Frequency of Herpes Virus (HSV) in Esophageal Muscle Samples among Patients with Achalasia under Heller Myotomy

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Abstract

Background

Despite years of research, the etiology of achalasia not well understood. Scientists suppose a role for autoimmunity, in this disorder, and probable viral agent, such as herpes virus (HSV). The aim was to find out the frequency of HSV in esophageal muscle samples in patients with achalasia under Heller's myotomy.

Methods

In this study, 60 patients with achalasia, after fulfilling the consent form, were underwent Heller’s myotomy surgery. Biopsy samples prepared for polymerase chain reaction (PCR) method for HSV DNA detection. After DNA-extraction, replication performed using specific primers.

Results

The mean age was 40.62 ± 5.08 years. Thirty-nine patients (65%) were female and 21 (35%) were male. Thirty-eight (63.3%) had no history but the else (36.7%) had a positive history of HSV. HSV-1 was positive in three patients (5%). Two females and one male were HSV-positive.

Conclusions

HSV-1 frequency is not notable among Iranian patients with achalasia. We suggest exploring other viruses, in special that involving the pathogenesis of achalasia, with a larger sample size.

Introduction

Achalasia, the low-esophageal sphincter dysfunction (LES), first described by Thomas Willis in 1674. As an incurable disease, achalasia treatment has often included sedatives, aimed at relieving solid food dysphagia. The prevalence of idiopathic achalasia is 1 per 100,000 estimated and geographically diverse. Most patients are in the age groups in 20 to 40 or 60 to 70 years[1, 2].

Despite years of research, the etiology of achalasia not well understood. Chagas is a similar multiorgan disease which its esophageal exhibitions have manometric characteristics like achalasia[3]. People with achalasia do not have adrenergic, noncholinergic and inhibitory ganglions which cause an imbalance in transmitting active potential in stimulatory and inhibitory nerves. The result is an increased pressure on the esophageal sphincter[4].

Histological evaluation of autopsy and myotomy showed an inflammatory response consisting of positive cytotoxic CD3/CD8 T-lymphocytes, a variable number of eosinophils and mast cells, loss of ganglion cells, and neurofibrosis. Therefore, there an area of autoimmunity for achieving achalasia, most likely because of viral stimuli[5]. Heller myotomy helps 90% of patients with achalasia. It can usually done with a laparoscopic aspects [6].

The best recommended manner was complete Heller's myotomy, known as Dor Fundoplication, which consists of a 180 to 200 degree anterior wrap around the esophagus. These results are good compared with Nissin fundoplication, which is associated with a higher prevalence of postoperative dysphagia[7]. Nearly, 67% of the world's population under 50 years-old have HSV-1. In the United States, about 47.8% and 11.9% of population have HSV-1 and HSV-2, respectively[8].

Viruses sometimes cause mild or unusual symptoms when they spread. After early infection, some infected people experience scattered parts of the virus reactivating or spreading. In the next outbreak, the virus is activated in a nerve cell and passed on to the skin through the neuron axon, where the virus multiplies and causes new wounds[9].

Achalasia is the result of a chronic inflammation involving the myenteric plexus. Neuritis and ganglionitis are evident in the early stages of the disease and lead to the gradual loss of ganglion cells and fibrosis. Immunohistochemical studies have shown the most inflammatory cells that invade the myenteric plexus are activated by positive CD3/CD8 lymphocytes. Among the potential infectious agents, the human herpes virus type-1 (HSV-1) exhibits some attractive features due to its achalasia[10]. The aim of this study was to find out the frequency of HSV in esophageal muscle samples in patients with achalasia under Heller myotomy.

Material And Method

In this study, people diagnosed with achalasia, based on clinical examinations and paraclinical evidences (radiography), after announcing their consent to evaluate and review in this study, underwent Heller myotomy surgery. Biopsy samples sent to a specialized polymerase chain reaction (PCR) laboratory for molecular studies. First, using DNA extraction solution, deoxyribonucleic acids extracted from muscle tissue and purified.

One milliliter of RNXTM-PLUS solution (Cinaclon, Iran) was added to each of the 6-cell plate wells and then incubated at room temperature for 5 minutes. Two hundred microliter of chloroform added to each microtube, shaken vigorously for 15 seconds, incubated for 5 minutes at 4°C, followed by centrifugation for 15 minutes at 4°C with 12000 RPM.

Using proprietary HSV-1 and HSV-2 primers (Table 1), amplification of the viral DNA evaluated in conventional PCR step and the number of copies were increased if the tissue samples containing the target genes. The PCR program was done on a Peqlab-peqSTAR apparatus (United Kingdom) as this

order: 95°C for 300 seconds, 40 cycle of 95°C for 20 seconds and 60°C for 20 seconds, and final extension with 75°C for 5 minutes.

Post-PCR step included the electrophoresis of the products on 1% agarose gel in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA, with a loading dye containing gel-red for visualization the amplified DNA in a florescent view box, if present. We used a 50 bp DNA ladder (Carl Roth GmbH) to determine the size of PCR products. The presence of virus DNA in the sample considered as a positive result and the absence as the HSV-1 negative tissue sample.

We have assessed the HSV serum immunoglobulin-G (IgG) presence and absence among studied participants using enzyme-linked immunosorbent assay (ELISA) (PISHTAZTEB, Iran).

Table-1: the sequence and amplicon size of specialized primers, used for amplification of HSV-1 and HSV-2 DNA samples

PCR size	Starting sequence	primer
280	TGCTAATCGTAGGGGTAAA	**HSV-1 forward**
	GTGCAGGGTCCGAGGT	**HSV-1 Reverse**
300	AGGCCGTCGATTCGCTC	**HSV-2 forward**
	GGAGGACCTGAAGACGTGC	**HSV-2 Reverse**

The sample size was determined using MedCalc statistical software with the maximum alpha error of 5% (minimum 95% reliability coefficient). According to a study by Kaufman et al. (2005) [11] at least 59 patients with inclusion criteria were enrolled for PCR evaluations. Considering the descriptive data, we presented data distribution and the percentage of frequencies using suitable curve and tables. SPSS ver.24 software also used to report the results.

Ethical consideration:

The permission was obtained from medical ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (registration no: IR.SBMU.MSP.REC.1398.244).

Results

In the present study, 60 patients were enrolled with a mean age of 40.62±5.08 years (30-50 years-old). Most individuals were between 36 to 40 years and 39 patients (65%) were female and other 21 (35%)

were male participants.

The patients were divided into two groups because of a history of herpes disease. According to the results, 38 people (63.3%) had no history of HSV, while 22 (36.7%) were positive for the history of HSV.

In the present study, patients were evaluated for serum IgG which showing that 38 (63.3%) negative and 22 (36.7%) positive results. Dedicated reproduction of HSV genome showed the 5% (3 patients) positive results belonging two females and one male. Therefore, no significant difference founded between male and female patients infected with HSV and suffering achalasia (P value = 0.4). The results showed that all three patients whose HSV-DNA tests were positive (100%) also harboring IgG serum antibody.

Discussion

Achalasia is a rare disorder of esophageal motility with an unknown cause that is characterized by loss of proper function of the lower esophageal sphincter, leading to dysphagia and poor quality of life. Studies have shown that achalasia is independent to the race or gender, may occur at any age, especially in the 3rd to 6th decades of life[12].

Internal and external causes that trigger and regulate the inflammatory responses are not fully understood. To address these drawbacks, several studies have suggested that some neurotransmitters and autoimmune agents may cause a response in the myenteric network and eventually inflammation, including Varicella zoster and HSV[13].

Researchers have tried to find the etiological role of these diseases in developing the neurological defects in achalasia by examining diseases leading to the T-cell proliferation. Although no infectious or pathogenic factors have yet been identified in myomectomy histological samples of patients with achalasia, the role of HSV has been noted, but not confirmed in various studies. Researchers believe the HSV type-1 has a role in the etiology of achalasia[14].

In some recent evidence, HSV-1 DNA has been identified in most patients. However, these studies confirm the inflammatory and immune response after HSV infection which leads in destroying of esophageal neurons, that predispose the patient to the achalasia and lower esophageal sphincter defects[15].

Boeckxstaens et al. reported that HSV DNA was detected in most of cases with ascites. The study infers the disease resulting from neuronal degeneration sought an immune-inflammatory response resulted from inflammatory response. The immune system can work against viral infections, especially HSV-1. Anyway, investigators believed that the immune-inflammatory response to exposure the HSV should also have a genetic basis[16].

Against many studies, the Birgisson and colleagues' study, measles and papilloma virus, in patients with achalasia did not report any positive PCR sample in control or patient groups. Unlike other studies, it has been suggested that disease has nothing to do with these infectious diseases, especially herpes

simplex[17]. Our results did not confirmed this theory and we believed that infectious disorder, especially HSV-1, can play an important role in achalasia.

Kaufman and coworkers, assessed the HSV-1 immunoassay and PCR tests on the tear and salivary samples. The percentage of positive eye and mouth swabs was almost equal (33.5% were positive for the eye and 37.5% for the mouth samples). Also, the prevalence of the virus in the tear samples of men and women was very similar (34.6% in men and 34.8% in women). They claim that 49 of 50 unmarked individuals (98%) tested positive for PCR with tears or saliva. They also described the positive results in PCR samples as much higher than ELISA method[18]. Therefore, evaluating the molecular epidemiology of infectious agent is preferable than serological measurements, as like as our results shown.

In 2000, Espy et al., From the Mayo Clinic of the United States examined 200 samples, including 160 genital samples, 38 skin samples, and 2 eye samples by PCR and cell culture. They also suggested the use of PCR to detect routine and rapid HSV[18].

Monica Facco et al., conducted a study on 59 patients with achalasia. Patients were evaluated using flow-cytometry and CDR3 lymphocyte analysis. In these patients, lymphocyte infiltration, by positive CD3 and CD8 markers T-cell cells, were found. Besides, the lymphocytes of achalasia patients extracted from LES responded to the HSV-1 antigen in vitro. The results of mentioned study showed the lymphocytic secretion in the lower esophageal sphincter could be representative of the cellular immune response to HSV-1 antigens[19].

In the study of Castagliulo et al., esophageal muscle tissue layers were evaluated in patients with achalasia. DNA was extracted from the muscular layer of esophageal tissue and the HSV-1 and HSV-2 antibodies were evaluated. The rate was 3.4 times that of the control group and the increase in interferon-gamma secretion, after HSV-1 incubation, was reported to be 1.4 times higher in achalasia patients is emphasized[20].

Conclusion

In the present study, amplification of HSV genome, in esophageal surgical specimens, using specific primers, showed that HSV-1 was positive in three patients. It seems, HSV-1 to not be prevalent among Iranian patients suffering achalasia. The other viruses, mentioned as potential infectious agents, may involve in pathogenesis of achalasia, and should be explored in a larger community size of Iranian achalasia patients.

Declarations

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

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Informed consent: Written informed consent was obtained from the patients for publication of this article and any accompanying images. A copy of the written consent is available for review by the editor-in-chief of this journal

References

1. Spechler, S. and D. Castell, *Classification of oesophageal motility abnormalities.* Gut, 2001. **49**(1): p. 145-151.

2. Spiess, A.E. and P.J. Kahrilas, *Treating achalasia: from whalebone to laparoscope.* Jama, 1998. **280**(7): p. 638-642.

3. Roucher-Boulez, F ,.et al., *Triple-A syndrome: a wide spectrum of adrenal dysfunction.* European journal of endocrinology, 2018. **178**(3): p. 199-207.

4. Cheatham, J.G. and R.K. Wong, *Current approach to the treatment of achalasia.* Current gastroenterology reports, 2011. **13**(3 :(p. 219-225.

5. Chuah, S.-K., et al., *2011 update on esophageal achalasia.* World Journal of Gastroenterology: WJG, 2012. **18**(14): p. 1573.

6. Deb, S., et al., *Laparoscopic esophageal myotomy for achalasia: factors affecting functional results.* The Annals of thoracic surgery, 2005. **80**(4): p. 1191-1195.

7. Rebecchi, F., et al., *Randomized controlled trial of laparoscopic Heller myotomy plus Dor fundoplication versus Nissen fundoplication for achalasia: long-term results.* Annals of surgery, 2008. **248**(6): p.1023-1030 .

8. Marrazzo, J.M., K. Stine, and A. Wald, *Prevalence and risk factors for infection with herpes simplex virus type-1 and-2 among lesbians.* Sexually transmitted diseases, 2003. **30**(12): p. 890-895.

9. Whitley, R.J. and B. Roizman, *Herpes simplex virus infections.* The lancet, 2001. **357**(9267): p. 1513-1518.

10. Clark, S.B., et al., *The nature of the myenteric infiltrate in achalasia: an immunohistochemical analysis.* The American journal of surgical pathology, 2000. **24**(8): p. 1153-1158.

11. Kaufman ,H.E., et al., *HSV-1 DNA in tears and saliva of normal adults.* Investigative ophthalmology & visual science, 2005. **46**(1): p. 241-247.

12. Farrokhi, F. and M.F. Vaezi, *Idiopathic (primary) achalasia.* Orphanet journal of rare diseases, 2007. **2**(1): p. 1-9.

13. Farr, C., *Achalasia: new thoughts on an old disease.* Journal of clinical gastroenterology, 1992. **15**(1): p. 2-4.

14. Gockel, H.R., et al., *Achalasia: will genetic studies provide insights?* Human genetics, 2010. **128**(4): p. 353-364.

15. Gockel, I., M. Müller, and J. Schumacher, *Achalasia—a disease of unknown cause that is often diagnosed too late.* Deutsches Ärzteblatt International, 2012. **109**(12): p. 209.

16. Boeckxstaens, G.E., *Achalasia: virus-induced euthanasia of neurons?* 2008, LWW.

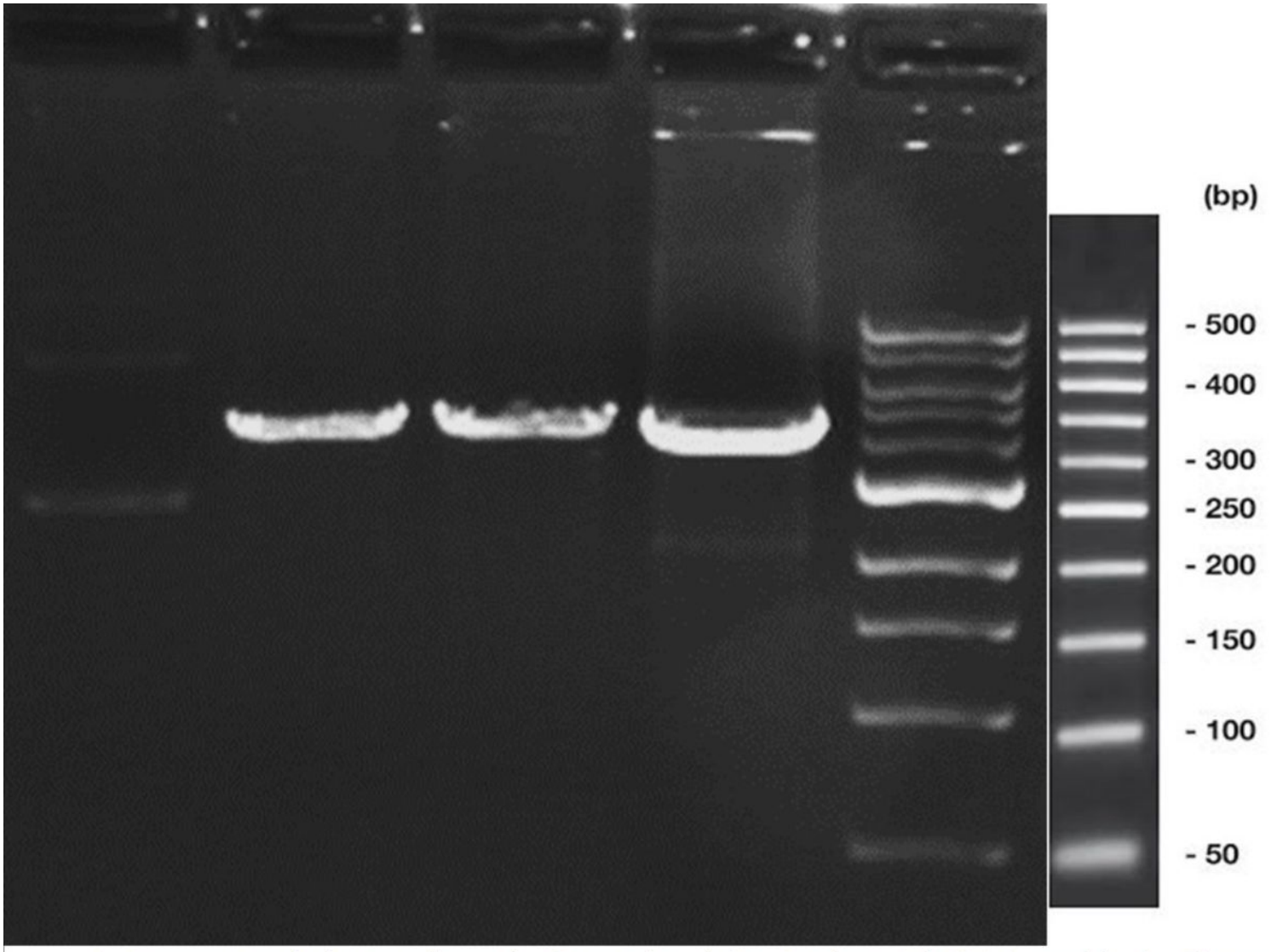
17. Aynaud, O., et al., *Frequency of herpes simplex virus, cytomegalovirus and human papillomavirus DNA in semen.* International journal of STD & AIDS, 2002. **13**(8): p. 547-550.

18. Espy, M.J., et al., *Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR.* Journal of Clinical Microbiology, 2000. **38**(2): p. 795-799.

19. Facco, M., et al., *T cells in the myenteric plexus of achalasia patients show a skewed TCR repertoire and react to HSV-1 antigens.* American Journal of Gastroenterology, 200 :(7)103 .8p. 1598-1609.

20. Castagliuolo, I., et al., *Esophageal achalasia: is the herpes simplex virus really innocent?* Journal of gastrointestinal surgery, 2004. **8**(1): p. 24-30.

Figures



1. Agarose gel electrophoresis. From the right: Patient 1 (HSV-1 positive), Patient 2 (HSV-1 positive), Patient 3 (HSV-1 positive) and an example of a negative patient. Amplified DNA showing around 300 bp size on 1% agarose gel-electrophoresis. DNA ladder is shown for comparison at the right side.

Figure

Figure 1

Agarose gel electrophoresis. From the right: Patient 1 (HSV-1 positive), Patient 2 (HSV-1 positive), Patient 3 (HSV-1 positive) and an example of a negative patient. Amplified DNA showing around 300 bp size on 1% agarose gel-electrophoresis. DNA ladder is shown for comparison at the right side.