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Frequency of Herpes simplex virus in patients with Stroke by PCR

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ABSTRACT

Herpes simplex virus (HSV) causes a wide range of pathologies in the human central nervous system (CNS) such as stroke. It was proposed that there is an association between HSV infection and stroke with different results reported by the researchers using different methods. This study aimed to investigate the frequency of HSV among stroke patient and control groups. We detected HSV in 28 stroke patients and 28 controls that admitted in Beheshti, Emam Reza, Rajaee and Emam Sajad Hospital in 2013 north of Iran. DNA was extracted from blood samples and the infection with HSV was examined by PCR technique. The results obtained by this study showed that 15(53.6%) out of 28 in patients were infected by HSV, while the number of control samples infected by this virus was 9(32.1%). Statistical analysis has shown that there is not any significant association between the frequency of HSV and stroke, while in other studies the significant association between HSV and stroke has been demonstrated. These contradictory results could be due to the use of different diagnosing techniques, sample type and epidemiological differences.

1. Introduction

Stroke is a common cause of mortality and morbidity and the second leading cause of death in worldwide. This disease is leading cause of functional impairments in adults which could affect both patients and their relatives in most regions (Hosseini et al., 2010). As populations are getting older, a dramatic increase in prevalence and burden of disability is expected in the future. In the Middle East and North Africa (MENA), stroke is progressively becoming a major health problem and it is estimated that its current mortality will double by 2030 (Fahimfar et al., 2012). Certain segments of the population have a disproportionately high risk of stroke. African-Americans have almost twice the risk of a first ever stroke as whites, and African-Americans and Hispanics are more likely to die after a stroke, compared to whites. Gender is also a risk factor and women are at higher risk than men (Go et al., 2013).

While the well-known risk factors for ischemic stroke has been shown to be aging, hypertension, diabetes, obesity, smoking, history of cardiovascular diseases (CVD), atrial fibrillation, left ventricular hypertrophy and hypercholesterolemia (Hosseini et al., 2010), viral infections have also emerged as risk factors for stroke (Lin et al., 2010; Heikinheimo et al., 2012; Sreenivasan et al., 2013; Liao et al., 2012; Muhammad et al., 2011). Herpes simplex virus (HSV) which is the member of herpes viridea family was shown to be associated with the disease of nervous system (Ebrahimi et al., 2011). Herpes viruses have double stranded DNA and icosahedral symmetry. This

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nucleocapsid is surrounded by a lipid (Izham et al, 2011).

HSV can cause severe systematic neonatal infection by transmission before or during birth (Tookey et al., 1996; Brown et al., 1997). Some research suggested that HSV is associated with stroke (Kis et al., 2007; Antonetta et al., 2009; Snider et al., 2014) while some other studies did not confirm this association (Paul et al.,1998). Also stroke should be considered a possible complication of HSV1 primary infection in childhood (Terelizzi et al., 2013). This contradiction may be due to the methods used in the diagnosis of HSV. This study aimed to investigate the frequency of HSV among stroke patient and control groups.

2. Materials and Methods

2.1. Sample collection

Blood samples of stroke patients (n=28) were collected from the ICU ward of hospitals of northern Iran including Emam Sajjad of Ramsar, Rajaee of Tonekabon, Emam Reza of Amol, Beheshti of Noshahr hospitals. Blood samples of control groups (n=28) were also collected from the North of Iran in 2013. Stroke patients were confirmed by a neurologist. Questionnaires and samples were collected with satisfaction of patient's attendance by observing medical ethics.

2.2. DNA extraction

Extraction of DNA from the tissue was implemented according to the instructions of the manufacturing company (Qiagene, Lot No : 11872584, Cat No: 51306). The purity of the extracted DNA was analyzed based on the absorbance of the extracted DNA at 260 and 280 nm wavelengths by biophotometer (Eppendorf-Germany).

2.3. PCR method

To ensure the health and safety of DNA extraction, human beta-globin gene was co-amplified with the target fragment as an internal amplification control. Specific Primers produced by TAG Copenhagen (Denmark) were used to amplify the beta-globin gene. The sequences of forward and reverse primers were 5'- TCC AAC ATC AAC ATC TTG GT-3' and 5'- TCC CCC AAA TTC TAA GCA GA-3' respectively (Zaravinos et al., 2009).

Reaction was performed in a total volume of 20 µl, which contained 10 µl master mix (Qiagene), 1 µl of forward and reverse PCR primers, 3 µl distilled water and 5 µl of DNA template. The negative control tube contained the same PCR regents as mentioned above but had 5 µl water substituted for the DNA template. PCR amplification condition on thermocycler (Biorad-Germany) for beta-globin gene were as follows: 95ºC for 5 min , followed by 35 cycles of 95 ºC for 45 sec , 55 ºC for 45 sec and 72 ºC for 40 sec, with a final extension at 72 ºC for 5 min (Eghbali et al., 2012).

For the detection of HSV genome in the samples, PCR was done by specific primers produced by TAG Copenhagen (Denmark) in order to amplify the HSV gene. The sequences of forward and reverse primers were 5'-CAG TAC GGC CCC GAG TTC GTG A-3' and 5'- TTG TAG TGG GCG TGG TAG ATG-3', respectively for HSV (Zaravinos et al., 2009).

Each reaction was performed in a total volume of 20, which contained 10 µl master mix (Qiagene-Germany), 1 µl of forward and reverse PCR primers, 3 µl distilled water and 5 µl of DNA template. The negative control tube contained the same PCR regents as above but had 5 µl water substituted for the DNA template. PCR amplification condition on thermocycler (Biorad-Germany) for herpes simplex virus were as follows: 95ºc for 5 min , followed by 35 cycles of 95 ºC for 50 sec , 64 ºC for 45 sec and 72 ºC for 40 sec, with a final extension at 72 ºC for 5 min. An aliquot of all PCR products was run on a 1.5% agarose gels with a 100 bp DNA ladder (Fermentas–Russia) and electrophoresed at 75 v for 40 min. The bands were visualized using ethidium bromide staining and photographed after UV treatment by a transilluminator (UV doc, England).

2.4. Statistical analysis

Chi Square test was used to determine whether there was any significant difference between the frequency of HSV in the ischemic patients and its relationship with the stroke (SPSS software 17).

3. Results

PCR of beta globulin gene was performed for all samples for the accuracy of DNA extraction. 122 bp band was observed in all samples (Figure 1).

In order to identify the DNA of virus in the blood samples, the PCR technique was used. The presence of a 267 bp band indicates the presence of HSV DNA in the sample (Figure 2). In summary, 15 samples (53.6%) were positive for the presence of HSV among 28 stroke group and 9 samples (32.1%) were positive for the presence of HSV among the 28 control group. No significant relationship between presence of HSV DNA and stroke was seen between two groups (P=0.526).

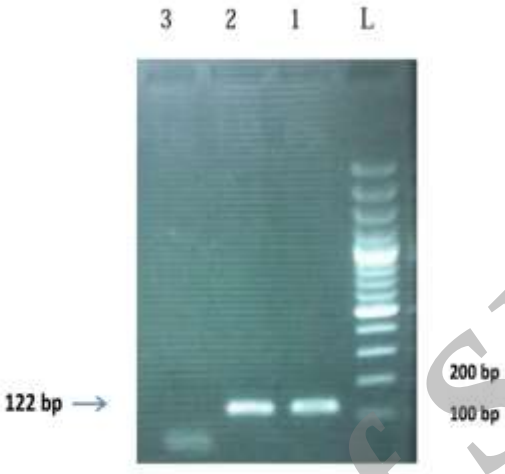

Figure 1. Beta globin gene amplification products electrophoresed on a 1.5% agarose gel. L: Ladder 100-1000 bp, 1 and 2 extracted DNA, 3 negative control

Figure 2. HSV amplification products electrophoresed on a 1.5% agarose gel L: Ladder 100-1000 bp, 1 positive control, 7-10 HSV positive sample,2-6 negative sample.11 negative control

In this study, among 15 samples (53.6%) were positive for the presence of HSV among the 28 patients, 6 individuals (40%) were women and 9 individuals (69.2%) were men (P=0.151). Among 9 samples (32.1%) were positive from the view point of the presence of HSV among the 28 control group, 5 individuals (33.3%) were women and 4 persons (30.76%) were men (P=0.604). In both group, a significant relationship was not observed between the presence of HSV DNA in the samples and sex (Table 1).

Table 1. Frequency of HSV and sex in stroke/control groups

sex	Man		Woman		p-value
	HSV+	HSV-	HSV+	HSV-	
stroke	9 (69.2%)	4 (30.8%)	6 (40%)	9 (60%)	0.151
control	4 (30.8%)	9 (69.2%)	5 (33.3%)	10 (66.7%)	0.604

In this study, among 15 samples, 53.6% were positive for the presence of HSV, among 28 patients, 3 individuals (50%) were in the age group of 40 to 60 years old and 12 individuals (57.1%) were in the age group over 60 years old (P=0.524). Among 9 samples, 32.1% were positive for the presence of HSV, among the 28 control group, one individuals was in the age group below 40 years (3.57%), 6 individuals (21.42%) were in the age group of 40 to 60 years old and 2 individuals (7.14%) were in the age group over 60 years old (P=0.344). In both groups, a significant relationship was not observed between the presence of HSV DNA in the samples and age class (Table 2). The statistical analysis did not show a significant relationship between the frequencies of this virus in the samples studied.

Table 2. Frequency of HSV and Age in stroke/control groups

age	<40		40-60		60<		p-value
	HSV+	HSV-	HSV+	HSV-	HSV+	HSV-	
stroke	0 (0%)	1 (100%)	3 (50%)	3 (50%)	12 (57.1%)	9 (42.9%)	0.524
control	1 (100%)	0 (0%)	6 (30%)	14 (70%)	2 (28.6%)	5 (71.4%)	0.334

4. Discussion

Stroke is the second leading cause of death in developed and developing countries (Hosseini et al., 2010). On average, one person in the U.S. has a stroke every 40 seconds, and every 4 minutes one people dies from one. Conservative estimates forecast that ischemic stroke alone will cost the U.S. an astounding $2.2 trillion from 2005 to 2050 (Go et al., 2013). Also Iran has high rates of stroke (Azadpoor et al., 2010). Viruses are risk factors for stroke. Although data are limited regarding the role of herpes viruses especially herpes simplex virus and stroke, but so far most of the viruses and pathogens associated with risk of stroke was examined in relation to communication and some of them have been proven including ophthalmic herpes zoster virus (Lin et al., 2010) HIV (Heikinheimo et al., 2012), VZV (Sreenivasan et al., 2013), HCV (Liao et al., 2012), HCMV, HHV-6, EBV (kis et al., 2007), Hepatitis A virus, Helicobacter pyloria, Chlamydia pneumonia (Smieja et al., 2004) and Influenza Virus (Muhammad et al., 2011).

Some studies including this study have shown no significant association between HSV and stroke (Paul et al., 1998). While the other studies were able to shown the significant association between HSV and stroke (Kis et al., 2007; Antonthal et al., 2009). Ussing ELISA, Kis et al in 2007 have shown that there is a significant association between ischemic stroke and IgA antibody of HSV-1 (Kis et al., 2007). Incidence of ischemic stroke in a 3-year-old girl was reported in 2009 that HSV-1 virus was isolated from her cerebrospinal fluid(Antonthal et al., 2009). These contradictory results could be from the usage of different diagnosing techniques, sample type and epidemiological differences.

The result by this study showed the high frequency of HSV in stroke patients. The high rate of mortality and disabilities were considered after occurrence of the cerebral stroke. Therefore, it is suggested that this research, considering the importance of its subject, is to be conducted in more extensive level and with more samples and in the various regions, and performance of empirical research is recommended. It seems that prevention from infection of this virus can play a more effective role in the reduction of the affliction with stroke. However, further studies are needed to confirm the role of HSV in stroke.

Refereces

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