Prognostic Value of Interleukin-18 in Children with Covid Respiratory Infection

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Abstract
Background: Coronavirus is one of the major pathogens that primarily targets the human respiratory system. A striking and consistent observation has been the difference in severity of COVID-19 at different ages: severity, the need for hospitalization and mortality rise steeply with older age while severe disease and death are relatively rare in children and young adults. Large epidemiological studies suggest that children comprise only 1 to 2% of all SARS-CoV-2 cases. However, more recent studies report that children are less likely to get infected after contact with a SARS-CoV-2 positive individual. Methods: A total of 100 children with covid respiratory infection were included in the study who were admitted to hospital from the period between end of December 2021 to March 2022. A Five ml of blood samples using disposable syringes under aseptic technique were collected and withdrawn from each patient, three ml were transferred to sterile Gel tube, and allow to clot at room temperature and centrifuge at 2500 rpm for 10 minutes and the separated serum was saved in Eppendorf tubes and immediately frozen at -20 C till further use to avoid repeated thawing and freezing for 1L-18 ELISA Kit test, and two ml of whole blood for direct complete blood count test. Results: The present study included 100 children with Covid-19 respiratory infection. The mean age was 1.45 ± 2.6 years. Median levels of IL-18 were 24.30 (21.60) ng/L and 9.90 (8.15) ng/L, in children with positive real time-PCR and negative real time-PCR respectively; the level was higher in children with positive real time-PCR in comparison with children with negative real time-PCR and the difference was highly significant ($P < 0.001$). Conclusion: There was significant correlation between COVID-19 and IL-18 and the level of interleukin was elevated during infection.
Keyword: SARS-CoV-2, Interleukin-18, ELISA and real time-PCR.

1. Introduction

Coronavirus is one of the major pathogens that primarily targets the human respiratory system. A striking and consistent observation has been the difference in severity of COVID-19 at different ages: severity, the need for hospitalization and mortality rise steeply with older age while severe disease and death are relatively rare in children and young adults (1). Large epidemiological studies suggest that children comprise only 1 to 2% of all SARS-CoV-2 cases. However, more recent studies report that children are less likely to get infected after contact with a SARS-CoV-2 positive individual (2). It has been suggested that children and adolescents have similar viral loads and may therefore be as likely to transmit SARS-CoV-2 as adults. In addition, the viral load may be similar in asymptomatic and symptomatic individuals. However, reassuringly, transmission in schools from children either to other children or to adults has been rare (3).

Understanding why children are generally less prone to develop severe COVID-19 and associated symptoms could help to define immune mechanisms of protection against SARS-CoV-2 infection in the general population. The way children respond to SARSCoV-2 is somewhat unusual, since the severity of infections with many other respiratory viruses, such as respiratory syncytial virus or influenza, is generally higher in children. This difference cannot be explained by a reduced viral load, as children have similar and sometimes higher viral copies in the first days of infection as compared to adults, but this viral load does not correlate with the severity of symptoms (4). There is also no clear evidence that an age-dependent variation in the ACE2 expression level correlates with reduced disease severity. The gene expression of ACE2 in the nasal cavity and lungs was initially shown to be lower in young infants and to increase with age, but in later studies it was found to be similar in infected adults and children (5).

Cytokine markers are a group of polypeptide signaling molecules that can induce and regulate many cellular biological processes by stimulating cell receptors at the surface (6). Primary and important cytokines include those that can play an important role in the types of adaptive immunity, pro-inflammatory cytokines, and interleukins and anti-inflammatory cytokines. However, host cells may secrete cytokines that can induce processes in the body as a defense response to cell metabolism (7). Studies have shown that SARS-CoV-2 infection has the ability to induce specific and disparate inflammatory responses in the body. Research has shown that an inappropriate immune response most often occurs in patients with certain diseases or other diseases such as diabetes, heart, and kidney

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disease. This condition increases the virus' ability to multiply and, in turn, increases its associated side effects (8).

Interleukin-18 is thought to play a broad role in defense against infections. Observational evidence concerning the role of interleukin-18 in reducing the risk of COVID-19 is limited and difficult to interpret because it concerns observational studies in patients where interleukin-18 could represent a protective response, a symptom or a cause of complications. Currently, to our knowledge, no experimental evidence concerning the role of interleukin-18 in COVID-19 exists (9). However, experimental evidence exists concerning the role of interleukin-18 in other viruses. Specifically, interleukin-18 was shown to protect mice against death from herpes simplex 1, possibly via natural killer cells or via IFN-γ. Interleukin-18 has also been shown to protect mice against murine coronavirus mouse hepatitis virus strain A59 by preventing viral replication via IFN-γ, although interleukin-1 was not similarly protective (10). More broadly, interleukin-18 protecting against infection is consistent with it increasing the risk of some auto-immune and atopic conditions. The serum concentrations of IL-18 correlate with COVID-19 pathogenesis (11).

2. Materials and Methods

This cross- sectional comparative study has been done with on 100 patients recruited from Maternity and Children Hospital in AL-Qadissiyah province with age range between 1–12 years, during the period of the end of December 2021 to March 2022. The patients were divided to Two groups according to the Real-time PCR results, 50 of which were PCR positive and 50 were negative. A Five ml of blood samples using disposable syringes under aseptic technique were collected and withdrawn from each patient, three ml were transferred to sterile Gel tube, and allow to clot at room temperature and centrifuge at 2500 rpm for 10 minutes and the separated serum was saved in Eppendorf tubes and immediately frozen at -20 C till further use to avoid repeated thawing and freezing for 1L-18 ELISA Kit test, and two ml of whole blood for direct complete blood count test. This study was in agreement with ethics of Maternity and Children Hospital and verbal informed consent was obtained from all participants.

3. Statistical Analysis

The Mean ± SE was used to display and analyze the data involving the probability level of p <0.05 for significant results. Statistical analyses were performed by using statistical analysis software (Statistical Package for the Social Science) (SPSS) version 25

4. Results

The present study included 100 children with Covid-19 respiratory infection. The mean age was 1.45 ± 2.6 years and Children under one years of age accounted for 67 (67.0 %) of cases table (1). The frequency distribution of children with covid respiratory infection according to the results of real time PCR is shown in figure (1). Positive results were reported in 50 (50.0 %); whereas, negative results were reported in 50 (50.0 %). The comparison of some blood parameters between children with positive real time-PCR and negative real time-PCR has been carried out and the results were demonstrated in table (2). Mean levels of white blood cell count were 11.54 ± 3.41 and 14.02 ± 3.65, in children with positive real time-PCR and negative real time-PCR respectively; the mean levels was lower in children with positive real time-PCR in comparison with negative real time-PCR and the difference was highly significant (P < 0.001). Mean levels of lymphocyte count were 35.25 ± 15.10 and 36.42 ± 20.10, in children with positive real time-PCR and negative real time-PCR respectively; the mean levels was slightly decreased in children with positive real time-PCR in comparison with negative real time-PCR but the difference was non-significant (P = 0.743). The comparison of serum Interleukin-18 (IL-18) level between children with positive real time-PCR and negative real time-PCR has been carried out and the results were demonstrated in table (3) and figure (2). Median levels of IL-18 were 24.30 (21.60) ng/L and 9.90 (8.15) ng/L, in children with positive real time-PCR and negative real time-PCR respectively; the level was higher in children with positive real time-PCR in comparison with children with negative real time-PCR and the difference was highly significant (P < 0.001).

Table 1: Mean age and frequency distribution of patients according to age

Age (years)	N	%
< 1 years, *n* (%)	67	67.0
1-3 years, *n* (%)	21	21.0
≥ 3 years, *n* (%)	12	12.0
Total	100	100.0
Mean ±SD	1.45 ± 2.6 years	
Range	4 days -8 years	

SD: standard deviation; *n*: number of cases

Table (2): Levels of some blood parameters (WBC and lymphocyte a) in children with covid respiratory infection.

Parameters	Real time-PCR		P
	Positive real time-PCR n = 50	Negative real time-PCR n = 50	
White Blood Cells			
Mean± SD	11.54 ± 3.41	14.02 ± 3.65	0.001 † HS
Range	7.30 – 20.80	7.30 – 18.50	
SE	0.51	0.48	
Lymphocytes			
Mean± SD	35.25 ± 15.10	36.42 ± 20.10	P= 0.743 † NS
Range	13.80 -68.20	7.80 -75.90	
SE	2.81	2.15	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

Table (3): Median levels of Serum IL-18 in children with covid respiratory infection.			
Serum IL-18	Real time-PCR		P
	Positive real time-PCR n = 50	Negative real time-PCR n = 50	
Range	4.90 – 52.80	1.00 – 26.10	< 0.001 † HS
Median (IQR)	24.30 (21.60)	9.90 (8.15)	
n: number of cases; IQR: inter-quartile range; †: Mann Whitney U test; HS: Highly significant at $P \leq 0.001$			

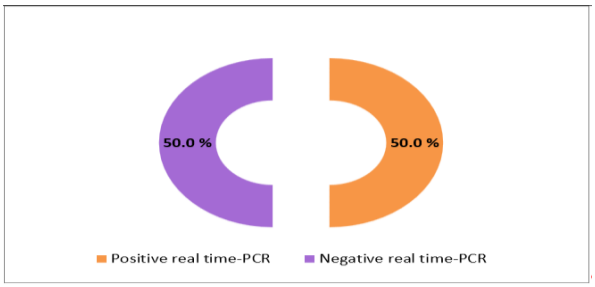

Figure (1): Pie chart showing the frequency distribution of children with covid respiratory infection according to the results of real time PCR

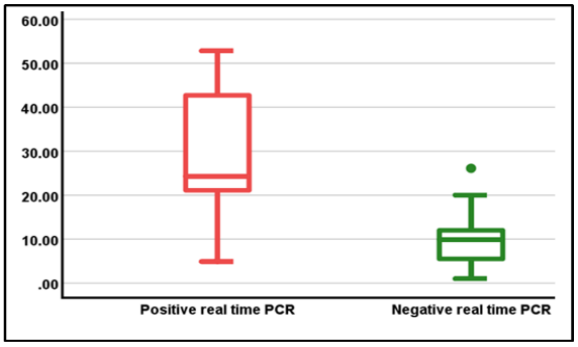

Figure (2): Distribution of children with covid respiratory infection according to the level of Serum IL-18.

5. Discussion

Respiratory tract infections (RTI) are the most common type of childhood disease. RTI represent a major global disease burden in children, particularly in children under five years of age (12). Their incidence is often difficult to estimate because ARTIs are typically treated in outpatient settings and the majority of available epidemiological data are collected in hospital settings and are referred to as the most severe respiratory illness (14). Since December 2019, the SARS-CoV-2 virus has had a significant impact on healthcare systems all over the world, with over 3.8 million directly associated deaths being reported. Due to this rapid worldwide disease spread and hospital overload, the World Health Organization (WHO) announced a global pandemic situation on 11 March 2020(14). Although the disease was initially reported as being most dangerous and fatal predominantly in the elderly population (over 60 years) with major comorbidities, a specific pediatric-related presentation of SARS-CoV-2 infection has been described, based on the multi-systemic inflammatory syndrome (15)

The present results shows the mean age of patients are 1.45 ± 2.6 years with range (4 days -8 years), these results agree with results of Diesner-Treiber et al, (16) which showed the median age of the patients at diagnosis time was 12 months (6-18 months). The present results slightly lower than the results of Mameli et al., (17) which showed the mean and standard deviation of age 2.39± 1.68 and their age ranged from 1 month to 5 years. But the present study disagree Saleh et al., (18) which showed the median age of 5.8 years (4 months–13.3 years).

Regarding age groups, the age range of patients at diagnosis was (between 4 days -8 years). Most patients with Covid respiratory infection were diagnosed at the age less than one year 67 (67.0 %), followed by the age group between 1-3 years 21 (21.0%) and the prevalence age groups was more than 3 years 12 (12.0%), table (1). Where, the incidence of infection varied among the 8 years of age analyzed, reaching the highest value in patients less than 1 year and gradually decreasing up to the age of 8 years. The present results observed an age-dependent decrease in the incidence of infection, which is consistent with data reported by other authors worldwide (19).This could be explained by the physiological postnatal maturation of both the innate and adaptive immune systems occurring during the first 6 years of a child's life, as a result of repeated environmental exposure to microbes(20).The clinical course of COVID-19 in children, in general, is less severe than in adults, but the recognized risk factor for severe complications and the need for hospital admission in children is age under 1 years (21).The present results can hypothesize on a number of potential factors influencing this result such as the highest incidence in infants could reflect the fact that parents of younger children could be more likely to contact an FP to schedule a medical visit than parents of older children(17). The present result consistence with results of Mameli et al.,(17) which demonstrates high prevalence of COVID-19 was in the first year of life among children hospitalized, 131 (27.1%).

The gold standard for SARS-CoV-2 detection is the viral nucleic acid findings by the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from the bio specimen obtained from the patient's upper or lower airway mucosal samples (nasopharynx, oropharyngeal swab, tracheal aspirate, bronchoalveolar lavage), even in the absence of clinical symptoms (22). The present results showed 45 (60.00 %) of children with Covid respiratory infection have positive Real time PCR, in compared to only 5 (20.0%) of healthy control showed positive Real time PCR, and the difference was highly significant (p = 0.001). The present results consistence with result of Ai et al., (23), which reported as much as 20% false-negative results for this type of test.

The negative PCR test in a symptomatic patient may be due to many reasons. Firstly, the molecular detection methods are based on the presence of the viral genome, which have to be amplified. A first limit can be linked to the viral load in the sample

collection site but also to the lack of the viral replication time window, which can provide false negative results (24). Secondly, an imperfect collection of the sample can produce false negative results, due to the presence of not enough virus in the swab sample (25). Thirdly, The results affect by mutations in viral genomes of SARS-CoV-2 (26). Furthermore, due to a possible delay of up to 5 days between the initial viral exposure and positive PCR detection, one negative RT-PCR test should not exclude the possibility of COVID-19 in symptomatic patients with a high likelihood of SARS-CoV-2 infection (the testing should be repeated 1–5 days after the initial negative result (27). Although PCR is considered the gold standard for SARS-CoV-2 detection accepted worldwide, a positive PCR test in an asymptomatic patient does not necessarily mean active COVID-19 disease. The positive results of asymptomatic individual can potentially due to infection several days or weeks previous, which went unnoticed, or the pre-symptomatic person in incubation period, where the developing symptoms within fewer than 10 days (28).

SARS-CoV-2 can cause changes in the number of white blood cells (29). WBCs were measured in all of the 100 patients and were significantly different between the two groups, where the mean levels of white blood cell count were 11.54 ± 3.41 and 14.02 ± 3.65, in children with positive real time-PCR and negative real time-PCR respectively; the mean levels was lower in children with positive real time-PCR in comparison with negative real time-PCR and the difference was highly significant (P < 0.001). The present results indicated a strong association between COVID-19-positive patients and a low WBC count. Consistence with the present results, Ferrari et al., (30), indicated a strong association between COVID-19-positive patients and a low WBC count (p-value <0.001), and Cheng et al., (31), which showed The median leukocyte counts in patients with positive results were lower than those in patients with negative results (leukocyte count, 4.38 vs 5.63 × 109 cells/L [p = 0.02]. Guan et al., (32), analyzed the peripheral blood cells (PBC) of 1099 COVID-19 patients, and the results showed that the number of white blood cells were decreased (<4.0×109/L).

Regarding to lymphocyte, the present results show the mean levels of lymphocyte count were 35.25 ± 15.10 and 36.42 ± 20.10, in children with positive real time-PCR and negative real time-PCR respectively; the mean levels was slightly decreased in children with positive real time-PCR in comparison with negative real time-PCR but the difference was non-significant (P = 0.743). Lymphopenia is a prevalent biomarker in SARS-COV-2 infected patients, and it indicates a faulty immunological response to the illness(18)..Consistence with the present results, Ferrari et al., (30), found the laboratory examination of 207 suspected COVID-19 patients that there was a difference in the number of white blood cells in the two groups of patients with SARS-CoV-2 rRT-PCR positive and rRT-PCR negative, and positive COVID-19 patients have a lower number of lymphocytes. Cheng et al., (31), which showed the median Lymphocyte count in patients with positive results were lower than those in patients with negative results (leukocyte count, 1.10 (1.03–1.33) vs 1.24 (0.98–1.65) [p = 0.42]. Qin et al., (33), conducted a study on 452 hospitalized COVID-19 patients and found that patients with severe infection are more likely to suffer from lymphopenia.

A robust immune response during viral infections, as well as a SARSCoV-2, may be considered essential for the resolution of COVID-19. However, persistent immune activation in severe patients can lead to hemophagocytosis like syndrome, with uncontrolled amplification of cytokine production (34). Upon viral infection, IL-18 release induces ferritin, explaining the frequently observed hyperferritinemia in viral infections. Moreover, serum concentrations of IL-18 might serve as a biomarker to predict disease outcome (11)

IL-18 is a member of the IL-1 family of cytokines which play roles in both the innate and adaptive immune responses, fibrosis and hematopoiesis (35). IL-18 is produced by macrophages at very early stages of viral infections and induces production of IL-6 and IFN-γ which are considered critical for optimal viral host defense (36). However, aberrant IL-18 production can also lead to severe pathological injury. The activity of IL-18 is balanced by IL-18 binding protein (IL-18BP) which is stimulated by IFN-γ, prompting a classical feedback loop whereby IL-18BP offsets exuberant IL-18 and attenuates the IFN-γ response (35). It is synthesized as an inactive precursor, pro-IL-18, requiring processing by caspase-1 into an active cytokine. IL-1β and IL-18 are mainly produced by monocytes/macrophages in response to harmful stimuli including viruses. Markedly elevated serum IL-18 levels have been linked to severe disease and mortality in some viral infections characterized by cytokine storm of Covid respiratory infection (11). To the best of our knowledge, this is the first study investigating IL-18 in patients with COVID-19 in Iraq.

The present findings showed a highly significant association between the concentration of IL-18 and Covid respiratory infection (P <0.001), table (3). Median levels of serum IL-18 was higher in children with positive real time-PCR in compared to children with negative real time-PCR, 24.30 (21.60) ng/L versus 9.90 (8.15) ng/L, respectively. From the point of biological action, IL-18 is a powerful inducer of the inflammatory cytokine IFN-γ and activation of Th1, NK cells and M1 (cytocidal and inflammatory) macrophages (37). This type of inflammation is very typical for Covid-19 which is thought to be mainly mediated by IL-18 due to marked hyperferritinemia (38). The stimulation of IL-18 depends on activation

of inflammasomes, particularly NOD-, LRR- and pyrin domain- containing protein 3 (NLRP3) inflammasome. Viral components as well as cytosolic danger signals, such as mitochondria injury, protein aggregates, and aberrant ion concentrations can activate the NLRP3 inflammasome which triggers the auto-cleavage of pro caspase-1 into active caspase-1, eventually leading to proteolytic activation of pro-IL- 1β, pro-IL-18 and the pyroptotic factor gasdermin D (GSDMD) (39). The secretion of IL-1β subsequently recruits neutrophils to the inflammatory site to defeat invading viruses. Both IL-1β and IL-18 are responsible for the induction of the adaptive immune response after the innate immune responses. Therefore, optimal activation of the NLRP3 inflammasome facilitates host defense against viruses, but excessive activation may result in pathologic consequences (40). The present results agree with the results of Satışa et al.,(11), which showed a COVID-19 patients had higher IL-18 levels compared to healthy subjects (103 [210] pg/mL vs 310 [502] pg/mL, p = 0.006).

Conclusions

Our results confirm that the majority of patients that was highly affected by covid respiratory infection was less than one year age. There was significant correlation between COVID-19 and IL-18 and the level of interleukin was elevated during infection.

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