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CD4 T-LYMPHOCYTES SUBSETS IN WOMEN WITH INVASIVE CERVICAL CANCER IN KENYA
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ABSTRACT

***Objective*: Invasive cervical cancer (ICC) and HIV are common in sub-Sahara Africa. Both ICC and HIV are immunosuppressive, and are associated with decreased CD4 and CD8 profiles. In a group of women with ICC starting radiotherapy, we determined their CD4 profiles.**
***Design*: A cross-sectional study.**
***Settings*: Kenyatta National Hospital, Nairobi, Kenya, radiotherapy unit.**
***Subjects*: Women with invasive cervical cancer (344) seeking radiotherapy treatment for the first time between January 2000 and March 2003, had blood samples analyzed for CD4 and CD8 cell counts by flow cytometry. Haemoglobin, white cell count, lymphocyte and platelet counts were determined using coulter machine. All patients had received pre- and post HIV counseling.**
***Results*: The mean age was 49+13 years. About 13.1% of the women with ICC were HIV positive. Overall, mean and median CD4 cell count was 829+355 cells/mm^3 and 792 cells/mm^3. Among HIV+ patients, mean and median CD4 cell counts were 451+288 cells/mm^3 and 405 cells/mm^3 respectively. The mean CD4 cell count for the HIV+ women was 886+329 cells/mm^3 with median of 833 cells/mm^3, range 147-2065 cells/mm^3. Only nine (20%) of the 45 HIV+ women had CD4 cell count of 0-200. HIV+ women had lower CD4 percentage and cell count and higher CD8 percentage and cell count as compared to HIV negative women, $p < 0.001$. HIV infection was significantly and independently associated with high proportion of women who had CD4 cell count of less than 200 cells/mm^3 or less than 350 cells/mm^3, $p < 0.0001$.**
***Conclusions*: Women with ICC and concurrent HIV infection have decreased CD4 cell subset. These results suggest HIV infection may be associated with more severe CD4 depletion in women with ICC.**

INTRODUCTION

Invasive cervical cancer (ICC) is common in populations with high HIV prevalence in sub-Sahara Africa (1,2). Depressed systemic as well as local immune responses have been observed in patients with advanced cervical cancer (3,4). Patients with iatrogenic CD4 cell depression following organ transplant develop malignancies among them ICC (5). T-lymphocyte derangements seem to be an important component in ICC tumorigenesis pathway. ICC and HIV are both immunosuppressive conditions which theoretically may be associated with profound immunosupression when they coexist.

The fundamental abnormality in women with HIV infection is T-lymphocyte derangement with decreased CD4cells. HIV is associated with increased risk of human papilomavirus (HPV) - associated cancers of the cervix (6), anus, oropharynx, penis, vagina and vulva (7). HIV infection is also associated with early onset of ICC (8,9). Other than the effect of immunosuppression from HIV infection in increasing standardised incidence ratios (SIRs) (7), in vitro studies suggest the HIV encoded tat protein

may enhance the expression of HPV E6- and E7-transforming proteins (10) or cell cycle progression (11) leading to development of ICC.

CD4 enumeration is important for the evaluation of HIV disease stage and progression. In resource limited environments, CD4 determination is the biomarker commonly used by clinicians together with the World Health Organization (WHO). HIV clinical staging system helps to decide when to initiate HIV infected patients on treatment as well as monitor the progress of treatment. Though there are several studies documenting CD4 and CD8 values in healthy individuals (12-14), such data is lacking for women with ICC. This study was undertaken to determine CD4 T-lymphocyte subsets in patients with ICC in Kenya.

MATERIALS AND METHODS

This cross-sectional study was conducted from January 2000 to March 2003 at the radiotherapy unit of Kenyatta National Hospital (KNH). Only patients seeking radiotherapy treatment for cervical cancer for the first time were included. All enrolled participants received HIV pre-test and post-test counseling and gave informed consent. A blood sample was obtained for HIV testing using enzyme-linked immunoassay (ELISA) (Biochem Immuno Systems Kit, Montreal, Canada) and positive samples were confirmed using double ELISA (Biotech Ltd, Cambridge, Ireland). From all enrolled patients, blood was obtained for CD4 and CD8 cell count using flow cytometry (FAC scan, Becton-Dickson). Flow cytometry is an accepted standard method for determination of absolute count of CD4, CD8+ T-lymphocytes.15 Blood specimens were collected by venipuncture in anticoagulated K-3 liquid vacutainer specimen bottle (Becton Dickson, Mountain View CA) between 9-12AM. CD4/CD8 absolute cell counts and their ratios were determined by two colour immunophenotyping on single platform fluorescence activated cells sorting (FACS) count system, using fluorochrome labeled monoclonal antibodies strictly following the manufacturers' instructions. Total white blood cell counts (WBCs), and hemoglobin values were determined using a Coulter machine. Study staff administered a structured questionnaire.

Statistical analysis was carried out using SPSS version 13.0 (SPSS Inc. Chicago, Illinois, USA) statistical package. Fisher exact test and Yates corrected Chi-square testing was used to compare proportions. Differences between means were tested by t-test. Odds ratio (OR) and 95% confidence interval (CI) was used to measure strength of associations. Multivariate logistic regression models included variables significant in univariate analysis or those that may have biological influence on the dependent variable. A P value (two-tailed test) of <0.05 was considered significant. All study participants gave informed consent. This study was reviewed and approved by KNH/University of Nairobi ethics and research committee before commencement.

RESULTS

None of the patients approached to participate in the study declined. A total of 344 patients with invasive cervical cancer (ICC) were evaluated. Table 1 shows sociodemographic characteristics. The mean age was 49+13, range 22-94 years. About 13.1% (45/344) women were HIV positive. Women less than 35 years, were eight times more likely to have HIV infection than women >35 years old (43.8% vs 8.3%, OR 8.6, 95% CI 4.2-17.5, $p<0.001$).

Table 1
Socio-demographic characteristics of all women included in the study

Variable	Number	Percent
Religion (344)		
Catholic	95	28
Protestant	240	70
Muslim	7	2
Marital status (344)		
Single	26	8
Married	206	60
Widowed	86	25
Divorced/separated	24	7
Polygamous (344)		
Yes	111	33

No	203	59
Unknown	28	8
Education attainment (344)		
None: Zero years	102	30
Primary: 1-7/8 years	183	54
Secondary: 8-12 years	56	17
University: 12-15 years	0	0
Occupation (276)		
Housewife	109	40
Farmer	144	52
Teacher	13	5
Unemployed	6	2
Others	4	1
Smoking (343)		
Yes	28	8
No	315	92
Family planning use (343)		
Yes	172	50
No	171	50

The mean CD4 cell count for the HIV negative women was 886+329 cells/mm^3 with median of 833 cells/mm^3, range 147-2065 cells/mm^3 as compared to mean CD4 cell count of 451+288 cells/mm^3 with median of 405 cells/mm^3, range 17-1275 cells/mm^3 for the HIV positive women, p<0.001. Of the 45 HIV positive women, nine (20%) had CD4 cell count of 0-200 compared to only 2 (0.7%) of the 299 HIV negative women (OR 37, 95% CI 7.7-166.7, p<0.0001). Using the revised WHO CD4 cell count criteria for initiation of HIV treatment, 18 (40%) of the 45 HIV positive women had CD4 cell count of 0-350 compared to nine (3%) of the 301 women who were HIV negative (OR 21, 95% CI 8.8-53, p<0.0001).

Table 2 shows selected parameters by HIV status. HIV positive women were significantly different from HIV negative women in most of the parameters except WBC, platelet count and lymphocyte percentage which were similar. HIV negative women had significantly lower CD4 percentage and count and higher CD8 percentage and count than HIV negative women, p<0.001.

Table 2
Comparion of selected numerical data among HIV positive and negative women

Variable	HIV+	HIV-	P value
Age in years	37.0 ± 9.0	50.0 ± 13.0	<0.001
CD4 percentage	19.0 ± 11.0	38.0 ± 8.0	<0.001
CD4 count	451.0 ± 288.0	885.0 ± 329.0	<0.001
CD8 percentage	53.0 ± 15.0	28.0 ± 9.0	<0.001
CD8 count	1378.0 ± 776.0	680.0 ± 392.0	<0.001
CD4/CD8 ratio	0.5 ± 0.5	1.5 ± 0.7	<0.001
WBC	7.4 ± 2.6	8.6 ± 4.4	0.091
Hemoglobin	9.0 ± 3.0	11.0 ± 2.0	<0.001
Platelets	312.0 ± 151.0	360.0 ± 155.0	0.052
Lymphocyte percentage	32.0 ± 14.0	31.0 ± 11.0	0.308

SD-standard deviation; WBC-While blood cells; ICC-Invasive cervical cancer

In univariate analysis, being HIV positive, reporting ever use of condoms for STD protection, reporting ever use of alcohol, and being younger than 40 years of age were significantly associated with severe immunossuppression, Table 3. In multivariate analyses adjusting simultaneously for age, age of first intercourse, condom use for STD protection, history of ever having had an STD, alcohol use, and number of viable pregnancies, being HIV positive and reporting ever use of alcohol was significantly and independently associated with high proportion of women with severe immunuossuppression (CD4 cell count of 0-200 cells/mm^3), Table 4. The same findings were obtained if CD4 cell count classification was changed to the new class of 0-350 cells/mm^3 and more than 350 cells/mm^3 (data not shown).

Table 3

Univariate analysis of factors associated with CD4 cell count for women with Invasive cervical cancer

Variable	CD4 cell count				OR	95%CI	p value
	0-200		201+				
HIV							
Positive	9	20	36	80	37	8-167	<0.0001
Negative	2	1	297	99			
Age in years							
>41	4	2	229	98	4.1	1-14	0.039
<40	7	7	97	93			
Marital status							
Single	2	8	24	93		0.6–14.0	0.175
Married or ever married	9	3	309	97	2.9		
Education attainment							
None	2	2	100	98	0.5	0.1–2.1	0.397
Any level of education	9	4	233	96			
Alcohol use							
Yes	4	16	21	84	8.5	2.3–31.3	0.005
No	10	3	305	97			
Smoking							
Yes	1	4	27	96	1.1	0.1–9.2	0.909
No	10	3	305	97			
Ever had an STD							
Yes	3	6	44	94	2.5	0.6–9.6	0.183
No	8	3	288	97			
Ever used condoms for STD protection							
Yes	5	10	46	90	5.1	1.5–17.5	0.014
No	6	2	283	98			
Ever use of family planning							
Yes	8	5	164	95	2.7	0.7–10.5	0.138
No	3	2	168	98			
Age at menarche							
Up to 15 years	9	5	187	95	3.3	0.7–15.5	0.131
16+ years	2	1	137	99			
Age at first sex							

Up to 18 years	8	3	228	97	1.1	0.3–4.2	0.903
19+ years	3	3	93	97			
Age at first pregnancy							
Up to 19 years	9	5	176	95	3.9	0.8–18.4	0.072
20+ years	2	1	157	99			
Age at first child							
Up to 19 years	9	5	171	95	4.1	0.8–19.4	0.067
20+ years	2	1	157	99			
Number of viable pregnancies							
0-4	5	5	97	95	2.0	0.6–6.7	0.315
5+	6	3	234	96			

OR Odds ratio; CI Confidence interval, STD Sexually transmitted infections/diseases

Table 4

Multivariate analysis of factors associated with CD4 cell count for women with Invasive cervical cancer

Variable	AOR	95%CI	p value
Being HIV positive	35.7	7.4-166.7	<0.0001
Age less than 40 years	0.6	0.1-3.4	0.532
Being never married ever	1.3	0.2-9.1	0.175
Reporting ever use of alcohol	5.5	1.2-25.0	0.027
Reporting history of ever smoking	1.3	0.1-13.4	0.851
Reporting history of ever having had an STD	2.1	0.4-12.0	0.405
Ever used condoms for STD protection	0.9	0.2-4.6	0.929
Age at first sex up to 18 years	0.6	0.1-3.4	0.588
5 or more viable pregnancies	0.6	0.1-3.2	0.527

AOR Adjusted odds ratio; CI Confidence interval, STD Sexually transmitted diseases.

DISCUSSION

Sub-Saharan countries, including Kenya, are characterised by high prevalence of HIV infection and high incidence of ICC. In such settings, HIV and ICC are common concurrent conditions. Invasive cervical cancer (ICC) is one of the three acquired immunodeficiency syndrome (AIDS) – defining cancers. Women who are HIV infected with ICC qualify to be initiated on HIV treatment. However, treating cancer and HIV in women with concurrent problems is likely to be a challenge because of potential drug interactions, side effects and potential negative effect of chemotherapy on CD4 count and HIV viral load (16, 17).

HIV treatment is now readily available in Kenya with treatment progress being principally monitored by CD4 determination. In our study, women with ICC and HIV+ had CD4 counts and percentage that were significantly lower than in women with ICC not HIV infected. These findings are similar to reports from other studies (18, 19). In Park and Kim's study, 18 out of 34 patients with ICC from Yonsei University, South Korea showed that ICC patients had lower CD4 percentage (36.3+10.1) as compared to controls (45.6+8.9, $p<0.05$).

In our study, the percentage of patients with CD4 cell count <200 cells/mm^3 were few, nine women (20.0%) and those with CD4 cell count <350 cells/mm^3 were 18 women (40%). These findings do suggest that women with ICC and concurrent HIV infection included in this study did not have severe immunosuppression. One would have expected to find severe immunosuppresion among ICC patients co-infected with HIV as compared to women without HIV infection, given that ICC and HIV are both immunossuppressive. A study by Leitao *et al* (20) in America, among 15 women with ICC and concurrent HIV infection demonstrated statistically significant lower CD4 cell count of 208 cell/microL in women

with ICC as compared to 445 cell/microL in women without ICC but HIV infected. There are notable differences in immunological status between the two study populations. In the study by Leitao *et al* (20), only 7% (1/15) of the patients had CD4 cell counts more than 200 while in our study 80% had CD4 cell count greater than 200. Although there is evidence that HIV infection is significantly associated with rapid progression of ICC lesions (2, 21) the temporal relationship of HIV and ICC is not well known. What comes first? ICC and premalignant lesions may facilitate HIV acquisition by the disruption of vaginal/cervical epithelium while having HIV infection would facilitate persistence of highly oncongenic HPV subtypes leading to the development of premalignant lesions and ICC. Therefore, the lack of demonstration of severe immunosuppression among ICC patients in our study was an unexpected finding. It is possible that these patients may have acquired HIV infection while they had cervical cancer accounting for the low degree of immunosuppression observed. The patients included in this study may be a cohort which has survived the tragedy of having the dual problem. Although all the ICC patients included in the study were in WHO stage 4 at the time of the study, none were not on HIV treatment. This was due to the lack of HIV treatment services in public health facilities in Kenya. This situation has changed and antiretroviral therapy medicines (ARVs) are now readily available.

The finding of no severe immunosuppression among ICC with HIV co-infection is significant for clinicians caring for women with ICC and concurrent HIV infection. From these results, CD4 cell count would be of value in women with ICC for monitoring HIV treatment response. Interpretation of CD4 counts, however, has to take into account the fact that CD4 cell count decrease when women with ICC are on radiotherapy/chemotherapy treatment with full recovery after treatment (16, 17).

The present study is subject to some limitations. This study was conducted at a tertiary facility located at the capital city which may have limited access for patients from far distances resulting in a biased population. Kenyatta National Hospital is the only public facility offering radiotherapy in Kenya. Another possible limitation of our study was its observational design. The dates of HIV infection in the women included in the study was unknown.

In conclusion, the results of this study show significant decrease in CD4 cell count among women with ICC and HIV co-infection. The expected severe immunosuppression in women with ICC and HIV infection was not demonstrated in this study. CD4 cell count determination can therefore be used in monitoring HIV treatment in women with ICC and HIV infection. Prospective cohort study including women with known dates of HIV infection will be needed to elucidate the relationship between ICC and HIV co-infections on the CD4 cell count.

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