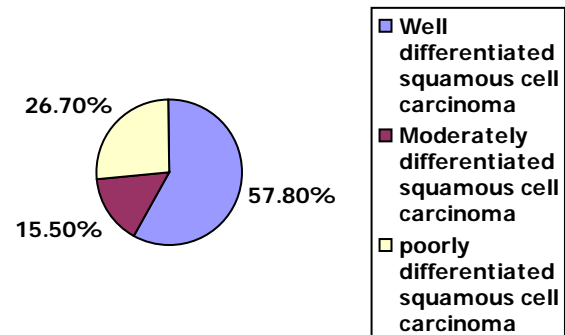


Fig 1: Histological types of Squamous cell CA.

4. Cases which were non-keratinizing on H&E as well as on immunoperoxidase analysis were 11 (24.4%).

Discussion

Our study shows well differentiated squamous cell carcinoma to be the major histological type. This is similar to the work done by Wu et al¹⁵ in Chinese population. Poorly differentiated cases comprised the second commonest group. The relatively large proportion of poorly differentiated tumours could be due to inadequate health education and incomplete awareness among our masses.

There is a male preponderance in our study. This is similar to the work done by others.¹⁶⁻¹⁷ Cady and Catlin¹⁸ did a twenty year survey on epidermoid cancer of the gum. Three quarters of the patients were males, again pointing to a higher male to female ratio in the West.

The maximum number of our patients were in the age range of 40-60 year. Gal et al showed an age range of 49-82 years¹⁹. Another study by Elman et al also showed an age range from 46-81 years.²⁰ Thus our patients belonged to a younger age group as compared to the western countries. In the well-differentiated tumours keratin antikeratin complexes were abundant. All the horn pearls were stained positive. The connective tissue and the vessels did not stain. This is similar to the results of Said et al.²¹

In well differentiated squamous cell carcinoma

staining positivity is 100%. This is comparable with the work done by Robinson and Gottschalk²². Tumours of moderate differentiation showed much less staining positivity as also seen by Corson et al²³. In the poorly differentiated tumours staining reaction was weak or focally positive. There were some cases, which did not stain at all. This is similar to the results of others⁵.

Our study helped in revising the original HE impression in 33.3% of patients. These included patients who showed doubtful keratinization on HE staining but revealed obvious keratinization on Immunoperoxidase staining, and patients who showed non keratinizing tumours on HE but showed keratin on Immunohistological analysis. Researchers²⁴ have also commented that immuno-staining confirmed, modified or in several instances, changed the original light microscopic impression. In 33.3% of the cases we gave revised diagnosis and this helped the oncologist in selection of a correct therapeutic regime and better prognostic evaluation of the patients.

In conclusion, cytokeratin is highly valuable in differentiating apparently non-keratinizing squamous cell carcinomas from the keratinizing squamous cell carcinomas. Thus the immunoperoxidase technique can help us in revising and modifying our original H and E impression. Cytokeratin is an epithelial cell marker which can be very well used as a tumour marker for squamous cell carcinoma of skin.

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