The Medical Journal of Tikrit University (2025) 31 (2): 329-341
DOI: http://dx.doi.org/10.25130/mjotu.31.2.35

IRAQI Academic Scientific Journals

ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at:www.mjotu.com

MJTU

The Medical Journal of Tikrit University

Molecular Study of Respiratory Syncytial Virus Concomitant with COVID-19 Infection in Children under 5 Years

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Keywords: *RSV , COVID-19 , SARS-CoV-2 , children, hygiene, RSV Serotype A, PCR , lower respiratory tract infection , epidemiology, influenza.*

ARTICLE INFO

Article history:

Received 01 Jul 2025
Accepted 01 Sep 2025
Available online 31 Dec 2025

Citation:

ABSTRACT

Background: Worldwide, an estimated 33 million children under the age of five years are projected to be infected with the respiratory syncytial virus (RSV), with 10% of those cases necessitating hospital admission and up to 199,000 deaths from the illness.

Patients and Method: We tested 100 suspected patients for RSV infection, with the help of pediatrician . The study was conducted at the Ramadi Teaching Hospital for Maternity and Children /Al-Anbar. The period of study was from 1 December 2023 to 15 March 2024.

Result:

In this study, we examined 100 cases of respiratory infection, 58 cases were positive for RSV. Out of 58 cases with positive RSV, 32 were positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antigen, and from cases positive for SARS-CoV-2 antigen, 18 were confirmed positive for SARS-CoV-2 by polymerase chain reaction (PCR). The age of patients ranged from 1 month to under 5 years and 57% were males while 43% were females. Statistical analysis was performed on data from RSV-infected patients as well as age subgroup populations. Patients were classified into the following age groups:1 to 6 months N=36(36%), 6 to 12 months N=24(24%), 12 to 18months N=9 (9%), 18 to 24 months N=17(17%), and more than24 months N=14(14%). The serotype of RSV were N=15(25.8%) of serotype A and were N=43 (74.1%) of serotype B.

Conclusion: RSV is the leading cause of sickness and death in newborns globally, especially in low- and middle-income nations. Palivizumab is crucial for preventing severe RSV LRTIs in high-risk newborns, but the excessively high cost prevents widespread use. During the Pandemic, primary preventative measures, such as hand hygiene and face masks, were more cost-effective in reducing RSV burden. In addition to the vaccination, non-pharmaceutical preventive hygiene measures should be implemented to reduce RSV spread globally, even after the coronavirus disease-19 (COVID-19) pandemic.

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INTRODUCTION

At the Walter Reed Army Institute of Research in the United States, the respiratory syncytial virus (RSV) was initially isolated from chimps suffering from respiratory illness in 1955 [1]. In 1957, Robert M. Chanock discovered the similar virus in youngsters with respiratory disease [2] . Investigations involving newborns and children revealed that the illness was frequent in the early stages of life [3]. The virus was later called human orthopneumovirus or human respiratory syncytial virus (hRSV) because of its propensity to produce cell fusion and the production of multinucleated syncytia [4]. The respiratory syncytial virus, or RSV, is becoming a major global cause of sickness and death, particularly in young children (under six months old). Respiratory syncytial virus is the most common cause of acute respiratory infections in infants and the primary reason for hospitalization throughout infancy. The virus has recently been revealed to afflict susceptible adults, including the elderly and those with impaired immune systems [5]. Respiratory syncytial virus is the most common cause of lower respiratory tract infections in low-income nations, accounting for more deaths than pneumococcal pneumonia and *Haemophilus influenzae* (*H. influenzae*) type B combined. Notably, 99% of RSV-related deaths globally occur in resource-constrained nations, where the disease is more than twice as common in severe cases as in wealthy ones [6].

Worldwide, an estimated 33 million children under the age of five years are projected to be infected with the respiratory syncytial virus (RSV), with 10% of those cases necessitating hospital admission and up to 199,000 deaths from the illness. An inflammatory response to the virus known as severe infantile RSV bronchiolitis is linked to wheezing in later childhood in certain susceptible individuals, according to mounting evidence; however, a direct causal link with asthma has not yetbeen shown. It is also becoming more well acknowledged as a contributing factor to morbidity and death in elderly people who are fragile, have immunocompromised conditions, and underlying airway diseases. As early as the 1970s, RSV was known to cause infections in adults, particularly in elderly adults. However, The introduction of reverse-transcription polymerase chain reaction in 2005 enabled a better knowledge of the burden of RSV in older persons[7]. Efforts were made to develop vaccines to prevent RSV infection and severe disease, but these early attempts were unsuccessful. In 1993, a pivotal trial of an RSV hyperimmunoglobulin was conducted, and in 1998, an RSV-specific monoclonal antibody against the RSV fusion F protein called palivizumab was developed and licensed [8,9]. This paved the way for passive prophylaxis as a method to prevent RSV in infants and young children[10].

While respiratory syncytial virus infection concomitant with COVID-19 infection in children under 5 years was during the first year of the COVID-19 pandemic, shortly after the deployment of non- pharmaceutical measures (NPIs) in February-March 2020, there was a significant decrease in RSV cases worldwide, as supported by several sources [11,12,13]. However, as these

interventions were gradually reduced, many countries throughout the world saw varying degrees of off-season upsurge in RSV cases [14]. Notably, Australia was among the first countries to experience an unprecedented increase in RSV incidence during the summer season, which occurred between December 2020 and February 2021. Interestingly, a shift was noticed in the afflicted age range, with more older children seeking medical attention for symptomatic RSV infections [15]. It is worth noting that this return of RSV cases was not fairly spread throughout the country, with maxima occurring in Western Australia between October and December 2020, and the Melbourne area reaching its peak around February 9th, 2021[16]. Other regions throughoutthe world followed suit, reporting resurgences of RSV infections with great heterogeneity in intensity and clinical severity [17]. The purpose of this article was to provide a thorough overview of the present research and explore the many theories that explain the changes in RSV epidemiology during the COVID-19 pandemic.

Infection with RVS is a common disease in humans, in part because there is no long-term immunity after infection, which leads to recurrent reinfections. The virus infects more than 90% of children during their first two years of life and regularly re-infects older children and adults. MostRSV patients have an upper respiratory infection, but a large minority have more severe lower respiratory tract illness, particularly bronchiolitis [18] . Lower respiratory involvement is most frequent in infants under one year old, with bronchiolitis accounting for up to 40% of original infections. Globally, RSV is predicted to cause about 33 million lower respiratory tract illnesses, 3 million hospitalizations, and up to 199,000 juvenile deaths, the bulk of which will occur in resource-limited settings. RSV incidence varies seasonally, with a notable winter-spring peak in temperate climes and less obvious seasonal spikes in tropical/equatorial locations. High-risk groups, such as premature infants, those with prior medical issues, and the elderly, face increased morbidity and mortality from RSV infections [19].

MATERIALS AND METHODS

We tested 100 suspected patients for RSV infection, with the help of pediatrician . This study wascarried out at Al Ramadi Teaching Hospital for Maternity and Children in Al-Anbar Governorate.The period of study was from December 1, 2023, to March 15, 2024. The study protocol was evaluated and approved by the Ethical Committee of the College of Medicine, University of Anbar (reference number 79 on 8-5-2024). Each participant has given informed consent. The inclusion criteria were suspects of both sexes who experienced RSV symptoms including fever, coughing, dyspnea, etc. for two to five days and who were under 5 years old , 57 suspects was male and 43 suspects was females. Data were gathered from every participant regarding the patient demographics, disease duration, symptoms at the time of presentation, and residency. Nasopharyngeal swabs were taken using different Swabs and sterile tubes with VTM (Virus Transport Medium-TM) for each specimen, using full aseptic procedure and precautions (personal protective equipment). Specimen collection involved obtaining of

both a Nasopharyngeal swab for rtRT-PCR for detection of RSV and the positive NP swab also tested for SARS-COV-2 Ag.

SARS-CoV-2 Ag Detection :

The Coronavirus nCoV Antigen test is used to qualitatively detect SARS-CoV-2 antigens in nasal swabs, nasopharyngeal swabs, or oropharyngeal swabs from people suspected of COVID

Principle

A two antibody sandwich technique is used in the immunochromatographic lateral flow device known as the coronavirus (SARS-CoV-2) antigen test. Colloidal gold-linked anti-SARS-CoV-2 antibodies that have been dry-immobilized are part of the test apparatus. The specimen rehydrates the gold conjugate complexes as it travels across the strip via capillary diffusion. SARS-CoV-2 viral antigens will react with the gold conjugate complexes to form particles that will migrate along the strip until they reach the Test Zone (T), where they will be captured by the immobilized anti-SARS-CoV-2 antibodies, leaving a visible red line if they are present at or above the limit of detection. There would be no red line in the Test Zone if the samples were free of SARS-CoV-2 viral antigens. The validity of the test is indicated by a red line that appears in the Control Zone (C), where the gold conjugate complexes travel until they are caught by an immobilized antibody.

Assay procedure

1. The test device was taken out of the pouch.were marked with the patient's or control's identification on the test apparatus.
2. Retum The white cap assembly were retumed to the extraction tube.
3. After inverting the sample extraction tube, was squeezed the extracted solution tube intothe sample window and was added two to three drops (about 50 to 75 µl) of test sample.
4. The results were read at 15-20 minutes.

Polymerase Chain Reaction (Reverse Transcriptase RT-PCR) (RSV AND CORONAVIRUS)

This technique was utilized to detect nucleic acid in nasopharyngeal swabs of coronavirus and respiratory syncytial virus in patients suspected of having SARS-CoV-2 and RSV. This was done in the Central Health Laboratory in the Al-Anbar Health Directorate's.

Reverse Transcriptase RT-PCR involves the detection of RNA. The procedure involves employing the enzyme reverse transcriptase to reverse-transcribe RNA or mRNA to complementary DNA (cDNA), then amplifying and detecting particular targets of this cDNA using a method known as rtRT-PCR. This test consists of two important steps; the first is the extractionof the nucleic acid of respiratory syncytial virus and coronavirus from the nasopharyngeal swabs. Second, amplification of the nucleic acid (N.A). Extracted from the first step in the rtRT-PCR System (QIAGEN and BIO-RAD).

Nucleic Acid Extraction of RSV:

The Quick-RNA™ Viral Kit is a quick,

purification of viral RNA from biopsies, cellular suspensions, plasma, serum, urine, cell culture medium, blood, saliva, swabs, and feces that have been preserved in DNA/RNA ShieldTM (for sample collection, nucleic acid preservation, and inactivation of pathogens).Additionally, the kit has a buffer mechanism that makes it easier to fully lyse virus particles for effective nucleic acid isolation. Both large (> 200 kb) and small (> 50 nt) DNA and RNA are eluted after being washed and bonded to the column. The high-quality total RNA that has been obtained is prepared for all subsequent uses, including RT qPCR detection, hybridization-based methods, and Next-Gen sequencing.

Two sets of primers and fluorescent probes are designed in this package for the respiratory syncytial virus subtypes A (FAM) and B (HEX). Both sets of primers and probes have a high degree of specificity in binding to the target sequences. A full-automatic fluorescent PCR detector can identify the fluorescent signal or signals produced by the RT-PCR amplification process, enabling real-time online monitoring of the RT-PCR reaction. The human gene functioned as a non-competitive internal control during the extraction and detection stage.

Nucleic Acid Extraction Kit of Coronavirus :

Nucleic Acid Extraction Kit (AeHealth-Product Name: (N.A. EXTRACTION, Model: NFASST32A, SN: AE0121062201, Germany). The active functional group which can specificallyadsorb nucleic acids is modified on the surfaces of magnetic beads, so they can bind to the target nucleic acids under specific conditions by using different lysis buffers, binding buffers, and washing buffers. At the same time, the magnetic beads can be easily removed and enriched under the action of the external magnetic field, to achieve the purpose of DNA/RNA extraction. The reagent plate was taken from the kit, and the test sample was shaken (VTM with a swab), 200µL of sample was putted in wells (1, 7). and the reagent plate was inserted into the designated position of the instrument and add the plastic sleeve was added into the instrument, after the accurate time the program was ended. Then elution plate was taken and the eluent was extracted from (6, 12) wells 15µL.

PCR tubes were taken(N=negative control number + RNA sample number + Positive control number) All the reaction solutions and all enzyme mixture and 16 µL was added to the rtPCR tube.then Fifteen microliter (15 µL) was added of RNA sample, negative control, and positive control to the PCR tube respectively. The tube was caped tightly and centrifuged at 6000 rpm for 10s.The caped rtPCR tubes were putting into a RT- PCR machine for amplification. After the target time, collection of the fluorescent signals at step3:60C^{o} was done. Fluorescent Channel: FAM for ORF1ab gene, VIC for N gene, CY5 for internal control.

RESULTS

Statistical analysis was performed on data from RSV-infected patients as well as populations of different ages. The following age categories were used to classify the patients:1-6 months, 6–12 months, 12–18 months , 18 to 24 months, and more than 24

months. In this study, we examined 100 cases of probable RSV infection, 58 cases were positive for RSV. Out of 58 cases with positive RSV, 32 were positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antigen, and from cases positive for SARS-CoV-2 antigen, 18 were confirmed positive for SARS-CoV-2 by PCR. In this study the percentage of positive RSV has been 58%, In this study, 55% of patients showed positive results for SARS-CoV-2 Ag tests while only (56.2%) of them were confirmed by RT-PCR which means the SARS-CoV-2 Ag test is inferior to the PCR assay.

DISCUSSION

Detailed demographic and clinical data were available on 100 patients suspected RSV infection The age of patients ranged from 1 month to under 5 years . In terms of gender, there is a correlation between RSV infection and male gender, with a greater infection rate and 57% were male while 43 were female , This is consistent with a study in the Bulgaria[20].

Incidence among patients 1–6 month ages was approximately 36% , than incidence among patients more than 24 months of age approximately14%. This study is consistent with a study in Germany[21]. In this study, the incidence was higher in cities than in rural areas . This is in the USA[22]. Also in the Bulgaria [23].

Our findings are comparable to those reported in Western Australia. Data from the NYC Department of Health's surveillance system are beginning to show the same pattern in RSV cases. Our findings indicated that younger infants have more severe illness, perhaps as a result of weakened immunity from not being exposed to RSV the season before. The disease may have spread less to older kids as a result of the ongoing closure of daycare facilities and online education [24]. In relation to the place of residence , there is an increase in the number of RSV in the urban areas, RSV was detected in 60 of 100 specimens (60%) from children in the urban site and in 40 of 100 specimens (40%)from those in the rural site. This is consistent with a study in the Bulgaria[25], although this study conflict with a study in the African[26].

The symptoms in RSV patients , the majority coughing and difficult in breath occurring in 92% of patients, with watery eyes being the least prevalent occurring in 48%. Our study focused on fever responses during RSV infection in children under the age of five years. In general, innate immune cells detect viral invasion and produce fever-generating substances [27]. However, innate immunity is underdeveloped and ineffective in early infancy due to persistent in-utero immunological tolerance and a lack of pathogen exposure. Infants in their early months of life tend to prevent fever onset and maintenance [28]. Attenuated fever responses slow the transition from innate to acquired immunity. As a result, Th1 responses, one of the acquired immune responses that provide critical defense against viral infection, prove to be less efficient [29]. A contemporaneous investigation conducted in the same participants as this study revealed consistent results: the titer of neutralizing antibody produced in response to RSV infection in children under 6 months old was notably low, and children's antibody responses

against RSV matured over months and years [30]. In this study, 67% of patients showed fever this agreement with study by Kawakami *et al* (31). Rhinorrhoea were present in 53% while in study conducted by Ramagopal *et al* [32], 100% of patients were with rhinorrhoea.

Watery eyes in children with RSV infections can be attributed to several interconnected factors. The virus primarily infects the upper respiratory tract, leading to inflammation and increased mucus production, which can obstruct nasal passages and cause sinus congestion. This congestion may trigger a reflex that stimulates excess tear production, resulting in watery eyes[33]. Furthermore, the inflammatory response associated with the viral infection can heighten sensitivity in the ocular region, potentially leading to conjunctival irritation. In some cases, secondary complications such as viral conjunctivitis may arise, further contributing to the symptom of watery eyes. Understanding these mechanisms highlights the multifaceted impact of RSV on pediatric health, extending beyond respiratory symptoms to include ocular manifestations[34].

In this study, 92% of patients showed cough symptoms this agreement with study by Milani [35] which found 88.5 % of patients have coughing also. Infection with RSV has been associated to the development of wheeze and asthma, and it may be a risk factor for recurrent wheezing sickness following recovery [36]. Observational studies cannot fully account for the influence of genetic susceptibility, underlying health state, and co-infections with other viruses, which confounds the link between RSV infection and subsequent wheeze or asthma [37]. In this study, 83% of patients showed wheezing symptoms this agreement with study by Jiang et al [38].

In this study, we examined 100 cases of probable RSV infection, 58 cases were positive for RSV. Out of 58 cases with positive RSV, 32 were positive for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antigen, and from cases positive for SARS-CoV-2 antigen, 18 were confirmed positive for SARS-CoV-2 by PCR. Infection with RSV occurred in 46% of children in WHO European nations. This ratio is comparable to that of other continents, such as Latin America, where RSV prevalence is 41.5% [39].

In this study the percentage of positive RSV has been 58%, Other authors identified a lower global positive rate of RSV infection than this study, such as 14.6% in Africa [40] and 16% in China [41]. Even studies conducted in European nations like France showed a lower rate of positive RSV of 12-18% [42], and in the US, the percentage of RSV infection in healthy children is only 1.8% [43].

In this study, 55% of patients showed positive results for SARS-CoV-2 Ag tests while only (56.2%) of them were confirmed by RT-PCR which means the SARS-CoV-2 Ag test is inferior to the PCR assay[44]. During the COVID-19 pandemic, rigorous PHSMs were established, and RSV prevalence fell globally. Furthermore, it is nearly extinct in numerous countries and regions. The overall RSV activity and the development of SARS-CoV-2 are shown; these declines can be linked to a decrease in pediatric hospitalized patients [14].

In this study, the percentage of positive RSV serotype B was higher than that of RSV serotype A, which is consistent with previous research showing that RSV-B infection is more severe [45]. prospectively assessed 105 children referred to a pediatric department in Copenhagen with RSV respiratory infections throughout three winter seasons (1993-1995) [45]. RSV strains were characterized and genotyped using PCR and nucleic acid restriction analysis. They identified an age-dependent variation in sickness severity: RSV B infections were associated with longer hospital stays, the usage of breathing aid, and the development of an infiltrate on a chest radiograph in infants aged 0 to 5 months. A study by Tran et al [46] in Vietnam with 235 RSV-A cases and 13 RSV B cases, revealed that subgroup B infection was related with a reduced hospitalization incidence and clinical severity score in children compared to subgroup A infection. Tran and colleagues' study looked at data collected during a single epidemic season, thus the results could be influenced by the prevalence of subgroups changing from year to year, altering immunity gained against previously circulating viruses, This contradicts with our findings.

CONCLUSION

Respiratory syncytial virus is the leading cause of sickness and death in newborns globally, especially in low - and middle - income nations. Palivizumab is crucial for preventing severe RSV LRTIs in high - risk newborns, but the excessively high cost prevents widespread use. During the pandemic, primary preventative measures, such as hand hygiene and face masks, were more cost-effective in reducing RSV burden. In addition to the vaccination, non - pharmaceutical preventive hygiene measures should be implemented to reduce RSV spread globally, even after the COVID-19 pandemic.

Ethical approval

The Medical Ethics Committee at the University of Al-Anbar in Iraq, approved this study in accordance with the Helsinki Declaration. Informed consent was obtained from participants, including the parents of the patients. (reference number 79, August 5, 2024).

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TABLES

Table 1: The frequency and percent of the study paramete

Variables		Frequent (n)	Percent (%)
RSV serotype (n=100)	Positive	58	58
	Negative	42	42
SARS-CoV-2 Ag (n=58)	Positive	32	55.17
	Negative	26	44.82
SARS-CoV-2 PCR (n=58)	Positive	18	31.03
	Negative	40	68.96

Table 2: The Positivity of RSV serotypes infection among children.

RSV serotype	Frequent (n)	Percent (%)
RSV serotype A	15	25.86
RSV serotype B	43	74.14
Total	58	100

FIGURES

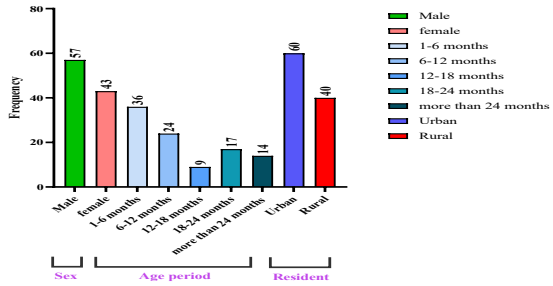

Fig.1: Frequency and percent for general characteristic of the study

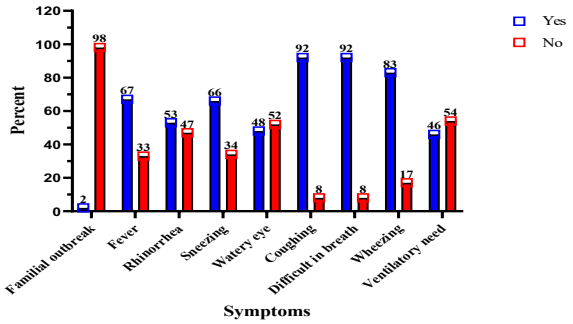

Fig 2: The frequency and percent of symptoms and disease outbreak for the study cases

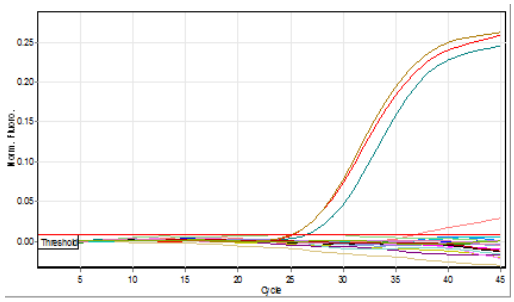

Fig 3: The positive result of the rtRT-PCR of the RSV serotype A.

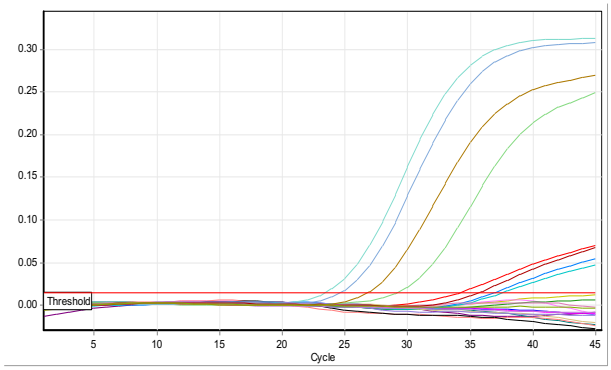

Fig 4: The positive result of the rtRT-PCR of the RSV serotype B.

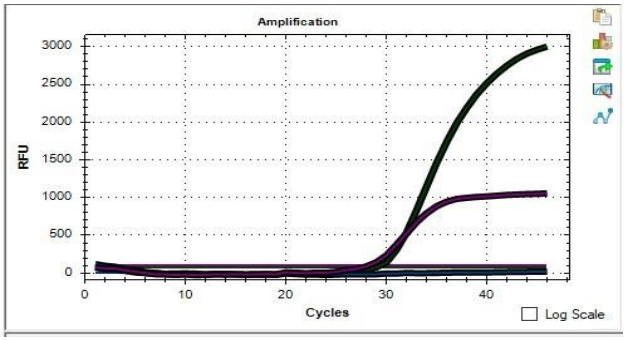

Fig 5: The positive result of the rtRT-PCR of the SARS-CoV-2.