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Immunohistopathologic Study of the Proliferative Expression Index Using Ki-67 Immunoperoxidase Protein on Diagnosed Variants of Basal Cell Carcinoma in a Tertiary Health Facility in South East Nigeria

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ABSTRACT

Background*: Basal cell carcinomas is one of the commonest cancer worldwide with a variable Nigerian prevalence of 7% to 20% of all histologically diagnosed cancers across Nigeria.*

Basal cell carcinoma has various histologic subtypes which can be divided into low risk and high risk subtypes. The low risk subtypes include Superficial basal cell carcinoma, Nodular basal cell carcinoma (adenoid, cystic and keratotic variant, Pigmented basal cell carcinoma, Fibroepithelial basal cell carcinoma and Infundibulocystic basal cell carcinoma (BCC with adnexal differentiation). They have low risk of recurrence, less proliferation and low potential for malignancy. While the high risk include; Superficial micronodular basal cell carcinoma, Infiltrating basal cell carcinoma, Sclerosing/morphoeaform basal cell carcinoma, Basosquamous basal cell carcinoma and Basal cell carcinoma with sarcomatoid differentiation. having higher ability to metastasize, proliferate rapidly and have invasive tendencies.

Aims: *This study analyzed the proliferative index using ki-67 immunoperoxidase protein of the histologic subtypes of basal cell carcinoma diagnosed in Federal Medical centre Umuahia, as to know the most dominant subtype of BCC and their proliferative index using Ki-67 in order to predict their biologic behaviors*

Methodology: *Archival FFPE blocks were retrieved alongside relevant clinical data. Hematoxylin and Eosin as well as immunohistochemistry using monoclonal antibody against Ki-67 (BioCare CRM325C(RM)) stains were done on fresh 4-micron sections of tumour specimens to determine the antigen expression.*

Result: *The Ki-67 expression among the nodular variant of BCC, ranges from 1.2-15.2% with a mean value of 9.3%. The superficial variant expresses a Ki-67 expression value ranges from 2-18% with a mean value of 11.2%. The infiltrating variant of BCC has Ki-67 expression of a range of value from 14.4-28.2% with a mean value of 19.6%. Basosquamous variant has Ki-67 expression ranges from 21-45.6% with a mean value of 35.4%. The Sclerosing (morpheaform) variant has a Ki-67 expression ranging from 18-32.7% with a mean value of 22.5%. The Proliferative index using Ki-67 nuclear stain of the low risk subtypes ranged from 1.2-18% with a mean value of 10%, while the high-risk subtypes have Proliferative index of 14.4-45.6% and a mean value of 25.1%.the most dominant variant is nodular BCC with the lowest proliferative index.*

Conclusion: *The high grade subtypes of cutaneous BCC, (infiltrating, Morpheaform and basosquamous BCC) possess a significant high proliferative index as high as 45.6% for basosquamous variant, a proliferative index that can be found in high grade carcinomas and sarcomas. Therefore, there is need for critical evaluation and follow up for all cases of BCC in order to isolate those with high proliferative index and manage accordingly.*

Keywords
Basal Cell Carcinoma, Ki-67, Immunohistochemistry, Proliferative Index, Immunoperoxidase Staining, Skin Cancer, Histopathology, Cancer Biomarkers.

Introduction
Basal cell carcinomas are among the most commonly encountered skin cancers worldwide. It assumes a significant proportion among the non-melanoma skin cancers of the skin [1]. The actual prevalence of BCC in Nigeria remains a matter of debate as various centre-based studies have demonstrated a range of 7% to 20% in conjunction with SCC of all histologically diagnosed malignancies with regional and geographic variations in local and global statistics [2]. This tumour exhibits various degree of differentiation and subtypes, which is invariably important in prognostication and predictability of aggression and tumour recurrence [3].

Basal cell carcinoma has various histologic subtypes which can be divided into low risk and high risk subtypes. The low risk subtypes which include Superficial basal cell carcinoma, Nodular basal cell carcinoma (adenoid, cystic and keratotic variant, pigmented basal cell carcinoma, Fibroepithelial basal cell carcinoma and Infundibulocystic basal cell carcinoma (BCC with adnexal differentiation), have low risk of recurrence, less proliferation and low potential for malignany [4].

Likewise, the high risk subtypes which are direct opposite of the lowrisk subtypes having higher ability to metastasize, proliferate rapidly and have invasive tendencies. They include; Superficial micronodular basal cell carcinoma, Infiltrating basal cell carcinoma, Sclerosing/morphoeaform basal cell carcinoma, Basosquamous basal cell carcinoma and Basal cell carcinoma with sarcomatoid differentiation [4]. These subtypes have higher risk of recurrence and proliferation, frequency of perineural invasion and lymphovascular invasion and increased metastatic rate; therefore, necessitating proper determination of surgical control margin in treatment [5].

Diagnosis of variants of BCC are made based on the identifiable histologic differences that exist between them.

Nodular Basal Cell Carcinoma; present as nodular island of basaloid cells with peripheral palisading extending into the dermis with haphazard nuclear arrangement, frequents apoptotic bodies and retraction artifact are often seen [3,4].

Superficial Basal cell carcinoma; appear as superficial lobules of basaloid cells that project from the epidermis, sides of follicles or eccrine ducts into the dermis, typically surrounded by loose myxoid stromal and lobules are confine to the papillary dermis [3].

Infiltrating Basal Cell Carcinoma; usally present as Variably sized, sometimes jagged nests of basaloid tumour cells infiltrate within a normal dermal collagen. The tumour has an irregular tentacle, infiltrates/permeating pattern of invasion at the deep tumour edge. There could be perineural invasion.

Morpheaform/sclerosing Basal Cell Carcinoma; Are Narrow cords of tumour compressed by sclerotic collagenous stromal, disrupting the normal dermis architecture. Retraction artifact is uncommon and they penetrate deeply and show an irregular tentacle deep infiltrating boarder with surrounding stromal [4].

Basosquamous Basal Cell Carcinoma; Present as Islands of basaloid cells combine with atypical squamous cells with abundant eosinophilic cytoplasm. The atypical squamous cells can be focal or scattered throughout the lesion [3,4].

Despite the fact that these tumours are all basal cell origin, their behaviour and aggressiveness differ and this can be measured by using immunoperoxidase proteins one of which is ki-67

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 of k1-67 tumour evaluation as follows; <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 and it has subsequently been improved upon. However, some authors used index >50% as strongly positive/ high proliferation rate for highly aggressive tumours [6].

Biologic proteins including Ki-67 nuclear expression are associated with the biological behavior of these tumours [7]. 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 keratinocytic tumours and this has become a useful tool in diagnosis and prognostication of cutaneous basal cell carcinoma as well as other aggressive and non-aggressive malignancies [7].

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 [8,9].

This study analyzed the proliferative index using ki-67 immunoperoxidase protein of the histologic subtypes of basal cell carcinoma diagnosed in Federal Medical Centre Umuahia, as to know the most dominant subtype of BCC and their proliferative index using Ki-67 in order to predict their biologic behaviors.

Materials and Method
Study Design
This is a descriptive retrospective study that involved the evaluation of all the skin biopsies histologically diagnosed as basal

cell carcinoma at the department of Anatomical Pathology, Federal Medical Centre (FMC) Umuahia, Abia State from 2012 to 2018.

The Study Population

The study involved all the skin biopsies with histologic diagnosis of basal at the department of Anatomical Pathology Federal Medical Centre (FMC) Umuahia, Abia State from 1st January 2012 to December 31st 2018.

Sampling Method

It involved the selection of all the consecutive skin biopsies that were histologically diagnosed within the study period for basal cell carcinoma.

Inclusion and Exclusion Criteria

The study involved formalin fixed paraffin embedded (FFPE) tissue block and H&E slides on histologically diagnosed cases of basal cell carcinoma received in the department within the study period. Cases with missing or damage blocks were excluded from the study.

Data Collection

The material that provided data for this study included duplicate copies of histopathologic reports that were issued within the study period, formalin fixed paraffin embedded tissue blocks, histopathology request cards and corresponding archival slides. Demographic data including age and sex, nature of specimen and histopathology diagnosis were obtained from these materials.

Methodology Archival FFPE blocks were retrieved alongside relevant clinical and social demographic data and hematoxylin and Eosin as well as immunohistochemistry using monoclonal antibody against Ki-67 (BioCare CRM325C(RM)) for antigen expression on fresh 4-micron sections of tumour specimens.

The H&E stained slides were interpreted under a light microscope and the immunohistochemical 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 bluish 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.

Result

A total of forty-two cases of basal cell carcinoma were histologically diagnosed in the department of Anatomical Pathology Federal Medical Centre Umuahia (FMCU) which constituted 28% of the total 150 cases of cutaneous malignancies within the study period.

Frequency of the Histological Subtypes of Cutaneous BCC

Of the 42 cases of BCC, nodular variant was the commonest and it accounted for 17 cases (40%), this was followed by Superficial variants accounted for 9 cases (21%) while infiltrating variant was 5 cases (12%). Morpheaform variant is the least common variant and it accounted for 4 cases (10%) while basosquamous yielded 7 cases (17%) respectively**.** Other histologic variants were not found within the study period in FMC Umuahia. See Figure 1.

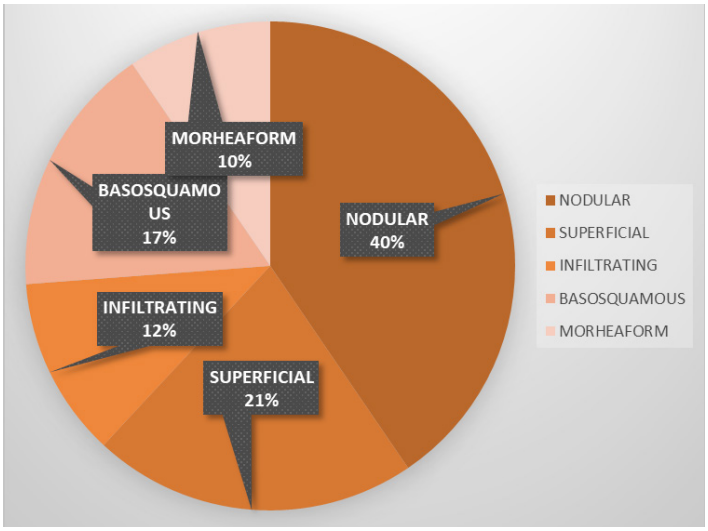

Figure 1: Showing the distribution of the variants of basal cell carcinoma and their frequencies.

Site Distribution of Cutaneous BCC and Variants

Of the 42 cases of BCC, 71% (30 cases) cases were seen in the head and neck region, 19% (8cases) cases were in the trunk and 10% (4cases) were seen in the extremities.

The highest frequency among the variants of BCC in the head and neck region was Nodular variant with number of cases amounted to 12. This is followed by superficial and basosquamous variant with a frequency of 6 cases each. The infiltrating and morpheaform recorded 3 cases each in the head and neck region. The face was the most frequently affected part in the head and neck region accounted for 53.3% (16 cases) of BCC as against the posterior auricular area which constituted the least 3.1% (1case) affected part in the head and neck region. Other areas of the head and neck affected by BCC in order of frequency included the scalp 23.3% (7 cases) and the neck 20% (6 cases).

In the Trunk, the highest occurring variant was also nodular variant amounted to 4 cases, while superficial, basosquamous, infiltrating and morpheaform variant recorded 1 case each. The peri-anal was the most common site for the truncal BCC with 50% (4 cases) frequency while the chest, vulva and back accounted for 25% (2 cases), 12.5% (1 case) and 12.5% (1 case) respectively.

The extremities had the lowest number of cases and superficial variant was the most occurred tumour with a number of 2 cases. The nodular and infiltrating variant recorded 1 case each. Other

variants recorded no case in the lower and upper limbs. The most affected part in this anatomical site is the arm with a frequency of 50% (3 cases), while the foot and hand accounted for 33.3% (2 cases) and 16.7% (1 case) respectively. Table 1.

Table 1: Site Distribution of Histologic Variants of Cutaneous Basal Cell Carcinoma.

Anatomic Sites	Histologic Variants of Cutaneous Basal cell carcinoma				
	Nodular	Superficial	Basosquamous	Infiltrating	Morpheaform
Head & Neck	12	6	6	3	3
Trunk	4	1	1	1	1
Extremities	1	2	-	1	-
Total	17	9	7	5	4

Ki-67 Expression Index Among the Variants of Cutaneous Basal Cell Carcinoma

The Ki-67 expression among the nodular variant of BCC, ranges from 1.2-15.2% with a mean value of 9.3%.

The superficial variant expresses a Ki-67 expression value ranges from 2-18% with a mean value of 11.2%.

The infiltrating variant of BCC has Ki-67 expression of a range of value from 14.4-28.2% with a mean value of 19.6%.

Basosquamous variant has Ki-67 expression ranges from 21-45.6% with a mean value of 35.4%.

The sclerosing (morpheaform) variant has a Ki-67 expression ranging from 18-32.7% with a mean value of 22.5%.

The Proliferative index using Ki-67 nuclear stain of the low risk subtypes ranges from 1.2-18% with a mean value of 10%.

These high-risk subtypes have a Proliferative index using Ki-67 nuclear stain range of values of 14.4-45.6% and a mean value of 25.1%. Table 2.

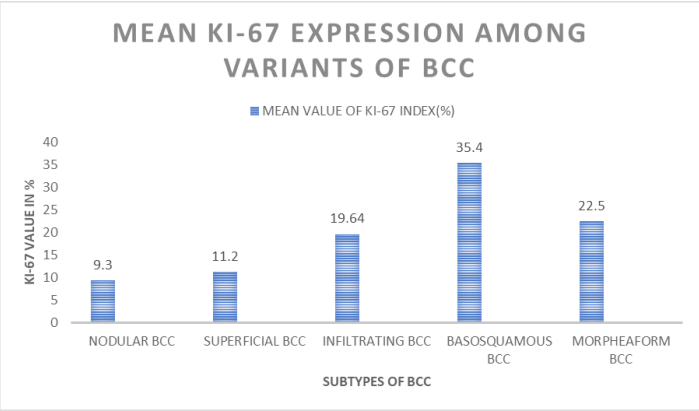

Figure 2: Showing the mean ki-67 proliferative index among variants of BCC.

KI-67 Staining Pattern Among the Grades and Variants of Basal Cell Carcinoma

The predominant staining pattern in low-grade subtypes of BCC (Nodular and Superficial variants) are focal with occasional peripheral stains.

High risk subtypes or aggressive variants (Basosquamous, Infiltrating and Morpheaform BCC) seen in this research have more diffuse and heterogeneous staining pattern. See Figures 2-4.

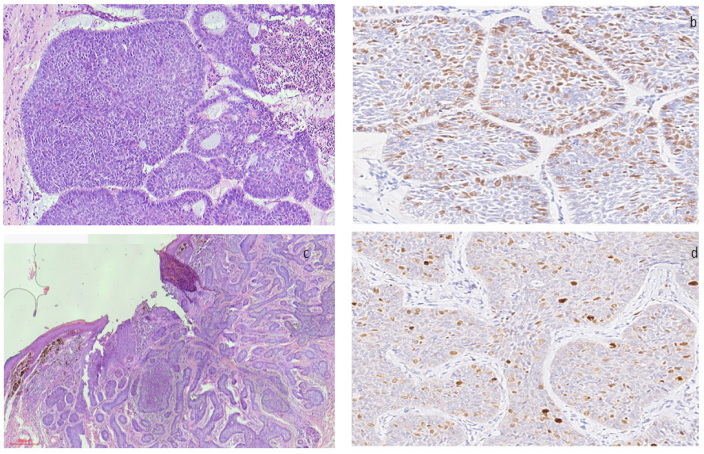

Figure 2: Showing NODULSR and Superficial Variants of BCC with their Pattern of KI-67 Immunoperoxidase Nuclear Stains at X40 Magnification.

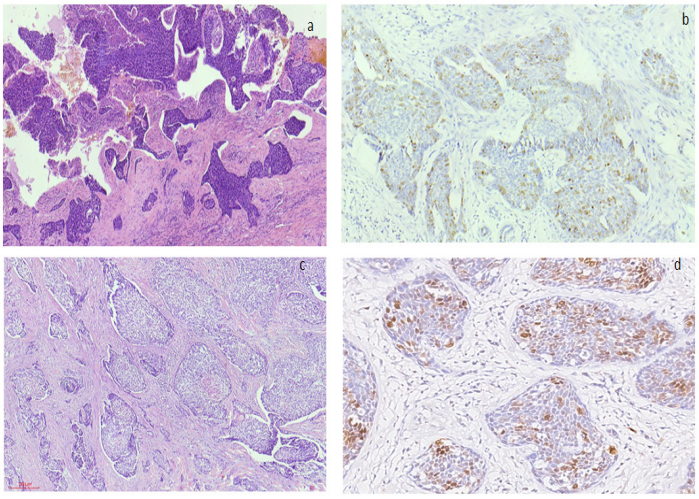

Figure 3: Showing The Histology of the Infiltrating ANF Basosquamous Variants of BCC and their KI-67 Immunoperoxidase Nuclear Stains at X40 Magnification.

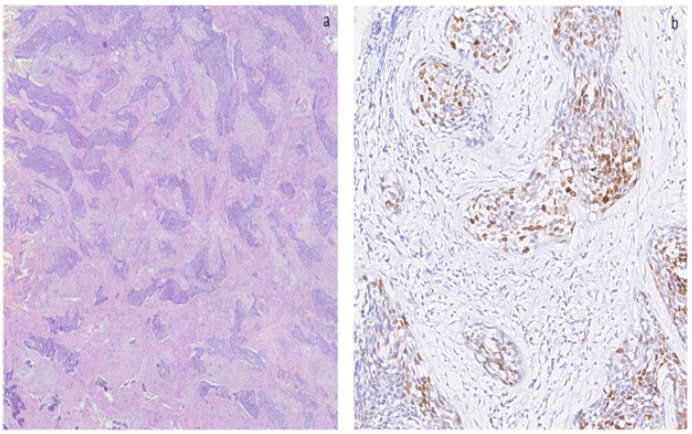

Figure 4: Showing the H&E stain and ki-67 immuno peroxidase nuclear stains of Sclerosing variant of BCC at X40 magnification.

Table 2: Mean Value of Ki-67 Expression Among Variants of Basal Cell Carcinoma.

Tumour Grade	Histologic Subtypes	Number of Cases	Percentages (%)	Range of Ki-67 Index (%)	Mean Value of Ki-67 Index (%)
LOW RISK SUBTYPES	NODULAR SUPERFICIAL	17 9	40 21	1.2-15.2 2-18	9.3 11.2
High Risk Subtypes	INFILTRATING BASOSQUAMOUS MORPHEAFORM	5 7 4	12 17 10	14.4-28.2 21-45.6 18-32.7	19.64 35.4 22.5

Discussion

Ki-67 expression index assessment in tumours can achieve the goal of predicting the considerable differences in biologic tumour behaviours like invading potential, growth an proliferative ability, distance and vascular metastasis. Tumour variants tend to behave differently despite belonging to the same family. Basal cell carcinoma (BCC) is not exceptional in these tumour characteristics.

The observed immunoreactivity pattern among the histologically aggressive subtypes of BCC (basosquamous, infiltrative and morpheaform) displayed more intense and diffuse staining pattern when exposed to nuclear stain of ki-67 immunoperoxidase protein. This staining pattern can be seen even among highly malignant tumours like melanoma, squamous cell carcinoma and other notable high grade tumours [6,10,11].

The nodular and superficial variants of BCC (low risk subtypes) displayed focal a less heterogeneous and peripheral staining pattern in areas of dense tumour formation and Regardless of the percentage staining of all the variants of BCC found in this study, the nuclear immunoreactivity was intense and unequivocal in all variants BCC. Tilli et al. and Mohebat et al. observed comparable similarities in pattern of distribution in their findings in their similar work [10,11].

The mean reactivity index of 15.8% and a range of 1.2-45.6% across all the variants of BCC observed in this study, its notably that BCC have significant proliferative potentials and should be a tumour of concern to clinicians whenever diagnosis is made. Tilli et al., Eya et al. and Al-Sader et al., had similar observations in their studies and they documented a mean of 14.9% for BCC with a range of 1- 61% for BCC respectively [7,8].

On the contrary, Albertine et al., Joonsoo et al., Vladimir et al., documented a mean and ranges of Ki-67 index for BCC in their studies that demonstrated wide variance with what we observed in this current study their finidings was of less significant with quite insignificant proiferative potentials [8,13,14]. However, Vladimir et al in his observation, documented that variable growth fraction could be seen among different histomorphologic subtypes of keratinocytes tumours and it could also vary from place to place [8].

The highest mean values of Ki-67 index for BCC was recorded for basosquamous to be 35.4% with a range of 21- 45.6% and morpheaform subtypes of BCC closely followed with a mean of 22.5% and a range of 18-32.7%. The lowest values for BCC were recorded for nodular variant which had a mean Ki-67 value of 9.3% with a range of 1.2-15.2%. Other variants of BCC like superficial, 2-18% with a mean of 11.2% and infiltrating 14.4-28.2% with a mean of 19.64

It has been documented of the high risk variants of BCC like Morpheaform and Adenoid variant of BCC having high Ki-67 nuclear stain of 34-100% while a Low-risk subtypes having as low as 0-22% respectively. These variables in staining was observed in this present study and had also been documented in researches done by Tilli et al., Amar et al., Haiying et al., Alexandru et al., Pietro et al., Mohebat et al., Mehrnaz et al., who in their independent research work observed that Ki-67 correlates with the aggressiveness of tumour [10,11,15-19].

Despite the general knowledge of the indolent nature of BCC, further buttress by Joonsoo et al. who regarded BCC as a neoplasia with high healing rate, less aggressive with little or no ability to metastasize, It surfices to say that these age long knowledge is not applicable to all variants of BCC, as findings in this research in corroboration with findings by Vladimir et al. and Tilli et al. gave a contrary view, describing BCC as a cancer with several tumour subtypes having variable histomorphological picture and variable biological behaviours [8,10]. They noted that some cases may ab initio have an aggressive behaviour with rapid infiltration in deeper tissue structures, recur after treatment and sometimes give rise to metastasis and exhibit greater proliferative activity [8,10]. Findings largely supported by the presence of variants with high proliferative index as measured by ki-67 anitigen nuclear stain, as high as 45.6%, proliferative index. Most implicated are basosquamous, infiltrating and sclerosing(morpheaform) BCC. 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 a high-grade human tumours ever known.

Conclusion

The high grade subtypes of cutaneous BCC, (infiltrating, morpheaform and basosquamous BCC) possess a significant high proliferative index as high as 45.6% for basosquamous variant, when compared with the low grade subtypes of BCC found in Umuahia which have proliferative index as low as 1.2 % for nodular BCC. Therefore, there is need for critical evaluation and follow up for all cases of BCC in order to isolate the types that are high grade as to manage accordingly.

References

1. Fiona B, Leonard JO, Chris S, et al. Trends in incidence of skin basalcell carcinoma. Additional evidence from a UK primary care database study. Int J Cancer. 2007; 121: 2105-2108.

2. Gerald DF, Adesuwa NO. Malignant Skin Tumors in Benin City, South-South, Nigeria. Oman Med J. 2013; 28: 311-315.
3. David EE, Daniela M, Richard AS, et al. World health Organisation classification of skin tumours. 4th edition. Lyon. IARC. 2018.
4. Crowson AN, Cynthia M, Martin C, et al. Biopsy interpretation of the skin. Philadelphia. Baltimore. Wolters Kluwer/ Lippincott Williams and Wilkins. 2009.
5. Eyal K, Oscar H, Jacob F, et al. Ki-67 as a Biologic Marker of Basal Cell Carcinoma: A Retrospective Study. IMAJ. 2014; 16: 229-232.
6. Amalia Elena A, Corina Daniela E, Monica N, et al. The relationship between interleukin 8 and Ki-67 in cutaneous malignant melanoma. International Journal of the Bioflux Society. 2015; 7: 149-154.
7. Eyal K, Oscar H, Jacob F, et al. Ki-67 as a Biologic Marker of Basal Cell Carcinoma: A Retrospective Study. Isr Med Assoc J. 2014; 16: 229-232.
8. Vladimir BA, Katarina A, Milada K et al. Immunohistochemical evaluation of proliferative activity (Ki-67 index) in different histological types of cutaneous basal cell carcinoma. Cellular and Molecular Biology. 2012; 67: 610-615.
9. Rakheja M, Singh P, Shergill AK, et al. Assessment of proliferative potential of tumour cell using Ki-67 expression and morphometrical analysis for prognostication of oral squamous cell carcinomas. Int j med Res Health Sci. 2015; 4: 820-826.
10. Tilli CM, Ramaekers FC, Broers JL, et al. Lamin expression in normal human skin, actinic keratosis, squamous cell carcinoma and basal cell carcinoma. Br J Dermatol. 2003; 148: 102-109.
11. Mohebet H, Gouda, Ghada AA, et al. Value of P53, CD10 and Ki-67 in differentiation between Keratoacanthoma and squamous cell carcinoma. Med J Cairo university. 2014; 82: 795-801.
12. Al Sader MH, Doyle E, Kay EW, et al. Proliferation indexes-a comparison between cutaneous basal and squamous cell carcinoma. J clin Path. 1996; 49: 549-551.
13. Joonsoo P, Youngil K, Jae Bok P, et al. Immunohistochemical evaluation of CD10, podoplanin and Ki-67 expression in basaloid epidermal proliferation with comparative analysis of basal cell carcinoma and trichoepithelioma. Int j clin exp Pathol. 2016; 9: 7188-7194.
14. Albertine L, Zenaida C, Mihai C, et al. Teofil Assessment of the aggressive features of basal cell carcinoma in the oral and maxillofacial region. OHDMBSC. 2006; 4: 62-64.
15. Mehrnaz A, Azadehzeinab T, Faezeh A. Comparative investigation on Ki-67 marker in oral squamous cell carcinoma and oral lichen planus by immunohistochemistry. Sch J Dent Sci. 2017; 4: 434-439.
16. Haiying Z, Yunfeng CA, Guoqiang W, et al. Expression of FOXC2, PinX1, Ki-67 and Cyclin D1 in cutaneous cell carcinoma. Oncol Lett. 2017; 14: 635-638.
17. Alexandru M, Alex ES, Claudiu M, et al. P53, p16 and Ki-67 immunoexpression in cutaneous squamous cell carcinoma and its precursor lesions. Rom J Morpho Embryo. 2016; 57: 691-696.
18. Pietro D, Giovanni P, Chiara P, et al. Another point of view on P16 and Ki-67 expression in melanotic and non-melanotic cutaneous lesions. Acta Dermatovenerol Croat. 2013; 21: 149-154.
19. Amer T, Charif B, Safa Z, et al. Ki-67 prognostic value in different histological grades of oral epithelia dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2018; 19: 3279-3286.