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Seroprevalence and associated factors of maternal cytomegalovirus in Southern Ethiopia: a cross-sectional study

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Abstract

Objectives The aim of this study was to assess the seroprevalence and associated factors of CMV among pregnant women in Southern Ethiopia.

Design Cross-sectional study.

Setting The study was conducted in Hawassa University comprehensive and specialized hospital. Hawassa, Southern Ethiopia.

Participants A total of 600 consecutive pregnant women attending the delivery ward were recruited for the study from August to October 2020.

Outcome measures The study assessed the rate of maternal anti-CMV IgG and IgM antibodies. The association of obstetric history, sociodemographic and behavioural characteristics with seropositivity of CMV was also evaluated based on the collected data using structured questioners.

Results Seropositivity for CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas the CMV IgG was 88.6% (532/600), (95% CI: 89.5 - 94.0%). Seroprevalence was higher in women of older age, currently unmarried and having nursery schooled children. Moreover, CMV seropositivity was significantly associated with any of the detected curable STIs. Seroprevalence was not significantly related to previous adverse pregnancy outcome, gravidity, being a child day care occupant mother, and birth weight of the newborn.

Conclusion In the present study, we identified a high rate of CMV IgM seropositivity among pregnant women in southern Ethiopia. Given that there is no existing CMV diagnosis, special attention should be designed for pregnant women in parallel to the existing antenatal care facility. Besides, training health care professionals will support awareness conception among pregnant women concerning the sequels of CMV infection during pregnancy.

Strengths and limitations of this study

- This study is the first to present the level of maternal cytomegalovirus infection in the region besides there is scarce of investigation throughout the country. The assessed anti-CMV IgM seropositivity also shown the possible threat of congenital CMV infection to the developing fetus.
- The detected anti-CMV IgM seropositivity was expected to be infection during pregnancy due to the fact that testing was conducted at the end of gestation (a nine month period). However, the study was unable to distinguish primary to secondary (reinfection or reactivation) as there was no base line data to decide about seroconversion.
- Being a hospital-based study, our finding will not be representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital.

Introduction

Cytomegalovirus (CMV) is the most common infection during pregnancy that poses the risk of congenital CMV infections (cCMV) worldwide.(1) In immunocompetent hosts, primary CMV infection may be asymptomatic or may cause mild self-limiting disease with fever, headaches, and myalgia and after primary infection the virus remains latent. Latency following a primary infection may relapse by periodic reactivations that give rise to recurrent infections later in life when the body immunity is suppressed.(2)

CMV infection or reactivation during pregnancy is mostly asymptomatic, however may lead to fetal infection and cCMV syndromes.(3) Congenital CMV infection of the fetus of mothers having pre-existing anti-CMV antibodies is also possible due to risk of reactivation or reinfection with a different strain of CMV during pregnancy.(4) Therefore unlike previous perception the high maternal CMV seroprevalence in developing countries like Ethiopia does not eliminate the threat of cCMV infection of the newborn. Worldwide cCMV following nonprimary maternal infections is more common in individuals of lower socioeconomic background.(5)

So far studies have shown maternal seropositivity rates of previous infection ranging from low (50 to 70%) in developed countries, to high (> 70%) in developing countries.(1) Presently, data on the prevalence of maternal CMV and associated risk factors are scanty in Ethiopia. The only available study conducted in Ethiopia had reported the prevalence of 15.5% for IgM and 88.6% for CMV IgG.(6)

In Africa the highest prevalence of CMV IgG was estimated ranging from 72 – 97.5% (7, 8) and of CMV IgM antibodies ranging from 0-15.5%.(9) However, for several reasons CMV infection among pregnant women in Africa have been overlooked.(10) One of the main reasons for

inattention is the perception that being infected in early childhood endures immunity for subsequent infection, so maternal reactivation or reinfection during pregnancy is unlikely to cause severe congenital infection.(11) However, in pregnant women the immune system is somehow suppressed. So ignoring maternal CMV and the subsequent effect of cCMV infection in Africa is short-sighted; furthermore, the possible confounding effects of HIV infection, malnutrition, tuberculosis, and a general higher disease burden of the continent must be taken into account.(12)

The objective of the study was to assess the seroprevalence of CMV among pregnant women and determine associated factors in Southern Ethiopia. Information about maternal the prevalence of CMV and associated risk factors is almost absent in Ethiopia and this study was the first in the southern region. We hope the study will attract health care professional attention and improve antenatal care in this domain. In the meantime it will generate awareness to the community, mainly pregnant women, regarding the consequence of CMV during pregnancy in Ethiopia.

Methodology

Study design and setting

From August to October 2020, a cross-sectional study was conducted among pregnant women who came for delivery in the obstetrics ward at Hawassa University Comprehensive and Specialized Hospital (HU-CSH), Ethiopia. The HU-CSH is one of the teaching hospitals serving as a referral centre for both public and private hospitals for more than 5 million inhabitants in the Southern Region and the neighbouring region of Ethiopia. The hospital has 500 beds, accommodating around 2,500 pregnant women for antenatal care (ANC) visits and conducting about 5,400 deliveries annually.

Participants

All pregnant women were recruited regardless of gestational age however, a mother with any critical illness (such as airway obstruction, current history of seizures or unconsciousness) that would deter them from participation in the study were excluded. Interrelated with the first phase of this project, where the initial 350 pregnant women had been tested for curable STI (*C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*) using GeneXpert (Xpert CT/NG and Xpert TV assays, Cepheid, Sunnyvale, California, USA), our manuscript being submitted and under review (manuscript number PONE-D-20-37668). In this second phase of the study, by including those initially enrolled 350 pregnant women, a total of 600 consecutively enrolled pregnant women were participated. A midwife at the obstetric ward provided general information about the study to all pregnant women before recruitment.

Sample size and sampling

The sample size was calculated based on the single population proportion formula by considering 15.5% prevalence of maternal anti-CMV IgM from a previous study conducted in central Ethiopia (6), a 3% margin of error and a 95% confidence level. Thus, the minimum sample size was 560. However, a total of 600 women were consecutively enrolled to signify the findings of seroprevalence of cytomegalovirus among pregnant women in the study settings. Sampling was based on convenience and continued until a total of 600 participants reached. If there is non-response during data collection, the study will be solved by taking subsequent participant until the intended sample size achieved.

Data collection

Socio-demographic, obstetric and behavioral data

Trained midwife at the obstetric ward provided general information about the study to pregnant women who came for delivery. Pregnant women agreeing to join in the study were interviewed using a structured questionnaire translated in Amharic, the language spoken by most people in the study area. The translated questionnaire was pre-tested on random mother at antenatal clinic to ensure the validity and feasibility of the questions as conducted in similar studies and principal investigator carefully checked the process of each data collection every day. Information related to socio-demographic characteristics (e.g., age, marital status, and educational level), obstetric history, and behavioral data were collected.

Sample collection and storage

The midwife-nurse aseptically collected a 3 ml blood sample from each subject. The collected samples were transported to the HU-CMHS microbiology laboratory within 12 hours of collection and the processed serums stored at -20 C^0 until analysis.

Laboratory methods

Testing was performed in Belgium, Ghent University hospital, department of laboratory medicine, using a commercially available enzyme immunoassay (ELISA) kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) for anti-CMV IgG and IgM according to the manufacturer's instructions. The sensitivity amounted to 99.2%, with a specificity of 100%. Results were evaluated semi-quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of the calibrator optical density at 450 nm. Seropositivity

was defined according to the guidelines given by the manufacturer, CMV negative when the ratio cut off value was < 0.8, borderline between 0.8 and 1.1, and positive if >1.1 for both IgG and IgM.

Ethical considerations

Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study.

The study was conducted in accordance with the Declaration of Helsinki, the significance of the study was clarified to each study participant and parents of a few minorities before obtaining informed consent. Permission attained from the minor (under the age of 18 years**)** participants parent or legal guardian according to the Ethiopian national research Ethics review guideline. Eventually, consent was granted from each participant including those who were under the age of 18 years with a participant confirmatory agreement to participate in the study. Confidentiality of the participant's information was ensured by anonymous typing.

Data analysis

Descriptive statistics were used to characterize the socio-demographic and obstetric and medical characteristics of the participants. We evaluated the seroprevalence of CMV and associated factors using a logistic regression model. Bivariate comparisons using Chi-square or Fisher's exact test where suitable were used to examine the relationships between participant characteristics and CMV test result. Finally, multivariable logistic regression was used to identify characteristics

independently associated with sero-status of CMV and adjusting for other factors. Variables with a significant level of ≤0.2 were included in the final model. P value < 0.05 is considered statistically significant. SPSS software version 20.0 (SPSS Inc. Chicago, IL, USA) was used for all analyses.

Patient and public involvement

No patients or the public were directly involved in the design, conduct, reporting or dissemination plans of this research.

Results

Participants

Six hundred pregnant women were assessed for seroprevalence of CMV and all women met the inclusion criteria and enrolled in the study, generating a 100% response rate. The mean of maternal age was 27.0 ± (SD) 5.2, with a ranging between 17 and 41. More than one-third of the study participant were under the age of 25. About one-fourth of the women were Primigravida. Out of the 600 participant 84 (14%) were currently unmarried, 475 (79.2%) were residing in urban setting, 377 (62%) were above or at secondary level of education. Forty-eight (8%) of the newborns were under-weighted (<2.5 Kg), and 64 (10%) of the births were preterm. Regarding STIs test result, 51 (14.6%) pregnant women were tested positive for any of curable STIs (Table 1).

In this study, around 96% of mothers had no knowledge of congenitally transmitted infection or the associated risks in pregnancy and about 9% of them had previous adverse pregnancy outcome. The chi- squire analysis shown that, seropositivity for CMV IgM significantly associated ($p<0.05$) with marital status, gestational age, having nursery school baby in the household, sharing a cup with children and having any of detected curable STIs. However, there was no significant

association with birth weight, gravidity and having previous adverse pregnancy outcome i.e. preterm birth, stillbirths and early neonatal death (Table 1).

Table 1 Maternal characteristics and associated factor with CMV IgM seropositivity in Southern Ethiopia

Characteristics	Total (N=600) n (%)	IgM-Positive (n=49) n (%)	IgM-Negative (n=551) n (%)	p-value
Age of mothers (years)				
<25	233 (38.8)	14 (28.6)	219 (39.7)	0.148
25-29	162 (27.0)	14 (28.6)	148 (26.9)	
30- 35	170 (28.3)	15 (30.6)	155 (28.1)	
>35	35 (5.8)	6(12.2)	29 (5.3)	
Marital status				
Married	516 (86.0)	37 (75.5)	479 (86.9)	
Currently unmarried	84 (14.0)	12 (24.5)	72 (13.1)	0.027
Residence				
Urban	475 (79.2)	44 (89.8)	431 (78.2)	0.056
Rural	125 (20.8)	5 (10.2)	120 (21.8)	
ANC follow up during pregnancy				
Yes	576 (96.0)	48 (98.0)	528 (95.8)	
no	24 (4.0)	1 (2.0)	23 (4.2)	0.712
Employed as daycare worker				
Yes	40 (6.7)	6 (12.2)	34 (6.2)	0.126
No	560 (93.3)	43 (87.8)	517 (93.8)	
Employed as health care				
Yes	32 (5.3)	6 (12.2)	26 (4.7)	0.055

no	568 (94.7)	43 (87.8)	525 (95.3)	
Education				
Primary and below	223 (37.2)	13 (26.5)	210 (38.1)	0.108
Secondary and above	377 (62.8)	36 (73.5)	341 (61.9)	
Gestational age				
Term	536 (89.3)	36 (73.5)	500 (90.7)	
Preterm	64 (10.7)	13 (26.5)	51 (9.3)	<0.001
Birth weight				
<2.5 Kg	48 (8.0)	3 (6.1)	45 (8.2)	0.789
>2.5 Kg	552 (92.0)	46 (93.9)	506 (91.8)	
Gravidity				
Primigravida	147 (24.5)	14 (28.6)	133 (24.1)	0.489
Multigravida	453(75.5)	35 (71.4)	418 (75.9)	
Previous adverse pregnancy outcome *				
Yes	39 (8.6)	2 (5.7)	37 (8.9)	0.756
No	414 (91.4)	33 (94.3)	381 (91.1)	
Knowledge on congenitally transmitted infections				
Yes	25 (4.2)	1 (2.0)	24 (4.4)	
No	575 (95.8)	48 (98.0)	527 (95.6)	0.712
Under-five children in the household				
Yes	396 (66.0)	31 (63.3)	365 (66.2)	0.673
no	204 (43.0)	18 (36.7)	186 (33.8)	
Daycare or Nursery school baby in the household				
Yes	259 (43.2)	31 (63.3)	228 (41.4)	0.003
no	341 (56.8)	18 (36.7)	323 (58.6)	

Sharing feeding cup with children				
Yes	107 (17.8)	14 (28.6)	93 (16.9)	0.040
no	493 (82.2)	35 (71.4)	458 (83.1)	
Sharing eating utensil with children				
Yes	88 (14.7)	8 (16.3)	80 (14.5)	0.732
No	512 (85.3)	41 (83.7)	471 (85.5)	
Sharing teeth brush with children				
Yes	42 (7.0)	3 (6.1)	39 (7.1)	0.999
No	558 (93.0)	46 (93.9)	512 (92.9)	
***N.gonorrhoeae* detected (n=350)**				
Yes	15 (4.3)	3 (10.0)	12 (3.8)	0.128
No	333 (95.7)	27 (90.0)	306 (96.2)	
***C. trachomatis* detected (n=350)**				
Yes	29 (8.3)	5 (16.7)	24 (7.5)	0.089
No	319 (91.7)	25 (83.3)	294 (92.5)	
***T. vaginalis* detected (n=350)**				
Yes	11 (3.1)	2 (6.9)	9 (2.8)	0.241
No	335 (96.8)	27 (93.1)	308 (97.2)	
Any of curable STI detected (n=350)				
Yes	51 (14.6)	10 (33.3)	41 (12.8)	0.005
No	299 (85.4)	20 (66.7)	279 (87.2)	

*previous adverse pregnancy includes; early neonatal death, stillbirth and preterm birth : STI; Sexually transmitted infections

Seroprevalence

Seropositivity of CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas seroprevalence of CMV IgG was 88.6% (532/600), (95% CI: 89.5 - 94.0%). Of 532 CMV IgG positive women, 483 (80.4%) were negative for IgM. Among all pregnant women, 68 (11.4%) were tested negative for both anti-CMV IgG and IgM, and none showed anti-CMV IgG negativity but IgM positivity (Table 2).

Table 2 Cytomegalovirus IgM and IgG test result of pregnant women

	Anti CMV IgG antibody n (%)		Total n (%)
Anti CMV IgM antibody	Positive	Negative	
Positive	49 (8.2)	0 (0)	49 (8.2)
Negative	483 (80.4)	68(11.4)	551 (91.8)
Total	532 (88.6)	68(11.4)	600

CMV seropositivity and associated factors

In bivariable analysis, seropositivity was more common in elder women (>35) compared to the youngest age group, in women who were currently unmarried, giving preterm birth, sharing a feeding cup with children or having nursery schooled children. Moreover women were positive for any of curable STIs also had a higher seroprevalence of CMV compared to those negative for STIs (Table 3).

Furthermore, in multivariable logistic regression maternal IgM seropositivity was associated with elder age groups (AOR = 4.9, 95% CI: 1.0–23.4), currently unmarried women (AOR = 3.8, 95%

CI: 1.7–7.9), preterm birth (AOR = 3.9, 95% CI: 1.5–10.3) and having nursery schooled children (AOR = 2.7, 95% CI: 1.1–6.4). Mothers with STIs has an association with seroprevalence (AOR = 4.1, 95% CI: 1.6–10.6) compared to mothers who were diagnosed negative for STIs.

Maternal seroprevalence was not significantly different in relation to residence, education level, to mother's occupation; being employed in child daycare or being health care worker (Table 3).

Table 3. Unadjusted and adjusted associated factors of maternal CMV IgM seropositivity in Southern Ethiopia

Characteristics	Un adjusted		Adjusted	
	OR 95% CI)	P-value	OR (95% CI)	P-value
Age of mothers (years)				
<25	1		1	
25-29	1.5 (0.7 -3.2)	0.318	1.2 (0.4 – 4.0)	0.739
30- 35	1.5 (0.7 -3.2)	0.283	3.0 (1.0 – 9.0)	0.048
>35	3.2 (1.2– 9.1)	0.026	4.9 (1.0 – 23.4)	0.047
Marital status				
Married	1		1	
Currently unmarried	2.2 (1.1 -4.3)	0.030	3.8 (1.3 -11.2)	0.015
Residence				
Urban	2.5 (1.0 -6.3)	0.064	2.3 (0.7 -7.9)	0.171
Rural	1		1	
Daycare worker				
Yes	2.1 (0.8 -5.3)	0.110	1.1 (0.2- 5.4)	0.857
No	1		1	
Health care worker				
Yes	2.8 (1.1 - 7.2)	0.031	1.2 (0.2 – 7.4)	0.841

no	1		1	
Education				
Primary and below	0.6 (0.3 -1.1)	0.111	0.7 (0.3 -1.8)	0.475
Secondary and above	1		1	
Gestational age				
Term	1		1	
Preterm	3.5 (1.8 – 7.1)	<0.001	3.9 (1.5 -10.3)	<0.006
Daycare or Nursery school baby				
Yes	2.4 (1.3 – 4.5)	0.004	2.7 (1.1 – 6.4)	0.027
no	1		1	
Sharing a cup with children				
Yes	2.0 (1.1 – 3.8)	0.044	2.2 (0.9 – 5.4)	0.074
no	1			
Any of curable STIs (n=350)				
Yes	3.4 (1.5- 7.8)	0.004	4.1 (1.6- 10.6)	0.003
No	1			

Discussion

In this study an overall 8.2% CMV IgM and 88.7% seroprevalence of CMV IgG were detected among pregnant women in southern Ethiopia. The associated factors of seropositivity were age, marital status, the presence of a curable STIs and sharing a cup with children. A statistically significant association was also observed between CMV seropositivity and preterm delivery.

The reported seropositivity of 88.6% CMV IgG in this study was comparable to a previous report of 88.5% h in central Ethiopia but a substantially higher rate (15.5%) of CMV IgM was detected

in this study.(6) Seropositivity of CMV IgG in our study was in line with a review done in Africa, with ranges from 60% to 100%.(9) Seropositivity rates of 77.3% for IgG and 8.1% for IgM in Kenya,(7) 93% for IgG and 11.1% for IgM in Nigeria,(13) 94% for IgG and 8.5% for IgM in Tanzania(14) were comparable to our finding.

In our study seroprevalence of CMV IgM is in concordance with several African studies.(7, 13-15) However, our rate was higher when compared to 0.4% in Tanzania,(16) 2.5% in Sudan,(17), and 7% in Egypt.(18) In the absence of maternal screening this high rate is alarming for policy makers. By far developed countries pregnant women are screened for CMV because of the consequence for the fetus and newborn.(19, 20) However, in most developing countries including Ethiopia, maternal CMV still lack awareness, overlooked and not diagnosed at least for pregnant women.(21) The high rate of positive CMV IgM may not only reflect primary infection but might also be attributed to reinfection or reactivation of CMV during pregnancy. So the reported high seropositivity in this study point to the existing negligence and the need to start screening to detect pregnant women at risk for congenital transmission of CMV.

In this study, elder age has significant association with seroprevalence. The same finding was reported in Sudan,(17) Kenya,(22), and Tanzania.(16) In addition a large scale study in London also reported that seropositivity increases with increasing age.(23) But, in a study in Egypt(18) and in Nigeria(24) age had no association with maternal CMV infection. The highest seroprevalence with age may indicate the more exposure of elders than youngsters or might be the existence of previous infection which can probably reactivated in the current pregnancy.

On the other hand, significant association of seroprevalence with having nursery schooled children among household was observed. For pregnant women the predictable source of CMV infection is

young children mainly exposure to nursery schooled children.(25) Children easily get infected in school and frequently shed CMV in their saliva or urine for many years continuously where spreads readily in preschool setting.(26) This places seronegative pregnant women who have a young child in the home or in day care at increased risk of seroconversion.(27, 28) Susceptibility to acquisition of CMV infection is high possibly through the direct contact with contagious secretions from their own children essentially in situation of poor hygienic practice like in Ethiopia.(23)

Among candidate predictors for maternal CMV seropositivity, occupation like being health care worker, or child day care worker; being multigravida, lower educational level and having other children at home were not detected. However, significant association with women who delivered preterm was reported. Although, preterm delivery might be due to the effect of maternal CMV infection we can not to say the risk factor as there are a lot of other confounding factors for preterm.(29)

Likewise, seropositivity was found to be significantly associated with STIs detected at delivery and currently being unmarried. Mothers who were positive for any of curable STIs had a four-time CMV seropositivity. It is also reported that STIs including CMV is more common in unmarried pregnant women.(23, 25, 30) Although cytomegalovirus is a virus that is transmitted through many body fluids, sexual transmission from a seropositive male partner is an additional established route by which women may be infected with CMV.(31) Indeed, it is somehow expected that sexual transmission is also responsible for reinfection of seropositive mothers with different virus strains in high-seroprevalence populations.(32)

This study lack differentiation of CMV IgM positivity of either primary or secondary (reinfection or reactivation) since we collected samples at the end of pregnancy and avidity test will not suitable. Moreover, it was a hospital-based study and not representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital. We lack also appropriate risk factors assessment tool due to cross-sectional nature of the study, hence more representative large scale survey is needed to identify possible risk factors prospectively.

Conclusion

In the present study, we documented a high rate of CMV seroprevalence among pregnant women in southern Ethiopia. The presence of a curable STIs, elder age, unmarried women, and having nursery schooled children have a significant association with seropositivity. Given that there is no existing CMV diagnostic facility, special attention should be designed for pregnant women in parallel to the existing antenatal care service. Besides, training health care professionals will support awareness conception for pregnant women concerning the sequels of CMV infection during pregnancy.

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Contributors: MH and EP contributed to the study design and conceptualisation. MH carried out the laboratory work. MH, EL, ZM and EP performed the statistical analysis and interpretation. All authors provided critical review and contributed to the write-up and approved the final version

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Patient consent: Obtained.

Ethics approval: Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study. The study was conducted in accordance with the Declaration of Helsinki.

Data sharing statement: Data are available upon reasonable request. All available data can be obtained by contacting the corresponding author.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalizability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Seroprevalence and associated factors of maternal cytomegalovirus in Southern Ethiopia: a cross-sectional study

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Abstract

Objectives The aim of this study was to assess the seroprevalence and associated factors of CMV among pregnant women in Southern Ethiopia.

Design Cross-sectional study.

Setting The study was conducted in Hawassa University comprehensive and specialized hospital. Hawassa, Southern Ethiopia.

Participants A total of 600 consecutive pregnant women attending the delivery ward were recruited for the study from August to October 2020.

Outcome measures The study assessed the rate of maternal anti-CMV IgG and IgM antibodies. The association of obstetric history, sociodemographic and behavioural characteristics with seropositivity of CMV was also evaluated based on the collected data using structured questioners.

Results Seropositivity for CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas the CMV IgG was 88.7% (532/600), (95% CI: 89.5 - 94.0%). Seroprevalence was higher in women of older age, currently unmarried and having nursery schooled children. Moreover, CMV seropositivity was significantly associated with any of the detected curable sexually transmitted infection (STIs). Seroprevalence was not significantly related to previous adverse pregnancy outcome, gravidity, being a child day care occupant mother, and birth weight of the newborn.

Conclusion In the present study, we identified a high rate of CMV IgM seropositivity among pregnant women in southern Ethiopia. Given that there is no existing CMV diagnosis, special attention should be designed for pregnant women in parallel to the existing antenatal care facility. Besides, training health care professionals will support awareness conception among pregnant women concerning the sequels of CMV infection during pregnancy.

Strengths and limitations of this study

- This study is the first to present the seroprevalence of maternal cytomegalovirus from the Southern region in Ethiopia and that provides the first awareness in medical, governmental and societal stakeholders.
- The study assessed both anti-CMV IgG and anti-CMV IgM seropositivity that can predict the possible threat of congenital CMV infection to the developing fetus.
- In this study, the factors associate with the level of seropositivity were explored among pregnant women.
- We were unable to distinguish primary from secondary (reinfection or reactivation) as there was no baseline data at the beginning of pregnancy to decide about seroconversion.
- Being a hospital-based study, our finding will not be representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital.

Introduction

Cytomegalovirus (CMV) is the most common infection during pregnancy that poses the risk of congenital CMV infections (cCMV) worldwide. (1) In immunocompetent hosts, primary CMV infection may be asymptomatic or may cause mild self-limiting disease with fever, headaches, and myalgia and after primary infection, the virus remains latent. Latency following a primary infection may relapse by periodic reactivations that give rise to recurrent infections later in life when the body immunity is suppressed. (2)

CMV infection or reactivation during pregnancy is mostly asymptomatic, however may lead to fetal infection and cCMV syndromes. (3) Congenital CMV infection of the fetus of mothers having pre-existing anti-CMV antibodies is also possible due to the risk of reactivation or reinfection with a different strain of CMV during pregnancy. (4) Therefore unlike previous perception the high maternal CMV seroprevalence in developing countries like Ethiopia does not eliminate the threat of cCMV infection of the newborn. Worldwide cCMV following nonprimary maternal infections is more common in individuals of lower socioeconomic background. (5) In addition, due to a high seroprevalence of CMV in the community of the developing countries, there would be a rare possibility of recurrent CMV infection as a result of reinfection. (6) As to the report from Portugal, maternal recurrent infections can have a significant impact on cCMV infections. (7)

So far previous studies have shown that maternal CMV seroprevalence rates ranging from low (50 to 70%) in developed countries to high (> 70%) in developing countries. (1) Presently, data on the prevalence of maternal CMV and associated risk factors are scanty in Ethiopia. The only available study conducted in Ethiopia had reported the prevalence of 15.5% for IgM and 88.6% for CMV IgG.(8)

In Africa, the highest prevalence of CMV IgG was estimated ranging from 72 – 97.5% (9, 10) and of CMV IgM antibodies to range from 0-15.5%. (11) However, for several reasons CMV infection among pregnant women in Africa have been overlooked. (12) One of the main reasons for inattention is the perception that being infected in early childhood endures immunity for subsequent infection, so maternal reactivation or reinfection during pregnancy is unlikely to cause severe congenital infection. (13) However, in pregnant women the immune system is somehow suppressed. So ignoring maternal CMV and the subsequent effect of cCMV infection in Africa is short-sighted; furthermore, the possible confounding effects of HIV infection, malnutrition, tuberculosis, and a general higher disease burden of the continent must be taken into account. (14)

The objective of the study was to assess the seroprevalence of CMV among pregnant women and determine associated factors in Southern Ethiopia. Information regarding the maternal prevalence of CMV and associated risk factors is almost absent in Ethiopia. Being the first study in the Southern region of Ethiopia, the finding will deliver the first awareness in medical, governmental and societal stakeholders in the region. Moreover, the study will attract health care professional attention and improve antenatal care in this domain. Indeed, it will generate awareness in the community, mainly pregnant women, regarding the consequence of CMV during pregnancy in Ethiopia.

Methodology

Study design and setting

From August to October 2020, a cross-sectional study was conducted among pregnant women who came for delivery in the obstetrics ward at Hawassa University Comprehensive and Specialized

Hospital (HU-CSH), Ethiopia. The HU-CSH is one of the teaching hospitals serving as a referral centre for both public and private hospitals for more than 5 million inhabitants in the Southern Region and the neighbouring region of Ethiopia. The hospital has 500 beds, accommodating around 2,500 pregnant women for antenatal care (ANC) visits and conducting about 5,400 deliveries annually.

Participants

All pregnant women were recruited regardless of gestational age however, a mother with any critical illness (such as airway obstruction, current history of seizures or unconsciousness) that would deter them from participation in the study were excluded. Interrelated with the first phase of this project, where the initial 350 pregnant women had been tested for curable STI (*C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*) using GeneXpert (Xpert CT/NG and Xpert TV assays, Cepheid, Sunnyvale, California, USA), our manuscript being submitted and under review (manuscript number PONE-D-20-37668). In this second phase of the study, by including those initially enrolled 350 pregnant women, a total of 600 consecutively enrolled pregnant women participated. A midwife at the obstetric ward provided general information about the study to all pregnant women before recruitment.

Sample size and sampling

The sample size was calculated based on the single population proportion formula by considering 15.5% prevalence of maternal anti-CMV IgM from a previous study conducted in central Ethiopia (8), a 3% margin of error and a 95% confidence level. Thus, the minimum sample size was 560. However, a total of 600 women were consecutively enrolled to signify the findings of seroprevalence of cytomegalovirus among pregnant women in the study settings. Sampling was

based on convenience and continued until a total of 600 participants reached. If there is non-response during data collection, the study will be solved by taking subsequent participant until the intended sample size achieved.

Data collection

Socio-demographic, obstetric and behavioral data

Trained midwife at the obstetric ward provided general information about the study to pregnant women who came for delivery. Pregnant women agreeing to join in the study were interviewed using a structured questionnaire translated in Amharic, the language spoken by most people in the study area. The translated questionnaire was pre-tested on random mother at the antenatal clinic to ensure the validity and feasibility of the questions as conducted in similar studies and the principal investigator carefully checked the process of each data collection every day. Information related to socio-demographic characteristics (e.g., age, marital status, and educational level), obstetric history, and behavioural data were collected.

Sample collection and storage

The midwife-nurse aseptically collected a 3 ml blood sample from each subject. The collected samples were transported to the HU-CMHS microbiology laboratory within 12 hours of collection and the processed serums kept at -20 C^0 until transported. Then frozen samples at −20°C were transported on dry ice packs to the testing laboratory.

Laboratory methods

Testing was performed in Belgium, Ghent University hospital, department of laboratory medicine, using a commercially available enzyme immunoassay (ELISA) kit (EUROIMMUN Medizinische

Labordiagnostika AG, Lübeck, Germany) for anti-CMV IgG and IgM according to the manufacturer's instructions. The sensitivity amounted to 99.2%, with a specificity of 100%. Results were evaluated semi-quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of the calibrator optical density at 450 nm. Seropositivity was defined according to the guidelines given by the manufacturer, CMV negative when the ratio cut off value was < 0.8, the borderline between 0.8 and 1.1, and positive if >1.1 for both IgG and IgM.

Ethical considerations

Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study.

The study was conducted in accordance with the Declaration of Helsinki, the significance of the study was clarified to each study participant and parents of a few minorities before obtaining informed consent. Permission attained from the minor (under the age of 18 years) participants parent or legal guardian according to the Ethiopian national research Ethics review guideline. Eventually, consent was granted from each participant including those who were under the age of 18 years with a participant confirmatory agreement to participate in the study. Confidentiality of the participant's information was ensured by anonymous typing.

Data analysis

Descriptive statistics were used to characterize the socio-demographic and obstetric and medical characteristics of the participants. We evaluated the seroprevalence of CMV and associated factors using a logistic regression model. Bivariate comparisons using chi- square or Fisher's exact test where suitable were used to examine the relationships between participant characteristics and CMV test result. Finally, multivariable logistic regression was used to identify characteristics independently associated with serostatus of CMV and adjusting for other factors. Variables with a significant level of ≤0.2 were included in the final model. P-value < 0.05 is considered statistically significant. SPSS software version 20.0 (SPSS Inc. Chicago, IL, USA) was used for all analyses.

Patient and public involvement

No patients or the public were directly involved in the design, conduct, reporting or dissemination plans of this research.

Results

Participants

Six hundred pregnant women were assessed for seroprevalence of CMV and all women met the inclusion criteria and enrolled in the study, generating a 100% response rate. The mean of maternal age was 27.0 ± (SD) 5.2, with a ranging between 17 and 41. More than one-third of the study participant were under the age of 25. About one-fourth of the women were Primigravida. Out of the 600 participant 84 (14%) were currently unmarried, 475 (79.2%) were residing in urban setting, 377 (62%) were above or at secondary level of education. Forty-eight (8%) of the newborns were

under-weighted (<2.5 Kg), and 64 (10%) of the births were preterm. Regarding STIs test result, 51 (14.6%) pregnant women were tested positive for any of curable STIs (Table 1).

In this study, around 96% of mothers had no knowledge of congenitally transmitted infection or the associated risks in pregnancy and about 9% of them had previous adverse pregnancy outcome. The chi- squire analysis showed that, seropositivity for CMV IgM significantly associated ($p<0.05$) with marital status, gestational age, having nursery school baby in the household, sharing a cup with children and having any of detected curable STIs. However, there was no significant association with birth weight, gravidity and having previous adverse pregnancy outcome i.e. preterm birth, stillbirths and early neonatal death (Table 1).

Table 1 Maternal characteristics and associated factor with CMV IgM seropositivity in Southern Ethiopia

Characteristics	Total (N=600) n (%)	IgM-Positive (n=49) n (%)	IgM-Negative (n=551) n (%)	p-value †
Age of mothers (years)				
<25	233 (38.8)	14 (28.6)	219 (39.7)	0.148
25-29	162 (27.0)	14 (28.6)	148 (26.9)	
30- 35	170 (28.3)	15 (30.6)	155 (28.1)	
>35	35 (5.8)	6(12.2)	29 (5.3)	
Marital status				
Married	516 (86.0)	37 (75.5)	479 (86.9)	
Currently unmarried	84 (14.0)	12 (24.5)	72 (13.1)	0.027
Residence				
Urban	475 (79.2)	44 (89.8)	431 (78.2)	0.056

Rural	125 (20.8)	5 (10.2)	120 (21.8)	
ANC follow up during pregnancy				
Yes	576 (96.0)	48 (98.0)	528 (95.8)	
no	24 (4.0)	1 (2.0)	23 (4.2)	0.712
Employed as daycare worker				
Yes	40 (6.7)	6 (12.2)	34 (6.2)	0.126
No	560 (93.3)	43 (87.8)	517 (93.8)	
Employed as health care				
Yes	32 (5.3)	6 (12.2)	26 (4.7)	0.055
no	568 (94.7)	43 (87.8)	525 (95.3)	
Education				
Primary and below	223 (37.2)	13 (26.5)	210 (38.1)	0.108
Secondary and above	377 (62.8)	36 (73.5)	341 (61.9)	
Gestational age				
Term	536 (89.3)	36 (73.5)	500 (90.7)	
Preterm	64 (10.7)	13 (26.5)	51 (9.3)	<0.001
Birth weight				
<2.5 Kg	48 (8.0)	3 (6.1)	45 (8.2)	0.789
>2.5 Kg	552 (92.0)	46 (93.9)	506 (91.8)	
Gravidity				
Primigravida	147 (24.5)	14 (28.6)	133 (24.1)	0.489
Multigravida	453(75.5)	35 (71.4)	418 (75.9)	
Previous adverse pregnancy outcome *				
Yes	39 (8.6)	2 (5.7)	37 (8.9)	0.756
No	414 (91.4)	33 (94.3)	381 (91.1)	
Knowledge on congenitally transmitted infections				

Yes	25 (4.2)	1 (2.0)	24 (4.4)	
No	575 (95.8)	48 (98.0)	527 (95.6)	0.712
Under-five children in the household				
Yes	396 (66.0)	31 (63.3)	365 (66.2)	0.673
no	204 (43.0)	18 (36.7)	186 (33.8)	
Daycare or Nursery school baby in the household				
Yes	259 (43.2)	31 (63.3)	228 (41.4)	0.003
no	341 (56.8)	18 (36.7)	323 (58.6)	
Sharing feeding cup with children				
Yes	107 (17.8)	14 (28.6)	93 (16.9)	0.040
no	493 (82.2)	35 (71.4)	458 (83.1)	
Sharing eating utensil with children				
Yes	88 (14.7)	8 (16.3)	80 (14.5)	0.732
No	512 (85.3)	41 (83.7)	471 (85.5)	
Sharing teeth brush with children				
Yes	42 (7.0)	3 (6.1)	39 (7.1)	0.999
No	558 (93.0)	46 (93.9)	512 (92.9)	
N.gonorrhoeae **detected (n=350)**				
Yes	15 (4.3)	3 (10.0)	12 (3.8)	0.128
No	333 (95.7)	27 (90.0)	306 (96.2)	
C. trachomatis **detected (n=350)**				
Yes	29 (8.3)	5 (16.7)	24 (7.5)	0.089
No	319 (91.7)	25 (83.3)	294 (92.5)	
T. vaginalis **detected (n=350)**				
Yes	11 (3.1)	2 (6.9)	9 (2.8)	0.241
No	335 (96.8)	27 (93.1)	308 (97.2)	

Any of curable STI detected (n=350)				
Yes	51 (14.6)	10 (33.3)	41 (12.8)	0.005
No	299 (85.4)	20 (66.7)	279 (87.2)	

*previous adverse pregnancy includes; early neonatal death, stillbirth and preterm birth : STI; Sexually transmitted infections

† Chi-square

Seroprevalence

Seropositivity of CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas seroprevalence of CMV IgG was 88.7% (532/600), (95% CI: 89.5 - 94.0%). Of 532 CMV IgG positive women, 483 (80.4%) were negative for IgM. Among all pregnant women, 68 (11.4%) were tested negative for both anti-CMV IgG and IgM, and none showed anti-CMV IgG negativity but IgM positivity (Table 2).

Table 2 Cytomegalovirus IgM and IgG test result of pregnant women

	Anti CMV IgG antibody n (%)		Total n (%)
Anti CMV IgM antibody	Positive	Negative	
Positive	49 (8.2)	0 (0)	49 (8.2)
Negative	483 (80.4)	68(11.4)	551 (91.8)
Total	532 (88.7)	68(11.4)	600

CMV seropositivity and associated factors

In bivariable analysis, seropositivity was more common in elder women (>35) compared to the youngest age group, in women who were currently unmarried, giving preterm birth, sharing a feeding cup with children or having nursery schooled children. Moreover women were positive for any of curable STIs also had a higher seroprevalence of CMV compared to those negative for STIs (Table 3).

Furthermore, in multivariable logistic regression, being over the age of 30 years had higher odds for CMV IgM seropositivity compared to being under 25 (AOR = 4.9, 95% CI: 1.0–23.4), currently unmarried women (AOR = 3.8, 95% CI: 1.7–7.9), preterm birth (AOR = 3.9, 95% CI: 1.5–10.3) and having nursery schooled children (AOR = 2.7, 95% CI: 1.1–6.4). Mothers with STIs had an association with seroprevalence (AOR = 4.1, 95% CI: 1.6–10.6) compared to mothers who were diagnosed negative for STIs.

Maternal seroprevalence was not significantly associated with residence, education level, occupation, being employed in a child daycare centre, or being a health care worker (Table 3).

Table 3. Unadjusted and adjusted associated factors of maternal CMV IgM seropositivity in Southern Ethiopia

Characteristics	Un adjusted *		Adjusted *	
	OR 95% CI)	P-value	OR (95% CI)	P-value
Age of mothers (years)				
<25	1		1	

25-29	1.5 (0.7 -3.2)	0.318	1.2 (0.4 – 4.0)	0.739
30- 35	1.5 (0.7 -3.2)	0.283	3.0 (1.0 – 9.0)	0.048
>35	3.2 (1.2– 9.1)	0.026	4.9 (1.0 – 23.4)	0.047
Marital status				
Married	1		1	
Currently unmarried	2.2 (1.1 -4.3)	0.030	3.8 (1.3 -11.2)	0.015
Residence				
Urban	2.5 (1.0 -6.3)	0.064	2.3 (0.7 -7.9)	0.171
Rural	1		1	
Daycare worker				
Yes	2.1 (0.8 -5.3)	0.110	1.1 (0.2- 5.4)	0.857
No	1		1	
Health care worker				
Yes	2.8 (1.1 - 7.2)	0.031	1.2 (0.2 – 7.4)	0.841
no	1		1	
Education				
Primary and below	0.6 (0.3 -1.1)	0.111	0.7 (0.3 -1.8)	0.475
Secondary and above	1		1	
Gestational age				
Term	1		1	
Preterm	3.5 (1.8 – 7.1)	<0.001	3.9 (1.5 -10.3)	<0.006
Daycare or Nursery school baby				
Yes	2.4 (1.3 – 4.5)	0.004	2.7 (1.1 – 6.4)	0.027
no	1		1	
Sharing a cup with children				
Yes	2.0 (1.1 – 3.8)	0.044	2.2 (0.9 – 5.4)	0.074
no	1			

Any of curable STIs (n=350)				
Yes	3.4 (1.5- 7.8)	0.004	4.1 (1.6- 10.6)	0.003
No	1			

* Logistic regression

Regarding CMV IgG seropositivity, elder women (>30), women with of primary or lower educational level and women having nursery schooled children shown association in bivariable analysis. There was no associated factor detected between CMV IgG seropositivity rate with maternal characteristic but, mother having nursery schooled children has an association with CMV IgG seroprevalence (AOR = 1.8, 95% CI: 1.0–3.0), (Table 4).

Table 4. Unadjusted and adjusted associated factors of maternal CMV IgG seropositivity in Southern Ethiopia

Characteristics	**Un adjusted ***		**Adjusted ***	
	OR 95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Age of mothers (years)				
<25	1			
25-29	1.2 (0.7 -2.3)	0.482	1.0 (0.5- 1.9)	0.991
30- 35	2.2 (1.1 -4.3)	0.028	1.8 (0.9- 3.8)	0.095
>35	1.3 (0.4– 3.9)	0.663	0.9 (0.3- 1.0)	0.877
Marital status				
Married	1			
Currently unmarried	0.8 (0.37 – 1.7)	0.573		
Residence				
Urban	0.8 (0.4- 1.5)	0.493		

Rural	1			
Daycare worker				
Yes	0.9 (0.3- 2.3)	0.810		
No	1			
Health care worker				
Yes	0.9 (0.3 – 2.6)	0.831		
no	1			
Education				
Primary and below	1.7 (1.0 – 2.8)	0.041	0.6 (0.4- 1.0)	0.037
Secondary and above	1			
Gestational age				
Term	1			
Preterm	0.9 (0.4-1.9)	0.756		
Daycare or Nursery school baby				
Yes	1.9 (1.1 – 3.1)	0.017	1.8 (1.0- 3.0)	0.045
no	1			
Sharing a cup with children				
Yes	1.1 (0.6 – 2,3)	0.705		
no	1			
Any of curable STIs (n=350)				
Yes	0.8 (0.3 – 1,9)	0.578		
No	1			

* Logistic regression

Discussion

In this study an overall 8.2% CMV IgM and 88.7% seroprevalence of CMV IgG were detected among pregnant women in southern Ethiopia. The associated factors of seropositivity were age, marital status, the presence of curable STIs and sharing a cup with children. A statistically significant association was also observed between CMV seropositivity and preterm delivery.

The reported seropositivity of 88.7% CMV IgG in this study was comparable to a previous report of 88.5% in central Ethiopia but a substantially higher rate (15.5%) of CMV IgM was detected in this study. (8) Seropositivity of CMV IgG in our study was in line with a review done in Africa, with ranges from 60% to 100%. (11) Seropositivity rates of 77.3% for IgG and 8.1% for IgM in Kenya, (9) 93% for IgG and 11.1% for IgM in Nigeria,(15) 94% for IgG and 8.5% for IgM in Tanzania (16) were comparable to our finding.

In our study seroprevalence of CMV IgM is in concordance with several African studies.(9, 15-17) However, our rate was higher when compared to 0.4% in Tanzania, (18) 2.5% in Sudan, (19), and 7% in Egypt. (20) In the absence of maternal screening this high rate is alarming for policy makers. By far developed countries pregnant women are screened for CMV because of the consequence for the fetus and newborn. (21, 22) However, in most developing countries including Ethiopia, maternal CMV still lacks awareness, is overlooked and not diagnosed at least for pregnant women. (23) The high rate of positive CMV IgM may not only reflect primary infection but might also be attributed to reinfection or reactivation of CMV during pregnancy. So the reported high seropositivity in this study point to the existing negligence and the need to start screening to detect pregnant women at risk for congenital transmission of CMV.

Earlier studies have shown that there was considerable debate regarding the relationship between maternal age and CMV seroprevalence. In this study, elder age had significant association with CMV IgM seroprevalence. The same finding was reported in Kenya, (24), in Nigeria, (25) and Tanzania. (18) However, a study in Ethiopia, (8) in Egypt (20) Sudan, (19), China, (26) and in Nigeria (27) have reported that age had no association with maternal CMV infection. Unlike our finding, studies in Iraq high rate of IgM in young women (28), and in USA CMV IgM seroprevalence varied by age with the highest IgM seroprevalence among younger and lower among those elderly were reported. (29) The highest seroprevalence with age in this study may indicate the more lifetime episodes exposure of elders than youngsters or might be the existence of previous infection which can probably reactivated in the current pregnancy. In fact, the observed difference in CMV seroprevalence by age may be useful to realize the risk of cCMV transmission and are useful for ascertaining target populations for intervention to reduce congenital CMV transmission. (29)

On the other hand, a significant association of seroprevalence with having nursery schooled children among households was observed. For pregnant women, the predictable source of CMV infection is young children mainly exposure to nursery schooled children. (30) Children easily get infected in school and frequently shed CMV in their saliva or urine for many years continuously that could spread readily even in a preschool setting. (31) This places seronegative pregnant women who have a young child in the home or in day care at increased risk of seroconversion. (32, 33) Susceptibility to the acquisition of CMV infection is high possibly through the direct contact with contagious secretions from their children essentially in a situation of poor hygienic practice like in Ethiopia. (34)

Among candidate predictors for maternal CMV seropositivity, occupations like being health care worker or child day-care worker; being multigravida, lower educational level and having other children at home did not shown any association. However, there was significant association for those with preterm delivery. Although, maternal CMV infection may result in preterm delivery, its isolated impact could not be assessed since we did not study other potential confounding factors.(35)

Likewise, seropositivity was found to be significantly associated with STIs detected at delivery and currently being unmarried. Mothers who were positive for any of curable STIs had a four-time CMV seropositivity. It is also reported that STIs including CMV to be more common in unmarried pregnant women.(30, 34, 36) Although cytomegalovirus is a virus that is transmitted through many body fluids, sexual transmission from a seropositive male partner is an additional established route by which women may be infected with CMV.(37) Indeed, it is somehow expected that sexual transmission is also responsible for the reinfection of seropositive mothers with different virus strains in high-seroprevalence populations.(38)

Although, CMV IgG avidity testing is a valuable laboratory tool for distinguishing primary from non-primary CMV infection, an avidity test was not performed in this study. Hence, this study lacks differentiation of CMV IgM positivity of either primary or non-primary (reinfection or reactivation) as we collected samples at the end of the pregnancy period that avidity test will not be suitable. Moreover, it was a hospital-based study and not representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital. We lack also appropriate risk factors assessment tool due to the cross-sectional nature of the study, hence a more representative large-scale survey is needed to identify possible risk factors prospectively. Furthermore, this is the first study from the Southern region in Ethiopia and that

makes the first awareness in medical, governmental and societal stakeholders. Thus, more studies may need to be accomplished before the introduction of appropriate measures.

Conclusion

In the present study, we documented a high rate of CMV seroprevalence among pregnant women in southern Ethiopia. The presence of curable STIs, elder age, unmarried women, and having nursery schooled children showed a significant association with seropositivity. Given that there is no existing CMV diagnostic facility, special attention should be designed to pregnant women in parallel to the existing antenatal care service. Besides, training health care professionals will support awareness conception for pregnant women concerning the sequels of CMV infection during pregnancy.

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Contributors: MH and EP contributed to the study design and conceptualisation. MH carried out the laboratory work. MH, EL, ZM and EP performed the statistical analysis and interpretation. All authors provided critical review and contributed to the write-up and approved the final version of the manuscript. MH had final responsibility to submit for publication. All authors read and amended drafts of the paper and approved the final version.

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Competing interests: no competing interest

Patient consent

The significance of the study was clarified to each study participant and parents of a few minorities prior to obtaining permission to participate in the study. Participation was fully voluntary and informed consent was obtained from all participants who were 18 years old or above. Informed consent was also obtained from the parents or legal guardians of the participants who were under the age of 18 years prior to their enrolment in the study. Confidentiality of the participant's information was ensured by anonymous typing.

Ethics approval

Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study. The study was conducted in accordance with the Declaration of Helsinki.

Data sharing statement

Data are available upon reasonable request. All available data can be obtained by contacting the corresponding author.

Competing interests

The authors declare that they have no competing interest.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalizability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Seroprevalence and associated factors of maternal cytomegalovirus in Southern Ethiopia: a cross-sectional study

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Abstract

Objectives The aim of this study was to assess the seroprevalence and associated factors of CMV among pregnant women in Southern Ethiopia.

Design Cross-sectional study.

Setting The study was conducted in Hawassa University comprehensive and specialized hospital. Hawassa, Southern Ethiopia.

Participants A total of 600 consecutive pregnant women attending the delivery ward were recruited for the study from August to October 2020.

Outcome measures The study assessed the rate of maternal anti-CMV IgG and IgM antibodies. The association of obstetric history, sociodemographic and behavioural characteristics with seropositivity of CMV was also evaluated based on the collected data using structured questioners.

Results Seropositivity for CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas the CMV IgG was 88.7% (532/600), (95% CI: 89.5 - 94.0%). Seroprevalence of CMV IgM was higher in women of older age, currently unmarried, having nursery schooled children and with any of the detected curable sexually transmitted infections (STIs). While seroprevalence of CMV IgG was significantly associated only with women having nursery schooled children. Seroprevalence was not significantly associated with previous adverse pregnancy outcome, gravidity, being a child daycare occupant mother, and newborn birth weight.

Conclusion In the present study, we identified a high rate of CMV IgM and CMV IgG seroprevalence among pregnant women in southern Ethiopia. Given that there is no existing CMV diagnosis, special attention should be designed to pregnant women in parallel to the existing antenatal care facility. Besides, training health care professionals will support awareness conception among pregnant women concerning the sequels of CMV infection during pregnancy.

Strengths and limitations of this study

- This study is the first to present the seroprevalence of maternal cytomegalovirus from the Southern region in Ethiopia and that provides the first awareness in medical, governmental and societal stakeholders.
- The study assessed both anti-CMV IgG and anti-CMV IgM seropositivity that can predict the possible threat of congenital CMV infection to the developing fetus.
- In this study, the factors associate with the level of seropositivity were explored among pregnant women.
- We were unable to distinguish primary from secondary (reinfection or reactivation) CMV infection as there was no baseline data to decide about seroconversion at the beginning of pregnancy.
- Being a hospital-based study, our finding will not be representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital.

Introduction

Cytomegalovirus (CMV) is the most common infection during pregnancy that poses the risk of congenital CMV infections (cCMV) worldwide. (1) In immunocompetent hosts, primary CMV infection may be asymptomatic or may cause mild self-limiting disease with fever, headaches, and myalgia and after primary infection, the virus remains latent. Latency following a primary infection may relapse by periodic reactivations that give rise to recurrent infections later in life when the body immunity is suppressed. (2)

During pregnancy CMV infection or reactivation is mostly asymptomatic, however, it might lead to fetal infection and cCMV syndromes. (3) Congenital CMV infection of the fetus of mothers having pre-existing anti-CMV antibodies is also possible due to the risk of reactivation or reinfection with a different strain of CMV during pregnancy. (4) Therefore unlike previous perception the high maternal CMV seroprevalence in developing countries like Ethiopia does not eliminate the threat of cCMV infection of the newborn. Worldwide cCMV following nonprimary maternal infections is more common in individuals of lower socioeconomic backgrounds. (5) In addition, due to a high seroprevalence of CMV in the community of the developing countries, there would be a rare possibility of recurrent CMV infection as a result of reinfection. (6) As to the report from Portugal, maternal recurrent infections can have a significant impact on cCMV infections. (7)

So far, previous studies have shown that maternal CMV seroprevalence rates ranging from low (50 to 70%) in developed countries to high (> 70%) in developing countries. (1) Presently, data on the prevalence of maternal CMV and associated risk factors are scanty in Ethiopia. The only

available study conducted in Ethiopia had reported the seroprevalence of 15.5% for CMV IgM and 88.6% for CMV IgG.(8)

In Africa, the highest prevalence of CMV IgG was estimated to range from 72 – 97.5% (9, 10) and of CMV IgM antibodies to range from 0-15.5%. (11) However, for several reasons CMV infections among pregnant women in Africa have been overlooked. (12) One of the main reasons for inattention is the perception that being infected in early childhood endures immunity for subsequent infection, so maternal reactivation or reinfection during pregnancy is unlikely to cause severe congenital infection. (13) However, in pregnant women the immune system is somehow suppressed. So ignoring maternal CMV and the subsequent effect of cCMV infection in Africa is short-sighted; furthermore, the possible confounding effects of HIV infection, malnutrition, tuberculosis, and a general higher disease burden of the continent must be taken into account. (14)

The objective of the study was to assess the seroprevalence of CMV IgM and IgG among pregnant women and determine associated factors in Southern Ethiopia. Information regarding the maternal prevalence of CMV and associated risk factors is almost absent in Ethiopia. Being the first study in the Southern region of Ethiopia, the finding will deliver the first awareness in medical, governmental and societal stakeholders in the region. Moreover, the study will attract health care professional attention and improve antenatal care in this domain. Indeed, it will generate awareness in the community, mainly pregnant women, regarding the consequence of CMV during pregnancy in Ethiopia.

Methodology

Study design and setting

From August to October 2020, a cross-sectional study was conducted among pregnant women who came for delivery in the obstetrics ward at Hawassa University Comprehensive and Specialized Hospital (HU-CSH), Ethiopia. The HU-CSH is one of the teaching hospitals serving as a referral centre for both public and private hospitals for more than 5 million inhabitants in the Southern Region and the neighbouring region of Ethiopia. The hospital has around 500 beds, accommodating more than 2,500 pregnant women for antenatal care (ANC) visits and conducting about 5,400 deliveries annually.

Participants

All pregnant women were recruited regardless of gestational age however, a mother with any critical illness (such as airway obstruction, current history of seizures or unconsciousness) that would deter them from participation in the study were excluded. Interrelated with the first phase of this project (15), where the initial 350 pregnant women had been tested for curable STI (*C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*) using GeneXpert (Xpert CT/NG and Xpert TV assays, Cepheid, Sunnyvale, California, USA). In this second phase of the study, by including those initially enrolled 350 pregnant women, a total of 600 consecutively enrolled pregnant women participated. A midwife at the obstetric ward provided general information about the study to all pregnant women before recruitment.

Sample size and sampling

The sample size was calculated based on the single population proportion formula by considering 15.5% prevalence of maternal anti-CMV IgM from a previous study conducted in central Ethiopia (8), a 3% margin of error and a 95% confidence level. Thus, the minimum sample size was 560. However, a total of 600 women were consecutively enrolled to signify the findings of seroprevalence of cytomegalovirus among pregnant women in the study settings. Sampling was based on convenience and continued until a total of 600 participants were reached. If there is non-response during data collection, the study will be solved by taking subsequent participants until the intended sample size is achieved.

Data collection

Socio-demographic, obstetric and behavioral data

Trained midwives at the obstetric ward provided general information about the study to pregnant women who came for delivery. Pregnant women agreeing to join in the study were interviewed using a structured questionnaire translated in Amharic, the language spoken by most people in the study area. The translated questionnaire was pre-tested on random mothers at the antenatal clinic to ensure the validity and feasibility of the questions as conducted in similar studies and the principal investigator carefully checked the process of each data collection every day. Information related to socio-demographic characteristics (e.g., age, marital status, and educational level), obstetric history, and behavioural data were collected.

Sample collection and storage

The midwife-nurse aseptically collected a 3 ml blood sample from each subject. The collected samples were transported to the HU-CMHS microbiology laboratory within 12 hours of collection

and the processed serums were kept at -20 C^0 until transported. Then frozen samples at −20°C were transported on dry ice packs to the testing laboratory.

Laboratory methods

Testing was performed in Belgium, Ghent University hospital, department of laboratory medicine, using a commercially available enzyme immunoassay (ELISA) kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) for anti-CMV IgG and IgM according to the manufacturer's instructions. The sensitivity amounted to 99.2%, with a specificity of 100%. Results were evaluated semi-quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of the calibrator optical density at 450 nm. Seropositivity was defined according to the guidelines given by the manufacturer, CMV negative when the ratio cut off value was < 0.8, the borderline between 0.8 and 1.1, and positive if >1.1 for both IgG and IgM.

Ethical considerations

Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study.

The study was conducted in accordance with the Declaration of Helsinki, the significance of the study was clarified to each study participant and parents of a few minorities before obtaining informed consent. Permission attained from the minor (under the age of 18 years**)** participants

parent or legal guardian according to the Ethiopian national research Ethics review guideline. Eventually, consent was granted from each participant including those who were under the age of 18 years with a participant confirmatory agreement to participate in the study. Confidentiality of the participant's information was ensured by anonymous typing.

Data analysis

Descriptive statistics were used to characterize the socio-demographic and obstetric and medical characteristics of the participants. We evaluated the seroprevalence of CMV and associated factors using a logistic regression model. Bivariate comparisons using chi- square or Fisher's exact test where suitable were used to examine the relationships between participant characteristics and CMV test result. Finally, multivariable logistic regression was used to identify characteristics independently associated with serostatus of CMV and adjusting for other factors. Variables with a significant level of ≤ 0.2 were included in the final model. P-value < 0.05 is considered statistically significant. SPSS software version 20.0 (SPSS Inc. Chicago, IL, USA) was used for all analyses.

Patient and public involvement

No patients or the public were directly involved in the design, conduct, reporting or dissemination plans of this research.

Results

Participants

Six hundred pregnant women were assessed for seroprevalence of CMV and all women met the inclusion criteria and enrolled in the study, generating a 100% response rate. The mean of maternal age was 27.0 ± (SD) 5.2, with a ranging between 17 and 41. More than one-third of the study participant were under the age of 25. About one-fourth of the women were primigravida. Out of the 600 participant, 84 (14%) were currently unmarried, 475 (79.2%) were residing in urban setting, 377 (62%) were above or at secondary level of education. Forty-eight (8%) of the newborns were under-weighted (<2.5 Kg), and 64 (10%) of the births were preterm. Regarding STIs test result, 51 (14.6%) pregnant women were tested positive for any of curable STIs (Table 1).

In this study, 95.8% of mothers had no knowledge of congenitally transmitted infection or the associated risks in pregnancy and 8.6% of them had previous adverse pregnancy outcome. The chi- squire analysis showed that, seropositivity for CMV IgM significantly associated ($p<0.05$) with marital status, gestational age, having nursery school baby in the household, sharing a cup with children and having any of detected curable STIs. However, there was no significant association with birth weight, gravidity and having previous adverse pregnancy outcome i.e. preterm birth, stillbirths and early neonatal death (Table 1).

Table 1 Maternal characteristics and associated factor with CMV IgM seropositivity in Southern Ethiopia

Characteristics	Total (N=600) n (%)	IgM-Positive (n=49) n (%)	IgM-Negative (n=551) n (%)	p-value †
Age of mothers (years)				
<25	233 (38.8)	14 (28.6)	219 (39.7)	0.148
25-29	162 (27.0)	14 (28.6)	148 (26.9)	
30- 35	170 (28.3)	15 (30.6)	155 (28.1)	
>35	35 (5.8)	6(12.2)	29 (5.3)	
Marital status				
Married	516 (86.0)	37 (75.5)	479 (86.9)	
Currently unmarried	84 (14.0)	12 (24.5)	72 (13.1)	0.027
Residence				
Urban	475 (79.2)	44 (89.8)	431 (78.2)	0.056
Rural	125 (20.8)	5 (10.2)	120 (21.8)	
ANC follow up during pregnancy				
Yes	576 (96.0)	48 (98.0)	528 (95.8)	
no	24 (4.0)	1 (2.0)	23 (4.2)	0.712
Employed as daycare worker				
Yes	40 (6.7)	6 (12.2)	34 (6.2)	0.126
No	560 (93.3)	43 (87.8)	517 (93.8)	
Employed as health care				
Yes	32 (5.3)	6 (12.2)	26 (4.7)	0.055
no	568 (94.7)	43 (87.8)	525 (95.3)	
Education				
Primary and below	223 (37.2)	13 (26.5)	210 (38.1)	0.108
Secondary and above	377 (62.8)	36 (73.5)	341 (61.9)	
Gestational age				
Term	536 (89.3)	36 (73.5)	500 (90.7)	
Preterm	64 (10.7)	13 (26.5)	51 (9.3)	<0.001
Birth weight				
<2.5 Kg	48 (8.0)	3 (6.1)	45 (8.2)	0.789
>2.5 Kg	552 (92.0)	46 (93.9)	506 (91.8)	
Gravidity				
Primigravida	147 (24.5)	14 (28.6)	133 (24.1)	0.489
Multigravida	453(75.5)	35 (71.4)	418 (75.9)	

Previous adverse pregnancy outcome *				
Yes	39 (8.6)	2 (5.7)	37 (8.9)	0.756
No	414 (91.4)	33 (94.3)	381 (91.1)	
Knowledge on congenitally transmitted infections				
Yes	25 (4.2)	1 (2.0)	24 (4.4)	
No	575 (95.8)	48 (98.0)	527 (95.6)	0.712
Under-five children in the household				
Yes	396 (66.0)	31 (63.3)	365 (66.2)	0.673
no	204 (43.0)	18 (36.7)	186 (33.8)	
Daycare or Nursery school baby in the household				
Yes	259 (43.2)	31 (63.3)	228 (41.4)	0.003
no	341 (56.8)	18 (36.7)	323 (58.6)	
Sharing feeding cup with children				
Yes	107 (17.8)	14 (28.6)	93 (16.9)	0.040
no	493 (82.2)	35 (71.4)	458 (83.1)	
Sharing eating utensil with children				
Yes	88 (14.7)	8 (16.3)	80 (14.5)	0.732
No	512 (85.3)	41 (83.7)	471 (85.5)	
Sharing teeth brush with children				
Yes	42 (7.0)	3 (6.1)	39 (7.1)	0.999
No	558 (93.0)	46 (93.9)	512 (92.9)	
***N.gonorrhoeae* detected (n=350)**				
Yes	15 (4.3)	3 (10.0)	12 (3.8)	0.128
No	333 (95.7)	27 (90.0)	306 (96.2)	
***C. trachomatis* detected (n=350)**				
Yes	29 (8.3)	5 (16.7)	24 (7.5)	0.089
No	319 (91.7)	25 (83.3)	294 (92.5)	
***T. vaginalis* detected (n=350)**				
Yes	11 (3.1)	2 (6.9)	9 (2.8)	0.241
No	335 (96.8)	27 (93.1)	308 (97.2)	
Any of curable STI detected (n=350)				
Yes	51 (14.6)	10 (33.3)	41 (12.8)	0.005
No	299 (85.4)	20 (66.7)	279 (87.2)	

*previous adverse pregnancy includes; early neonatal death, stillbirth and preterm birth : STI; Sexually transmitted infections

† Chi-square

Seroprevalence

Seropositivity of CMV IgM antibodies was 8.2% (49/600) (95% CI: 6 -10.5%), whereas seroprevalence of CMV IgG was 88.7% (532/600), (95% CI: 89.5 - 94.0%). Of 532 CMV IgG positive women, 483 (80.4%) were negative for IgM. Among all pregnant women, 68 (11.4%) were tested negative for both anti-CMV IgG and IgM, and none showed anti-CMV IgG negativity but IgM positivity (Table 2).

Table 2 Cytomegalovirus IgM and IgG test result of pregnant women

	Anti CMV IgG antibody n (%)		Total n (%)
Anti CMV IgM antibody	Positive	Negative	
Positive	49 (8.2)	0 (0)	49 (8.2)
Negative	483 (80.4)	68(11.4)	551 (91.8)
Total	532 (88.7)	68(11.4)	600

CMV seropositivity and associated factors

In bivariable analysis, seropositivity of CMV IgM was more common in elder women (>35) compared to the youngest age group (<25), in women who were currently unmarried, giving preterm birth, sharing a feeding cup with children or having nursery schooled children. Moreover women were positive for any of curable STIs also had a higher seroprevalence of CMV compared to those negative for STIs (Table 3).

Furthermore, in multivariable logistic regression, being over the age of 30 years had higher odds for CMV IgM seropositivity compared to being under 25 (AOR = 4.9, 95% CI: 1.0–23.4), currently

unmarried women (AOR = 3.8, 95% CI: 1.7–7.9), preterm birth (AOR = 3.9, 95% CI: 1.5–10.3) and having nursery schooled children (AOR = 2.7, 95% CI: 1.1–6.4). Mothers with STIs had an association with seroprevalence (AOR = 4.1, 95% CI: 1.6–10.6) compared to mothers who were diagnosed negative for STIs.

Maternal seroprevalence was not significantly associated with residence, education level, occupation, being employed in a child daycare centre, or being a health care worker (Table 3).

Table 3. Unadjusted and adjusted associated factors of maternal CMV IgM seropositivity in Southern Ethiopia

Characteristics	Un adjusted *		Adjusted *	
	OR 95% CI)	P-value	OR (95% CI)	P-value
Age of mothers (years)				
<25	1		1	
25-29	1.5 (0.7 -3.2)	0.318	1.2 (0.4 – 4.0)	0.739
30- 35	1.5 (0.7 -3.2)	0.283	3.0 (1.0 – 9.0)	0.048
>35	3.2 (1.2– 9.1)	0.026	4.9 (1.0 – 23.4)	0.047
Marital status				
Married	1		1	
Currently unmarried	2.2 (1.1 -4.3)	0.030	3.8 (1.3 -11.2)	0.015
Residence				
Urban	2.5 (1.0 -6.3)	0.064	2.3 (0.7 -7.9)	0.171
Rural	1		1	
Daycare worker				
Yes	2.1 (0.8 -5.3)	0.110	1.1 (0.2- 5.4)	0.857
No	1		1	

Health care worker				
Yes	2.8 (1.1 - 7.2)	0.031	1.2 (0.2 – 7.4)	0.841
no	1		1	
Education				
Primary and below	0.6 (0.3 -1.1)	0.111	0.7 (0.3 -1.8)	0.475
Secondary and above	1		1	
Gestational age				
Term	1		1	
Preterm	3.5 (1.8 – 7.1)	<0.001	3.9 (1.5 -10.3)	<0.006
Daycare or Nursery school baby				
Yes	2.4 (1.3 – 4.5)	0.004	2.7 (1.1 – 6.4)	0.027
no	1		1	
Sharing a cup with children				
Yes	2.0 (1.1 – 3.8)	0.044	2.2 (0.9 – 5.4)	0.074
no	1			
Any of curable STIs (n=350)				
Yes	3.4 (1.5- 7.8)	0.004	4.1 (1.6- 10.6)	0.003
No	1			

* Logistic regression

Regarding CMV IgG seropositivity, women within the age group of 30-35 have shown a significantly higher risk of CMV IgG positivity compared to women of <25 years in bivariable analysis. However, the detected significant risk in the bivariable analysis in this study is not sustained in the multivariable analysis. The discrepancy possibly due to the fact that most of the exposure to CMV infection were in early childhood in developing countries like Ethiopia so that CMV IgG seroprevalence seem comparable in all age category . In addition, participants might

have a similar type of behaviour at different ages. Furthermore, women having primary or lower educational levels are associated with a higher risk of CMV IgG seropositivity than having secondary or above educational levels in bivariable analysis (OR 1.7). But not associated in adjusted analysis this might be due to the common exposure and awareness level of the study participants.

In this study, women having nursery schooled children shown association both in bivariable and multivariable analysis (AOR = 1.8, 95% CI: 1.0–3.0). However, the other maternal characteristic were not associated with CMV IgG seropositivity (Table 4).

Table 4. Unadjusted and adjusted associated factors of maternal CMV IgG seropositivity in Southern Ethiopia

Characteristics	Un adjusted *		Adjusted *	
	OR 95% CI)	P-value	OR (95% CI)	P-value
Age of mothers (years)				
<25	1			
25-29	1.2 (0.7 -2.3)	0.482	1.0 (0.5- 1.9)	0.991
30- 35	2.2 (1.1 -4.3)	0.028	1.8 (0.9- 3.8)	0.095
>35	1.3 (0.4– 3.9)	0.663	0.9 (0.3- 1.0)	0.877
Marital status				
Married	1			
Currently unmarried	0.8 (0.37 – 1.7)	0.573		
Residence				
Urban	0.8 (0.4- 1.5)	0.493		
Rural	1			

Daycare worker				
Yes	0.9 (0.3- 2.3)	0.810		
No	1			
Health care worker				
Yes	0.9 (0.3 – 2.6)	0.831		
no	1			
Education				
Primary and below	1.7 (1.0 – 2.8)	0.041	0.6 (0.4- 1.0)	0.037
Secondary and above	1			
Gestational age				
Term	1			
Preterm	0.9 (0.4-1.9)	0.756		
Daycare or Nursery school baby				
Yes	1.9 (1.1 – 3.1)	0.017	1.8 (1.0- 3.0)	0.045
no	1			
Sharing a cup with children				
Yes	1.1 (0.6 – 2,3)	0.705		
no	1			
Any of curable STIs (n=350)				
Yes	0.8 (0.3 – 1,9)	0.578		
No	1			

* Logistic regression

Discussion

In this study an overall seroprevalence of 8.2% for CMV IgM and 88.7% for CMV IgG were detected among pregnant women in southern Ethiopia. Factors associated with CMV IgM seropositivity were age, marital status, the presence of curable STIs and sharing a cup with children. A statistically significant association was also observed between CMV seropositivity and preterm delivery.

The reported seropositivity of CMV IgG (88.7%) in this study was comparable to a result found in previous study of pregnant women in central Ethiopia (88.5%) however, a substantially higher rate of CMV IgM (15.5%) was documented compared to our finding. (8) Furthermore, seropositivity of CMV IgG in our study was in line with a review done in Africa, with ranges from 60% to 100%. (11) Seropositivity rates of 77.3% for IgG and 8.1% for IgM in Kenya, (9) 93% for IgG and 11.1% for IgM in Nigeria,(16) 94% for IgG and 8.5% for IgM in Tanzania (17) were also comparable to our finding.

In our study seroprevalence of CMV IgM is in concordance with several African studies.(9, 16-18) However, our rate was considerably higher when compared to 0.4% in Tanzania, (19) 2.5% in Sudan, (20), and 7% in Egypt. (21) In the absence of maternal screening this high rate is alarming for policy makers. By far in most of the developed countries, pregnant women are screened for CMV due to the panic effect and consequence to the developing fetus and newborn. (22, 23) However, in most developing countries including Ethiopia, maternal CMV still lacks awareness, is overlooked and not diagnosed at least for pregnant women. (24) The high rate of CMV IgM may not only reflect primary infection but might also be attributed to reinfection or reactivation of CMV during pregnancy. So the reported high seropositivity in this study point to the existing negligence

and the need to start screening to detect pregnant women at risk for congenital transmission of CMV.

Earlier studies have shown that there was considerable debate regarding the relationship between maternal age and CMV seroprevalence. In this study, elder age had significant association with CMV IgM seroprevalence but no association observed in CMV IgG. The same finding was reported in Kenya, (25), in Nigeria, (26) and Tanzania. (19) However, a study in Ethiopia, (8) in Egypt (21) Sudan, (20), China, (27) and in Nigeria (28) have reported that age had no association with maternal CMV infection. Unlike our finding, studies in Iraq a high rate of CMV IgM in young women was reported (29), whereas, in USA CMV IgM seroprevalence varied by age with the highest among younger and lower among those elder age was reported. (30) The increment of association of CMV IgM seroprevalence with age in this study may indicate the more lifetime episodes exposure of elders than youngsters or might be the existence of previous infection which can probably reactivated in the current pregnancy. In fact, the observed difference in CMV IgM seroprevalence by age may be useful to realize the risk of cCMV transmission and are useful for ascertaining target populations for intervention to reduce congenital CMV transmission. (30)

On the other hand, mother who have nursery schooled children among households has shown a significant association with seroprevalence of both CMV IgM and IgG. For pregnant women, the predictable source of CMV infection is young children mainly exposure to nursery schooled children. (31) Children easily get infected in school and frequently shed CMV in their saliva or urine for many years continuously that could spread readily even in a preschool setting. (32) This places seronegative pregnant women who have a young child in the home or in day care at increased risk of seroconversion. (33, 34) Susceptibility to the acquisition of CMV infection is

high possibly through the direct contact with contagious secretions from their children essentially in a situation of poor hygienic practice like in Ethiopia. (35)

Among candidate predictors for maternal CMV seropositivity, occupations like being health care worker or child day-care worker; being multigravida, lower educational level and having other children at home did not shown any association. However, there was significant association for those with preterm delivery. Although, maternal CMV infection may result in preterm delivery, its isolated impact could not be assessed since we did not study other potential confounding factors.(36)

Likewise, CMV IgM seropositivity was found to be significantly associated with STIs detected at delivery and currently being unmarried. Mothers who were positive for any of curable STIs had a four-time CMV IgM seropositivity. It is also reported that STIs including CMV to be more common in unmarried pregnant women.(31, 35, 37) Although cytomegalovirus is a virus that is transmitted through many body fluids, sexual transmission from a seropositive male partner is an additional established route by which women may be infected with CMV.(38) Indeed, it is somehow expected that sexual transmission is also responsible for the reinfection of seropositive mothers with different virus strains in high-seroprevalence populations.(39)

Although, CMV IgG avidity testing is a valuable laboratory tool for distinguishing primary from non-primary CMV infection, an avidity test was not performed in this study. Hence, this study lacks differentiation of CMV IgM positivity of either primary or non-primary (reinfection or reactivation) as we collected samples at the end of the pregnancy period that avidity test will not be suitable. Moreover, it was a hospital-based study and not representative of all pregnant mothers in the locality since a significant portion of mothers may not deliver in the hospital. We

lack also appropriate risk factors assessment tool due to the cross-sectional nature of the study, hence a more representative large-scale survey is needed to identify possible risk factors prospectively. Furthermore, this is the first study from the Southern region in Ethiopia and that makes the first awareness in medical, governmental and societal stakeholders. Thus, more studies may need to be accomplished before the introduction of appropriate measures.

Conclusion

In this study, we identified a high rate of both CMV IgM and CMV IgG seropositivity among pregnant women in southern Ethiopia. The presence of curable STIs, elder age and unmarried women showed a significant association with CMV IgM seropositivity. Furthermore, having nursery schooled children showed a significant association with CMV IgM and IgG seropositivity. Given that there is no existing CMV diagnostic facility, special attention should be designed to pregnant women in parallel to the existing antenatal care service. Besides, training health care professionals will support awareness conception for pregnant women concerning the sequels of CMV infection during pregnancy.

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interpretation. All authors provided critical review and contributed to the write-up and approved the final version of the manuscript. MHZ had final responsibility to submit for publication. All authors read and amended drafts of the paper and approved the final version.

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Competing interests: no competing interest

Patient consent: The significance of the study was clarified to each study participant and parents of a few minorities prior to obtaining permission to participate in the study. Participation was fully voluntary and informed consent was obtained from all participants who were 18 years old or above. Informed consent was also obtained from the parents or legal guardians of the participants who were under the age of 18 years prior to their enrolment in the study. Confidentiality of the participant's information was ensured by anonymous typing.

Ethics approval: Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study. The study was conducted in accordance with the Declaration of Helsinki.

Data sharing statement: Data are available upon reasonable request. All available data can be obtained by contacting the corresponding author.

Competing interests

The authors declare that they have no competing interest.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalizability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.