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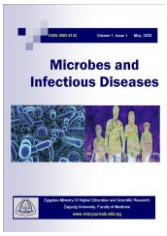

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Original article

Molecular and immunological diagnosis of human respiratory syncytial virus (HRSV) A and B in respiratory infection patients in Mosul city / Iraq

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ABSTRACT

Background: Human respiratory syncytial virus (HRSV) is considered one of the most critical causes of Respiratory Tract Infections (RTI) across all age categories, particularly in infants, the elderly, and those with compromised immune systems. Rapid HRSV detection helps decrease antibiotic use, laboratory testing, and hospitalization time. There is no data available on diagnosing HRSV using the sandwich enzyme-linked immunosorbent assay (ELISA), so the major goal of the examination turned into to determine the superiority of HRSV among patients with RTI with the use of sandwich ELISA and Real-time Polymerase chain reaction (Rt-PCR) techniques. In addition, the results of these two diagnostic methods will be compared to determine the efficacy of ELISA for detecting the virus. **Methods:** One hundred samples of nasopharyngeal secretions and throat swabs were accumulated from patients with RTI of various ages and genders who were admitted to Mosul hospitals throughout the period March - July at concluded 2023. HRSV-Ag and HRSV-RNA were detected in each sample using ELISA and Rt-PCR, respectively. **Results:** The prevalence rates of HRSV-Ag and HRSV-RNA were 56% and 34%, respectively. For both tests, males were more likely to be infected with the virus than females. When comparing the ELISA and PCR results, statistical analysis demonstrated that the ELISA had 100% sensitivity, 66.67% specificity, 60.71% positive predictive value, and 100% negative predictive value. The accuracy rate, kappa value, and percent-positive agreement between the detection techniques were 78%, 78%, 0.65, and 76.6%, respectively. **Conclusion:** We consider that HRSV infection can be diagnosed using both tests. The sandwich ELISA is characterized by way of being a highly sensitive, rapid, inexpensive, and simple procedure, which can be used for routine diagnostics.

Introduction

Worldwide, respiratory tract infections are a major public health concern that affects people of all ages [1]. RTIs are classified as upper or lower infection. Upper infections can cause mild illnesses, such as the common cold, which includes cough, runny nose, fever, and sore throat, whereas lower infections, especially bronchiolitis, and pneumonia,

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are more dangerous and are the main cause of hospitalization, morbidity, and mortality globally [2]. According to a 2016 study, the fatality rate of severe acute respiratory infections was 9.5%, with a higher percentage of infections occurring in children and those over the age of 60 [3]. Viruses and bacteria are the most significant pathogens that can infect the respiratory system [4].

The human respiratory syncytial virus is one of the most important viruses that cause upper and lower respiratory diseases worldwide, particularly in infants, the elderly, and those with chronic illnesses [5]. The RSV previously belonged to the Paramyxoviridae family, but recently, it was reclassified by ICTV into the Pneumoviridae family [6]. RSV is characterized by a large envelope, single-stranded, negative-sense ribonucleic acid (RNA) non-segmented genome consisting of 10 genes encoding 11 glycoproteins. Based on antigenic variation, RSVs have been divided into two distinct and specific subgroups: A and B. Close contact with infected respiratory droplets during speech, coughing, and sneezing facilitates the person-to-person transmission of RSV. The peak viral infectivity occurs during the winter months in temperate regions [7-9].

Several methods or techniques are available for detecting respiratory viruses, including cell culture, serological methods (ELISA, western blotting analysis, immunofluorescence assay), and Molecular methods (PCR) [10]. From an immunological point of view, the body’s immune system cannot provide complete immunity, and it is possible to get re-infected with HRSV following the first infection. Furthermore, no effective vaccines or therapeutic alternatives are available [11].

There are no previous studies on HRSV diagnosis using sandwich ELISA, and no data comparing the results of HRSV detection using sandwich ELISA and Rt-PCR are available. In addition, the detection of the virus is not common in Iraqi hospital laboratories, although it is highly contagious, especially in infants and the elderly. To avoid numerous tests and reduce the use of missed antibiotics, it is necessary to conduct accurate, rapid, and sensitive tests to detect HRSV. Hence, the design of our study included the implementation of sandwich ELISA and Rt-PCR techniques to identify HRSV in samples from patients with RTI admitted to Mosul hospitals.

Materials and methods

Ethical approval

The study was conducted following the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before a sample was taken. The study protocol, the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 4189 to get this approval.

Samples and patients

The present study was conducted in Mosul / Iraq between March and July 2023 at Al-Salam Teaching Hospital and Al-Shifaa Hospital for Infectious Diseases (Advisory Clinic for Chest and Respiratory Diseases).

Patients with RTI of both sexes and of various ages were selected. For the samples, 100 nasopharyngeal secretions and throat swabs were collected from patients and placed in a sterile viral transport medium (VTM) to protect the samples from desiccation. Samples were then transported to the laboratory and deep frozen at 70°C until tests were performed to identify antigens and viral nucleic acids.

ELISA-based HRSV antigen detection

All samples were subjected to a sandwich ELISA test for the detection of HRSV antigen (Human Respiratory Syncytial Virus antigen (ADV-AG) ELISA Kit) according to the instructions provided by the kit's manufacturer (SunLong Biotech Co., LTD).

Rt-PCR-based detection of HRSV nucleic acid

- Nucleic acid extraction

Viral RNA was extracted from all swab samples using a QIAamp MinElute Virus Spin Kit, as instructed by the kit manufacturer (Qiagen-Germany).

- cDNA preparation

Following the instructions provided by the product manufacturer Pioneer Inc. of South Korea, the RNA extracts were reverse transcribed into cDNA using Accu Power® RT Pre Mix.

- Rt-PCR amplification

An Rt-PCR kit (Fast-Track FTD Respiratory Pathogens 33, Luxembourg) was used to amplify cDNA. In brief, the following cycling circumstances were applied: 15 min at 50°C, 1 min at 94°C, 40 cycles for 8 s at 94°C, and 1 min at 60°C.

Viral load in clinical samples was quantified using detected cycle threshold (Ct) values [12].

Results and Discussion

- **Frequency of Human Respiratory syncytial virus**

As illustrated in **Table 1**, sandwich ELISA and Rt-PCR assays were performed on 100 nasopharyngeal secretions and throat swab samples from patients with RTI of both sexes and different ages to identify antigen and viral RNA for HRSV. Results revealed that the presence of HRSV was 56(56%) and 34(34%) patients, respectively. Additionally, ELISA and PCR data revealed that males had higher HRSV-positive rates than females 29 (51.79%) and 19 (55.88%) compared with 27 (48.21%) and 15(44.12%), respectively. The study population was divided into four age groups. For the ELISA test, patients aged 20-29 years had the highest number of infections (26 out of a total of 56 positive samples), whereas for the PCR test, patients older than 39 years were the most likely to be infected with HRSV (19 out of a total of 34 positive samples), and patients under 20 years had the lowest number of infections for both tests. The virus was directly detected during the amplification process of Rt-PCR, with Ct values ranging from 28.4 to 35.33.

Bacteria and viruses are the main causes of respiratory infections, and diagnosing an infection clinically can be challenging because all patients experience extremely similar clinical symptoms. As a result, detecting these pathogens requires a quick, accurate, sensitive, and effective method to provide proper care and prevent the misuse of antibiotics, especially in the absence of necessary vaccines [11]. The sandwich ELISA has several advantages, including high sensitivity, good specificity, and simplicity of work, in addition to the possibility of testing many samples in a short time and is considered more accurate in diagnosis than indirect ELISA, which relies on the presence of antibodies as a result of a body's immune response, whereas the sandwich ELISA relies on the presence of viral antigens [13,14]. Given the lack of previous studies to detect HRSV in this way, we decided in this study to use sandwich ELISA to determine the extent of the virus's spread in Mosul city, Iraq. In addition, several diagnostic methods have been used in Iraq to detect HRSV, but no previous studies have been conducted in the city of Mosul to diagnose this virus using the Rt-PCR technique, which is regarded as the gold standard for HRSV diagnosis. It is characterized by its exceptional sensitivity and accuracy and is considered better than traditional PCR because it is less time-consuming and conducts the test in a closed system, reducing the risk of sample contamination [15,16].

In the current study, the prevalence of HRSV was 56% according to ELISA. This result is lower or higher than the results of other studies in which other methods were used to detect the viral antigen, including lateral flow chromatography, indirect immunofluorescence, Respi, and direct fluorescence assays at ratios of 85%, 61.7%, 44%, and 24.5%, respectively [17-20]. In contrast to ELISA, many studies have been conducted to detect viral RNA using Rt-PCR, and these studies covered a wide range of nations, including Iraq, Jordan, Saudi Arabia, Iran, the United States, Europe, the Philippines, China, and Brazil, with virus dissemination rates ranging from (1%–64%) [15,21-28]. Differences in viral spread rates between countries, or even within the same country, may be due to environmental and seasonal factors, sample-related issues (size, type, time of collection), patient-related issues (age, immune status), or differences in diagnostic methods [17,29].

Regarding sex, the results of our study showed that males were more likely to be infected with the virus than females. These results are consistent with the findings reported by several studies [28-30]. This may be attributed to the anatomical, physiological, genetic, and immunological differences between males and females [30].

Our findings also revealed that adults are more susceptible to viral infection than children. Results of multivariate logistic regression tests conducted on one study revealed that there was no relationship or any noticeable effect between age, gender, and symptoms and the propagation of the virus [15]. Nutrition, immune status, presence of chronic illnesses, severity of virus infection, and the quantity of samples collected from each group have a major role in the extent to which a particular group is infected.

- **Comparison between sandwich ELISA and Real-time PCR**

Among 100 samples, 56 had HRSV-Ag positivity by ELISA, and 34 of them had HRSV-RNA positivity by PCR; as a result, they were considered true positives, whereas 22 samples had PCR negativity; they were considered false positives. Forty-four samples showed a negative result in both tests, and these were considered true

negative, while there were no false negative results (a negative ELISA result revealed a positive PCR result).

When comparing the results of ELISA and PCR, statistical analysis revealed that ELISA had 100% sensitivity, 66.67% specificity, 60.71% positive predictive value, and 100% negative predictive value. The accuracy rate, kappa value, and percent positive agreement between these two detection techniques were 78%, 0.65, and 76.6%, respectively **(Table 2)**.

No previous studies have compared the results of HRSV detection using sandwich ELISA and Rt-PCR. Discordant results between the two techniques were also noted by Fan et al. and Wang et al who used the same techniques to detect Porcine Deltacoronavirus in faecal samples from new-born pigs and found sensitivity (93.5%, 80.8%), specificity (98.1%, 95.6%), accuracy rate (95.7%, 89.8%), and kappa value (0.914, 0.827), respectively [13,31]. Abdullah and ALTaie used sandwich ELISA and traditional PCR to detect the herpes complex virus in infected skin swab samples. The sensitivity and specificity rates of ELISA compared with PCR were 93% and 91%, respectively [32].

According to the findings of our study, only 34 out of all ELISA-positive samples were PCR-positive, while the remainder of the samples gave a negative result. This might be attributed to very early infection since the amount of viral RNA is minimal because replication is in its early stages. Furthermore, late infection can also result in erroneous findings [33,34]. Viral RNA is unstable and more susceptible to degradation and mutation, in addition to the presence of inhibitory substances in saliva, which have been shown to increase Ct values, potentially affecting the test's sensitivity and accuracy and leading to false-negative results. Furthermore, the viral load is determined by detecting specific gene copies, which do not always indicate the quantity of packed mature viral particles and may better reflect the probability of infection and the risk of disease progression [34-37].

The use of sensitive, specific, and rapid laboratory diagnostic tests to detect HRSV will improve clinical management. The RT-PCR test is the gold standard for detecting respiratory viruses, but it is not routinely performed in laboratories for several reasons, including its high cost, time requirements, and specialized staff and technical expertise [17,19]. Therefore, it is necessary to find an alternative test that is rapid, simple, efficient, and less time-consuming. Therefore, sandwich ELISA was used because positive viral antigens provide strong evidence of the existence of a viral infection [22,34,38]. This will allow prompt provision of appropriate therapy and avoid the erroneous use of antibiotics, which are ineffective in treating the disease. Furthermore, it will operate to enhance their resistance to germs, leading to an increase in their ferocity and progress, which has become an important scientific issue in communities [39-41].

Table 1. Frequency of Human Respiratory Syncytial Viruses by using two diagnostic methods: Sandwich ELISA and Real-time PCR.

Age/ Year	HRSV+ve (ELISA)			HRSV+ve (Rt-PCR)		
	No.	Male	Female	No.	Male	Female
> 20	5	4	2	2	0	2
20-29	26	18	8	7	5	2
30-39	14	5	9	6	4	2
<39	11	3	8	19	10	9
Total	56(56%)	29(51.79%)	27(48.21%)	34(34%)	19(55.88%)	15(44.12%)

HRSV (Human respiratory syncytial virus), +ve (positive), ELISA (Enzyme-linked immunosorbent assay), Rt-PCR (Real-time Polymerase chain reaction), No. (Number).

Table 2. Comparisons and statistical analysis of the sandwich ELISA with the Real-time PCR assay.

Category		Real-time PCR		
		HRSV +ve	**HRSV -ve**	**Total**
Sandwich ELISA	**HRSV +ve**	34	22	56
	HRSV -ve	0	44	44
	Total	34	66	100
Statistical analysis	**Sensitivity**	100%		
	Specificity	66.67%		
	Positive predictive value	60.71%		
	Negative predictive value	100%		
	Accuracy rate	78%		
	Kappa value	0.65		
	Percent-positive agreement	76.6%		

The statistical analysis was conducted by the Statistical Package for the Social Sciences (SPSS) version 22 software.

Conclusion

The current study demonstrated the frequency of HRSV in patients with RTI in Mosul, Iraq. This is the first study to highlight the detection of HRSV-Ag using sandwich ELISA as well as to compare the results of this technique with those of Rt-PCR. The study showed that sandwich ELISA has high sensitivity, specificity, and accuracy compared with Rt-PCR and can be used for routine laboratory diagnosis.

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