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Research Article

Risk Association between Human Leucocyte Antigens (HLA) and Cervical Neoplasia in Kenyan Women

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Background: Epidemiological studies have shown a strong link between human papilloma virus (HPV) infection and the development of cervical cancer. Certain Human Leucocyte Antigen (HLA) alleles may influence the susceptibility and outcome of HPV infection in carriers of those alleles. It is therefore important to investigate any potential protective and/or risk association between HLA and cervical neoplasia.

Objective: To describe the risk association between HLA and cervical neoplasia in Kenyan women.

Setting: Tigoni, Kiambu in Central Kenya.

Method: Nested case-control study in a cervical cancer screening study. DNA was extracted from blood buffy coat cells. HLA typing was done by PCR to determine Class I and II gene frequencies.

Results: In sixty six women (43 cases, 23 controls) aged 25-60 years (mean 36.98 (SD ± 8.7), HLA class I **B35** allele increased risk for cervical neoplasia, odds ratio (OR) 11.5 (95% CI 1.32-99.46). Individuals with HLA class II allele DR1 were 4 times more likely than controls to have a lesion OR=3.7 (95% CI 1.01-13.65) while HLA class I allele **B35** increased by 22 times the risk of LSIL OR=22.5 (95% CI 2.30-219.89); HLA class I **CW7** allele was protective , OR=0.1 (95% CI 0.003-0.72). HLA class I **B7** allele increased by 5 times the likelihood of a lesion OR=5.2 (95% CI 10.6-25.13). HLA class II alleles **DR1** and **DQ5** increased risk for HSIL, OR 6.4 (95% CI 1.53-26.89) and 3.4 (95% CI 1.02-11.81) respectively. HLA class I **BW6** appeared protective, OR 0.2 (95% CI 0.04-095).

Key words: Cervical neoplasia, Human Leucocyte Antigen

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1. Introduction

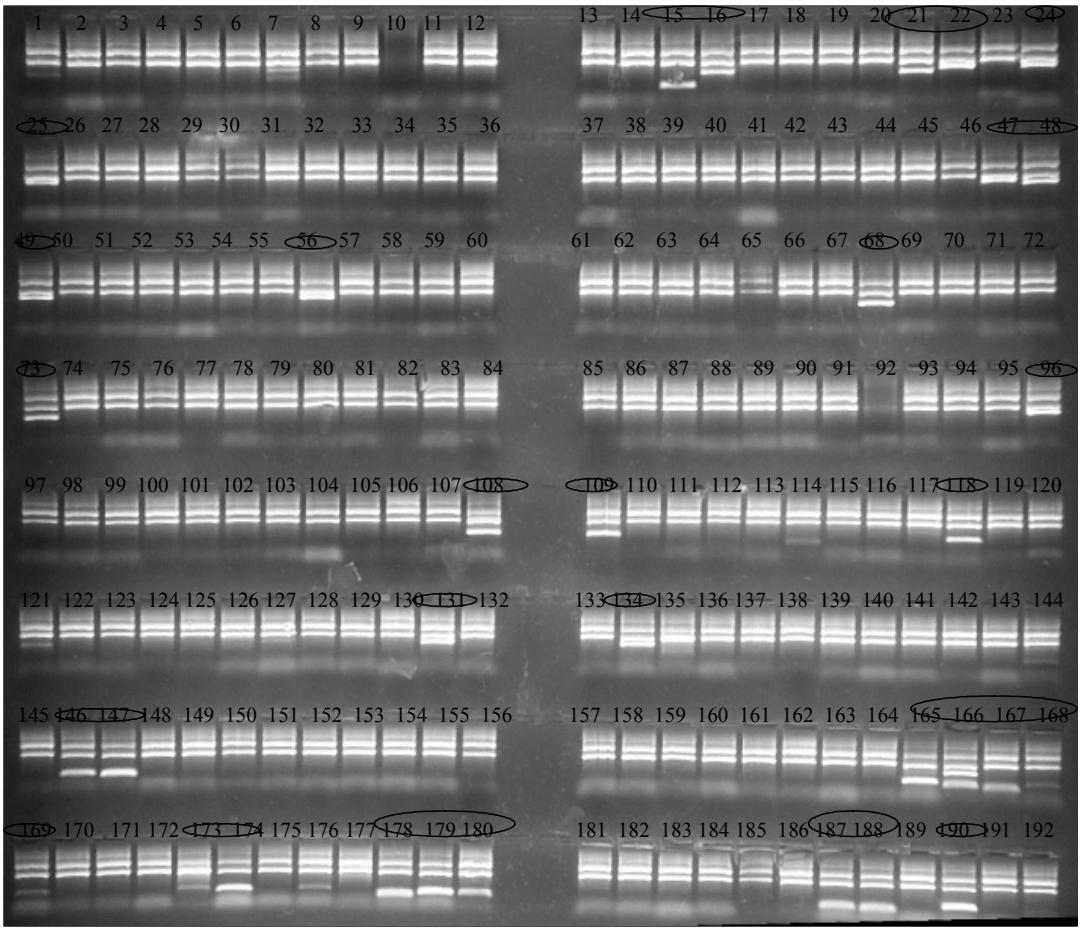
Cervical cancer is the second most common cause of cancer mortality among women worldwide (Franco et al, 2003). Epidemiological studies have shown a strong link between human papilloma virus (HPV) infection and the development of cervical cancer (Franco et al, 1999; Walboomers et al, 1999; Baseman et al, 2005). HPV type 16 and 18 are responsible for over 70% of all cervical cancers. Although HPV infection is considered to be necessary or a major risk factor, it is not sufficient for the development of cervical cancer. Other risk factors such as host genetic background, and environmental factors may also contribute to the

carcinogenic process (Chan et al, 2005). Most HPV infections are transient and regress spontaneously and only a minority of women develops persistent infection that with time may evolve into cervical intraepithelial neoplasia and/or progress to invasive cervical cancer (Villa, 1997; Franco et al, 1999).

Given that host immune response to HPV is thought to be an important determinant of HPV acquisition and progression to high-grade cervical lesions and cancer, it is plausible that human leucocyte antigen (HLA) variations may affect pathogenesis of cervical neoplasia (Beskow et al, 2005; Clerici et al, 1997; Hildesheim et al, 1997). The major histocompatibility complex is a highly polymorphic gene cluster on the short arm of chromosome six. The genes in this cluster are divided into three classes with different roles in immune responses. HLA gene polymorphisms result in variations in peptide-binding cleft, therefore influencing the antigens bound and presented to T cells (Beskow et al, 2005; Wang e al, 2005). The HLA class I genes (HLA-A, -B, and –C) present foreign antigens to CD8+ Cytotoxic T lymphocytes, while class II genes (HLA-DR, -DQ and –DP) present antigenic peptides to CD4+ T helper cells and are important in host immune responses to viruses and other pathogens (Wang et al, 2001).

Figure 1: HLA typing by PCR-SSP showing gel lanes

ID:2740	Study: TDS
Date: 20/04/07	
HLA-A: 30, 66	DRB1: 13
HLA-B: 45, 5802/06	DRB3: 0209/03
HLA-C: 06, 1601	DQB1: 06
Bw: 04, 06	Report Date 20/04/07



It has been shown that certain HLA class II alleles increase the risk for cervical carcinoma, primarily because the carriers of those alleles are rendered more susceptible to HPV type 16, the most common type of HPV found in cervical carcinomas. A plausible hypothesis is that HLA is involved in the cellular

immunity against HPV and that certain HLA alleles make carriers more susceptible to HPV infections and less efficient in viral clearance. A meta-analysis has demonstrated a protective effect of some alleles and haplotypes, specifically DRB1*1301 and DQB1*0603 against development of cervical carcinoma in 18 out of 19 studies (Beskow et al, 2005). This consistently strong association indicates that it would be important to test the protective effect and the risk of HLA class II haplotypes on HPV viral load for some of the most frequent HR-HPV types.

The host immune system is critical in controlling HPV infection and determining disease outcome. HLA plays an important role in regulating cell-mediated immunity against pathogens. Because HLA class I and class II molecules are highly polymorphic, individuals expressing different alleles may differ in their ability to handle a given set of HPV-derived peptides, and hence HLA alleles may influence the outcome of persistence infection (Chan et al, 2005).

The aim of this study was to describe the risk association between HLA and cervical neoplasia in group of Kenyan women.

Table 1: Overall age distribution of the study population

Age range	Frequency	Percent	Cumulative percent
25-29	14	21.2	21.2
30-34	14	21.2	42.4
35-39	16	24.2	66.7
40-44	12	18.2	84.8
45-49	5	7.6	92.4
50+	5	7.6	100.0
Total	**66**	**100.0**	**100.0**

2. Methods

2.1 Study population

This was a case-control study of a subset of women who had been screened for cervical neoplasia and HIV in Tigoni, Limuru Division of Kiambu District in Central Kenya. Initially, all women aged 25 to 60 years who were eligible for cervical screening were invited. A subset of these women who had an abnormal smear was randomly selected and each case had an age-matched pap smear negative control. A purposive sample size of 100 (2 for 1) was recruited, but only 66 (46 cases and 23 controls had all results for analysis.

2.2 Grading of cervical lesions

All women had previously been screened for cervical cancer by pap smears, and those who had a cervical lesion were graded as negative for intraepithelial neoplasia (normal), Low grade squamous intraepithelial neoplasia (LSIL), and High grade intraepithelial neoplasia (HSIL). Those who had a pap smear reported as Atypical squamous cells of undetermined significance (ASCUS) were re-classified as normal or LSIL after colposcopy and biopsy.

2.3 HLA Typing

For HLA typing, DNA was extracted from buffy coat cells by an automated DNA extraction Qiagen™ EZI DSP DNA blood system to yield highly pure DNA. The HLA class I and class II typing profiles were determined by PCR-SSP (sequence-specific primer) with single-stranded oligonucleotide primers (SSOPs) method followed by amplification and gel electrophoresis. Interpretation was done on gel photography to determine the positive lanes and match with specific PCR products in the lot-specific Interpretation and Specificity Tables in order to obtain the HLA typing of the sample DNA (**Figure 1**) (Olerup et al, 1991; Olerup et al, 1992.

This work was done in the Kenya AIDS Vaccine Initiative (KAVI) laboratory in the Department of Medical Microbiology, University of Nairobi.

2.4 Data and statistical analysis

HLA class I and II phenotype frequencies were determined by direct counting. Subgroup analyses were done according to the grade of cervical lesion. Class I and class II gene frequencies were calculated and statistical differences between cases and controls evaluated by use of χ^2 test and Fisher's exact test as appropriate, for significance (Bland et al, 1995). The magnitude of the statistical significance of association was determined by means of odds ratios (ORs) and 95% confidence intervals (CIs). A p-value of <0.05 was considered significant.

2.5 Ethical considerations

Ethical approval to carry out this study was granted by the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON-ERC Study approval No: **P7144/12/2003**).

Blood samples for HLA typing were obtained after they provided written, informed consent. The participants were also informed that they would not receive individual HLA typing results since, at the time, the results had no clinical utility.

Table 2: Cervical lesions results in the study population

Cervical abnormality	Number (N=43)	Percent (%)
ASC-US	16	24.2
ASC-H	6	6.1
LSIL	10	15.2
HSIL	11	16.7

3. Results

A total of 66 women were enrolled for this case-control study (43 cases, 23 controls). The ages ranged from 25 to 60 years with a mean age of 36.98 (SD ± 8.71 years). The age range frequencies are shown in **Table 1**.

A total of forty three (43) or 62.2% of women had a cervical abnormality by pap smear or cervical biopsy (**Table 2**), of whom 24.2% had atypical squamous cells of undetermined significance (ASCUS), 6.1% had atypical squamous cell – cannot exclude a high grade lesion (ASC-H), 15.2% had a low grade squamous intraepithelial lesion (LSIL), and 16.7% had a high grade squamous intraepithelial lesion (HSIL). A total of 37.8% had normal smears. For analysis, only biopsy confirmed cervical neoplastic lesions were considered, and grouped into LSIL and HSIL.

Table 3 shows selected HLA class I and II alleles that were associated with increased risk for cervical neoplasia. The HLA class I **B35** allele was associated with increased risk for cervical neoplasia (LSIL or HSIL) with an OR of 11.5 (95% CI 1.32-99.46) compared to controls. In addition, individuals who had HLA class II allele **DR1** were almost 4 times more likely than controls to have a cervical neoplastic lesion (LSIL, HSIL), OR of 3.7 (95% CI 1.01-13.65).

When only LSIL alone was considered (**Table 4**), HLA class I allele **B35** was associated with increased risk, OR=22.5 (95% CI 2.30-219.89) and HLA class I **CW7** allele was associated with a protective role, OR=0.1 (95% CI 0.003-0.72)

When the risk of having HSIL alone was considered, those with HLA class I **B7** allele appeared to be about 5 times more likely than controls to have a cervical lesion OR=5.2 (95% CI 10.6-25.13); similarly HLA class II alleles **DR1** and **DQ5** also appeared to increase the risk for HSIL with ORs of 6.4 (95% CI 1.53-26.89) and 3.4 (95% CI 1.02-11.81) respectively. The HLA class I **BW6** appeared to play a protective role with OR of 0.2 (95% CI 0.04-095) (**Table 5**).

The full list of HLA allele frequencies for cases and controls as well as the corresponding odds ratios, confidence intervals and *p*-values can be found in the **Supporting Information** (available online at http://www.uonbi.ac.ke/journals/kesobap/).

Table 3: Risk association between HLA alleles and cervical neoplasia

Alleles	Cervical neoplasia (LSIL, HSIL)	Control	O.R (95%CI)	*P* value
HLA class I				
B35	7 (24.1%)	1 (2.7%)	11.5 (1.32-99.46)	0.018
BW6	21 (72.4%)	34 (91.9%)	0.2 (0.06-0.97)	0.048
CW7	7 (24.1%)	21 (56.8%)	0.2 (0.08-0.71)	0.012
HLA class II				
DR1	9 (31%)	4 (10.8%)	3.7 (1.01-13.65)	0.040

Table 4: Risk association between HLA alleles and LSIL

Alleles	LSIL	Control	O.R (95%CI)	*P* value
HLA class I				
B35	5 (38.5%)	1 (2.7%)	22.5 (2.30-219.89)	0.030
CW7	2 (15.4%)	21 (56.8%)	0.1 (0.03-0.72)	0.012

Table 5: Risk association between HLA alleles and HSIL

Alleles	HSIL	Control	O.R (95%CI)	*P* value
HLA class I				
B7	5 (31.3%)	3 (8.1%)	5.2 (10.6-25.13)	0.045
BW6	11 (68.8%)	34 (91.9%)	0.2 (0.04-0.95)	0.045
HLA class II				
DR1	7 (43.8%)	4 (10.8%)	6.4 (1.53-26.89)	0.011
DQ5	10 (62.5%)	12 (32.4%)	3.4 (1.02-11.81)	0.041

4. Discussion

In the past most research on the association between HLA and cervical neoplasia has evaluated class II alleles in different populations such as American-white and -Indian women, Caucasian women in Europe, non-Hispanic white, Hispanic white, African-American, Brazilian, Japanese, (Zoodsma et al, 2005), and Chinese women (Chan et al, 2007), and only one in African women in Senegal (Lin et al, 2001). The importance of HLA class I antigens in cervical neoplasia is well-established (Wang et al, 2002). Although Koehler et al (2010) have described HLA class I genotyping in some populations in the East African region, to our knowledge this study is the first to assess the risk association between HLA class I and II alleles and cervical neoplasia in women in the region.

This study demonstrated that both HLA class I and II alleles increase the risk for cervical neoplasia of any grade among a group of Kenyan women. The HLA class I allele **B35** increased the risk for any grade of cervical neoplasia by up to eleven times, and by twenty-two times for LSIL. This strong association between a HLA class I allele and cervical neoplasia has not been demonstrated before. Wang et al (2002) demonstrated a decreased risk for cervical cancer, HSIL or LSIL for women with the haplotypes HLA-CW*0202 allele across two ethnic groups in the US and Costa Rica, although according to their study, some women with cervical cancer also had the same allele, suggesting that the role of HLA molecules is only one of many factors in disease development.

Our results show that the HLA class II allele **DR1** was significantly associated with cervical neoplasia of any grade with odds of up to four times; other studies have shown that the presence of a single allele (DRB1*1301) (Wang et al, 2001) was associated with risk reduction while multiple haplotypes DRB1*1301/DQB1*0302 had protective association (Zoodsma et a,l 2005; Sastre-Garau et al, 1996; Hildesheim et al, 1998). However, 4-digit haplotyping was not done for this study therefore comparison with haplotypes in other studies cannot be made. As in other studies (Wang et al, 2001), our results confirm the risk reduction as well as risk increase with multiple alleles.

Wang et al (2002) separated as well as merged analysis for two distinct ethnic groups in the US and Costa Rica involving a 24,000-women cohort in Portland, Oregon, and 10,077 women in Guanacaste, Costa Rica. They observed that the extreme polymorphisms of both class I and II alleles are fairly evenly distributed, making single allele-disease associations difficult to observe. Different populations have different genetic pools that can also be influenced by ethnicity (Wang et al, 2002). Koehler et al (2010) indicates that there is very low representation of the East African population in global HLA databases, and given that the HLA genes are the most diverse of human genes, comparisons and inferences with gene frequencies and variants with disease associations are somewhat limited. However, with availability of high-through-put high-resolution HLA typing methods such as PCR-SSP that can provide HLA typing information with the needed level of molecular detail in a timely and cost-effective fashion, conduction of large epidemiological studies is now possible. Future studies should probably include the complete haplotypes analyses for HLA class I and II alleles, assessment of HPV type specificity in order to identify protective or risk alleles specific to oncogenic HPV types in disparate genetic pools in Kenya as exist in different ethnic groups. The limitations in this study included the small numbers which reduced the power to make generalizable conclusions as well as the potential for confounding by ethnicity in a merged analysis.

Efforts to develop vaccines and improved treatments for major diseases such as cervical cancer are hindered by our poor understanding of the molecular events and cellular mechanisms that determine clinical outcome. Genetic epidemiology provides a potentially powerful tool that may identify hitherto unknown mechanisms and improve our understanding of critical events in the evolution of disease. High throughput genotyping technology is projected to make it feasible to screen for genetic factors that determine susceptibility to infections such as HPV and help identify critical pathways of host defense and therefore generate novel strategies for disease prevention (Kwiatkowski, 2000).

5.0 Conclusion

This study has demonstrated that both HLA class I and II alleles increase the risk for cervical neoplasia of any grade among a group of Kenyan women. The strong association between a HLA class I allele and cervical neoplasia has not been demonstrated before. Future studies should include the complete haplotypes as well as 4-digit haplotypes analyses for HLA class I and II alleles, and assessment of HPV type specificity in order to identify protective or risk alleles specific to oncogenic HPV types in disparate genetic pools in Kenya as they exist in different ethnic groups. The study limitations in this study included the small numbers which reduced the power to make generalizable conclusions as well as the potential for confounding by ethnicity in a merged analysis. However, it was an important methodological advancement for a local Kenyan research laboratory to be able to perform HLA typing.

Conflict of Interest declaration

The authors declare no conflict of interest

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