

Distribution of HSV-1 IgG antibodies by two methods comparing in Turkish atopic children

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SUMMARY

Herpes Simplex Virus Type 1 (HSV-1) is a ubiquitous pathogen. Other than known diseases, HSV-1 may have an important role in the pathogenesis of atopy by causing immortality of th2 cells.

From June 1st to July 31st 2006, seventy five blood samples were collected from atopic children referred to the allergy clinic of the hospital. The bloods samples were used to detect HSV-1 IgG antibodies by Enzyme-Linked Immunosorbent Assay and Virus Neutralization Test. HSV-1 IgG antibody seroprevalence in atopic children was found high, 62.6% by Enzyme-Linked Immunosorbent Assay and 57.3% by Virus Neutralization Test. Thus Virus Neutralization Test sensitivity was 92.15% and specificity was 100% regarding to the Enzyme-Linked Immunosorbent Assay technic. Although Enzyme-Linked Immunosorbent Assay was more sensitive than Virus Neutralization Test, there was no significant difference between two technics ($p>0.05$) in detecting HSV-1 IgG antibodies in serum.

KEY WORDS: Herpes Simplex Virus Type 1, allergy, ELISA, Neutralization Test, prevalence

Received November 10, 2006

Accepted February 14, 2007

INTRODUCTION

Herpes Simplex Virus Type 1 (HSV-1) is an ever-present pathogen that usually causes either a symptomatic infection or skin and mucosal diseases. The major clinical manifestations associated with HSV-1 infections are gingivostomatitis, keratitis and conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and lethal infections of newborns.

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Other than those known diseases, it is also suggested that HSV-1 may have an important role in the pathogenesis of atopy. One of the demonstrated examples of a virus causing the immortality of the T-helper type 2 (Th2) cells is HSV type 1. It infects mouse or human Th2 cells and although it does not multiply, causes immortality by increasing FAS-mediated apoptosis of t-cells directed against the infected cells (Hamid QA, Minshall EM. 2000; Jayaraman S, *et al.* 1999; Spinozzi *et al.*, 1998; Baskin *et al.*, 2005). Thus it is possible to speculate that HSV-1 could make immortal Th2 clones driving specific atopies. For this reason, we think that HSV-1 may cause atopy development and prevalence of this virus would be higher in atopic children. To determine this hypothesis, the epidemic proportion of HSV-

1 in atopic children should be tested by seroprevalance assays. This study aimed at studying seroprevalance of herpes simplex type 1 in atopic children for the age group 5-17 years old, using two different methods: enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT) and comparing the sensitivity of these two methods.

MATERIAL AND METHODS

Serum specimens

In this study, seventy five blood samples were taken from 5-17 year old atopic children between June 1st and July 31st 2006 according to the approval of the Institutional Ethic Committee and written informed consent was given by parents. Children detected with at least one IgE positivity against allergens found by prick test panel that was routinely used in our allergy clinic, were accepted as atopic. Each blood sample was centrifuged at 3000 rpm for 20 minutes at 4°C and serum was obtained. After centrifugation, each serum was poured into another tube (Eppendorf, Germany), heat inactivated at 56°C for 30 minutes and stored at -20°C until tested.

Cell culture

Human laryngeal epithelial carcinoma cell line (HEp-2) was grown in a 75 cm² cell culture flask (Orange Scientific, Belgium) in Eagle's minimum essential medium (EMEM, Sigma GmbH, Germany) with 5% fetal calf serum (FCS, Sigma GmbH, Germany) and 1% penicillin/streptomycin (Biochrome, KG). The cells were maintained in 5% CO₂ atmosphere with 95% humidity at 37°C. Cells were periodically screened for contamination.

Virus strain

The KOS strain of HSV-1 was used in this study. Virus stock was prepared by infection of HEp-2 cells and incubation in EMEM containing 2% fetal calf serum at 37°C in 5% CO₂. Cells were monitored by daily microscopic examination until the cytopathic effect (CPE), i.e., rounding up and fusion of infected cells to form multinucleated giant syncytia reached 80-100%. After freezing and thawing for three times, cell lysates were aliquoted and stored at -80°C as virus stocks.

Infectivity Assay for HSV-1

The infectivity value (TCID₅₀) of the virus was determined by microtitre technique on HEp-2 cells as described by Kaerber (Kaerber, G. 1964). Briefly, virus samples were prepared in serial ten fold dilutions and placed in 96-well tissue culture plates (Greiner, Germany). 100 µl of each concentration of viruses was made on four replicate wells. Subsequently, 50 µl of an HEp-2 cell (300 000 cells/ ml) were added to diluted viruses and incubated in humidified atmosphere at 37°C. Virus titre was calculated on the basis of CPE determination as 50% tissue culture infective dose (TCID₅₀/0.1 µl).

Virus Neutralisation Tests

VNT protocol described by Frey and Liess (Frey, H.R., Liess B., 1971) was performed using HEp-2C cells in 96 well microplates (Grainer, Germany). Test sera were diluted 1/2 in EMEM. Fifty µl of each diluted serum were added to wells, mixed with an equal volume of 100 TCID₅₀ dilutions of the HSV-1 and incubated for 1h at 37°C. Afterwards 50 µl of HEp-2 cells (300000 cells/ml) were distributed into wells. Test plates were incubated at 37°C in 5% CO₂ atmosphere for 3 days until a 100% CPE was detected in control virus wells.

ELISA test for determination of IgG antibodies against HSV1

For the detection of antibodies against HSV-1, ELISA was performed using a commercial ELISA test kit (Dia. Pro, Milan, Italy) according to the test procedure. Briefly, 100 µl of serum samples diluted with sample diluent were transferred into the antigen coated wells and the plates were incubated at 37°C for 60 min. The wells were washed three times with PBS-Tween 20. After these washing steps, 100 µl of horseradish peroxidase conjugate was added to each well and incubated at 37°C for 60 min. The wells were rewashed three times with PBS-Tween 20. 100 µl of chromogen/substrate solution were added to each well and incubated at 24°C for 20 min. Finally, 100 µl of stop solution were added to each well and the reaction stopped. The wells were read on a plate reader (DAF, Italy) and optical densities (OD) of wells were determined at 450 nm. For the test validation, the mean OD of blank wells should be <0.050 and the mean OD of the Cal 1 (0arbU/ml), Cal 2 (5 arbu/ml) and Cal 6 (100

arbU/ml) should be <0.150 , $> \text{Cal } 1+0.100$ and >1.000 , respectively. For the interpretation of the results, the serum samples with a concentration lower than 5 arbU/ml were accepted as negative for anti HSV-1 IgG and the serum samples with a concentration higher than 5 arbU/ml were accepted as positive for anti HSV-1 IgG.

Statistical analysis

Seropositivity proportion of the two technics was compared using Pearson's chi-square test and $p < 0.05$ was considered statistically significant.

RESULTS

HSV-1 grew rapidly in HEp-2 cells with its characteristic CPE at 48 hours post infection (PI) with syncytia formation, rounding and ballooning of the infected cells and terminated by total cellular lysis on the third day PI. The virus infectivity was determined as $\text{TCID}_{50} 10^{-5.00}/0.1 \text{ mL}$ at 72 hours PI. Seventy five serum specimens were tested for the presence of HSV-1 IgG antibodies by ELISA and VNT.

Seroprevalence of HSV-1 IgG antibodies in atopic children was 62.6% with ELISA and 57.3% with VNT respectively.

All specimens positive by ELISA were also positive by VNT, except four specimens which were positive only by ELISA. In other words, out of 47 specimens positive by ELISA only four of them were missed by VNT, which had sensitivity a 92.15% regarding the ELISA technique. All of the 28 specimens negative by ELISA were also negative by VNT, thus VNT's specificity was 100% compared with the ELISA technique (Table 1). There was no significant difference between the

frequency of seropositivity of HSV-1 IgG antibodies in atopic children with ELISA and VNT (χ^2 : 0.444, df: 1, $p > 0.05$).

DISCUSSION

The detection of HSV-1 IgG provides a powerful, rapid and economical method for investigation of HSV-1 past infection.

Comparison between different assay methods with respect to specificity and sensitivity shows ELISA to be the most suitable of the available techniques. Therefore, ELISA is more readily applied in routine laboratories but it also has some disadvantages such as the possibility of contamination, length of time and it is more expensive than VNT.

This study is the first seroprevalence investigation of HSV-1 IgG antibodies in atopic children in Turkey. Serum IgG antibodies against HSV-1 were determined in atopic children by two different methods to investigate the prevalence of HSV-1 infection and to determine the sensitivity and specificity of VNT by comparing ELISA. We found high a seroprevalence of HSV-1 IgG antibodies in atopic children with both ELISA (62.6%) and VNT (57.3%). We also found that the sensitivity and specificity of VNT was 92.15% and 100% respectively compared with ELISA. Although VNT was less sensitive than ELISA, there was no significant difference between the two methods ($p > 0.05$) and VNT could be used safely as ELISA to investigate the prevalence of HSV-1 IgG antibodies. An increase in HSV-1 antibody titers in atopic children may reflect basic immunoregulatory disturbances in atopy but it is also possible that HSV-1 may play a role in the pathogenesis of atopic diseases.

Among children, HSV-1 prevalence was particularly high in Eriyeria: 97% of those >5 years old were HSV-1 positive (Ghebrekidan *et al.*, 1999). Among New Mexico Navajo children HSV-1 prevalence was 59% in those aged 1-5 years and 79% in those aged 1-5 years old (Becker TM, *et al.*, 1988). Similar results were found among children and young adults in Syria (55% among 1-5 years olds, 80% among 11-20 year olds) (Ibrahim *et al.*, 2000). In Germany, HSV-1 prevalence was lower among children 1-5 years (31%), increasing to 46%-49% among those 6-

TABLE 1 - Results of tests of 75 specimens from atopic children for the presence of HSV-1 Ig G antibodies in the serum by ELISA and VNT.

VNT (n)	ELISA (n)		Total
	Positive	Negative	
Positive	43	0	43
Negative	4	28	32
Total	47	28	75

16 years old (Wutzler *et al.*, 2000). Among hospital attendees in Israel, HSV-1 prevalence among children aged 2-4 years was 38% and 54% among those 15-17 years old (Isacsohn *et al.*, 2002). In England and Wales, HSV-1 prevalence was 46%-49% among newborns, probably reflecting, at least in part maternal antibody status, and 17%-27% among children aged 1-14 years (Vyse *et al.*, 2000). In a cross sectional study of 0-19 year old Swedish children, the HSV-1 seroprevalence was 31% in 2003 (Tunback *et al.*, 2003). In a European study using samples collected in 1989-2000, the seroprevalence for HSV-1 for the age group of 5-9 years in Belgium, England and the Netherlands was 25-35% and the seroprevalence for the age group of 0-19 years in Bulgaria was 14-15%, in the Czech Republic and Finland it was 23-27%, in Slovenia it was 12% (Pebody *et al.*, 2004). Recently in another study from Israel, the seroprevalence of HSV-1 increased with age in both sexes, and reached 57% by the age of 18 years (Davidovici *et al.*, 2006).

As is seen, while the seroprevalence of HSV-1 is high among African children, it is relatively low in Europe. Although Turkey is found in between these countries there is no study representing the seroprevalence of HSV-1 among Turkish children and adults except one study performed in pregnant women (Arseven *et al.*, 1992).

In conclusion while making certain decisions without comparison with a healthy control group is not correct, the data show that there may be a higher exposure to HSV-1 in atopic children and VNT immunoassay is as reliable as ELISA to detect HSV-1 IgG antibodies for seroprevalence studies. Further studies should be undertaken to clarify the relation between HSV-1 infection and atopy in children.

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