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RESEARCH ARTICLE

THE EXPRESSION OF CEA AND E-CADHERIN IN THE PROGRESSION TO SCC OF THE CERVIX

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ABSTRACT: **Introduction**: Cervical cancer may be a serious ill health, with nearly 500 000 women developing the disease annually worldwide. Cases occur mostly in less developed countries where no effective screening systems are available. Risk factors include exposure to human papillomavirus, smoking, and immune-system dysfunction. **Aim**: The aim of this study is to observe and study the expression of CEA and E-cadherin in normal cervix, Cervical Intraepithelial Neoplasia (CIN) and Squamous Cell Carcinoma (SCC) of the cervix and determine if these markers can be used as predictive markers for SCC of the cervix progression. **Materials and Methods**: A total of 50 formalin fixed, paraffin embedded tissue blocks were obtained including: 5 normal cervical tissue, 10 CIN 1, 15 CIN 2 &3 and 20 confirmed SCC of the cervix cases. Immunohistochemistry technique was carried out in this study. **Result**: The expression of CEA showed increase in positivity with the stages to the SCC of the cervix with the expressions in CIN 2&3 and SCC cases having the most marked expressions and CIN 1 having weak reaction. Meanwhile, E-cadherin expression showed higher grades of positivity in normal cervical tissues and CIN 1 as compared to CIN 2&3 and SCC cases. E-cadherin decreases in positivity with the stages in the progression to SCC of the cervix. E-cadherin expression was found to be inversely relational to the progression to SCC of the cervix while CEA expression was found to be directly relational to the progression to SCC of the cervix. **Conclusion**: Therefore, based on the findings in this study, both CEA and E-cadherin are confirmed as predictive markers to the progression to SCC of the cervix.

KEYWORD: SCC, CIN, CEA, E-cadherin, IHC

INTRODUCTION:

Cervical cancer is a sexually transmitted disease caused by the human papillomavirus (HPV), particularly high risk HPV strains such as HPV-16 and -18. Cervical cancer, although a preventable disease, is the second most common malignancy affecting women worldwide and a major cause of mortality particularly in developing countries where it accounts for 15% of all female cancers, with an estimated 500 000 new cases and 275 000 deaths[21]. The squamocolumnar junction (SCJ) is the point where the endocervical and ectocervical epithelium meet. The SCJ is particularly susceptible to HPV infection and most squamous cell carcinoma develops at this point [18, 7]. Epidemiological studies supported by molecular technology have provided sufficient evidence on the causal role of some Human Papillomavirus (HPV) infections in the

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development of cervical cancer [2]. Low-risk HPV types include: 6, 11, 40, 42–44, 54, 61, 72, 81 while High-risk HPV types include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82 [11]. Richart introduced the concept that each one sorts of precursor lesions to epithelial cell carcinoma of cervix represented one disease process, which he termed cervical intraepithelial neoplasia (CIN). The CIN terminology divided cervical cancer precursors into three groups. CIN 1 corresponded to lesions previously diagnosed as mild dysplasia, CIN 2 corresponded to moderate dysplasia, and CIN 3 to both severe dysplasia and carcinoma in place, since pathologists could not distinguish between the two. At the time of its introduction, CIN was thought to define a spectrum of histological changes that shared a common etiology and natural history. Furthermore, the diagnostic term CIN implied that such lesions, if untreated, had a big, albeit individually unknown, risk of developing into invasive carcinoma within the future. It was presumed that when the histological changes of CIN were diagnosed and therefore the lesion adequately treated, the event of invasive cancer might be prevented. Although the CIN terminology allowed lesions to be subdivided into three separate categories, it had been anticipated that the utilization of a unified concept of one disease process would deemphasize lesion grade as a determinate of clinical management[22].

Immunohistochemistry is the preferred technique in this study whereby there is identification of cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified by direct labelling of the antibody, or by use of secondary labelling method. Although histological analysis of Haematoxylin & Eosin stained tissue sections remains at the core of the practice, immunohistochemistry has become a powerful tool in the armamentarium of the pathologist [14]. Immunohistochemical studies have traditionally focused on markers of specific cell and/or tumour type as aids within the diagnosis of specific tumours. Traditionally, the goals of diagnostic immunohistochemical studies have been to explore and certify diagnosis by identifying the pathway of differentiation of a given tumour [13, 9].

Carcinoembryonic Antigen (CEA) is a glycoprotein of molecular weight approximately 180KD found in normal fetal gastrointestinal tissues. It is normally found in small concentrations in adult plasma but its concentration is increased in the presence of many tumours. Studies have focused on CEA for over 40 years, suggesting it be a useful marker in cancer diagnosis as it is a cancer cell adhesion marker [3].

Epithelial Cadherin (E-cadherin) are molecules that participate in adhesion which determine the architecture and differentiation of keratinocytes in the epithelium. Flat cells of the cervical epithelium are attached fairly strongly to at least one another and to the basal membrane via the high expression of those adhesion molecules. It is known that, in intraepithelial cervical neoplasia, E-cadherin expression changes. The decrease in its expression interferes with the cells' ability to adhere to each other and increases their malignant potential, capacity for invasive growth, and metastasis. In particular, the decreased expression of E-cadherin expression is supposed to be an efficient marker of the enhanced aggressiveness of invasive cervical cancer [17].

The ability of CEA and E-cadherin to predict squamous cell carcinoma of the cervix from samples of the normal and various transitional stages of CIN was evaluated by relating their immunoreactivity to the lesion severity in the progression to SCC of the cervix. These IHC markers may prove to be valuable in clinical practice if they are found to be predictors of SCC of the cervix.

MATERIALS AND METHODS

Case Selection: Cervical tissue blocks were selected from the pathology archives of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). All samples were fixed in formalin and embedded in paraffin wax by conventional techniques. Haematoxylin and eosin stained slides of all samples were reviewed and classified. Confirmed cervical tissue blocks of non-malignant, CIN and invasive squamous cell carcinoma (SCC) were selected. In total, 50 biopsy samples were taken. Among these, 5 cervical tissue blocks were normal cervical tissues, 10 cervical tissue blocks had CIN 1 diagnosis, 15 cervical tissue blocks were diagnosed with CIN 2 and 3, and 20 cervical tissue blocks were diagnosed with SCC of the cervix.

PREPARATION OF SECTIONS FOR IMMUNOHISTOCHEMISTRY

The formalin fixed paraffin embedded tissues were sectioned at a thickness of 4 micron and each tissue block was stained with H&E to confirm the presence of lesions and grade of the lesions present. IHC analysis was carried out with the following procedure;

Deparaffinization of tissue sections on poly lysine coated slides was carried out with xylene followed by rehydration through decreasing grades of alcohol. Quenching of endogenous enzymes using 3% hydrogen peroxide (H_2O_2) was carried out. Antigen retrieval methods was done by heating the sections for 10 mins in citrate buffer at 121°C. After which blocking of non-specific sites was carried out by addition of Triton X. Incubation with primary antibodies was done for 30 min at room temperature. The primary antibodies used in this study are CEA (Dako, CD66e, 1:100) and E-cadherin (Dako,clone NCH-38, ready to use). Then binding with biotinylated secondary antibody (streptavidin) was carried out. This is then followed by the addition of diaminobenzidine (DAB). The slides were counterstained with haematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in Dibutylphtalate polystyrene xylene (DPX) mountant [8]. Staining expression was evaluated optically using the light microscope at ×100 magnification. Photomicrographs of relevant fields were taken at × 100 magnifications with the use of a light microscope and camera. Expression of CEA and E-cadherin was determined through a semi-quantitative method.

IMMUNOSTAINING ASSESSMENT

The immunoreactivity of these markers was determined by accessing the cytoplasmic staining intensity per field. The cytoplasmic staining intensity of CEA and E-cadherin was graded as mild, moderate and strong [12].

PHOTOMICROGRAPHY

The Stained sections were examined under a LEICA research microscope (LEICA DM750, Switzerland) interfaced with digital camera (LEICA ICC50). Digital photomicrographs of stained sections for the histomorphology, immunohistochemistry on the tissue sections studied were taken at various magnifications, and reported for Morphological changes.

DATA ANALYSIS

Statistical analysis of obtained results was carried out using Tables and Micrographs.

RESULTS

Table. 1: A table indicating the staining intensity of CEA in the progression of Squamous Cell Carcinoma of the Cervix

Staining intensity	Normal(5)	CIN1(10)	CIN2&3(15)	SCC(20)
Weak	3(60)	2(20)	2(13.33)	2(10)
Moderate	2(40)	6(60)	5(33.33)	6(30)
Marked	-	2(20)	8(53.33)	12(60)

Table 1 depicts the increase in staining intensity from cases with normal cervical tissue to SCC. It was observed that more cases in CIN 2&3 had more of moderate and marked cytoplasmic staining intensity while the normal cervical tissues had weakly to moderately stained cytoplasm and CIN 1 had weak, moderate and marked staining intensity. This therefore indicates that the staining intensity of E-cadherin CEA increases with stages of progression of the SCC of the cervix.

Table 2: A table indicating the staining intensity of E-Cadherin in the progression of Squamous Cell Carcinoma of the Cervix

Staining intensity	Normal(5)	CIN1(10)	CIN2&3(15)	SCC(20)
Weak	-	2(20)	3(20)	11(55)
Moderate	1(20)	4(40)	9(60)	8(40)
Marked	4(80)	4(40)	3(20)	1(5)

Table 2 depicts the decrease in staining intensity from cases with normal cervical tissue to SCC. It was observed that normal cervical tissues blocks had the highest percentage of marked staining intensity, followed by CIN 1 cases, which had a lower percentage. Meanwhile, CIN 2&3 cases had more of weak to moderate staining intensity for E-cadherin and few marked samples. Lastly, in SCC cases, there was a significant drop in staining intensity percentage due to loss of cell adhesion. This therefore indicates that the staining intensity of E-cadherin decreases with the stages of progression to the SCC of the cervix.

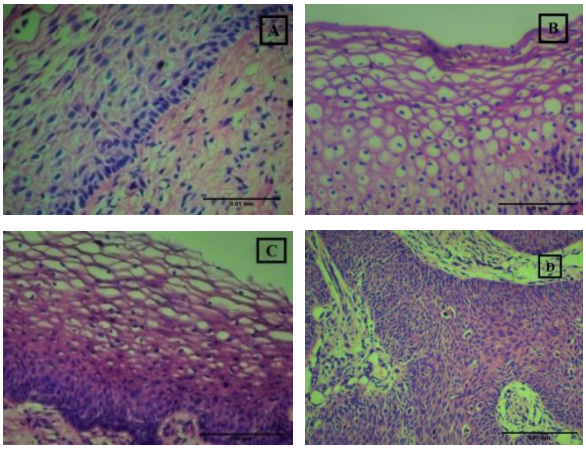

Plate 1: micrographs of the uterine cervical sections stained with H&E illustrating: (A) (Normal H&E x100) showing parabasal cells located just above the basal cells which has slightly more cytoplasm than the basal cells. (B) (CIN 1 H&E x100) showing the most distinctive feature which is koilocytosis and also nuclear atypia. (C) (CIN 2&3 H&E x100) showing hyperchromasia and significant nuclear atypia and (D) (SCC H&E x100) showing infiltrating nests of neoplastic squamous epithelium in the stroma

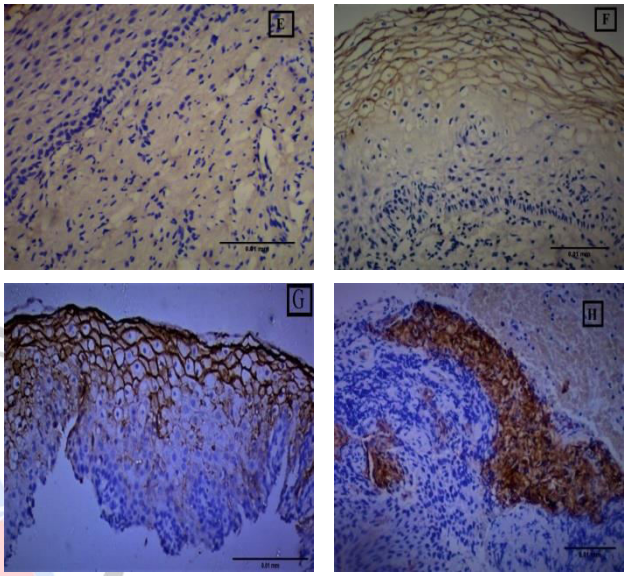

Plate 2: micrographs of the uterine cervical sections stained with CEA illustrating: (E) (Normal CEA x100) showing the presence of basal cells and parabasal cells and illustrating very weak staining intensity. (F) (CIN 1 CEA x100) showing koilocytosis with a weak staining intensity (G) (CIN 2&3 CEA x100) showing the abnormal parabasal-like cell proliferation with loss of polarity and (H) (SCC CEA x100) showing hyperchromasia, anisonucleosis, loss of polarity due to dysplasia and marked staining intensity as CEA expression is increased with the stages of progression of SCC of the cervix

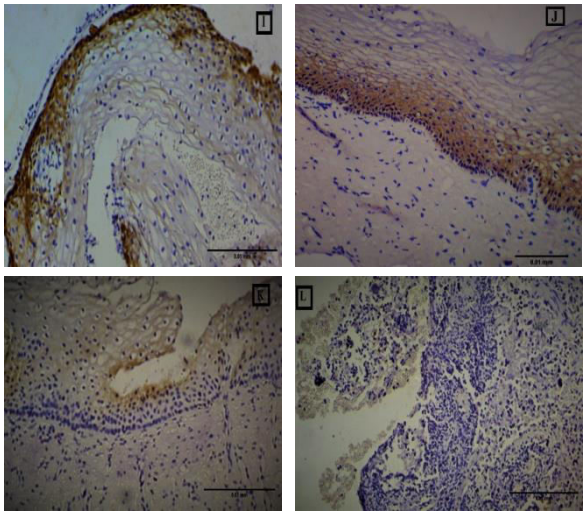

Plate 3: micrographs of the uterine cervical sections stained with E-CADHERIN illustrating: (I) (NORMAL E-CAD x100) showing the intactness of the basal cells and also the absence of nuclear atypia and it also depicts marked staining. (J) (CIN 1 E-CAD x100) showing koilocytosis and a moderate to marked staining reaction. (K) (CIN 2&3 E-CAD x100) showing parabasal cell atypia and overlapping nuclei (L) (SCC E-CAD x100) showing cells having marked dysplasia that have invaded the basement membrane and thereby loss of polarity and significant hyperchromasia.

DISCUSSION:

Cervical cancer is the second most common malignancy in women worldwide and a major cause of morbidity and mortality particularly in developing countries where it accounts for 15% of all new female cancers [21]. Invasive squamous cell cervical cancers are preceded by a long phase of pre invasive disease, collectively referred to as cervical intraepithelial neoplasia (CIN). CIN could also be categorized into grades 1, 2 and three depending upon the proportion of the thickness of the epithelium showing mature and differentiated cells. More severe grades of CIN (2 and 3) reveal a greater proportion of the thickness of the epithelium composed of undifferentiated cells. This can be identified through histochemical techniques [20]. In this study the immunological expression of specific tumour markers in confirmed normal cervical tissue, CIN 1, CIN 2&3 and SCC were observed microscopically and evaluated in order to determine if they were relevant in predicting the progression of SCC of the cervix.

Carcinoembryonic Antigen (CEA) is a glycoprotein of molecular weight approximately 180KD found in normal fetal gastrointestinal tissues. Studies have focused on CEA for over 40 years, suggesting it be a useful tool in the diagnosis of cancer, as it is a cancer cell adhesion marker [3]. CEA expression has the potential of being a useful diagnostic tool and a useful marker for identifying those at risk for progressive cervical neoplasia [1]. The results showed that there was a direct relationship between the stages of the progression of SCC of the cervix and the immunoreactive expression of CEA as the expression of the CEA was increased as the stages progressed from CIN 2&3 cases to SCC of the cervix; meanwhile, there was little to no expression of CEA in normal cervical tissue and CIN 1 cases. CEA was present in normal cervical tissue, but the expressions were very faint and was thereby classified under weak to moderate reaction. From this result, there is an agreement with Aron *et al*. who stated that CEA expression increased most significantly between CIN grades 2 to 3. He suggested that lesional CEA expression increases in CIN 3 and SCC thereby making CEA expression a useful diagnostic tool and a useful marker for identifying those at risk for progressive cervical neoplasia [1]. Disaia*et al*., 1977 also studied a group of patients with invasive squamous cell carcinoma of the cervix and found that there was a progressive increase in the percentage of patients with positive CEA values correlating with advancing stage of the disease from 26% in stage 1 to 88% in stage 2 thereby also agreeing with this study [6]. CEA functions by organizing tissue architecture and regulating different signal transduction, while aberrant expression leads to the development of human

malignancies. It functions as a pan-inhibitor of cell differentiation and cell polarization.Overexpression of CEA therefore modulates cancer progression through aberrant cell differentiation, anti-apoptosis, cell growth and resistance to therapeutic agents [19].

Epithelial-cadherin (E-cadherin; encoded by CDH1) may be a member of the classical cadherins (the others being neural cadherin (N-cadherin) and vascular endothelial cadherin (VE-cadherin)). These single pass transmembrane glycoproteins are expressed by a spread of tissues and are involved in Ca2+-dependent cell–cell adhesion. Loss of cell adhesion molecules or altered expression of these molecules plays an important role in tumour progression in epithelial tissues. Cadherins are one of these molecules that mediate via interactions with their cytoplasmic domain catenins which bind to actin cytoskeleton [5]. From the results obtained in this study, it was observed that there was a decrease in the immunohistochemical expression of E-cadherin with the stages of progression of SCC of the cervix. This is due to the loss of cell to cell adhesion. There was a strong expression of E-cadherin in normal cervical tissues and CIN 1 cases; meanwhile, there was a decrease in the expression in CIN 2&3 cases and a decrease was noticed in SCC cases. Here, the expression of E-cadherin can be said to be directly relational to the progression to SCC of the cervix therefore, it can be used as a useful diagnostic marker in predicting SCC of the cervix progression. Kaplanis*et al.,* 2005 described in his work that it is presumed that down-regulation reduces the capacity of cells to adhere each other and facilitates their shutdown of primary tumour and metastasis. Therefore, the decrease in the expression of E-cadherin is a useful parameter in evaluating the potential for malignancy of cervical cancer [15]. Therefore, from the information from this existing literature and carrying out this work, it is evident and confirmed that the work of Kaplanis*et al*.,2005 is in agreement with this work. Also, another work explains the reason for its reduced expression which entails that loss of adhesion or reduction of this protein staining can be caused by deletion or mutation silencing by CpG methylation or by altered gene expression of E-cadherin [16, 4]. The squamous cells of cervix epithelium are strongly attached to every other and to the basement membrane through an outsized number of molecules of adhesion. Thus, E-Cadherin is one among the key molecules of adhesion that outline the architecture and differentiation of keratinocytes therein epithelium. It is known that in intra-epithelial cervical cancer, there is an altered expression of these molecules. This suggests that the decrease or loss of expression of E-cadherin can be correlated with aggressive behaviour and progression of cancer [15]. Gupta *et al*., 2018 stated that it can be used as a significant biomarker for diagnostic and prognostic purpose in cervical premalignant and malignant lesions [10] thereby, it is in agreement with this study that E-cadherin is a relevant tumour marker of predictive value for the progression to SCC of the cervix. The marker therefore recommended from this study is E-cadherin and this is due to its consistency in the progression squamous cell carcinoma of the cervix.

CONCLUSION:

The data that has been compiled from this study has made it evident that CEA can be expressed in normal cervical tissues, CIN 1 cases, CIN 2&3 cases and SCC of the cervix cases but due to staining intensity, it is noticed that this expression varies based on the stages of cancer progression where it significantly increases. Meanwhile, E-cadherin on the other hand are also expressed in normal cervical tissues, CIN 1 cases, CIN 2&3 cases and SCC of the cervix cases with a significant decrease as the cancer progresses from pre malignant to malignant phase. These data therefore prove that variations in the expression CEA and E-cadherin are valuable markers for predicting the progression towards squamous cell carcinoma of the cervix from normal cervical tissue and pre malignant phase.

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