Original Article

Role of Nonstructural Proteins and Hemagglutinin Antigens among Patients Suffering from Influenza B Virus

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Abstract

Introduction: Influenza B is a single-negative-sense, ribonucleic acid (RNA) virus possessing an RNA-dependent RNA polymerase of viral origin for replication. These properties enable it to switch antigens. Research focuses on influenza B because of its significant public health impact. The study aims to determine the disease and the speed of its spread in young patients and tries to limit its spread through immunization. **Materials and Methods:** The study included 50 patients aged from 10 to 30 years old. The specimens were collected from patients who were admitted to Al-Hilla Teaching Hospital in the city of Babylon, Baylon, Iraq. A total of 20 symptomatic patients had documented influenza B virus infection. The virus was diagnosed first by the rapid test method [immunoglobulin (Ig)M and IgG] and then by polymerase chain reaction (PCR) using diagnostic genes [nonstructural protein (*NSP*)] and hemagglutinin (*HA*) and reverse transcription PCR to determine the amount of virus. **Results:** The results showed that 20 out of 50 gave a positive result for (IgM) in the rapid test, whereas the conventional PCR showed that 8 (40%) out of 20 patients carried the *NSP* gene (NSP) and showed 18 (85%) out of 20 carrying *HA* genes. **Conclusion:** Influenza B is a common and rapidly spreading disease. Therefore, it is recommended to develop techniques to limit the spread of the disease, considering the speed of its spread and the antigenic transformation capacity possessed by this family must be taken into account. It is also recommended to find more effective vaccines to reduce their spread. Our observations showed that young children are at risk for infection with the influenza B virus compared with adults.

Keywords: Hemagglutinin, nonstructure proteins, the global influenza B virus

Introduction

The family Orthomyxoviridae includes an important virus, influenza B, and it is single-stranded, negative-sense, and segmented. Genetic segments are divided into 11 proteins.[1] Studies have found that the clinical symptoms caused by the influenza B virus are similar to influenza A virus, where many studies have shown that population immunity as well as the sporadic influenza B virus had a role in the spread of the virus.[2] Advanced research has focused on the hemagglutinin (*HA*) gene. This gene was found to consist of two separate strains a year ago in 1983.[3] Studies have found that the segmented genome of the influenza B virus allows genetic exchange through the process of reassortment[4] contributing to the diversity of B viruses. Evolutionary studies of influenza B also confirmed some genes and their relationships with each other, as it was found that when nucleic acid changes as a result of the reclassification process, the vaccine antigen changes.[5] The nonstructural protein (NSP) encoded by the virus genome suppresses the induction of interferons (IFNs)-α/β.[6] The NSP of influenza B viruses has the effect of interfering with the production of INF.[7] NSP disturbs the mimicry of IFNs by first inhibiting the intracellular sensor retinoic acid-inducible gene I (RIG-I), which plays the main role in single-stranded ribonucleic acid (RNA) detection during influenza B virus infection. Activation of RIG-I

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leads to binding to the downstream adapter interferon-beta promoter stimulator 1, which leads to interferon regulatory factor 3 phosphorylation and subsequent transcriptional activation of IFN-β, NSP can associate with host messenger RNA and polyadenylation through interaction with U6 small nuclear RNA and polyadylate cleavage factor 30, respectively. In addition to inhibiting *IFN-γ* gene transcription, NS1 promotes the accumulation of viral transcripts. Global influenza B virus study is a study launched in 2012 on global influenza B to gather information on epidemiology over the years.[8]

Pandemic influenza infections occur in the southern and northern hemispheres during the months from October through April. The full season is considered the period of the full capacity of the virus's peak activity. They found that through numerous studies, each country and season has a certain percentage of infections with this virus according to the location of the country. It was also found that it varies from one season to another, and this is due to the epidemiology of the season in the year.[9]

Materials and Methods

Patients and study design

The study included 50 patients aged from 10 to 30 years old. Specimens were collected from patients who were admitted to Al-Hilla Teaching Hospital, Babylon, during the period from June 2021 to October 2021. The collected specimens included mouth or nose swabs, nasopharyngeal washings, and blood.

RNA extraction

Deoxyribonucleic acid (DNA) was extracted from 100 µL of the virus using (RNeasy; Qiagen Co., Hilden, Germany) and 40 µL of RNase-free distilled water was supplemented. Reverse transcription polymerase chain reaction (RT-PCR) was performed by the layered coding transport protocol (RT-PCR Kit; Takara, Shiga, Japan). The RT reaction consisted of 20 pmol of influenza B virus and 9.5 µL of the RNA sample/20 µL of the RT reaction mixture. About 5 µL of the resulting complementary DNA was used in all PCR amplifications comprising: initial denaturation at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and final extension at 72°C for 10 min. The *HA* gene reaction conditions (initial denaturation at 95°C for 2 min followed by 34 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 1 min, and final extension at 72°C for 10 min. The resulting amplicons were purified and directly sequenced by previously described methods (PerkinElmer, Waltham, MA, USA). Oligonucleotide primers for PCR and sequencing reactions were designed with annealing temperatures ranging from 52°C to 55°C. As for a device, we can determine the amount of the specific virus for infection, and it depends on the severity of the infection.

Table 1: Primer and thermal cycler products

Primer name	Polymerase chain reaction product (bp)	Annealing temperature (°C)	Results (%)
Nonstructural protein gene	241	55	40
Hemagglutinin gene	353	53	85

The amplification products were separated in 1% agarose. DNA ladder (500–1500 pb) for *NSP* gene and DNA ladder (100–1500 pb) for *HA* gene. After electrophoresis, the gel was photographed under ultraviolet light. The primer and thermal cycler products are listed in Table 1 for *NSP* and *HA* genes.

Results

Through the current study, it was found that 20 of 50 samples carried IgM through the rapid test, and it was found through the results of conventional RT-PCR that 8 (40%) of 20 samples carried the *NSP* gene and 17 (85%) samples from 20 samples carried the *HA* gene. As shown in Figures 1 and 2 and the amplicon results for PCR to NSP (241) pb and (353) pb for the *HA* gene. As for RT-PCR, it was used to determine the amount of virus causing infection as shown in Figures 3 and 4 for the two genes, Table 2, for the *NSP* gene, and Table 3, for the *HA* gene (HA) as well as genetic factors and other factors related to the host.

The results showed that the *HA* gene is the most prevalent in the samples, as it was considered diagnostic, which was indicated by many studies, and the current study showed it, where Figure 4 dependent on the results of RT-PCR showed that the presence of the gene in a high curve, whereas the samples that did not contain it were in a straight line and the specific conditions for obtaining the curve according to Table 3 in RT-PCR and due to its effective role in causing the infection through the virus penetration into the host, it was considered one of the strongest factors of the virus, that is, viruses that do not contain it are less powerful and unable to penetrate.

As for the *NSP* gene, it was found that its percentage is low or absent as shown in Figure 1 and in RT-PCR shown in Figure 3 and Table 2, its percentage is low in most of the isolates and its absence in the form of a straight line indicates its absence. This study confirmed that its role is represented by the efficiency of the virus during its growth and to induce the production of INF, and it is not considered one of the factors of virulence. Thus, the *NSP* gene is considered to be less relevant than the *HA* gene, and due to the active role of the HA gene, it is considered to be more dominant than the first.

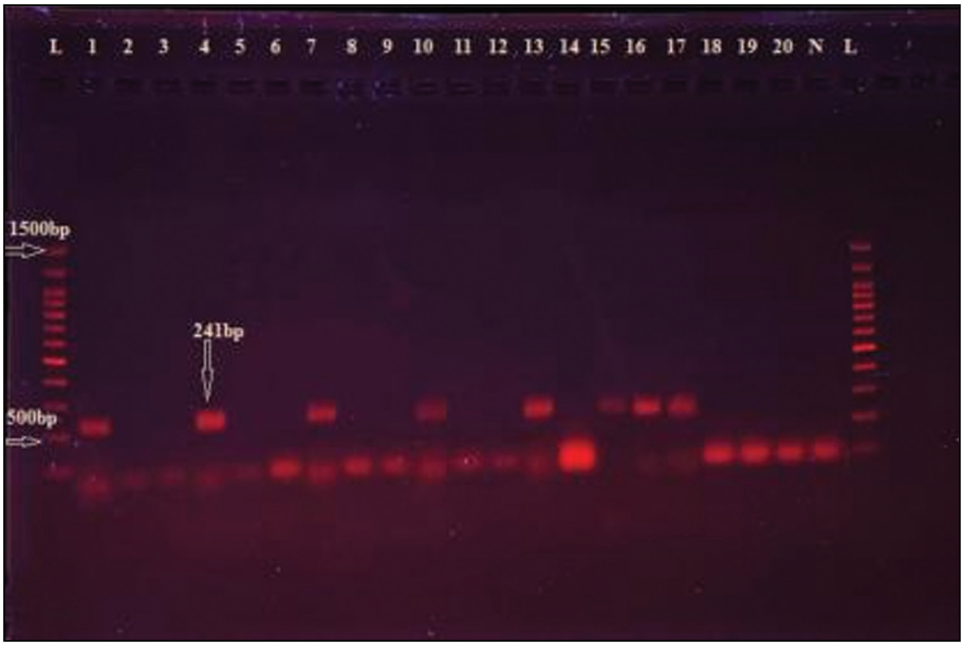

Figure 1: Gel electrophoresis for polymerase chain reaction product of *NSP* gene which showed 241 bp at 55°C (agarose 2%, 15 min at 100 V, then lowered to 70 V, 60 min) Visualized under ultraviolet light after staining with ethidium bromide. Lane L: DNA ladder (1500–100) bp. Lanes (1, 4, 7, 10, 13, and 15–17) represented positive results. Lanes (2, 3, 5, 6, 8, 9, 11, 12, 14, and 18–20) represented negative results. Lane (N) represented negative control

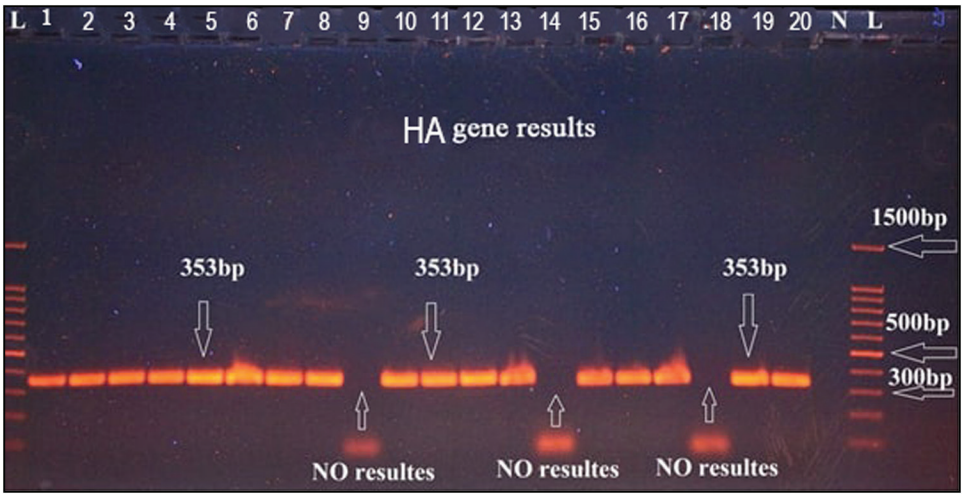

Figure 2: Gel electrophoresis for polymerase chain reaction product of *HA* gene showed 353 bp. TM at 53°C (agarose 1%, 10 min at 100 V and then lowered to 70 V, 60 min) visualized under ultraviolet light after staining with ethidium bromide. Lane L: DNA ladder (100–1500 bp). Lanes (1–8, 10–13, 15–17, and 19 and 20) represented positive results of the *HA* gene. Lanes (9, 14, and 18) represented negative results

Discussion

They have developed a special concept for the epidemic of influenza B, with a focus on specific areas of the world. The areas in which infection is frequent are rural, poor, and population centers due to the ease of spread of the disease and the speed of its spread. In addition to the average age to receive the disease, young people are more likely to receive infection and spread it through gatherings.

Schools, especially for ages between 5 and 17 years and adults from (18 to 30) years old, and several authors have shown the rate of infection with pandemic influenza B at younger ages than at older ages. The NSP was indicated by Jia *et al*.[10] and by the study conducted by Yin *et al*.,[11] whereas Blaurock *et al*.[12] indicated that its percentage is low in samples and its presence may be high or low due to genetic variations. Wolff and Veit[13] referred to the importance of the *NSP* gene in the process of reproduction to produce the required proteins.

The current study showed that the proportion is approximately 40% and that 8 of the 20 samples carry the

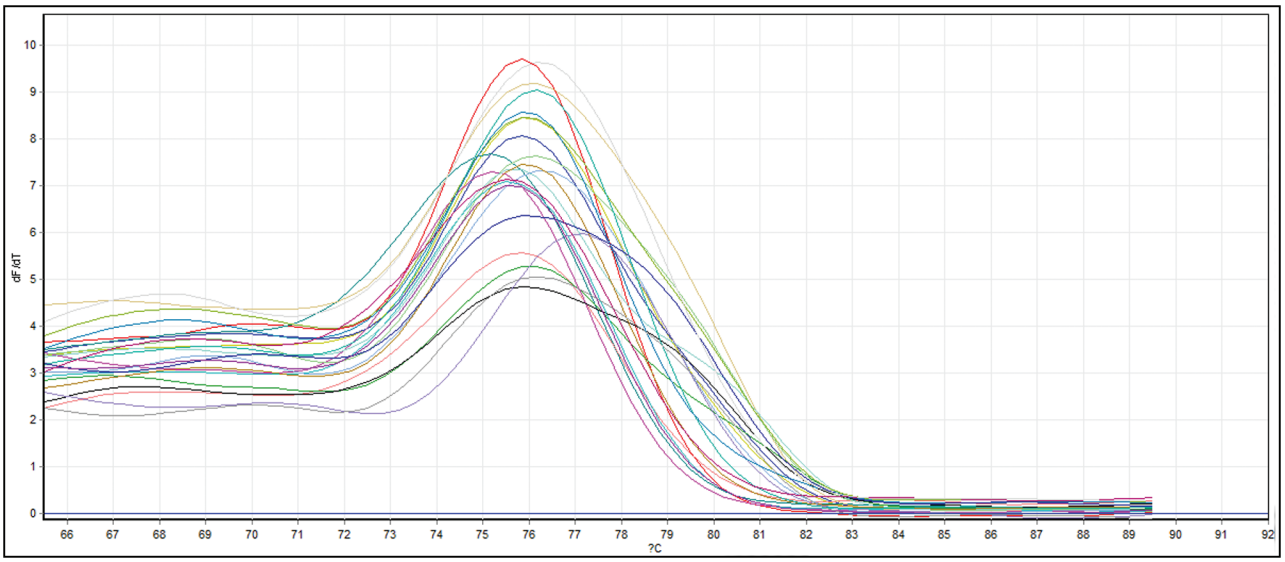

Figure 3: Nonstructural protein dissociation curves by quantitative polymerase chain reaction (qPCR). Samples included all studies. The photograph was taken directly from the qPCR machine

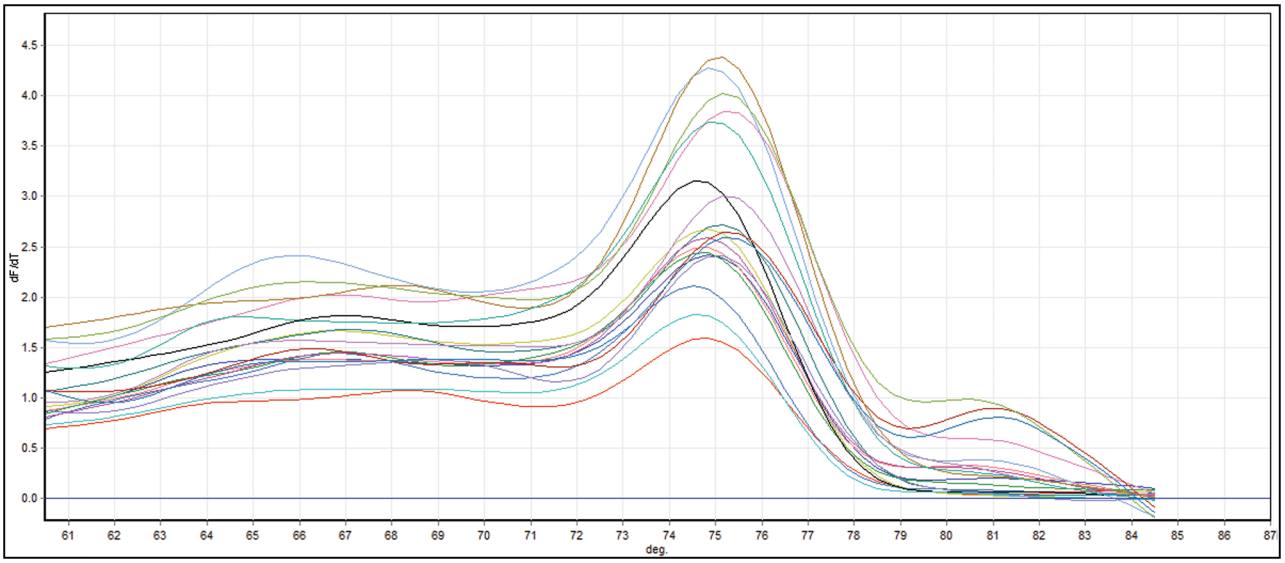

Figure 4: Hemagglutinin dissociation curves by quantitative polymerase chain reaction (qPCR). Samples included all studies. The photograph was taken directly from the qPCR machine

Table 2: Stages and temperature of quantitative polymerase chain reaction for nonstructural protein gene

	Stages	Temperature (°)	Time	Cycle
Stage 1	Denaturation	94	30	1
Stage 2	Denaturation	94	5	
	Annealing	52	15	35
	Extension	72	20	
Stage 3	Dissociation	78-98	1	1

gene as shown in Figure 1. Other studies showed that the *NSP* gene has a small proportion in the influenza B virus,[12] whereas the study conducted by Hao *et al.*[14] explained its effective role in virus replication and translation and in causing virus infection, as it was considered a virulence factor as well as in virus penetration into the host. It was considered a driving force for the virus.

As for RT-PCR, the results in Figure 3 show that the virus has varying proportions in its presence in the samples, as the high percentage indicates the height of the curve, whereas the low ones are less high, and the nonexistent ones have a straight line, and this proves that the high percentage increases the severity of the virus because of its active role in the viral life of the virus.

As for the gene *HA*, it has the highest percentage at a rate of 85%, as shown in Figure 2. This protein plays an active role in the binding of the virus to cell receptors and mediates the fusion of the virus into the cytoplasm during

Table 3: Stages and temperature of quantitative polymerase chain reaction for hemagglutinin gene

Stages		Temperature (°)	Time	Cycle
Stage 1	Denaturation	94	30	1
Stage 2	Denaturation	94	5	
	Annealing	59	15	35
	Extension	72	20	
Stage 3	Dissociation	78–98	1	1

membrane fusion. Several authors[15,16] have pointed out its importance and effective role through its distinctive structure and immune function, which was confirmed by the results of this study that showed a high percentage is due to its active role.

The majority of viral species possess this gene[17] and the extent of similarity between the surfaces of the gene and human cells helped the virus to penetrate before the immune system distinguishes it, thus enabling the virus to increase its strength. Murchu *et al*.[18] investigated the efficacy, effectiveness, and safety of recombinant hemagglutinin seasonal influenza vaccines for the prevention of laboratory-confirmed influenza in individuals ≥18 years of age.

Parker *et al*.[19] revealed that the effectiveness of the gene does not reduce the efficacy of the vaccine, whereas Hu *et al*.[20] found that the diagnosis of the virus can rely on the gene sequence in diagnosing the cause of infection, and it is a diagnostic sign that it exists at high rates, which is proven by its study. The study conducted by Shrestha *et al*.[21] showed that genes trigger a different immune response. Several authors worldwide showed that HA genes trigger a different immune responses among different viral species.[21-23]

As for RT-PCR, Figure 4 showed that the presence of the gene was in the form of a high curve in the majority of isolates, while the missing isolates were in the form of a horizontal line, indicating its absence in the virus. Table 3 shows the conditions of the RT-PCR to search for the virus by the quantitative and qualitative counting methods.

Conclusion

The study made it clear that it is important to consider influenza as one of the causes of seasonal influenza, and it constitutes about one-fourth of the total cases in the world, studies seek to limit its spread by finding vaccines (developing the influenza vaccine) despite the occurrence of genetic mutations in it. It is also recommended to consider the influenza B vaccine among the recommended vaccines. The necessary seasonality in different regions of the world and recommending the establishment of vaccination campaigns according to the requirements of the country.

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Conflicts of interest

There are no conflicts of interest.

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