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Determination of Esptein-Barr Virus in the Plasma of Adult Patients with Otitis Media with Effusion

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Abstract: To investigate the epidemiological characteristics of the Esptein-Barr Virus (EBV) in Otitis Media with Effusion (OME) and its role in the pathogenesis of OME. The plasma samples of 65 patients with OME and 70 healthy people were tested for EBV by real-time quantitative PCR technique. Genomes of EBV positive rate in OME patients are 38.5% (25/65) while only 2.8% (2/70) in control group (p = 0.0001). EBV infection rate in patients with B type tympanogram is higher than that of C type (p = 0.006). No difference of EBV-DNA infection rate was found between the male and female (p = 0.5) or patients with unilateral and bilateral OME (p = 0.076). According to the treatment effect grouping, the EBV infection rate is 17.5% in patients with the subjective symptoms disappeared in three months after treatment, significant lower than that not disappeared or worsen in 3 months (72%, p = 0.001). EBV infection may contribute as a cause of OME and seems to affect the treatment effect. More extensive study are required to elucidate the role of the EBV and the relationship of OME.

Key words: Esptein-Barr virus, otitis media with effusion, real-time fluorescent quantitative PCR, sympoms, male, female, China

INTRODUCTION

Otitis Media with Effusion (OME) is a non-purulent inflammatory disease of auris media and is mainly characterized by hearing loss and tympanic effusion but with the absence of symptoms of acute infection (Stool *et al*., 1994). OME frequently occurs in children but is also common in adult patients. The etiopathogenesis of the diseases has not been completely elucidated despite numerous published reports. Possible multifactorial factors include allergy, Eustachian tube dysfunction, gastroesophageal reflux and infection with viruses or bacteria and so on (Dodson *et al*., 2012; Van Zon *et al*., 2013; Holder *et al*., 2012).

Epstein-Barr Virus (EBV) is a B-lymphocytic virus of human herpes family, past studies have shown that EBV plays a major role in the pathogenesis of many diseases, such as Nasopharyngeal Carcinoma (NPC), Burkitt's lymphoma and Hodgkin's disease and the level of EBV-DNA in the plasma is related to the prognosis of those diseases (Liang *et al*., 2012; Ohshima *et al*., 1999). Epstein-Barr viruses were also confirmed at a high rate in middle ear fluids of the patients of Acute Otitis Media (AOM) which showed the potential role of EBV infection in the pathgeneous (Shinogami and Ishibashi, 2004; Savenko *et al*., 2008). However, compared to bacterial agents and respiratory viruses, the presence of Epstein-Barr virus in case of OME is still limited and controversial.

For example, one study (Storgaard *et al*., 2004) has showed that none of the Epstein-Barr virus was detected in the middle ear fluids in the children with mean age 3.7 years but in another study in school age children (Mean age, 7.35 years), this result was not verified on the contrary, it showed a high rate of Epstein-Barr virus in middle ear fluids of OME (Bulut *et al*., 2007). These two completely different results may due to the different age group for the incidence of herpesvirus infections is relative to the age and may increase at school age in children (Bulut *et al*., 2007; Becker *et al*., 1988).

Therefore, the objective of the present study was to determine the rate of EBV infection in adult OME patients which has not been well reported in English literature. Researchers test the EBV-DNA by PCR from the sample of

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the plasma in place of middle ear fluids to more directly define the evidence of systemic EBV infection. In addition, researchers also investigate the possible influence of this infection to the clinical character and prognosis to further demonstrate the EBV infection in OME patients.

MATERIALS AND METHODS

Clinical data: The samples used in the current study were taken from 65 adult patients first diagnosed with OME at the Department of Otolaryngology of the Daqing Oil Field General Hospital from Jun, 2011 to Feb, 2012. The study was approved by the Ethics Committee in the hospital and written informed consent was provided by the patients. The 33 of them were male and 32 female ranging in age from 24-52 years with a mean age of 38.8 years. The illness courses of the patients ranged from 1-30 days without any other treatment before, no history of flying or diving in all patients. The 70 normal adults of similar age and sex were used as controls. Both patients and controls underwent clinical otologic and audiological evaluation including medical history, pneumatic otoscopy, tympanometry (Madsen Zodiac 901) and standard pure-tone audiometry. The diagnosis of otitis media with effusion was established when findings in at least three of them were positive (Balatsouras *et al.*, 2012). Nasal endoscopies were performed to exclude nasopharyngeal carcinoma. None of the patients had clinical symptoms accepted as specific for the EB virus infections.

A period of secretolytic drug gelomyrtol forte and steroid nasal spray rhinocort were routinely administered to the OME patients. After 3 month, all OME patients underwent a 2nd otoscopic exam and tympanometry and any improvement or worsening of complaints was directly asked from the patients. Surgical treatments were recommended if necessary.

Real-time fluorescent quantitative PCR (RQ-PCR): Plasma samples were collected from OME patients at their first admission to the clinic before treatment. Epstein-Barr virus PCR fluorescent detection kit was purchased from Da An Gene Co., Ltd. of Sun Yat-Sen University. These steps were as follows: Transfer 1 mL of the whole blood into a dry glass tube, add 1 mL of normal saline and gently mix well. Transfer 500 μL of lymphocyte separation medium into another dry glass tube, add slowly the diluted whole blood into the test tube containing lymphocyte separation medium with a pipette. Place the whole blood into the centrifuge tube, centrifuge for 20 min at 2000 r min^{-1}, remove the supernatant, draw white blood cell layer into a 1.5 mL centrifuge tube, centrifuge for 5 min at 12000 r min^{-1}, remove the supernatant, add 50 μL of DNA extract liquid into the precipitate and thoroughly mix, place it at 100°C constant temperature for 10±1 min. Place it in 4°C refrigerator for 10-20 min, centrifuge for 5 min at 10000 r min^{-1}, transfer 5 μL of the supernatant into PCR reaction tube and load. The cycling conditions after loading the PCR reaction tube: initial denaturation at 93°C for 2 min; 93°C for 45 sec→55°C for 60 sec, first perform 10 cycles; 93°C for 30 sec→55°C for 45 sec, perform 30 cycles. The copy number of EBV-DNA in the plasma was analyzed by using the Model 5700 fluorescence quantitative instrument and automatic analysis software system, growth curve S curve and the number $>10^3$ was considered as positive, growth curve was not S curve and the number 10^3 was considered as negative.

Patients grouping: Grouping was made according to the clinical character of the patients, including sex, unilateral or bilateral ear, tympanogram type, B or C (Bilateral ear with 2 types each was looked as B type) and medical treatment effect. Treatment effect grouping was made as following:

- Group A: The subjective symptoms disappeared in 3 months after treatment
- Group B: The subjective symptoms were not disappeared or worsen after 3 months

Statistical analysis: The experimental data was analyzed by the χ^2 test using SPSS13.0 Software. Difference was considered statistically significant if the p-value was <0.05.

RESULTS AND DISCUSSION

PCR analysis of the plasma samples showed that Genomes of EBV positive rate in OME patients are 38.5% (25/65) while only 2.8% (2/70) in control group (p = 0.0001). EBV infection rate in patients with B type tympanogram is higher than that of C type (p = 0.006). No difference of EBV-DNA infection rate was found between the male and female (p = 0.5) or patients with unilateral and bilateral OME (p = 0.076).

According to the treatment effect grouping, the EBV infection rate in group A is 17.5%, significant lower than that in group B. (40%, p = 0.001) (Table 1).

In the present study, the results of PCR analysis of plasma indicated a statistical significance higher rate of EB-virus presence in adult OME patients than control group and the EB-virus infection may affect the prognosis of OME patients. This result is similar to a previous study

Table 1: EBV-DNA detection in the plasma of OME patients

Groups	EBV-DNA (-)	EBV-DNA (+)	p-values
Patients	40 (61.5%)	25 (38.5%)	0.0001
Control	68 (97.2%)	2 (2.8%)	
Male	19 (57.6%)	14 (42.4%)	0.5
Female	21 (65.6%)	11 (34.4%)	
Unilateral ear	28 (70%)	12 (30%)	0.076
Bilateral ear	12 (48%)	13 (52%)	
B tympanogram	22 (50%)	22 (50%)	0.006
C tympanogram	21 (87.5%)	3 (12.5%)	
Group A	33 (82.5%)	7 (17.5%)	0.001
Group B	7 (60%)	18 (40%)	

that in school age children patients which shown a high positive rate of EBV DNA within the middle ear fluids of OME. Therefore, the EBV monitoring may be helpful for physicians to determine the prognosis and to select a more powerful treatment to those EBV positive patients of OME.

Because of the sophisticated growth of the EBV, it is some difficult to detect EBV from sample culturing (Bulut *et al*., 2007; Germi *et al*., 2012). Due to PCR assay relies on the detection of genetic material but not living virus, it should be note that although some of the past studies have found the PCR-positive sample from the middle ear effusions, EBV in the pathogenesis of OME still cannot fully demonstrated at present. Unfortunately, this disadvantage also exists in this study. Furthermore, PCR were performed in 65 plasma samples but not effusion of the middle ear, one reason is the aim of the present study is to test the systemic EBV infection, the other is effusion liquid was impossible to taken from each OME patient, not all the OME patients need the treatment of auripuncture or surgery, sometimes, auripucture may be harmful for causing infection, especially in early course of the disease.

Plasma EBV DNA has been studied in several other conditions, such as infectious mononucleosis (Papesch and Watkins, 2001), acute lymphoproliferative disorder, EBV-associated lymphoma and Nasopharyngeal Carcinoma (NPC). In NPC patients, the higher level of EBV DNA, the greater the risk of recurrence, monitoring of plasma EBV DNA levels has been demonstrated be useful in detecting NPC disease recurrence and metastases (Spano *et al*., 2003).

In OME patients, nasopharyngeal carcinoma must be excluded as a differential diagnosis, especially for unilateral adult patients. In clinical, intensive nasopharyngeal examination, EBV detection and nasopharynx biopsy when needed were usually the routine for suspicious NPC patients. Thus as the results of the reports, EBV-DNA can be detected in the plasma of 38.5% OME patients without NPC. The positive result of EBV-DNA should be carefully valued for patients with OME but negative result of nasopharynx to avoiding the panic of the patients.

CONCLUSION

In this study, EBV DNA were determined at a high rate in plasma of adults with OME. However, the present study is a preliminary clinical study, more extensive study, especially with virus culture in middle ear effusion and experimental animal studies are required to elucidate the role of the EBV and the relationship of OME.

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