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

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ABSTRACT

Background: Squamous cell carcinomas are tumours originating from keratinocytes and they are among the most commonly encountered skin cancers worldwide. This tumour exhibits various degree of differentiation and subtypes, which is invariably important in prognostication and predictability of aggression and tumour recurrence.

The true prevalence of SCC in Nigeria is still debatable. However, The high frequency of cutaneous SCC significantly impacts the health system, making it a public health issue despite its low mortality rates and rare occurrence of metastases

Aims: The aim of this study was to examine the proliferative index using ki-67 immuno peroxidase protein among the variants of squamous cell carcinoma diagnosed through routine biopsies at federal medical centre, umuahia, Abia state between 2012 and 2018

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)) for antigen expression on fresh 4-micron sections of tumour specimens was used.

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):

Result: In Overall, the Ki-67 index of all the cutaneous SCC in this study ranges from 2.3-80%, with a mean value of 24.7%.The Ki-67 index was variably expressed among the variants of SCC, of which the squamous cell carcinoma NOS have Ki-67 index range from 9.2-35.7% with a mean value of 20.2%, Adenosquamous variant have Ki-67 index range 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, has Ki-67 index range of 2.3-10.5% with a mean value of 7.7%, that of Keratoacanthoma variant ranges from 2.4-10.7% with a mean value of 7.0%

Conclusion: These findings may be helpful in prognostication as well as predictability of SCC tumour recurrence and for the purpose of therapy and

clinical follow up. Although a high proliferative index alone may not be the only factor responsible for the more aggressive behaviors exhibited by SCC, however, ki-67 nuclear stain is an important prognostic tool for predictability of the biologic behavior of SCC.

INTRODUCTION

Squamous cell carcinomas are tumours originating from keratinocytes and they are among the most commonly encountered skin cancers worldwide and combined with cutaneous Basal cell carcinoma, amount to 75% of non-melanoma malignant skin tumours [1][2]. This tumour exhibits various degree of differentiation and subtypes, which is invariably important in prognostication and predictability of aggression and tumour recurrence.

Cutaneous squamous cell carcinoma arises in the epidermis from the malignant transformation and proliferation of keratinocytes. It appears as patches, plaques, and nodules that enlarge and develop central areas of inflammation, induration and necrosis. SCC metastasizes by direct, lymphatic and hematogenous spread. Metastasis is most likely to occur in a cutaneous SCC that grows rapidly to larger than 2cm.[3] Squamous cell carcinoma can present as slightly elevated slowly growing red scaly patch, found most often on the scalp and ears of men and on the lower limbs of women or as a chronic non-healing ulcer.[4]

Over the years, the number of SCC cases has surged [3] and this incidence is expected to double in European countries [4]. There is ongoing debate about whether this increase is due to an actual rise in cases or improved early detection [5] and some parts of the United States, SCC mortality rates is very high with the second leading cause of death from skin cancer after melanoma and is responsible for most skin cancer deaths in individuals over 85 years old [3]

The true prevalence of SCC in Nigeria is still debatable. [6] For instance, Lagos has reported a 22% prevalence, while Oshogbo has reported 32.7% of all cutaneous malignancies as SCCs [7]. The high frequency of cutaneous SCC significantly impacts the health system, making it a public health issue despite its low mortality rates and rare occurrence of metastases [8].

Criteria for diagnosing the various histologic variants are based on the recognizable features that are detectable under hematoxylin and eosin stain. These criteria are discussed as follows:

Squamous cell carcinoma nos: This lesion varies from well differentiated to poorly differentiated based on the presence of extracellular and intracellular keratin produced by these squamous cell. They composed of atypical squamous cells which could be classic SCC insitu to tumours showing obvious deep invasion. **Keratoacanthoma**: Features may vary by clinical stage. Early proliferative stage may show symmetrical invaginations of interconnecting follicular infundibula/isthmus–type squamous epithelium. The squamous cells contain pale, glassy eosinophilic cytoplasm with irregular atypical keratinocytes at the base of the tumour.

Acantholytic SCC: Thickened overlying epidermis and hyperkeratotic or ulcerated and full-thickness carcinoma in-situ with invasive tumour showing acantholysis resulting in variable sized and spaces within the invasive tumour lobules.

Verrucous SCC: Lesion show exophytic and endophytic architecture with hyperkeratosis and characteristics deep tongues of intradermal growth that are club-like in contour with minimal cytologic atypia.

Adenosquamous SCC: Composed of small to large interconnecting nests of anaplastic squamoid cells displaying cytoplasmic cornification and keratinizing cyst with desmoplastic stroma. These tumour nests are connecting with the epidermis. Glandular differentiation is variable and could include both ductular and glandular elements

The aim of this study was to examine the proliferative index using ki-67 immuno peroxidase protein among the variants of squamous cell carcinoma diagnosed through routine biopsies at federal medical centre, Umuahia, Abia state between 2012 and 2018

MATERIALS AND METHOD

STUDY DESIGN: This is a descriptive retrospective study that involved the evaluation of all the skin biopsies histologically diagnosed as squamous 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 squamous cell carcinoma 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 squamous 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 squamous cell carcinomas as 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 data. Hematoxylin and Eosin as well as immunohistochemical stain using monoclonal antibody against Ki-67 (BioCare CRM325C(RM)) for antigen expression on fresh 4-micron sections of tumour specimens were used. 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 Fifty-five cases of squamous cell carcinoma were histologically diagnosed between January 1st 2012 to December 31st 2018. This constituted 36%, of all the malignant skin tumours.

HISTOLOGIC VARIANTS

Squamous cell carcinoma NOS was the commonest variant of SCC in this study which accounted for more than half 53% (29 cases) of the SCC diagnosed within the study period. This proportion was followed by Adenosquamous variant which accounted for 16% (9 cases). Other variants included; Acantholytic SCC and Keratoacanthoma squamous cell carcinoma and Verrucous squamous cell carcinoma which accounted for 11%(6 cases), 11%(6cases) and 9%(5 cases), respectively. **See fig.1**

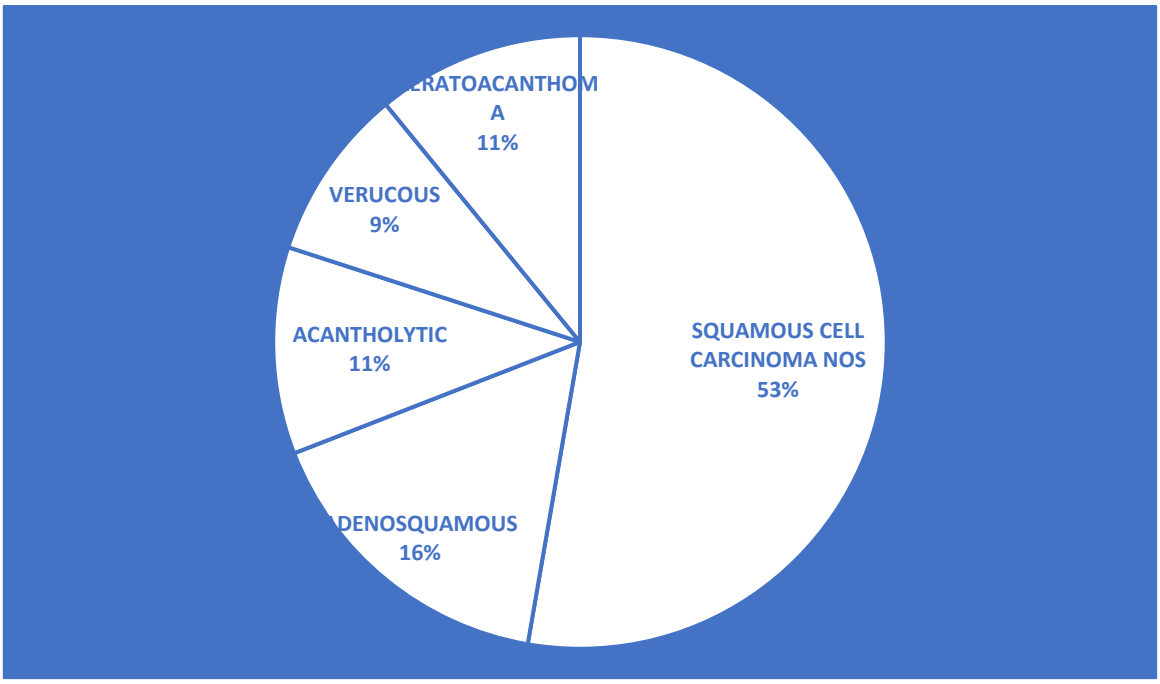

FIGURE 1. Showing Histologic Variants Of Cutaneous Squamous Cell Carcinoma And Their Percentages

SITE DISTRIBUTION OF SCC.

Out of the 55 cases of SCC, 29% (16cases) were located in the head and neck region, 31% (17 cases) were located on the body while 40% (23cases) were located on the upper and lower limbs (extremities).Among the variants of Cutaneous SCC, 4 cases of Adenosquamous, 2 cases of Acantholytic, 9 cases of SCC NOS, and 1 case of Keratoacanthoma were found within the head and neck region. Verrucous SCC recorded no case within this region. The face was the most affected part in the head and neck with a frequency of 50% (8cases). Other sites in the head and neck region affected by SCC were scalp 37.4% (6 cases) and the pinna 6.3% and the mandible 6.3% (1 case). On the body, SCC NOS is the most occurred tumour with a frequency of 9 cases. This is followed by Keratoacanthoma with 3 cases, then Adenosquamous and verrucous SCC had 2 cases each. Acantholytic variant recorded just 1 case. The commonest site for SCC on the body was the scrotum which accounted for 29.4 %(5cases). Other areas included the vulva 23.5% (4 cases), the abdomen 11.8% (2 cases), the groin 17.6% (3 cases) and back 17.6% (3 cases). The extremities recorded 11 cases for SCC NOS while Adenosquamous, Acanatholytic and verrucous SCC recorded 3 cases each. The least common occurred

tumour in this region was Keratoacanthoma with a frequency of just 2 cases. In the upper and lower limbs, SCC was found mostly on the forearm which accounted for 45.5% (10cases). Other affected sites included the foot, thigh, knee, leg and hand which accounted for 22.7%, (5 cases), 13.6% (3 cases), 9.1% (2 cases) 4.5% (1 case) and 4.5% (1 case) respectively. **See Table 1**

TABLE 1. Site Distribution Of Cutaneous Squamous Cell Carcinoma

ANATOMIC SITES		NUMBER OF CASES	PERCENTAGE (%)
HEAD & NECK	SCALP	6	37.4
	CHIN	1	6.3
	FACE	8	50
	EAR	1	6.3
TRUNK	GROIN	3	17.6
	SCROTUM	5	29.4
	BACK	3	17.6
	ABDOMEN	2	11.8
	VULVA	4	23.5
ACRAL	THIGH	3	13.6
	LEG	1	4.5
	FOREARM	10	45.51
	HAND	1	4.5
	FOOT	5	22.7
	KNE	2	9.1

KI-67 PROLIFERATIVE INDEX EXPRESSION SCORE AMONG VARIANTS OF SQUAMOUS CELL CCARCINOMA.
In Overall, the Ki-67 index of all the cutaneous SCC in this study ranges from 2.3-80%, with a mean value of 24.7%.
The Ki-67 index was variably expressed among the variants of SCC, of which the squamous cell carcinoma NOS have Ki-67 index range from 9.2-35.7% with a mean value of 20.2%, Adenosquamous variant have Ki-67 index range 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, has Ki-67 index range of 2.3-10.5% with a mean value of 7.7%, that of Keratoacanthoma variant ranges from 2.4-10.7% with a mean value of 7.0%. The histologically designated low-grade variants of SCC (keratoacanthoma, Verrucous carcinoma and Squamous cell carcinoma NOS), have Ki-67 index mean value of 16.7% and a range of value of 2.3-35.7%, while the histologically designated high-grade variants (Adenosquamous and Acantholytic) have a Ki-67 index mean value of 46.0% with a range of 23.5-80%. The nuclear staining pattern for low grade SCC was less heterogenous with occasional focal stain while the high-grade variants maintained diffuse and overt heterogenous staining pattern. **See table 2, figure 2**

TABLE 2. Mean Value of Ki-67 Expression among the Different Tumour Grades of Cutaneous Squamous Cell Carcinoma

TUMOUR GRADE	VARIANTS	NUMBER OF CASES	PERCENTAGES (%)	RANGE VALUE OF KI-67 INDEX (%)	MEAN VALUE OF KI-67 INDEX (%)
LOW GRADE VARIANTS	SCC NOS	29	53	9.2-35.7	20.2
	VERUCOUS	5	9.0	2.3-10.5	7.7
	KERATOACANTHOMA	6	11	2.4-10.7	7.0
AGGRESSIVE VARIANTS	ADENOSQUAMOUS	9	16	40-80	55.3
	ACANTHOLYTIC	6	11	23.5-45.2	32.0

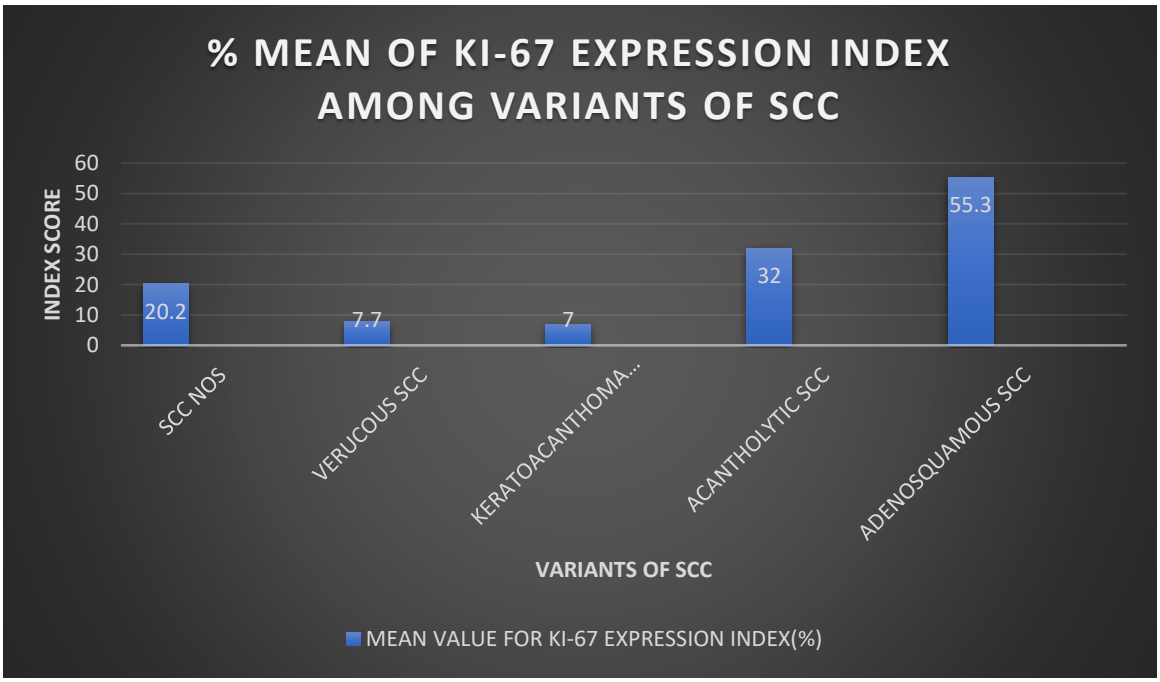

FIGURE 2: Showing The Mean Ki-67 Expression Value Among The Variants Of Squamous Cell Carcinoma

KI-67 STAINING PATTERN

The application of Ki-67 immunoperoxidase, a proliferative marker on SCC cohort, yielded an intense and diffuse observable brownish nuclear stains in areas of dense proliferating tumour cells on all the variants of squamous cell carcinoma. **See figure 3-5**

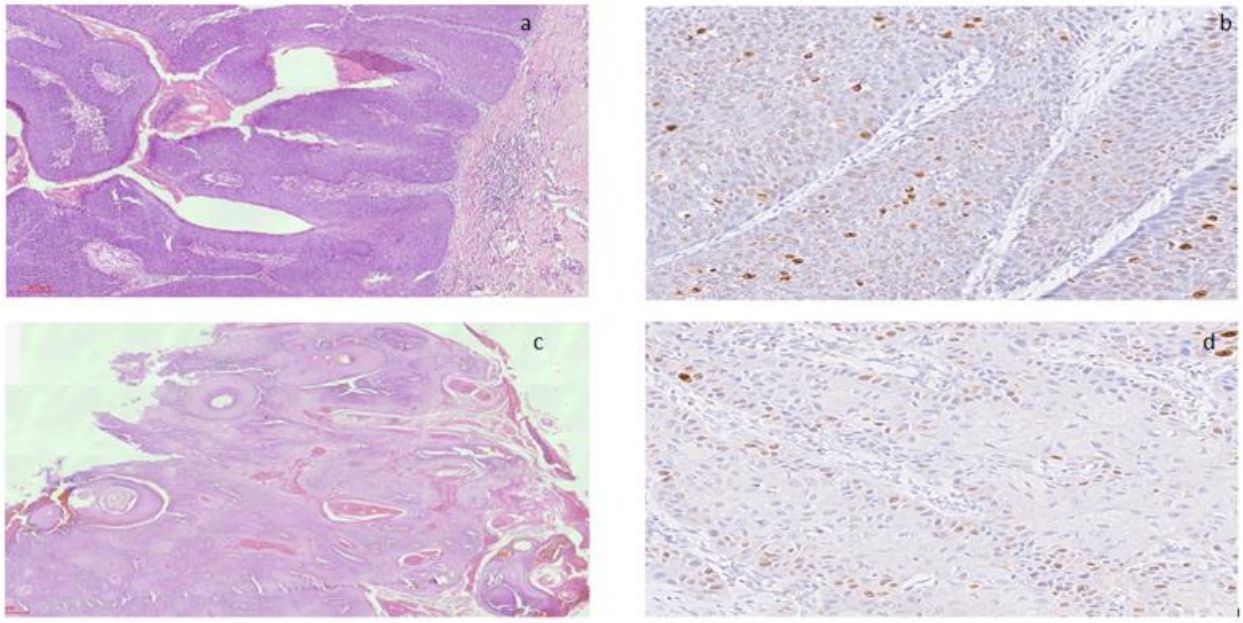

FIGURE 3: Showing The Histology And Ki-67 Immunoperoxidase Staining Pattern Of Scc Nos And Keratoacanthoma Scc And Their Corresponding Immune Stains at x40 Magnification

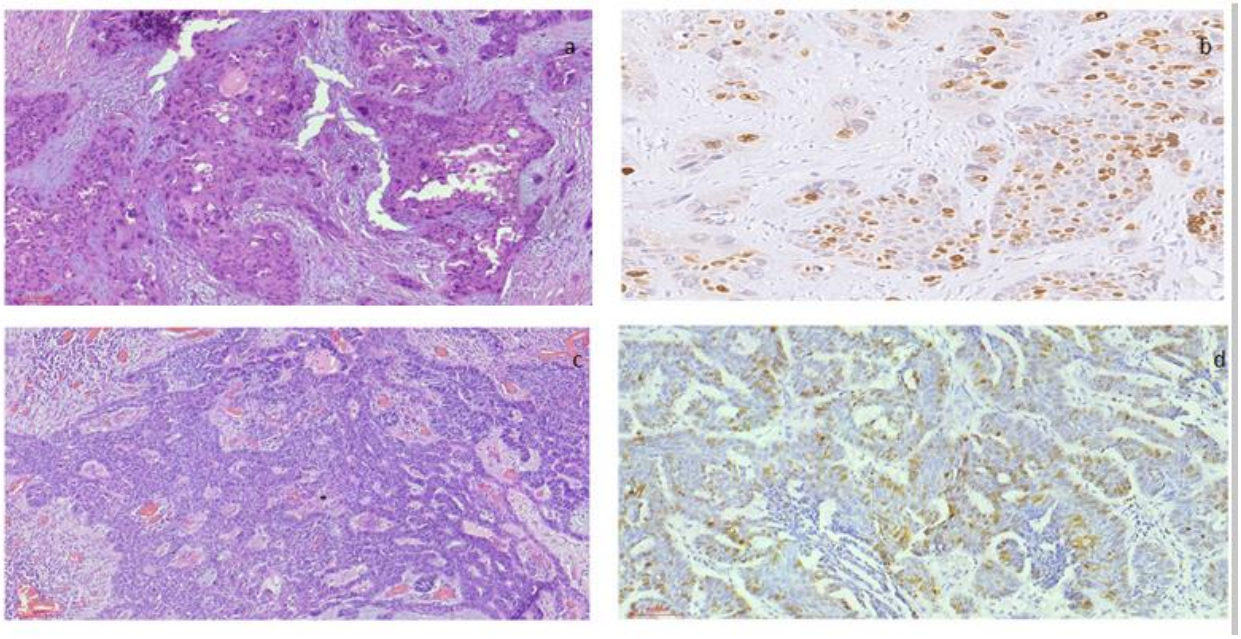

FIGURE 4: Showing The Histolohy And Ki-67 Immunoperoxidase Staining Patter Of Acantholytic And Adenosquamous Scc And Their Immune Stain Counterparts At x40 Magnification

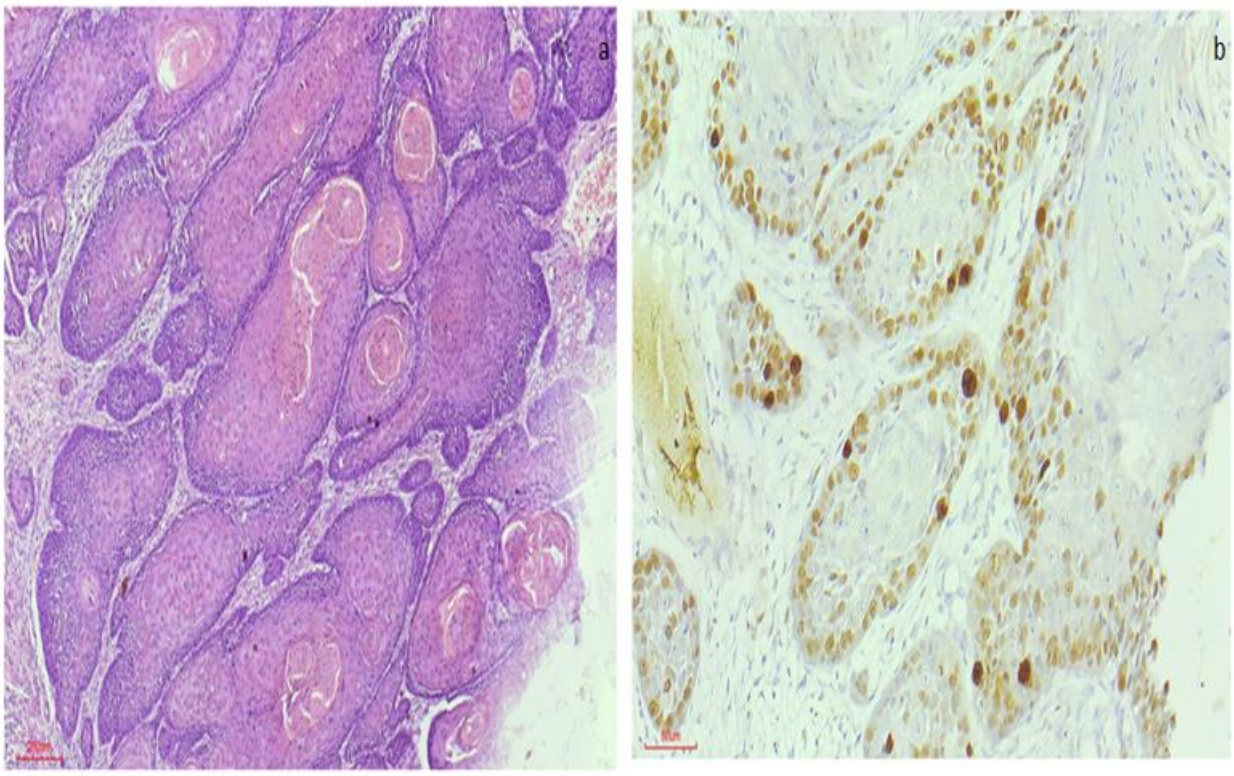

FIGURE 5: Showing The Histology And Ki-67 Immunoperoxidase Staining Pattern Of Squamous Cell Carcinoma Nos And Its Immune Staining At x40 Magnification.

DISCUSSION

Regardless of the percentage range of Ki-67 staining, the nuclear immunoreactivity in this study was intense in all SCC cases and there was no tumour without Ki-67 nuclear reaction in this study, a characteristics that depict growth, aggressiveness and metastatic potentials of tumours [11]. This pattern of stains in SCC are similar to what have been observed in previous studies by Tilli et al and Mohebat et al who 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 SCC evaluated in their studies in support of 100% found in our study [8][12].

All the SCC analyzed in this work displayed a mean value of 24.7% and a range of 2.3-80% of Ki-67 nuclear index. Reports differ among authors on the ranges and mean of Ki-67 index of SCC as observed in many literatures. Tilli et al, Eya et al and Al-Sader et, all had similar observations in their studies and they documented means of 20% , 14.9% for and a range of 1- 61% and 0-71% for SCC respectively [8][12][13][14].

Among the individual variants of SCC seen in this study, Adenosquamous , a histologic high-grade variant of SCC, had the highest mean value of Ki-67 nuclear stain of 53.3% with a range of value of 40-80%.This was closely followed by Acantholytic variant, another histologic high grade variant , with a mean value of 32.0% and a range of value of 23.5-45.2%. The least proliferative index of SCC in this present study was Keratoacanthoma, a histologic lowgrade variant, having a mean value of 7.0% and a range of value of 2.4-10.7%. Other histologic variant observed in this study (SCC NOS and Verrucous SCC) recorded a mean and range Ki-67 index in-between these values. These findings corroborate with documented values of tilli etal, Eyal and Al-Sader whose findings dinstinguished high grade SCC having high nuclear proliferative indes and low grade SCC having low nuclear stains values of ki-67 respectively [12][13][14]. These correlation of grade in response to their values for nuclear stain values is widely documented across various findings in the world

The Staining variability in SCC is according to their level of aggressiveness as well differentiated SCC possesses sparse nuclear stains and low ki-67 value and this is well demonstrated in this research. Eyal et al, Alexandru et al, Pietro et al, Amer et al and Vulkelic et al all noted that the well differentiated the SCC is, the less the value of their Ki-67 and that in effects explains their biologic behaviors [14][15] [16][17][18]. Further demonstration in this study is revealed by low grade SCC demonstrating ki-67 nuclear stain value as low as 2.3% while the high grade SCC demonstrating a value as high as 80%. Therefore, the lesser the grade of the tumour, the lower their score and well differentiated SCC possess negligible ki-67, this invariably affected their aggressiveness and metastatic potentials.

SCC although an aggressive keratinocytes tumour, on the contrary, SCC have significant number of variants that are less aggressive when compared with the ones with high aggressive variants. In this study, more than half (58%) of the cutaneous SCC had moderate proliferative index (PI 10-30%) and 29% had high proliferative index (>30%).The percentage of tumour that were low proliferative index (<5%) and mild proliferative index (5-10%) combined up to 12%. This demonstrated more numbers of SCC with

moderate, mild and low proliferative index combined, when compared with those with high proliferative index. Therefore, SCC still have higher number of low aggressive variants than aggressive ones [19][20].

CONCLUSION

This study demonstrated a significant higher percentages of low ,mild and moderate proliferative index combine for SCC than aggressive variants of SCC. Although aggressive variants of SCC exist by virtue of their proliferative index, the low and moderate proliferative index variants are still significant and more in number. However, the percentages of high proliferative aggressive SCC are significant. These findings may be helpful in prognostication as well as predictability 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 only factor responsible for the more aggressive behaviors exhibited by SCC, however, ki-67 nuclear stain is an important prognostic tool for predictability of the biologic behavior of SCC.

RECCOMENDATION

A proper profiling and clinical follow up for all cases of SCC is recommended because of the existence of aggressive variants possessing significantly high proliferative index.

SOURCE OF FUNDING

No funding was received for the study

CONFLICT OF INTEREST:

Authors declare no conflict of interest

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