Seroprevalence of Rubella Virus, Cytomegalovirus and Herpes Simplex Virus–2 among Pregnant Women at the Komfo Anokye Teaching Hospital, Ghana

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Abstract

Background: Rubella virus, Cytomegalovirus (CMV) and Herpes simplex virus-2 (HSV-2) are important viruses associated with adverse outcomes in neonates. Their occurrence and frequencies are, however, least studied in many African countries including Ghana.

Methods: A cross-sectional study was designed to investigate the prevalence and risk factors associated with rubella virus, CMV and HSV-2 exposures among pregnant women attending the ante-natal unit of the Komfo Anokye Teaching Hospital. The study was carried out between January 2013 and March 2013. IgM and IgG antibodies were tested to assess the current and past history of virus exposure.

Results: Ninety one subjects were screened for both CMV and HSV-2 infections, and 89 for rubella virus infection. CMV infection had the highest IgG and IgM seropositivity of 95.6% (95% CI: 89.1% - 98.8%) and 38.5% (95% CI: 28.4% - 49.2%) respectively. This was followed by rubella virus infection with IgG positivity of 92.3% (95% CI: 87.4% - 98.2%), IgM of 6.6% (95% CI: 2.5% - 13.8%), and HSV-2 IgG positivity of 68.1% (95% CI: 57.5% - 77.5%). Fifty four (60.7%) subjects were co-infected with all the three viruses while 32 (36%) were co-infected by a combinations of two viruses. There was no association between virus exposure and various socio-demographic indicators and risk factors.

Conclusions: This study has provided pilot data on herpes and rubella viruses infections among pregnant women in Ghana. A larger prevalence study is recommended to inform policy makers and health stakeholders.


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INTRODUCTION

Viruses associated with congenital infections are not widely studied in developing countries including Africa. The occurrence of three of such viral infections - rubella virus, cytomegalovirus (CMV), and herpes simplex virus type-2 (HSV-2) - have been documented in some African countries [1-4]. The infection is usually mild in immunocompetent individuals, and, in most instances, the cases are asymptomatic. However, infections in pregnant women, especially at certain stage of gestation, is associated with complications including miscarriage, and developmental abnormalities in the newborn baby that include hearing loss, mental retardation, developmental delay, cerebral palsy, epilepsy, ocular abnormality, microcephaly, hydrocephaly, skin lesions and psychomotor retardation [1,5-7]. Although the incidence of these congenital infections (especially rubella infections) has reduced in industrialised countries due mainly to the implementation of comprehensive vaccination programmes, such vaccination programmes are not available in most of the sub-Saharan Africa [8]. Little is known about the prevalence of these viruses in Ghana. Only 25 published articles on CMV infections are available for sub-Saharan Africa, and most of the studies included healthy adult populations or HIV patients [9]. A few studies conducted among pregnant women in some Africa countries like Egypt, Sudan and Burkina Faso demonstrated high CMV and rubella IgG seroprevalence of between 50% and 90% [2,10]. In Ghana, three studies conducted among healthy adults and HIV patients showed CMV and rubella IgG seroprevalence rates of 55-90% [11-13]. Another study reported congenital rubella syndrome rate of 0.8 per 1000 live births [14]. We, therefore, conducted this study to describe the history of exposure to CMV, rubella and HSV-2 using IgG as a marker, and to identify current state of these infections using IgM seromarker.

MATERIALS AND METHODS

Study Site and Design

This research was a cross-sectional study and was conducted at the Antenatal Clinic (ANC) of the Komfo Anokye Teaching Hospital (KATH) between January 2013 and March 2013. The hospital is the second largest tertiary medical facility in Ghana with approximately 1000 bed capacity.

Study Population and Sample Size

Each individual attending the antenatal care who consented to the study was sampled. Sampling was done in a systematic order until the required sample size was achieved. Pregnant women in need of emergency care or having high risk during pregnancy were excluded from the study. Prior to the study, a sample size of 96 was determined by using an average reported rubella prevalence of 90% [4], a marginal error of 6%, and a confidence limit of 95%.

Laboratory Investigations and Sampling Methods

Five millilitres of blood samples were collected from all subjects who were enrolled in the study. The samples were centrifuged at 3500 rpm for 5 minutes at the virology department of the KATH after which the sera was aliquoted into cryo-tubes and stored at -20°C until testing. The sera were transported in cold chain to the virology laboratory of the Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), where they were screened for antibodies to CMV (IgM and IgG), Rubella virus (IgM and IgG) and HSV-2 (IgG) using commercially available ELISA kits. The tests were performed according to the manufacturer’s instructions (Human Diagnostic Worldwide, Germany).

Briefly, 10µl of serum was diluted with 1ml of the dilution buffer and incubated at room temperature for 5 minutes. 100µl of the resultant solution was pipetted into ELISA microwell plates and incubated for a further 1 hour at room temperature. Three cycles of washing were performed on the mixture and 100µl of IgM or IgG conjugate was added to each well for each virus and incubated for 30 minutes. Washing was repeated for another 3 cycles and 100µl of conjugate was added to each well and incubated for 30 minutes at room temperature. The washing procedure was repeated after which 100µl of substrate was added. The reaction was stopped with 100µl of stopping solution and the
absorbance was read at 450nm within 30 minutes.

**Ethical Considerations**

The study protocol was approved by the Committee for Human Research, Publications and Ethics of KATH and the School of Medical Sciences, KNUST, Kumasi, Ashanti region, Ghana before commencement of the study. The protocol was first explained to every subject and those who consented were asked to thumbprint or sign a participant consent form depending on their literacy level. Assent was obtained from one subject who was below 18 years of age and written informed consent was taken from her parents.

**Statistical Analyses**

The data were entered into a Microsoft Excel Spreadsheet version 2010. All categorical variables were compared for the different viruses using contingency analysis and Chi-square or Fisher’s exact test. Continuous variables were expressed as means ± standard deviation. Unpaired t-test was used to compare the means and a p-value of <0.05 was considered significant. All statistical analyses were performed using R statistical software [15] version 3.0.2.

**RESULTS**

A total of 91 out of the estimated 96 subjects (94.7%) were enrolled in this study. The mean age of study subjects was 31 years (standard deviation ±5.3). A large proportion of the subjects who were interviewed were literates with the following educational levels: Junior High School (36, 39.6%), Secondary School (17, 18.9%) and Tertiary (20, 22%). The main occupation of the study subjects was trading (38, 41.8%) followed by hairdressing (24, 26.4%), and teaching or administration (21, 23.1%). The most frequent stage of pregnancy was the 3rd trimester (65, 71.4%) followed by 2nd trimester (22, 24.2%) and 1st trimester (4, 4.4%).

**Serological Profile of Subjects**

IgG and IgM tests were performed for CMV (91 subjects), HSV-2 (91 subjects) and rubella virus (89 subjects) infections. Among the IgG seropositive subjects, CMV infection rate was the highest (95.6%; 95% CI: 89.1% - 98.8%), followed by rubella (92.3%; 95% CI: 87.4% - 98.2%) and HSV-2 infection (68.1%; 95% CI: 57.5% - 77.5%). Similarly for IgM, CMV infection rate was the highest (38.5%; 95% CI: 28.4% - 49.2%) followed by rubella virus (6.6%; 95% CI: 2.5% - 13.8%). We did not test for HSV-2 IgM antibody. Of the positive CMV infection subjects, 52 (57.1%) were positive for only IgG and 35 (38.5%) were positive for both IgM and IgG antibodies. Among the rubella infection positive subjects, 78 (87.6%) were positive for only IgG and six (6.7%) for both IgG and IgM.

We observed multiple infections among our study subjects. Three subjects (3%) had single virus infections, 32 (36%) had dual virus infections and 54 (60.7%) had the three viral infections. Single virus infections occurred for CMV (1.1%) and rubella virus (2.2%) while dual virus infections occurred for CMV and rubella (29.2%), and CMV and HSV-2 (4.5%). Figure 1 highlights the various combinations of viruses and their counts.

![Figure 1. IgG Seropositive Viruses](image)

To identify the factors associated with IgM and IgG seropositivity, various socio-demographic parameters including age, occupation, and gestation stage were compared for each virus. Table 1 gives the comparative account of socio-demographic and clinical characteristics of pregnant women enrolled in the study. There was no significant difference among subjects positive for either rubella IgM or IgG. A comparison of the IgM and IgG serostatus for CMV infected subjects also showed no
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1. Rubella Seropositivity</th>
<th>2. CMV Seropositivity</th>
<th>3. HSV-2 Seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n=5)</td>
<td>IgG Positive (n=78)</td>
<td>Both IgG and IgM Positive (n=6)</td>
</tr>
<tr>
<td>Age, mean (SD) (years)</td>
<td>28 (3)</td>
<td>31.4 (5.5)</td>
<td>28.7 (2)</td>
</tr>
<tr>
<td>Marital status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>0</td>
<td>8 (10.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Married</td>
<td>5 (100)</td>
<td>69 (88.5)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>Single</td>
<td>0</td>
<td>1 (1.3)</td>
<td>0</td>
</tr>
<tr>
<td>Educational level, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>6 (7.7)</td>
<td>0</td>
</tr>
<tr>
<td>Primary</td>
<td>0</td>
<td>10 (12.8)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Junior high school</td>
<td>3 (60)</td>
<td>31 (39.7)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Senior high school</td>
<td>2 (40)</td>
<td>14 (17.9)</td>
<td>0</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0</td>
<td>17 (21.8)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Occupation, n (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair dressers</td>
<td>2 (40)</td>
<td>20 (25.6)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>2 (40)</td>
<td>5 (6.4)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Teacher or Admin</td>
<td>0</td>
<td>18 (23.1)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Trader</td>
<td>1 (20)</td>
<td>35 (44.9)</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>
Table 1 (cont). Socio-demographic and Clinical Characteristics of Pregnant Women and their Association with the Viral Infections

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1. Rubella Seropositivity</th>
<th>2. CMV Seropositivity</th>
<th>3. HSV-2 Seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n=5)</td>
<td>IgG Positive (n=78)</td>
<td>Both IgG and IgM Positive (n=6)</td>
</tr>
<tr>
<td><strong>Gestation stage, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Trimester</td>
<td>0</td>
<td>3 (3.8)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>0</td>
<td>19 (24.4)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>5 (100)</td>
<td>56 (71.8)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td><strong>Still birth, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4 (80)</td>
<td>50 (64.1)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (20)</td>
<td>28 (35.9)</td>
<td>3 (50)</td>
</tr>
<tr>
<td><strong>Blood transfusion, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5 (100)</td>
<td>67 (85.9)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>11 (14.1)</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>

CMV – Cytomegalovirus; HSV-2 - Herpes Simplex Virus–2
* Overall p-value for occupation was significant at p = 0.039 for CMV Seropositivity
significant difference, except for the types of occupations (p=0.039). Logistic regression analysis using the occupation types as references did not show any statistical significance. The proportion of subjects who were either positive or negative for HSV-2 infection also showed no significant difference for the various socio-demographic factors.

**DISCUSSION**

Although rubella, CMV, and HSV-2 infections are reported to be associated with pregnancy related complications, data on their occurrence in Africa are limited. The current study identified rubella and CMV viruses as having the highest IgG seroprevalence among the pregnant women in our study. This high seroprevalence is not surprising as many studies from West African countries including Nigeria, Benin and Gambia have reported prevalence of 87% to 97% [16-18]. Two previous studies conducted in Ghana identified IgG seroprevalence for CMV infection to be between 76-96% among healthy voluntary blood donors [11,12]. No case of CMV IgM was identified. The high prevalence of IgG antibodies to CMV confirms the general perception that such viruses are common in developing countries including Africa. The identification of around 39% (35/91) of pregnant women as having both IgM and IgG antibodies is quite important and could suggest sustained and ongoing active CMV infection among the pregnant women in Ghana. With the reported 40% maternal-to-child transmission rate [19], neonatal infections with this virus among Ghanaians cannot be over emphasised.

Similarly, our finding of 86% rubella IgG seropositivity and around 7% of both IgG and IgM is consistent with previously documented rubella studies in Ghana and other West African countries [3,13,14]. Although we did not follow up on the IgM positive pregnant women to look out for the final outcome of these viral detections, previous reports of clinical identification of congenital rubella syndrome in Ghana could suggest such happenings cannot be underestimated [14]. The current study also found 68% HSV-2 IgG seroprevalence among the study subjects. This finding is quite unique and to our knowledge, the first of its kind conducted among the pregnant women in Ghana. Unlike rubella and CMV infections, HSV-2 infection is usually acquired at the time of delivery with the highest maternal-to-child transmission rates occurring mostly among non-immune mothers [20,21]. We also observed in our study that subjects were exposed to multiple infections at a high rate.

As part of describing the risk factors of rubella, CMV, and HSV-2 infections, socio-demographic and other clinical factors were compared for subjects with single or dual IgM or IgG exposures and those without such exposures. All study variables were not statistically significant except for the type of occupation among CMV infected subjects. Logistic regression analysis of the occupation types however did not show any significant association with other factors. Other studies have documented occupations including business and farming as significantly associated with exposure to herpes virus infections [22].

The present study did not show significant association between any of the infectious agents and age (both as continuous and categorised). The relationship between maternal age and rubella or herpes viruses has attracted varied views. Whereas some investigators have observed higher risk of infections for elderly mothers [4,23] others reported the opposite outcomes [3,10]. It is possible that the small sample size of this study may have contributed to our inability to detect significant differences. Although we sampled close to our estimated sample size, recruiting large numbers would have revealed significant comparisons and a more precise virus estimates.

Based on this pilot data and evidence from other works [14], we recommend active public health education in order to sensitise policy makers and health stakeholders about the need to fully implement the rubella vaccination programmes in Ghana and other African countries. Further investigations may also be necessary to help understand the dynamism of HSV-2 in Ghana and its impact on pregnant women. Future studies could also consider using larger sample sizes from different populations to have more precise estimates for the different viruses.
CONCLUSIONS

The present study has demonstrated the occurrence of rubella, CMV, and HSV-2 antibodies among a small population of pregnant women in Ghana. This indicates the possible persistence and risk of transmission of such infections to children. Immunisation of women at risk and screening for rubella, CMV and HSV-2 infections during ante-natal care should be considered in Ghana as a means of reducing congenital rubella or herpes virus syndrome. The study has also highlighted the need for public education and awareness on the possible transmission routes of these viruses.

AUTHORS’ CONTRIBUTIONS

FA, MM, PA, CAT and TBK collected the data and contributed to laboratory analysis of the samples. SBN and MO performed statistical analysis of the data and contributed to data interpretation and writing of the manuscript. TBK and MO planned and initiated the study, and contributed to writing of the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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15. R Development Core Team: A language and environment for statistical computing. R
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