Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 among Egyptian Children Aged from One to 15 Years old: A Comparative Study

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Background and study aim: Recent research has highlighted the importance of monitoring trends in herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) seroprevalence and their etiological surveillance. In this study, we aim to evaluate the seroprevalence of HSV1 and HSV2 among Egyptian children aged from one to 15 years old.

Materials and Methods: This study was based on a sample of children who regularly attended Misr University for Science and Technology University hospital's outpatient clinics. The participants in the study ranged in age from 1 to 15 years old. An ELISA kit tested blood samples for HSV-1 and HSV-2 IgG and IgM.

Results: In the present survey, 123 children were included. Overall, the median IgM and IgG values for the HSV type I study cohort were 0.39 (range, 0.0-3.07) and 1.50 (range, 0.02-3.89), respectively. The median IgM and IgG values for the HSV type II study cohort were 0.32 (range, 0.00-2.11) and 0.42 (range, 0.00-2.87), respectively. The prevalence of HSV type I among study candidates was 56.1%. However, the prevalence of type II was 4.1%. In comparison with the different age groups, it was found that the IgM levels in both HSV I and II have no difference (P-value = 0.701 and P-value = 0.576, respectively). In HSV I patients, there was a significant difference in IgG levels between age groups (P-value = 0.001), but no difference was found in HSV II patients (P-value = 0.870).

Conclusion: HSV1 and HSV2 seroprevalence is prevalent among Egyptian children.

INTRODUCTION

The final stage of the liver's Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) are widespread human pathogens that can cause orolabial and vaginal infections, with the majority of infections occurring during childhood **[1,2]**. Mainly, HSV is asymptomatic and self-limited; however, in the presence of symptoms, ulcerative lesions develop at the infection site, particularly in neonates, young children, and immunocompromised hosts **[3,4]**. HSV-1 is traditionally considered a vesicular lesion pathogen. Nevertheless, increasing evidence suggests that adolescents and young adults develop genital HSV-1 infections **[5]**. HSV transmission from infected moms to newborns could result in significant neurological disorders or an increase in neonatal deaths **[6]**. Furthermore, in wealthy nations, HSV ocular infection is considered the most common cause of corneal blindness **[7]**. HSV infection can cause mild to severe diseases, including encephalitis, gingivostomatitis, and meningitis **[8]**. Moreover, a significant link between HSV infection and Alzheimer's disease and other dementias was proposed **[9]**.

The global prevalence of HSV is 3.6 billion people infected orolabially and half a billion genitally **[10]**. Due to ongoing improvements in hygiene and living conditions, HSV-1 antibodies seroprevalence appears to be declining in western countries and around 30% among adolescents in the United States over the last three decades **[11,12]**. In the Middle East, it

was estimated that the seroprevalence of HSV1 was 80.5% among Palestinians, 81.4% among Lebanese, 82.3% among Qataris, and 88.5% among Syrians, 92.6% among Yemenis, and 97.5% among Egyptians **[13]**. Furthermore, seroprevalence among Egyptians was continuously above 90% at all ages. Another study found that HSV1 and HSV2 were found in 80% of healthy Egyptian children **[14]**. These records highlight the importance of monitoring the trends in HSV1 and HSV2 seroprevalence and their etiological surveillance. As a result, this study aimed to determine the seroprevalence of HSV1 and HSV2 among Egyptian children aged 1 to 15 years old.

MATERIALS AND METHODS

Patients and study design:

The present study follows the STROBE (Standards for Strengthening the Reporting of Observational Studies I Epidemiology) guidelines **[15]**. This study was based on a sample of children who attended outpatient clinics at Misr University for Science and Technology University hospital over time. The participants in the study ranged in age from 1 to 15 years old. Each participant had a venous blood sample obtained to determine blood lipid concentrations. At room temperature, the blood samples were allowed to coagulate. After that, each sample was centrifuged two hours after harvesting. The serum was then transferred to a storage container and stored at -20°C.

Serologic testing:

ELISA was used to test blood samples for HSV-1 and HSV-2 IgG and IgM, as per the manufacturer's recommendations (HerpeSelect, Focus Technologies, Inc., Cypress, CA) [16]. After centrifugation, samples with index values between 0.8 and 1.2 were re-evaluated using the sample supernatant (15000 rpm for 5 min on an Eppendorf 5804 microfuge). Because it was regulated using a reference serum that the manufacturer had modified to obtain a cut-off value that was erroneously assigned the Index value of 1, the OD was incorrectly assigned the Index value of 1. An immunoblot was utilised to eliminate undecided samples (HerpesSelect 1 & 2 Immunoblot, Focus Technologies, Inc).

Statistical analysis:

SPSS version 22.0 for Windows was used to analyse the data. The mean and standard deviation were used to determine the results (SD). The Kolmogorov-Smirnov test was used to determine the normality for continuous variables. A P-value of less than 0.05 was considered statistically significant (two-sided testing). A one-way ANOVA test was used to compare the outcomes of the different age groups' tests. The Chi-square test was used to examine if there were any significant differences between proportions and categorical variables.

RESULTS

In this survey, the 123 children approved the participation and were included in this study through our outpatient clinic in Misr University for Science and Technology University hospital's outpatient clinics. The study Cohort was segmented regarding age groups into three groups. Group one had children under five years old (n = 41). Group two included children aged between five and ten (n = 41). Group three included children aged more than ten and less than fifteen years old (n = 41).

Seroprevalence of HSV Type I and Type II:

Overall, the median IgM and IgG values for the HSV type I study cohort were 0.39 (range, 0.0-3.07) and 1.50 (range, 0.02-3.89), respectively. The median IgM and IgG values for the HSV type II study cohort were 0.32 (range, 0.00-2.11) and 0.42 (range, 0.00-2.87), respectively. The prevalence of HSV type I among study candidates was 56.1%. However, the prevalence of type II was 4.1% **(Figure 1).**

Seroprevalence of HSV and age groups:

Regarding age group one (less than five years old), the median IgM and IgG values of HSV type I was 0.35 (range, 0.00 - 2.11) and 0.54 (range, 0.02 - 2.70), respectively. In HSV type II, the median IgM and IgG were 0.30 (range, 0.00 - 0.81) and 0.41 (range, 0.00 - 0.94), respectively. The prevalence of HSV type I in this group was 17.1%. However, the prevalence of type II was 0.0%. Concerning the second group (five to ten years old), the median IgM and IgG values of HSV type I were 0.30 (range, 0.01 - 3.00) and 1.33 (range, 0.03 - 3.87), respectively. In HSV type II, the median IgM and IgG were 0.42 (range, 0.01 - 0.85) and 0.45 (range, 0.03 - 0.85),

respectively **(Table 1).** In this group, the prevalence of HSV type I was 61.0%. However, the prevalence of type II was 0.0%. Regarding group three (more than ten and less than fifteen years old), the median IgM and IgG values of HSV type I were 0.40 (range, 0.01 - 3.07) and 2.56 (range, 0.12 - 3.89), respectively. In HSV type II, the median IgM and IgG were 0.29 (range, 0.00 - 2.11) and 0.34 (range, 0.01 - 2.87), respectively. The prevalence of HSV type I among this group was 90.2%. However, the prevalence of type II was 12.2% **(Table 2).** Compared with the different age groups, the IgM levels in both HSV I and II have no difference (*P*-value = 0.701 and *P*-value = 0.576, respectively). Regarding IgG levels, there was a significant difference between the different age groups for HSV I (*P*-value = 0.001). However, no difference was detected in HSV II patients (*P*-value = 0.870).

Table (1): The serum levels of IgM and IgG of HSV in the study cohort.

Parameters	1 to 5 years (n=41)	More than 5 and less than 10 (n=41)	More than 10 and less than 15 years (n=41)	P value
HSV-I IgM, ng/mL	0.35 (0.0 – 2.11)	0.30 (0.01 – 3.0)	0.41 (0.01 – 3.07)	0.701
HSV-I IgG, ng/mL	0.54 (0.02 – 2.7)	1.33 (0.03 – 3.87)	2.56 (0.12 – 3.89)	**0.001**
HSV-II IgM, ng/mL	0.30 (0.0 – 0.81)	0.42 (0.01 – 0.85)	0.29 (0.00 – 2.11)	0.576
HSV-II IgG, ng/mL	0.41 (0.0 – 0.94)	0.45 (0.03 – 0.85)	0.34 (0.01 – 2.87)	0.870

Table (2): The prevalence of HSV in the different age groups

Prevalence	1 to 5 years old (n=41)	More than 5 and less than 10 years old (n=41)	More than 10 and less than 15 years old (n=41)	P value
HSV type I, %	17.1%	61.0%	90.0%	**0.01**
HSV type II, %	0.0%	0.0%	12.2%	**0.01**

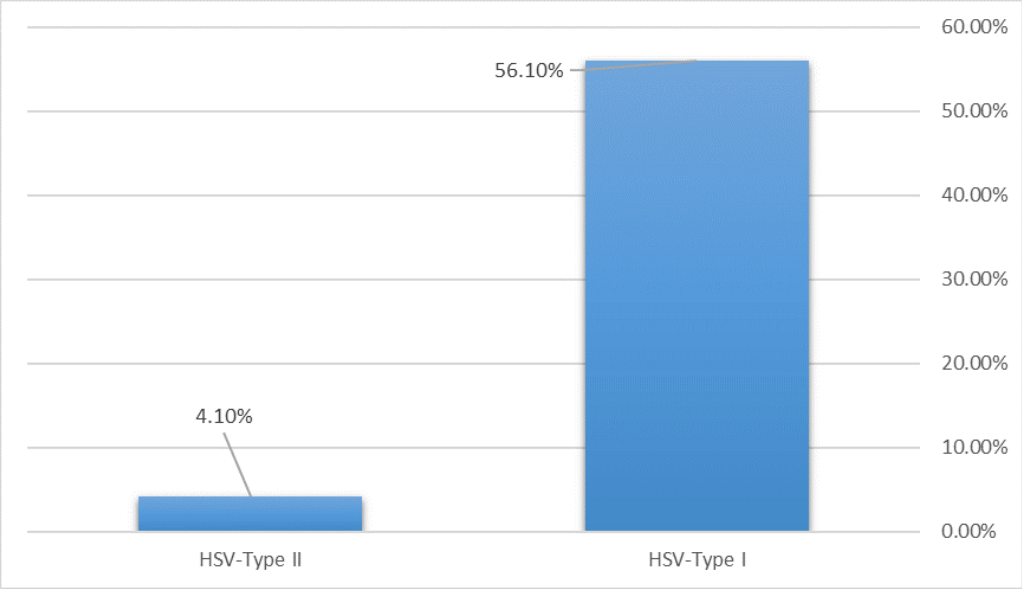

Figure (1): The prevalence of *HSV* in the study cohort.

DISCUSSION

In this observational study, we evaluated the seroprevalence of HSV-1 and HSV-2 in Egyptian children, which proved to be widespread, especially among those over the age of ten. This is the first Egyptian study that we are aware of that looked at HSV seroprevalence in healthy children. Loutfy et al. **[14]**, The seroprevalence of HSV-1 and 2 (IgG) was 69% in children with acute leukaemia and 80% in the standard control

group. Moreover, they found that the prevalence was higher in children aged 5-10 years and older than ten years, supporting our findings. On the other hand, they showed that the prevalence was comparable between both genders (70% in males and 66.6% in females).

Regarding HSV-2, we found that the prevalence in children for more than ten years and less than 15 years was 12.2%. According to large-scale studies in Asia, the overall prevalence of HSV-2 seropositivity is around 10% **[17,18]**. A seropositive HSV-2 prevalence of 15 to 29% was reported in several investigations around the end of the twentieth century. However, these studies were largely hospital-based or focused on the adult population **[19–21]**.

Many studies were conducted in the Middle East to estimate the seroprevalence of HSV; however, the data regarding healthy children were scanty. In Netherlands, the seroprevalence of HSV-1 ranged between 42.7% to 47.7% **[22]**. The authors have also found that adults who ever had sexual intercourse were more often seropositive for HSV-1 [adjusted Odds Ratio (aOR) 1.69 95 % CI 1.33-2.16] and HSV-2 (aOR 2.35 95 % CI 1.23-4.52). Age at sexual debut was the only sexual risk determinant associated with HSV-1 seropositivity. In another study conducted by Woestenberg et al. **[23],** Adults who ever had sexual intercourse were more often seropositive for HSV-1 [adjusted Odds Ratio (aOR) 1.69 95 % CI 1.33-2.16] and HSV-2 (aOR 2.35 95 % CI 1.23-4.52). Age at sexual debut was the only sexual risk determinant associated with HSV-1 seropositivity.

In the middle East and North African male populations residing in Qatar, country-specific HSV-1 seroprevalence was estimated for 10 national populations: 97.5% among Egyptians, 92.6% among Yemenis, 90.7% among Sudanese, 88.5% among Syrians, 86.5% among Jordanians, 82.3% among Qataris, 81.4% among Iranians, 81.4% among Lebanese, 80.5% among Palestinians, and 77.0% among Pakistanis **[23]**. Seroprevalence increased with age among Fertile Crescent and Qatari nationals. Seroprevalence increased from 70.0% among those aged ≤ 24 years up to 98.0% among those aged ≥55 years among Fertile Crescent nationals **[23]**. According to a recent comprehensive analysis, HSV-1 seroprevalence was 65 % in Middle Eastern youngsters **[24]**. This high level of HSV-1 indicates considerable HSV-1-related morbidity in these populations. The bulk of Middle East studies found seroprevalence increases with age at younger ages, which was consistent with most infections occurring in childhood. The average seroprevalence in Asian children was over 60%, while in adults, seroprevalence was roughly 30% higher than in children **[25–27]**. The age of children was also the strongest predictor of HSV-1 seroprevalence **[28].** Although socioeconomic conditions can affect the prevalence of HSV-1, Chaabane et al. showed that 44% of the variation in seroprevalence is due to age **[24].** According to Yousuf et al. **[29]**, age accounts for roughly half of the range in seroprevalence in other countries, with the striking exception of Africa, where age accounts for 80% of the variation **[29]**. However, the role of early speculation cannot be neglected, especially in children.

The situation is slightly different in adults; young people had much lower HSV-1 seroprevalence than older adults **[24]**. Remarkably, In the Middle East and North Africa (MENA) region, there was inadequate evidence for HSV-1 sexual transmission to play a role. According to Chaabane et al., they were unable to find a single study in this region that looked into the role of HSV-1 in the aetiology of genital herpes **[24]**. However, in North America, the detection of HSV-1 in genital herpes was 34% higher than HSV-2 **[25]**. The differences in sexual culture between North America, Europe, and MENA can be attributed to differences in sexual transmission. The most common route for HSV-1 infection in early infants may be via non-sexual transmission. The most common mechanism of viral transmission among people was close contact between young children (for example, in daycare centres or among family members) and adults and children. HSV-1 serostatus may be affected by childhood hygiene and living conditions as well **[30]**.

In wealthy countries, seroprevalence was found to be lower, showing a global link between HSV-1 infection and socioeconomic position [31]. Compared to those wealthy countries in MENA, such as Iran, Jordan, and Qatar, low to middle-income countries like Egypt, Sudan, and Yemen had the highest seroprevalence **[32]**. Asia's faster modernisation can explain this disparity between the Middle East and North Africa.

This epidemiological situation may end up constituting a public health risk. Genital herpes has been related to a higher risk of HIV infection

[33]. Second, pregnant women with primary genital herpes can transfer HSV-1 to their newborns during labour and delivery, and neonatal HSV infections have a high risk of significant morbidity **[34,35]**. A rise in the frequency of ocular infection, in addition to vaginal infection, may be connected to age-specific HSV-1 seroprevalence **[9]**.

Recommendations:

Since we evaluate only the seroprevalence of HSV1 and HSV2 among Egyptian children aged from one to 15. We recommend adding more questions in sociodemographic data to be more valuable in the study to find its relation to the seroprevalence of HSV1 and HSV2 among Egyptian children aged 1 to 15. Also, larger study with bigger sample sizes should be the logical forward step following the currently available literature.

In conclusion, HSV-1 and HSV-2 seroprevalence is high in Egypt, particularly among older children (10-15 years old), highlighting the necessity for ongoing surveillance of HSV-1 and 2 seroprevalences, as well as etiological surveillance of linked morbidities.

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Ethical considerations:

All participants were required to sign a consent before withdrawing the blood samples. This consent involves the potential risk and the detailed information about the study but not principally for the assays of Herpes simplex antibodies. The Institutional Review Board of MUST University authorised the usage of these serum samples.

HIGHLIGHTS

- Herpes Simplex Virus -1 and 2 seroprevalence is high in Egypt among children aged from one to 15 years old.
- The prevalence is particularly high among children (10-15 years old).
- The study is highlighting the necessity for ongoing surveillance of HSV-1 and 2 seroprevalences.
- Etiological surveillance of linked morbidities should be addressed in HSV patients.

REFERENCES

1. Gnann JW, Whitley RJ. Genital Herpes. Solomon CG, editor. *N Engl J Med* 2016; 375: 666–674.
2. Gupta R, Warren T, Wald A. Genital herpes. *Lancet*. 2007; 370: 2127–2137.
3. Muller W, Jones C, Koelle D. Immunobiology of Herpes Simplex Virus and Cytomegalovirus Infections of the Fetus and Newborn. *Curr Immunol Rev*. 2010; 6: 38–55.
4. Gantt S, Muller WJ. The Immunologic Basis for Severe Neonatal Herpes Disease and Potential Strategies for Therapeutic Intervention. *Clin Dev Immunol.* 2013;2013:1–16.
5. Shen JH, Huang KY, Chao-Yu C, Chen CJ, Lin TY, Huang YC. Seroprevalence of Herpes Simplex Virus Type 1 and 2 in Taiwan and Risk Factor Analysis, 2007. Suvas S, editor. *PLoS One.* 2015;10:e0134178.
6. Corey L, Wald A. Maternal and Neonatal Herpes Simplex Virus Infections. *N Engl J Med.* 2009;361:1376–1385.
7. Azher TN, Yin XT, Tajfirouz D, Huang AJ, Stuart PM. Herpes simplex keratitis: challenges in diagnosis and clinical management. *Clin Ophthalmol.* 2017; 11: 185–191.
8. Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: Epidemiology, pathogenesis, symptomatology, diagnosis, and management. *J Am Acad Dermatol.* 2007; 57:737–763.
9. Bahbah EI, Fathy S, Negida A. Is Alzheimer's disease linked to Herpes simplex virus type 1 infection? A mini-review of the molecular correlation and the possible disease connections. *Clin Exp Neuroimmunol.* 2019; 10: 192–196.
10. Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. DeLuca NA, editor. *PLoS One*. 2015; 10:e0140765.
11. Xu F, Lee FK, Morrow RA, Sternberg MR, Luther KE, Dubin G, et al. Seroprevalence of Herpes Simplex Virus Type 1 in Children in the United States. *J Pediatr*. 2007; 151: 374–377.

12. Bernstein DI, Bellamy AR, Hook EW 3rd, Levin MJ, Wald A, Ewell MG, et al. Epidemiology, Clinical Presentation, and Antibody Response to Primary Infection With Herpes Simplex Virus Type 1 and Type 2 in Young Women. *Clin Infect Dis.* 2013; 56: 344–351.

13. Nasrallah GK, Dargham SR, Mohammed LI, Abu-Raddad LJ. Estimating seroprevalence of herpes simplex virus type 1 among different Middle East and North African male populations residing in Qatar. *J Med Virol.* 2018; 90: 184–190.

14. Loutfy SA, Alam El-Din HM, Ibrahim MF, Hafez MM. Seroprevalence of herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus in children with acute lymphoblastic leukemia in Egypt. *Saudi Med J.* 2007;27:1139–1145.

15. Elm E v, Altman D G, Egger M, Pocock S J, GÃ¸tzsche P C, Vandenbroucke J P et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement : Guidelines for reporting observational studies. *Int J Surg.* 2014; 12:1495–1499.

16. Hogrefe W, Su X, Song J, et al. Detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in African sera by using recombinant gG2, Western blotting, and gG2 inhibition. *J Clin Microbiol.* 2002.

17. Hogrefe W, Su X, Song J, Ashley R, Kong L. Seroprevalence of Herpes Simplex Virus 1 and 2 in a Population-Based Cohort in Japan. *J Epidemiol.* 2009; 19: 56–62.

18. Lin H, He N, Su M, Feng J, Chen L, Gao M. Herpes simplex virus infections among rural residents in eastern China. *BMC Infect Dis.* 2011; 11:69.

19. Memish ZA, Almasri M, Chentoufi AA, Al-Tawfiq J, Al-Shangiti A. Al-Kabbanim K, et al. Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 and Coinfection With HIV and Syphilis. *Sex Transm Dis*. 2015;42:526–532.

20. Issakwisa HM, Mbwile GR, Mbwanji GF, Nassoro DD, Ntinginya NE, Nsojo AA. Seroprevalence of herpes simplex virus type 1 among people living with HIV in Mbeya, Tanzania. *BMC Infect Dis*. 2020;20:577.

21. Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. DeLuca NA, editor. *PLoS One* 2015; 10: e0140765.

22. Woestenberg PJ, Tjhie JH, de Melker HE, van der Klis FR, van Bergen JE, van der Sande MA, et al. Herpes simplex virus type 1 and type 2 in the Netherlands: seroprevalence, risk factors and changes during a 12-year period. *BMC Infect Dis*. 2016;16:364.

23. Nasrallah GK, Dargham SR, Mohammed LI, et al. Estimating seroprevalence of herpes simplex virus type 1 among different Middle East and North African male populations residing in Qatar. *J Med Virol*. 2018;90:184–190.

24. Nasrallah GK, Dargham SR, Mohammed LI, Abu-Raddad LJ. Herpes simplex virus type 1 epidemiology in the Middle East and North Africa: systematic review, meta-analyses, and meta-regressions. *Sci Rep*. 2019;9:1136.

25. Khadr L, Harfouche M, Omori R, Schwarzer G, Chemaitelly H, Abu-Raddad LJ. The Epidemiology of Herpes Simplex Virus Type 1 in Asia: Systematic Review, Meta-analyses, and Meta-regressions. *Clin Infect Dis.* 2019;68:757–772.

26. Roberts CM, Pfister JR, Spear SJ. Increasing Proportion of Herpes Simplex Virus Type 1 as a Cause of Genital Herpes Infection in College Students. *Sex Transm Dis.* 2003;30:797–800.

27. Samra Z, Scherf E, Dan M. Herpes Simplex Virus Type 1 Is the Prevailing Cause of Genital Herpes in the Tel Aviv Area, Israel. *Sex Transm Dis.* 2003;30:794–796.

28. Gilbert M, Li X, Petric M, Krajden M, Isaac-Renton JL, Ogilvie G, et al. Using centralized laboratory data to monitor trends in Herpes Simplex Virus type 1 and 2 infection in British Columbia and the changing Etiology of genital Herpes. *Can J Public Heal.* 2011.

29. Yousuf W, Ibrahim H, Harfouche M, Abu Hijleh F, Abu-Raddad L. Herpes simplex virus type 1 in Europe: systematic review, meta-analyses and meta-regressions. *BMJ Glob Heal.* 2020; 5: e002388.

30. Nasrallah GK, Dargham SR, Abu-Raddad LJ. Negative epidemiological association between HSV-1 and HSV-2 infections. *Heliyon*. 2019; 5:e02549.

31. Ryder N, Jin F, McNulty AM, Grulich AE, Donovan B. Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992-2006. *Sex Transm Infect.* 2009;85:416–419.

32. Bradley H, Markowitz LE, Gibson T, McQuillan GM. Seroprevalence of Herpes Simplex Virus Types 1 and 2--United States, 1999-2010. *J Infect Dis.* 2014; 209: 325–333.

33. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: Systematic review and meta-analysis of longitudinal studies. *AIDS*. 2006.

34. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J, Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *J Am Med Assoc.* 2003.

35. Sappenfield E, Jamieson DJ, Kourtis AP. Pregnancy and susceptibility to infectious diseases. *Infect. Dis. in Obstet. Gynecol* 2013.