ORIGINAL ARTICLE

Evaluation of Cytokine Profiles (IL-6 & IL-10) in Pediatric Patients with RSV Infection Diagnosed by RT-PCR

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ABSTRACT

Background: *Respiratory syncytial virus (RSV) ranks high among the most prevalent and consequential causes of viral lower respiratory tract infections (LRTIs). All patients, regardless of age, exhibiting symptoms of LRTI should have RSV as a possible differential diagnosis. RSV infection is common in infants and toddlers younger than two years old.* ***Objective:*** *This study aimed to evaluate the levels of IL6 and IL10 in cases that confirmed diagnosis by RT-PCR as RSV infection.* ***Methodology:*** *This cross-section study was conducted from November, 2024 to the end of January, 2025. Total sample size was 150 (102 males, 48 females) within the one to five years old. From all participants blood and nasopharyngeal swabs were collected for hematological, ELISA test and RT-PCR detection respectively. SPSS and GraphPad software was used to analyze research data using suitable tests.* ***Results:*** *there was a highly significant increase in the serum IL6 among RSV positive infection children (60.41± 12.81) when compared with RSV negative infection groups (16.25± 3.86), with (P= <0.001). Also, there was a highly significant increase in the serum IL10 among RSV positive children (49.38± 6.18) when compared with RSV negative groups (9.09± 3.41), with (P= <0.001).* ***Conclusion:*** *high IL-6 and IL10 levels in RSV-infected children indicate a prolonged subacute inflammatory process and complex role in modulating inflammation.*

INTRODUCTION

Worldwide, respiratory viral infections are the second most common cause of infant mortality and the top reason for hospitalisation for infants and young children[1]. Low respiratory tract infections (LRTIs) in young children are most commonly caused by the Respiratory Syncytial Virus (RSV)[2,3]. In the family Paramyxoviridae, RSV is an enveloped, non-segmented, negative-strand RNA virus[4].

Antigenic subtypes A and B of RSV are defined by the degree to which monoclonal antibodies react with the F and G surface proteins, respectively[5]. An estimated 33 million people worldwide suffer from RSV-associated acute LRTI every year; this leads to more than 3 million hospitalisations, hospital fatalities in children below 5 years old reaching 59,600 fatalities, overall accounting for 6.7% of all infant deaths in the first year of life[6].

Depending on the severity of the infection, RSV can cause anything from a minor URI to a life-threatening pneumonia or bronchiolitis that requires hospitalisation, and can cause significant consequences including respiratory failure, along with related sequalae (i.e., hyperreactive airways, asthma, and wheezing) extending into childhood and even adulthood[7]. Severe RSV illness is most common in children under the age of two, with the peak incidence occurring in newborns around three months of age and a subsequent progressive drop as the child ages[8].

IL-6 is a soluble mediator mainly produced by epithelial cells and macrophages among other sources[9]. After production, interleukin-6 (IL-6) travels to the liver via the circulation, where it exerts a pleiotropic influence on inflammation and immunity[10]. Naïve CD4+ and CD8+ T cells are promoted to differentiate by this cytokine. another function of IL-6 is linking acquired and innate immunity[11]. Nasal lavage fluids from children with LRTI, especially those who required oxygen therapy, also showed elevated levels of IL-6 along with other cytokines[12].

The majority of immune system cells, which includes certain regulatory T cells, are capable of producing IL-10. By preventing macrophages and monocytes from producing and releasing inflammatory cytokines, it suppresses the immune system and maintains mucosal areas' normal immune quiescence. Increased clearance of mediators and cell debris from inflammatory sites is another benefit of IL-10's enhancement of phagocytic activity[13].

It is worth mentioning that IL-10 can be detected in RSV mouse model and the serum and nasopharyngeal secretions of newborns with severe bronchiolitis. Further evidence of the relevance of IL-10 during human RSV bronchiolitis has been provided by a recent study that suggests an association between post-

bronchiolitis wheezing and local IL-10 levels during the first infection[14].

METHODOLOGY

Subjects & Study design

A cross-section study was conducted on the following study groups during the period from November 2024 to the end of January 2025. Total sample size was 150 (102 males, 48 females) within the (1-5) years age group. Under the supervision of Pediatrician, this study was carried out at Al Zahraa Teaching Hospital in Al-Najaf, Iraq. It was subjected to evaluate IL-10 and IL-6 by ELISA technique and RSV detection by RT-PCR.

Ethical Considerations

Approval for the study was obtained from the Institutional Ethics Committee of the Faculty of Medicine, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences

Sample collection

Five ml of venous blood has been drawn from patients and (2 ml) collected in sterile test tubes (gel tube) and allow sample to clot for few minutes at room temperature then followed by separation of serum from the clot by centrifugation for 10 minutes at 3000 xg and stored at -20 C for ELISA. The rest amount of blood was transfer directly on EDTA tube for CBC estimation. Placing a sterile fine plastic stick into the nasopharynx until it becomes challenging was the method used to capture nasopharyngeal aspirates from each patient. The samples were then placed on viral transport media (VTM). Afterwards, the samples were brought from the hospital to the pharmacy department in an ice bag and kept at a temperature of -70˚C until the molecular tests were conducted.

Molecular diagnosis

RNA extraction

The extraction based on modern method called Magnetic beads method using full automated extractor analyzer. The use of Lysis Buffer allows for the release of nucleic acid from blood samples. The magnetic beads bind the released nucleic acid, which can be DNA or RNA. While the magnetic material binds to the nucleic acid (RNA), the wash buffer removes impurities After the automatic purification is over, transfer the Elution Buffer in columns 5 and 11 to a clean nuclease-free 0.5mL centrifuge tube.

Primers and thermal condition

RSV was detected using real-time RT-PCR assay[15]. The probe was labeled with 5 reporter dye FAM and the 3 at a non-fluorescent dye BHQ1. The primers were provided by (Bioneer Company, Korea), as shown in table.

Table 1: Sequence of primers that used in the RT-PCR of the respiratory syncytial virus

Primer	Sequence
RSV-F primer	5′- AACAGATGTAAGCAGCTCCGTTATC-′3
RSV-R primer	5′-CGATTTTTATTGGATGCTGTACATTT-′3
Probe	5′-TGCCATAGCATG ACACAATGGCTCCT -′3 (The probe was labeled with 5´ reporter dye FAM and the 3´at a non-fluorescent dye BHQ1)

Amplification and detection were done using CFX96 Real-Time system (Bio-Rad, USA),Taq R-stepRT9PCR (Promega/USA) was the master mix utilized for RSV detection by one step RT-PCR. We added to 5µl qPCR master mix, 0.25ml of RT mix, 0.25ml MgCl, 0.5ml forward primer, 0.5ml reverse primer and 3.5ml of RNA, the total volume 10ml. Briefly, one cycle for 15min at 37c° and 5min at 95 c°, followed by 40 cycles for 20s at 95c° and 20min at 63c°.

Estimation of IL6 and IL10 levels

This ELISA kit uses the Sandwich-ELISA principle supplied for (Elabscience company, USA). This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-10 and IL-6. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-10 and IL-6 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The optical density (OD) is measured spectrophotometrically at a wavelength of 450±2 nm. The OD value is proportional to the concentration of Human IL-10 and IL-6.

Statistical Analysis

Data review and analysis were conducted using Statistical Package for the Social Sciences (v.21.0) program (IBM Corp., Armonk, NY, USA), while the figures were created using GraphPad Prism (ver.9). Medians (interquartile range) are used to represent numerical variables, whereas percentages and frequencies (n) are used for categorical variables. In order to analyse normality, the Kolmogorov-Smirnov test was used. The distribution of categorical variables distribution was compared between groups using the chi-square test. The continuous variables were compared between the two groups using the

Independent Samples t-Test test. Statistical significance was determined by accepting a p-value less than 0.05.

RESULTS

The majority of the study samples were obtained from children below 6 month old, 18(50.0%), followed by 12(33.3%) were between 6-11 months while RSV negative group were 51(44.7%) under 6 months and 25(21.9%) were 6-11 respectively, with no statistically significant difference when compared with RSV negative group as shown in Table (2). Among the 36 pediatric patients with respiratory syncytial virus, 22(61.1%) were males, whereas RSV negative showed 80(70.2%) were males with no statistical significant variances between groups. History of prematurity was found in 12(33.3%) patients and in 16(14.0%) in RSV negative group, with statistically significant difference when compared with RSV negative group (P=<0.05), whereas the type of feeding was formula fed 13(36.1%) with statistically significant difference when compared with RSV negative group (P=<0.05), and family history of smoking found in 23(63.9%).

Table (3) revealed the presence of highly significant (p=<0.001) decrease in the mean of hemoglobin among children with RSV infection (11.23± 2.06), when compared with RSV negative group (13.06± 1.02). In comparison to the RSV (−) group, the RSV (+) group had higher white blood cell and platelet counts (p= < 0.001). Mean lymphocyte count was (4.37) in the RSV (+) group and (2.66) in the RSV (−) group. In comparison to the RSV (−) group, the lymphocyte count in the RSV (+) group was statistically significantly higher, whereas the mean of neutrophil count had no significant difference when compared with RSV (−) group.

Table 2: Demographic data of the study population.

Variables	RSV positive *n*=36	RSV negative *n*=114	P. value
Age (months)			
<6 *n*, (%)	18(50.0%)	51(44.7%)	χ^2=5.933 P= 0.115
6-11 *n*, (%)	12(33.3%)	25(21.9%)	
12-24 *n*, (%)	5(13.9%)	18(15.8%)	
25-60 *n*, (%)	1(2.8%)	20(17.5%)	
Sex			
Male	22(61.1%)	80(70.2%)	χ^2=1.033 P= 0.309
Female	14(38.9%)	34(29.8%)	
Family history of smoking			
Yes	23(63.9%)	75(65.8%)	χ^2=0.044 P= 0.835
No	13(36.1%)	39(34.2%)	
Type of feeding			
Breast feeding	12(33.3%)	20(17.5%)	χ^2=4.467 P= <0.05
Formula	13(36.1%)	58(50.9%)	
Mixed	11(30.6%)	36(31.6%)	
History of prematurity			
Mature	24(66.7%)	98(86.0%)	χ^2=6.711 P= <0.05
Premature	12(33.3%)	16(14.0%)	
χ^2 : chi-square test; p. value less than 0.05 considered significant.			

Table 3: Results of laboratory testing of groups infected and uninfected with RSV.

Laboratory findings	RSV positive Mean ±SD	RSV negative Mean ±SD	*P. value*
Hb, g/dl	11.23± 2.06	13.06± 1.02	**<0.001**
WBCs, ×10⁹/L	9.92 ± 1.52	7.89 ± 2.63	**<0.001**
Neutrophil count, ×10⁹/L	3.67± 1.11	3.73± 1.24	0.814 NS
Lymphocyte count, ×10⁹/L	± 1.41 4.37	2.66± 0.66	**<0.001**
Platelet count, ×10⁹/L	242.33± 48.36	192.57± 38.41	**<0.001**
SD: standard deviation**; Hb:** hemoglobin**; WBCs:** white blood cells**;** p. less than 0.05 considered significant.			

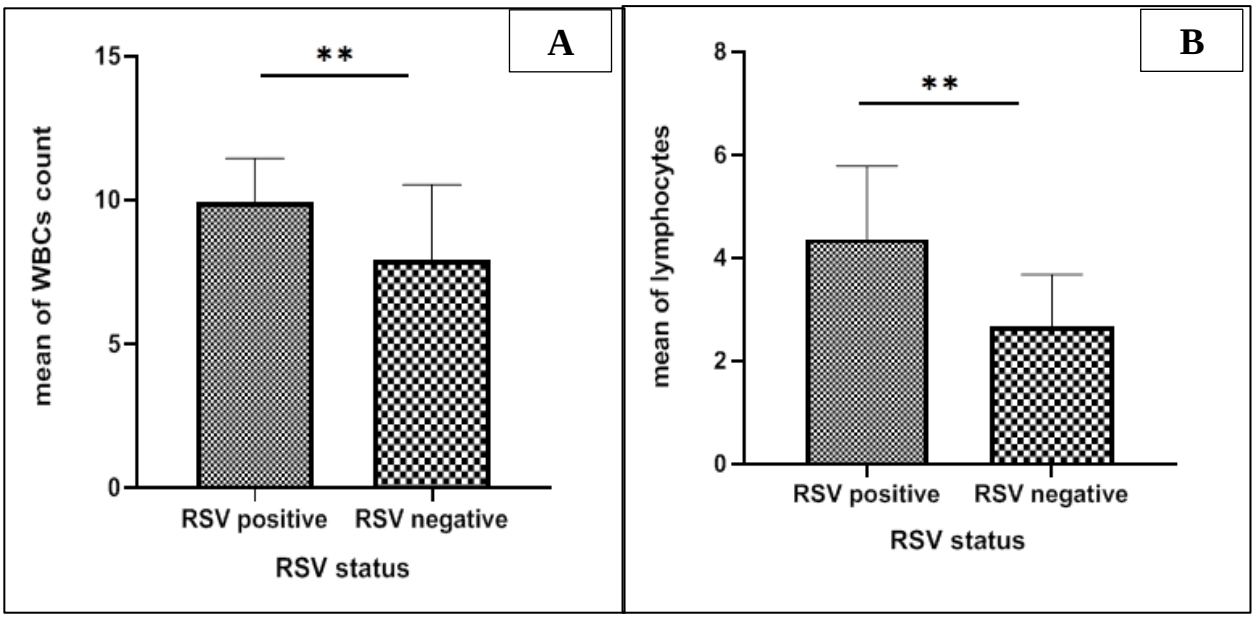

Fig.1: The mean and standard deviation of laboratory findings among RSV positive and RSV negative groups where; **A:** WBCs count; **B:** Lymphocyte count

Figure (2), shows positive nasopharyngeal swabs samples' real-time PCR amplification plot of RSV based nucleoprotein gene primers and probe (FAM) dye with a positive amplification from 21.87 into a threshold cycle (CT) of 33.22.

The results in Table (4) and Figure (3), shows a highly significant increase in the serum IL6 among RSV (+) children (60.41± 12.81) when compared with RSV (−) groups (16.25± 3.86), with (P= <0.001). The results in Table (5) and Figure (4), showed a highly significant increase in the serum IL10 among RSV (+) children (49.38± 6.18) when compared with RSV (−) groups (9.09± 3.41), with (P= <0.001).

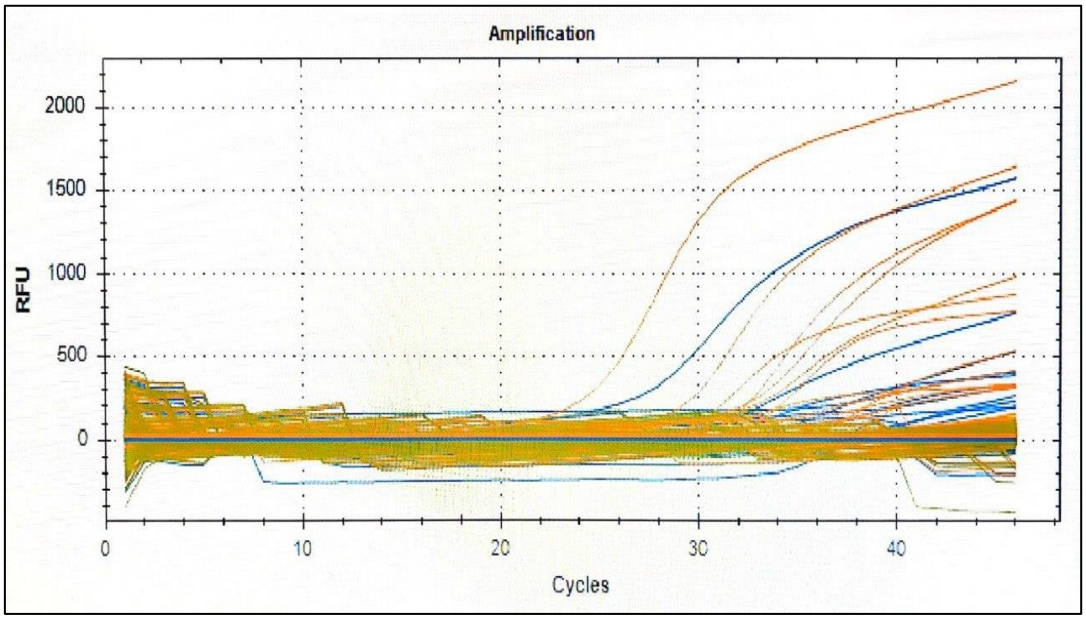

Fig.2: RT-PCR positive RSV detection.

Table 4: Serum level IL6 pg/ml among study groups.

IL6 pg/ml	Groups comparison		*P*
	RSV positive *n*=36	RSV negative *n*=114	
Mean	60.41	16.25	**<0.001****
Range	30-80	10-26	
Median (IQR)	61.91 (21)	14.85 (6)	
95% C.I	56.07- 64.75	15.53-16.97	
Standard deviation of the mean	12.81	3.86	

SD: standard deviation**; C.I:** confidence intervals**; IL6:** interleukin6**; IQR:** Interquartile range**; **:** significant at p. value <0.05.

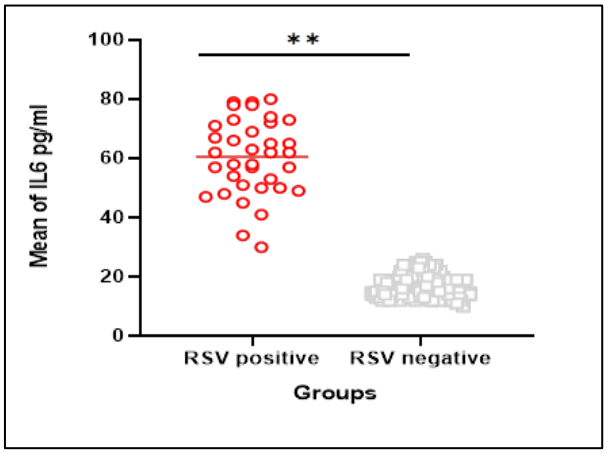

Fig. 3: serum IL6 pg/ml level among RSV (+) and RSV (-) groups, p. value (<0.001).

Table 5: Comparison of serum IL10 among studied groups.

IL10 pg/ml	Groups comparison		*P*
	RSV positive *n*=36	RSV negative *n*=114	
Mean	49.38	9.09	**<0.001****
Range	38-58	3-16	
Median (IQR)	49.30 (8)	8.32 (6)	
95% C.I	47.28-51.47	8.45-9.72	
Standard deviation of the mean	6.18	3.41	

SD: standard deviation**; C.I:** confidence intervals**; IL10:** interleukin10**; IQR:** Interquartile range**; **:** significant at p. value <0.05.

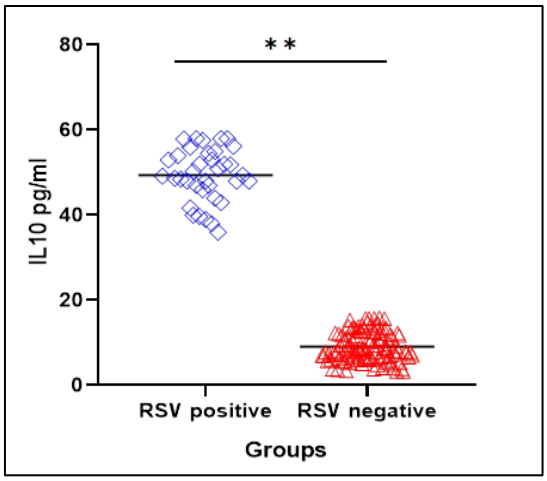

Fig. 4: Serum IL10 pg/ml level among RSV (+) and RSV (-) groups, p. value (<0.001).

DISCUSSION

It was not surprising that the RSV positive children in this study were under a year old, with an average age of 6 months for the positive cases. This finding sheds light on the potential effect of age on RSV infection. This is consistent with previous studies[16], which verified that RSV hospitalisations increased with decreasing patient age, reaching a peak in the first year of life. RSV is a common cause of respiratory illness in people of all ages, but it is especially associated with significant morbidity and mortality in infants and the elderly[17].

Consistent with previous research, our results did not show that gender was a major determinant of RSV infection[18]. However, Ogunsemowo *et al.*,[19], found males to be more predominantly infected. According to the available data, adolescent asthma was more common in boys who had contracted RSV as infants[20].

In this study, 23(63.9%) family member were smokers in the families. Children exposed to smoking have been found to have a higher risk of hospitalisation for severe RSV infections, including asthma, wheezing, and SIDS according to other researchers who found similar results[21]. Nicotine seems to have an effect on lung development, which may explain the association between smoking and RSV[22].

In consistent with most published studies[23], which provide more evidence that the risk of respiratory failure and the number of hospitalisations caused by RSV are both decreased in infants who are breastfed compared to infants without breast feeding (bottle fed)[24]. Although the majority of severe cases occur among previously healthy term infants[25], clinical studies have shown that infants with a history of prematurity are at higher risk of RSV infection requiring hospitalization than infants born at term[26].

The current study results indicated to presence a highly significant decrease in the level of hemoglobin among RSV positive children when compared to RSV negative group. The result agrees with Okuyan *et al.*, [27]. Anemic children are at risk of developing various consequences of anemia including infections[28]. Studies on ALRI reported a significant correlation between ALRI and low hemoglobin[29].

In the current study, lymphocyte and WBC counts were significantly higher in the RSV (+) group compared to the RSV (−) group with no significant variances in neutrophil counts among studied groups. In febrile patients hospitalised with RSV LRTI, the likelihood of an abnormal white blood cell count (WBC) of less than 5000 or between 15,000 and 30,000 being linked to a concomitant significant bacterial infection was extremely low, and it was not different from the likelihood of a normal WBC count[30].

IL-6 promotes the development of Th2 cells while inhibiting the polarisation of Th1 cells; it is a crucial proinflammatory cytokine. In acute and chronic inflammatory responses, TNF-α plays a significant role as a proinflammatory cytokine[31]. Patients infected with RSV have higher IL-6 serum levels than control group, although the difference was not statistically significant[14]. Infants infected with RSV had blood cytokine concentrations that were significantly correlated with birth weight rather than delivery method; this finding may help to elucidate the possible mechanism. Compared to patients with low IL-6 levels, those with high IL-6 had substantially decreased lung function and experienced more frequent exacerbations of asthma[32]. Circulating IL-6 is elevated in asthmatic patients and in bronchoalveolar lavage fluid of patients in whom asthma is clinically active. IL-6 levels probably reflect an activated state of the lung, and could potentially serve as an asthma biomarker[33].

The levels of IL-10 were found to be significantly higher in patients who tested positive for RSV. there's evidence that IFN-γ and IL-4 synthesis and response can be inhibited by IL-10[34]. Researchers found that monocyte-derived IL-10 played a role in post-bronchiolitis wheezing in newborns infected with RSV[35]. Sputum from individuals experiencing acute asthma attacks also showed that other viruses, such rhinovirus, might trigger the IL-10 gene expression[36]. The primary cytokine responsible for airway hyperresponsivity in an animal model of asthma has been identified as IL-10[37].

CONCLUSION

The study concluded that, high IL-6 levels in RSV-infected children indicate a prolonged subacute inflammatory process, suggesting ongoing inflammation despite clinical improvement. High IL-10 levels in RSV-infected children may indicate a complex role in modulating inflammation, potentially contributing to both protective and exacerbatory responses, as elevated IL-10 is associated with severe lower respiratory tract infections and subsequent asthma development.

Conflict of interest

The authors insured there was no conflict of interest in this study

Assignment of the family member

The assignment of the family member, either the father or the mother was to be designated as the legal guardian of the patient on behalf of each child in this study

Publication rule

All authors declare their consent to the publication of this study, and the researchers confirm that it has not been previously published in any other journal. Also, all authors equally participated in sample collection,

conducting the practical experiments, writing the paper, performing statistical analysis, and interpreting the results.

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