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Comprehensive Analysis of Ki67 Proliferative Index in Basal Cell Carcinoma: Correlation with Clinical and Histopathological Subtypes

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تحليل شامل لمؤشر تكاثر الخلايا *Ki67* في سرطان الخلايا القاعدية: علاقته بالأنواع الفرعية السريرية والنسيجية المرضية

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Abstract:

Background: Basal cell carcinoma (BCC) is the most common type of non-melanoma skin cancer in the whole world with about 75 percent of all skin-related cancers. It has a close pathogenesis associated with cumulative exposure to ultraviolet (UV) radiation, and major variation in clinical behaviour based on the histopathological subtype. Ki67, a nuclear proliferation, has already become one of the useful markers in differentiating between biologically aggressive and indolent BCC subtypes, but its systematic evaluation among the population of Iraq is still minimal.

Objective: The study aims to examine the clinical pathology features of BCC among patients who visit hospitals of the Wasit Health Directorate and specifically to explore the Ki67 proliferative index of the histological types and the possible application as a prognostic marker.

Methods: A prospective cross-sectional study was carried out in the period between May 2022 and May 2024 at the hospitals of the Wasit Health Directorate. Eighty BCC cases were recruited histopathologically. Demographic information (age, sex, occupation), the location of the tumor and histopathological subtypes were noted. Hematoxylin and eosin (H&E) tissue sections were used. The immunohistochemical assay of Ki67 was done by investigating with MIB-1 monoclonal antibody clone; Ki67 was considered low (less than 10% nuclear staining) and high (greater than 10% nuclear staining) staining. Fisher exact test was used to measure statistical significance.

Results: BCC was higher among the male population (57.5% vs. 42.5 per cent), although the highest incidence was found in the group of patients of 61 years and above (45 per cent). The risk factor of outdoor occupational UV exposure was very high and was found in 42.5% of male patients and 17.5% of female patients. The most prevalent histopathological subtype was nodular (55%), then there were superficial (22.5%), pigmented (8.75%), basosquamous (7.5%), and infiltrative (6.25%). Nine percent percent of aggressive subtypes (basosquamous

and infiltrative combined) had high Ki67 expression, versus 16 of non-aggressive subtypes (nodular, superficial, and pigmented, p = 0.001). Most affected anatomical sites were at the nose (55%) and the medial canthus (25%).
Conclusion: The Ki67 immunohistochemical marker expression has proved to be statistically significant regarding BCC histopathological aggressiveness, making it possible to objectively distinguish between indolent and aggressive subtypes. Ki67 analysis included in standard pathology of BCC can help to improve the generalization of prognosis and adjust treatment choices. Such results also highlight a necessity of special attention to effective public health strategies to deal with UV exposure among the occupationally vulnerable Iraqi population.

Keywords: Basal Cell Carcinoma; Ki67; Proliferative Index; Immunohistochemistry; Histopathological Subtypes; UV Radiation; Iraq.

الملخص
خلفية الدراسة :تُعد سرطانة الخلايا القاعدية (BCC) النوع الأكثر شيوعاً من سرطانات الجلد غير الميلانينية في العالم، حيث تمثل حوالي 75% من جميع السرطانات المرتبطة بالجلد. ويرتبط نشوؤها المرضي ارتباطاً وثيقاً بالتعرض التراكمي للأشعة فوق البنفسجية(UV) ، كما تُظهر تبايناً كبيراً في السلوك السريري بناءً على النوع الفرعي النسيجي. وقد أصبح بروتين Ki67 (علامة تكاثر نووي) أحد المؤشرات المفيدة في التمييز بين أنواع سرطانة الخلايا القاعدية العدوانية بيولوجياً والأنواع الحميدة (الخاملة)، إلا أن تقييمه المنهجي بين سكان العراق لا يزال محدوداً.
الهدف :تهدف الدراسة إلى فحص الخصائص السريرية المرضية لسرطانة الخلايا القاعدية بين المرضى المراجعين لمستشفيات دائرة صحة واسط، واستكشاف مؤشر التكاثر Ki67 للأنواع النسيجية وتطبيقه المحتمل كعلامة إنذارية.
المنهجية :أُجريت دراسة مقطعية مستعرضة في الفترة ما بين مايو 2022 ومايو 2024 في مستشفيات دائرة صحة واسط. شملت الدراسة 80 حالة مشخصة نسيجياً بسرطانة الخلايا القاعدية. تم تسجيل المعلومات الديموغرافية (العمر، الجنس، المهنة)، موقع الورم، والأنواع الفرعية النسيجية. استُخدمت مقاطع نسيجية مصبوغة بالهيماتوكسيلين والإيوسين(H&E) ، وأُجري الفحص المناعي الكيميائي للنسيج لبروتين Ki67 باستخدام الجسم المضاد أحادي النسيلة(MIB-1) ؛ واعتُبر تعبير Ki67 منخفضاً (أقل من 10% صبغ نووي) أو عالياً (أكثر من 10% صبغ نووي). استُخدم اختبار "فيشر" الدقيق لقياس الأهمية الإحصائية.
النتائج :كانت نسبة الإصابة بسرطانة الخلايا القاعدية أعلى بين الذكور (57.5% مقابل 42.5%)، ووجدت أعلى نسبة إصابة في الفئة العمرية 61 عاماً فما فوق (45%). كان عامل الخطر المتمثل في التعرض للأشعة فوق البنفسجية بحكم المهنة (العمل في الخارج) مرتفعاً جداً، حيث وُجد لدى 42.5% من المرضى الذكور و17.5% من الإناث. كان النوع الفرعي النسيجي الأكثر شيوعاً هو "العقدي" (55%)، يليه "السطحي" (22.5%)، ثم "المتصبغ" (8.75%)، و"القاعدي الحرشفي" (7.5%)، و"الارتشاحي" (6.25%). أظهرت الأنواع الفرعية العدوانية (القاعدي الحرشفي والارتشاحي معاً) تعبيراً عالياً لـ Ki67 بنسبة 90%، مقابل 16% للأنواع غير العدوانية (العقدي، السطحي، والمتصبغ) بقيمة احتمالية (p = 0.001).كانت أكثر المواقع التشريحية تضرراً هي الأنف (55%) والمآقي الإنسي.(25%)
الاستنتاج :أثبت تعبير المؤشر الكيميائي المناعي Ki67 دلالة إحصائية فيما يتعلق بالعدوانية النسيجية لسرطانة الخلايا القاعدية، مما يجعل من الممكن التمييز بموضوعية بين الأنواع الفرعية الخاملة والعدوانية. إن إدراج تحليل Ki67 في الفحص المرضي القياسي لسرطانة الخلايا القاعدية يمكن أن يساعد في تحسين التنبؤ بالمرض وضبط خيارات العلاج. كما تسلط هذه النتائج الضوء على ضرورة إيلاء اهتمام خاص لاستراتيجيات الصحة العامة الفعالة للتعامل مع التعرض للأشعة فوق البنفسجية بين الفئات السكانية العراقية المعرضة للخطر بسبب طبيعة عملها.

الكلمات المفتاحية :سرطانة الخلايا القاعدية؛Ki67 ؛ مؤشر التكاثر؛ الكيمياء النسيجية المناعية؛ الأنواع الفرعية النسيجية؛ الأشعة فوق البنفسجية؛ العراق.

1. Introduction

In the whole world, basal cell carcinoma (BCC) is the most widespread type of cutaneous malignancy with the proportion of 75% of all non-melanoma skin cancers [1]. The BCC is a disease that emerges in response to cumulative ultraviolet (UV) radiation-induced damage to DNA, especially leading to impairments in the tumor suppressor TP53 pathway and as well as the unregulated activation of hedgehog signaling cascade [2], which is caused by the pluripotent basal cells of the epidermis and its derivatives. Despite the fact that BCC does not spread much, its ability to invade local tissues greatly when not properly treated makes early diagnosis and proper management a clinical necessity.

BCC is on the increase in most parts of the world with the highest rates seen in the fair skinned population which is found in high UV index areas. Old age, male gender, and long-term occupational or recreational exposure to sun are already determined demographic risk factors [3]. The older adults (more than 50 years old) are particularly susceptible to this, as UV-induced genomic damage is cumulative; men are less commonly exposed, in large part because of the increased incidence of outdoor work-related exposure [3].

Histologically, BCC is divided into various distinct subtypes such as nodular, superficial, pigmented, infiltrative and basosquamous ones with different biological behavior and clinical prognosis. Nodular subtype which comprises 80 percent of BCC cases in most series is usually considered the least aggressive [4, 5]. On the contrary, infiltrative and basosquamous variants have much higher local recurrence and deep tissue invasion rates and require more intensive treatment methods [4].

Ki67 is a cellular nuclear protein that is expressed in proliferating cells in all active periods of the cell cycle (G1, S, G2 and M) making it a good immunohistochemical surrogate of cellular proliferative activity [6]. Ki67 labeling index is a prognostic and predictive biomarker that has been used in a variety of solid tumors. The Ki67 index has been demonstrated to be related to histopathological subtype in BCC and is becoming a more useful tool to predict tumor aggressiveness and risk of recurrence [6, 7].

The current research seeks to describe the clinicopathologic features of BCC in cases seen in the Wasit Health Directorate hospitals as well as to critically compare the Ki67 expression of the various histopathological subtypes with an aim to establishing its prognostic value and whether it can be used to provide individualized patient care or not.

2. Materials and Methods

2.1 Study Design and Setting

The prospective cross-sectional analytical research was carried out at the Wasit Health Directorate hospitals during the period of May 2022 to May 2024. Primary clinical diagnoses were made by dermatologists and all the cases were later confirmed using histopathological biopsy analysis done in the diagnostic laboratory of the hospital.

2.2 Patient Selection

Eighty patients who had ever had histopathic confirmed BCC were recruited sequentially. Demographic data (age, sex, occupational category, indoor or outdoor) on a patient basis was taken systematically. Occupation was categorized as outdoor whereby the patient stated that most of their working hours were under direct sun exposure (e.g., agriculture, construction, and transportation).

2.3 Histopathological Analysis.

The skin biopsy samples were preserved in 10 percent neutral-buffered formalin and subjected to routine paraffin-wax histological processing. Thick serial sections with a thickness of 3 5 of 5 0 mm were cut and stained using hematoxylin and eosin (H&E) to perform routine

histopathological assessment. A board-certified consultant histopathologist carried out microscopic examination and subtype classification. Histopathological subtypes were subdivided as: nodular, superficial, pigmented, basosquamous and infiltrative types.

2.4 Ki67 Immunohistochemical Study.

The Ki67 immunohistochemical (IHC) staining was applied on representative formalin fix, paraffin embedded (FFPE) sections of all cases that were confirmed to have BCC, with the use of the MIB-1 monoclonal antibody against the Ki67 according to the standard IHC protocols [9, 10]. Ki67 labeling index was calculated by dividing the percentage of the positively stained tumor cell nuclei by 500 or more cells counted in hotspots under high power magnification (x 400). Ki67 level was further reduced to dichotomous: Low Expression (10% nuclear staining) and High Expression (>10% nuclear staining), confirmed in previous BCC studies [11, 12]. To analyze them, histopathology subtypes were divided into aggressive (basosquamous and infiltrative) and non-aggressive (nodular, superficial, and pigmented) types.

2.5 Statistical Analysis

The study cohort demographic and clinical profiles were used to describe data in terms of categorical variables, using frequencies and percentages. Fisher exact test was used to measure the relationship between the Ki67 expression and BCC histopathological subtype (aggressive vs. non-aggressive). The statistically significant p-value was taken to be under 0.05. The statistical analyses were all done using IBM SPSS Statistics version 26.0.

3. Results

3.1 Distribution of BCC According to Sex

As presented in Table 1, BCC was more prevalent among male patients (n=46; 57.5%) compared to female patients (n=34; 42.5%), a distribution consistent with global epidemiological trends linking BCC to occupational UV exposure in outdoor professions [8].

Table 1: Distribution of BCC Cases According to Sex (n=80)

Sex	Number of Cases	Percentage (%)
Female	34	42.5
Male	46	57.5
Total	80	100.0

3.2 Distribution of BCC According to Age

Table 2 demonstrates that BCC incidence increased progressively with advancing age. The highest case concentration was observed in the age group 61 years and above (n=36; 45%), followed by the 41–50-year cohort (n=24; 30%). No cases were recorded in patients aged 20 years or younger, consistent with the cumulative nature of UV-induced carcinogenesis.

Table 2: Distribution of BCC Cases According to Age Group (n=80)

Age Group (years)	Number of Cases	Percentage (%)
≤20	0	0.0
21–30	2	2.5
31–40	6	7.5
41–50	24	30.0
51–60	12	15.0
≥61	36	45.0
Total	80	100.0

3.3 Distribution of BCC According to Occupational UV Exposure

Occupational data revealed that 42.5% of male patients were engaged in outdoor occupations compared with 17.5% of female patients (Table 3), underscoring the significant contribution of occupational UV exposure to BCC development, particularly in male subjects.

Table 3: Distribution of BCC Cases According to Sex and Occupational UV Exposure (n=80)

Sex	Indoor Occupation n (%)	Outdoor Occupation n (%)
Female	20 (25.0)	14 (17.5)
Male	12 (15.0)	34 (42.5)

3.4 Distribution of BCC According to Clinical Type

The nodular clinical subtype was the most frequent presentation, accounting for 80% of cases (n=64), consistent with international literature (Table 4). Histopathological analysis revealed characteristic peripheral palisading of basaloid cells, varying growth patterns, and stromal changes across subtypes.

Table 4: Distribution of BCC Cases According to Clinical Subtype (n=80)

Clinical Subtype	Number of Cases	Percentage (%)
Nodular	64	80.0
Cicatrized	2	2.5
Pigmented	6	7.5
Cystic	4	5.0
Superficial	4	5.0
Total	80	100.0

3.5 Distribution of BCC According to Anatomical Site

The nose (55%; n=44) and medial canthus of the eye (25%; n=20) were the predominant tumor locations, corroborating the established predilection of BCC for chronically sun-exposed facial areas (Table 5).

Table 5: Distribution of BCC Cases According to Anatomical Site (n=80)

Anatomical Site	Number of Cases	Percentage (%)
Nose	44	55.0
Medial Canthus	20	25.0
Temporal Region	8	10.0
Cheek	4	5.0
Trunk	4	5.0
Total	80	100.0

3.6 Distribution of BCC According to Histopathological Subtype

Among the histopathological variants, nodular BCC was the most prevalent (n=44; 55%), followed by superficial (n=18; 22.5%), pigmented (n=7; 8.75%), basosquamous (n=6; 7.5%), and infiltrative (n=5; 6.25%) subtypes (Table 6). Representative histopathological photomicrographs of nodular and infiltrative BCC are shown in Figures 1A and 1B, respectively.

Table 6: Distribution of BCC Cases According to Histopathological Subtype (n=80)

Histopathological Subtype	Number of Cases	Percentage (%)
Nodular	44	55.00
Superficial	18	22.50
Pigmented	7	8.75
Basosquamous	6	7.50
Infiltrative	5	6.25
Total	80	100.0

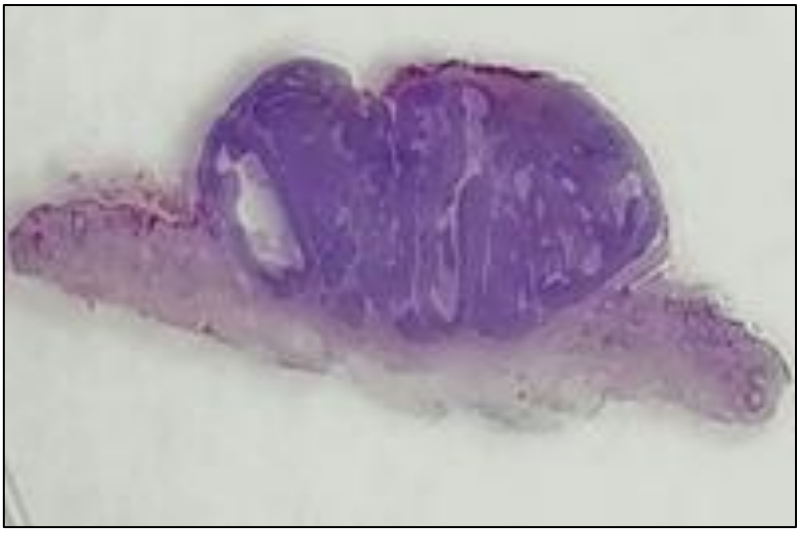

Figure 1A: Nodular BCC characteristic peripheral palisading of basaloid cells and peri-tumoral clefting (H&E, ×100)

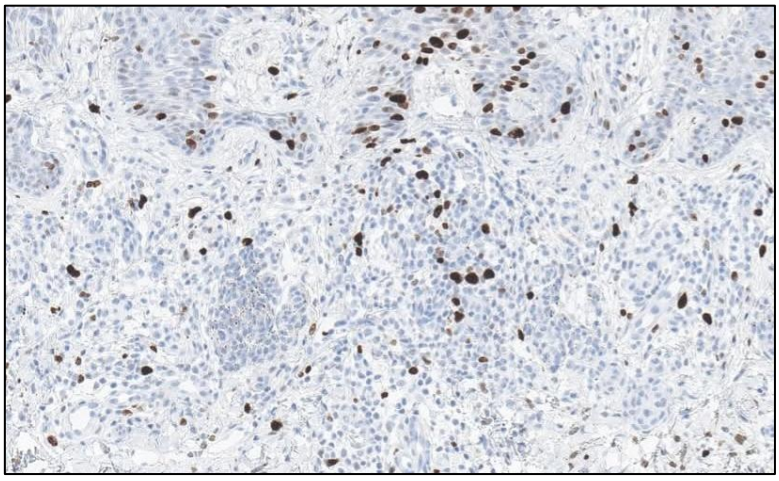

Figure 1B: Infiltrative BCC irregular strand-like invasion into the dermis without peripheral palisading (H&E, ×100)

3.7 Ki67 Expression Across Histopathological Subtypes

Ki67 immunohistochemical expression differed markedly between aggressive and non-aggressive BCC subtypes (Table 7). Among the aggressive subtypes (basosquamous and infiltrative; n=11), high Ki67 expression (>10% nuclear staining) was identified in 91% of cases (n=10). Conversely, among non-aggressive subtypes (nodular, superficial, and pigmented; n=69), low Ki67 expression was predominant, present in 84% of cases (n=58). The difference in Ki67 expression between aggressive and non-aggressive groups was highly statistically significant (Fisher's exact test, $p = 0.001$).

Table 7: Ki67 Expression According to BCC Histopathological Subtype Grouping

Histopathological Group	Low Ki67 n (%)	High Ki67 n (%)	Total n (%)	p-value
Aggressive (Basosquamous + Infiltrative)	1 (9%)	10 (91%)	11 (100%)	0.001
Non-Aggressive (Nodular + Superficial + Pigmented)	58 (84%)	11 (16%)	69 (100%)	
Total	59 (73.75%)	21 (26.25%)	80 (100%)	

Representative photomicrographs of Ki67 immunohistochemical staining in non-aggressive (low expression) and aggressive (high expression) BCC subtypes are illustrated in Figures 2A and 2B.

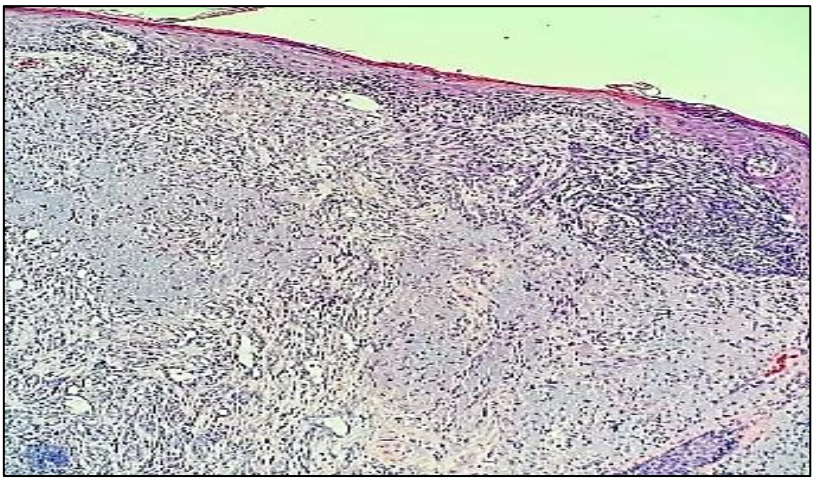

Figure 1: Comparative Ki67 expression (%) between aggressive and non-aggressive BCC subtypes

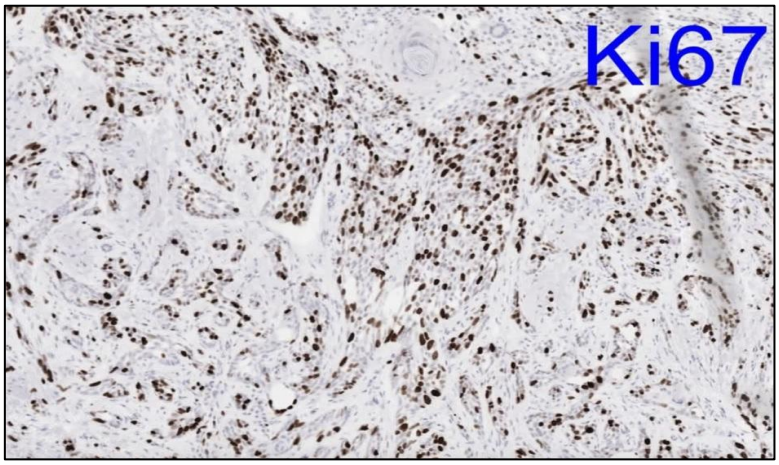

Figure 2: Ki67 immunohistochemical staining (A) Low Ki67 expression in non-aggressive BCC (nodular subtype); (B) High Ki67 expression in aggressive BCC (basosquamous subtype) (Anti-Ki67/MIB-1, ×400)

4. Discussion

4.1 Demographic and Occupational Risk Factors

This male predominance (57.5) is in line with the epidemiological trends in the world [13, 14]. Exposure to UV radiation through outdoor working conditions has continued to be one of the greatest modifiable environmental risk factors of BCC and this research also supports such an association: 42.5 percent of male patients had worked in outdoor jobs as opposed to 17.5 percent of the female patients [15]. However, the increasing ratio of women patients in this series could be associated with the changes in societal trends, such as the rising rates of women engagement in outdoor activities as well as the lack of photoprotective behavior.

The presence of the age distribution of the cohort of 45% of cases occurring in patients aged 61 years and above supports the well-known cumulative model of UV carcinogenesis. The cumulative growth of DNA damage during decades in combination with the age-associated deterioration of DNA repair mechanisms and immunosenescence are all factors that explain such a dramatic rise in the number of BCC causation with age progression [3].

4.2 Clinical and Histopathologic Variants.

The nodular type of BCC (55% histopathological; 80% clinical) found in this paper is in agreement with other literature worldwide, where nodular BCC is always the most common form of BCC [16]. Clinically, nodular BCC is a typical pearly, telangiectasis papule which may have a central fissure when it is subjected to sun. The superficial type of BCC (22.5%) is usually found in younger patients and is more likely linked to the regions of the UV intermittence exposure, including the trunk [17]. Pigmented BCC (8.75) is somewhat rare and is commonly seen in those people with darker skin phototyping.

Aggressive subtypes basosquamous and infiltrative constituted, in total, 13.75% of histopathological cases, in line with the prevalence rates in respective population groups [18]. This finding is supported by the fact that the nasal (55) and medial canthal (25) location was the most common in this group of participants as a demonstration of the fact that BCC develops in a region where UV exposure is the most cumulative, namely the central face, and is also an anatomically problematic area with regards to achieving adequate surgical margins [19].

4.3 Ki67 Expression and Subtype of histopathology.

Massive difference in Ki67 proliferative index was observed between BCC histopathological subtypes. In the case of non-aggressive forms (nodular, superficial, and pigmented), 84 percent showed a low level of Ki67 expression in line with their clinically insensitive nature and low likelihood of local recurrence [20]. On the other hand, aggressive subtypes (basosquamous and infiltrative) had high Ki67 in 91% of cases, which can be related to the increased cellular proliferation activity in line with their aggressive clinical course.

Basosquamous carcinoma, a mixed neoplasm that has morphological characteristics of BCC and squamous cell carcinoma is characterized by a high local recurrence rate and local and distant metastasis [10]. Infiltrative BCC where the irregular thin strands of tumor cells infiltrate the dermis that lack peripheral palisading cells and the stromal retraction creates an ill defined clinical border that makes complete surgical excision technically challenging. The Ki67 labeling index in these aggressive subtypes is very high, and this objective quantifies the proliferation capacity of these subtypes and the reason why greater excision margins and more vigilant postsurgery protocols should be implemented [21].

4.4 Clinical Implication of Ki67 expression.

Ki67 proliferative index has a high prognostic and therapeutic value in the treatment of BCC. In low Ki 67-expressing non-aggressive subtypes, non-aggressive subtypes with conservative treatment modalities such as surgical curettage with electrodessication, cryotherapy, or photodynamic therapy are valid and well-supported treatment options. On the other hand, basosquamous and infiltrative BCC that exhibit high levels of Ki67 expression should be aggressively excised with histologically verified clear margins, Mohs micrographic surgery

should be considered and a structured post-surgical follow-up to enable prompt local recurrence [22].

These results substantiate the importance of incorporating Ki67 immunohistochemical staining as a complementary adjunct of conventional histopathological subtyping especially in those situations where the histologic appearance is gray or where the clinical behavior does not correlate with the histopathological grade. We find that our findings are consistent with the reports of Cameron et al. [15] and Gundalli et al. [16], who also reported high Ki67 indices in aggressive BCC variants, and thus, we can conclude that Ki67 is a reproducible and a clinically significant biomarker in this case [23, 24].

4.5 Environmental and Behavioral Determinants.

Extensive work-related UV exposure, especially in farming, building and transport sectors, is one of the major environmental causes of BCC carcinogenesis among this group of individuals. Some of the causes include low knowledge of the photoprotection measures and difficulty in accessing sunscreen products in rural and peri-urban populations. These findings underscore the urgent requirement of community-based health education on the occupational groups at high risk and policy interventions to guarantee access to the right photoprotective materials [24].

4.6 Global Epidemiological Data Comparison.

The identified epidemiological trends in this cohort are generally consistent with the global data. Areas that have high UV indexes ambiently (especially in Australia) record one of the highest incidence rates of BCC in the world. It is also important to note that the morpheaform and infiltrative subtypes were comparatively lower in the current series than in some of the Western series, which might also be due to genetic differences, referral patterns or a variation in diagnostic methods. More population descriptive research should be conducted to determine the entire range of BCC histopathological subtype distribution in Iraq [25].

The importance of early detection and prevention is also described (4.7).

BCC diagnosis at an early stage is the most important one, as later disease progression is characterized by the increased morbidity, more complicated surgical treatment, and higher healthcare expenses. Awareness on warning signs of BCC at the population level, along with systematic skin surveillance programs aimed at high-risk occupational populations is highly encouraged. Nonpharmacological measures (behaviors) such as frequent application of the broad-spectrum sunscreen, protective clothing, and wide-brimmed hats have been proven to be effective in preventing UV-based cutaneous carcinogenesis [26].

4.8 Limitations to the Study and Future Direction.

This research is limited in a number of ways. The single-center nature and the rather small sample size (n=80) may hinder the conclusions to other areas and healthcare environments in Iraq. The Ki67 cutoff of 10% came out of the earlier literature, but further research employing the continuous analysis of the index of Ki67 can give more subtle prognosis results. Future multicenter research should be encouraged to include more cohorts as this would help us to have stronger epidemiological evidence on BCC in Iraq. In addition, molecular profiling of BCC such as p53 mutational status, Bcl-2 expression and hedgehog pathway activation would provide further understanding of the pathobiological mechanisms behind the subtype-specific behavior and may help in finding other therapeutic targets [16].

5. Conclusion

This paper outlines the patterns of expression of Ki67 proliferative index distinctly in BCC histopathological subtypes in an Iraqi patient cohort. Non-aggressive forms such as nodular, superficial, pigmented BCC, are associated with low Ki67 levels, which is in line with their insidious course. Conversely, basosquamous and infiltrative subtypes of BCC have significantly higher levels of Ki67 labeling index, which indicates their aggressive biological

characteristics and increased likelihood of recurrence. The Ki67 immunohistochemistry assay has been a useful addition to standard histopathological subtyping with the ability to enhance prognostic stratification, evidence-based therapeutic decision-making, and eventually patient outcome.

Future studies ought to aim at confirming the prognostic value of Ki67 in larger multicenter BCC cohorts as well as provide insights on the complementarity of Ki67, p53, Bcl-2 and hedgehog pathway biomarkers in establishing the molecular nature of BCC aggressiveness. Simultaneously, the focus on UV protection education and early diagnosis measures is an urgent national health concern to limit the BCC morbidity in Iraq.

6.Conflict of Interest

The author declares no conflict of interest in relation to this study.

7.Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

8.Ethical Approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Institutional ethical approval was obtained from the Wasit Health Directorate, and informed consent was obtained from all study participants prior to enrollment.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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