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Low CD4 cells and viral co-infection increase the risk of VaIN: Use of SCCA1 and Ki67 as diagno-prognostic biomarkers

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ABSTRACT

This study evaluated the correlation of SCCA1, Ki67 and CD4 cell expressions and classified vaginal smears in individuals co-infected with Human immunodeficiency virus (HIV), Herpes simplex virus 2 (HSV2), Epstein Barr virus (EBV) and Human Papilloma virus (HPV). This crossectional study included 173 participants within the age range of 20–70 years. Vaginal smears were stained by Papanicolaou technique and classified into high-grade squamous cell intraepithelial lesion (HSIL), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells of undetermined significance (ASCUS) and negative for intraepithelial lesion (NIL). Presence of immunoglobulin M and G antibodies for EBV, HIV, HPV and HSV2, and SCCA1 and Ki67 antigens were determined by ELISA method. Result showed that biomarkers SCCA1 had higher sensitivity (87.5%) to vaginal lesions when compared with Ki67 which had a sensitivity of 70.8% ($p > .01$). Assays revealed viral co-infections of 96.0% and 16.8% in smears positive and negative for vaginal lesions, respectively ($p < .01$) with HIV, HSV2 and EBV as the most prevalent type of co-infection (36%). The findings of this study suggest that low CD4 cells and viral co-infection could increase the risk of developing vaginal lesions. This study also suggests that SCCA1 and Ki67 could be used as diagnostic and prognostic biomarkers for vaginal intraepithelial neoplasia (VaIN).

1. Introduction

Vaginal cancer is a malignant growth on the epithelial lining of the vagina and comprises of approximately 1–3% of all gynecologic malignancies worldwide [[10],[11–14]]. Cancer of the vagina which can either be primary or metastatic are linked to risk factors including: smoking of cigarette, human immunodeficiency virus (HIV), herpes simplex virus 2 (HSV-2), Epstein Barr virus (EBV) and Human Papilloma virus (HPV) infections [3] and history of other malignant urogenital diseases. Herpes simplex virus 2 has been reported to increase HIV acquisition in men and women [4]. Human immunodeficiency virus (HIV) infected individuals are at a greater risk of some variants of cancer compared with uninfected individuals who are within the same age range [5]. This is because infection with HIV adversely affects the body's immune system and diminishes its ability to eliminate infecting organisms, especially viruses which can increase the risk of cancer [6,7]. Report has it that HPV types increase the chances of HIV infection in Africa [8]. The high prevalence of HIV in Ogun State (11.7%) as reported by Motayo et al. [9] as against the national value of 3.34% [10] suggests that vaginal cancer could be high in the State. Furthermore, HSV-EBV co-infection has been implicated in anal cancer especially among individuals co-infected with HPV + HIV [11–14]. However, to this day, there is little or no information on the prevalence of such viral co-infections in vaginal lesions, particularly in Nigeria. This could be due to poor surveillance and monitoring of associated clinical presentations in the country [15]. Thus, this study is warranted.

Proliferation antigen, Ki67, found in the nuclei of developing cells, has been used as a marker of cell proliferation and for grading dysplastic anal, cervical, vaginal and vulva biopsies [16,17]. On the other hand, squamous cellular carcinoma antigen (SCCA), an aggressive cancer marker, is a member of the (serpins) serine protease inhibitors. High serum and tissue levels in hepatocellular carcinoma, head and neck, cervix, lung, oropharynx and other epithelial cancers have been reported [18]. Still, no study has attempted to use and compared the diagnostic value of SCCA1 and Ki67 in grading vaginal smears, hence the need for this study.

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Table 1
Sensitivity of SCCA1 and Ki67 in vaginal lesions among HIV seropositive and seronegative participants.

Classification of smears	№ of Ppts	HSIL n = 7	LSIL n = 9	ASCUS n = 8	Total № of lesions	NIL n = 149	SCCA1 positive	Ki67 Positive
HIV Positive	103	6 (5.8)	5 (4.9)	4 (3.9)	15 (14.7)	88 (85.4)	13 (86.7)	10 (66.7)
HIV Negative	70	1 (1.4)	4 (5.7)	4 (5.7)	9 (12.9)	61 (87.1)	8 (88.9)	6 (75.0)
SCCA1 positive	–	6 (85.7)	9 (100)	6 (75.0)	21 (87.5)	–	–	–
Ki67 positive	–	6 (85.7)	6 (66.7)	5 (62.5)	17 (70.8)	–	–	–

2. Material and methods

2.1. Study area, inclusion and exclusion criteria

This study was carried out at the HIV clinic in State Hospital Ijaye, Abeokuta (latitude 07°03′N and longitude 03^{0}19′E) which serve people in Ogun state and other bordering states with a population of 494,700 in the year 2015 [19]. In this crossectional study, a total of 173 female participants (103 HIV seropositive and 70 HIV seronegative participants) within the age range of 20–70 years were included using the non-probability sampling technique, irrespective of any clinical features of malignancy. However, participants who were diabetic, pregnant, were menstruating, had undergone radiation therapy or chemotherapy, had hysterectomy, organ transplantation or have been diagnosed of any other cancer other than gynaecological cancers were excluded from this study. The HIV infected participants who were recruited in this study were all on highly active anti-retroviral therapy (HAART).

2.2. Blood sample collection and assays

Six (6) ml of whole-blood samples were collected from participants by venipuncture. Three ml were discharged into an EDTA vacutaner while three ml were discharged into a plain tube and allowed to stand for two hours (for clotting), sera were separated into plain bottles and stored at −20 °C until when analyzed. The EDTA whole-blood samples were investigated for TCD4 cells using CD4 easy count kit (Sysmex Partec GmbH, Germany) and Sysmex Partec Cyflow Counter IVD flow cytometer. Separated sera were tested, using commercial ELISA kits, for IgG and IgM antibodies of EBV (Calbiotech Inc, El Cajon, USA), HPV (Qingdao Hightop Biotech Co. Ltd, China), HSVII (Qingdao Hightop Biotech Co. Ltd, China). Antibodies for HIV-1 and HIV-2 and P24 antigen were detected using ELISA kit (Qingdao Hightop Biotech Co. Ltd, China). Quantitative determination of proliferation-related Ki-67 antigen concentration was carried out by ELISA kit (Melsin Medical Co. Ltd, China) while that of Human Squamous Cell Carcinoma Antigen 1 (SCCA1) was also carried out by ELISA kit produced by Elabscience Biotechnology Inc, USA. All investigations were carried out according to manufacturers' instruction. All laboratory investigations were carried out at the laboratory complex of Babcock University Teaching Hospital.

2.3. Vaginal sample collection, handling and classification of smears

Samples were collected from the vagina using Ayres spatula following dilation of the external genitalia with disposable speculum and Colposcopy. Smears were made from the Ayres spatula on labelled, grease free, clean slides, spray-fixed accordingly and stained by the Papanicolaou techniques. The stained vaginal smears were classified by two pathologists based on the recommendations of the International Society for the Study of Vulvovaginal Disease Terminology: 1. Negative for intraepithelial lesion or malignancy (NIL), 2. Atypical squamous cells of unknown significance (ASCUS), 3. Low-grade squamous intraepithelial lesion (LSIL), 4 High-grade squamous cell intraepithelial lesion (HSIL). Participants with positive smears (HSIL) were biopsied for confirmation.

2.4. Statistical analysis

Descriptive statistics were carried out on the data and are given as frequency distribution for categorical variables. Data generated were analyzed by Chi-square or Analysis of variance using statistical package for social sciences (SPSS) version 16. Significance levels were set at $p < .01$ and $p < .05$.

2.5. Ethical consideration

Ethical approvals were obtained from Babcock University Health Research and Ethics Committee (BUHREC665/16) and State Hospital Abeokuta Ethics Committee (SHA/RES/VOL.2/147) before the commencement of this research work. Written informed consent was also obtained from the participants before the collection of samples and other relevant information. Participants who were positive for vaginal lesions were informed, counseled and referred to gynecologist for expert management.

3. Results

Table 1 below shows that the prevalence of LSIL and ASCUS are higher among HIV seronegative participants compared with HIV seropositive participants ($p > .05$). This suggests that HAART may have conferred immunity against some oncogenic virus. However, the table reveals that the prevalence of HSIL is more frequent among HIV positive when compared with HIV seronegative participants ($p > .01$). This suggests that other factors, possibly genetic factors, other than HIV may have favoured the development of HSIL. Overall, the prevalence is vaginal lesions are higher among HIV seropositive participants as shown by their lower percentage of negative vaginal smears (85.4%) when compared with that of their seronegative counterparts ($p > 0.05$). Biomarkers SCCA1 and Ki67 had sensitivities of 87.5% and 70.8%, respectively. This suggests that SCCA1 is a better biomarker than Ki67 in detecting vaginal lesions. Despite the fact that there are some biomarker negatives among the smear positives, both SCCA1 and Ki67 complemented each other in detecting positive smears. Furthermore, SCCA1 and Ki67 are more sensitive to positive vaginal smears of HIV seronegative participants when compared with HIV seropositive participants ($p > .05$).

Table 2 shows that the average age for the development of HSIL is higher when compared with the age of those with and without other types of vaginal lesions ($p > .05$). It also shows that those who are positive for HSIL had lower CD4 counts and had their first sexual intercourse at early ages when compared with other groups ($P > .05$). The insignificant in TCD4 cell counts could be due the fact that all participants who were HIV positive were on HAART. SCCA1 and Ki67 exhibited descending pattern of expression in LSIL, HSIL and ASCUS. The values $\leq$1650 and $\leq$7.849 were used are cut-off values for SCCA1 and Ki67 positivity, respectively. Significant correlation between Ki67 and Age was also observed ($p = .023, r = 0.470$).

As shown in Table 3, the seroprevalence of IgG antibodies for EBV, HPV and HSV2 were observed highest in HSIL, ASCUS, LSIL while that of IgM antibodies for EBV, HPV and HSV were highest

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