Iranian Journal of Pathology (2010)5 (1), 14 - 17

Original Article

Presence of Cytomegalovirus in Sinunasal Mucosa of Patients with Chronic Sinusitis and Without Sinusitis

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ABSTRACT

Background and Objective: **Sinusitis is one of the most common hygienic problems and a large part of the budget has been allocated to its diagnosis and treatment yearly. In this study, the presence of cytomegalovirus in sinuses of patients, undergone operation for treatment, with or without sinusitis, was studied.**

Materials and Methods: **This study was an applied and case-control study, which had been performed on 44 HIV negative patients in ENT clinic of Shahid Mostafa Khomeini Hospital in Tehran during 4 months in 2005. Biopsy specimens were taken from left and right of uncinet process mucosa of middle meatus of 22 patients with chronic sinusitis and 22 patients without sinusitis undergo operation for nasal septal deviation. After purification of DNA, PCR test was done for replication of early gene in cytomegalovirus DNA by two kits, which was purchased from Cinnagen Co. and Gen Fanavaran Co.**

Results: **After the electrophoresis of PCR product on agarose gel, neither of samples has shown DNA band same the positive control enclosed in kits. Therefore, all specimens were considered negative for cytomegalovirus DNA.**

Conclusion: **Cytomegalovirus has not been detected in sinunasal mucosa of patients with chronic sinusitis and without sinusitis. Test with more specimens and other diagnostic procedure are recommended for prove of absence of cytomegalovirus in sinunasal mucosa.**

Key words: Cytomegalovirus, Sinusitides, Polymerase Chain Reactions

Introduction

Rhinosinusitis is one of the most common medical complaints affecting nearly 31 million US citizens annually (1). Respiratory viruses can play a pathologic role in airway infection allowing secondary bacterial overgrowth (2). Sinusitis is usually considered a complication of viral rhinitis. Virus infection in the upper respiratory tract leads to mucosal swelling, which may obstruct paranasal sinus outflow, resulting in infection in the paranasal sinuses (3).

Chronic sinusitis is the one of the most common hygienic problems and a large part of the budget has been allocated to its diagnosis and treatment yearly

Received: 25 May 2009
Accepted: 13 Augest 2009
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(4,5). Role of bacteria and fungi in sinusitis were more studied but recently role of viruses was also noted (6-9). Cytomegalovirus (CMV) is a member of herpesviruses that its possible role in creation of sinusitis and latency in sinunasal mucosa is shown in a few literatures (10-15). CMV DNA can be detected by PCR from infected tissues (16).

In this study the presence of cytomegalovirus in paranasal sinuses of patients with chronic sinusitis undergoes operation for treatment were studied. In addition, the presence of cytomegalovirus in sinuses of patients without sinusitis undergoes operation for nasal septal deviation was studied by polymerase chain reaction (PCR).

Materials and Methods

Patient Selection

This study was an applied and case-control study, which had been performed on 44 HIV negative patients in ENT clinic of Shahid Mostafa Khomeini Hospital in Tehran during 4 months in 2005. Ethics Committee of Shahed University has been approved this work.

The case group in this study included 22 patients with chronic sinusitis (confirmed by coronal CT-scan of paranasal sinuses) that did not respond to standard medical treatment and undergoing functional endoscopic sinus surgery.

The control group in this study included 22 patients undergone septoplasty for deviated nasal septum without any sign of sinusitis (in coronal CT-scan from paranasal sinuses).

Specimens

All tissue specimens were taken from the mid-third of uncinate process in middle meatus and lateral side of middle turbinate (by sterile Beleckesly). Two samples were taken from each side of nasal cavity of the patients. Samples were located in phosphate buffer saline and transferred to Department of Microbiology, Shahed University, Tehran, for diagnostic test. During transportation of samples, cold chain was protected.

DNA Extraction

DNA extraction was carried out by DNP™ kit (Cinnagen, Iran). 25-50 mg of specimens was added to 5 µl protease and 100 µl protease buffer. After incubation in 55ºC for 2 hours, this treated were mixed with 400 µl lysis solution and mixed 15-20 s, then mixed with 300 µl precipitation solution. Tubes were placed at -20 ºC for 20 min and then centrifuged at 12000 g for 10 min. After decanting, tubes were washed twice with 1ml wash buffer and pellets were dried at 65ºC. Purified DNAs were suspended in 50 µl of solvent buffer and saved in -70 ºC.

Polymerase Chain reaction (PCR)

PCR for detection of CMV DNA was performed by using two Kits. First PCR was done with CMV detection kit purchased from Cinnagen, Iran, and then retested by CMV kit obtained from Gen Fanavaran Co., Iran, according to Manufacture's procedures.

In the cinnagen Kit, after defrosting the reagents, 0.3 µl Taq-DNA polymerase and 20 µl 1X PCR MIX were added to the tubes. Then 10 µl purified DNA of samples or positive and negative reagents were added to the separate labeled tubes and mixed well. Tubes were transferred to thermocycler (Techne, UK) with following program: 95 ºC- 180s, 62 ºC- 40s and 72 ºC-40s for 1 cycle and 93 ºC- 40s, 61 ºC-40s and 72 ºC-40s for 45 cycles. After the completion of program, 10 µl of amplified samples were electrophoresed by Tris-acetate buffer in 2% agarose gel without adding the loading buffer. The presence of 222 bp fragments indicated as positive results as shown in the positive control but not in the negative control. Ladder 100 bp was used for weight marker.

In Gen Fanavaran Co. Kit, 10 µl of PCR diluent was added to each ready to use tubes for test and controls and mix well. Then 10 µl of purified DNA of samples were added to test tubes and 10 µl of double distilled water to each control tubes. After mixing, tubes were transferred to thermocycler with following program: 95 ºC-120s, 62 ºC-60s and 74 ºC-120s for 1 cycle, 95 ºC-60s, 60 ºC-50s and 74 ºC- 60s for 1 cycle, 95 ºC-50s, 58 ºC-40s and 74 ºC- 60s for 42 cycles and 74 ºC-120s for 1 cycle. The presence of 451 bp fragments indicated as positive results.

Data analysis

SPSS software (version 11.5) was used for analysis of data obtained from studied individuals.

Results

In this study, 44 patients (24 males, 54.5% and

20 females, 45.5%) between 7 to 76 years old were surveyed. Twenty two patients were suffered from chronic sinusitis (15 males, 7 females with an average age of 39.817.1± years) were considered as case group. Frequency of CT-scan finding in case group was shown in Table 1. In 5 persons, maxillary sinuses, in 1 person etmoid sinuses and in 4 persons maxillary and etmoid sinuses simultaneously were involved. Pansinusitis was shown in 12 persons. Frequency of sinusitis sign and clinical finding of sinusitis in case group were shown in Fig. 1 and 2, respectively. As control group, 22 patients with deviated nasal septum (9 males and 13 females with an average age of 25.97.3± years) and without chronic sinusitis, were studied.

Table 1: Frequency of CT-scan finding in case group

CT Scan finding	percent
Mucosal thickness	95.5
Air fluid level	86.4
Opacification of involved sinus	100
Osseous erosion	31.8

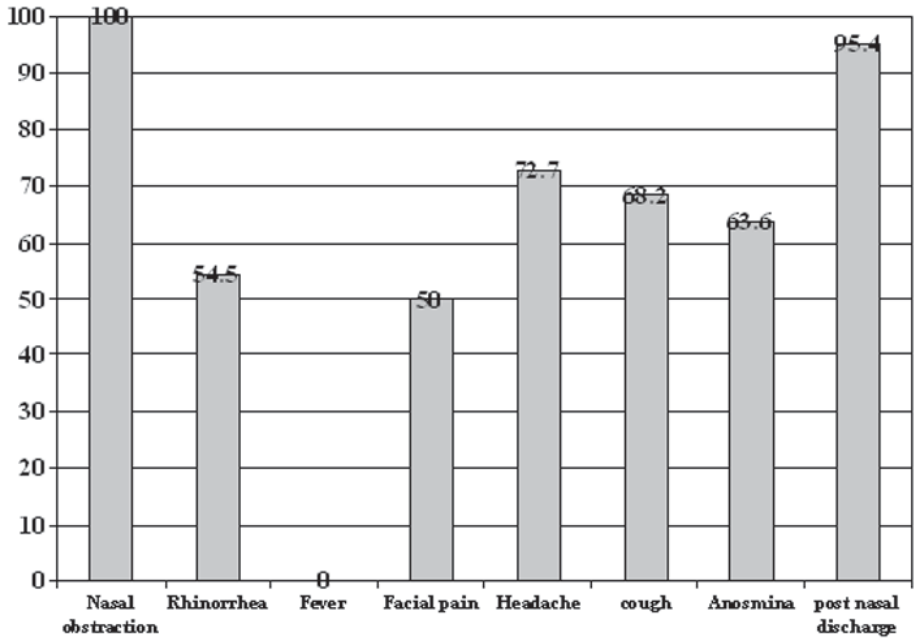

Fig 1: Frequency of sign of sinusitis in case group

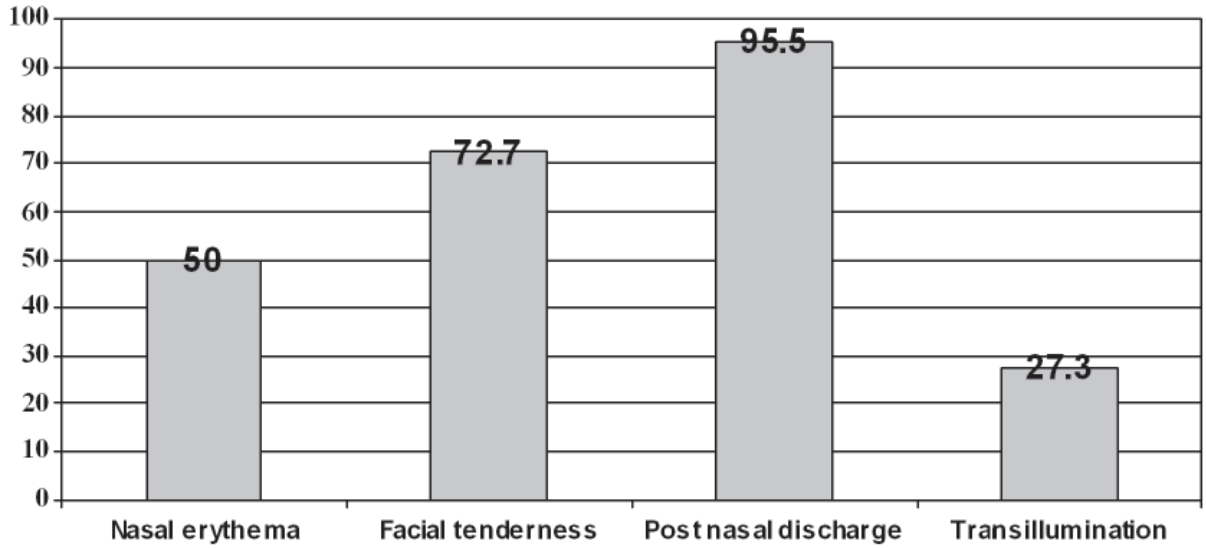

Fig 2: Frequency of clinical finding of sinusitis in case group

The results of PCR tests in both case and control groups were negative, no band were observed, which was indicative of absence of CMV.

Discussion

There are literatures about the presence of cytomegalovirus in sinunasal mucosa of patients with acute sinusitis, which proposed the creating of this disease by this virus (11-14). In the present study, by using PCR tests, CMV is not detected in sinonasal mucosa of case group (22 patients with chronic sinusitis). In other studies, detecting CMV in sinunasal samples of patients suffering from acute sinusitis, all patients were simultaneously infected with HIV; which may affect immune system and persistence of CMV in sinunasal mucosa, but we studied patients without HIV infection. Also, in the study of Tarp and coworkers no relation between CMV and acute sinusitis was shown in immunocompetent individuals (13).The relation of CMV and chronic sinusitis was shown only in one study so CMV could not create chronic sinusitis (14).Various prevalence of CMV in different populations leads in acquiring distinctive results of this study and other studies detecting CMV in sinonasal samples.

Since cytomegalovirus can infected and persistence in various cells and tissues, the possible role of sinonasal mucosa as reservoir of CMV is proposed by some investigators. In the present study, by using PCR tests, CMV is not detected in sinonasal mucosa of control group (22 patients without sinusitis); as it had shown in other study (13). Therefore, it is proposed that sinunasal mucosa is not the reservoir of CMV.

Conclusion

Overall, in this study, which is the first study to investigate the presence of CMV in HIV negative sinusitis patient in Iran, we could not show CMV in the sinunasal mucosa of selected patients, although the possible presence of CMV cannot be ruled out. Nevertheless, more sensitive diagnostic techniques and further investigations on more patients are recommended.

Acknowledgement

This paper is the result of medical student thesis and has been financially supported by Research Council of Shahed University. The authors declare that they have no conflicts of interest.

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