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Original Research Paper

"Correlation of Interferon-gamma (IFN-γ) and C-Reactive protein Levels with Clinical Diagnosis and Disease Severity in Pediatric Patients with RSV Infection"

Saad mohammed1 saad[1], Duha Abdullah kadhim [2], Zahraa mohammed majeed [3]

[1,2,3] *faculty of medicine, Jabir Ibn Hayyan University for medical and pharmaceutical sciences, Iraq.*

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*Corresponding Author: *saad mohammed jabir ibn hayyan university for mesical and pharmaceutical sciences,faculty of medicine,Iraq.*
Email: saad.m.saad@jmu.edu.iq

Abstract: Objective: To analyze the relationship between IFN-γ, CRP and clinical diagnosis of Respiratory Syncytial Virus (RSV) infection.
Method: Clinical data of RSV positive children admitted to the pediatrics department of Al-Zahraa Teaching Hospital, AL-Najaf, Iraq, from November 2024 to February 2025. They were divided into three groups according to different ages, <6months, 6-11 months, 12-24 months, 25-60 months, and male group and female group according to the sex. The clinical characteristics of the children in different groups were compared, and the significance of clinical diagnosis of the children with RSV pneumonia, acute Bronchitis and Bronchiolitis was analyzed by measuring the serum INF-γ, CRP and other hematological parameters levels.
Results: INF-γ is significantly higher in pneumonia patients compared to acute bronchitis and bronchiolitis. While, WBC levels are slightly higher in bronchitis and bronchiolitis compared to pneumonia, though the overall differences are relatively low. Furthermore, CRP, a key inflammatory marker, is higher in pneumonia than in bronchitis or bronchiolitis. The mean of interferon gamma showed there were a highly significant increase in the level among RSV (+) children (181.8±47.8) when compared with RSV (−) groups (18.21±3.34), with (P= <0.001).
Conclusion: the current study concluded that, the significant differences in INF-γ, WBCs, CRP, and ESR levels suggest that biomarkers can aid in distinguishing between these respiratory conditions, contributing to more accurate clinical decision-making and may also indicate to the clinical severity of disease in those patients.

Keywords: :interferon-gamma, C-reactive protein, Respiratory syncytial virus.

1. Introduction

Among children in developing nations, viral respiratory infections are the leading cause of illness and death [1]. Children under the age of five accounted for the majority of these cases [2]. In children, Respiratory Syncytial Virus (RSV) is a leading cause of acute upper and/or lower respiratory infections. It accounts for around 50% of pneumonia cases and 90% of bronchiolitis cases globally [3]. Hospitalization is necessary in nearly half of these cases, and infants younger than six months old account for the vast majority of fatalities [4]. In children, an RSV infection nearly invariably causes symptoms, which can range from nearly undetectable or moderate, nonspecific symptoms to a life-threatening sickness affecting the lower airways [5].

Almost all children contract RSV during the first two

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years of their lives, and almost 70% of infants have had an infection by the time they turn one year old [6]. Reinfection rates with RSV, which are often less severe, range from 6% to 83% annually in developing nations [7]. Due to antigenic diversity or an insufficient immune response, RSV can re infect newborns even when maternal antibodies are present [8]. Although no RSV vaccine has been licensed by the FDA as of May 17, 2022, Palivizumab, an authorized passive antibody treatment, is available. Concerning the virology of the virus, RSV is enveloped, negative-sense, non-segmented, single strand RNA virus (-ssRNA) of the family Pneumoviridae. The genome is approximately 16 kb and is comprised of 10 genes encoding for 11 proteins [9]. There are many different genotypes of RSV; genetic analysis has separated the virus into two subgroups, A and B. Of the two subgroups, 15 genotypes of RSV-A and 37 genotypes of RSV-B have been found so far [10]. various subgroups not only rotate in dominance but can also co-circulate during the same season, as determined by the examination of the glycoprotein (G) gene's nucleotide sequence [11,12]. Type I interferon-gamma (IFN-γ) cytokines have direct antiviral effects, enhance antigen presentation by triggering the production of major histocompatibility complex molecules (MHC), and boost the cytotoxic activity of T lymphocytes that are specific to viruses and natural killer cells [13]. Thus, the severity of RSV disease and the type II cytokine responses observed during reinfection in neonates and adults, respectively, may be greatly influenced by the early IFN-γ response strength, and a decrease in IFN-γ production may prolong the infection duration and have negative effects on lung function. On the other hand, delayed RSV infection resulted in increased IFN-γ production and less severe disease during reinfection [14].

2. Methodology

Study population & Design:

A cross-section study was conducted on the following study groups during the period from November 2024 to the end of January 2025. In this study patients group composed of (100), (70) of them are males and (30) females with age range 1-5 years old. Under the supervision of Pediatrician, this study is carried out at Al Zahraa Teaching Hospital in Al-Najaf, Iraq. This study was subjected to evaluate INF-γ by ELISA technique.

Sample collection:

After drawing 5 ml of blood from the veins and transferring 2 ml to sterile test tubes (gel tubes), the next step is to let the sample clot for a few minutes at room temperature. After that, separate the serum from the clot using centrifugation at 3000 xg for 10 minutes. and stored at -20 C for ELISA the rest amount of sample transfer directly for EDTA tube for CBC estimation.

Estimation of INF-γ and CRP levels:

This ELISA kit uses the Sandwich-ELISA principle supplied for (Elabscience company, USA). The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Interferon-gamma (INF-γ). CRP reagent is an in vitro diagnostic medical device intended to be used by healthcare professionals for the quantitative immunoturbidimetric measurement of C-Reactive Protein (CRP) in human serum and plasma using the automated Beckman Coulter (AU480, USA) analyzers.

Statistical Analysis:

Statistical Package for the Social Sciences version 21.0 software package for Windows (IBM Corp., Armonk, NY, USA) was used for data evaluation and analysis. Categorical variables are presented as frequencies (n) and percentages (%), and numerical variables are presented as medians (interquartile range). The Kolmogorov–Smirnov test was applied for normality analysis. The chi-square test was used to compare the distribution of categorical variables between groups. The Independent Samples t-Test test was used to compare continuous variables between two independent groups and one way ANOVA test was used for three groups comparison. A value of $p < 0.05$ was accepted as statistically significant.

3. Results and discussion

The majority of the participant were below 6 month of age, 12(40%), followed by 8(26.6%) were between 6-11 months while RSV negative group were 22(31.5%) under 6 months and 20(28.5%) were 6-11 respectively, with statistically significant difference (p=<0.05) when

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compared with RSV negative group as shown in Table (1). Among the 30 pediatric patients with respiratory syncytial virus, 18(60%) were males, whereas RSV negative showed 44(62.8%) were males with no statistical significant variances between groups.

History of prematurity found in 8(26.6%) patients and in 13(18.5%) in RSV negative group, with statistically significant difference when compared with RSV negative group (P=<0.001), whereas type of feeding was formula fed 10(33.4%) with statistically significant difference when compared with RSV negative group (P=<0.05). Totally, 16(53.4%) of the patients were reported to have urban residence with considerable highly significant in comparison with RSV negative group (P=<0.001). Among the clinical diagnoses of the patients 10(33.3%) had bronchiolitis, 12(40%) had acute bronchitis, 8(26.7%)had pneumonia with statistically significant variance with RSV negative group of patient (P=<0.05) as it presented in Table (1).

Table 1: characteristics of the studied samples.

Variables	RSV positive *n*=30	RSV negative *n*=70	*P. value*
			Age (months)
<6 *n*, (%)	12(40%)	22(31.5%)	χ^2=6.533 P= <0.05
6-11 *n*, (%)	8(26.6%)	20(28.5%)	
12-24 *n*, (%)	6(20%)	10(14.3%)	
25-60 *n*, (%)	4(13.4%)	18(25.7%)	
			Sex
Male	18(60%)	44(62.8%)	χ^2=1.055 P= 0.207
Female	12(40%)	26(37.2%)	
			Type of feeding
Breast feeding	12(40%)	20(28.6%)	χ^2=4.566 P= <0.05
Formula	10(33.4%)	34(48.6%)	
Mixed	8(26.6%)	16(22.8%)	
			History of prematurity
Mature	22(73.4%)	57(81.5%)	χ^2=5.611 P= <0.05
Premature	8(26.6%)	13(18.5%)	
			Residence
Urban	16(53.4%)	52(74.3%)	χ^2=8.066 P= <0.001
Rural	14(46.6%)	18(25.7%)	
			Clinical diagnosis
Pneumonia	8(26.7%)	18(25.7%)	χ^2=6.308 P= <0.05
Acute Bronchitis	12(40%)	21(30%)	

Table 2: laboratory findings of RSV positive and Negative cases.

Laboratory findings	RSV positive Mean ±SD	RSV negative Mean ±SD	*P. valu*
Hb, g/dl	10.75± 1.9	13.07± 1.01	<0.00
WBCs, ×10⁹/L	10.12 ± 1.5	8.12 ± 2.78	<0.00
Neutrophil count, ×10⁹/L	3.72± 1.01	3.86± 1.2	0.716 N
Lymphocyte count, ×10⁹/L	± 1.4 4.26	2.63± 0.64	<0.00
Platelet count, ×10⁹/L	243.17± 48.8	193.27± 32.1	<0.00
CRP, mg/L	37.4± 6.03	4.60± 0.70	<0.00
ESR, mm/1h	± 15.6 67.7	32.29± 8.5	<0.00

SD: standard deviation**; Hb:** hemoglobin**; WBCs:** white blood cells**; CRP:** C-reactive prot
ESR: erythrocytes sedimentation rate**;** p. value less than 0.05 considered signific

Table (2) indicated to presence of highly significant (p=<0.001) decrease in the mean of hemoglobin among children with RSV infection (10.75± 1.9), when compared with RSV negative group (13.07± 1.01). WBC count was higher in the RSV (+) group compared to the RSV (−) group (10.12 ± 1.5) (8.12 ± 2.78) (p= < 0.001). The mean of lymphocyte count was (4.26) in the RSV (+) group and (2.63) in the RSV (−) group. Lymphocyte count was statistically significantly higher in the RSV (+) group than in the RSV (−) group, whereas the mean of neutrophil count had no significant difference when compared with RSV (−) group. CRP content was higher in the RSV (+) group compared to the RSV (−) group (37.4± 6.03; 4.60± 0.70; p=<0.001) respectively. In addition, the mean of ESR level indicated to a highly significant increase in RSV positive group when compared with RSV negative group.

Table 3: levels of studied parameters across clinical diagnosis of RSV positive cases.

Laboratory findings	RSV positive Mean ±SD	RSV negative Mean ±SD	*P. valu*
Hb, g/dl	10.75± 1.9	13.07± 1.01	<0.00
WBCs, ×10⁹/L	10.12 ± 1.5	8.12 ± 2.78	<0.00
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SD: standard deviation**; Hb:** hemoglobin**; WBCs:** white blood cells**; CRP:** C-reactive prot
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Table (3) presents the mean ± standard deviation (SD) values of different inflammatory and immune response parameters among patients with pneumonia (n=8), acute bronchitis (n=12), and bronchiolitis (n=10). A one-way ANOVA test was used to determine statistical significance, and all parameters have a p-value < 0.001, meaning the differences between the groups are statistically significant. INF-γ is significantly higher in pneumonia patients compared to acute bronchitis and bronchiolitis as it shown in Figure (1). WBC levels are slightly higher in bronchitis and bronchiolitis compared to pneumonia, though the overall differences are relatively low. CRP, a key inflammatory marker, is higher in pneumonia than in bronchitis or bronchiolitis as it shown in Figure (2). ESR is highest in bronchiolitis, followed by bronchitis, and lowest in pneumonia.

Parameters	**Clinical diagnosis**		
	Bronchiolitis *n*=10 mean± SD	**Acute Bronchitis *n*=12** mean± SD	**Pneumonia *n*=8** mean± SD
INF-γ (ng/ml)	166.09± 10.4	170.46± 14.32	214.8± 37.8
P. value	**<0.001†**		
WBCs $\times 10^9$ /L	10.49± 1.1	10.32± 0.12	9.38± 1.9
P. value	**<0.001†**		
CRP (mg/L)	35.01± 4.03	37.33± 9.08	40.28± 13.2
P. value	**<0.001†**		
ESR mm/1h	72.3± 19.9	67.36± 13.09	62.6± 17.5
P. value	**<0.001†**		
SD: standard deviation**; WBCs:** white blood cells**; CRP:** C-reactive protein**; ESR:** erythrocytes sedimentation rate**; †:** one way ANOVA test**;** p. value less than 0.05 considered significant.			

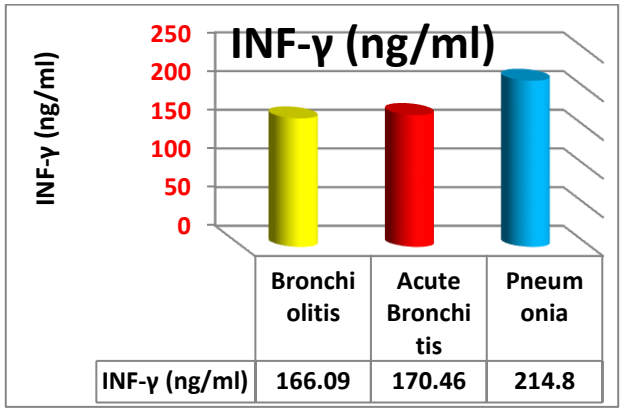

Fig 1: INF-γ level related to clinical diagnosis of RSV patients.

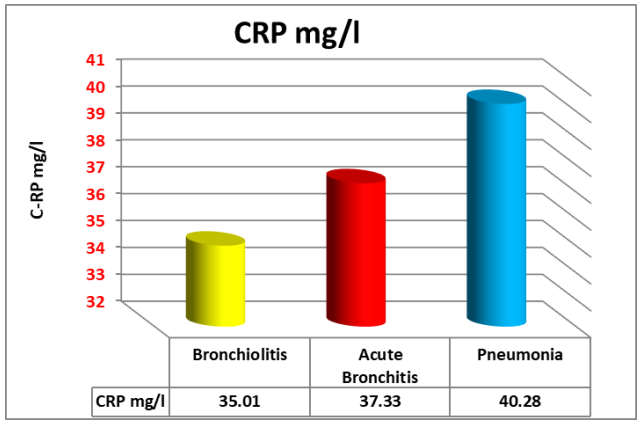

Fig 2: CRP level related to clinical diagnosis of RSV patients.

The mean of interferon gamma showed there were a highly significant increase in the level among RSV (+) children (181.8±47.8) when compared with RSV (−) groups (18.21±3.34), with (P= <0.001) as it shown in Table 4 and Figure 3.

Table 4: serum level of INF-γ among RSV positive children in comparison with RSV negative.

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	Groups		*P. value*
	RSV positive *n*= 30	**RSV negative *n*=70**	
Interferon-gamma (INF-γ)			
Mean± SD	± 47.8 181.8	± 3.34 18.21	**< 0.001 HS†**
Range	100.77 – 291	12.2 – 23.6	

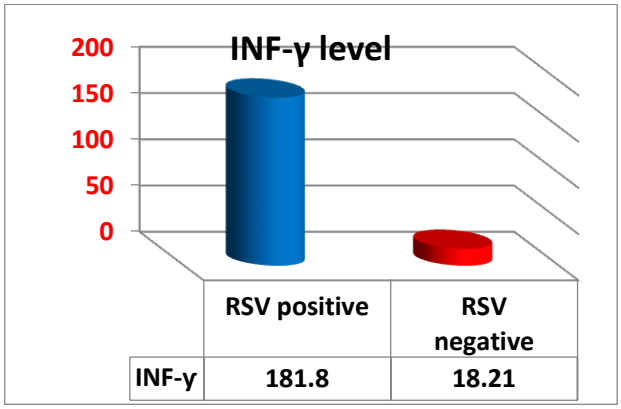

Fig 3: INF-γ among RSV positive and negative cases.

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 [15], which verified that RSV hospitalisations increased with decreasing patient age, reaching a peak in the first year of life. Although RSV can cause respiratory infection at any age, it is more dangerous for newborns and the elderly, where it can cause serious complications and even death [16]. Consistent with previous research, our results did not show that gender was a major determinant of RSV infection [17]. Although the majority of severe cases occur among previously healthy term infants [18], 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 [19]. The study in agreement with what were published as Cunningham (2019), who reported that bronchiolitis is typically caused by RSV in about 70% of cases (Cunningham, 2019). According to [20]. bronchiolitis was the primary symptom experienced by most RSV positive cases. The results of current study 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 [21]. Anemic children are at risk of developing various consequences of anemia including infections [22]. Compared to the RSV (−) group, the RSV (+) group had considerably greater lymphocyte and white blood cell counts in the present study. However, there were no significant differences in neutrophil counts among the groups observed. The likelihood of a severe bacterial infection being linked to aberrant white blood cell counts below 5,000 or between 15,000 and 30,000 was extremely low and was similar to a normal white blood cell count in febrile patients admitted with RSV LRTI[23]. One of the most important cytokines for both innate and adaptive immunity in fighting off bacterial and viral infections is INF-γ. Elevated INF-γ levels in pneumonia suggest a robust immune activation, possibly due to the severity and depth of lung tissue involvement. While specific studies directly comparing INF-γ levels across these conditions are limited, the heightened levels in pneumonia align with the understanding that more invasive infections elicit stronger immune responses [24]. IFNs molecules bind to specific cellular receptors, which in turn activate interferon-inducible genes and establish an efficient antiviral state and modulate immune responses through the activation of signal transduction pathways (STP) [25]. CRP is an acute-phase protein that rises in response to inflammation. Elevated CRP levels are associated with bacterial infections like pneumonia. A study found CRP concentrations of 6.5 ± 7.3 mg/dL in lobar pneumonia, 1.9 ± 2.0 mg/dL in bronchiolitis, and 2.1 ± 2.4 mg/dL in bronchopneumonia . Another study indicated that CRP levels were significantly higher in pneumonia patients compared to those with acute asthma, chronic obstructive bronchitis, and acute bronchitis . The elevated CRP levels across all groups in our data suggest notable inflammatory responses, with the highest levels in pneumonia aligning with its typically greater severity [26].

Conclusion

The study concluded that, these results demonstrate the differential immune and inflammatory responses in pneumonia, bronchitis, and bronchiolitis, reinforcing the need for targeted diagnostic and therapeutic approaches.

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The significant differences in INF-γ, WBCs, CRP, and ESR levels suggest that biomarkers can aid in distinguishing between these respiratory conditions, contributing to more accurate clinical decision-making. Future studies with larger sample sizes and more specific cytokine profiling may provide deeper insights into the immunopathology of these respiratory diseases.

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Ethics

This study was conducted under approval by the medical ethics committee at the Jabir ibn hayyan university, faculty of medicine Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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