Research Article

Herpes Simplex Virus Type 2 (HSV-2) and Cytomegalovirus (CMV) among Women with Macerated Stillbirth: A Cross-Sectional Hospital-Based Study from Mwanza, Tanzania

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Background. Stillbirth adversely affects pregnancy outcomes in low- and middle-income countries (LMICs). Viral infections have been implicated as one of the causes of stillbirths. Despite high rates of stillbirths and high viral prevalence in LMICs, there is limited information regarding their association. This study investigated the magnitude of herpes simplex 2 virus (HSV-2) and human cytomegalovirus (HCMV) among women with macerated stillbirth. Methods. A cross-sectional hospital-based study was conducted involving 279 women with macerated stillbirth between July and August 2018 at different health facilities in Mwanza, Tanzania. Detection of HSV-2 was done by immunochromatographic test while that of HCMV was done using enzyme-linked immunosorbent assay (ELISA). Descriptive data analysis was done using STATA version 13. Results. A total of 28 (10.04%, 95% CI: 6.8-13.9) tested positive for HSV-2 IgG antibodies with only 4 (1.43%, 95% CL: 0.3-2.8) testing positive for HSV-2 IgM antibodies. HCMV IgG antibodies were detected in 131 (77.98%, 95% CI: 71-84) of 168 women tested. By multivariate logistic regression analysis, advanced age (OR: 0.93, 95% CI: 0.87-0.99, \( p = 0.025 \)) was significantly associated with negative HSV-2 IgG antibodies. By log multinomial regression analysis, only urban residence (RRR: 4.43: 95% CI 1.53-12.80, \( p = 0.006 \)) independently predicted HCMV IgG seropositivity among women with stillbirth. Twenty-one (30.9%) of women with positive HCMV IgG antibodies had low avidity index (<40%) indicating recent infection.

Conclusion. Significant proportion of women with macerated stillbirth residing in urban and with low age have HCMV and HSV antibodies, respectively. This calls for the need to consider introducing screening of these infections in the Tanzanian antenatal package and further studies to explore the role of these viruses in causing stillbirth in Tanzania.

1. Background

Vertical transmission of cytomegalovirus (CMV) and human simplex viruses (HSV) has been associated with disabling and potentially fatal effects on the fetus. Worldwide, cytomegalovirus (CMV) is regarded as one of the most common congenital infections and can cause hearing loss and neurodevelopmental disorders. Worldwide, it is estimated...
about 2-3% of pregnant women are infected with HSV [1]. Furthermore, based on the systematic review and meta-analysis, 83% of general population are seropositive for CMV with estimated seroprevalence of 86% being reported in women of childbearing age [2]. Vertical transmission during pregnancy is rare occurring in less than 1% of cases, but for those with active lesions or shedding the virus asymptomatically, the risk of vertical transmission intrapartum is high. Stillbirth is among the most devastating obstetric complications in resource-constrained countries [3]. By 2015, there were 2.6 million cases of stillbirth worldwide with high prevalence reported in low- and middle-income countries (LMICs) [4–6]. In recent years, there has been reduction of stillbirth cases in high-income countries (HICs) due to improved antenatal care (ANC) as compared to LMICs. In most of sub-Saharan African countries including Tanzania, the rates of stillbirths are still high with approximately 47,000 cases annually in Tanzania [7]. Despite high number of cases in most of these countries, causes have not been well studied. In Tanzania, much has been done to reduce stillbirth cases; however, there are infections which are known to contribute to cases of stillbirths elsewhere [8–10]. Maternal infections account for up to 25% and 50% of stillbirth cases in HICs and LMICs, respectively [11–13].

Viruses such as rubella, parvovirus B19, herpes simplex 2 virus (HSV-2), and human cytomegalovirus (HCMV) are common in Mwanza, Tanzania [14–17]. Primary human cytomegalovirus (HCMV) infection is associated with transplacental transmission in about 30%-40% of maternal infection [18]. HCMV and HSV-2 have been associated with cases of stillbirth worldwide with high rates reported in LMICs [8, 9]. This study for the first time in Mwanza is aimed at determining seroprevalence of these viruses among women with macerated stillbirth.

### 2. Materials and Methods

#### 2.1. Study Design and Study Settings

A cross-sectional hospital-based study was conducted between July and August 2018 in nine health facilities located in rural and urban areas in Mwanza. These health facilities were Bugando Medical Centre, Sekou Toure Regional Referral Hospital, Nyamagana District Hospital, Sengerema Designated District Hospital, Misungwi District Hospital, Ngudu District Hospital, and other rural and urban health facilities in Mwanza, Tanzania.
Table 3: Univariate and multivariate logistic regression analyses of the factors associated with HSV-2 IgG seropositivity among 279 women with stillbirth in Mwanza, Tanzania.

| Variables          | Mean (SD) (%) | Univariate, OD (95%CL) | P value | Multivariate OD (95%CL) | P value |
|--------------------|---------------|------------------------|---------|-------------------------|---------|
| Age                | 27.76 ± 7.22  | 0.92 (0.86-0.98)       | 0.015   | 0.93 (0.87-0.99)        | 0.025   |
| Residence          |               |                        |         |                         |         |
| Rural              | 16 (10.26%)   | 1.0                    |         |                         |         |
| Urban              | 12 (7.76%)    | 0.95 (0.43-2.08)       | 0.890   |                         |         |
| Parity             | 2.93 ± 2.18   | 0.92 (0.76-1.12)       | 0.414   |                         |         |
| Education          |               |                        |         |                         |         |
| Primary            | 24 (10.48%)   | 1.0                    |         |                         |         |
| Secondary          | 3 (8.33%)     | 1.18 (1.11-12.42)      | 0.889   |                         |         |
| Tertiary           | 1 (7.14%)     | 1.52 (0.19-12.15)      | 0.692   |                         |         |
| Occupation         |               |                        |         |                         |         |
| Unemployed         | 4 (12.50%)    | 1.0                    |         |                         |         |
| Business           | 4 (9.09%)     | 1.15 (0.19-6.77)       | 0.877   |                         |         |
| Employed           | 2 (8.0%)      | 1.64 (0.27-9.78)       | 0.586   |                         |         |
| Farming            | 18 (10.11%)   | 1.28 (0.28-5.94)       | 0.741   |                         |         |
| Marital status     |               |                        |         |                         |         |
| Single             | 8 (15.69%)    | 1.0                    |         |                         |         |
| Married            | 20 (8.77%)    | 1.93 (0.79-4.68)       | 0.143   | 1.45 (0.58-3.64)        | 0.418   |
| Fever              |               |                        |         |                         |         |
| No                 | 21 (10.19%)   | 1.0                    |         |                         |         |
| Yes                | 7 (9.59%)     | 0.93 (0.38-2.29)       | 0.882   |                         |         |
| H/rash             |               |                        |         |                         |         |
| No                 | 26 (10.24%)   | 1.0                    |         |                         |         |
| Yes                | 2 (8.00%)     | 0.76 (0.17-3.42)       | 0.723   |                         |         |
| Headache           |               |                        |         |                         |         |
| No                 | 21 (10.94%)   | 1.0                    |         |                         |         |
| Yes                | 7 (8.05%)     | 0.71 (0.29-1.74)       | 0.458   |                         |         |
| H/abortion         |               |                        |         |                         |         |
| No                 | 25 (10.96%)   | 1.0                    |         |                         |         |
| Yes                | 3 (5.88%)     | 0.51 (0.15-1.75)       | 0.283   |                         |         |
| H/low birth weight |               |                        |         |                         |         |
| No                 | 23 (9.66%)    | 1.0                    |         |                         |         |
| Yes                | 5 (12.20%)    | 1.29 (0.46-3.63)       | 0.619   |                         |         |
| Stillbirth         |               |                        |         |                         |         |
| No                 | 21 (11.35%)   | 1.0                    |         |                         |         |
| Yes                | 7 (7.45%)     | 0.62 (0.25-1.54)       | 0.308   |                         |         |
| HIV status         |               |                        |         |                         |         |
| Negative           | 26 (9.96%)    | 1.0                    |         |                         |         |
| Positive           | 2 (11.11%)    | 1.12 (0.25-5.19)       | 0.8     |                         |         |

Hospital, Sumve and Butimba Hospitals, and Magu District Hospital (Table 1).

2.2. Study Participants. Study participants were women aged 18 years and above presenting with stillbirths.

2.3. Sample Size Estimation and Sampling Technique. The sample size was calculated by Kish Leslie formula using the prevalence of 20.7% [19]. The minimum sample size estimated was 252. Serial sampling of women who met the inclusion criteria was performed until the sample size was reached.

2.4. Inclusion Criteria. The study included women aged 18 years and above presenting with stillbirths.

2.5. Exclusion Criteria. The women whose cause of stillbirth was known such as antepartum hemorrhage, hypertensive disorder, labor related, and severe anemia were excluded.
2.6. Data Collection and Sample Collection from the Participants. Data were collected using a pretested questionnaire. Variables collected included social demographic, maternal, foetal health systems, and ANC and intrapartum characteristics (Table 2). Four-five millilitres of whole blood was collected and transferred to plain vacutainer tubes (Becton, Dickinson, and company, Nairobi, Kenya). Sera was separated and then stored in cryovials at -40°C freezer until processing.

2.7. Diagnostic Tests and Laboratory Procedures and Case Definition. Detection of HSV-2 was done using immunochromatographic tests following manufacturer’s instructions (Exact Diagnostic Devices, USA), while detection of HCMV IgM and IgG antibodies was done using enzyme-linked immunosorbent assay as per the manufacturer’s instructions (PishtazTeb, Tehran, Iran). All IgG samples with high titters were subjected to avidity assay as previously described [20, 21]. In this study, positive IgG and IgM for HSV-2 and HCMV were defined as case, therefore outcome of this study.

2.8. Statistical Analysis. Data were cleaned, coded, and analyzed using STATA version 13.0. Percentage/fraction was used to summarize categorical variables, while mean (STD)/median (IQR) was used to summarize continuous variables. T-test and Wilcoxon’s rank-sum (Mann-Whitney) tests were used to compare means and medians among various groups, respectively. Logistic regression analysis and log multinomial were used to show association between dependent and independent variables for HSV and HCMV seropositivity, respectively. P value of < 0.05 at 95% confidence interval was considered as statistically significant.

2.9. Ethical Statement. Ethical clearance for conducting this study was sought from CUHAS/BMC Research Ethics and Review Committee (CREC), CREC/613/2018. Permission
of doing the study was obtained from relevant government authorities and specific health facilities.

3. Results

3.1. Sociodemographic Characteristics of the Enrolled Women with Macerated Stillbirth. A total of 279 women with macerated stillbirth were enrolled in this study with median age of 27 and IQR of 22-34 years. More than a half 93(55.36%) of women were from rural areas. Other characteristics are shown in Table 2.

3.2. Seropositivity of HSV-2 IgG and IgM Antibodies among Women with Macerated Stillbirth in Mwanza, Tanzania (n = 279). Out of 279 women tested, 28 (10.04%, 95% CI: 6.8-13.9) tested positive for HSV-2 IgG antibodies, while 4 (1.43%, 95% CI: 0.3-2.8) tested positive for HSV-2 IgM antibodies. The median age of IgG seropositive women was significantly lower than that of IgG seronegative women (24.5, IQR: 19.5-29 vs. 27, IQR: 22-34 years, p = 0.009).

3.3. Factors Associated with the HSV-2 IgG Seropositivity among Women with Macerated Stillbirth in Mwanza Tanzania (n = 279). On univariate analysis, advanced age (OR: 0.92, 95% CI: 0.86-0.98, p = 0.015) significantly protected women from being IgG seropositive. Advanced age (OR: 0.93, 95% CI: 0.87-0.99, p = 0.025) remained significantly associated with HSV-2 IgG protection on multivariate logistic regression analysis (Table 3).

3.4. Seropositivity of HCMV IgM and IgG Antibodies among Women with Macerated Stillbirth in Mwanza, Tanzania (n = 168). Out of 168, 131 (77.98%, 95% CI: 71-84) tested positive for HCMV IgG antibodies, while none of them was IgM seropositive.

3.5. Factors Associated with HCMV IgG Seropositivity among Women with Macerated Stillbirth in Mwanza Region. On univariate log binomial regression analysis, residing in urban areas (RRR: 7.34; 95% CI 2.61-20.03 p = 0.000) was significantly associated with HCMV IgG seropositivity and remained significant on log multinomial regression analysis (RRR:4.43; 95% CI 1.53-12.80, p = 0.006) (Table 4).

3.6. HCMV IgG Avidity Index Values. Avidity assay of HCMV IgG antibodies positive samples revealed that 21 (30.9%) of the samples had low avidity index indicating recent infections (Figure 1).

4. Discussion

About one-tenth of study population tested positive for HSV-2 IgG antibodies with about one percent testing positive for HSV-2 IgM antibodies. Furthermore, it was confirmed that the majority of study participants were positive for HCMV IgG antibodies with about a third having low avidity index (<40%) indicating recent infection. Urban residence independently predicted HCMV IgG seropositivity among women with stillbirth.

This is the first study to investigate maternal immunoreactivity to HSV-2 and HCMV among women with macerated stillbirth in Mwanza, Tanzania. The seropositivity of HSV-2 IgG antibodies was found to be 10.4% comparable to a previous study in Nepal that reported seropositivity of 10% among women with spontaneous abortion [22]. In comparison to previous studies among women with abortions and stillbirth in Iraq and Nigeria, the seropositivity in the current study is significantly low [8, 23]. This could be due to the fact that these previous studies used ELISA assays which are more sensitive than immunochromatographic test. In contrast to a previous study in the same setting, only decrease in age was associated with HSV-2 IgG seropositivity [8]. This could be explained by the fact that as the age increases, antibody titters tend to decrease as reported previously [24, 25].
Regarding HCMV IgG seropositivity, it was found to be high (77.98%) as in previous studies among pregnant women in Mwanza as well as among pregnant women in Sudan [26, 27]. This observation is also similar to a previous study among pregnant women and women with recurrent abortion in Russia [28]. Only residing in urban areas was significantly associated with IgG seropositivity which is similar to previous studies conducted among pregnant women in Mwanza and among women of child bearing age in India [26, 29]. High populated areas have been associated with high HCMV transmission due to poor living conditions [30]. About a third (31%) of women had low avidity index reflecting recent infections signifying high transmission of HCMV in Mwanza and possible cause of poor pregnancy outcomes [9].

5. Limitation of the Study

The study was done in Mwanza, Tanzania; therefore, this study may not be representative of the whole country at large. In addition, this study used rapid immunochromatographic tests which have been found to have low sensitivity compared to ELISA assay; therefore, prevalence of HSV-2 IgG and IgM might have been underestimated.

6. Conclusion and Recommendations

The seropositivity of HSV-2 IgG antibodies among women with macerated stillbirth and low age in Mwanza is high. In addition, the HCMV infections are significantly high among women with stillbirth residing in urban areas in Mwanza. There is a need of including HSV-2 and HCMV screening services in Tanzania antenatal package.

Data Availability

All data generated during this study are included in this manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

MMM, EC, HAN, and SEM participated in the designing of the study. MMM, EC, FM, LM, AS, BM, and VS participated in the data/sample collection. MMM, VS, BM, LM, AS, and SEM participated in the laboratory analysis of samples. SEM did the data analysis. MMM, DK, HN, AEC, and SEM participated in the data interpretation. HN and MMM wrote the first draft of the manuscript. SEM and MM did the critical review of the manuscript. All authors approved the last version of the manuscript.

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References

[1] W. A. B. Hammad and J. C. Konje, “Herpes simplex virus infection in pregnancy - an update,” European Journal of Obstetrics & Gynecology and Reproductive Biology, vol. 259, pp. 38–45, 2021.
[2] O. B. Navti, M. Al-Belushi, and J. C. Konje, “Cytomegalovirus infection in pregnancy - an update,” European Journal of Obstetrics & Gynecology and Reproductive Biology, vol. 258, pp. 216–222, 2021.
[3] S. Saleem, S. S. Tikmani, E. M. McClure et al., “Trends and determinants of stillbirth in developing countries: results from the global network’s population-based birth registry,” Reproductive Health, vol. 15, no. S1, pp. 23–30, 2018.
[4] J. Lawn, H. Blencowe, P. Waiswa et al., “For the Lancet Ending Preventable Stillbirths Series study group with The Lancet Stillbirth Epidemiology investigator group. Stillbirths: rates, risk factors, and acceleration towards 2030,” Lancet, vol. 387, no. 10018, pp. 587–603, 2016.
[5] M. Aminu, R. Unkels, M. Mdegela, B. Utz, S. Adaji, and N. van den Broek, “Causes of and factors associated with stillbirth in low- and middle-income countries: a systematic literature review,” BJOG: An International Journal of Obstetrics & Gynaecology, vol. 121, pp. 141–153, 2014.
[6] World Health Organization, World Health Statistics 2016: Monitoring Health for the SDGs Sustainable Development Goals, World Health Organization, 2016.
[7] P. Karin, B. Katarina, B. Roger et al., “Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998–99,” Acta Obstetricia et Gynecologica Scandinavica, vol. 81, no. 4, pp. 284–292, 2002.
[8] I. A. Naqid, S. H. Yousif, and N. R. Hussein, “Seroprevalence of rubella and herpes simplex virus in women with miscarriage and stillbirth in Zakho city, Kurdistan region, Iraq: a cross-sectional study,” Women’s Health Bulletin, vol. 7, no. 1, pp. 18–22, 2020.
[9] J. M. H. J. Iwasenko, S. Arbuckle, N. Graf, B. Hall, M. E. Craig, and W. D. Rawlinson, “Human cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy,” Journal of Infectious Diseases, vol. 203, no. 11, pp. 1526–1533, 2011.
[10] W. D. H. B. Rawlinson, C. A. Jones, H. E. Jeffery et al., “Viruses and other infections in stillbirth: what is the evidence and what should we be doing?,” Pathology, vol. 40, no. 2, pp. 149–160, 2008.
[11] E. McClure, M. Nalubamba-Phiri, and R. Goldenberg, “Stillbirth in developing countries,” International Journal of Gynecology & Obstetrics, vol. 94, no. 2, pp. 82–90, 2006.
[12] R. L. Goldenberg, E. M. McClure, S. Saleem, and U. M. Reddy, “Infection-related stillbirths,” The Lancet, vol. 375, no. 9724, pp. 1482–1490, 2010.
[13] R. L. Goldenberg and C. Thompson, “The infectious origins of stillbirth,” American Journal of Obstetrics and Gynecology, vol. 189, no. 3, pp. 861–873, 2003.
[14] M. M. Mirambo, S. Aboud, M. Majigo, U. Groβ, and S. E. Mshana, “Adverse pregnancy outcomes among pregnant women with acute Rubella infections in Mwanza city,
Tanzania,” *International Journal of Infectious Diseases*, vol. 78, pp. 72–77, 2019.

[15] M. M. Mirambo, F. Maliki, M. Majigo et al., “The magnitude and correlates of parvovirus B19 infection among pregnant women attending antenatal clinics in Mwanza, Tanzania,” *BMC Pregnancy and Childbirth*, vol. 17, no. 1, p. 176, 2017.

[16] M. Mirambo, E. Chibwe, M. Mushu, M. Majigo, and S. Mshana, “Cytomegalovirus, parvovirus B19 and rubella co-infection among pregnant women attending antenatal clinics in Mwanza City: the need to be considered in Tanzanian antenatal care package,” *Epidemiology (Sunnyvale)*, vol. 6, no. 230, article 1000230, p. 2161, 2016.

[17] M. M. Mirambo, C. Isdori, and S. E. Mshana, “Serological profiles of herpes simplex virus type 2 among HIV negative population in Mwanza City, Tanzania,” *Tanzania Journal of Health Research*, vol. 19, no. 2, 2017.

[18] D. S. Ross, S. C. Dollard, M. Victor, E. Sumartojo, and M. J. Cannon, “The epidemiology and prevention of congenital cytomegalovirus infection and disease: activities of the centers for disease control and prevention workgroup,” *Journal of Women’s Health*, vol. 15, no. 3, pp. 224–229, 2006.

[19] K. I. Yahya-Malima, B. Evjen-Olsen, M. I. Matee, K. Fylkesnes, and L. Haarr, “HIV-1, HSV-2 and syphilis among pregnant women in a rural area of Tanzania: prevalence and risk factors,” *BMC Infectious Diseases*, vol. 8, no. 1, p. 75, 2008.

[20] H. E. Prince, M. Lapé-Nixon, and S. M. Novak-Weekley, “Performance of a cytomegalovirus IgG enzyme immunoassay kit modified to measure avidity,” *Clinical and Vaccine Immunology*, vol. 21, no. 6, pp. 808–812, 2014.

[21] M. Bodéus, S. Feyder, and P. Goubau, “Avidity of IgG antibodies distinguishes primary from non-primary cytomegalovirus infection in pregnant women,” *Clinical and Diagnostic Virology*, vol. 9, no. 1, pp. 9–16, 1998.

[22] D. Acharya, A. Shrestha, B. Bogati, K. Khanal, S. Shrestha, and P. Gyawali, “Serological screening of TORCH agents as an etiology of spontaneous abortion in Dhulikhel Hospital, Nepal,” *American Journal of Biomedical and Life Sciences*, vol. 2, no. 2, pp. 34–39, 2014.

[23] E. Kalu, C. Ojide, A. Chuku et al., “Obstetric outcomes of human herpes virus-2 infection among pregnant women in Benin, Nigeria,” *Nigerian journal of clinical practice*, vol. 18, no. 4, pp. 453–461, 2015.

[24] A. Kamali, A. Nunn, D. Mulder, E. Van Dyck, J. Dobbins, and J. Whitworth, “Seroprevalence and incidence of genital ulcer infections in a rural Ugandan population,” *Sexually Transmitted Infections*, vol. 75, no. 2, pp. 98–102, 1999.

[25] H. Weiss, A. Buve, N. Robinson et al., “The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations,” *AIDS*, vol. 15, pp. S97–S108, 2001.

[26] E. Chibwe, M. M. Mirambo, A. Kihunrwa, and S. E. Mshana, “Magnitude of the cytomegalovirus infection among pregnant women attending antenatal clinics in the city of Mwanza, Tanzania,” *BMC Research Notes*, vol. 10, no. 1, p. 489, 2017.

[27] H. Z. Hamdan, I. E. Abdelbagi, N. M. Nasser, and I. Adam, “Seroprevalence of cytomegalovirus among pregnant women and hospitalized children in Palestine,” *BMC Infectious Diseases*, vol. 13, no. 1, p. 528, 2013.