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Seroprevalence of Anti-HIV Antibodies in Women with Abnormal Pap Smears in Jamaica

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NMA and HF coordinated the study. All the authors participated in the conception and design, analysis and interpretation of data, drafting the article, final approval of the version to be published.

Research Article

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ABSTRACT

The Human Papilloma Virus (HPV) and Human Immunodeficiency Virus (HIV) are both sexually transmitted infections, which have impacted the prevalence of cervical dysplasia and cancer in women. Infections with one of these viruses can facilitate infection with the other. In Jamaica cervical cancer is seen in 27.5 per 100, 000 women making it the second leading cause of cancer death in this population only to breast cancer as a cause of death in women with cancer. Our study investigates the seroprevalence of anti-HIV antibodies in women with abnormal pap smears in Jamaica to determine the influence of HIV on cervical dysplasia. Only patients with positive confirmatory tests were classified as HIV positive. Enzyme-Linked Immunosorbent Assay (ELISA) was used for screening while the Western blot was used for confirmation. Sero-prevalence of anti-HIV antibodies in women with abnormal pap smears was 0.85%. The preliminary results of HIV

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seroprevalence in women with abnormal pap smears may be low in Jamaica because of the success of the HIV/AIDS programme. A larger study can be done in the future and be representative of the Jamaica population, since the present study has as a limitation a smaller number of controls in comparison to cases. The findings reported do not support the hypothesis that HPV infection facilitates HIV infection in the studied population. It is the first study of its class reported in the Caribbean. It has been postulated that HPV infections may account for the cervical dysplasia despite the low prevalence of HIV association in the women with abnormal pap smears and that persistent HPV and to a lesser extent the HIV is responsible for the prevalence of abnormal pap smears in Jamaica. A limitation of the study was that the control group was smaller than that expected for 3 million's population but a larger study can be done in the future.

Keywords: Cervical cancer; cervical dysplasia; Jamaica; HIV; HPV; ELISA; seroprevalence.

1. INTRODUCTION

The Joint United Nations Programme on human immunodefficiency virus/ acquired immunodefficiency syndrome (HIV/AIDS; UNAIDS) has reported that the Caribbean is second only to Sub-Saharan Africa in world AIDS prevalence in 2001 [1].

Cervical cancer is one of the leading causes of death from cancer among women worldwide and is the most common female cancer in developing countries. In Jamaica, at 27.5 per 100, 000 it is second only to breast cancer as a cause of cancer death in women [2].

HIV infection is related primarily with unsafe sexual behaviour, whereas cervical carcinoma is multifactorial and risk factors are early initiation of sex, high parity, infection with oncogenic human papillomavirus (HPV). In **HIV**-positive women, plasma **HIV** RNA level and CD4+ count in combination appear to have a strong and statistically interactive association with incident detection or reactivation of HPV [3]. This present study investigated the seroprevalence of anti-HIV antibodies in women with abnormal pap smears in a cross section of subjects from Jamaica and also investigated the hypothesis of association between HPV and HIV infection.

2. MATERIALS AND METHODS

2.1 Human Subjects and Variables

Women who participated in this case-controlled study were recruited from the Gynaecology and Colposcopy clinics, at the University College Hospital of the West Indies (UCHWI) in 2004. After giving informed consent for the study, blood specimens were collected from 102 controls (women with normal pap smears) and 234 cases women with abnormal pap smears) and stored at –20ºC prior to laboratory analysis. The cases were classified as cervical intraepithelial neoplasia: CIN I, CIN II, CIN III and carcinoma based on stage and severity of the condition. The mean age ± SD of women with cervical dysplasia was 39.1 ±SD 11.8 years and the mean age of the control women was 38.1 ± SD 10 years. All women with abnormal pap smears (100%) were human papilloma virus positive. Several variables were analysed in relation with the progression of the disease and comparison with the control group (102 subjects) including socio-economical status, alcohol consumption, parity,

number of biological fathers, number of Pap smear done, use of hormonal contraceptive and number of sexual partners.

2.2 Indirect Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HIV Antibodies in Human Serum

The 96 well polystyrene microplates (U-shaped bottom; Sigma-Aldrich) were coated with 50 ng of a mixture of synthetic peptides (including the fragment 579-601 of the HIV gp41 and fragments 254-274 and 308-331 of the HIV gp120) for 4 h at 37ºC and blocked (3% non-fat milk in PBS, 25 µl/well, 1h) at room temperature (RT). The microplates were washed 4X (PBS-Tween-20) and duplicates of 25 µl of 1:16 diluted human sera were added. After incubation for 90 min at RT the microplates were washed 4X (PBS-Tween 20) and 25 µl of a chimeric commercially-prepared recombinant pLA-HRP conjugate (Sigma-Aldrich) diluted 1:5000 was added. After incubation for 90 min at RT and washing steps 25 µl TMB was added to each well for 15 min in the dark, the reaction was stopped with 3M H_2SO_4 and read in a microplate reader at 450 nm. In the ELISA was included a pooled human sera with high titre of anti-HIV antibodies as positive control, a pooled sera from healthy individuals as negative control and 0.9% normal saline solution was used as the blank. The cut-off point was 0.165.

2.3 Western Blotting

Aliquots of 3-5 µl of the serum were applied to the gel and run on a protein electrophoresis (SDS-PAGE). Gels were transferred to nitrocellulose membranes (Immobilon-Nc, pore size 0.45 µm, during 75 min at 40 mAmps using a semi-dry electroblotter, HEP-1 Model, Owl Scientific Inc). The running buffer contained 25mM Tris, 192mM glycine pH 8.3 and 20% methanol. The nitrocellulose membranes were blocked overnight in 10% nonfat skim milk in PBS with 0.05% Tween-20 pH 7.4 and then washed 4 times for 10 minutes with PBS-Tween 20. Peroxidase-labeled anti-HIV conjugate was added and incubated at 4ºC overnight. Membranes were washed as above and then tetra-methyl-benzidine was added and the reaction was stopped with deionised water. A positive test displays two or more HIV proteins. Only patients with positive confirmatory tests were classified as HIV positive. Enzyme-Linked Immunosorbent Assay (ELISA) was used for screening while the Western Blot was used for confirmation.

2.4 Statistical Analysis

Statistical analyses were conducted using the statistical package for social sciences (SPSS) software (version 18). Differences between cases and controls were tested by the student t-test. Logistic regression was employed to estimate the odds ratio (OR) of a diagnosis of cervical dysplasia and a diagnosis of high risk lesions. The 95% confidence intervals (CIs) of the odd ratios are presented.

3. RESULTS AND DISCUSSION

The sero-prevalence of anti-HIV antibodies in women with abnormal pap smears was 0.85% among (CIN) I= 71, CIN II= 59, CIN III= 51 and carcinoma= 53. These results reflect a low seroprevalence of anti-HIV antibodies in women with abnormal pap smears, despite the high prevalence of HIV infection/AIDS in Jamaica [1]. All cases of dysplasia were HIV negative and only two cases of cervical carcinoma were HIV positive and they were being treated with

anti-retrovirals. All women with abnormal pap smears (100%) were human papilloma virus positive by cytological and histological analysis.

Bazuaye et al. reported [2] that sexual lifestyle factors by stage of disease and compares them to those in healthy controls. The mean number of children (2.4 ±1.9 to 4.0 ±2.8), mean number of biological father (1.4 ±1.0 to 1.9 ±2.8) and number of sexual partners (3.9 ±2.3 to 5.3 ±4.6) were significantly higher with progression of the disease and not associated with HIV infection. There was a significant lineal trend for number of children and biological father (p=0.001).This study also revealed that socio-economic status, alcohol consumption and parity were statistically significantly associated with the development of cervical dysplasia when compared to healthy controls (P=0.020, 0.019 and 0.008 respectively) but they were not statistically associated with HIV infection in women with cervical carcinoma. Zero parity was found to be a protective factor in controls compared to cases (p=0.000). Severity of the disease was associated with number of sexual partners and HIV infection.

Bazuaye et al. [2] also reported in multivariate analysis with age as a co-variate, use of hormonal contraceptive was associated both with the disease and the severity of the disease (OR 2.04, CI 1.18, 3.50; P=0.010 and 2.21, CI 1.07, 4.57; p=0.033) respectively. A limitation of the study was that the control group was smaller than that expected for 3 million's population. Anti-retroviral therapy is not associated with a decrease in the incidence of cervical cancer, and it is not known whether HPV vaccination of HIV-positive women will reduce their incidence of cervical cancer.

Infection with oncogenic Human papillomavirus (HPV) has now been firmly established as the etiological agent for invasive cervical cancer (ICC). Globally there are 270,000 deaths from this disease per annum with over 85% of these occurring in low resource countries. In sub Saharan Africa HPV infection is known to be augmented by HIV, which is endemic in this region. HPV and HIV are both sexually transmitted and infection with either is reported to facilitate infection with the other [4] but it is observational evidence, far from being conclusive.

A low prevalence of anti-HIV antibodies has been found in several populations of women with abnormal Pap smears. Few evidences can be mentioned in this paper. For example two hundred African patients with cervical carcinoma in Kenya, were screened for HIV antibodies and a seroprevalence rate of only 1.5% was obtained. This was comparable to the 2% found in the general population, but much lower than the 18-59% recorded in recognized high-risk groups [5]. Another example is a Nigerian study that showed that only 6 patients (2.7%) were positive for anti-HIV antibodies [6]. The table below shows associations between Human immunodeficiency virus (HIV), Human papilloma virus (HPV) and abnormal Pap smear. There was a strong association between HPV and abnormal pap smears, other associations were not significant.

In this study the seroprevalence of HIV antibodies in female with cervical dysplasia/carcinoma was low. This could indicate the success of the HIV/AIDS programme carried out by The Ministry of Health in Jamaica, where the population is educated about the lifestyle and behavioral factors to prevent HIV infection including protected sex. Table 1 shows the strong association between HPV and abnormal pap smears, other associations were not significant.

Table 1. Associations between Human immunodeficiency virus (HIV), Human papilloma virus (HPV) and abnormal pap smear

HIV-HPV	Cases	p> 0.05
HIV-HPV	Control	p> 0.05
HPV-Abnormal pap smear	Cases	p< 0.001
HIV- Abnormal pap smear	Cases	p> 0.05

The present table shows the strong association between HPV and abnormal pap smears, other associations were not significant.

4. CONCLUSION

The preliminary results of HIV seroprevalence in women with abnormal pap smears may be low in Jamaica because of the success of the HIV/AIDS programme. A larger study representing Jamaica population should be done in the future, since the present study has a limitation of smaller number of controls. The findings reported do not support the hypothesis that HPV infection facilitates HIV infection in the studied population. It is the first study of its class reported in the Caribbean. We found that HPV infections majorly contribute to the cervical dysplasia despite the low prevalence of HIV association in the women with abnormal pap smears, and persistent HPV is responsible for the prevalence of abnormal pap smears in Jamaica, and HIV contributes to a lesser extent.

CONSENT

The patients gave their informed consent for this study to be published. Journal editorial office may ask for the copies of the consent documentation at any time.

ETHICAL APPROVAL

Ethical approval for the study was granted by the Faculty of Medical Sciences, The University of the West Indies Mona Campus Ethics Committee and all experiments and procedures were performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared no competing interests exist

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