Analysis of Herpes Simplex 1 and 2 IgG and IgM Antibodies in Pregnant Women and their Neonates.

A. J. Alzahrani[1] MSc, PhD, O. E. Obeid[1] MSc, PhD, A. A. Almulhim[2] AB ObGyne, Jordanian Board ObGyne, B. Awari[3], Atya Taha, [4]Feryal. Al-Ajmi[2], Hatim K. Al-Turkistani[3]

[1]Department of Microbiology; [2]Department of Obstetrics and Gynecology; [3]Department of Pediatrics; [4]Department of FAMCO
College of Medicine, King Faisal University, Dammam, Saudi Arabia

Abstract

Objectives: (1) Assessment of prevalence of herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) antibodies in **pregnant mothers** visiting the **antenatal clinic** and **delivery room** in King Fahd Hospital of the University (KFHU) in Al-Khobar, Saudi Arabia and (2) assessment of prevalence of HSV-1 and HSV-2 antibodies in **cord blood** in the delivery room in KFHU.

Material and methods: Laboratory methods used included the type-specific enzyme linked immunosorbent assay to assess HSV-1 and HSV-2 IgM and IgG antibodies in the sera.

Results: generated from the samples of pregnant mothers (N=459) showed that 90.5% have detectable levels of HSV-1 IgG antibodies, 6.5% have detectable level of HSV-2 IgG antibodies, 4.3% have detectable levels of HSV-1 IgM antibodies and 0.5% have detectable level of HSV-2 IgM antibodies. As for the cord blood samples (N=459), the IgG antibody reactivity was exactly the same as the corresponding mothers for both HSV-1 and HSV-2. However, only three cord blood samples have detectable levels of HSV-1 IgM antibodies, and none have detectable antibodies for HSV-2 IgM antibodies.

Conclusions: Because both HSV-1 and HSV-2 can infect pregnant women and their neonates, assessment of HSV infection in pregnant women and neonates will help in proper management of HSV infection and will also be useful for epidemiological purposes.

Keywords: Herpes simplex virus 1 and 2, antibody screening, pregnant women.

INTRODUCTION

Herpes simplex virus (HSV) is classified in the alpha virinae subfamily within the family Herpesviridae. Two closely-related viruses are designated HSV types 1 and 2. HSV-1 is the usual cause of orolabial infection (gingivostomatitis or herpes labialis), whereas HSV-2 is the major cause of genital infection. However, either virus can infect either location (1-4).

Several modalities are available for the diagnosis of HSV infections (5-7). The benchmark method is viral culture, but it is not widely available in Saudi Arabia. Serology can establish current and past infection with HSV. It has also been used in research studies of the epidemiology of HSV and is very useful in unusual clinical situations (8-15).

The antibody response to HSV glycoprotein G (gG) is highly specific, and gG-based assays can accurately determine whether individuals have past infection with HSV-1 and/or HSV-2 (12-14).

Because genital HSV-2 infection is much more likely to recur than genital HSV-1 infection, the presence of antibody to HSV-2 and a compatible clinical history would be strong presumptive evidence that the disease was recurrent genital herpes (17-19).

Testing of pregnant women for HSV antibodies is usually done with a type-specific assay for HSV antibodies (20). Studies of neonatal HSV infections have generally shown that most infected infants are born to women who have no clinical history of recurrent genital herpes but who are HSV-2 antibody positive at term (21). Early identification of these women by serologic testing might be used as part of a strategy to prevent some perinatal transmission of HSV. It is generally agreed that identification of both unrecognized HSV-2-positive pregnant women and pregnant women who are HSV antibody negative but in danger of becoming infected is essential (22).

When a primary HSV-2 infection is contracted during pregnancy, the fetus is at high risk of acquiring HSV-2 infection at delivery. In the study population of almost 200 infants, an estimated 5% of neonatal HSV infections were intrauterine (23-25).

In one study specific antibody to type 2 herpes simplex virus was detected in 439 of 1355 pregnant women without a clinical history of genital herpes (32%)(18). 280 Asymptomatic shedding of virus in late pregnancy and at delivery was detected in 5 of 1160 cultures (0.43%). During the pregnancy 43 of the women who had antibody

to type 2 virus recognized their first symptomatic genital infection.

Detection of HSV IgM antibody in cord blood obtained by cordocentesis and in the blood of the neonate during the first week of life is also diagnostic of in-utero HSV infection (26).

Worldwide the number of individuals seropositive for HSV-1 and HSV-2 increases with age (27-28). Various studies have been published that evaluate the prevalence of HSV-1 and HSV-2 antibody in Saudi population [29-35].

Hossain A (36) investigated the seroepidemiology of infection due to HSV-1 in 224 Saudi children and 452 adults (healthy male blood donors and pregnant women) indirect immunofluorescence. The overall prevalence of antibodies was 60 per cent for HSV-1 in children whereas about 90 per cent of the adults showed the presence of antibodies to HSV-1. However, the author did not assess antibodies to HSV-2 and no comparison was made between HSV-1 and HSV-2 in this population.

Hossain A et al (37) found genital HSV infections in asymptomatic pregnant women and in male patients to vary between 61.1% and 16.6% depending on the technique. However, the authors did not assess antibodies to HSV-1 or HSV-2 and no comparison was made between HSV-1 and HSV-2 in this population.

Ghazi et al (38) assessed the seroprevalence of TORCH agents in 926 pregnant Saudi women and detected HSV-1 IgG antibodies in 90.9% of samples and detected HSV-2 IgG antibodies in 27.1% of samples. The authors did not assess IgM antibodies to either HSV-1 or 2 and they did not analyze the cord blood of the neonates born to the pregnant women in the study.

To our knowledge, there is no published data about the seroprevalence of HSV-1 and HSV-2 antibody in Saudi pregnant women AND their neonates. Because both HSV-1 and HSV-2 can infect pregnant women and neonates, assessment of HSV infection in both will help in proper management of HSV infection and will also be useful for epidemiological purposes

The current paper presents a hospital-based assessment of prevalence of HSV-1 and HSV-2 antibodies in pregnant mothers and their neonates who delivered in King Fahd Hospital of the University (KFHU) in Al-Khobar.

MATERIALS AND METHODS:

(a) Type of study:

A hospital-based cross-sectional study

(b) Study area, population and period of study:

All pregnant mothers who delivered in the third trimester at KFHU were included in the study. In addition, all neonates delivered at KFHU were included in this study. The period of the study was one year.

(c) Techniques Used for Data Collection:

Blood samples were collected from the subjects by venepuncture and serum samples were analyzed for HSV-1 and HSV-2 IgG and IgM antibodies using type-specific ELISA (Gull, USA). Briefly, patient serum is diluted with specimen diluent. The diluted serum is incubated with purified HSV antigens bound to ELISA plate wells. If antibodies to HSV are present they bind to the antigen and do not rinse off. Subsequently when enzyme labeled anti-human IgG/IgM is added to the reaction site, it binds to the immobilized antibodies. After washing and the addition of a chromogenic substrate and stopping reagent, specimens containing antibodies to HSV produce a color endpoint reaction which can be read with a standard ELISA reader. Results were interpreted according to the manufacturer's recommendations.

(d) Statistical Analysis:

Data were entered to a d-Base file. Bivariate analysis between the dependent and the independent variables were done through Chi-square, t-test and paired t-test as appropriate. Analysis of variance ANOVA was used to test difference in means for qualitative variables. Level of significance were set to be < 0.05 throughout the study.

RESULTS:

HSV-1 and HSV-2 IgG Antibodies in pregnant mothers:

Samples of pregnant mothers (N=459) were analyzed for IgG antibody levels against HSV-1 and HSV2 using ELISA.

As shown in Figure 1, 90.5% of samples have detectable levels of HSV-1 IgG antibodies wherease 6.5% of the samples have detectable level of HSV-2 IgG antibodies.

HSV-1 and HSV-2 IgM Antibodies in pregnant mothers:

Samples of pregnant mothers (N=459) were analyzed for IgM antibody levels against HSV-1 and HSV2 using ELISA.

As shown in Figure 2, 4.3% of samples have detectable levels of HSV-1 IgM antibodies and 0.5% have detectable level of HSV-2 IgM antibodies.

HSV-1 and HSV-2 IgG and IgM Antibodies in cord blood:

Samples of cord blood (N=459) were analyzed for IgG antibody levels against HSV-1 and HSV2 using ELISA.

The IgG antibody reactivity was exactly the same as the corresponding mothers for both HSV-1 and HSV-2.

However, only three cord blood samples had detectable levels of HSV-1 IgM antibodies, and none had detectable antibodies for HSV-2 IgM antibodies (Figure 3).

DISCUSSION:

When a primary HSV infection is contracted during pregnancy, the fetus is at high risk of acquiring HSV infection either intrauterine or at delivery. Therefore, screening of pregnant mothers for HSV-1 and HSV-2 is an important part of the antenatal check.

The seroprevalence of HSV-1 antibody in pregnant women in France was 68%. This is considerably lower than the values obtained in our study (90.5% for HSV-1). This could well be due to the fact that all our samples were from adults, where as the French study was conducted in general population of all ages. HSV-1 seropositivity is known to increase with age. The seropevalence of HSV-2 in the French study in the same population was 17.3%[28] where as it was 6.5% in our study. Since HSV-2 is mainly sexually transmitted, the difference in the HSV-2 seropositivity between the two studies could be explained by the differences in the sexual habits between the two communities. The Saudi community is a conservative community.

In Zaire, the seroprevalence of HSV-1 and HSV-2 antibodies in pregnant women was 85% and 32% respectively. The difference in the HSV-2 positivity between our studied population and the Zaire study could well be due to the assay used. The ELISA used in our report is highly specific to HSV-2.

The seropositivity for HSV-1 IgG antibodies in our study (90.5%) confirms the findings of previous investigators (33, 38).

The estimated seropositivity of HSV-2 IgG antibodies in our study (6.5%) is significantly less than the HSV-2 IgG antibodies reported by Ghazi et al (27.1%). This could well be due to regional differences within Saudi Arabia.

Using cell culture, Hossain et al estimated the positivity of HSV isolation in pregnant women to be 50%. The difference in positivity between our data and data generated using the cell culture may well be due to the difference in sensitivity between

serological diagnosis of HSV and detection of HSV by cell culture. Serological assays are far more sensitive than cell culture techniques.

Our data showed that the seropositivity of HSV IgG antibodies in pregnant women and their babies are identical. This is due to the placental transfer of IgG. Only three cord blood samples have detectable HSV-1 IgM antibodies. This is considerably less than the IgM positivity in their corresponding mothers because IgM does not cross the placental barrier. These three neonates may have HSV-1 infection inutero. Follow up of these babies is currently underway.

Detectable IgM antibodies in HSV infection usually reflect current or recent infection. In fact, most IgM-positive pregnant mother gave symptoms and signs suggestive of HSV infections although there were some IgM-positive asymptomatic mothers. The IgM-negative symptomatic mothers may well be suffering from reactivation of a latent HSV infection. IgM is usually negative in HSV reactivation

CONCLUSION:

HSV-1 and HSV-2 can infect pregnant women and their neonates, assessment of HSV infection among them will help in the proper management of HSV infection, besides being useful for epidemiological purposes.

ACKNOWLEDGEMNT:

The study was supported by a grant from the Deanship of Scientific Research, King Faisal University. The authors would like to thank all the members of the Department of Obstetric and Gynecology for allowing us to include their patients in the study.

REFERENCES:

1. Whitley RJ, Roizman B: Herpes simplex viruses, In Richman DD, Whitley RJ, Hayden FG, editors: *Clinical virology,* New York, 1997, Churchill Livingstone.
2. Reeves WC, Corey L, Adams HG, et al: Risk of recurrence after first episodes of genital herpes: relation to HSV type and antibody response, *N Engl J Med* 305:315, 1981.
3. Wald A, Corey L, Cone R, et al: Frequent genital herpes simplex virus 2 shedding in immunocompetent women: effect of acyclovir treatment, *J Clin Invest* 99:1092, 1997.
4. Alvin AM, Prober CG: Herpes simplex viruses. In Murray PM, Baron EJ, Pfaller

MA, et al, editors: *Manual of clinical microbiologt,* ed 7, Washington, DC, 1999, ASM Press.

5. Blank H, Burgoon CF; Baidridge GD, et al: Cytologic smears in the diagnosis of herpes simplex, herpes zoster, and varicella, JAMA 146:1410,1999.
6. Nahass GT, Goldstein BA, Zhu WY, et al: Comparison of Tzanck smear, viral culture, and DNA diagnostic methods in detection of herpes simplex and varicella - zoster infection, *JAMA* 268:2541, 1992.
7. Kimberlin DW, Lakeman FD, Alvin AM, et al: Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease: National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group,*J Infect Dis* l74:1162, 1996.
8. Ashley RL, Militonij, Lee F; et al: Comparison of Western blot (immunoblot) and glycoprotein G specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human *sera, J Clin Microbiol 2:662,* 1988.
9. Ashley R, Benedettij, Corey L: Humoral immune response to HSV-1 and HSV-2 viral proteins in patients with primary genital herpes,*J Med Virot* 17:153, 1985.
10. Goldstein LC, Corey L, McDougall JK, et al: Monoclonal antibodies to herpes simplex viruses: use in antigenic typing and rapid diagnosis,J InfrctDis 147:829,1983.
11. Goodyear HM, Wilson P Cropper L, et al: Rapid diagnosis of cutaneous herpes simplex infections using specific monoclonal antibodies, *Clin Exp Dermatol* 19:294, 1994.
12. Ashley RE, Militonij, Lee F; et al: Comparison of Westem blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera, *] Clin Microbiol* 26:662, 1988.
13. Lee FK, Coleman RM, Pereira L, et al: Detection of herpes simplex virus type 2-specific antibody with glycoprotein G, J *Clin Microbiol* 22:641, 1985.
14. Ashley RE, Wu L, Pickering JW, et al: Premarket evaluation of a commercial glycoprotein G-based enzyme immunoassay for herpes simplex virus type-specific antibodies, J*Clin Microbiol* 36:294, 1998.
15. KurtzJB: Specific IgG and IgM antibody responses in herpes-simplex virus infections, *Med Microbiol* 7:333, 1994.
16. Koutsky LA, Stevens CE, Holmes KK, et al: Under-diagnosis of genital herpes by current clinical and viral-isolation procedures, *N Engl J Med* 326:1533,

1992.

17. Corey L, Adams HG, Brown ZA, Homes KK: Genital herpes simplex virus infections: clinical manifestations, course, and complications, *Ann Intern Med* 98:958, 1983.

18. Kulhanjianj A, Soroush **V,** Au DS, et al: Identification of women at unsuspected risk of primary infection with herpes simplex virus type 2 during pregnancy, *N Engl j Med* 326:916, 1992.

19. Frenkel LM, Garratty EM, Shen JP, et al: Clinical reactivation of herpes simplex virus type 2 infection in seropositive pregnant women with no history of genital herpes, *Ann Intern Med* 118:414, 1993.

20. Brown ZA, Selke S, Zeh J, et al: The acquisition of herpes simplex virus during pregnancy, *NEngi] Med*

337:509, 1997.

21. Brown ZA, Benedettij, Ashley R, et al: Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor, *N Engl] Med* 324:1247, 1991.

22. Koutsky LA, Stevens CE, Holmes KK, et al: Under-diagnosis of genital herpes by current clinical and viral-isolation procedures, *N Engl] Med* 326:1533, 1992.

23. Hutto C, Alvin A, Jacobs R, et al: Intrauterine herpes simplex virus infections, *]Pediatr* 110:97, 1987.

24. Prober CG, Hensleigh PA, Boucher FD, et al: Use of routine viral cultures at delivery to identify neonates exposed to herpes simplex virus, *N Engl] Med* 318:887, 1988.

25. Grose C: Congenital infections caused by varicella zoster virus and herpes simplex virus, *Semin Pediatr Neuroll:43,* 1994.

26. Whitley R, Alvin A, Prober C, et al: Predictors of morbidity and mortality in neonates with herpes simplex virus infections: the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group, *NEngl]Med* 324:450, 1991.

27. Johnson RE, Nahmias AJ, Magder LS, et al: A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States, *N Engl JMed32l:7,* 1989.

28. Whitley RJ: Herpes Simplex Viruses. In Knipe DM & Howley PM, editors: *Fields Virology,* New York, 2001, Lippincott.

29. Al-Ahdal MN, Kessie G, Taha MA, al-Shammary FJ, Ettayebi M.J: Genomic variation among herpes simplex virus type 1 strains: virus DNA analysis of isolates from Saudi patients. *Med Virol* 38:16, 1992.

30. Zakzouk SM, Hossain A**.** Hearing impairment among children in Saudi Arabia: familial incidence and potential risk factors. *Int J Pediatr Otorhinolaryngol* 29:111, 1994.

31. Qutub MO, Klapper PE: Polymerase chain reaction and its value in the diagnosis of Herpes Simplex Complex, *Ann Saudi Med* 23:29, 2000.

32. Altaf FJ: Pattern of cervical smear cytology in the Western Region of Saudi Arabia, *Ann Saudi Med* 23:35, 2000.

33. Agarwal PK: Pattern of skin diseases in Al-Jouf region, *Ann Saudi Med 18*:21, 1996.

34. Parthasaradhi A, Al-Gufai AF: Pattern of skin diseases in Hail region, Saudi Arabia, *Ann Saudi Med 19*:15, 1997.

35. Hossain A, Bakir TM, Ramia S.J: Immune status to congenital infections by TORCH agents in pregnant Saudi women, *Trop Pediatr* 32:83, 1986.

36. Hossain A.: Herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) infections in Saudi Arabia , *J Trop Pediatr* 35:171, 1989.

37. Hossain A, Bakir TF, Siddiqui MA, Khawaja MM, De-Silva S, Sengupta BS, el-Sheikh MM, Madani ME: Genital herpes simplex virus infections: laboratory confirmation in diverse patient groups. *Int J Gynaecol Obstet* 29:51, 1989.

38. Ghazi HO, Telmesani AM, Mahomed MF. TORCH agents in pregnant Saudi women. Med Princ Pract. 2002 Oct-Dec;11(4):180-2.