

The Immunohistochemical expressions and diagnostic values of E-cadherin and β -Catenin in suspected cervical lesion or cervical cancer

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Abstract: Background: Cervical lesions are the cellular changes of a pathologic process with a bacterial and viral etiology ranging from cervicitis, cervical intraepithelial neoplasm and invasive cancer. The integrity of the cervical epithelium is maintained by complex cell adhesion molecules. This work is aimed at determining the expression pattern diagnostic value of the adhesion molecules to enhance early detection of cancer.

Methods: The study population was from 10 years retrospective archival tissue blocks of cervical cancer among women in Abuja metropolis from 2005 to 2015. Histopathological diagnosis made, from the 80 cases examined 20(25%) cases were cervicitis (CC), 15(18.8%) were cervical intraepithelial neoplasia (CINI), 15(18.8%) were cervical intraepithelial neoplasia (CIN2) while 30(37.3%) cases were squamous cell carcinoma SCC. They were examined immunohistochemically by Avidin Biotin Complex (ABC) method. E-Cadherin and Beta-Catenin, (cell adhesion molecules).

Results: Statistically significant rate of positivity were found in the expression of β -catenin 100% among all the categories of the studied cases. β -catenin expression was significantly higher in CINI than in SCC, while in E-cadherin, there were no statistically significant difference ($p < 0.05$) between the distribution of high expression and low expression of E-cadherin in both CINI and SCC. In expression of E-cadherin among the cases studied, there was a significant difference ($p < 0.05$) between rate of positivity and negativity for the expression of E-cadherin. A statistically significant rate of positivity was found in the expression of β -catenin among all the categories of the studied cases. In the expression E-cadherin, there was a gradual decrease in high expression from cervicitis to CINI, CIN2 and SCC. There was Significant ($p < 0.05$) high degree of expression of E-cadherin in cervicitis and CINI against CIN2 and SCC, while a significant ($p < 0.05$) low degree expression of E-cadherin in CIN2 and SCC against Cervicitis and CINI. In β -catenin expression, there was a gradual decrease in high expression from cervicitis to CIN2 and SCC except in CINI where all had high expression.

Conclusion: This study showed that most of the immunological markers examined can be used for identification of different stages involved in cancer progression, also for diagnosis of cancer, predictive prognosis and can be explore as therapy targets for cervicitis and Squamous cell carcinoma.

Keywords: Immunochemistry, E-cadherin, Beta-Catenin, cervical cancer

Introduction

The cervix comes from Latin words cervix uteri meaning “neck of the uterus” due to its role as the narrow connection between the larger bodies of the uterus above the vagina. It is 2- 3cm in length and cylindrical in shape in women that are not pregnant. The cervix is the lower part of the uterus, an organ of the female reproductive tract. The cervix acts as a gateway in that it connects the vagina with the main body of the uterus. The cervix is the prominent and dynamic part of the uterus and yet, the most inferior of the uterus of the female reproductive system. The cervix is formed from the two paramesonephric ducts after six weeks of embryogenesis where the outer parts of the two ducts are fused together to form a single urogenital canal which becomes the vagina, cervix and uterus (Gasner and Aatsha, 2022).

Cervical cancer is a cancer arising from the cervix. It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body (AlHarfiet al., 2019). Early on, typically no symptoms are seen. Later symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during sexual intercourse.

There were 500,000 new cases and 265,000 deaths across the globe in year 2018 (Globocan, 2012). Estimated deaths of 90% of this death occur in low-income country. Cervical cancer (CC) is the fourth most common cancer in women. According to existing data, an estimated 100,000 are diagnosed annually with cervical cancer in Sub-Sahara African, of which without treatment 62% of these women would have died from cervical cancer. (Plummer et al., 2016). Most patients with early CC have good prognosis, by contrast, patients with a later cancer stage or metastatic cervical cancer have poor survival rate because of less effective treatments available (Dizon et al., 2014). Therefore, additional studies on late cancer development and prognosis methods are necessary.

Aberrant activation of the Wingless-type (Wnt)/ β -catenin (canonical Wnt pathway) is a very common pathway in human CC (Chen et al., 2015). Recent molecular testing has demonstrated that the CC biological behavior may arise as a multistep gene process. Specifically, infection with human papillomavirus (HPV) could be “the first hit, while the deregulation of canonical Wnt pathway may be required as “the second hit” in cervical oncogenesis. However, the mechanism involving Wnt pathway in CC is still not well understood and

requires additional studies. In the canonical Wnt pathway, the binding of Wnts to a heterodimeric receptor complex stabilizes the β -catenin expression and leads to the activation of β -catenin target genes inside the nucleus (Liset al., 2015). Furthermore, Wnt inhibitory factor 1 (WIF1) is an upstream secreted Wnt antagonist, first identified as highly conserved gene in the human retina. WIF1 main function is to bind the extracellular Wnt ligands, disturbing the Wnt interaction with the receptors and consequently leading to β -catenin degradation, therefore inhibiting the canonical Wnt pathway. Currently, there are dozens of studies regarding the correlation with β -catenin and various types of cancer. In most of these cases, elevated levels of β -catenin have been strongly correlated with poor cancer prognosis. However, so far, there have been limited reports focusing on the association between β -catenin, WIF1, and the clinicopathological characteristics in cervical cancer.

The disruption of intercellular adhesions is an important component of the acquisition of invasive properties in epithelial malignancies. Alterations in the cell-cell adhesion complex, E-Cadherin/ β -Catenin, have been implicated in the oncogenesis of carcinomas arising from various anatomic sites and have been correlated with adverse clinico-pathological parameters, Epithelial Cadherin (E-Cadherin) is a 120 kDa transmembrane glycoprotein which is involved in both homotypic and heterotypic Ca^{2+} -dependent cellular adhesions, (Vergaraet al., 2019). Inactivation of E-Cadherin may occur through mutations, methylations or deletions of the E-cadherin gene, suppression of the E-Cadherin gene promoter, or posttranslational modification of the protein leading to cytoplasmic delocalization, (Decourtye-Espiard, 2022). The strength of E-Cadherin-mediated intercellular adhesion is significantly increased by interactions between the cytoplasmic tail of E-cadherin and the cytoskeletal network (Decourtye-Espiard, 2022). This interaction is mediated through the cytoplasmic proteins β -Catenin, α -Catenin and γ -Catenin (Vergaraet al., 2019). β -Catenin is a 92 kDa protein that, in addition to its cell-adhesion properties, also plays a role as a transcriptional co-activator in the Wnt signaling pathway; the latter is involved in cellular development, differentiation and oncogenesis (Tanne Van der Wal and Renee van Amerongen, 2020). Deregulation of the Wnt pathway may occur through an activating mutation of the β -Catenin gene, leading to accumulated levels of β -Catenin in the cytoplasm and nucleus, and culminating in the altered transcription of a variety of critical genes, (Vergaraet al., 2019).

The cervix, in which a dysplasia-to-carcinoma sequence is well-established, offers a useful medium to comparatively study the expression of proteins involved in cell-to-cell adhesion in dysplastic and invasive epithelium. Previous studies have shown that the expression of E-Cadherin and β -Catenin, as evaluated immunohistochemically, is inversely proportional to the histologic grade in squamous intraepithelial lesions (SIL) (CIN) of the cervix: expression of both markers is generally maintained in low-grade lesions (CIN1) and is lost in high grade lesions (CIN2).

B-catenin (also known as Armadillo in *Drosophila*) is one of the very first examples of moonlighting; that is, a protein that performs more than one radically different cellular function. Thus, β -catenin is a dual purpose protein, involved in regulation and management of cell–cell adhesion and gene transcription. In human beings, the CTNNB1 protein is programmed by the CTNNB1 gene. B-catenin is a subunit of the cadherin protein complex and functions as an intracellular signal transducer in the Wnt signaling pathway. B-catenin belongs to the catenin protein family and homologous to β -catenin, otherwise called plakoglobin. β -catenin is commonly seen in many tissues. In cardiac muscle for example, β -catenin localizes to adherence junctions in intercalated disc structures; and these are very (Shenet al., 2019)

Beta-catenin (β -catenin) is the mammalian homologue of the *drosophila* armadillo gene. β catenin serves both as a transcriptional co-regulator and an adaptor protein for intracellular adhesion (Daraghmehet al., 2021). B-catenin is crucial for the formation and preservation of epithelial layers and offers a mechanical connection between intracellular junctions and cytoskeletal proteins. Wnt/signaling is the principal regulator of β -catenin (Li et al., 2020).

The role of b-catenin and E-cadherin has not been extensively studied in cervical cancer. Catenin gene expression was observed altered and associated with absent or reduced E-cadherin levels in

cervical cancer-derived cell lines (Akhmetkaliyevet al., 2023). Normal mRNA levels of E-cadherin, and a- and B-catenin were observed in primary tumours from the cervix, compared with low levels in more advanced tumours (Pandeyet al., 2023). On the contrary, increased h-catenin mRNA levels were found in 5 analyzed by micro-array technology. Previous results from our laboratory showed that nearly 50% of tumours from the uterine cervix exhibited increased levels of expression or/and altered patterns of localization of h-catenin (Daniele et al., 2020).

The localization of β -catenin either on the membrane or in the cytoplasm is regulated by various extracellular signals. The phosphorylation of Y654 (a specific tyrosine residue) results to the stoppage of the catenin/E-cadherin interaction, which leads to the separation of the complex and the subsequent degradation of E-cadherin and β -catenin. The separation of the E-cadherin– β -catenin complex results in the loss of epithelial apico-basal polarity. However, it is the presence of other signals that determine cellular response to this alteration (Saifet al., 2022).

The accumulation of β -catenin within the nucleus or cytoplasm has been established in more than half of all cancer cases and is associated to increased tumourigenicity. The hallmark of colon cancer is cytoplasmic β -catenin. This is because it is capable of prompting tumourigenic traits in normal cells, and also enables both the multiplication of cancer cell and their survival. Stem-like cell populations in cancers that are resistant to chemotherapeutics and could bring about new tumours are typical of high-level cytoplasmic expression, and

nuclear localisation of beta-catenin. β -catenin also enables a suitable environment for cancer progression by modulating cancer microenvironment. The suppression of several cancer hallmarks leads to the inhibition of β -catenin activity (Chaichian et al., 2019).

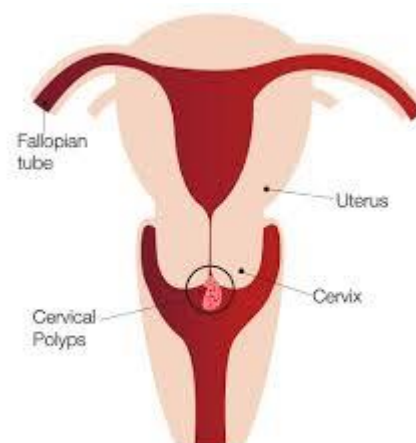


Figure 1. Image of the Female Reproductive Organ. Source: rafflesmedicalgroup.com [19]

Materials and Methods

Study area

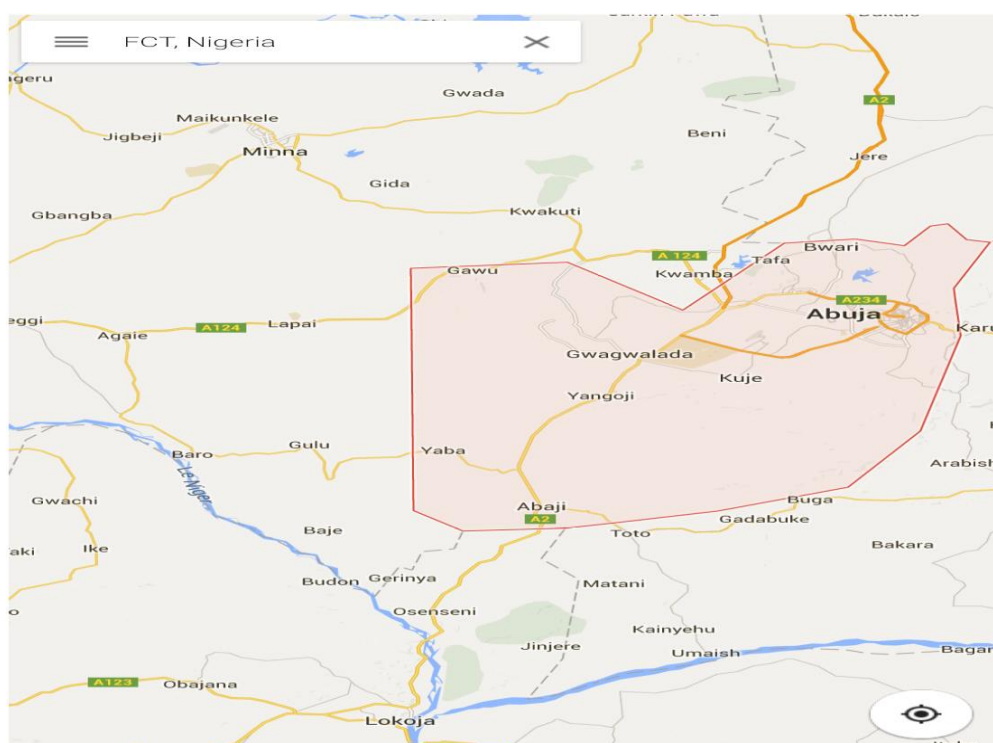


Figure 2. Map of Federal Capital Territory (FCT), Abuja Nigeria (NGIMS, 2016).

The research was carried out in these health Institutions: University of Abuja Teaching Hospital (UATH), Mataima General Hospital, Gwarinpa General Hospital, National Hospital in FCT Abuja. And also in Cachar cancer hospital and Research Centre India where the analysis was done. Data was collected to enhance effective and credible results from the research.

ANALYTICAL SITE



Figure 3. Showing the Map of Indian (mapsofindian.com)

Cachar Cancer Hospital and Research Center is a DSIR SIRO (Govt. of India) recognized not for profit comprehensive cancer care centre situated in the outskirts of Silchar on land provided by the Govt. of Assam. It is administered by a non-profit society The Cachar Cancer Hospital Society. Cachar Cancer Hospital and Research Centre (CCHRC), established (in 1996) and administered by the Cachar Cancer Hospital Society, a non-profit NGO registered under the Societies Registration Act, is located in the outskirts of Silchar town in the Barak Valley of Assam in India. The society came into existence in 1992 as a result of a desperately felt need for a cancer hospital (since the only cancer hospital in the entire north east was in faraway (Guwahati) with three principal objectives:

- (1) To make people aware of cancer, adopt preventive measures and seek early detection
- (2) To establish a full-fledged cancer hospital to provide meaningful services to all suffering people and
- (3) To set up a cancer research Centre.

It provides affordable and accessible standard cancer care to more than 3500 new and 16,000 follow-up patients every year for the last 22 years. Patients come from all the northeastern states and nearly 70% of them belong to low socio-economic strata earning on an average Rs 8,000 a month. Cachar Cancer Hospital and Research Center provides standard cancer care without any discrimination to all of them through its low-cost clinical services, subsidized medicine, free food, lodging, adhoc employment and active resource mobilization by connecting them to Government (Prime Minister National Relief Fund, Atal Amrit Abhyan) and private schemes (Indian Cancer Society, Sai Trust, Budhrani Trust) that potentially would cover their cost of treatment.

Sample technique

The study samples shall be grouped into four study groups into four groups and the control group. They study group is made up of one those diagnosed for squamous cells carcinoma, cervicitis, CIN and control (Taro Yamane, 1967 updated 2008).

Taro-yamane formula shall be applied to calculate sample size. When n signifies sample size, N signifies the population under study, e signifies the margin error

$$\begin{aligned}
 n &= \frac{N}{1+(e)^2} \\
 &= \frac{160}{1+0.5000} \\
 n &= 160/(1+1) \\
 n &= 160/2 \\
 n &= 80
 \end{aligned}$$

Sample collection

Sections of about 3 – 5 microns are cut from the selected samples (blocks) using microtome and microtome blade, it will be picked on IHC special slides and preserved for IHC staining. Prior to staining slides are placed in oven at 60° for 1 hour to avoid wash-off-during the IHC Staining.

Sample analysis

IHC analysis was carried out for β -Catenin antibody from Novasca/ Leica shall be use, immune activity target of this marker is cytoplasmic.

IHC Analysis was carried out for E-cadherin using novastra/leica.

Immune reactivity target of this marker is membranous to cytoplasmic.

Statistical Analysis:

The results of case and control was collated and manage statically using EPI info, statically package for social science (SPSS) and Microsoft access soft wares. The SPSS shall be used for data analysis; descriptive statistic was used to analyze all variable in order to access association of independent variables within the study.

Summary of ethical issue involved in the research.

The potential subject must be adequately informed of the aim, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. The right of every subject must be respected. The subject shall also be informed that they have the right to opt out of the study if they want to do so. The interest of the subjects was placed above that of the research work.

Consequences of the Study for The Local Community, Environment and Participants: There was no adverse consequences for the local community; environment and participants since safety measures was put in place to ensure that any waste generated are adequately disposed of by the health facilities.

Dissemination of results of study: The results of study was for academic purpose; however, the findings of the results will be used to prefer solution for the management, prevention, treatment of cancer patient and awareness creation to the general public on issues relating to cervical cancer.

Confidentiality and privacy: To ensure confidentiality and privacy, the consent forms bearing the bio data of the participants shall be immediately destroyed after the collection of the results of the analysis. Participants' names will not be used only the age, sex and the nature of the sample.

The cost and sources of funding of research: The research work was funded from personal effort and financial support from my Employee University of Abuja.

Ethical consideration

Study was approved by the Federal Capital Territory Health Research Ethics Committee, Abuja with reference number FHREC/2019/01/93/07.10.19. A copy is attached at the appendix.

Inclusion criteria:

All Haematoxylin and Eosin-stained slides were reviewed to confirm diagnosis of cervical cancers. Furthermore, all tumourcases that were selected were ensured to have adequate tumor tissue representation.

The age range must be from 17 years and above.

Exclusion criteria:

- i) Inadequate tissue sections and cases not diagnosed as cervical lesion or cervical cancers were excluded from the study.
- ii) Cases with no clinical information in the records or for which the tissue blocks are missing or damaged were excluded.

Procedure for Handling and Treatment of Archived Tissue Blocks

The archived tissue blocks representing the cancer diagnosis were retrieved from the archive storage room. Proper selections were made by evaluating the existing state of the tissue, which consists of checking the gross tissue adequacy on the paraffin blocks, the orientation of the tissue, the presence or absence of dust particles and molds. Re-embedding was done for the tissues that required it.

All samples selected were sectioned at 3 microns using a rotary microtome and stained with standard Haematoxylin and Eosin staining method. The slides were reviewed independently by two pathologists and diagnosis were classified into Cervicitis, CIN1, CIN2 and squamous cell carcinoma.

Four sections of 3microns were further cut for each sample to represent the four antibody markers that were used in this study. Also sections were cut 3microns for specify positive and negative control for the four difference antibody.

The tissue sections were brought down to water through deparaffinization in xylene, hydration in descending grades of alcohol and finally washed in water. Antigenic sites of tumour cells were retrieved using heat mediated antigen retrieval method that required pressure cooker and citric acid of PH 6.0. Tissue sections were cooled in water and ready for further steps of the immunohistochemical technique

Principle of Haematoxylin and Eosin staining (Titford, 2005; Chan, 2014)

Haematoxylin and Eosin stain is the most widely used stain in histology and Histopathology Laboratories for the purpose of demonstrating a wide range of normal and abnormal cells and tissue components. The Haematoxylin component stains the cell nuclei blue-black showing good intra-nuclear details while eosin stains cell cytoplasm and most connective tissue fibres in varying shades and intensities of pink, orange and red.

The principle is based on the acidic component of the cell which has the affinity to basic dye and the basic component of the cells which have the affinity to acidic dye. In Haematoxylin and Eosin stains the acidic part of the cell is the nucleus. Thereforehaematoxylin is called a nuclear stain while eosin act as an acidic stain and bind with the basic part of the cell – the cytoplasm and staining pink.

The Immunohistochemical method

The method is the Avidin Biotin Complex (ABC) method also referred to as the Avidin biotin Imuunoperoxidase method microns thick of formalin fixed and paraffin embedded tissue was cut for the IHC. Tissue antigenic sites were retrieved using citric acid solution PH 6.0 and pressure cooker. Peroxidases, protein and biotin blocks were done using Hydrogen peroxide, avidine and biotin respectively. Sections were incubated with the different antibodies for the study. These were followed by the biotylated secondary antibody, streptavidine, DAB/substrate reaction and haematoxylin counterstain.

The antibody dilution factor used were as follows: 1:150 for E-cadherin, 1:50 for p16 while ready to used mouse monoclonal antibody was used for ki-67 and beta-catenin antibody markers. Below is the detail of the IHC protocol

The processed tissue were sectioned at 2microns on the rotary microtome and placed on the hot plate at 70 degree for at least 1hour, sections were brought down to water by passing the on 2 changes of xylene, then 3 changes of descending grades of alcohol and finally to water. Antigen retrieval was performed on the sections by heating them on a citric acid solution of PH 6.0 using the Microwave at power 100 for 15minutes. The sections were equilibrated gradually with cool water to displace the hot citric acid for at least 5min for the section to cool.

Peroxidase blocking were done on the sections by simply covering section with 3% hydrogen peroxide (H₂O₂) for 15min, sections were washed with PBS and protein blocking were performed using avidin for 15min, sections were washed with PBS and endogenous biotin in tissue were blocked using biotin for 15min, after washing with PBS sections were incubated with the respective diluted primary antibody for example E-cadherin antibody diluted 1:100 for 60 min, excess antibody were washed off with PBS and a secondary antibody (LINK) were applied on section for 15min. Sections were washed and the (LABEL) which is the horseradish peroxidase (HRP) were applied on the sections for 15min.

A working DAB solution is made up by mixing 1 drop (20microns) of the DAB chromogen to 1ml of the DAB substrate. This working solution is applied on sections after washing off the HRP with PBS for at least 5min. The brown reactions begins to appear at this moment especially for a positive target. Excess DAB solution and precipitate are washed off with water. Sections were counterstained with Haematoxylin solution for at least 2min and blued briefly. Sections are dehydrated in alcohol, cleared in xylene and mounted in DPX

Cells with specific brown colours in the cytoplasm, cell membrane or nuclei depending on the antigenic sites are considered to be positive. The haematoxylin stained cells without any form of brown colours are scored negative. Non-specific binding/brown artifacts on cells and connective tissue are disregarded.

Results

This study involved Eighty (80) female subjects including Cervicitis 25%, CIN1,18.8%, CIN2,18.8% and 37.50% for SCC as shown in figure 3. Table 1 shows the rate of expression (positivity and negativity) of E-cadherin among the cases studied, there was a significant difference ($p < 0.05$) between rate of positivity and negativity for the expression of E-cadherin. A statistically significant rate of positivity was found in the expression of β -catenin among all the categories of the studied cases as shown in Table 2. Figure 4 shows the degree of expression for E-cadherin, there was a gradual decrease in high expression from cervicitis to CIN1, CIN2 and SCC. There was significant ($P < 0.05$) high degree of expression of E-cadherin in cervicitis and CIN1 against CIN2 and SCC, while a significant ($p > 0.05$) low degree expression of E-cadherin in CIN2 and SCC against Cervicitis and CIN1. Figure 5 shown the

degree of expression B-catenin, there was a gradual decrease in high expression from cervicitis to CIN2 and SCC except in CIN1 were all had high expression.

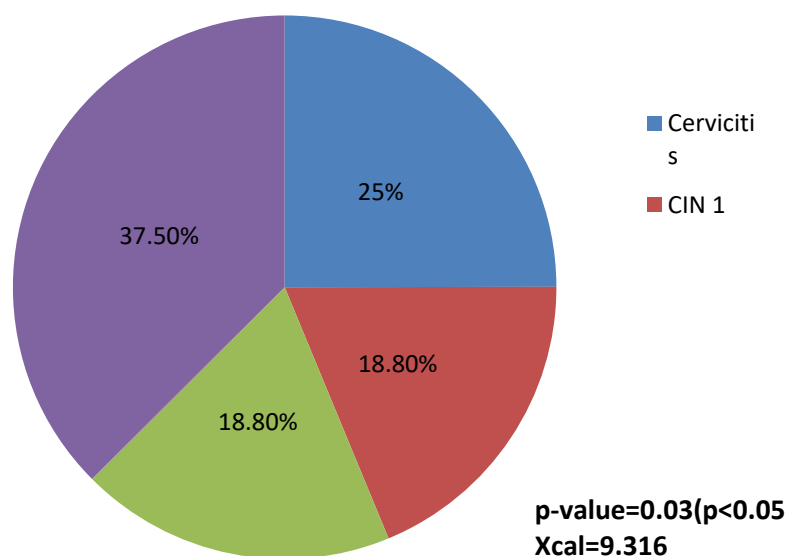


Figure 4. Classification of the Cases of the Subjects Examined in this study.

Table 1. Rate of Expression of E-Cadherin among the difference cases in the study

Diagnosis	Number examined	Positive Expression n(%)	Negative Expression n(%)	p-value
Cervicitis	20	20(100)	0(0.0)	<0.0001
CIN 1	15	15(100)	0(0.0)	<0.0001
CIN 2	15	15(100)	0(0.0)	<0.0001
SCC	30	30(100)	0(0.0)	<0.0001
Total	80	80(100)	0(0.0)	<0.0001

KEYS: CIN intraepithelial neoplasm, SCC; squamous cell carcinoma, %; percentage of expression, P-value; significant level

Table 2. Rate of Expression of B-Catenin among the difference cases in the study

Diagnosis	Number examined	Positive Expression n(%)	Negative Expression n(%)	p-value
Cervicitis	20	20(100)	0(0.0)	<0.0001
CIN 1	15	15(100)	0(0.0)	<0.0001
CIN 2	15	15(100)	0(0.0)	<0.0001
SCC	30	26(86.7)	4(13.3)	<0.0001
Total	80	76(95.0)	4(5.0)	<0.0001

KEYS: CIN intraepithelial neoplasm, SCC: squamous cell carcinoma, % : percentage of expression, P-value: significant level.

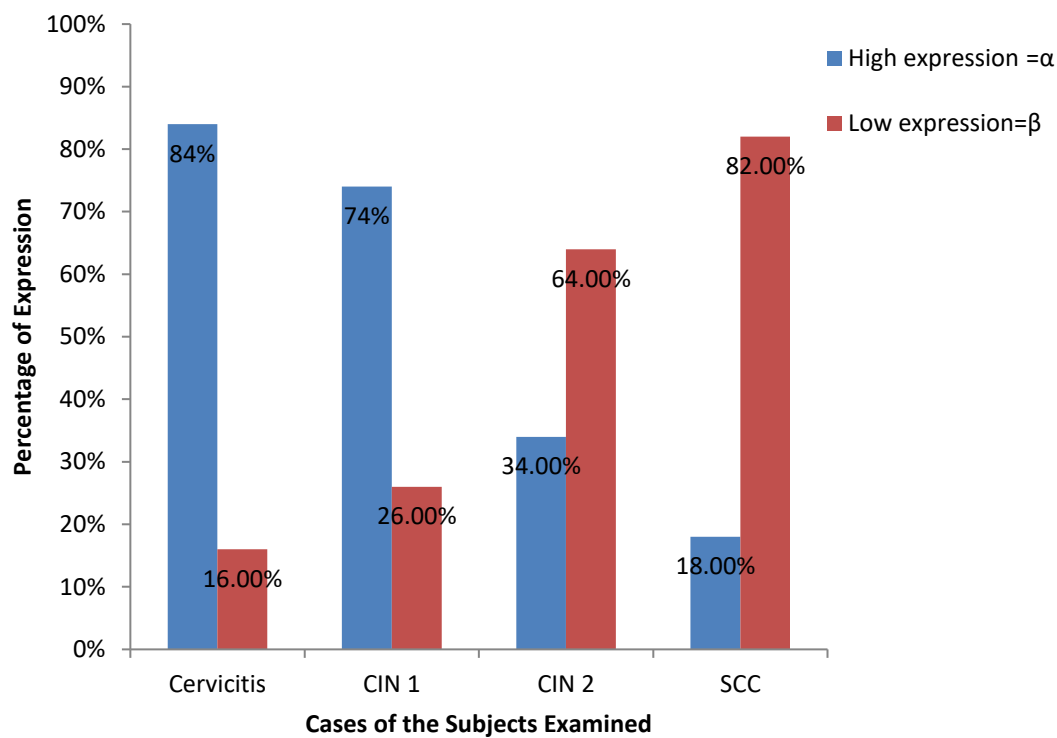


Figure 5. Degree of Expression of E-Cadherin across the difference cases, Cervicitis $\alpha > \beta$ ($P < 0.05$), CIN 1 $\alpha > \beta$ ($P < 0.05$), CIN 2 $\beta > \alpha$ ($P < 0.05$), SCC $\beta > \alpha$ ($P < 0.05$)

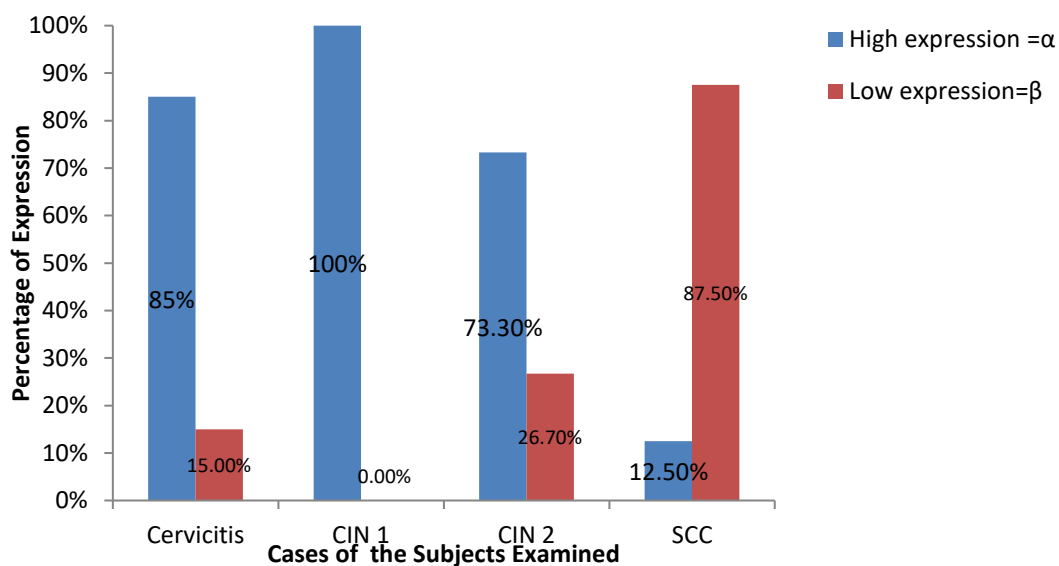


Figure 6. Degree of Expression of B-Catenin across the difference cases. Cervicitis $\alpha > \beta$ ($P < 0.05$), CIN 1 $\alpha > \beta$ ($P < 0.05$), CIN 2 $\alpha > \beta$ ($P < 0.05$), SCC $\alpha < \beta$ ($P < 0.05$)

Photomicrograph Of Heamatpoxylin And Eosin Stain Slides

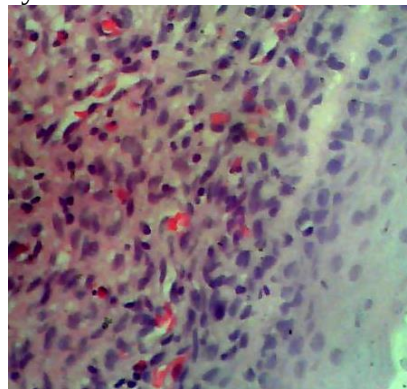
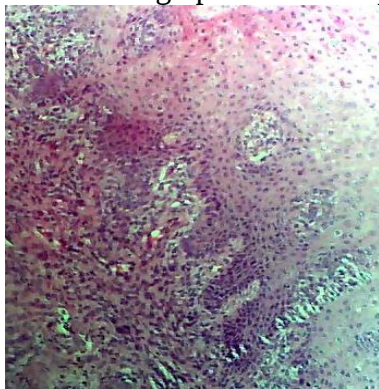
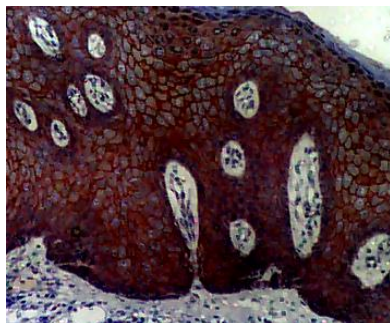


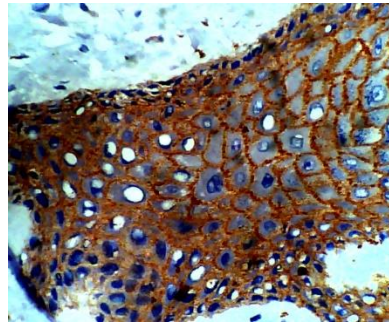
Plate. 1 CERVICITIS X10 and x40

The expression of E cadherin
Cervicitis



High expression

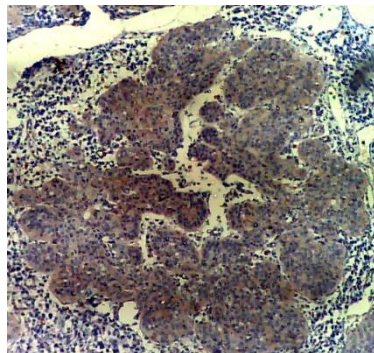
CIN 2



High expression

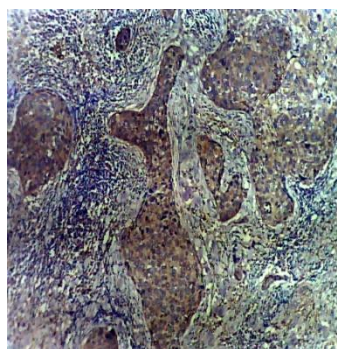
Plate 2:

The expression of B- catenin
SCC



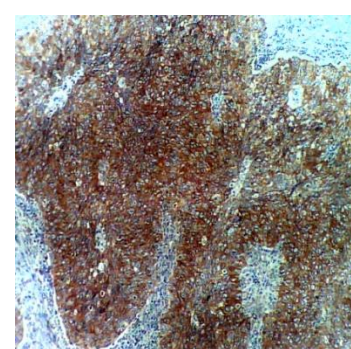
Low expression

SCC



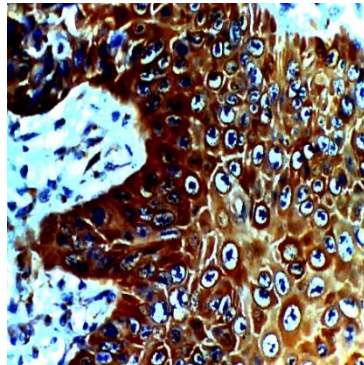
High expression

SCC



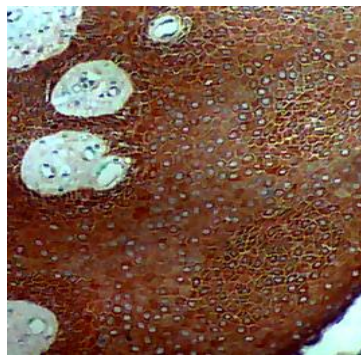
Low expression

Pate 3
Cervicitis



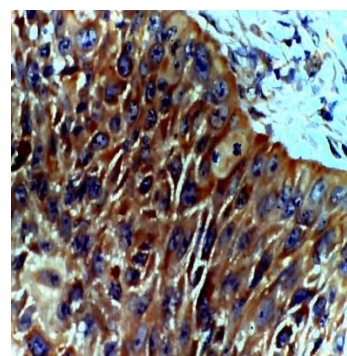
High expression

CIN1



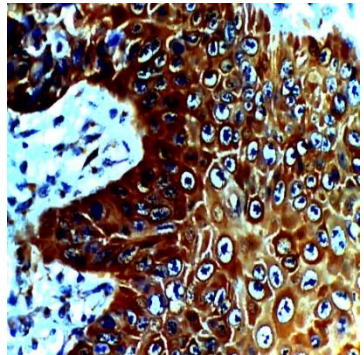
High expression

CIN2



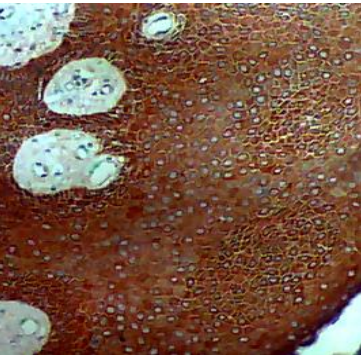
High expression

Plate 4.
Cervicitis



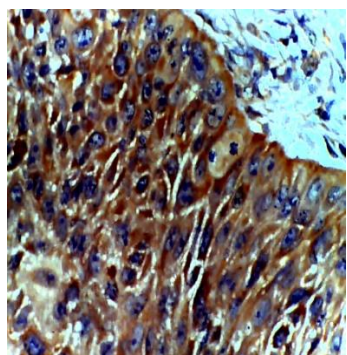
High expression

CIN1



High expression

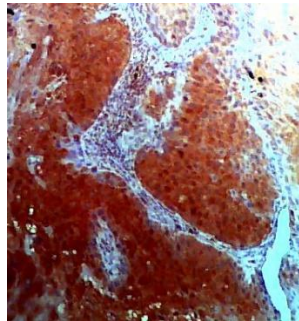
CIN2



High expression

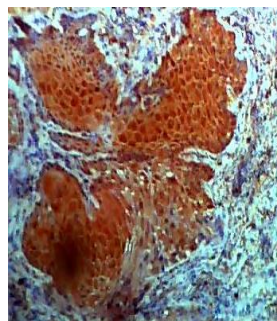
Plate 5

SCC



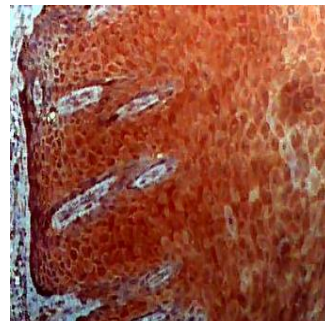
High expression

SCC



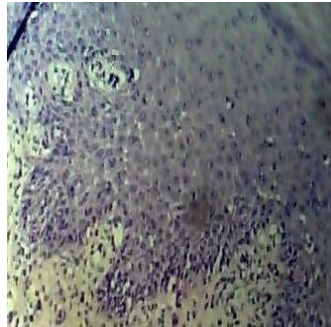
High expression

CIN2

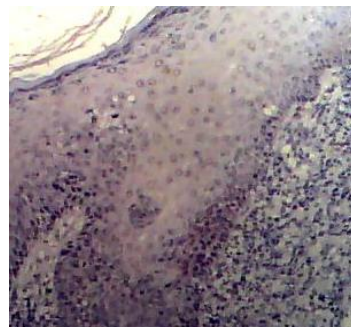


High expression

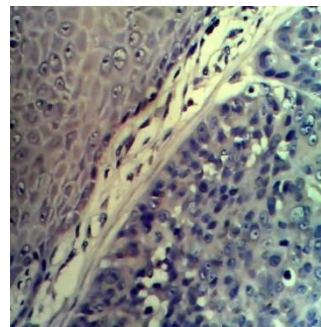
Plate 6
Negative Immunohistochemistry
Cervicitis



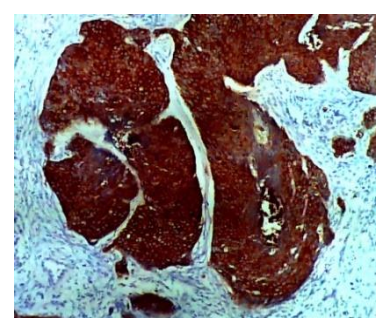
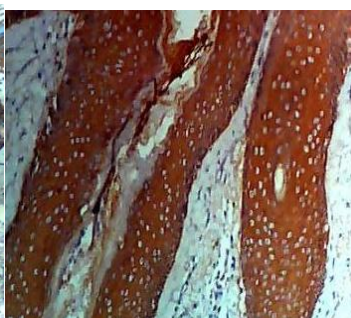
CIN1



CIN2



Positive Control Immunohistochemistry Photos



Skin for E- Cadherin

Skin for Beta- Catenin

A known positive Cervical Cancer

Discussion

This study shows a progressive decrease in E-cadherin staining from SILs to SCCs, with minor expression in SILs and practically no expression in SCCs. It clearly demonstrates that SILs lose E-cadherin immunoexpression as they progress towards severity, which is in agreement with previous studies (Cavalcante *et al*, 2014). More interestingly, we verified that this adhesion molecule exhibits a different expression pattern according to the epithelial thickness layer.

The normal epithelium adjacent to the SIL areas showed greater negative E-cadherin expression than the lesions themselves. These results were unexpected, and there is no definitive answer for this observation so more research is needed on this aspect.

We observed a statistical difference with decreasing /loss of e-cadherin expression in cervical carcinogenesis. Cervicitis 84%, CIN1 75%, CIN2 34 % and SCC 18% respectively. These results are similar to those of Cavalcante *et al*, 2014, who found that membrane E-cadherin exhibited a tendency to disappear in 40% of SILs.

The progressive reduction in cell adhesion molecule (CAM) expression in neoplastic cervical tissue indicates that it may participate in the epithelial stratification process. (Mendez and Bosch 2011; Cavalcante *et al*, 2014). The lack of differentiation and epithelial organization seen in SILs may be due to disrupted expression of adhesion

molecules such as E-cadherin (Wang *et al.*, 2018), which confirmed that E-cadherin plays a pivotal role in inducing cell polarity and epithelial organization.

The importance of defining thresholds in any investigations reporting the loss of the immunohistochemical expression of a biomarker cannot be overemphasized. An orderly, membranous expression E-Cadherin and β -Catenin is found in the normal cervix. The loss of expression of both proteins in proportion of high-grade squamous intraepithelial lesions (Khieu and Butler, 2022) suggests that dysregulation of this pathway is an early event in cervical carcinogenesis. We investigated cytoplasmic staining as a manifestation of impaired expression of cervicitis, dysplastic cervix and SCC. Normally low expression of β -Catenin was significantly associated with advanced pathologic b stage. This is associated disease-free survival in another study by (Sefidbakht *et al.*, 2021). High expression was evident in cervicitis 85%, and CIN1 100% and CIN2 73.30%. The low expression seen in SCC 12.50% is also similar with the expression of E-cadherins in SCC. In the typical normal cell β -Catenin's complex with E-Cadherin and the cytoskeletal network is inversely proportional to the association of β -Catenin with the adenomatous polyposis coli protein, a large multifunctional cytosolic protein.

Conclusion

E-cadherin and beta- Catenin are essential cell adhesion molecules during the process of cervical carcinogenesis and in this context exhibits a different low expression pattern according to the dysplastic lesion.

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