Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)
Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Serological Diagnosis of Respiratory Syncytial Virus by ELISA Technique among Children with Respiratory Tract Infections in Beiji City, Iraq

Elaph M. Shuwaikh, Nihad A. Jafar, Sana S. Ahmed
Department of Microbiology, College of Veterinary Medicine, Tikrit University, Tikrit, Iraq
https://doi.org/10.25130/tjps.v28i1.1258

ARTICLE INFO.

Article history:
-Received: 26 / 7 / 2022
-Accepted: 11 / 10 / 2022
-Available online: 20 / 2 / 2023

Keywords: HRSV, Respiratory tract infection, ELISA.

Corresponding Author:
Name: Elaph M. Shuwaikh
E-mail: Elaf_madallah@tu.edu.iq
Tel:

ABSTRACT

The study have been done to detect Human Respiratory syncytial virus (HRSV) infections among hospitalized children under 5 years old in Beiji city Salah-Aldin Governorate, in addition to identify the risk factors associated with the virus. Whole blood samples were taken from 300 hospitalized children between the end of October 2021, to the end of June 2022, each sample was analyzed for HRSV by immunoglobulin M (IgM) specific to HRSV determining from blood specimens in serum using enzyme-linked immune sorbent assay (ELISA) technique. Of the 300 serum samples, Respiratory syncytial virus (RSV) was found in 120 (40%), 63 (42%) females, and 57 (38%) males. The age group was more susceptible to infection between 7-12 months for RSV at a rate of 25%. The study revealed that 61.66% of children exposed to smoke from one or both parents was positive for RSV, while 38.33% showed negative results for the presence of RSV. Considering the type of feeding, the current study showed that RSV infection was demonstrated in a higher percentage in patients with bottle feeding 60%, while a low percentage was demonstrated in patients with breastfeeding at 13.3%. Our results indicated the prevalence of RSV among children after imposing vaccination and reducing non-pharmaceutical interventions.

1. Introduction

Due to their high prevalence, ease of transmission, and severe morbidity and death, respiratory viral infections are the second largest cause of infant mortality and the primary reason for hospitalization in babies and young children around the world [1, 2]. Of all respiratory viruses that affect young children, RSV represents the main cause, because of its implications for acute disease severity. Nearly all children get at least one RSV infection by the time they are two years old [3].

On March 11, 2020, the World Health Organization (WHO) proclaimed the novel coronavirus illness which called coronavirus disease-19 (COVID-19) a worldwide pandemic [4]. Since then, social interactions have been restricted globally, and methods to prevent infection, such as handwashing, wearing masks, and avoiding close contact with others, have been strengthened. Other than COVID-19 and rhinovirus (RV), these efforts decreased the frequency of respiratory virus infections in 2020, and the number of reported cases has significantly decreased [5, 6, 7]. After that as a consequence of vaccination and reducing non-pharmaceutical interventions (NPIs) (measures socioeconomic activity and movement has increased), therefore, the overll risk for RSV in infants and young children expected to increase.

The greatest cause of pulmonary death in children under 5 is RSV, which causes more than three million annual hospital admissions and up to 118 000 deaths in this age group [8]. Although the infection may only cause minor, common-cold symptoms, the effects could be disastrous, especially in children with relatively underdeveloped immune systems, which would result in severe morbidity and a significant rise in medical expenses [9, 10].

An enzyme-linked immunosorbent assay (ELISA) can detect RSV-specific antibodies in sera of a patient

with respiratory tract infections (RTIs). Enzyme-linked immune sorbent assay was shown to be more sensitive and reliable than the traditional neutralization test [11, 12]. Even in environments with limited resources, ELISA is very inexpensive and simple to use compared to molecular approaches [13].

2. Materials and Methods

2.1. Studying groups

Three hundred serum samples from children with RTIs who needed medical attention were collected for this study at Beiji General Hospital between the beginning of October 2021 and the end of June 2022 (both sexes were included).

2.2. Collection of Specimens

Three milliliters of fresh venous blood were collected from each child and was kept in a test tube without anticoagulant and centrifuged for 15 minutes at 3000 revolutions per minute; the serum put into one aliquot stored in one microcentrifuge (eppendrof) tube labeled with patient's code number and frozen upon reaching the deep freezer at -70℃ until tested by ELISA. Once thawed refreezing was avoided.

2.3. Detection of respiretory syncytial virus antibody by ELISA

The ELISA kit used in this study was supplied from SunLong Biotech Cat.No. SL2980Hu, and the procedure of detection of HRSV Ab was done as described in the manufacturer's instructions.

2.4. Statistical Analysis Methods

Utilizing a statistical package for science services, computerized statistical analysis was performed. Comparative implementation techniques using probability and Chi-square (P value and X2). If the P value was less than 0.05, it is considered statistically significant (S) and if the P value was greater than 0.05, is considered statistically non-significant(NS).

3. Results

***Respiratory syncytial virus* detection**

Serum specimens collected from patients' children, and subjected to ELISA showed that the prevalence of RSV was 120 out of 300 (40%) serum specimens as shown in **Table 1.**

Table 1: *Respiratory syncytial virus* detection rate by ELISA technique

ELISA	Count (%)	Total (%)
Positive (%)	**120(40%)**	
Negative (%)	**180(60%)**	**300 (100%)**

Table 2: Frequency of *respiratory syncytial virus* infection and relation to risk factors

Risk factor	No. of patients (%)	No. of RSV-IgM +ve (%)	Sig/Non Sig= singnificant.
Sex			
Male	150(50%)	57(38%)	Non P-Value =0.291
Female	150(50%)	63(42%)	
Total	300(100%)	120(100%)	
Age group(month)			
≤6	48(%)	27(22.5%)	Sig P-Value =0.045
7-12	63(%)	30(25%)	
13-18	24(%)	12(10%)	
19-24	66(%)	18(15%)	
25-36	51(%)	18(15%)	
37-60	48(%)	15(12. 5%)	
Total	300(100%)	120(100%)	
Smoking Exposur			
Yes	187(62.33%)	74(61.66%)	Sig P-Value = 0.009
No	113(37.66%)	46(38.33%)	
Total	300(100%)	120(100%)	
Type of feeding			
Breastfeeding	52(17.33%)	16(13.33)	Sig P-Value = 0.006
Bottle feeding	94(31.33%)	72(60%)	
Mixed feeding	154(51.33%)	32(26.66%)	
Total	300(100%)	120(100%)	

Risk factors for the spread of RSV infection among infants and young children with RTIs were analyzed. Data were collected by using a special questionnaire including the sex, age, exposure to smoke (both or one parents), and type of feeding as shown in **Table 2.**

According to sex, in the present study, a slight female predominance, out of 120 (40%) RSV-positive children, 63(42%) were females and 57(38%) were males, but this difference was not statistically significant ($P > 0.05$). The age group that is more susceptible to infection is between 7-12 months at a rate of 25%, with statistically significant was found at $P < 0.05$. A comparison of infants and young children with exposure to parental smoking showed that 61.66% of patients with RSV belong to patients with

exposure to parental smoking. Significant association between children exposed to smoking and RSV infection at $P < 0.05$.

Considering the type of feeding, the current study showed that RSV infection was demonstrated in a higher percentage in patients with bottle feeding 60%, while a low percentage was demonstrated in patients with breastfeeding at 13.3%, and statistical analysis indicated a significant difference between the age groups with $P < 0.05$.

Discussion

In the present study, RSV was detected in 120(40%) children presenting with RTIs using ELISA.

In 2015, 2018, and 2019 RSV was found in different areas in Iraq at 17%, 55.91%, and 17.33% respectively [8, 14, 15]. The study conducted in 2015 was in the same area as the current study, but for children under 10 years old. According to our simple knowledge no studies has been conducted in Iraq measuring the frequency of RSV in children during the COVID-19 pandemic and after vaccination and socioeconomic activity and movement have increased. There is evidence available in neighboring countries such as Turkey that during the epidemic period for children with respiratory infections, the positivity rate for respiratory viruses was calculate 36% and RV was the most common agent (62.7%), and RSV was not detected. They noted that there was a decrease in positivity rate compared to previous years. According to study conducted in the same center including cases between April 2011 and April 2018, the positivity rate was at 58.7%, and the most common respiratory viral pathogens were RV (36.2%) and RSV (19.0%) [5]. While after vaccination and socioeconomic activity and movement increased, among 404 patients who were diagnosed with RTI, was found RSV and RV at 15.3% and 8.7% respectively, and 43.8% of patients diagnosed with RSV needed hospitalization [16].

Another country like Finland revealed that the incidence of RSV declined by 98%. after the initiation of local COVID-19 restrictions compared with data of prepandemic years [7]. and non-pharmacological interventions interrupted completely the circulation of all other respiratory viruses than rhinovirus. This difference in detection between RSV and RV viruses might be related to their stability because RSV enveloped while RV non-enveloped viruses [17]. After restrictions were relaxed in September 2021, a high RSV season was atypically early. RSV season peaked especially among children aged under 5 years [18].

The current study revealed an of RSV in rate 40%, which confirms the currently circulating studies that report the spread of RSV among children after immunization was imposed and life returned to normal.

In addition, the parents of younger children may seek medical attention earlier in the course of the disease due to parental anxiety. These variations in incidence between studies reflect different countries, which in turn may be related to environmental factors, geographical factors, differences in host genetic susceptibility, immune status, type and size of sample, detection techniques, and different viral strains circulating in different geographical areas [14, 19].

Due to the physiological, hormonal, and genetic variations between males and females, it is typically discovered while researching the association between sex and viral infection that males tend to infect at a higher rate than females. Innate and adaptive immunity are higher in females than in males, which may speed up the clearance of viruses [20]. In our current study, there was a slight female predominance, but statistically non-significant at $P > 0.05$. Out of 120(40%) RSV-positive children, 63(42%) were females and 57(38%) were males. These variations may be attributed to the small number of patients reported in our studies.

The current study revealed that peak age incidence for RSV was between 7-12 months at a rate of 25%, **Table.2**. This may be due to exclusive or partial breastfeeding in the first six months, also transplacentally acquired immunity may prevent infection in the first six months of life. Due to their comparatively undeveloped adaptive immune system as compared to older children and adults, infants are typically more prone to infections. Additionally, the risk of infections, particularly respiratory tract infections, is increased by the deterioration of maternal antibodies, stopping of breastfeeding, and staying in daycare facilities [21]. Other reasons include narrow airway, inadequate lung development and a very small bronchial tree [22].

A comparison of infants and young children with or without exposure to parental smoking showed 61.66% in patients with RSV. There significant association between children expose to smoking and RSV infection ($P < 0.05$). This finding was in agreement with that mentioned by other studies [23, 24, 25], but in disagreement with [26, 27, 28, 29]. In addition to raising the rate of cross-infection from the parents who smoke, triggering an allergic reaction, or irritating the infantile passageways, a study discovered numerous consequences of passive smoking and biomass fuels in newborns and young children [30].

Considering the type of feeding, the current study showed that RSV infection was demonstrated in a higher percentage in patients with bottle feeding 60%, while a low percentage was demonstrated in patients with breastfeeding 13.3%, statistical analysis revealed a difference between the age groups that was significant at $P < 0.05$. This finding was in agreement with studies confirm a protective role of breastfeeding against respiratory infections compared to those on bottle feeding and mixed feeding [31, 32, 33, 24], and this role mainly effect in the long term, is often measured after 6 months of age, showing a

persistent protective effect even after breastfeeding has been stopped.

Our findings also support the notion that breastfeeding has a protective effect against respiratory infection. The possible explanation for this partial protection of breastfeeding includes: that lymphocytes in the colostrums or milk that are sensitized to this virus and colonize in the nasopharynx of infants, or to stimulation immune response of the infants by transfer of sensitized T cells or of antigen on macrophages or may be because breast milk causes suppress to the IgE response which may be important in the pathogenesis of bronchiolitis [25, 30]. Some studies found that RSV-IgA and lactoferrin in the breast milk which promotes maturation probably through the influence of prolactin [34]. In order to intentionally improve maternal nutrition and/or supplement alternate sources of nutrition for infants who receive mixed feedings or no breast milk globally, it is crucial to have a better understanding of the protective components in breast milk.

According to certain research, the chance of developing RSV LRTI is greatly increased when breastfeeding is not practiced in conjunction with other risk factors such crowding, exposure to passive smoking, or low socioeconomic position [35, 36]

Conclusion

The frequency of RSV in children under five years of age after the COVID-19 pandemic vaccination and reducing NPIs. Children who were exposed to smoke from the environment were more likely to be infected with RSV infection than non-exposed. Children with breastfeeding are less likely to be infected with RSV infection than those with Bottle-feeding. The current study showed the importance of using the ELISA technique to diagnose RSV infection, especially when examining a large number of samples. The procedure is simple, easy to perform, requires minimal equipment, and is considered an excellent method with sufficiently high sensitivity. A further study is required to detect the RSV by using a molecular technique.

References

[1] AL-Bashar, S. H., Badawy, A. S., & Mohammed, B. A. R. (2022). Laboratory diagnosis of human adenovirus associated with respiratory tract infection in children. *Tikrit Journal of Pure Science*, *27*(1), 6-18.

[2] Heinonen, S., Rodriguez-Fernandez, R., Diaz, A., Rodriguez-Pastor, S. O., Ramilo, O., & Mejias, A. (2019). Infant immune response to respiratory viral infections. *Immunology and Allergy Clinics*, *39*(3), 361-376.

[3] Mejias, A., & Ramilo, O. (2015). New options in the treatment of respiratory syncytial virus disease. *Journal of Infection*, *71*, S80-S87.

[4] World Health Organization. (2020). WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020 [WWW Document]. URL https://www. who. int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020 accessed, 11, 20.

[5] Şık[1], N., Başerdem, K. A. Ç., Başerdem, O., Appak, Ö., Sayıner, A. A., Yılmaz[1], D., & Duman[1], M. (2022). Distribution of Viral respiratory pathogens during the COVID-19 pandemic: A Single-Center Pediatric study from Turkey. *Turkish Archives of Pediatrics*, *57*(3), 354.

[6] Ujiie, M., Tsuzuki, S., Nakamoto, T., & Iwamoto, N. (2021). Resurgence of respiratory syncytial virus infections during COVID-19 pandemic, Tokyo, Japan. *Emerging Infectious Diseases*, *27*(11), 2969.

[7] Yeoh, D. K., Foley, D. A., Minney-Smith, C. A., Martin, A. C., Mace, A. O., Sikazwe, C. T., ... & Moore, H. C. (2021). Impact of coronavirus disease 2019 public health measures on detections of influenza and respiratory syncytial virus in children during the 2020 Australian winter. *Clinical Infectious Diseases*, *72*(12), 2199-2202.

[8] Abduljabbar, H. L., Hussein, A. A., Al-Mayah, Q. S., & Aufi, I. M. (2019, July). Phylogenetic analysis of respiratory syncytial virus isolated from children with respiratory tract infections in Baghdad City, Iraq. *In Journal of Physics: Conference Series 1234*(1), p. 012082). IOP Publishing.

[9] Yassine, H. M., Sohail, M. U., Younes, N., & Nasrallah, G. K. (2020). Systematic review of the respiratory syncytial virus (RSV) prevalence, genotype distribution, and seasonality in children from the Middle East and North Africa (MENA) region. *Microorganisms*, *8*(5), 713.

[10] Madaniyazi, L., Seposo, X., Ng, C. F. S., Tobias, A., Toizumi, M., Moriuchi, H., ... & Hashizume, M. (2022). Respiratory syncytial virus outbreaks are predicted after the COVID-19 pandemic in Tokyo, Japan. *Japanese Journal of Infectious Diseases*, *75*(2), 209-211.

[11] Alhajj, M., & Farhana, A. (2022). Enzyme linked immunosorbent assay. In StatPearls [Internet]. StatPearls Publishing.

[12] Ostroff, R., Ettinger, A., La, H., Rihanek, M., Zalman, L., Meador III, J., ... & Polisky, B. (2001). Rapid multiserotype detection of human rhinoviruses on optically coated silicon surfaces. *Journal of* Clinical Virology, *21*(2), 105-117.

[13] Zhang, Y., Hung, T., Song, J., & He, J. (2013). Electron microscopy: essentials for viral structure, morphogenesis and rapid diagnosis. *Science China Life Sciences*, *56*(5), 421-430.

[14] Al-Shuwaikh, A. M. A., Ali, S. H., & Arif, H. S. Detection of respiratory syncytial virus in infants and young children with chest infection: a comparison of reverse transcription-PCR technique to

chromatographic immunoassay and enzyme linked immunosorbent assay. *Iraqi JMS. 2018; 16* (3): 319-326.

[15] Al-Bashar, S.H .(2015). Diagnosis of human adenovirus and human respiratory syncytial virus among children suffering from respiratory tract infections in Salahaldin Governorate-Iraq.Ph.D.thesis, College of Science. Tikrit University.

[16] BEDİR DEMİRDAĞ, T. U. Ğ. B. A., ATAY ÜNAL, N. U. R. S. E. L., TAPISIZ, A., KARA ULU, N. U. R. S. E. L., GÜDELOĞLU, E., TEZER, H., & BOZDAYI, G. (2022). Distribution and clinical features of viral respiratory infections in children after face-to-face education in 2021-2022 Winter Period. *Journal of pediatric infection*, *16*(1).

[17] Takashita, E., Kawakami, C., Momoki, T., Saikusa, M., Shimizu, K., Ozawa, H., ... & Kawaoka, Y. (2021). Increased risk of rhinovirus infection in children during the coronavirus disease- 19 pandemic. *Influenza and* Other Respiratory Viruses, *15*(4), 488-494.

[18] Kuitunen, I., Artama, M., Haapanen, M., & Renko, M. (2022). Respiratory virus circulation in children after relaxation of COVID- 19 restrictions in fall 2021–a nationwide register study in Finland. *Journal of Medical Virology*.

[19] Panayiotou, C., Richter, J., Koliou, M., Kalogirou, N., Georgiou, E., & Christodoulou, C. (2014). Epidemiology of respiratory syncytial virus in children in Cyprus during three consecutive winter seasons (2010–2013): age distribution, seasonality and association between prevalent genotypes and disease severity. *Epidemiology & Infection*, *142*(11), 2406-2411.

[20] Klein, S. L. (2012). Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. *Bioessays*, *34*(12), 1050-1059.

[21] Yilgwan, C. S., John, C., Abok, I. I., & Okolo, S. N. (2013). Pattern of acute respiratory infections in hospitalized children under five years of age in Jos Nigeria. *Nigerian Journal of Paediatrics*, *40*(2), 150-153.

[22] Wert, S. E. (2004). Normal and abnormal structural development of the lung. *Fetal and Neonatal Physiology*, *5*, 627-641.

[23] Al-Ayed, M. S., Asaad, A. M., Qureshi, M. A., & Ameen, M. S. (2014). Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction. *Saudi Medical Journal*, *35*(11), 1348.

[24] Rida, M. F. (2011). Risk factors for Respiratory Syncytial Virus (RSV) bronchiolitis in children: a hospital based study. *The Iraq Postgraduate Medical Journal*, *10*(3), 305-310.

[25] Albargish, K. A., & Hasony, H. J. (1999). Respiratory syncytial virus infection among young children with acute respiratory tract infection in Iraq. *EMHJ-Eastern Mediterranean Health Journal*, *5* (5), 941-948, 1999.

[26] Ali, S. H., Al-Shuwaikh, A. M. A., & Arif, H. S. (2019). An investigation of risk factors associated with respiratory syncytial virus infection in a sample of infants and young children from baghdad. *Journal of Biotechnology Research Center*, *13*(1), 29-34.

[27] Al-bahadily, A. K. J. M., Al-Omrani, A. A. A. M., & Atiya, A. A. (2017). Hypertonic 3 Saline in Comparison with 0.9 (Normal) Saline in Treatment of Acute Bronchiolitis. *International Journal of Pediatrics*, *5*(1), 4209-4216.

[28] Halasa, N., Williams, J., Faouri, S., Shehabi, A., Vermund, S. H., Wang, L., ... & Khuri-Bulos, N. (2015). Natural history and epidemiology of respiratory syncytial virus infection in the Middle East: Hospital surveillance for children under age two in Jordan. *Vaccine*, *33*(47), 6479-6487.

[29] Hall, C. B., Weinberg, G. A., Iwane, M. K., Blumkin, A. K., Edwards, K. M., Staat, M. A., ... & Szilagyi, P. (2009). The burden of respiratory syncytial virus infection in young children. *New England Journal of Medicine*, *360*(6), 588-598.

[30] Etiler, N., Velipasaoglu, S., & Aktekin, M. (2002). Incidence of acute respiratory infections and the relationship with some factors in infancy in Antalya, Turkey. *Pediatrics International*, *44*(1), 64-69.

[31] Etrhuni, S., Omar, R., & Hadid, I. (2020). Risk factors of acute respiratory infections in children in Tripoli, Libya. *Ibnosina Journal of Medicine and Biomedical Sciences*, *12*(3), 200.

[32] Dixon, D. L. (2015). The role of human milk immunomodulators in protecting against viral bronchiolitis and development of chronic wheezing illness. *Children*, *2*(3), 289-304.

[33] Chantry, C. J., Howard, C. R., & Auinger, P. (2006). Full breastfeeding duration and associated decrease in respiratory tract infection in US children. *Pediatrics*, *117*(2), 425-432.

[34] Sommer, C., Resch, B., & Simões, E. A. (2011). Suppl 2: risk factors for severe respiratory syncytial virus lower respiratory tract infection. *The Open Microbiology Journal*, *5*, 144.

[35] Al-Zayadneh, E., Assab, D. M. A., Arabiat, E. A., Al-Iede, M., Kayed, H. A., & Daher, A. (2021). The burden of influenza and other respiratory viruses in hospitalized infants and children in a university hospital, Jordan. *Multidisciplinary Respiratory Medicine*, *16*(1).

[36] Resch, B., Manzoni, P., & Lanari, M. (2009). Severe respiratory syncytial virus (RSV) infection in infants with neuromuscular diseases and immune deficiency syndromes. *Paediatric Respiratory Reviews*, *10*(3), 148-153.

التشخيص المصلي للفيروس المخلوي التنفسي بواسطة تقنية الاليزا بين الأطفال المصابين بعدوى الجهاز التنفسي في مدينة بيجي، العراق

إيلاف مدالله شويخ , نهاد عبدالحسين جعفر , سناء سعود أحمد

فرع الاحياء مجهرية , كلية الطب البيطري , جامعة تكريت , تكريت , العراق

الملخص

أجريت هذه الدراسة لتشخيص فايروس HRSV في الاطفال الراقدين في المستشفى تحت الخمس سنوات في مدينة بيجي محافظة صلاح الدين إضافة الى تحديد عوامل الخطر المرافقة للإصابة بالفايروس. أخذت عينات الدم الكلي لـ 300 طفل للفترة بين بداية تشرين الاول لعام 2021 لغاية نهاية أيار لعام 2022 وكل عينة تم تحليلها بواسطة الاجسام المضادة نوعIgM لفايروس HRSV من عينات المصل المفصولة عن الدم بواسطة تقنية الاليزا. من بين 300 عينة مصل تم العثور على الفيروس في 120 (40%) ، 36 (42%) من الإناث و 57 (38%) من الذكور. كانت الفئة العمرية الأكثر عرضة للإصابة بالفيروس بين 7–12 شهرًا بنسبة 25%. كان 61.66% من الاطفال المعرضين لتدخين احد الوالدين او كلاهما ايجابيين لـ HRSV بينما اظهر 38.33% نتائج سلبية لوجود الفيروس. بالنظر إلى نوع التغذية ، أظهرت الدراسة الحالية أن الإصابة HRSV ظهرت بنسبة أعلى 60% في المرضى الذين تم إرضاعهم صناعياً، بينما ظهرت نسبة منخفضة 13.3% في المرضى الذين تلقوا الرضاعة الطبيعية. تشير نتائجنا إلى انتشار HRSV بين الأطفال بعد فرض التطعيم وتقليل تدابير الوقاية غير صيدلانية.