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Comparative Analysis of Ki-67 Protein as a Proliferative Expression Index in Cutaneous Basal and Squamous Cell Carcinoma in Federal Medical Centre Umuahia, Nigeria

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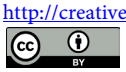
Abstract

Background: Evaluating the tumor proliferative index helps predict clinical behavior and provides prognostic insights for cutaneous basal cell carcinoma (cBCC) and squamous cell carcinoma (cSCC). **Objective:** This study aimed to identify differences in the proliferative indices among variants of cBCC and cSCC diagnosed at a tertiary healthcare center. **Method:** Skin biopsies histologically diagnosed as cBCC and cSCC between 2012 and 2018 at the Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria, were analyzed. Archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved along with clinical data, and were prepared on charged microscope slides and the immunohistochemical staining was carried out. The primary antibody used in this study was clone BioCare CRM325C (RM) and adenotonsillar tissue blocks/slides served as positive controls. Ki-67 immunohistochemistry was performed on fresh 4μm sections of the tumor specimens. **Results:** The application of Ki-67 immunoperoxidase on both BCC and SCC cohort, yielded an intense observable brownish nuclear stain in areas of dense proliferating



tumour cells on both cutaneous tumours. The average Ki-67 index for all cSCC cases was 24.7%, with a range of 2.3% - 80%, while the mean for cBCC was 15.8%, ranging from 1.2% - 45.6%. Variants with high proliferative indices were observed in 11.9% of cBCC cases and 29.1% of cSCC cases. Among the low proliferative index category, cSCC accounted for 5.4%, while cBCC represented 14.3%. For mild proliferative indices, cSCC cases made up 7.3% and cBCC, 11.9%. The majority of cases showed moderate proliferative indices, with 61.9% for cBCC and 58.2% for cSCC. Overall, there was a significant difference in proliferative indices between cSCC, cBCC, and their variants. **Conclusion:** The study found a significantly higher rate of cell proliferation, measured by Ki-67 immunostaining, in cSCC and its variants compared to cBCC. However, certain variants of cBCC also exhibited high Ki-67 expression, indicating they can be as aggressive as some cSCC variants.

Keywords

Ki-67 Expression, Cancer, Proliferation, Histological Variants, Squamous Cell Carcinoma, Basal Cell Carcinoma

1. Introduction

Cutaneous basal cell carcinoma (cBCC) and squamous cell carcinoma (cSCC) share a common histogenetic origin, arise from the epidermis and similar aetiologic factors, however, considerable differences abound in their behavior. BCC will rarely metastasize, whereas SCCs are not only more locally aggressive, but also have a much greater metastatic potential. Why BCCs have a more indolent course remains an enigma [1].

Recent advances in molecular pathology and biology have identified various biomarkers in tumour tissue that significantly participate in carcinogenesis and they have proven to be of great importance in the prediction of clinical behavior of cutaneous basal and squamous cell carcinoma and other malignant conditions [2].

Biologic proteins including Ki-67 nuclear expression are associated with the biological behavior of these tumours [3]. It has been documented that Ki-67 antigen (a proliferative marker) assessment is superior by far to the mitotic count for the assessment of proliferation of cutaneous basal and squamous cell carcinoma and this has become a useful tool in diagnosis and prognostication of cutaneous basal cell and squamous cell carcinoma as well as other aggressive and non-aggressive malignancies [3].

Cellular proliferation in malignant lesions generally correlates with rapid tumour growth, poor tumour cell differentiation and aggressive clinical and biological behavior. These proliferative activities of malignant cell can be detected immunohistochemically by antibodies against Ki-67 antigen [2]. Ki-67 antigen is a non-histone nuclear protein expressed during active phases of the cell cycle (G1, S, G2 and mitotic phase) and it is absent in "resting" (G0) phase. Under normal condition, this protein resides predominantly in the nucleolus and is present on the surfaces

of condensed chromatin and chromosomes during mitosis. It is however relocated to the nucleoplasm before localizing into the nucleolus after cell division [2].

Immunohistochemical assessment of nuclear Ki-67 expression (Ki-67 index) in neoplastic cells allows a quantitative measure of their proliferation status and index, constituting one of the basic prognostic indicators in a routine histopathological report as its assessment may help in early and precise diagnosis and prognostication of cutaneous basal and squamous cell carcinomas. In addition, a percentage of Ki-67 immunoreactivity can also serve as one of the cut-off criteria for malignancy in numerous neoplasms [2] [4].

Ki-67 score in malignant tumours is assessed by the intensity of the antibody immunohistochemical staining. The earliest publications on ki-67 assessment regarded percentage score <5% as inconclusive/negative/or low proliferation rate, 5% - 25% as weak positive/mild proliferation rate, values >25% - 30% to be moderate and values above 30% as high proliferation rate and therefore termed as aggressive tumours [5] [6]. This has been replicated in subsequent publications with variations and modifications; However, some authors used index >50% as strongly positive/ high proliferation rate for highly aggressive tumours [6].

Researchers in most parts of the western world have made several enquiries into the differences in biological behavior of BCC and SCC, by studying their proliferative indices using nuclear antigen marker like Ki-67. However, no documented data on the proliferative indices of cutaneous BCC and SCC using Ki-67 nuclear antigen in Federal Medical Center (FMC) Umuahia, Abia State and Southeastern region of Nigeria at large exists. This study therefore sought to determine the differences in the proliferative indices among the variants of cutaneous basal and squamous cell carcinoma seen in Federal Medical Centre Umuahia, Abia State.

2. Methods

2.1. Study Area

The study was carried out in the Department of Anatomical Pathology, Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria. FMC Umuahia is one of the leading tertiary health care providers in the entire South-Eastern Nigeria with a 327-bed space capacity and an average yearly sample turnover of about 850 - 1000 patient tissue specimens.

2.2. Study Design

This was a cross-sectional retrospective seven year (2012-2018) hospital-based study that compared the proliferative indices of cutaneous basal cell carcinoma and its variants with those of cutaneous squamous cell carcinoma as measured by Ki-67 immunoperoxidase nuclear stain.

2.3. Study Population

The study involved all the skin biopsies with histologic diagnosis of cutaneous

basal and squamous cell carcinoma at the Department of Anatomical Pathology, FMC Umuahia, Abia State from 1st January 2012 to December 31st 2018.

2.4. Inclusion and Exclusion Criteria

The study involved formalin fixed paraffin embedded (FFPE) tissue blocks and haematoxylin and eosin (H&E) slides of histologically diagnosed cases of cutaneous basal and squamous cell carcinomas as received in the department within the study period. Cases with missing or damaged blocks were excluded from the study.

2.5. Data Collection

The list of all histologically diagnosed cutaneous squamous and basal cell carcinomas in the departmental histology register from 2012-2018 were retrieved from the patients' database. Relevant histology numbers and their biodata, clinical information, including age, sex, and nature of specimen and sites of tumour location of both cutaneous BCC and SCC diagnosed during the study period were extracted and used to retrieve the archival H&E slides and corresponding FFPE tissue block for review. In cases where the slides were faded, fresh sections were prepared form FFPE

2.6. Microtomy and Tissue Preparation for Staining

Representative thin sections at 4 μm of the paraffin-embedded tissue block of the selected cases of cutaneous basal and squamous cell carcinoma were cut on a microtome, floated on charged slides and incubated for 10 mins at 55˚C to melt the wax and de-paraffinized in two changes of xylene lasting 10 mins each to completely remove the wax. In order to prevent residual wax artifacts, the amount of xylene used was limited to 50 ml per fifty slides. The tissue sections were passed through two changes of 100% ethyl alcohol each for 3 mins to remove the xylene. Thereafter, the tissue sections were passed through 95% and 70% of ethyl alcohols respectively for 3 mins each and subsequently aqueous wash buffer for 5 mins.

2.7. Antigen Retrieval

Antigen retrieval was by heat-induced epitope retrieval (HIER) using conventional water bath. This antigen retrieval method served to unmask antigenic sites in order to allow the antibodies to bind. Citrate buffer (0.01 M citrate buffer, pH 6.0) was pre-heated in water bath to 95˚C and thereafter the rehydrated tissues on slides were immersed in the pre-heated solution and incubated for 20 mins at 98˚C. The tissue sections were then removed from the water bath and allowed to cool, while still in retrieval solution for 20 mins at room temperature. The tissue sections were then rinsed in phosphate buffered saline (PBS) at room temperature for 5 mins in readiness for immune-histochemical staining.

2.8. Blocking of Endogenous Peroxide Activity

The tissue sections on the slides were demarcated with a hydrophobic marker pen to prevent run-off of reagents and 2 drops of endogenous enzyme block (3% hydrogen peroxide) were applied to the tissue sections and incubated in humidified chamber for 10 mins. The endogenous enzyme block neutralized the actions of endogenous peroxidase and the slides were then washed in buffer.

2.9. Immunohistochemical Staining

Using a primary monoclonal antibody (clone BioCareCRM325C (RM)) against Ki-67 with a dilution of 1:100 dilution, each section was then covered with the 100 µl of the diluted primary antibody and incubated in the humidified chamber at room temperature for 1 hour along with the control. Excess primary antibody was rinsed off with wash buffer 2 - 3 times for 5 mins each. Afterwards, sections were incubated with 100 µl of BioCare MACH4 mouse probe for 30 mins at room temperature as secondary antibody and then washed with wash buffer. Thereafter incubated in 3, 3-diaminobenzidine substrate solution (BioCare DAB Chromogen) and the slides were rinsed in running tap water. This was followed by counterstaining with Mayer's hematoxylin for 10 seconds, the slides were then dehydrated in 70%, 95% and two changes of 100% alcohol for 5 minutes each. Exactly 2 drops of xylene-based mounting medium, DPX (distyrene, plasticizer and xylene) were added to the sections using a glass rod, the sections were covered with glass coverslip and allowed to dry.

2.10. Immunostaining Evaluation

The slides were viewed with Olympus CX22LED light microscope and brown nuclear staining was interpreted as positive staining for Ki-67 regardless of staining intensity. While blue staining of the nucleus was interpreted as negative for Ki-67. The sections were examined at high power (x40) and 10 fields were chosen in the area showing most proliferation (areas showing most positive nuclear staining with Ki-67): 100 cells were assessed in each field. The quantitative estimate of the Ki-67 immunoreactivity was made by scoring positive nuclei per 1000 nuclei per sections. The Ki-67 index was calculated manually by quantitatively evaluating 1000 cells and determining the number of Ki-67 positive tumour cells divided by total number of cells multiplied by 100 [5]. The histologic sections of tonsillar tissue stained with Ki-67 was used as positive control while the Ki-67 stain of the stratum basalis of the epidermis served as positive internal control for individual sections of cutaneous BCC and SCC. The intensity of immunohistochemical staining as well as the categorization of the proliferative index using Ki-67 were as follows: Low cell proliferation rate (<5%), mild cell proliferation rate (5% to 10%), moderate cell proliferation rate (>10% to 30%) and high cell proliferation rate (>30%). [7]

2.11. Data Analysis

The data obtained was analyzed using the Statistical Package for Social Sciences

SPSS version 25.0 (IBM Corp., Armonk, N.Y., USA). The student's t test was used for comparison of the mean of continuous variables with a p value of <0.05 considered as significant.

2.12. Ethical Considerations

Ethical approval for this study was obtained from the Health and Research Ethics committee of Federal Medical Centre Umuahia Abia State, Nigeria with approval number: FMC/QEH/596/Vol.10/421.

3. Results

3.1. General Overview

The application of Ki-67 immunoperoxidase, a proliferative marker on both BCC and SCC cohort, yielded an intense observable brownish nuclear stain in areas of dense proliferating tumour cells on both cutaneous tumours. Overall, the Ki-67 index of all the cutaneous SCC in this study ranged from 2.3% - 80% with a mean value of 24.7% while those of cutaneous BCC in ranged from 1.2% - 45.6% with a mean value of 15.8% (Figure 1).

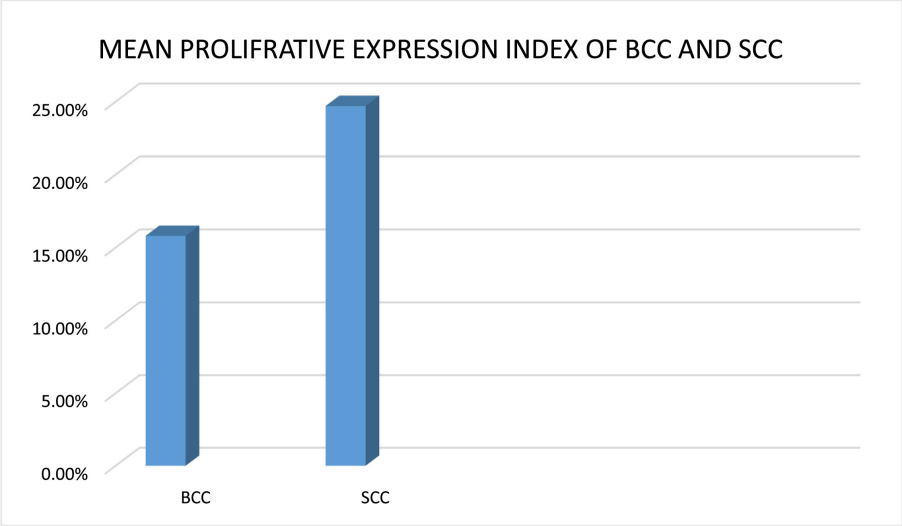


Figure 1. Showing mean expression of proliferative index of BCC and SCC.

3.2. Ki-67 Staining Pattern and Proliferative Expression of Cutaneous SCC

In this research, the proliferative marker on SCC cohort, yielded a more intense observable brownish nuclear stains, much more than that of cutaneous BCC in areas of dense proliferating tumour cells. The histologically designated low-grade variants of SCC (keratoacanthoma, Verrucous carcinoma and Squamous cell carcinoma NOS) had Ki-67 index mean value of 16.7% and a range value of 2.3% - 35.7%, while the histologically designated high-grade variants (adenosquamous and acantholytic) had a Ki-67 index mean value of 46.0% with a range of 23.5-

80%.

Despite the variability in stains among all the variants of SCC, the nuclear staining pattern for low grade SCC was less heterogeneous with occasional focal stain while the high-grade variants maintained diffuse and overt heterogeneous staining pattern. See Figures 2-4.

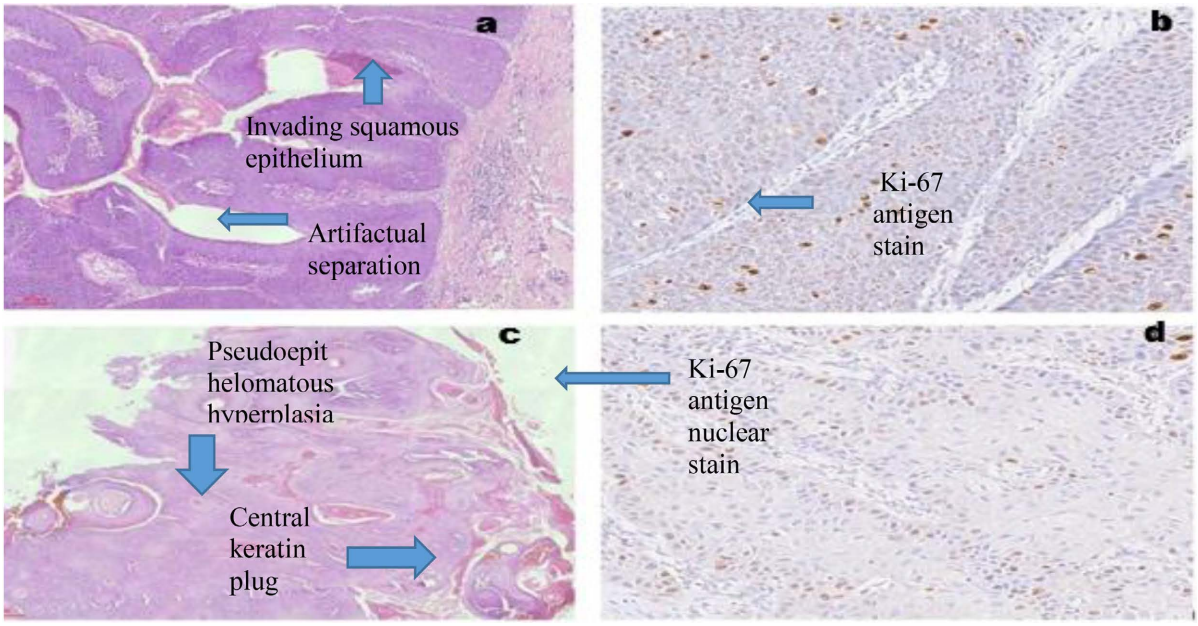


Figure 2. Photomicrograph of H&E stain of Verrucous and Keratoacanthma SCC at x40 magnification: (a) Photomicrograph showing H & E stain of verrucous SCC, (b) Photomicrograph showing ki-67 positive nuclear staining technique of a mild proliferative index verrucous SCC, (c) Photomicrograph showing H & E stain of keratoacanthoma SCC, (d) Photomicrograph showing ki-67 positive immunoperoxidase nuclear staining of a mild proliferative index keratoacanthoma SCC.

3.3. Ki-67 Staining Pattern and Proliferative Expression among Cutanous Basal Cell Carcinoma

The application of Ki-67 immunoperoxidase, a proliferative marker on BCC cohort, yielded an intense observable brownish nuclear stains in areas of dense proliferating tumour cells. The staining pattern as well as the histologic slides of all variants of basal cell carcinoma seen in this research (nodular BCC, infiltrating, Basosquamous, sclerosing (morpheaform)) are shown in Figures 5-7 respectively.

The predominant staining pattern in histologically designated low-risk subtypes of BCC (Nodular and Superficial variants) were focal and peripheral, with a Ki-67 index ranged from 1.2% - 18% and a mean value of 10.0%. The histologically designated high-risk subtypes or aggressive variants of BCC (Basosquamous, Infiltrating and Morpheaform BCC) had more diffuse and heterogeneous staining pattern combined with occasional peripheral staining pattern with expression index ranged from 14.4% - 45.6% and a mean value of 25.1%. See Figures 5-7.

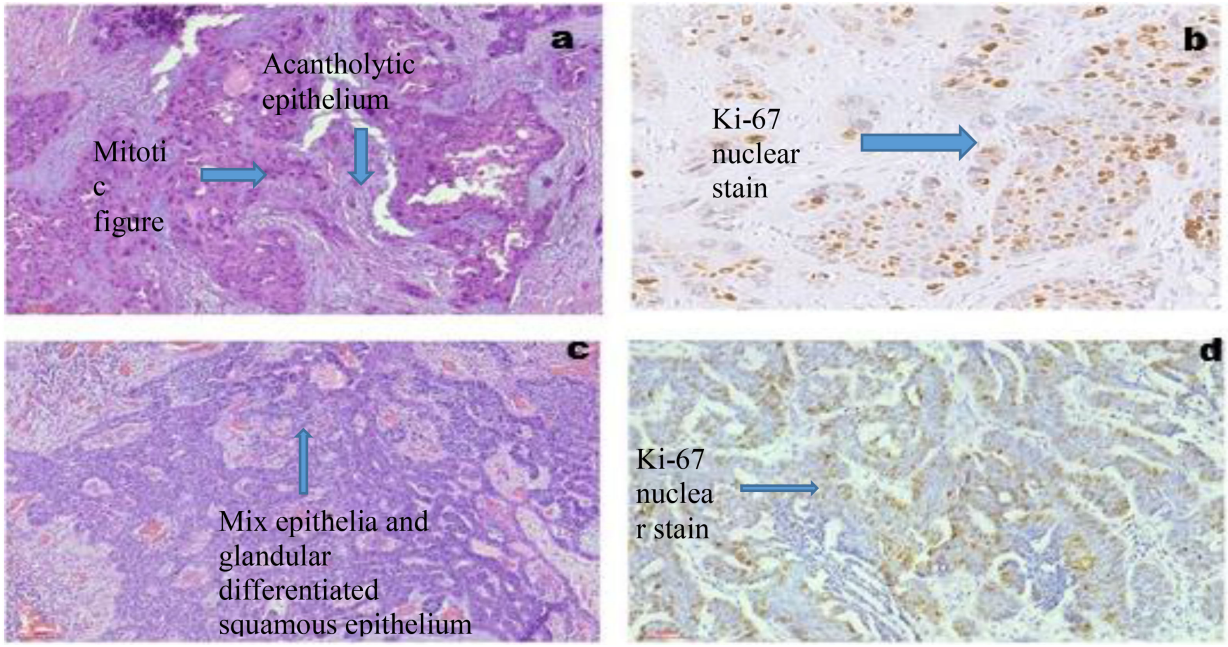


Figure 3. Photomicrograh of acantholytic and adenosquamous variants of SCC at x40 magnifications: (a) Photomicrograph showing H & E stain of acantholytic SCC, (b) Photomicrograph showing ki-67 positive staining of a high proliferative index acantholytic SCC, (c) Photomicrograph showing H&E stain of adenosquamous SCC, (d) Photomicrograph showing ki-67 positive nuclear staining of a moderate proliferative index adenosquamous SCC.

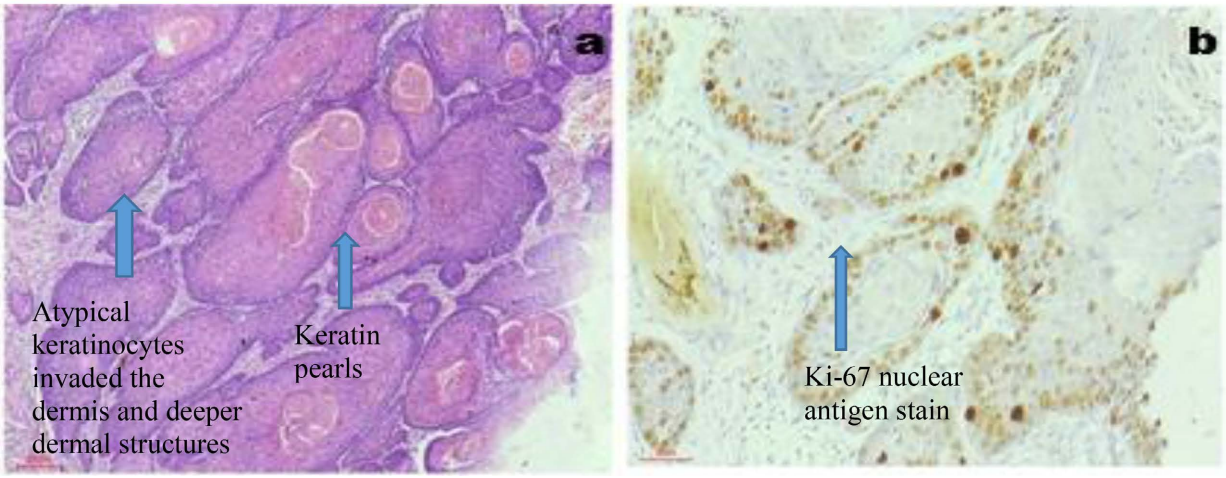


Figure 4. Photomicrograph of squamous cell carcinoma NOS at x40 magnifications: (a) Photomicrograph showing high power view of squamous cell carcinoma NOS, (b) Photomicrograph showing ki-67 positive immunoperoxidase nuclear staining of a moderate proliferative index of squamous cell carcinoma NOS.

3.4. Proliferative Index Score among Variants of BCC

The Ki-67 index of nodular BCC variant ranged from 1.2% - 15.2% with a mean value of 9.3 while those of superficial BCC variant were between 2% - 18% with a mean value of 11.2, the infiltrating variants of BCC were between 14.4% - 28.2% with a mean value of 19.6. The Ki-67 index of basosquamous variant ranged from 21.2% - 45.6% with a mean value of 35.4 while the morpheaform (sclerosing) variant 18% - 32.7% with a mean value of 22.5%. **See** Table 1.

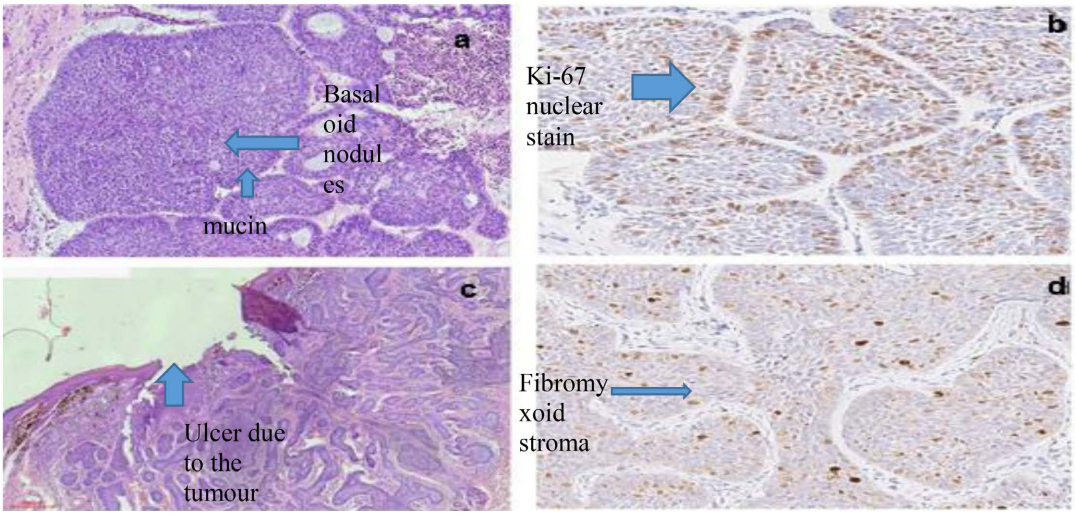


Figure 5. Photomicrographs of nodular and superficial variants of basal cell carcinoma at x 40 magnifications: (a) Photomicrograph showing H & E stain of nodular BCC, (b) Ki-67 positive immunoperoxidase nuclear stain of a moderate proliferative index nodular BCC, (c). Photomicrograph showing H & E stain of superficial BCC, (d) Photomicrograph showing ki-67 positive immunoperoxidase nuclear stain of a moderate proliferative index superficial BCC.

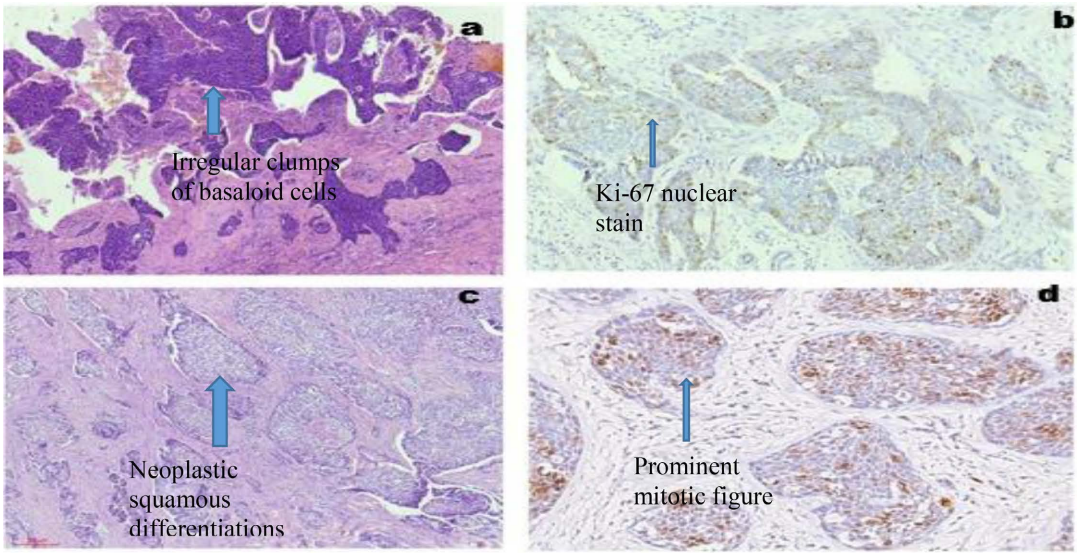


Figure 6. Photomicrographs of Infiltrating and Basosquamous subtypes of BCC at X 40 magnification: (a) Photomicrograph showing H & E of infiltrating BCC, (b) Photomicrograph showing ki-67 positive stain of a moderate proliferative index infiltrating BCC, (c) Photomicrograph showing H & E stain of basosquamous BCC, (d) Photomicrograph showing ki-67 positive nuclear stain of a high proliferative index basosquamous BCC.

Table 1. Ki-67 index scores of the variants of cutaneous basal cell carcinoma.

BCC Variants	Range of score (%)	Mean Ki-67 score
Basosquamous	21.2 - 45.6	35.4
Infliterating	14.4 - 28.2	19.6
Morphaeform	18 - 32.7	22.5
Nodular	1.2 - 21	9.3
Superficial	2 - 18	11.2

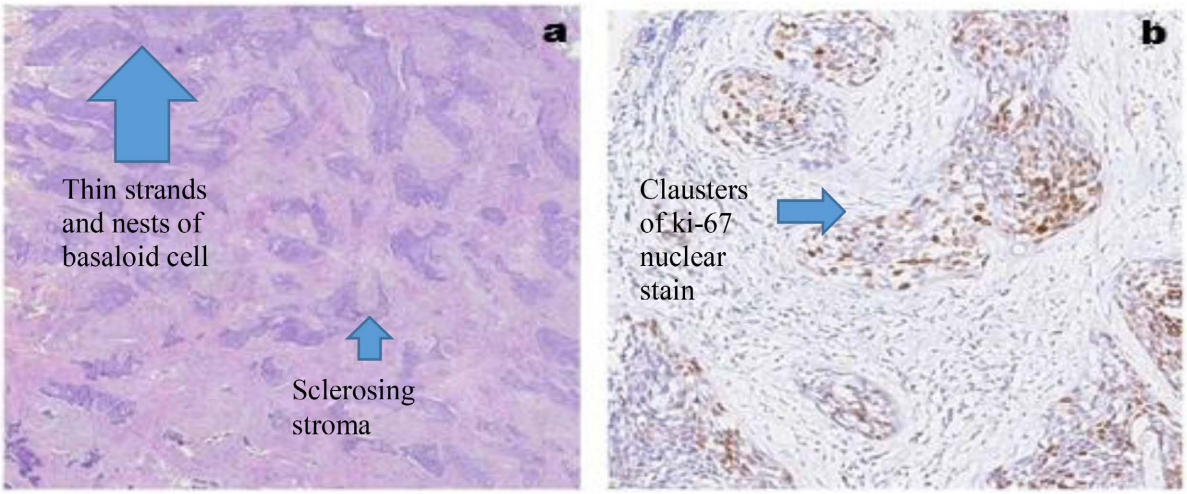


Figure 7. Photomicrographs of sclerosing (morphaeform) variant of BCC at x 40 magnification: (a) Photomicrograph showing H & E of sclerosing (morpheaform) BCC, (b) Photomicrograph showing ki-67 positive immunoperoxidase nuclear staining of a high proliferative index sclerosing (morpheaform) BCC.

The predominant staining pattern in histologically designated low-risk subtypes (subtypes with low proliferative indices) of BCC (Nodular and Superficial variants) were localized and predominantly peripheral, with a Ki-67 index ranging from 1.2% - 4.9% and a mean value of 2.6% (Table 2). Those with mild proliferative index had scores ranging from 5% - 10% with a mean index score of 8.1. BCC tumour variants regarded as having moderate proliferative index recorded scores ranging from 10.2% - 29.1% with an average of 16.5%. The histologically designated high-risk subtypes or aggressive variants of BCC (basosquamous, infiltrating and morpheaform BCC) had more diffuse and heterogeneous staining pattern combined with occasional peripheral staining pattern with a mean of 25.1% and a range of 14.4% - 45.6%.

Table 2. Ki-67 index scores among the tumour grade of BCC.

BCC tumour variants grades	Range of score (%)	Mean Ki-67 score
Low proliferative index	1.2 - 4.9	2.6
Mild proliferative index	5 - 10	8.1
Moderate proliferative index	10.2 - 29.1	16.5
High proliferative index	14.4 - 45.6	25.1

3.5. Index Score among the Variants of SCC

The Ki-67 index was variably expressed among the variants of SCC of which the squamous cell carcinoma NOS had Ki-67 index range from 9.2% - 35.7% with a mean value of 20.2%. Adenosquamous variant ranged from 40% - 80% with a mean value of 55.3%, Acantholytic variant, 23.5% - 45.2% and a mean value of 32.0%, Verrucous squamous cell carcinoma, 2.3% - 10.5% with a mean value of 7.7%, while those of Keratoacanthoma variants ranged from 2.4% - 10.7% with a mean value of 6.8% (Table 3).

Table 3. Ki-67 index scores of the variants of cutaneous squamous cell carcinoma.

SCC variants	Range of score (%)	Mean Ki-67 score
Acantholytic	23.5 - 45.2	32.0
Adenosquamous	40 - 80	55.3
Keratoacanthoma	2.4 - 10.7	6.8
SCC NOS	9.2 - 35.7	20.2
Verrucous	2.3 - 10.5	7.7

3.6. Proliferative Index Score of SCC Tumour Grade

The histologically designated low proliferative variants of SCC (keratoacanthoma and verrucous squamous cell carcinoma) had Ki-67 index mean value of 2.7% and a range value of 2.3% - 3.5% (Table 4), the SCC variants which recorded mild proliferative indices (SCC NOS, keratoacanthoma and verrucous squamous cell carcinoma) had Ki-67 index mean value of 7.8 with 6.6% - 9.2% range Ki-67 index scores respectively. The subtypes with moderate proliferative indices had mean Ki-67 score of 18.3 while their minimum and maximum index scores were 10.2% and 30% respectively, while the histologically designated high-grade (high proliferative index) variants had a Ki-67 index mean value of 46.2 with a range of 31.2% - 80% (Table 4). The nuclear staining pattern for low grade SCC was less heterogeneous with occasional focal stain while the high-grade variants maintained diffuse and overt heterogeneous staining pattern. See Table 4.

Table 4. Ki-67 index scores of squamous cell carcinoma tumour grades.

SCC tumour variant grades	Range of score (%)	Mean Ki-67 score
Low proliferative index	2.3 - 3.5	2.7
Mild proliferative index	6.6 - 9.2	7.8
Moderate proliferative index	10.2 - 30	18.3
High proliferative index	31.2 - 80	46.2

3.7. Comparison of Ki-67 Expression between Cutaneous SCC and BCC

As shown in Figure 8 the number of cutaneous SCC and cutaneous BCC with a low proliferative index (Ki-67 score <5%) as observed by their Ki-67 nuclear stain accounted for 5.6% (3) and 14.3% (6) respectively, while those that fell under mild proliferative index (Ki-67 score between 5% - 10%) were 7.4% (4) for SCC and 11.9% (5) for BCC. Cases with moderate proliferative index (Ki-67 score between 10% - 30%) were 61.9% (26 cases) for BCC and SCC 57.4% (31 cases). BCC had only 11.9% (5 cases) with high proliferative index (Ki-67 score >30%) and these were the histologically designated aggressive subtypes of BCC while SCC had 29.6% (16) with high proliferative index which were predominantly the histologically designated high-grade variants of SCC. This vividly showed that SCC has more aggressive variants that BCC.

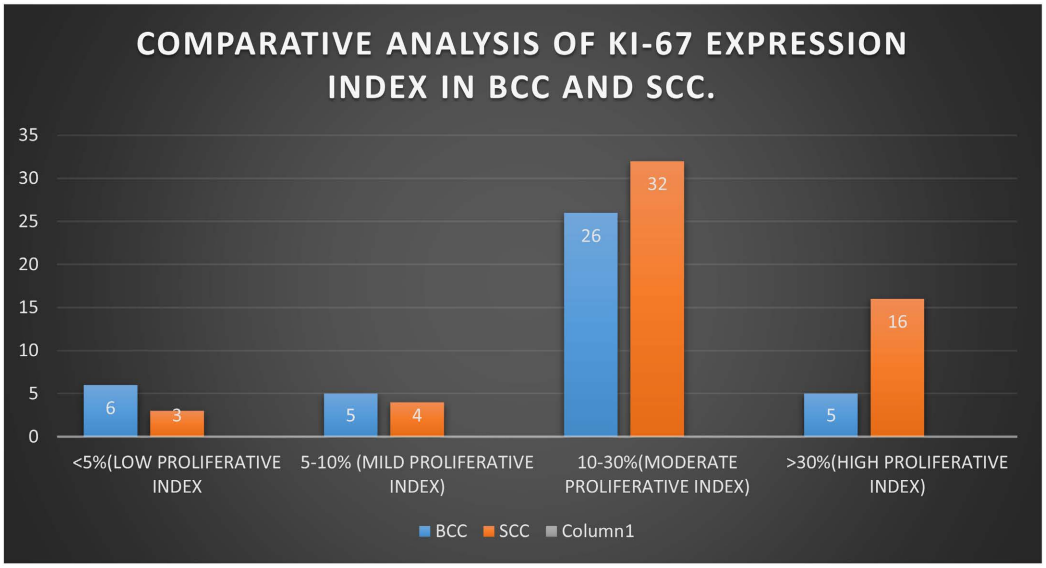


Figure 8. Showing the proliferative index distribution between BCC and SCC.

4. Discussion

The assessment of tumour proliferative index has proven to be of great importance in the prediction of clinical behavior as well as tumour prognostication even in cutaneous basal and squamous cell carcinoma, therefore Ki-67 marker can achieve the goal of explaining the considerable differences in biologic behaviors that exist between cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) [2] [4].

This study all together evaluated 96 cases of BCC and SCC which accounted for 64% of all cutaneous malignancies in FMC Umuahia over the 7 year period. The observed immunoreactivity pattern among the histologically aggressive subtypes (basosquamous, infiltrating and morpheaform) of BCCs and the high-grade subtypes (Adenosquamous and Acantholytic variants) of SCCs displayed more heterogeneous and diffuse pattern of staining. Conversely, most of nodular and superficial variants of BCC (low risk subtypes) and the low-grade variants of SCC (Squamous cell carcinoma NOS, Verrucous carcinoma and keratoacanthoma), displayed focal and less heterogeneous and peripheral Ki-67 staining pattern in areas of dense tumour formation. Regardless of the percentage range of Ki-67 staining, the nuclear immunoreactivity was intense in almost all BCC and SCC cases and there was no tumour without Ki-67 nuclear reaction in this study. This pattern of stains in SCC and BCC are similar to what have been observed in previous studies. Tilli *et al.* and Mohebat *et al.* observed comparable similarities in pattern of distribution in their findings and Ki-67 stain was detected in more than 83.3% of all cases of BCC and SCC evaluated in their studies [8] [9].

Reports differ among authors on the ranges and means of Ki-67 index of BCC and SCC as observed in many literatures. Tilli *et al.*, Eya *et al.* and Al-Sader *et al.*, had similar observations in their studies and they documented a mean of 20% Ki-67 index for SCC, 14.9% for BCC with a range of 1% - 61% and 0% - 71% for BCC

and SCC respectively [1] [3] [8]; findings which are similar to what we observed for SCC and BCC in this current study.

On the contrary, findings of Mehrnaz *et al.* recorded a mean of 75.4% for SCC while Albertine *et al.*, Nuran *et al.*, Joonsoo *et al.*, Vladimir *et al.*, documented a mean and ranges of Ki-67 index for BCC that showed wide variance with what we observed in this current study [2] [10]-[13]. Vladimir *et al.* documented a variable growth fraction between different histomorphologic subtypes of keratinocytes tumours and therefore noted that this could vary from place to place [2]. Therefore, it suffices to say that the range of proliferative index of both BCC and SCC can vary from place to place.

This staining variability of BCC and SCC according to their level of aggressiveness had also been documented in previous studies on BCC and SCC Ki-67 expression [1] [2] [5] [12]. Furthermore, earlier studies by Florescu *et al.* and Pietro *et al.* showed that high risk variants of BCC like Morpheaform and Adenoid variant of BCC have very high Ki-67 nuclear stain and Low-risk subtypes can be as low as 0% - 22% while the high-risk subtypes can be as high as 34% - 100% [5] [14]. These variabilities in staining were observed in this present study and had also been documented by Tilli *et al.,* Mohebet, *et al.*, Mehrnaz, *et al.*, Pietro *et al.*, Alexandru *et al.*, Amar *et al.*, Haiying *et al.*, who in their independent researches observed that Ki-67 correlates with the aggressiveness of tumour. They noted that the well differentiated the SCC, the lesser the value of their Ki-67 and that affects its biological behavior [8]-[10] [14]-[17].

It is worthy of note that these proliferative indices observed in this study reached and even exceeded the prognostic proliferative index of some of the aggressive human malignancies like melanoma and glioblastoma similar to the findings of Vladimir *et al.* and Amalia *et al.* [2] [6]. Most (62%) of the variants of BCC in the present study fell within moderate proliferative index while only (12%) were recorded as high proliferative index which was about the same number that were observed for the mild proliferative index and low proliferative index accounting for 14.3% (PI < 5%). On the contrary, more than half (57.4%) of the cutaneous SCC had moderate proliferative index and 29.6% had high proliferative index. The percentage of tumour that had low proliferative index and mild proliferative index combined, formed 13% of the population. This demonstrated more number of SCC with high Ki-67 nuclear stain when compared to the number of BCC that had their proliferative index greater than 30%. The proliferative indices of cutaneous squamous cell carcinoma were significantly higher than those of cutaneous basal cell carcinoma with a T static value of −2.89 and p-value of 0.006.

Although BCC is regarded as a neoplasia with high healing rate, less aggressive with little or no ability to metastasize [5] [12] [13], there is a contrary view, describing BCC as a cancer with several tumour subtypes having variable histomorphological picture and biological behaviors [2] [7]. Some cases may ab initio have an aggressive behavior with rapid infiltration in deeper tissue structures, recur after treatment and sometimes give rise to metastasis and exhibit greater proliferative activity [2] [8]. This fact is further buttressed by the presence of some variant

of BCC in the current study which had Ki-67 index up to 45.6%. It must be kept in mind therefore that although BCC is largely a less aggressive tumour, there exist certain variants that can be as aggressive as SCC and other high-grade human tumours. Overall, there was higher proliferative index in SCC when compared with BCC and this could be responsible for the more aggressive behaviors in SCC.

5. Conclusion

In conclusion, this study demonstrated a significant proliferative index as measured by Ki-67 immunoperoxidase nuclear staining in cutaneous SCC than BCC. The aggressive subtypes of both BCC and SCC have higher proliferative index than the low-grade subtypes and also variants of SCC have higher proliferative index than the variants of BCC. These findings may be helpful in prognostication as well as probability of tumour recurrence after treatment and for the purpose of therapy and clinical follow up. Although a high proliferative index alone may not be the factor responsible for the more aggressive behaviors exhibited by SCC as against the more indolent behavior of BCC, one must also recognize that BCC is a relevant factor as some of its variants also exhibit high Ki-67 expression.

6. Recommendations

In view of our observation, there is a need to do a proper clinical follow up for cutaneous BCC as much as we do for SCC because of the existence of aggressive variants of BCC with high Ki-67 immunoperoxidase expressions and as such can have similar clinical and biologic behaviors to that of SCC in terms of invasion, metastasis and recurrence.

7. Limitations and Shortcomings

This study is not able to correlate the proliferative index of these tumors with their clinical presentation and outcome. This, therefore, serves as window for further study. The fund was also a limitation for this study

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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