Expression of Ki-67 antigen in various histological types of BASAL CELL CARCINOMA

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(Ann Coll Med Mosul 2022; 44 (2):103-108).
Received: 29th Octo. 2022; Accepted: 19th Nove. 2022.

ABSTRACT

Background : Basal cell carcinoma (BCC) is the most commonest malignant tumor of the skin especially fair-skinned individuals makes up roughly 70–80% of all malignant tumors. In certainty, this malignancy had a variety of many tumor sub-types, through a distinct histo-morphological appearance and biological function. Unlike other tumors, usually has an affirmative clinical course, developing slowly and just locally. Antibodies against “Ki-67 antigen” or proliferating Cell nuclear antigen (PCNA) are typically used in immunohistochemistry to detect the proliferative activity (growth fraction) of malignancies.

Aim of the study : This study aims to examine the tumor cells' proliferative status utilizing an immunohistochemistry analysis of the “Ki-67” antigen in skin biopsy specimens of different sub-type of BCCs.

Material and methods : A Case series study enrolling 35 biopsies sampled from retrospectively selected cases of cutaneous BCCs were registered in the research.

Results : Thirty five biopsy (31 Primary, 4 Recurrent)of different histological types from 35 patients (11 male, 24 female) were included. The patient’s age ranged from 36 to 75 years (with mean of 59.2 year) .In this study results showed that commonest site of involvement was the face in 71.4% of cases , and there was significant association between tumor size and the Ki67 level and grade, the study reported a non-significant association between the tumor histological subtype and Ki67 level.

Discussion : The data showed that there were a wide range of ki67 level (2 to 70%) among various histological growth subtype of basal cell carcinoma, higher proliferative index indicate more malignant potential and lower proliferative index mean less aggressive tumor. So the wide range of proliferation which had been reported in the BCC illuminate the wide spectrum of biological activity of tumor.

Keywords : BCC , lesion , Ki67, skin, tumor.

التعبير عن مستضد كي-٦٧ في أنواع نسيجية مختلفة من سرطان الخلايا القاعدية

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الخلاصة

سرطان الخلايا القاعدية هو ورم الجلد الخبيث الأكثر انتشارا وخاصة في الأفراد ذوي البشرة الفاتحة يشكل ما يقرب من ٧٠-٨٠ ٪ من جميع الأورام الخبيثة ، يقينا ، هذا الورم الخبيث هو طيف من العديد من أنواع فرعية للورم ، ولكل منها مظهر نسيجي متميز. على عكس الأورام الأخرى ، فإنه عادة ما يكون له مسار سريري مميت ، يتطور ببطء وينتشر موقعيا فقط. تستخدم الأجسام المضادة للمستضد النووي للخلايا المتكاثرة (مستضد كي-٦٧) أو المستضد النووي للخلايا المتكاثرة عادة في الكيمياء المناعية للكشف عن النشاط التكاثري (جزء النمو) للأورام الخبيثة ، الهدف من هذه الدراسة هو فحص الحالة التكاثرية للخلايا السرطانية باستخدام تحليل الكيمياء المناعية للمستضد كي-٦٧ في عينات خزعة الجلد من مختلف انواع سرطان الخلايا القاعدية. تم تسجيل دراسة مستعرضة ل ٣٥ عينة خزعة من حالات مختارة بأثر رجعي من الخلايا القاعدية الجلدية (٣١ الابتدائية ، ٤ المتكررة) من أنواع نسيجية مختلفة من ٣٥ شخصا (١١ رجلا ، ٢٤ امرأة) في هذه الدراسة. تراوح عمر المرضى من ٣٦ إلى ٧٥ سنة (متوسط العمر ٥٩.٢ سنة). في هذه الدراسة, أظهرت النتائج أن الموقع الأكثر شيوعا للمرض كان الوجه في ٧١.٤٪ من

الحالات ، وجد أن هناك علاقة كبيرة بين حجم الورم ومستوى كي ٦٧ ، ودلت الدراسة على وجود علاقة غير كبيرة بين النوع النسيجي للورم ومستوى كي ٦٧.

الكلمات المفتاحية : مستضد كي ٦٧ ، جلد ، سرطان الخلايا القاعدية .

INTRODUCTION

The skin is the largest organ of the human body and it is the site from which various types of tumors may arise, from benign ones to malignant masses, Basal cell carcinoma “BCC” is the commonest malignant skin tumor especially individuals with fair-skinned constitutes approximately 70 to 80% of all malignant tumors.[1,2] In certainty, this malignancy has a variety of many tumor sub-types, each with distinct histo-morphological appearance and biological function. Unlike other tumors, it typically has an affirmative clinical course, slowly escalating and only locally spreading.[3]

Nevertheless, certain cases may exhibit aggressive behavior from the beginning, quickly penetrating deeper tissue structures, returning after therapy, and occasionally (albeit extremely rarely) leading to metastatic dissemination.[3] Currently, there is a growing need to identify tumors that have a worse prognosis and have a more significant negative influence on patients' general health state. As a result, it aims to pinpoint the risk factors for development of more aggressive disease with higher chances of recurrence& metastasis. Latest developments in molecular pathology have identified a number of markers in tumors that show substantial role in tumorigenesis& this discovery is crucial for predicting the clinical course of cancer. Antibodies against “Ki-67 antigen” or proliferating cell nuclear antigen (PCNA) are typically used in immune-histochemistry to detect proliferative activity “growth fraction” of malignancies. During the active stages of the cell cycle “G1, S, G2, & mitosis”, which are absent in the "resting" (G0) phase, the nuclear protein known as Ki-67 expressed.[4] One of the primary prognostic markers in a standard histopathological report is the immunohistochemical evaluation of nuclear “Ki-67”Expression (Ki-67 index) in cancer cell, which provides a quantitative assessment of tumors proliferation state. Furthermore, one of the indicators of malignancy in specific tumors is the proportion of Ki-67 positivity.[5,6]

The Aim

The research aims to examine tumor cells proliferative status utilizing an immunohistochemistry analysis of the “Ki-67 antigen” in skin biopsies of different subtype ‘’BCCs’’.

PATIENTS & METHOD

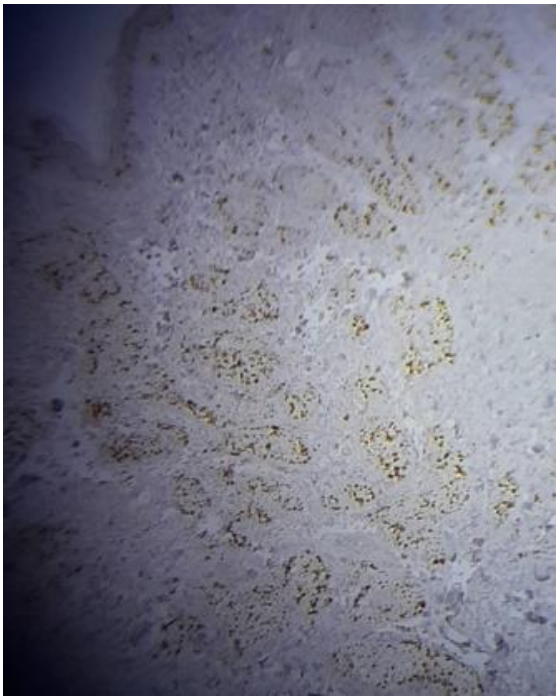
Case series study enrolling 35 biopsies from retrospective cases of cutaneous ’BCCs’ was designed .The patients had been carefully chosen from dermatological unit at Ibn-Sina teaching hospital, Mosul, Iraq. They had been diagnosed clinically with basal cell carcinoma during the period from Jan 2020 - Jan 2021. Three Board certificated dermatologists had diagnosed the lesions clinically and reported all the patients’ demographics information , disease -duration, the shape ,subtype , lesion location, the diameter & topographical distribution of lesions. Then excisional biopsies had been done and examined for histo-pathological assessment to confirm the diagnosis .

Staining of the slides : The blocks of tissues (embedded with paraffin) were sectioned &stained with ((Hematoxylin and Eosin)). Another sections were stained using “mice antihuman antibodies” against “Ki-67” antigen after the tissues were sliced into (four µm in thickness) then de-parafinized in XYLENE followed by rehydration steps using different concentrations of ethanol .

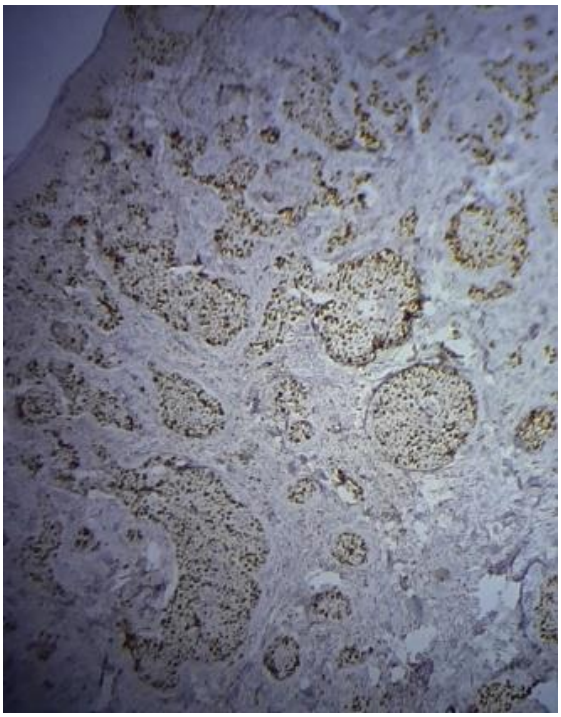
Assessment of the slides: The percentage of cells (with nuclear staining only) that stained positively in 10 high power fields in the scored area was used to express the Ki-67 index. The grade of staining was given similar to ‘Lee’ et al method, [7] as following classification:

- Grade zero : mean no staining at all ,
- Grade plus / minus: mean there is positive nuclear stain in 1–5% of tumors cells,
- Grade plus one: mean there is positive nuclear stain in 6–25% of tumors cells,
- Grade plus two: mean there is positive nuclear stain in 26–50% of tumors cells,
- Grade plus three: mean there is positive nuclear stain in more than 50% of tumors cells, as it shown in pictures (1,2,3).

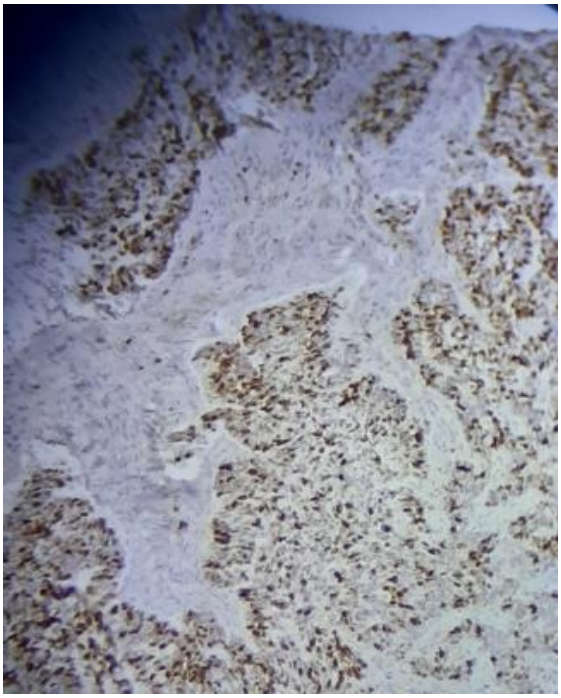
Statistical analysis was done using chi-square test with p -value < 0.05 was considered significant association.



Pic1:primary nodular BCC immunohistochemical staining for Ki-67 antigen. There is irregular positivity approximately 21% of all neoplastic cells (grade 1+), while a certain part of tumor is strikingly positive, another regions are practically negative



Pic2:Primary nodular BCC immunohistochemical staining for Ki-67 antigen. There is irregular positivity approximately 35% of all neoplastic cells (grade 2+)



Pic3:Primary nodular BCC immunohistochemical staining for Ki-67 antigen. There is irregular positivity approximately 65% of all neoplastic cells (grade 3+)

RESULTS

In this study, 35 patients registered with total 35 biopsy had been done(31primary & 4recurrent). There were eleven male and twenty four female. The mean age was (59.2 years).

Regarding the topographical disease distribution, it was considerably found that 71.4% of the cases on the face as it shown in table(1) . While contemplating to histological subtypes of basal cell carcinoma, the nodular infiltrate growth pattern account for (48.6% of cases) as it shown in table (1) also.

Table (1): Clinicopathological Data of BCC biopsies

Parameters	**NO. of lesions**	**Percentage %**
Age		
30-45	9	25.7
46-60	12	34.3
61-75	14	40.0
TOTAL	35	100.0
Gender		
Female	24	68.6
Male	11	31.4
TOTAL	35	100.0
Topographical distribution		
Face	25	71.4
Neck	4	11.4
Nose	3	8.6
Ear	2	5.7
Eye	1	2.9
TOTAL	35	100.0
Histological subtype		
Nodular	6	17.1
Ulcerative	5	14.2
Nodular infiltrative	17	48.6
Superficial	2	5.7
Morphea	1	2.9
Mixed (basalosquamous)	1	2.9
BCC with adnexal differentiation	3	8.6
TOTAL	35	100.0
Infiltration		
No	7	20
Yes	28	80
TOTAL	35	100.0

Then assessment of the association between the proliferating cell nuclear antigen (Ki-67) with different parameters showed significant relation between tumor size and the Ki 67 level and grade as it shown in table (2).

Table (2) : Association between Ki67 grade of expression and the tumor size .

BCC Size	Ki-67 antigen grade					
	G0	G+/-	G1+	G2+	G3+	P-VALUE
Less than 20 mm	0	1	4	2	2	0.01
Equal or More than 20 mm	0	0	2	16	8	
TOTAL	0	1	6	18	10	

The study reported a non-significant association between the tumor histological subtype and Ki 67 level as it shown in table (3).

Table(3): Association between the histological subtype of BCC and Ki67 grade of expression.

BCC subtypes	Ki-67 antigen grade					
	G 0	G+ /-	G1 +	G2 +	G3 +	P - VALUE
Nodular	0	1	2	2	1	0.1
Ulcerative	0	0	1	3	1	
Nodular infiltrative	0	0	3	9	5	
Superficial	0	0	0	1	1	
Morphea	0	0	0	0	1	
Mixed (basalosquamous)	0	0	0	0	1	
BCC with adnexal differentiation	0	0	0	3	0	
TOTAL	0	1	6	18	10	

This study reveal no significant association between disease duration and the Ki67 level as shown in table (4).

Table (4): Association between the disease duration and Ki67 Expression grades.

Disease duration	Ki-67 antigen grade					
	G0	G+/-	G1+	G2+	G3+	P-VALUE
Less than 6 months	0	1	2	7	3	0.2
More than 6 months	0	0	4	11	7	
TOTAL	0	1	6	18	10	

The data showed that range of ki67 level was (2 to 70%) with mean value of (34%) among various histological growth subtype of basal cell carcinoma.

DISCUSSION

Basal Cell Carcinoma of skin is distinct from majority of other carcinomas in that it has a disproportionately high proportion of proliferative active tumor cells. [8] The"Ki-67" antigen, which encodes 2 protein iso-forms was identified by ' Scholzer and Gerdes' forty years ago. It is not found in resting cells, but during all active phases

of cell cycle it is present. Studies on the predictive usefulness of Ki67 have revealed its potential as a reliable indicator in malignancies of the breast, soft tissue, lung, prostate, cervix &CNS.[9]

The data showed that there were a wide range of ki67 level (2 to 70%). Higher proliferative index indicate more malignant potential and lower proliferative index mean less aggressive tumor. So the wide range of proliferation which had been reported in the BCC illuminate the wide spectrum of biological activity of the tumor. [10]

Despite the fact that there is probably no substantial association between the "Ki-67"index and the histological BCCs type, the data showed aggressive tumor types stereotypically come with higher levels of cell proliferation for example Nodular infiltrative tumor showed high level of Ki67 (63%), Superficial lesion with ulceration reported (59%) and basalosqumamous lesion conveyed the highest level (74%), the same results had been informed by other researches as in Barrett et al., Horlock et al.,Mazareli et al. , Misiuk Hojlo et al. & Ionescu et al. [11-15] In nodular BCCs, s. Cabral et al. identified 2 types of "Ki-67" immunoreactivity. Proliferation restricted to BASAL PALISADING CELLS within tiny nodular patterns, but missing at the basal-membrane &dispersed throughout the lesion in vast nodular patterns. Both patterns were present side-by-side in several lesions. They proposed the hypothesis that it may be due to loss of differentiation in tumor & certain mutations can lead to loss of cellular micro-architecture and proliferation control related to stroma of the tumour interactions as it approved in this research. [16]

A special focus should be placed on the skin's BCC, which has a disproportionately high percentage of proliferating cells. It is inconsistent with clinical signs and symptoms that is typically slowly progressing, inert cancer with poor metastatic activity. This phenomena could be explained by either a protracted cell cycle or significant & continuous loss of cells followed by their perpetual rebirth. Previously in 1972, Kerr postulated that slowly growing of BCC might be explained by the huge rate of cancer cell apoptosis. Mooney et al. later provided evidence in favor of this theory. Currently, the imbalance between the proliferative and apoptotic mechanisms of the tumor is what really causing the variances in biological activity & advancement of Cutaneous BCC. [3,17- 22]

Toward the end, The effect of "Ki-67" expression as a prognostic factor has received mixed data up to this point. Despite the fact that several writers (Misiuk Hojlo et al. [14] ,Chuprov.,[20] Salman [6] et al.) asserted that the degree of Ki-67 expression was a helpful and accurate indicator of the severity of the disease, other researchers (Horlock et al.[12] , Correa et al. [23]) came to the conclusion that there was no significant differences statistically in the BCC growth fraction between prognostically "favorable" and "unfavorable" tumors & that the "Ki-67 index" couldn't be regarded as a respectable prognostic antigen.

In conclusion, this study finding suggests that it is likely insufficient to simply quantify BCC proliferation activity in order to forecast future behavior, evolution & fate of that tumor .

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