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

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ABSTRACT

The study was carried out to detect Human Respiratory Syncytial Virus (HRSV) infections among hospitalized children under 5 years old in Beiji city, Salah-Aldin Governorate, Iraq. In addition, it aimed at identifying the risk factors associated with this virus. Whole blood samples were taken from 300 hospitalized children during the period from the beginning of October 2021 to the end of June 2022. Each sample was analyzed by using immunoglobulin (IgM) specific to HRSV from serum samples separated from blood using enzyme-linked immune sorbent assay (ELISA) technique. Of the 300 serum samples, Respiratory Syncytial Virus (RSV) was found in 120 (40%) samples, divided into 63 (42%) females and 57 (38%) males. The 7-12 months age group was more susceptible to the RSV infection at a rate of 25%. The study revealed that about 61.66% of children exposed to smoking from one or both parents showed positive results for RSV, while about 38.33% of them 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 as represented by 60%, while a low percentage was demonstrated in patients with breastfeeding at a rate of 13.3%. The current 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 among babies and young children around the world [1, 2]. Of all respiratory viruses that affect young children, RSV represents the main cause of illness, 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, termed as coronavirus disease-19 (COVID-19) and considered 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 have 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 the reduction of non-pharmaceutical interventions (NPIs), measures of socioeconomic activity and movement have increased. Therefore, the overall risk for RSV in infants and young children has been expected to increase.

The greatest cause of pulmonary death in children under 5 years old 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). ELISA has been 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 during the period from the beginning of October 2021 to the end of June 2022 (both genders were included).

2.2. Collection of Specimens

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

2.3. Detection of Respiratory 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) were utilized. If the P value was less than 0.05, it was considered statistically significant (S) and if this value was greater than 0.05, it was considered statistically non-significant (NS).

3. Results

Respiratory Syncytial Virus Detection

Serum specimens collected from patients (children) and subjected to ELISA showed the prevalence of RSV in 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%)**	**300 (100%)**
Negative (%)	**180(60%)**	

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

Risk factor	No. of patients (%)	No. of RSV-IgM +ve (%)	Sig/Non Sig= significant
Gender			
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 Exposure			
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 gender, age, exposure to smoking (both parents/ or one of them) and type of feeding, as shown in **Table 2.**

According to gender, the present study showed a slight female predominance, as out of 120 (40%) RSV-positive children, 63(42%) were females and 57(38%) were males. However, this difference was not statistically significant ($P > 0.05$). The 7-12 months age group was more susceptible to infection at a rate of 25% with a statistically significant P value

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 were exposed to parental smoking. A significant association between children exposed to smoking and RSV infection was found 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 as represented by 60%, while a low percentage was demonstrated in patients with breastfeeding by 13.3%. The statistical analysis indicated a significant difference between the age groups at $P < 0.05$.

Discussion

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

In 2015, 2018, and 2019, RSV was found in different areas in Iraq at the rates of 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 it was for children under 10 years old. According to the researchers' knowledge, no studies were conducted in Iraq measuring the frequency of RSV in children during the COVID-19 pandemic as well as after the increase in vaccination and socioeconomic activity and movement. 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 represented by 36% and RV was the most common agent by about (62.7%), while 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 represented by about 58.7%, and the most common respiratory viral pathogens were RV by (36.2%) and RSV by (19.0%) [5]. After the increase in vaccination and socioeconomic activity and movement, among 404 patients who were diagnosed with RTI, about 15.3% of them had RSV and about 8.7% of them had RV. While approximately 43.8% of patients diagnosed with RSV needed hospitalization [16].

Another country like Finland revealed that the incidence of RSV had declined by 98% after the initiation of local COVID-19 restrictions compared with data of pre-pandemic years [7]. 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 is an enveloped virus, while RV is a non-enveloped virus [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 the presence of RSV at the rate of 40%, confirming the currently circulating studies that reported the spread of RSV among children after the imposition of immunization and the return of normal life.

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 were found in different countries, which in turn could 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, the search for the association between gender and viral infection revealed that males tend to infect at a higher rate than females. Innate and adaptive immunity was higher in females than in males, which could speed up the clearance of viruses [20].

In the current study, there was a slight female predominance, but it was statistically non-significant at $P > 0.05$. Out of 120 (40%) of RSV-positive children, 63 (42%) patients were females and 57 (38%) patients were males. These variations may be attributed to the small number of patients reported in this study. The current study revealed that peak age incidence for RSV was between 7-12 months at a rate of 25% (see **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/ without exposure to parental smoking showed that 61.66% of patients had RSV. There was a significant association between children exposed to smoking and RSV infection at ($P < 0.05$). 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 among newborns and young children [30].

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

finding was in agreement with the studies that confirm a protective role of breastfeeding against respiratory infections compared to those on bottle feeding and mixed feeding [31, 32, 33, 24]. This role mainly affect in the long term, and is often measured after 6 months of age, showing a persistent protective effect even after breastfeeding has been stopped.

Our findings also supported the notion that breastfeeding has a protective effect against respiratory infection.

The possible explanation for this partial protection of breastfeeding includes lymphocytes in the colostrum or milk that are sensitized to this virus and colonized in the nasopharynx of infants; stimulating immune response of the infants by transferring sensitized T cells or antigen on macrophages. Moreover, it 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 promote 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 as crowding, exposure to passive smoking, or low socioeconomic position [35, 36]

Conclusion

The frequency of RSV in children under five years of age increased after the COVID-19 pandemic vaccination and the reduction of NPIs. Children who were exposed to smoke from the environment were more likely to be infected with RSV infection than non-exposed ones. Children with breastfeeding were 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. In this study, this technique was simple, easy to perform, required minimal equipment, and was considered excellent with sufficiently high sensitivity. This study therefore recommends conducting a further research to detect the RSV by using a molecular technique.

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

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

الملخص

أجريت هذه الدراسة لتشخيص فايروس HRSV في الاطفال الذين تقل أعمارهم عن خمس سنوات والراقدين في المستشفى في مدينة بيجي, محافظة صلاح الدين, العراق. بالإضافة الى ان هذه الدراسة هدفت الى تحديد عوامل الخطر المرافقة للإصابة بالفايروس. أخذت عينات الدم الكلي لـ 300 طفل للفترة من بداية تشرين الاول لعام 2021 ولغاية نهاية أيار لعام 2022. وتم تحليل كل عينة 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 (7–12 شهرًا) الأكثر عرضة للإصابة بالفيروس 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 بـ HRSV ظهرت بنسبة أعلى 60% في المرضى الذين تم إرضاعهم صناعياً، بينما ظهرت بنسبة منخفضة 13.3% في المرضى الذين تلقوا الرضاعة الطبيعية. اشارت نتائج الدراسة الحالية إلى انتشار HRSV بين الأطفال بعد فرض التطعيم وتقليل تدابير الوقاية غير الصيدلانية.