Original article

Value of avidity index of IgG and polymerase chain reaction in diagnosis of herpes simplex 2 virus in pregnancy

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Abstract

Objective: The goal of the present study is to highlight the significance of the determination of IgG avidity to herpes simplex2virus(HSV2) compared to detection of viremia by polymerase chain reaction(PCR) in relation to recurrent abortions. **Methods**: Serum samples from pregnant patients with bad obstetric histories and control subjects were analyzed for quantitative determination of IgG specific for HSV2 by using ELISA with and without sample treatment with 8 M urea as protein-denaturing agents for measurement of avidity index (AI). PCR was performed for samples to determine HSV2 viremia. **Results**: Regarding avidity index 11.5% had avidity index =100.0, 11.5% has avidity index = 47% and 76.9% had avidity index > 60%. Control group had AI > 70%. There was statistically insignificant difference between OD of IgG in patients with viremia before urea treatment and after urea treatment compared to healthy control. Also there was statistically insignificant difference in OD before urea and after urea in patients with viremia. **Conclusion**: From this study we can conclude that viremia for HSV2 is common finding in patients with bad obstetric history. Low avidity immunoglobulin G was not common finding in those patients. This may be explain that even reactivation of latent HSV 2 infection is associated with fetal loss.

Keywords: Herpes simplex 2; PCR; IgG avidity test

INTRODUCTION

Genital herpes simplex virus (HSV) infection is one of the most common viral sexually transmitted infections. During infection this virus establishes latency in the nuclei of nerve cells in the local dorsal root ganglion. At intervals throughout the life of the host the virus may reactivate and is either shed silently or produces symptoms of recurrent infection. The majority of women with genital herpes will have a recurrence during pregnancy. Transmission of the virus from mother to fetus typically occurs by direct contact with virus in the genital tract during birth[1].

Few (10%) infected individuals report symptomatic disease, typically consisting of painful genital sores, although forward transmission is possible with no reported symptoms. In addition, HSV-2 seropositivity is a known risk factor for human immunodeficiency virus (HIV) acquisition[2,3].

Thus, serological tests serve as a useful adjunct not only for epidemiological and etiological studies but also for clinical studies to elucidate HSV infections. Generally, in the diagnosis of viral diseases, confirmation of primary infections depends on the detection of immunoglobulin M (IgM) or IgG seroconversion comparing the titers of acute and convalescent-phase serum samples. However, well-timed collection of paired sera is often an impractical requirement. Furthermore, in HSV infection, the IgM response is not unique to the primary phase, but the persistence or reappearance of IgM is often observed during recurrences or even in subjects without clinical symptoms[4]. To overcome these difficulties, a unique serodiagnostic approach which differentiates

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primary HSV infections by the characteristic presence of the polymeric form of specific IgA antibody was developed[5]. This technique, which requires sedimentation and fractionation procedures with a supercentrifuge, however, still could not be regarded as the most practical method in clinical laboratories.

It has been demonstrated that the early specific IgG response after primary infections consists of low-avidity antibodies, with maturation of avidity in a few months[6]. Low-avidity-antibody denaturation techniques have been applied effectively to the diagnosis of a variety of human infectious diseases including rubella[7,8], varicella zoster virus,[9,10], cytomegalovirus[11,12], Epstein-Barr virus[13], human herpesvirus 6[14], and toxoplasma infections[15].

Few studies discuss if even latent viral HSV2 infection with viremia is associated with bad obstetric history with fetal loss.

The goal of the present study is to highlights the significance of the determination of home- made IgG avidity to HSV2 compared to detection of viremia by Polymerase chain reaction (PCR) in relation to recurrent abortions.

MATERIALS AND METHODS

The study was conducted on 64 pregnant females. The patients were recruited from an obstetric outpatient clinic at the Mansoura University Hospital. Two different groups were evaluated. The first group (n = 52) consisted of patients with herpes simplex 2 infections diagnosed with clinical symptoms and PCR. Patients were complaining of bad obstetric histories with recurrent unexplained medical abortions and early neonatal death. Other medical and pathogens that can lead to such condition were excluded.

The second group (n = 12) consisted of pregnant women without a history of HSV2 infection and with pregnancy duration of more than 32 weeks´gestation. The demographic, medical, and clinical data were collected in each case based on personal interviews and medical examination.

The women signed an informed consent before they were included in this study and the study was approved by the ethical committee of Mansoura University.

Sera collection

Blood samples were obtained from each patient and centrifuged, and the sera were kept frozen in aliquots at −70°C until analysis.

Serologic study for HSV2

Microtiter strip wells are coated with HSV gG2c recombinant antigens. The solid phase is first treated with the diluted sample. After the washing steps, the IgG specifically bound to antigen, are detected with anti-human IgG antibodies labelled with peroxidase (HRP). A substrate/chromogen solution is added and the intensity of the generated color is proportional to the amount of anti-HSV 2 IgG antibodies in the sample.

For avidity measurement, serum samples from each patient and control subjects were analyzed for qualitative specific IgM for HSV2 (ELISA-Equipar Via G, Ferrari, Saronno, Italy), and quantitative determination of Herpes simplex IgG avidity was measured by using the same kit with sample treatment with 8 Murea as protein-denaturing agents. Quadruplicate microplates were washed with phosphate-buffered saline (PBS) and 8 M urea containing PBS solution following incubation of the serum specimens in antigen-coated plates. After an 8-min exposure to the agent at room temperature, the plates were washed and processed as described above. HSV-specific IgG antibody activities in the wells washed with the elution agents or PBS only were used to calculate the avidity index (AI), where AI (HSV-specific IgG antibody activity of the wells washed with an elution agent)/ (that of the wells washed with PBS) × 100.

PCR for HSV2

DNA was extracted with the commercially available Qiagen kit (GmbH, Hilden). Primers were designed to bracket a well-conserved region in the DNA polymerase gene. Primer pair HSV-P1 (5′-GTGGTGGACTTTGCCAGCCTGTACCC-3′) and HSV-P2 (5′-TAAACATGGAGTCCGTGTCGCCGTAGATGA-3′) was used to amplify HSV2[16].

Taq (0.25 μL) and extracted DNA (10 μL) were added to each premixed supplied tube. Negative control was analyzed by adding water instead of DNA and positive control was performed by 5.0 μL of HSV-1 positive control and 5.0 μL of positive control HSV-2 DNA. The following program was used for the thermal cycle: 1 cycle at 94°C for 2 min, 35 cycles (94°C for 30 seconds, 56°C for 30

seconds, 72°C for 30 seconds), and 1 cycle at 72°C for 5 min.

From amplified product we added 1 μL DNA and 0.25 μL of Taq polymerase in premixed tubes supplied with the kit. The program used in the thermal cycles was 1 cycle at 94°C for 2 min, 30 cycles (of 94°C for 30 seconds, 58°C for 30 seconds, 72° C for 30 seconds), and 1 cycle at 72°C for 5 min. Following PCR, the amplicon 100 bp for HSV-2 was resolved on a 1.5% agarose gel and visualized using ethidium bromide (0.5 μg/mL) under ultraviolet illumination.

Statistical analysis

Values were represented as means ± SD, median (range), or the number of subjects and proportions. One-way analysis of variance test and independent samples Student t test were used for group comparisons of normally distributed variables, and the Kruskal-Wallis test and Mann-Whitney U test were used for comparisons of variables with skewed distribution. The chi-square test was used to compare proportions.

The receiver operator characteristic method was used to determine the best possible cutoff values for HSV2 IgG as a predictor of viremia according to PCR; the curves were obtained by plotting sensitivity on the y-axis against the false-positive rate (1-specificity) on the x-axis for all possible cutoff values of the tests. From this curve, the best or optimal cutoff value was determined. Significance was considered when $P < 0.05$.

RESULTS

Fifty tow patients with genital herpes simplex as diagnosed by clinical symptoms and were positive by PCR for HSV2 and 12 healthy pregnant women were enrolled in the present study. Forty cases (80%) were in the first trimester and 12 cases (20%) were in the third trimester. The mean age ± SD of the patients was 26.8 ± 5.61 years and the mean age of the control group was 26.2 ± 6.25 years with a statistically insignificant difference between both groups ($P = 0.80$). The range of gravidity for both groups was 2 to 7 times.

Regarding avidity index 6/52 (11.5%) of patients had avidity index = 100.0, 6/52 (11.5%) had avidity index = 47% and 40/52 76.9% had avidity index > 60% control group had AI > 70% (Table 1).

There was statistically insignificant difference between OD of IgG in patients with viremia before urea treatment and after urea treatment compared to healthy control. Also there was statistically insignificant difference in OD before urea and after urea in patients with viremia (Table 2)

The best AI that was associated with viremia was found at 65% with sensitivity 55.6, specificity 91.9% (Figure 1).

Table 1 Avidity Index for IgG specific for HSV2. among studied subjects.

Avidity index	No	%	Avidity Index
Patients ($n = 52$)	6	11.5	100
	6	11.5	47
	40	76.9	>60%
Control ($n = 12$)	12	100.0	70 – 100

Table 2 Optical density for IgG before treatment with urea and after treatment.

	OD Mean ± SD	*t* statistic 2-tailed *P*
Total IgG		
Control($n = 12$)	1.4 ±0.35	
Patients($n = 52$)	1.6 ± 0.47	−1.54 $P = 0.128\ 7$
IgG with urea elution		
Control ($n = 12$)	0.98 ±0.25	
Patients ($n = 52$)	1.2 ± 0.61	−1.43 $P = 0.157\ 2$

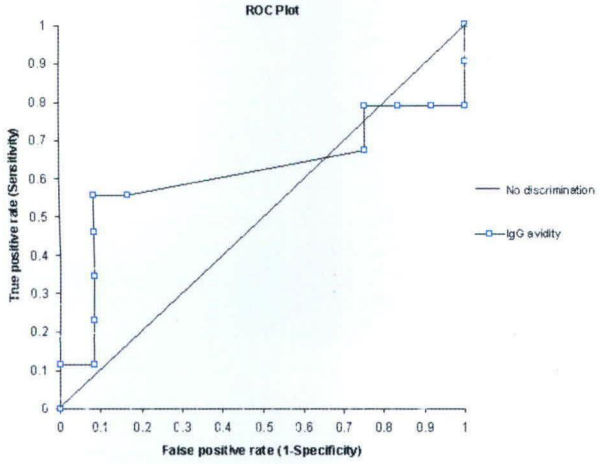

Figure 1 Receiver operative curve for IgG avidity.

DISCUSSION

Risk factors for neonatal herpes simplex virus (HSV) infection associated with maternal herpes are prematurity, prolonged rupture of membranes (PROM), and primary as opposed to recurrent maternal infection.

Infants born to primary infected mothers develop systemic HSV infection in up to 75% of cases. In contrast, newborns delivered to mothers with long-lasting infection the risks of their infection are not determined especially if their mothers had viremia.

Both the serological status of the mother and the titer of HSV antibodies at the time of birth are risk factors for neonatal infection. Consequently, precise characterization of maternal immune status is needed. However, serological analyses carried out for establishing the diagnosis during the acute phase of the disease are often misleading[17]. However in house made avidity test depending upon elution solution as is easy, rapid and inexpensive method for determination of low avidity IgG.

Cross-reactions with other viruses or vigorous nonspecific immune stimulations often result in spurious presence or increments of herpes-" specific" IgM and IgG. Therefore, the diagnosis of HSV infection is usually based on the direct identification of the virus in clinical or autopsy specimens by culture and/or PCR with variably delayed results[18].

In the present study all pregnant patients with bad obstric histories had positive PCR results for HSV 2 viremia. This finding high lights the occurrence of HSV2 viremia as risk factor for bad obstetric history. This viremia leads to grater chance for fetal infection. It should be mentioned that HSV seem to be able to cross the placenta and cause, as described by several investigators, placental infection manifested by deciduitis and villitis. These placental pathological changes may increase fetal damage[19].

In study of avidity index, the majority of patients (69.9%) had avidity index > 60. Online with our results, Brown et al., (2007)[20] reported that 4 of 8 women (50%) with avidity 40 or greater transmitted herpes simplex virus to the neonate. Herpes simplex virus 2 antibody avidity increased over time after genital herpes simplex virus-2 acquisition, as has been previously observed for herpes simplex virus-2. Patients with recurrent episodes and established HSV2 infections (median, 6.1 years' duration) had higher avidity antibodies (median, 92.7; range, 55.1-100) than patients after first episodes (median, 33.7; range, 6.4-73.9; $P < 0.001$)[21].

For a variety of viral infections, the usefulness of measuring specific IgG avidity is recognized for distinguishing primary infection with low specific IgG avidity from past, long-lasting or recurrent infection, in which the increase in IgG avidity correlates with time after infection.[17]. For HSVs, the clinical value of IgG antibody avidity measurements has also been established. In case of active diseases, AI measurements make clear whether symptoms are due to recurrences and reactivations or whether they result from primary infection. Avidity test can separate primary and non primary infections by the presence of specific IgG of low and high avidities[22,23] and showed that the avidity of HSV-specific IgG increases up to 100 days after infection before reaching a plateau. Although in our study small percentage of patients with viremia had low avidity index giving clue to recent infections the majority of patients had high avidity index showing that reactivation of latent HSV 2 was also a leading cause for bad obstetric history in those patients.

The wider range of AIs for samples with positive PCR may be explained by the influence of cross-reactive antibody avidity against HSV-1 which had infected those patients previously, by the immunological or genetic factors of the hosts with high avidity antibody characteristic of the response at the time of the repeated episodes.

In study of cut of value for AI which can be used as marker for presence of viremia, 65% was found suitable with sensitivity 55.5% and specificity 91.9%. The clearest separation was obtained with 6 M urea treatment, giving mean avidity indices of 0.

398 for sera < or = 100 days after the infection and 0.879 for sera > 100 days after the infection ($P < 0.001$)[24]. However higher avidity index was reported for primary herpes simplex Index values that tended to rise after infection, peaking a median of 9-10 weeks post-infection (range 8-323 days) with at least one AIs value (>1.1 to < or =3.5)[25].

From this study we can conclude that HSV2 viremia is common finding in patients with bad obstetric history. Low avidity immunoglobulin G was not common finding in those patients. This may be explain that even reactivation of latent HSV 2 infection is associated with fetal loss. Furthermore search for detection of viremia in vulnerable patients can be valuable by molecular methods.

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