Background and objectives: Different environmental factors, such as infection, can cause Alzheimer’s disease (AD). Herpes simplex virus types 1 (HSV1) and 2 (HSV2) and cytomegalovirus (CMV) are related to AD. This study explores the potential role of HSV1, HSV2 and CMV in AD progression.

Methods: Plasma samples were taken from 100 AD patients (47 women and 53 men). After isolating viral DNA, PCR was performed using specific primers for the detection of the viruses.

Results: The prevalence of CMV, HSV1 and HSV2 was 27%, 8% and 4%, respectively. Although CMV was most prevalent in AD patients, HSV1 and HSV2 were found in patients with advanced AD. The prevalence of HSV1 and HSV2 was significantly associated with dysphoria, hallucination, insomnia and depression (P<0.05), while CMV was significantly associated with hallucination and dysphoria (P=0.001). AD symptoms were higher in patients with HSV1 and HSV2.

Conclusion: It seems that HSV and CMV infections may be related to the severity of AD.

Keywords: Alzheimer’s disease, Epidemiology, Cytomegalovirus.
INTRODUCTION
Since the discovery of Alzheimer’s disease (AD) over 100 years ago, many studies have reported the pathological signs of this devastating disease (1,2). Development of AD is thought to be related to extracellular deposition of amyloid beta (Aβ) and intracellular hyperphosphorylated tau protein (3) that leads to synaptic dysfunction and decreased cognitive performance (4). It was believed that genetic and environmental factors may affect the onset of AD. Recently, it is hypothesized that AD may be linked to dysbiosis of microbes in the intestine. The hypothesis suggests that the intestinal flora is capable of affecting brain activity (3).
The prevalence of mild to severe dementia is 5% among individuals aged 65 years and above. It is expected that the number of people with AD will reach 74.7 and 131.5 million by 2030 and 2050, respectively (5). In some patients with AD herpes simplex virus type 1 (HSV-1) weaken the immune system (6). Further experimental data proposed that other viruses, like cytomegalovirus (CMV), may also be involved in the pathogenesis of AD (7).
HSV types 1 (HSV-1) and 2 (HSV-2) are DNA viruses that encode more than 84 different proteins (8). These genes permit virus entry into cells for expression of certain genes that stop cellular and immune responses against viral infection. Both HSV-1 and HSV-2 protect cells against apoptosis (9). Initiation of apoptosis pathways occurs in a cell-type specific manner (10, 11, 12).
CMV is common in older adults and associated with mortality and cardiovascular disease (13, 14). Similar to HSVs, CMV constitutes a state of chronic infection, and viral reproduction must be concealed from the host immune response (15, 16). Most CMV brain infections occur in immunocompromised patients, such as transplant recipients, HIV patients and congenital CMV cases (17). Considering the importance of AD and its diagnosis, the present study aimed to investigate the prevalence of common DNA viruses in serum samples of AD patients in Tehran, Iran.

MATERIALS AND METHODS
Plasma samples were taken from 47 women and 53 men with AD in hospitals in Tehran, Iran. The samples were collected in sterile tubes and transferred to the microbiology laboratory. The samples were kept at -80 °C until DNA extraction. Quantitative and qualitative assessments of the extracted DNA were performed by spectrophotometry and agarose gel electrophoresis, respectively. Three primer pairs specific for HSV-1, HSV-2 and CMV were used to detect the viruses (Table 1) (19).
HSV1 and HSV2 were grown in a Vero cell line (Pasteur Institute of Iran) kept in RMPI medium.
CMV was extracted from serum with a specific DNA titer. The PCR reaction was performed using PCR Master Mix, primers and extracted DNA samples. The results were analyzed on 2% agarose gel stained with SYBR green dye (SinaClon, Iran).
Statistical analysis was performed using SPSS 16.0 software. The results were expressed as mean ± standard deviations (SD). Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett’s new multiple range test at significance of 0.05.

Table 1-The primers used for the detection of HSVs and CMV by PCR

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primer Sequence</th>
<th>Product size for PCR (bp)</th>
<th>Target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV1</td>
<td>H1F 5-TGGGACACATGCTTCTTGGG-3</td>
<td>147</td>
<td>Glycoprotein D</td>
</tr>
<tr>
<td></td>
<td>HIR 5 CCCCCAGTGTTACCTTACC-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV2</td>
<td>H2F 5-GTACAGACCTTCGGGAGG-3</td>
<td>227</td>
<td>Glycoprotein D</td>
</tr>
<tr>
<td></td>
<td>H2R 5-CGCTTCATCCTGGG-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>CMF 5-GTACACGCACGGTCTGTTACC-3</td>
<td>256</td>
<td>IRL 11</td>
</tr>
<tr>
<td></td>
<td>CMR 5-GTAAAGCCCTCGACATC-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Demographic profile and medical records of patients with AD are presented in table 2. HSV-1 and HSV-2 were significantly associated with dysphoria, hallucination, insomnia and depression (P<0.05), and CMV frequency was significantly associated with hallucination and dysphoria (P=0.001). People with underlying diseases such as diabetes, hypertension, and dyslipidemia were also more susceptible to CMV infection. Results of PCR detection of HSV-1, HSV-2 and CMV are presented in figures 1-3.

Table 2- Demographic data and medical records of the subjects

<table>
<thead>
<tr>
<th>Male (n=53)</th>
<th>Blood types</th>
<th>Dysphoria (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n=7)</td>
<td>A+ (n=24)</td>
</tr>
<tr>
<td>AB- (n=5)</td>
<td>AB+ (n=11)</td>
<td></td>
</tr>
<tr>
<td>B+ (n=17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O+ (n=35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n=47)</td>
<td>History of infectious diseases (n=10)</td>
<td>Hallucination (n=33)</td>
</tr>
<tr>
<td>Married</td>
<td>Anemia (n=3)</td>
<td>Blood fats (n=38)</td>
</tr>
<tr>
<td>Mean age: 70.72±7 years</td>
<td>Fe deficiency (n=10)</td>
<td>Brain stroke (n=10)</td>
</tr>
<tr>
<td>Duration of disease: 10.34±7.23 months</td>
<td>P (normal), Ca (normal), Na (normal), K (normal)</td>
<td>Thyroid (n=15)</td>
</tr>
<tr>
<td>Insomnia (n=25)</td>
<td>Depression (n=44)</td>
<td>Heart attack (n=28)</td>
</tr>
<tr>
<td>Hypertension (n=37)</td>
<td>Diabetes (n=27)</td>
<td>CMV (n=27)</td>
</tr>
</tbody>
</table>

HSV-1 and HSV-2 were significantly associated with dysphoria, hallucination, insomnia and depression (P<0.05), and CMV frequency was significantly associated with hallucination and dysphoria (P=0.001). People with underlying diseases such as diabetes, hypertension, and dyslipidemia were also more susceptible to CMV infection. Results of PCR detection of HSV-1, HSV-2 and CMV are presented in figures 1-3.

Figure 1- Gel electrophoresis of PCR products for detection of HSV-1 in samples collected from AD patients. M: 1kb DNA ladder (bioflux), C+: positive control, C-: negative control, lanes 51-75: negative samples.

Figure 2- Gel electrophoresis of PCR products for detection of HSV-2 in samples collected from AD patients. C+: positive control (231bp), C-: negative control, lanes 51,52,53,55-75: negative samples, lane 54: positive samples.
samples of healthy individuals were IgG positive for HSV. Kristen et al. investigated HSV-2 infection with neurological symptoms such as AD in human neuroblastoma cells, and authors reported that HSV-2 infection leads to severe accumulation of phosphorylated peptides such as Aβ40 and Aβ42 amyloid peptides in human SK-N-MC neuroblastoma cells. A study in England showed that inflammation may be caused by a CNS infection or environmental infection (31). There are various microorganisms such as bacteria (mainly Troponium species), viruses (HSV-1) and yeasts (Candida species) in brain of subjects with AD. Oral infection is also known as another possible cause of AD (31, 32). Viral agents of these infections occupy the central nervous system (32, 33). In another study, five different types of HSV were detected simultaneously using multiplex PCR in 86 patients with symptoms of meningitis and encephalitis. HSV-1 was detected in 3.5% of patients with meningoencephalitis, while HSV-2 was found in a new-born. In addition, varicella-zoster virus was detected in four patients with meningitis symptoms (4.6%) and in a new-born (19, 35).

CONCLUSION
CMV infection is associated with increased risk of AD and a quick rate of cognitive decline in elderly populations. Experimental data suggest participation of a polymicrobial community in the pathogenesis of AD. Furthermore, neuronal infection with HSV-1, HSV-2 and CMV may help accumulate amyloid beta deposits and hyperphosphorylated tau that are related to AD pathology.

DISCUSSION
This hypothesis that microorganisms might have an essential role in the development of AD was suggested by the Itzhaki’s group (3). It has been proposed that latent HSV-1 in the trigeminal ganglia could ascend along known nerve paths into the limbic system and regions of the brain most influenced in AD. HSV-1 seropositivity is associated with cognitive impairment in children and defective reading and visuospatial processing in middle-aged adults (22, 28).

CMV spreads from person to person via body fluids. Older adults have increased levels of IgG antibodies to CMV compared with younger individuals (20, 21, 22). Changes in cell-mediated immune parameters often happen with aging and can cause subclinical CMV reactivation (3).

Recent studies have provided further support for the recurrence of HSV-1 in brain in relation with the pathogenesis of AD (23). A review reported that these viruses can interact with other disease-modifying agents for initiation and/or progression of neurodegenerative disease (24).

Apolipoprotein E is also involved in infection with HSV-1, hepatitis C and immune deficiency virus (24, 25). Bourgade et al. show that pathogens may be the most important contributor to the development of AD, especially in the case of HSV infection, that is often important at the same brain sites (26).

Itzhaki et al. confirmed the role of HSV-1 in AD in the last 8 years. Major advances in humans and mice have shown possibility of a hidden virus in the brain (20). Epidemiologic studies have shown the role of HSV-1 in AD (28, 29). Olsson et al. reported the prevalence of HSV to be 11.5% in human brain tumours. In addition, they showed that 86% of serum samples of healthy individuals were IgG positive for HSV.

Kristen et al. investigated HSV-2 infection with neurological symptoms such as AD in human neuroblastoma cells, and authors reported that HSV-2 infection leads to severe accumulation of phosphorylated peptides such as Aβ40 and Aβ42 amyloid peptides in human SK-N-MC neuroblastoma cells. A study in England showed that inflammation may be caused by a CNS infection or environmental infection (31). There are various microorganisms such as bacteria (mainly Troponium species), viruses (HSV-1) and yeasts (Candida species) in brain of subjects with AD. Oral infection is also known as another possible cause of AD (31, 32). Viral agents of these infections occupy the central nervous system (32, 33). In another study, five different types of HSV were detected simultaneously using multiplex PCR in 86 patients with symptoms of meningitis and encephalitis. HSV-1 was detected in 3.5% of patients with meningoencephalitis, while HSV-2 was found in a new-born. In addition, varicella-zoster virus was detected in four patients with meningitis symptoms (4.6%) and in a new-born (19, 35).

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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