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HLA-DR, p16 and p53 in Cervical Cancer in Southwestern Nigeria

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Abstract

Background: Cervical cancer remains the most common female genital tract malignancy, despite being preventable and possibly curative. The burden is enormous in resource-poor nations, where organized preventive screening methods are yet to be developed. Research efforts geared toward finding immunological and possibly therapeutic and prognostic markers are on-going and constitute the basis of this study.

Objectives: To determine the expression of the immune marker HLA-DR in cervical cancer patients as well as its possible associations with p16 and p53 in cervical cancer patients in Southwestern Nigeria.

Methods: Thirty and-eight cases of cervical cancer seen within a period of two years at two tertiary health institutions in Nigeria were processed for immunohistochemistry with HLA-DR, p53 and p16. Semi quantitative immunohistochemistry scoring was performed, and the results were analyzed *via* SPSS version 25. The expression of HLA-DR was correlated with that of p53 and p16, with the level of significance set at $p<0.5$. Pearson correlation analysis of independent variables was performed.

Results: The peak age of cervical cancer incidence was 50-59 years. Thirty patients had squamous cell carcinoma. High and moderate expression of HLA-DR was observed in 23.7% of the patients, 28.9% of the p16 patients and 7.9%of the p53 patients. There was no relationship between HLA-DR expression and age ($r=-0.23$, $p=0.159$), or p16 ($r=-0.159$, $p=0.340$), but there was a strong negative relationship with p53 ($r=0.92$, $p=0.581$).

Conclusion: Among the three markers used, p16 was most strongly associated with cervical cancer, followed by HLA-DR and p53. HLA-DR is likely not a reliable biological marker of cervical cancer and may not be a useful therapeutic target in cervical cancer.

Keywords: Cervical cancer; HLA-DR; p16; p53; Nigeria

Introduction

Cervical Cancer (CC) is the fourth most common malignancy in females and the most common female genital tract malignancy worldwide. Globally, an estimated 604,127 new cases of CC and 341,831 CC-related deaths occurred in 2020. Although the incidence has decreased in some countries, increasing incidence has been observed in many parts of sub-Saharan Africa [1]. In middle and low-income countries of sub-Saharan Africa, where the incidence is high, a lack of effective prevention and treatment strategies as well as the burden of Human Immunodeficiency Virus (HIV) infection may worsen the prognosis [2]. A recent study revealed that most women are unaware of CC, risk factors and symptoms of the disease [3]. Whereas routine screening for precancerous cervical lesions has led to a decrease in the incidence of CC in developed nations, in many low-income countries, the national screening program for CC has not been successfully implemented. CC has been strongly associated with persistent HPV infection worldwide, although there are other risk factors and co-factors involved in cervical carcinogenesis. Attention is currently being focused on the possible role of Human Leukocyte Antigen (HLA) in immunosurveillance and as a candidate tumor suppressor in malignancies [4]. HLA, the expression product of the human MHC, is located on chromosome 16p21.31. Class II antigens are constitutively expressed on professional antigen-presenting cells

such as B-lymphocytes, dendritic cells, macrophages, monocytes, Langerhans cells and endothelial cells. Many cell types, including some tumors, are also capable of expressing class II MHC, an expression that may determine the prognosis in some cases [5]. There is evidence that some HLA class II alleles may be involved in CC [6].

P16 (CDKN2A) is a member of the INK4 cell cycle inhibitor family. It is a tumor suppressor protein, and a CDK inhibitor essential for regulating the cell cycle [7]. P16 is considered a surrogate marker for HPV-related head and neck squamous cell carcinomas. de Wispelaere, et al., in an analysis of 124 different tumor types by IHC, noted that the highest p16 positivity rates (94.4%) were in Squamous Cell Carcinoma (SCC) of the cervix. HPV was noted in 80.4% of p16-positive and 20.6% of p16-negative cancers [8]. They concluded that p16 may not be a surrogate marker for HPV except in tumors of the cervix and penis.

The p53 gene is another tumor suppressor gene and cell cycle regulator; it is the guardian of the genome and is encoded by TP53 on chromosome 17. Loss of wild-type p53 activity leads to deregulation of the p53 signaling pathway. p53 is mutated in 50% of human cancers. This involves inactivation of its pathway, including MDM2 amplification, loss of p14ARF and mutations in activating kinases such as ATM and Chk2. Loss of p53 function gives cancer cells a survival advantage to bypass the resolution of oncogenic signals and DNA damage to continue proliferation [9]. This study investigated the expression of the immune markers HLA-DR as well as, p16 and p53 in CC and the relationships between these markers in CC with the goal of contributing to the literature on the biology of CC.

Materials and Methods

Thirty eight anonymized cases of CC out of forty cases seen within a period of two years (January 2022–December 2023) at two tertiary health institutions in two states in southwestern Nigeria, the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) in Ile-Ife, Osun State, and the Federal Medical Center, Abeokuta, in Ogun State, were included in this study. The tissues were fixed in formalin and embedded in paraffin. Immunohistochemical staining was performed on 4 µm thick deparaffinized sections with HLA-DR (Clone MA5 5-11966 from Thermo-Fisher Scientific), p16 (Clone 6154652 from BD Pharmingen) and p53 (Clone MS 18P DO-7 from Epredia) *via* the indirect immunoperoxidase and avidin-biotin method and TA-125-ADQ antibody diluent (Thermo Fisher Scientific). Appropriate positive and negative controls were used. Semi-quantitative IHC scores were obtained *via* multiplication of the intensity of nuclear or cytoplasmic staining (0–3 where 0 indicates no staining, 1-indicates weak, 2-indicates moderate and 3-indicates strong staining) by the score of the percentage of positively stained cells (0-30–3 where 0 indicates ≤ 5%, 1 indicates 6%–25% positive cells, 2–26%–50% positive cells and 3 ≥51% positive cells). Protein expression was considered low if the product of the staining intensity and percentage of stained tumor cells score was ≤ 3 and high if the product was ≥ 4.25. Negative expression was assigned a score of 0. The expression pattern of HLA-DR was correlated with p16 and p53 scores *via* SPSS Version 25. The level of significance was set at $p<0.05$.

Results

The ages ranged from 35 years to 83 years (mean 52.3 years; SD 12.1). Thirty two patients (84.2%) had squamous cell carcinoma (nineteen with keratinized cancer and thirteen without keratinized cancer). The remaining patients had four adenocarcinomas, one each of transitional cell carcinoma and poorly differentiated carcinoma. Table 1 shows the percentage frequency by age group.

Table 1: (N=38) percentage by age group.

Variable	Frequency	Percentage
Age groups (in years)		
30–39	5	13.1
40–49	8	21.1
50–59	15	39.5
60–69	6	15.8
≥ 70	4	10.5

The categories of the degree of positivity for each antibody are shown in Figure 1. P16 had the highest degree of positivity in terms of moderate and high expression. Figure 2 shows the various intensities of antibody staining.

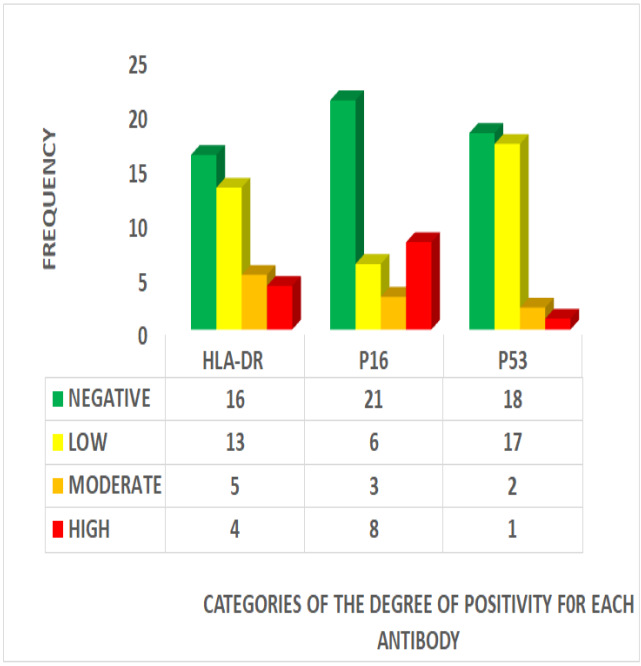

Figure 1: Degree of positivity for each antibody.

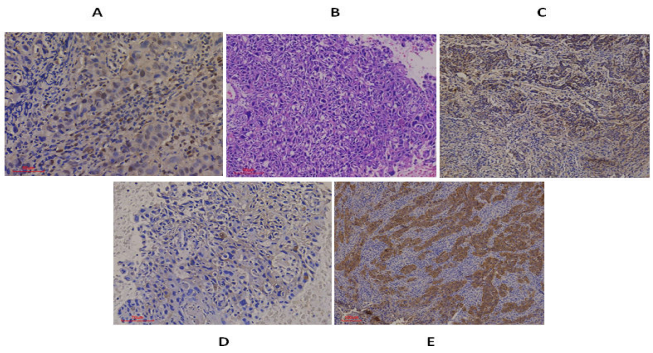

Figure 2: Intensity of antibody staining. (A) SCC p53 staining of 30% of the cells, (B) SCC H and E staining, (C) SCC weak staining with HLA-DR, (D) Weak staining for p16, (E) Strong membrane and cytoplasmic staining for p16. (SCC–Squamous Cell Carcinoma).

Pearson correlation analysis revealed that there was no relationship between HLA-DR independent variables, such as age (r=-0.23, p=0.159), or p16 (r=-0.159, p=0.340), but there was a strong negative relationship with p53 (r=0.92, p=0.581) (Table 2).

Table 2: Associations between HLA-DR and the independent variables (age and p16 and p53 scores) *via* Pearson correlation analysis (n=38).

Variables	Correlation statistics with HLA-DR	
	Correlation coefficient	p value
Age (in years)	-0.233	0.159
p16 Score	-0.159	0.34
p53 Score	0.92	0.581

Discussion

Despite being preventable through routine screening, early detection and HPV vaccination, morbidity and mortality from cervical cancer still constitute an enormous burden globally, mostly in resource limited nations. The incidence and mortality of this disease have drastically decreased in high income nations as a result of effective primary prevention strategies. Some cases of CC in the developing world do not present at the hospital before death or are seen but cannot afford the cost of preliminary investigations and treatment. Therefore, cases of CC in low-income countries may be grossly underreported and therefore underestimated. The peak age incidence of 50-59 years in this study is a welcome departure from the 40-49 years reported in a study [10] from One of the Two Centers (OAUTHC) in 2004, two decades earlier than the present study. A peak age of 40-69 years, a rather wide age range, was reported in another study from another part of Nigeria [11]. Only 26.3% of CCs had moderate or high expression of HLA-DR in this study. Madeleine and Brumback [12], in their study of 315 patients, demonstrated that some HLA-DR alleles are associated with an increased risk of SCC of the cervix, whereas other alleles or allele combinations confer a low risk. These findings underscore the importance of molecular studies using different HLA alleles in the risk stratification of CC. A study by Chambuso, et al., [13] also revealed an association of some HLA-DR alleles with cervical cancer patients with concurrent HIV infection, an association that was rare or absent in females with non-malignant cervical disease. It is not known how many of the patients in this study had concurrent HIV infection or other sexually transmitted viral

infections, which may be relevant in CC carcinogenesis. Unless suspected or indicated, routine screening for HIV is not performed for CC patients in our study centers. The role of herpes simplex virus type II in cervical cancer is controversial at present [14]. In the study by Musa et al in North Central Nigeria, 8.2% of patients with CC tested positive for HIV [15]. The increased expression of HLA genes has been found to be associated with prolonged survival in patients with tumors studied by Shaafsma, et al. [16]. Samuels, et al., concluded that the upregulation of HLA-DRA is significantly related to increased disease-free survival and disease survival in patients with cervical adenocarcinoma [17].

However, that observation may have limited application in this study since the majority of our patients had squamous cell carcinoma. P53 was strongly negatively associated with HLA-DR in this study. Frier, et al., [18] reported that 66% of cancers of the cervix studied expressed p53. The p53-positive patients also had better survival, implying a better response to chemotherapy. The role of p53 loss in cancer cells is enormous. It enables cancer progression and presumably affects the response of cancer cells to different chemotherapeutic regimens [19]. While p53 is important in preventing cancer, inappropriate p53 activation can also have detrimental effects by promoting various pathological states and developmental phenotypes [20]. A balanced p53 activity is therefore imperative in the development of p53-based therapy. The overexpression of p53 is a significant prognostic factor in luminal/HER2-negative breast cancer [21]. No comparable finding has been noted in CC. Various therapeutic strategies targeting mutant p53 (mutp53) and unresolved obstacles facing mutp53 targeted therapy for cancer have been described by Zhang, et al. [22]. Despite these uncertainties, p53 is still an important determinant of cell fate in response to chemotherapy, under appropriate treatment conditions [23]. The low expression of p53 in this study likely implies that the tumors are highly malignant and may not have a good response to therapy. Most cases in Nigeria, as in many resource-limited countries, present late to the hospital at advanced disease stages for many reasons. In Lagos, for example, 72.81% of cervical cancer patients are seen in hospitals at late stages. The reasons for this include fear, misconception, sometimes misdiagnosis, ignorance and prolonged investigation time [24]. The same pattern is recorded in some parts of Nigeria, such as Zaria at 78% [11], North Central China at 72.3% [15] and Ghana at 95% [25]. Financial constraints and distance to a healthcare facility are some of the reasons adduced for this. This study revealed the expression of HLA-DR in cervical cancer. With the use of different HLA alleles, a clearer picture may emerge. No significant associations of p16 or p53 with cervical cancer or HLA-DR were detected in this study. The implications of these findings with respect to CC biology, chemotherapy, immunotherapy and prognosis are not clear. Okonofua, et al. [26], in a recent editorial, emphasized the prioritization of primary prevention of CC with the HPV vaccine as a more cost-effective method than secondary and tertiary prevention strategies. Fortunately, HPV vaccination has taken a foothold in many African countries. The African populace must be enlightened as to the usefulness and efficacy of the vaccine in view of the numerous conspiracy theories of directed at the vaccine.

Conclusion

P16 was expressed at the highest level in cervical cancer, followed by HLA-DR and p53. Further studies using different HLA-DR alleles are recommended. Primary prevention of cervical cancer with HPV vaccination is most appropriate in resource-limited countries.

Ethics Approval

National ethics approval No NHREC/ 01/01/2007-24/10/2019.k

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data And Materials

Available on upon request from the corresponding author

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Authors' Contributions

KAA, NOA, GOO, and DAO initiated the study. KAA wrote the manuscript. AOO contributed cases from his center. AAA and AOA were responsible for the histopathology and immunohistochemistry aspects of the study. ALB performed the statistical analysis. NOA is shown in the bar chart. All the authors read the completed manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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