

## Low CD4 Counts and Early Sexual Debut Predict Genital Cytomegalovirus Infection among HIV Infected Women in Mwanza, Tanzania

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### Abstract

**Objective:** Here we report the prevalence and predictors of genital CMV infection among HIV infected women in Mwanza, Tanzania.

**Methods:** A total of 255 HIV seropositive women were investigated between August and October, 2014. Demographic and clinical data were collected using standardized data collection tool. Exfoliated cells from endocervix were obtained and detection of CMV DNA was done using real-time polymerase chain reaction (RT-PCR).

**Results:** A total number of 255 HIV infected women were enrolled with the mean age of  $39.2 \pm 9.1$ . The overall prevalence of genital CMV among HIV infected women was 43(16.7%, 95% CI: 12-21). Inflammatory changes and dysplasia did not show any association with genital CMV infection ( $p > 0.05$ ). Only good socio-economic status (OR=2.36, 95% CI: 1.1-5.02,  $p = 0.027$ ), young age at first sexual intercourse (OR=2.18, 95% CI: 1.04-4.62,  $p = 0.04$ ) and lower CD4+ counts (OR=2.07, 95% CI: 1.23-3.48,  $p = 0.027$ ) were found to be independent predictors of genital CMV infection among HIV infected women.

**Conclusion:** A significant proportion of HIV infected women are genitally infected with CMV in the genital tract. More studies to ascertain the clinical role of this virus in the female genital tract are recommended especially in HIV infected women.

**Keywords:** Genital cytomegalovirus; HIV infected women; CD4 counts and sexual debut

### Introduction

Cytomegalovirus (CMV) is known to be one of the commonest opportunistic pathogen among HIV/AIDS patients especially before the era antiretroviral therapy [1,2]. In the era of antiretroviral (ARV), the prevalence of CMV infections has significantly decreased [3]. CMV has been known to cause congenital infections as well as morbidity and mortality among HIV patients however; its clinical significance in the female genital tract remains poorly understood. Genital CMV infections has been documented in some reports with the incidence ranging between 4 and 12% being reported [4,5]. Genital CMV in HIV/AIDS patients was first reported in 1988 [6]; since then several reports [5-8] have been documented. The correlation of genital CMV and cervical dysplasia is not well understood emphasizing more research on this area. In Tanzania, there is limited data on the magnitude of CMV infections among HIV infected patients with no single study focusing on HIV infected women. This study for the first

time in Mwanza, Tanzania was designed to determine the prevalence and predictors of genital CMV among HIV infected women; the information that may provide insights for further studies that may explore and add knowledge on the role of this pathogen in female genital tract pathology.

### Methodology

A cross sectional hospital based study involving 255 HIV seropositive women was conducted between August and October, 2014 at Bugando Medical Centre (BMC), HIV care and treatment clinic (CTC). BMC-CTC receives an average of 80 revisiting HIV infected women per day. The clinic attends patients who are on antiretroviral drugs and those who have not started therapy [9]. The study involved all HIV infected women aged  $>18$  attending BMC-CTC during the study period. The study included all consented to participate and excluded all women who underwent total hysterectomy or wedge resection of the cervix. Socio-demographic, clinical and obstetric information were collected using standardized data collection tool.

## Sample collection and laboratory procedures

Pelvic assessment was done to all women who met the inclusion criteria and consented to participate in the study. The cervical exfoliated cells from the ectocervix and endocervix were obtained using a cytobrush. The tip of the cytobrush, which contains a cervical cells was placed into a transport medium and stored at -100C for analysis. After the cervical swab was taken; before removal of the speculum; specimen for Papanicolaou (PAP) smear was taken and processed as previously described [10].

CMV DNA from swabs was extracted using the MagNaPure LC DNA Large Volume Kit on a MagNaPure LC instrument (Roche Diagnostics, Mannheim, Germany) following manufacturer instructions with elution volume set at 100µl. HCMV real-time PCR was done using CMV 5': 5'-GAG CAG ACT CTC AGA GGA TCG G-3', CMV 3':5'-AAG CGG CCT CTG ATA ACC AAG-3' and CMV Probe 5'-CAT GCA GAT CTC CTC AAT GCG GCG-3' to detect the presence of CMV DNA as previously described [7].

## Data analysis

Data were entered in the computer using excel software and analysed using STATA version 11(STATA Corp LP, USA). Categorical variables were summarized as proportion while continuous variables were summarized as mean with standard deviation. Socioeconomic status (SES) was defined using income generating activities and education level; whereby individuals with sustainable income generating activities and attained secondary education and above were categorized as having good SES. Univariate and multivariate logistic regression analysis were done to determine factors that independently predict genital CMV infections among HIV infected women. Predictors with p-values of less than 0.05 were considered statistically significant.

## Ethical clearance

The protocol for the study was approved by the CUHAS/BMC research ethics and review committee and permission to conduct the study was obtained from BMC CTC authority. Written informed consent was sought from each participant prior enrolment.

## Results

### Socio-demographic characteristics

A total of 255 HIV infected women was enrolled in the study with mean age of 39.2 ± 9.1419. Out of 255 women, 111(43.5%) were married. Of 255 enrolled women, 138(54.1%) had good SES. Ninety seven (38.03%) and 158(61.9%) of women were residing in urban and rural areas respectively.

### Prevalence and predictors of genital cytomegalovirus among HIV infected women

The prevalence of genital CMV was found to be 43(16.7%, 95% CI: 12-21) among HIV infected women. The prevalence was found to be higher among women with lower education than those with higher education, however, the difference was not statistically significant (p>0.05). As the age increases the risk of acquiring genital CMV was found to decrease (OR: 0.95, 95% CI: 0.91-0.98, p=0.011).

Of 22 patients with CD4+ counts less than 100 cells/µl, 11 (50%) had genital CMV compared to 18 (13.4%) and 14 (14.1%) of those who had CD4+ counts >200 and 100-200 cells/µl respectively. The odds of acquiring genital CMV were higher among the women with lower CD4 counts (<100 cells/µl) than those with higher CD4 counts (>100 cells/µl) (OR; 2.07, 95%; 1.23-3.48, p=0.006) Table1. In addition the median duration of ARV treatment among patients with genital CMV was 2 years (IQR; 0.25-5) while the median duration for those with no genital CMV was 4 years (IQR, 1-6). On Wilcoxon rank-sum test the difference of the media of ARV duration was statistically significant (p=0.043). Similarly, women who had their first sexual intercourse at young age were at higher risk of getting genital CMV than those who had first sexual intercourse >18 years (OR; 2.18, 95%; 1.04-4.62, p=0.040) (Table 1). In addition, good socio-economic status was significantly found to be associated with genital CMV infections (OR; 2.22, 95% CI, 1.09-4.49, p=0.02).

On histopathology; cervical inflammatory changes and dysplasia did not show any association with genital CMV infections (p>0.05). Low CD4 counts, young age at first sexual intercourse and good socioeconomic status were found to be independent predictors of genital CMV infections among HIV infected women.

Characteristics	CMV positivity (N%)	Univariate analysis	P value	Multivariate analysis	
		OR(95% CI)		OR(95% CI)	P value
Age(years)	35 ± 1.4	0.95(0.91-0.98)	0.011	0.96(0.92-1)	0.096
<b>Residence</b>					
Rural (158)	21(13.3%)	1			
Urban(97)	22(22.7%)	1.91(0.98-3.70)	0.054	1.88(0.92-3.85)	0.086
<b>Socio-economic status</b>					
Poor(117)	13(11.1%)	1			
Good(138)	30(21.7%)	2.22(1.09-4.49)	0.02	2.36(1.1-5.02)	0.027
<b>Parity</b>					
0(24)	7(29.2%)	1			

≥ 1(195)	36(15.6%)	0.4(0.173-1.158)	0.098	2.22(0.5-9.88)	0.293
<b>Age of first sex</b>					
>18(122)	13(10.6%)	1			
<18(111)	23(20.7%)	2.2(1.1-4.5)	0.037		
Not stated(22)	7(31.8%)	3.9(1.3-11.4)	0.012	2.18(1.04-4.62)	0.04
<b>History of STI</b>					
No(213)	33(15.5%)	1			
Yes(42)	10(23.8%)	1.7(0.7-3.8)	0.192		
<b>CD4 counts</b>					
>200 (134)	18(13.4%)	1			
<200 (99)	14 (14.1%)	1.1(0.5-2.2)	0.877		
<100 (22)	11(50%)	6.4(2.4-17)	<0.001	2.07(1.23-3.48)	0.006
ARV duration	2(IQR 0.25-5)	0.91(0.82-1.01)	0.094	0.98(0.88-1.11)	0.824

**Table 1:** Univariate and multivariate logistic regression analysis of factors associated with genital cytomegalovirus among HIV seropositive women

## Discussion

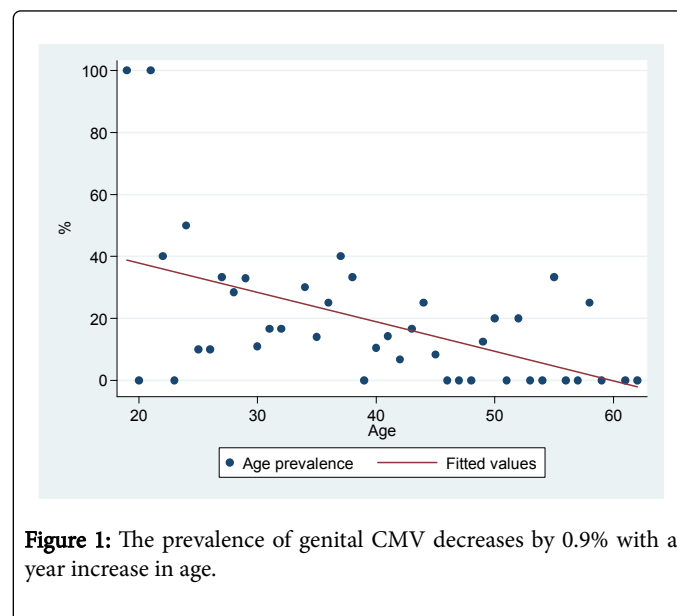
Cytomegalovirus (CMV) is one of the opportunistic pathogen in immune compromised individuals and a frequent cause of congenital infections when acquired during pregnancy. However, its role in causing genital infection among HIV infected women is not well elucidated. As previously reported [4,11,12], this study has documented significant high proportion of genital CMV among HIV infected women. In addition, this study has confirmed the association of genital CMV with low CD4 counts [12]. However; in contrast to previous report [13] genital CMV did not show any association with cervical inflammatory changes despite being significantly common in patients with low CD4+ counts (Figure 1).

CMV has been found to cause different manifestations in HIV infected patients [14,15]. This is due to impaired cell mediated immunity which exposes these women to a number of opportunistic infections. This is further supported by the fact that patients who had longer duration on ARV were less likely to develop genital CMV.

The risk of acquiring genital CMV was 2 times more among women with relatively good socio-economic status as compared to those with poor socio-economic status. This is contrary to previous reports where poor socio-economic status was found to influence sexual behaviours and predict sexually transmitted infections (STIs) including CMV [16-18]. In this study most of patients with good social-economic status were residing in urban hence at higher risk of sexual behaviours in seeking good life. This is further supported by previous study which reported that migration from rural to urban for seeking good life was significantly found to be associated with STIs infection among women [19].

On the other side, the odds of having genital CMV were significantly higher among young women than in older women which is comparable to what was observed in previous studies [12,13]. In addition, in the current study as it was observed previously, young age at first sexual intercourse was significantly found to predict genital

CMV infections [20]. At young age most of women are forced to participate in sexual activities out of curiosity without knowing the consequences and the fact that the virus is sexually transmitted.



**Figure 1:** The prevalence of genital CMV decreases by 0.9% with a year increase in age.

Therefore, these young vulnerable women are prone to get STIs including CMV. This has been suggested by previous report whereby other factors such as physiologic susceptibility, cognitive development, peer pressure, specific sexual behaviours and other logistic issues were linked to high rates of STIs among young women [21]. This observation is also supported by previous study in Mwanza which showed that most of the women get pregnancy at young age indicating early sexual debut [22]. Additionally, recent study in Mwanza has shown significantly high prevalence rates of STIs among adolescents which may explain the increased risk of acquiring genital CMV [23].

Though not statistically significant, patients with history of STI in the present had 1.7 times risk of acquiring genital CMV. These findings confirm what has been reported earlier whereby majority of women were found to have multiple STIs including CMV [12,24,25]. There is high possibility that participants in this study were concurrently infected with other STIs.

In conclusion, a significant proportion of HIV infected women with low CD4+ counts, good social economic status and history of early sexual debut is infected with CMV in the genital tract. However, clinical implications of this virus in the genital tract are yet to be determined since the pathological changes observed in this study did not show any association with genital CMV infection. More studies focusing on the role of this virus in the female genital tract pathology are recommended.

### Authors' contributions

MMM, FM AMand SEM participated in the design of the work. FM DM and MF participated in the collection of specimens and clinical data. FM, AM and KK performed laboratory analysis of the specimens. MMM, MM and SEM analyzed and interpreted the data. MMM and SEM wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

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