Original Article

DIFFERENTIATION OF KERATOACANTHOMA FROM SQUAMOUS CELL CARCINOMA BY ARGYROPHILIC NUCLEOLAR ORGANIZER REGION (AgNOR) STAINING

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ABSTRACT

***Objectives*:** The objective was to evaluate the differentiation of keratoacanthoma from squamous cell carcinoma by using argyrophilic nucleolar organizer region staining.

Methodology: Thirty one cases of keratoacanthoma and 31 cases of squamous cell carcinoma (S.C.C) were selected. The AgNOR staining carried out. Counting of AgNOR was done in 100 cells of each tumor. Two criterias including M.AgNOR (mean number of AgNORs) and P.AgNOR (percentage of nuclei with five or more than five AgNORs per nucleous) were used. Statistical analysis was done by Mann- Whitney test.

Results: Significant increase in M.AgNOR and P.AgNOR was found in S.C.C compared with keratoacanthoma (M.AgNOR =16.52 ± 10.1 and P.AgNOR = 84.12 ± 23.4 in S.C.C compared with M.AgNOR = 6.58 ± 4.4 and P.AgNOR = 52.29 ± 26.7 in keratoacanthoma, P=0.000). Exceptionally overlapping was seen.

Conclusion: This study indicated that the AgNOR counting is a valuable diagnostic criterion for differentiation of keratoacanthoma and S.C.C especially in cases with borderline *histologic* features.

KEY WORDS: Keratoacanthoma, Squamous Cell Carcinoma, AgNOR.

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INTRODUCTION

Squamous cell carcinoma (S.C.C) is a common malignancy of skin and most commonly arises in sun exposed areas.[1] Histologically it is composed of downgrowth proliferation of atypical squamous cells.[1-2] Tumor differentiation has inverse relation with the severity of atypia.[1] Dyskeratosis, keratinization in the form of keratin pearls and mitoses are the features of this tumor.[1-2] An important differential diagnosis of S.C.C is keratoacanthoma, which is a benign skin tumor.[1] This tumor is also frequently seen in sun exposed areas. Microscopically, it composed of a symmetrically shaped lesion with central crater, filled with keratin. At the base, strands of atypical squamous cells extend into

the dermis. Dyskeratosis, keratinization and occasionally many mitoses are seen.[2-3] The architecture of the lesion in a keratoacanthoma is as important as the cellular characteristics. Therefore, if the lesion can not be excised completely, excision of a fusiform specimen including center of the lesion and at least one of the lateral margins is advised.[1]

Clinically and histologically, distinction of these two tumors exhibiting different biologic nature is occasionally very difficult and even impossible.[1,2,4] Recently, counting of NOR by colloid silver staining is used as a marker of proliferative activity and the degree of ploidy and so distinction of benign and malignant tumors and also tumor grade and prognosis.[4-6] Nucleolar organizer regions (NORs) are loops of DNA occurring within nucleoli that encode for ribosomal RNA.[4] NORs are closely associated with nonhistone proteins, known as AgNOR which can be visualized by a histochemical technique relying on their argyrophile properties.[4] Some studies showed that different malignant tumors such as breast, lung and skin tumors contain more AgNOR in comparison with their benign counterparts.[7-9]

In France, AgNOR counting performed on unequivocal cases of S.C.C (n=20) and keratoacanthoma (n=16). It was higher in S.C.C (6.29 ± 0.091) compared with keratoacanthoma (3.80 ± 1.62).[4] In Italy, two different methods were used to assess the activity of cell proliferation: MIB1 immunohistochemical detection and AgNOR protein of silver staining. Eighteen cases of keratoacanthoma compared with 10 cases of S.C.C. Analysis of variance showed a significancant difference of both parameters: P<0.003 for MIB1 and P<0.001 for AgNOR.[10]

These studies suggest that there is a good relation between AgNOR counting and proliferative activity. Thus this method can be used for differentiation of malignant tumors from their benign counterparts.[4]

This study was *conducted* to asses the value of AgNOR staining as a useful technique for distinction of S.C.C from keratoacanthoma.

METHODOLOGY

Thirty one cases of S.C.C and 31 cases of keratoacanthoma were retrieved from files of pathology departments of Urmia and Tehran universities of medical sciences. These specimens included whole lesion or center and at least one margin of the lesion. Histopathology diagnoses were unequivocal. After cutting the paraffin embedded blocks, hematoxylin- eosin staining (for confirmation of the diagnosis) and colloid silver staining by using the technique of Ploton (for AgNOR counting) was done.[4] Then the cells examined were chosen within representative areas of the tumor and were located at the peripheral portions of the tumor, corresponding to the more proliferative cells. A total of 100 cells were counted in each case. All separated black dots counted as individual AgNOR by using oil immersion lens *(×100)*.[4] After that, mean value, standard deviation and AgNOR count of each cell (M.AgNOR), mean value, standard deviation and percent of cells with five or more than five AgNOR (P. AgNOR) were calculated in each case. Mann- Whitney test was used for statistical analysis.

RESULTS

Both M.AgNOR and P.AgNOR were significantly high in S.C.C than in keratoacanthoma. M.AgNOR was 16.52 ± 10.1 for S.C.C compared with 6.58 ± 4.4 seen in keratoacanthoma (P = 0.000) and P.AgNOR was 84.12 ± 23.4 in S.C.C compared with 52.29 ± 26.7 seen in keratoacanthoma (P = 0.000). The results are seen in Tables-I and II completely.

DISCUSSION

Keratoacanthoma was first described by Hutchinson, more than 115 years ago as a "crateriform ulcer of the face" and since then it continues to be a source of debate as to the exact pathogenesis and preferred management.[3] keratoacanthoma is typically a self- healing, rapid onset skin lesion, whereas S.C.C is conventionally a malignant lesion with stromal invasion that progress continuously without spontaneous resolution.[2]

Table-I: Comparison of Mean. AgNOR between keratoacanthoma and S.C.C

	Keratoacanthoma	*S.C.C*
Mean	6.58	16.52
Minimum	1.50	2.27
Maximum	21	35.05
95% CI *	4.94-8.22	12.80-20.23
S.D**	4.46	10.12
P	0.000	

* Confidence Interval ** Standard Deviation

Table-II: Comparison of Percentage. AgNOR between keratoacanthoma and S.C.C

	Keratoacanthoma	*S.C.C*
Mean	52.29	84.12
Minimum	0	10
Maximum	100	100
95% CI	42.47-62.10	75.54-92.71
S.D	26.7	23.4
P	0.000	

As the clinical behavior and prognosis are different for these two lesions , there must be a reliable way to differentiate them.[2] Many studies concluded that the distinction is impossible by routine Hematoxylin- Eosin staining alone.[2] Recently, using of AgNOR counting as a marker of cell proliferation in many benign and malignant tumors has been supported.[5-6] Some investigations also show the higher number of AgNOR in S.C.C than in keratoacanthoma.[10-12] According to the results seen in Tables-I and II there is a significant difference between these two tumors in our study, too. It should be pointed out that M.AgNOR was below 10 in all cases of keratoacanthoma except four cases and for S.C.C above 10 in all cases except five cases. Since AgNOR staining is more simple and less expensive than the other methods such as immunohistochemistry and flow cytometry, it can be performed in all pathology centers. We suggest this technique can be used as a useful method for distinction of keratoacanthoma and S.C.C, especially in a small biopsy samples which are not including entire architecture of the lesion.

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