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Immunohistochemical Evaluation of β-catenin Expression in Archived Cervical Premalignant Lesions From Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Ezeofor C. P*[1], Ilegbedion I.G[2], Ezejiofor I. F[3], Ogenyi S. I[1], Olaofe O.O[4], Osonwa C. N[5], Lawani-Luwaji E.[6]

*,[1]Department of Medical Laboratory Science, Faculty of Health Science and Technology, *Nnamdi Azikiwe University, Awka, Anambra State,Nigeria.*

[2,6]*Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.*

[3]Department of Internal Medicine, Nnamdi Azikiwe University, Nnewi Campus, *Awka, Anambra State, Nigeria.*

[4]Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria.

[5]Histopathology Department, Nnamdi Azikiwe University Teaching Hospital, Nnewi Anambra State, Nigeria.

Author's correspondence: Ezeofor C. P.

Email: ezeoforchinelo@gmail.com

Phone: +234 8038947770.

ABSTRACT:

Loss of membranous β-catenin (B. catenin) and increase in cytoplasmic expression of these markers by the effect of the oncogens leads to alteration in the structural adhesion. Alterations in the β-catenin cells adhesion complex have been involved in the pathogenesis of cervical carcinomas even at their earliest stage of the disease (Fadare et al., 2015). The aim of this study is to evaluate expression pattern of β-catenin and the role in progression of cervical intraepithelial neoplasm or lesions (CIN/CILs). A five-year retrospective research carried out at the Histopathology Department of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria, from 2016 to 2020 to confirm the expression pattern of E.cadherin, B. catenin in relation to P16 immunohistochemical stains in CILs using monoclonal antibodies to, β-catenin . Eighty-six (86) cases of archival paraffin-embedded tissue blocks was used for the analysis. Pathology reports of all CILs and their blocks were retrieved. The age range of presentation was 18-80 years with median age of 58 years. The blocks were recut, immunostained and reviewed to determine each lesion. Expressions of markers were analysed using the immunoreactivity scores. Eighty-six (86) cases of histopathologically diagnosed cervical intraepithelial lesion with β-catenin protein expression were studied. Out of the 86cases of CILs subjected to β-catenin monoclonal antibodies,

55.8%(48/86cases) had an initial, pre-immunohistochemical diagnosis as CIN 1, while 44.2%(38/86cases) were high-grade squamous intraepithelial lesions consisting 13(15.1%)CIN 2, 16(18.6% CIN3 and 9(10.5%) CIS . However, on application of monoclonal antibodies of E β-catenin, majority of CIN1 had a change of diagnosis to Negative squamous intraepithelial lesions (NSIL) 21/48 (43.8%) and chronic cervicitis 11/48 (27.1%) while 16/48 cases (35.4%) were finally retained as CIN1. Of initially diagnosed as HSIL lesions {CIN2, CIN3, and Carcinoma In-situ; (CIS)} 35 cases (92.1%) 38were concordant with previous diagnosis while three cases were regrouped as CIN 1 (one case) and chronic cervicitis (2 cases).Therefore, the total number of CIN 1 was 17 cases and chronic cervicitis was 13 cases. The rate of β-catenin membranous expression in non neoplastic and LSIL lesion were; 85.3% and 100% respectively; In HSIL the rate of the β-catenin cytoplasmic expression increased with the increased degree of dysplasia, from 70% in CIN2 to 87.5% in CIN3 and 88.9 % in CIS. The rate of the β-catenin cytoplasmic expression positively correlated with histopathology grades of intraepithelial neoplasm**.**

Keywords: β-Catenin expression, Cervical premalignant lesion, Intraepithelial neoplasm, Achived tissues.

INTRODUCTION

Loss of membranous β-catenin (B. catenin) and increase in cytoplasmic expression of these markers by the effect of the oncogens leads to alteration in the structural adhesion. Alterations in the β-catenin cells adhesion complex have been involved in the pathogenesis of cervical carcinomas even at their earliest stage of the disease (Fadare *et al.,* 2015).

Early and specific diagnosis of high–risk HPV infected cervical lesion and the premalignant features in cervical lesions, appropriate and aggressive targeted therapy are sure promise to reduction of premalignant cervical lesions progressing to cervical cancer. The comprehensive approach to preventing, detecting and treating of cervical malignant disease has been on increase in the recent years (WHO, 2022), though has gone a long way to improving cervical cancer incidence and reduction in its mortality rate. However, despite the recommended screening program for cervical cancer and aggressive treatment, cervical cancer still remain a global challenge with more effect in the underdeveloped and developing countries. The burden is still very high in Africa and some parts of Asia (Brisson *et al*., 2020; Canfell *et al*., 2006). Nigeria has the highest no of incident cases of cervical cancer in Africa, with 12,075 new cases and 7968 death making Nigeria one of the eight countries with largest number of incident cases in the world (Nigeria Fact Sheet, 2020). It is necessary to carry out more research in other to obtain a high diagnostic accuracy to control cervical cancer in Nigeria and entire Africa. Hence, it is important to evaluate the mechanism of initiation and development of CILs, a precursor of cervical cancer and to determine the factors that are involved in its formation.

The expression of catenin delta 1 (CTNND is significantly altered and is closely correlated with the the degree of tissue differentiation and pathologic tumors stage of patient with pancreatic carcinoma (Fei *et al* 2010). Catenin delta-1 gene help keep E.cadherin in its proper place in the cell membrane preventing it's broken down premature or being taken into cells by process of endocytosis.

METHODOLOGY

This is a retrospective study of 86 diagnosed cases of CILs in the Histopathology Department of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria, between January 2016

and December 2020'. 'Female patients diagnosed with cervical intraepithelial lesions (CILs) between January 2016 and December 2020, whose histopathology reports contained complete data (age, specimen site, clinical diagnosis, and type of surgery), were included in this study.'. The paraffin blocks of all 86 available cases of CILs 'that met the inclusion criteria during the study period.

This study was approved by our local Ethics Review Committee of the hospital. Biopsy samples and total hysterectomy specimens were routinely processed using 10% neutral buffered formalin (10% neutral buffered formalin) for fixation, graded alcohol for dehydration, xylene for clearing and finally, paraffin infiltration, embedding and sectioning. Sections (4 µm thick) were prepared from each paraffin block for routine H&E staining and immunohistochemistry.

Immunohistochemical studies were done by the Avidin Biotin immunoperoxidase method on formalin-fixed paraffin embedded sections (FFPE) using Novocastra Histology Kit (LEICA, BioSB, California). Four micrometer (4µm) thick sections of FFPE tissue blocks were made and mounted on a positively charged glass slides. The tissue sections were deparaffinised by passing them through different grades of xylene and then rehydrated in decreasing ethanol concentrations. The antigens were appropriately retrieved by steam treatment in a citrate buffer at P_H 6.0 using microwave at power 100 for 15 minutes and subsequently washed three times with physiologic buffer saline (PBS) for 15 minutes. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 15 minutes at room temperature in the dark. The tissues were incubated with primary antibodies (Mouse monoclonal antibodies to B.catenin for 60 minutes at room temperature and washes 3 times with PBS. Sections were also incubated for 15 minutes in secondary antibodies (biotinylated goat antimouse). Diaminobenzene (DAB) a chromogen was used as a final substrate. The primary antibody was omitted for the negative control. Invasive squamous cell carcinoma was used as a positive external control and it was included with each batch of staining while basal epithelial cell layer was used as internal control. Stained slides were screened by the candidate and the consultant pathologists. Photomicrographs of the stained slides were taken to display the staining pattern of B.catenin in different grades of cervical squamous intraepithelial lesion.

DATA ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) Incorporated, version 21 Chicago, Illinois, USA. Quantitative continuous variables such as age were summarised using mean and standard deviation while categorical variables such as sex were summarised using percentage. Results were presented in tables and charts. Pearson's Chi-square was used to test the association between two categorical variables, and $P < 0.05$ was considered statistically significant.

RESULTS

Eighty-six (86) cases of cervical intraepithelial lesions (CIL) were studied. The classification of CILs were based on *WHO Tumour Pathology and* Bethesda System (TBS) of histopathological diagnosis. The age range of presentation was 18-80 years with median age of 58. Of the 86 cases of CILs, 55.8% (48/86 cases) had an initial, pre-immunohistochemical diagnosis as CIN 1 (Low-grade squamous intraepithelial lesion; LSIL) while 44.2% (38/86 cases) were high-grade squamous intraepithelial lesions (HSIL) **(Table 1)**. LSIL were commoner in lower age groups than HSIL. HSILs were common within ages of 51 to 60 year **(Table 2)**

Table 1 Frequency and rate of distribution of cervical squamous intraepithelial lesions with H and E (Preimmunohistochemical studies)

Histological grades of lesion	Frequency	Percent(%
CIN 1	48	55.8
CIN 2	13	15.1
CIN 3	16	18.6
CIS	9	10.5

Keys: IHC = immunohistochemistry, Immuno = immunohistochemistry, P-value = significant level, P values < 0.05 is considered statistically significant, P> 0.05 is statically not significant, NILM = negative Intraepithelial lesion/malignancy, CIS = carcinoma in situ, CIN 1= cervical intraepithelial lesion/neoplasia 1, CIN 2 = cervical intraepithelia lesion/neoplasia 2, CIN 3= cervical intraepithelia lesion/neoplasia 3

Table 2: Age groups versus histopathological diagnosis of cervical intraepithelial lesions before application of immunohistochemistry.

Age Groups	Percent (%) within Age Group		
	HSIL	LSIL	Total
11-20	0 (0.0%)	1 (100.0%)	1(100.0%)
21-30	1 (33.3%)	2 (66.7%)	3 (100.0%)
31-40	4 (28.6%)	9 (64.3%)	14 (100.0%)
41-50	10 (31.2%)	22 (68.8%)	32 (100.0%)
51-60	13 (65.0%)	7 (35.0%)	20 (100.0%)
61-70	5 (62.5%)	3 (37.5%)	8 (100.0%)
71-80	5 (55.6%)	4 (44.4%)	9 (100.0%)
Total	38 (43.0%)	48 (55.8%)	86 (100.0%)

Key; HSIL High grade squamous intraepithelial lesion, **;** LSIL Low grade squamous intraepithelial lesion

Microscopic evaluation of β - catenin immunohistochemistry in normal, and cervical SIL.

Expression of β - catenin were considered positive when brown colourations were shown in the nuclear membrane or cytoplasm of the cervical epithelium. Scored as Negative, membranous staining and cytoplasmic staining.Expression of β - catenin cytoplasm were considered as positive using known **immunoreactivity scoring system (IRS)** (Klein *et al.,* **2001;** Gua *et al* 2021)

Expression pattern of β - catenin immunohistochemistry in cervical intraepithelial lesions, (Figure 1) showed membranous stain in non-neoplastic cervical lesions (chronic cervicitis and in normal squamous intraepithelial cells) and in cervical intraepithelial neoplasm (CIN 1). The membranous staining weakens with increase in dysplasia of cervical intraepithelial lesion. The membranuos staining were simultanously replaced with increase in the β - catenin cytoplasm expression from CIN 2, CIN 3 and CIS (Figures. 1 to 3).

Therefore the IHC of β - catenin expression in different grades of squamous intraepithelial lesions with majority of nonplastic lesions and CIN 1 showed consisted, strong staining of β - catenin through all membrane while the CIN 2, CIN3 and CIS showed staining in the cytoplasm with increased intensity.

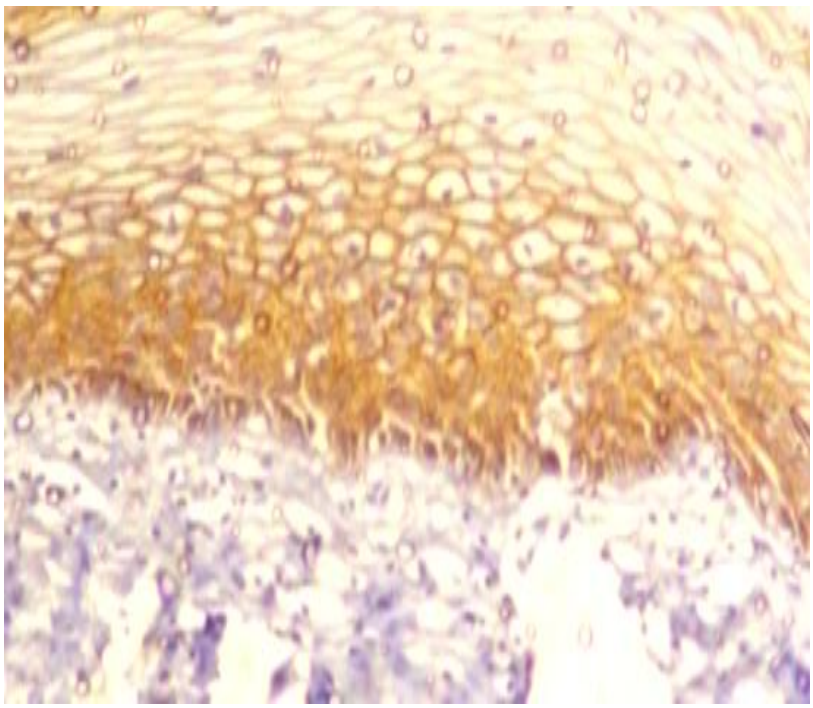

Fig1; Photomicrograph showing β - catenin membrane expression in epithelial cell in CIN 1 X400 magnifications.

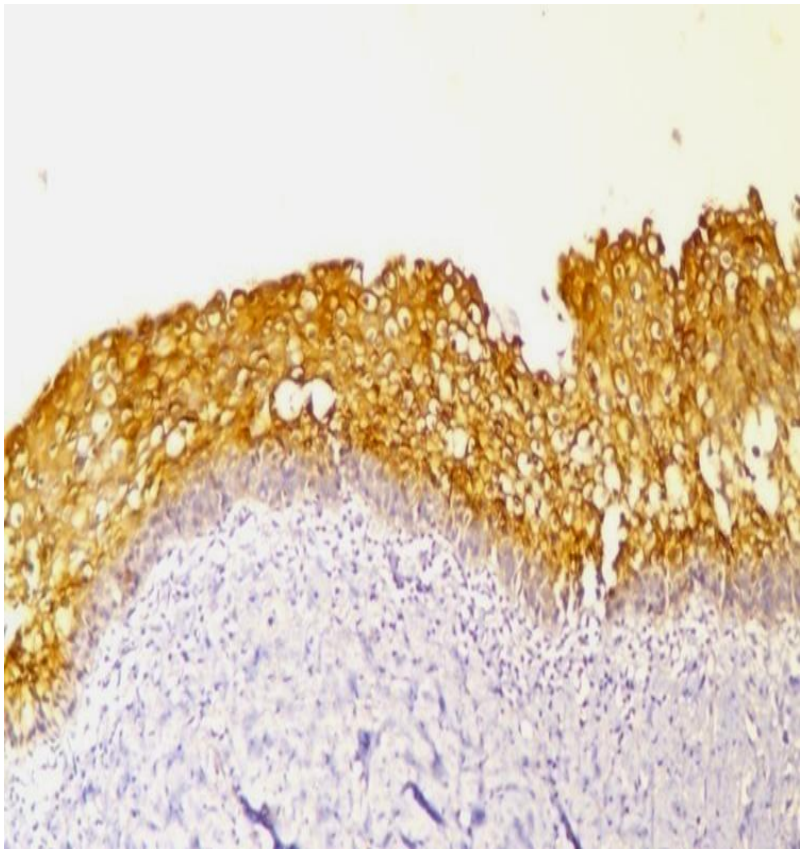

Fig2; Photomicrograph showing **β -** catenin cytoplasm expression in CIN 3 x200 magnifications.

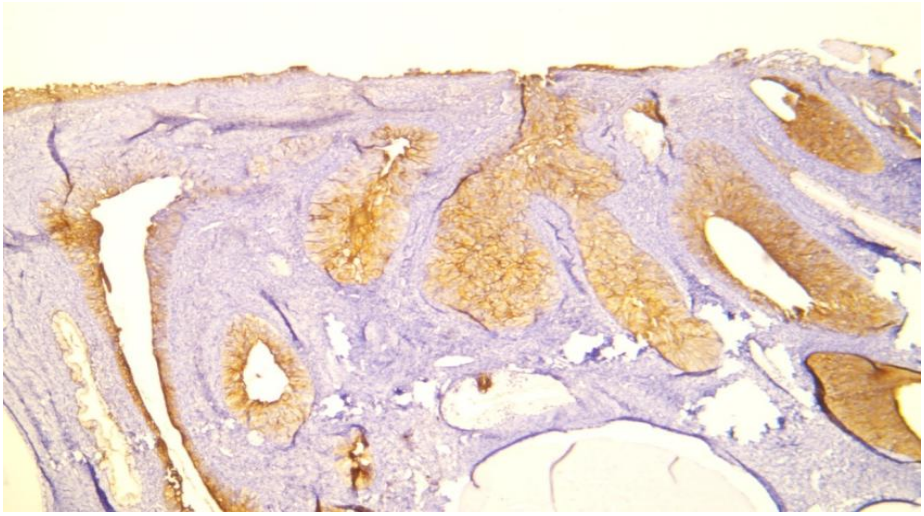

Fig3; Photomicrograph showing **β -** catenin cytoplasm staining in CIS with grandular epithelium metaplasia x100 magnification

Immunoreactivity of β - catenin in normal and neoplstic cervical lesion

The frequency and rate of the β - catenin immunoreactivity was evaluated, as shown below (Table 4.7). 85.3% and 100% of non neoplastic lesions (chronic cervicity and NILM) and CIN1 respectively showed strong β - catenin membranous staining while 14.7% (5/34) of non neoplastic lesions showed negative β - catenin expression. However, the membranous β - catenin immunoreactivity weakens with significant increase in the β - catenin cytoplasm staining in the HSIL (P = 0.00). The rate of the β -catenin cytoplasmic increased with the degree of dysplasia, from 70% in CIN2 to 87.5% in CIN3 and 88.9 % in CIS. We also noted that 17.1% (6/35) of HSIL showed β - catenin negative stain. There is statistical significant difference in the rate of the β - catenin expression in CIN2 CIN3 and CIS with CIN1 but not in CIN1 compared with non neoplastic lesions (chronic cervicitis and NILM). Therefore loss of β - catenin membranous staining respectively were noted as the SIL progresses to high grade lesion, with simultanous increase in the cytoplasm expression of high grade lesion..

We also found that strong association existed between expression of β - catenin and the histopathology grades of cervical squamous intraepithelial lesion,

Table 3; Distribution and rate β - catenin expression in normal and histopathology grades of squamous intraepithelial lesions

β - catenin staining	Histopathology grades of SIL				
Intensity of expression	Control	CIN 1	CIN 2	CIN 3	CIS
Frequency/rate of BC staining	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)
Negative	1(8.3%)	0(00.0%)	2(25.0%)	2(16.7%)	1(8.3%)
SC	0(00.0%)	0(00.0%)	7(100.0%)	14(48.3%)	8(27.6%)
SM	12 (26.7%)	17(37.8%)	0(00.0%)	0(00.0%)	0(00.0%)
P -value		0.00			

Keys; %= Percentage, BC= B. catenin, SC = cytoplasm staining, SM= Membranous staining, NILM= Negative intraepithelial lesion or malignancy, CIN 1= cervical intraepithelial neoplasm 1, CIN2= cervical intraepithelial neoplasm 2, CIN3= cervical intraepithelial neoplasm 3, CIS= Carcinoma in situ, SM = staining, SC= cytoplasm staining, %= percent.

DISCUSSION

Our study examined β-catenin expression in 86 cases of CILs using immunohistochemistry. We then compared the diagnosis of cervical squamous intraepithelial lesion using the histopathology analysis alone and adding immunohistochemistry of -catenin β-catenin as adjunct diagnosis and found out that by combining the immunohistochemistry of β-catenin, to the same morphological diagnosed cervical squamous intraepithelial lesion that 7.9% of high grade squamous

intraepithelial lesion was also misdiagnosed as low grade squamous intraepithelial lesion This finding is concordance with the report of Ike et al (2022), where 13.8% of cervical squamous intraepithelial lesion were misdiagnosed on the combination of IHC of P16 INK4a (P16) and Ki-67 proteins to histomorphology accessement of cervical SIL (Ike et al., 2021).

As a tumour suppressor gene, β - catenin prevents cells from growing and dividing too rapidly or uncontrollably (Maitre, 2015) and play great role in maintaining epithelial cells adhesion and the tissue architecture (Tsanou et al., 2008) suggesting that abnormal expression of Beta.- catenin may be regarded as important molecular events in dysfunction of the cell adhesion system, triggering SIL progression and consequently contributes to proliferation of cervical SIL to cervical cancer invasion and metastasis.

The expression pattern of Beta catenin in cervical SIL is found to have strong association degree of loss of membranous expression to increase in cytoplasm expression in cervical squamous intraepithelial lesion. According to Clever and Nurse (2015), the deregulation of Beta-catenin activates the forces that up-regulates oncogens during tumourigenesis. Therefore, weakening of Beta-catenin membranous expression and simultaneous increase in cytoplasm Beta-catenin expression may lead to deregulation of Wnt/β-catenin signalling (Falero-Rodrigues and Lopes, 2004) suggesting that cytopasmic expression of Beta-catenin may be an early event in the progression of ceervical squamous intraepithelial lesion to cervical carcinoma. Loss of membranous Beta- catenin and simultaneous increase in cytoplasmic expression of markers may also leads to alteration in the structural adhesion, process which is closely associated with progression of squamous intraepithelial lesion. The findings of this study are consistent with the past reports (Kawasaki et al., 2007; Pelosi et al., 2005).

This study noted increase in intensity of Beta. cateninin staining in CN2, CIN3 and CIS indicating the increased risk of these group lesions of lesion transformimg to malignant tumour.

Immunostaining may improve the diagnostic reproducibility and accuracy of the cervical lesion (Klaes et al., 2006). This study observed the positive predictive values, Sensitivity and Specificity of Beta-catenin were higher in HSIL compared to LSIL while the negative predictive value of Beta-catenin expression in LSIL were higher than of HSIL suggesting that with the use of immmunohistochemistry of β - catenin, as ancillary diagnosis offers greater specificity and sensitivity most especially in detecting the HSIL. This study is concordance with the past research (Lim *et al*., 2016). Therefore, overcoming the variability encountered in the use of only histopathology diagnosis alone and offers a better diagnostic accuracy of cervical squamous intraepithelial lesion.

The expression of Beta-catenin were also positively correlated with patient's age group, histopathology grades of the cervical SIL, HSIL being highest at the age group 51-60 years and LSIL at age group 31-40 years.

CONCLUSION

Ancillary use of β - catenin immunohistochemistry offers greater precision in diagnosis of premalignant cervical lesion. The Sensitivity and specificity of B-catenin IHC gave high percentage in HSIL and the positive predictive value is highest in HSIL. We here by conclude that there was no overall agreement of IHC expression of Beta-catenin with histopathogical diagnosis. The use of hematoxylin and Eosin in diagnosis of premalignant cervical lesions still remain the gold standard, but ancillary use of immunohistochemistry of β - catenin is advocated for as routine diagnostic tool for management of cervical squamous intraepithelial lesions. Since about 90% of squamous cervical carcinoma originates from cervical intraepithelial lesions therefore cervical

cancer is a preventable disease if detected early, detecting the patient with high- risk HPV group of high grade intraepithelial lesion which have higher tendency to develop to cervical cancer with proper and targeted therapy will ensures great reduction in morbidity and mortality as a result of cervical cancer in Nigeria.

RECOMMENDATIONS

Further research is advocated for on the use of these biomarkers for diagnosis of cervical intraepithelial lesion to determine more information on the targeted therapeutic use of this marker determination its role in targeted therapy management of premalignant as well in malignant cervical disease.

CONFLICT OF INTEREST: There is no conflict of interest whatsoever in this work to disclose.

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