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Detection of Respiratory Viruses by Enzyme-Linked Immunosorbent Assay from Respiratory Patients in Mosul City, Iraq

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ABSTRACT

Respiratory tract infections (RTIs) are an essential source of disease and mortality, especially in high-risk populations. Respiratory virus diagnosis must be accurate and timely to give optimum patient care and control infection. The enzyme-linked immunosorbent assay (ELISA) is a rapid assay that can detect the antigen/antibodies' reaction of the respiratory pathogens in a diagnostic test. A total of 150 serum samples were collected from RTI patients who were admitted to Mosul hospitals between October and December 2023. The inclusion criteria were RTI diagnosed clinically with fever, cough, or shortness of breath. Exclusion criteria were bacterial infection confirmed or non-viral respiratory infection. The most commonly detected virus was human respiratory syncytial virus (HRSV) with (20 cases, 13.3%), followed by human rhinovirus (HRV) with (14 cases, 9.3%), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with (12 cases, 8.0%) and human enterovirus (HEV) with (10 cases, 6.6%). The other viruses detected were influenza a virus (IAV) with (8 cases, 5.3%), human metapneumovirus (HMPV) with (7 cases, 4.6%), human coronavirus OC43 (HCoV-OC43) with (4 cases, 2.6%), and human coronavirus NL63 (HCoV-NL63) with (3 cases, 2.0%). Age group analysis established that HRSV was greatest in individuals between 60-69 and 20-29 years and that the greatest cases of HRV were in the 40-49 age group. Females presented a greater infection when compared to males. The study highlights the existence of HRSV, HRV, and SARS-CoV-2 as prevailing viral etiologies of RTIs in Mosul.

Keywords: Coronavirus, enterovirus, ELISA, influenza A, metapneumovirus.

INTRODUCTION

The respiratory system is a major biological system that allows the body to breathe, which is a process of exchanging carbon dioxide and oxygen between the organism and its surroundings. It is made up of the following parts: The pharynx, larynx, nasal cavity, and paranasal sinuses are all part of the upper respiratory system; the trachea, bronchi, and lungs are part of the lower respiratory system (Peate, 2021; Peate, 2018; Nunn, 2013).

Respiratory infections (RTI) are widespread and are a primary cause of social visits for doctors in primary care, especially in the winter months (Ho *et al*., 2023; Satia *et al*., 2020).

In the United States, acute respiratory tract infection (ARI) contributes to a problem with high illness and health care, with millions of outpatient trips and significant indirect costs (Cui *et al*., 2024). In addition, a surplus of antibiotics for Aris is a common problem, which contributes to antibiotics, an increasing public health problem. (Shaver *et al*., 2019; Coxeter *et al*., 2015).

Human rhinovirus (HRV), adenovirus, parenfluenzavirus, IAV, HRSV, HMPV, and human bocavirus are the usual causes of URTs. HRV is the most widespread among them. In addition to specific symptoms of colds, HRV can cause up to 50% of pneumonia, bronchiolitis, pharyngitis, and asthma. They are spread by self-cinema from aerosol drops, foams, and secretions on surfaces. (Thomas *eat al*., 2023; Kahbazi *et al*., 2011).

Aim of the study

- To detect and analyze the prevalence of common respiratory viruses among patients with respiratory tract infections (RTIs) in Mosul City, Iraq, using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.
- The study seeks to evaluate the distribution of viral infections across different age groups and genders.
- To assess the effectiveness of ELISA as a diagnostic tool in identifying viral pathogens in RTI cases.

MATERIALS AND METHODS

The present study was an observational cohort study that sought to assess the frequency of respiratory viruses in respiratory tract infection patients in Mosul, Iraq, using the ELISA technique. A total of 150 serum specimens were taken from patients with respiratory symptoms from Al-Shifa Hospital, Al-Jumhory Teaching Hospital (where the research was carried out), Ibn-Sena Teaching Hospital, and the Advisory Clinic for Chest and Respiratory Diseases in Al-Fasalea from October to December 2023. Also, 10 control samples were purchased from asymptomatic persons to confirm the specificity of the diagnostic assay and determine the status of antigen/antibody interaction in a healthy population.

These control samples were obtained at the time of sampling and with no reported respiratory tract infection (RTI) symptoms in the past four weeks. The control samples were divided into two groups, 5 males and 5 females of middle age.

The qualitative enzyme immunoassay method is the foundation of the ELISA. A specific antibody has already been applied to the microplate included in this kit, making it a solid-phase antibody. Samples and the particular antibody are mixed in the microplate wells. The antibody-antigen-enzyme-labeled antibody complex is then created by adding the virus-specific horseradish peroxidase (HRP)-conjugated antibody to each microplate well and letting it sit for a while.

Each well is filled with the TMB substrate solution after any unbound reagent has been washed off. When the stop solution is added, only the wells containing the virus and HRP-conjugated virus antibodies will first appear blue before turning yellow. Using spectrophotometry, the optical density (OD) is determined at a wavelength of 450 nm. Comparing the virus to the CUTOFF value yields the qualitative determination of the virus (according to SunLong Biotech kits, Catalog Number: SL3210Hu, SL2127Hu, SL0925Hu, SL2479Hu, SL0655Hu, SLY3968Hu and SLY3967Hu).

The procedure of the test

According to the human respiratory syncytial virus (HRSV) ELISA kit: Catalog number: SL2127Hu, human rhinovirus (HRV) ELISA Kit: Catalog number: SL3210Hu, human influenza A virus (FLU-A) ELISA kit catalog number: SL0925Hu, human enterovirus ELISA kit: Catalog number: SL0655Hu, human coronavirus ELISA kit: Catalog number: SL2479Hu, human metapneumoniavirusIgM (HMPV-IgM) ELISA kit: Catalog number: SLY3968Hu and human metapneumoniavirusigg (HMPV-IgG) ELISA kit: Catalog number: SLY3967Hu, sunlong biotech, China.

RESULT AND DISCUSSION

The ELISA assay results are useful for determining the prevalence of viral antigens within age groups and gender. Out of the total 150 patients tested, 78 were positive. The total number of male-positive cases is 31 (20.7%), while the total number of female-positive cases is 47 (31.3%). Of the viruses detected, HRSV was the most prevalent with 20 cases (13.3%), followed by HRV with 14 cases (9.3%), SARS-CoV-2 with 12 cases (8%), and HEV with 10 cases (6.6%), IAV with 8 cases (5.3%), HMPV with 7 cases (4.6%), HCoV-OC43 with 4 cases (2.6%) and HCoV-NL63 with 3 cases (2%). From the results, it can be seen that HRSV was mostly detected in participants aged 60-69 years and 20-29 years, indicating high exposure among both young adults and the elderly. In contrast, HRV was relatively evenly distributed among various age groups, but it was most frequently found in the 40-49 age group (Table 1).

Table 1: Shows the distribution of infection groups according to the age group using ELISA analysis.

Ages/years	Rhino. Antigen	%	RSV antigen	%	Influ. An antigen	%	Entero. antigen	%	Metapneumovirus antigen	%	Coronavirus					
											OC43 antigen	%	NL63 antigen	%	SARS-Cov2 antigen	%
10-19	Zero	0.0	3	2.0	Zero	0.0	zero	0.0	Zero	0.0	zero	0.0	zero	0.0	1	0.6
20-29	2	1.3	4	2.6	2	1.3	zero	0.0	Zero	0.0	zero	0.0	zero	0.0	1	0.6
30-39	3	2.0	2	1.3	2	1.3	3	2.0	2	1.3	1	0.6	zero	0.0	2	1.3
40-49	4	2.6	3	2.0	2	1.3	5	3.3	1	0.6	2	1.3	1	0.6	5	3.3
50-59	3	2.0	3	2.0	1	0.6	1	0.6	2	1.3	1	0.6	2	1.3	zero	0.0
60-69	2	1.3	5	3.3	1	0.6	1	0.6	2	1.3	zero	0.0	zero	0.0	3	2.0
Total	14	9.3	20	13.3	8	5.3	10	6.6	7	4.6	4	2.6	3	2.0	12	8.0

The IAV and HEV antigens were detected less often, with a slight increase in age groups 30-39 and 40-49, respectively. The HMPV was more common in age groups 30-39 and 50-59, while the different coronavirus antigens had differing prevalence. SARS-CoV-2 was most often detected in the age group 40-49 (5 cases), while HCoV-OC43 was slightly more common in older age groups. The comparatively low detection of HCoV-NL63 indicates that it might not have been extensively present in this population during the observation period.

For the gender comparison (Table 2), women identified more HRSV (12 compared to 8 in males), HEV (6 compared to 4), IAV (5 compared to 3), and HMPV (7 compared to 0), females generally exhibit stronger immune responses to viral infections compared to males. This is partly due to stronger type I interferon (IFN) responses, which are crucial for antiviral defense. These responses are influenced by sex hormones and genes on the X chromosome, contributing to the observed differences in immunity between sexes (Cheung *et al*., 2023; Pujantell and Altfeld, 2022; Jacobsen and Klein, 2021). SARS-CoV-2 was present equally in males and females (6 each). HRV and HCoV-OC43 detection were fairly even between the sexes, with no difference.

Age is a key determinant of susceptibility to specific viruses. Young adults and the elderly are more prone to RSV, while middle-aged adults show higher rates of HRV and SARS-CoV-2. Gender plays a significant role, with females exhibiting higher positivity rates for several viruses, especially HMPV and HRSV.

Some infections reflect a compound pattern of age and gender, HMPV's exclusive detection in females and concentration in 30-59 years suggests a potential gender-age synergy (Cheung *et al*., 2023). RSV in the elderly (60-69) was seen in both genders but with a slight female predominance. SARS-CoV-2 in 40-49 years was equally distributed across genders, suggesting that exposure was a stronger factor than gender biology.

Table 2: Shows the distribution of infection groups according to gender using ELISA analysis.

Gender	Rhino.	%	RSV	%	Influ. A	%	Entero.	%	Metapneumo.	%	Coronavirus					
											OC 43	%	NL63	%	SARS-Cov2	%
Male	6	4.0	8	5.3	3	2.0	4	2.6	Zero		2	1.3	2	1.3	6	4.0
Female	8	5.3	12	8.0	5	3.3	6	4.0	7	4.6	2	1.3	1	0.6	6	4.0
Total	14	9.3	20	13.3	8	5.3	10	6.6	7	4.6	4	2.6	3	2.0	12	8.0

Overall, the data reveal that HRSV and HRV were most commonly detected in this population, with SARS-CoV-2 also showing a strong presence in middle-aged adults. Detection of IAV is low and may be representative of seasonal patterns, pre-exposure vaccination, or reduced incidence rates at the time of testing.

Sandwich immunoassay, which uses a pair of antibodies that bind to an antigen, has a high specificity and is frequently used for antigen detection. This technique offers a high sensitivity and specificity for identifying viral pathogens. On the other hand, genetic variety, lineage identification, and the discovery of evolutionary links between them are all made possible by the application of molecular techniques (Jin *et al.*, 2021; Fan *et al.*, 2020).

The low concentration of viral genetic materials can cause samples that achieve a positive ELISA result, but a negative PCR result. PCR current is very sensitive to the amount of viral genetic material. If the viral load is extremely low, it cannot be detected by PCR, even if antibodies or antigens are present and are discovered by ELISA (Ye *et al*., 2021; Al-Azzawy, 2019).

The benefits of ELISA include speed, low costs, ease of use in an environment with limited resources, fitness to analyze a large number of samples, and the ability to use less strict techniques (Wang *et al*., 2021).

CONCLUSION

Sandwich ELISA has several benefits, including high sensitivity, good uniqueness, and simplicity in the work, except for the possibility of testing more samples in some time, and it is considered more accurate in diagnosis than indirect ELISA, which depends on the presence of antibodies as a result of a body's immune response.

This study highlights the significant presence of respiratory viral infections among patients in Mosul city, Iraq, with human respiratory syncytial virus (HRSV), human rhinovirus (HRV), and SARS-CoV-2 being the most commonly detected pathogens using ELISA-based diagnostic kits. A higher prevalence of several viruses was observed among females, likely due to sex-related differences in immune response. ELISA proved an effective, rapid, and accessible diagnostic method, especially in resource-limited settings. However, the findings also underscore the limitations of ELISA compared to molecular methods, especially in cases with low viral load.

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الكشف عن الفيروسات التنفسية باستخدام اختبار المقايسة الامتصاصية المناعية للانزيم المرتبط لدى مرضى الجهاز التنفسي في مدينة الموصل، العراق

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الملخص

تُعد التهابات الجهاز التنفسي مصدرًا مهمًا للأمراض والوفيات، وخاصةً في الفئات السكانية المعرضة للخطر. يجب أن يكون تشخيص فيروس الجهاز التنفسي دقيقًا وفي الوقت المناسب لتقديم الرعاية المثلى للمريض والسيطرة على العدوى. يُعد اختبار المقايسة الامتصاصية المناعية للأنزيم المرتبط اختبارًا سريعًا يمكنه الكشف عن تفاعل المستضد/الأجسام المضادة لمسببات الأمراض التنفسية في اختبار تشخيصي. تم جمع ما مجموعه 150 عينة مصلية من مرضى التهاب الجهاز التنفسي الذين تم إدخالهم إلى مستشفيات الموصل بين أكتوبر وديسمبر 2023. كانت معايير الإدراج هي تشخيص التهاب الجهاز التنفسي سريريًا بالحمى أو السعال أو ضيق التنفس. كانت معايير الاستبعاد هي تأكيد الإصابة بعدوى بكتيرية أو عدوى تنفسية غير فيروسية. كان الفيروس الأكثر شيوعًا الذي تم اكتشافه هو الفيروس فيروس الجهاز التنفسي المخلوي (20 حالة، 13.3%)، يليه الفيروس الانفي (14 حالة، 9.3%)، وفيروس كورونا نوع SARS-CoV-2 (12 حالة، 8.0%) والفيروس المعوي (10 حالات، 6.6%). الفيروسات الأخرى التي تم اكتشافها هي فيروس الانفلونزا نوع أ (8 حالات، 5.3%)، وفيروس التهاب الرئة البشري (7 حالات، 4.6%)، وفيروس كورونا نوع OC43 (4 حالات، 2.6%)، وفيروس كورونا نوع NL63 (3 حالات، 2.0%). أثبت تحليل الفئات العمرية أن الفيروس التنفسي المخلوي كان أكثر لدى الأفراد الذين تتراوح أعمارهم بين 60–69 و20–29 عامًا وأن أكثر حالات الفيروس الانفي كانت في الفئة العمرية 40–49 عامًا. أظهرت الإناث إصابة أكبر مقارنة بالذكور. تسلط الدراسة الضوء على وجود فيروس كورونا نوع-SARS CoV-2، الفيروس الانفي والفيروس التنفسي المخلوي كمسببات فيروسية سائدة لالتهابات الجهاز التنفسي في مدينة الموصل.

الكلمات الدالة: فيروس كورونا، الفيروس المعوي، المقايسة الامتصاصية المناعية للأنزيم المرتبط، فيروس الانفلونزا أ، فيروس الجهاز التنفسي المخلوي.