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Gene expression of cytokines IFN-γ, TGF- β, IL-4 and IL-10 in children infected with HMPV in Al-najaf city

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ABSTRACT

Human metapneumovirus (HMPV) can be defined as one of the Pneumoviridae respiratory viruses that has been initially discovered in the respiratory tracts of children in the Netherlands in the year 2001. In addition, HMPV is a major pathogen in newborn respiratory infections; almost 50% of all children are infected with this virus before the age of two, and the majority are infected before the age of five. In immunosuppressed people, children, and the elderly, HMPV is one of the most common acute respiratory tract infections (ARTI) causes. Also, this pathogen is considered as a leading mortality cause in the children who are younger than five years old. The present study does focus on the investigation of cytokines IFN-γ, TGF- β, IL-4 and IL-10 gene expression in the positive pediatric patients for HMPV were recruited from inpatients of Al zahraa hospital, Najaf City, Iraq. Studies conducted in various countries allover the world has verified that the human metapneumovirus (HPMV) is the most etiological agent of respiratory infections among children. In this context, this study encompassed 44 children infected with human metapneumovirus. The recruited patients were those routinely do visit the external clinics of Al zahraa hospital, Najaf City from September 2020 to April 2021. The presumptive diagnosis by the pediatrician was viral respiratory infection. Data of participants including clinical symptoms, age and family history were collected by using a questionnaire format specially designed for this purpose. Fourty four blood samples were positive with human metapneumovirus of different ages were enrolled, Real-time PCR assay was used for molecular detection of cytokines IFN-γ, TGF- β, IL-4 and IL-10 gene expression and PCR primers set for this cytokines were used. Statistical analysis for all data was done using Statistical Package of Social Sciences (SPSS), version 27, (Inc., Chicago, IL, USA) computer software. Statistical comparison between study groups analyzed using chi-square test and T test. $P<0.05$ was regarded as statistically significant. Regarding the antiviral immune response in terms of IL-10, IL-4, TGF beta, and INF-y were monitored in cases of human metapneumovirus (HMPV) in this study. The gene expression of TGF beta, IL-4, and INF-y were significantly higher among the positive pediatric patients when compared to those among the health control group, while the gene expression of IL-10 were degress observed among positive cases infected with human metapneumovirus (HMPV). IL-10 occurred in median concentration of 0.0083 in patient group and 1 in control group. It was significantly and higher different in two groups when compared with each other ($P<0.05$).

IL-4 occurred in median concentration of 2.78 in patient group and 1 in control group. It was significantly and higher different in two groups when compared with each other (P<0.05). IFN-γ occurred in median concentration of 2.38 in patient group and 1 in control group. It was significantly and higher different in two groups when compared with each other (P<0.05). TGF- β occurred in median concentration of 4.481 in patient group and 1 in control group. It was significantly and higher different in two groups when compared with each other (P<0.05).

1. INTRODUCTION

Human metapneumovirus (HMPV) can be defined as one of the Pneumoviridae respiratory viruses that has been initially discovered in the respiratory tracts of children in the Netherlands in the year 2001 [1]. HMPV is most commonly transferred from infected individual to others via secretions from sneezing and coughing, close personal contact, like shaking hands, and touching surfaces or objects which have the viruses on them before touching the eyes, nose, or mouth. HMPV is circulating throughout the United States in various annual seasons. The circulation of HMPV starts begins in the winter and continues till or through spring [2].

The symptoms of HMPV infection are indistinguishable from those of other respiratory virus diseases. Wheezing, cough, shortness of breath, expectoration, bronchitis, runny nose, bronchiolitis, asthmatic bronchitis, and pneumonia are all symptoms of HMPV in the lower or upper respiratory tract [3].

HMPV infection was linked to the development of neurological symptoms like febrile seizures and encephalitis [4]. Also, the symptoms with most severity related to HMPV infections are frequently indicated in newborns less than one year of age, although they are also common in early childhood up to the age of five. Pre-term birth, asthma, and previous infections by some of the respiratory viruses, like Human Respiratory Syncytial Virus (HRSV, lately renamed into human ortho-pneumovirus), are all high-risk factors in infants, and such factors predispose infants to a more severe disease manifestation following an infection with the HMPV [1].

Interferon-gamma (IFN-γ) is a proinflammatory cytokine that activates effector immune cells and enhances antigen presentation in vivo [5].

IFN-γ is secreted by natural killer (NK) cells as part of the innate immune response, and by CD4+ Th1 cells and CD8+ cytotoxic T lymphocytes (CTLs) once adaptive immunity has been triggered [6].

Interferons induced by viral infection constitute the first line of the innate antiviral immune defense. There are three types of interferons: type I IFN, a multi-gene cytokine family that includes several subtypes (α, β, ε, δ, κ, τ, ω, and ζ) [7]. One of the HMPV proteins that regulate the IFN response in HMPV infection is the attachment (G) protein. G protein is a type II transmembrane glycoprotein. Albeit the fact that the postulated functional role of G protein is for attachment to a host cell receptor, it is not essential for viral replication [8].

Transforming growth factor b (TGF-b) is a pleiotropic cytokine with potent regulatory and inflammatory activity [9].

Interferon-g (IFN-g) potently inhibits TH17 development [10]. Given the suppressive actions of TGF-b on IFN-g production, we speculated that TGF-b might contribute to TH17 development by limiting inhibitory actions of IFN-g [11].

Interleukin 10 (IL-10) also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans, interleukin 10 is encoded by the IL10 gene, also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans, interleukin 10 is encoded by the IL10 gene [12].

IL-4 is a pleiotropic cytokine involved in a number of immunoregulatory pathways. It plays a central role in IgE regulation by triggering isotype switching from IgM to IgE and IgG4. Furthermore, it enhances the expression of surface molecules, such as the IL-4 receptor chain α, the low-affinity receptor for IgE and MHC class II. Moreover, IL-4 is necessary for the promotion of its own production and thus critical for the induction and maintenance of allergy. Taken together, these facts indicate that IL-4 is crucial for the development of various pathologic mechanisms important in allergic disease [13].

2. Materials and Methods

2.1 Patients and study design

A total of fourty four inpatients of children who have been infected with human metapneumovirus, the data-base of those patients has been registered in the present work, involving the patient's name, their age, gender as well as major clinical RTI symptoms, like the cough, fever, nasal discharges (i.e. rhinorrhea), sneezing, and asthma attacks which have been principally evaluated, by pediatricians consultant through taking main clinical characteristics of asthma, encompassing (dyspnea and wheezing), the selected patients' age has been 6 months to 12 years old of both genders, from Hospitals at Al najaf city between Dec. 2020 and Apr. 2021. Every patient's information has been obtained according to a questionnaire format. The inclusion criteria (Pediatric patients have respiratory tract illness, Wheezing, shortness of birth, and pediatric ages were 6 months to 12 years and Exclusion criteria (Immunocompromised, Diabebitic, severly ill child) and Control 50 pediatric controls were appearantly healthy.

2.2 Samples collections

The clinical specimens were blood sample, which are taken by add 1 ml trizol to 250 microliter blood, then the samples were transported by an ice bag from the hospital into the blood bank department for storage in -70˚C.

2.3 Gene expression study primers

The gene expression study was performed for the estimation of relative gene expression of some cykokines genes (IL-4, INF gamma, IL-10 and TGF-beta), which induced by HMPV infection and in only cDNA template of positive samples by using Real-Time PCR.

Table 1

primer	sequence		amplicon
Housekeeping gene (actin)	F	GGAGCGAGATCCCTCCAAAAT	197 bp
	R	GGCTGTTGTCATACTTCTCATGG	

IFN-Gamma	F	GGCCTCTACCACTAACTTCTCTC	117bp
	R	ACACTGCTGAATTGACAAGGTTT	
IL-4	F	TCTTTGCTGCCTCCAAGAACA	173bp
	R	GTTCCTGTCGAGCCGTTTCA	
IL-10	F	CCTCCGTCTGTGTGGTTTGAA	174bp
	R	CACTGCGGTAAGGTCATAGGA	
TGF beta	F	CGGCTACCTAGTCTACGCC	186bp
	R	AAGTCGCCGCCAATGTTGA	

2.4 Statistical Analysis

Statistical analysis for all data was done using Statistical Package of Social Sciences (SPSS), version 27, (Inc., Chicago, IL, USA) computer software. Statistical comparison between study groups analyzed using chi-square test and T test. $P<0.05$ was regarded as statistically significant (Al-Ukaelii and Al-Shaeb, 1998).

3. Result

This study encompassed 44 children positive with human metapneumovirus (HMPV). The recruited patients were suffered from respiratory infections with clinical manifestations like cough, shortness of breath, fever, rhinorrhea, and nasal congestion those routinely do visit the external clinics of Al zahraa hospital, Najaf City from September 2020 to April 2021.

3.1 Antiviral immune response among positive cases of HMPV

The profile of antiviral immune response in positive cases of human metapneumovirus was monitored in this study. The tested parameters of antiviral immune response were IL-10, TGF beta, IL-4, and INF-y as shown in Tables (2, 3, 4 and 5). For IL-10, degress gene expression of IL-10 were observed among positive cases infected with human metapneumovirus (HMPV) compared to the gene expression of IL-10 among control group as shown in Table 2.

Table (2): IL-10 expression among positive cases of HMPV

Groups	**IL-10 level mean± SD**
Control	1±0
Patients	0.0083±0.02
T test value	71.57*
P value	0

*Significant difference at $P<0.05$

Differently, the gene expression of IL-4 was significantly higher among patients when compared to its gene expression among the healthy individuals (control groups) (Table 3).

Table (3): IL-4 level among positive cases of HMPV

Groups	**IL-4 level mean± SD**
Control	1±0
Patients	2.78±1.16
T test value	1.536*
P value	0.045

*Significant difference at P<0.05

For IFN-y, a significant difference in the gene expression of INF-y was existed compared to that gene expression recorded among control group (Table 4).

Table (4): INF- γ level among positive cases of HMPV

Groups	**IFN-y mean± SD**
Control	1±0
Patients	2.38±0.60
T test value	2.293*
P value	0.032

*Significant difference at P<0.05

For TGF-β a significant difference in the gene expression of TGF-β was existed compared to that gene expression recorded among control group (Table 5).

Table (5): TGF- β expression among some positive cases of HMPV

Groups	**TGF-β mean± SD**

Control	1±0
Patients	4.481±1.53
T test value	2.270*
P value	0.038

*Significant difference at P<0.05

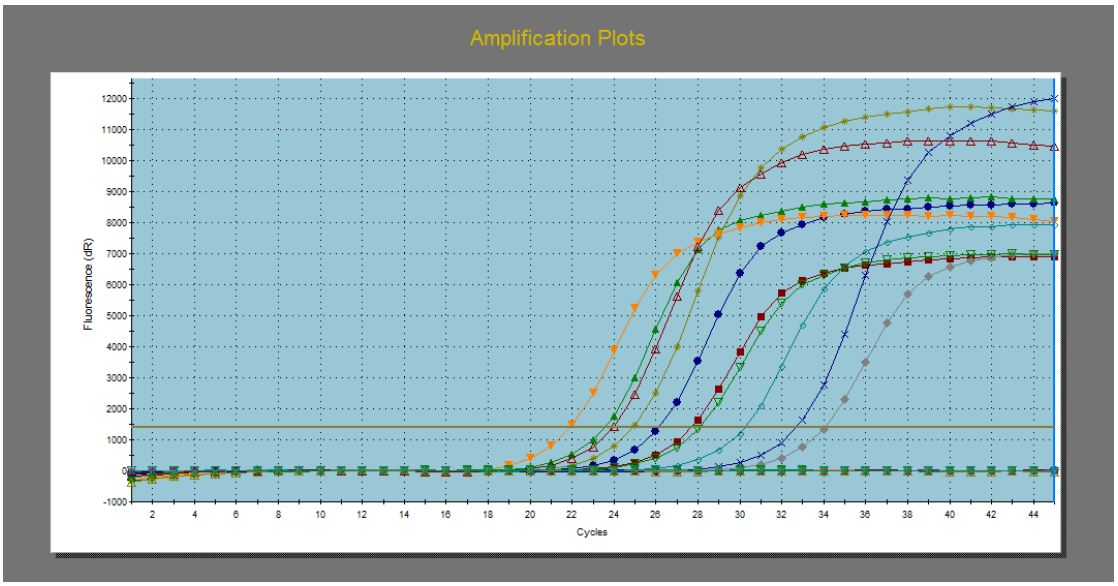

Fig S1: Amplification curves of RT-real time PCR experiment for estimating the four immune parameters responses among positive cases infected with HMPV and control group.

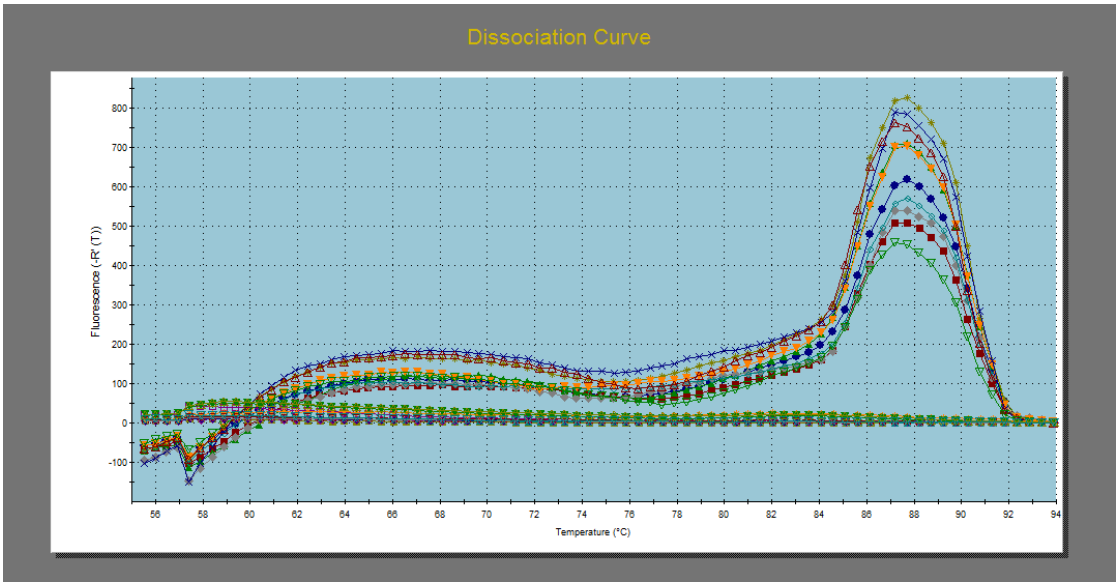

Fig S1: Melting curves of RT-real time PCR experiment for estimating the four immune parameters responses among positive cases infected with HMPV and control group.

4. Discussion

The present finding regarding the immune response in terms of INF- γ is in disagreement with that of Pencham and his co-workers who demonstrated that the infection with human metapneumovirus (HMPV) would trigger a defective immune response of INF-y among pre-mature children [14]. Guerrero-Plata, and co-workers reported that human metapneumovirus (HMPV) did induce both IL-1 and INF-y among children with positive infection with the virus [15]. Leitich and Mogaka reported induced levels of IL-4 in positive cases for human metapneumovirus (HMPV) with levels of 30.94, higher than those reported in the present study (2.78±1.16) as mentioned in (Table 3) [16]. The observed IL-4 hyper responsiveness might be evidenced of Th2 bias. Consequently, it is probable that IL-4 is supposed to play a major role in the pathogenesis of HMPV infection as opposed to the clearance of the virus from the body. Normally, CD8+ T cells (synonymously called cytotoxic lymphocytes (CTL)) do secrete IFN-γ as an antiviral response. Consequently, IFN-γ + CTLs did accumulated in the lungs of human metapneumovirus (HMPV) infected mice accompanied with higher levels of soluble IFN-γ in infected mice compared to those in uninfected mice (control) were also increased in the lungs compared to uninfected mice [17]. Interferons (IFNs) are probably the most comprehensively investigated antiviral factors produced as a response of the body due to the presence of viral infections. These cytokines can activate transcriptional programs in infected and non-infected cells, ultimately restricting viral replication [18]. IFN-γ could be either absent or minimally detected in human metapneumovirus (HMPV) infected children [19]. An elevation in the production of IFN-γ, IL-1β, IL-2, IL-4, and IL-6 was described in nasopharyngeal aspirates taken from children infected with human metapneumovirus (HMPV); this in line with what was previously recorded in other studies conducted on mouse model [20], [21]. For other types of interferons, Type I IFNs (IFN-α and IFN-β), were reported as key mediators in epithelial cells infected with human metapneumovirus (HMPV) [22]. Park and his co-workers evidenced increased levels of IFN-γ and IL-4 in children infected with human metapneumovirus (HMPV) compared to the control group [20].

Alvarez and Tripp reported increased interleukin-10 expression associated with persistent viral replication in the lungs of a BALB/c mouse model, infected with human metapneumovirus (HMPV) [23]. A controversy study stated that human metapneumovirus (HMPV) failed to induce traceable levels of IL-10 in the lungs of a BALB/c mouse model, infected with human metapneumovirus (HMPV) [15]. Other study revealed strong virus-driven IL-10 and INF-γ among children infected with human metapneumovirus (HMPV) [24]. Leitich and Mogaka reported level of IL-10 of 26.98 among children infected with human metapneumovirus (HMPV) [16]. The study of [25] stated that there were detected levels of TGF-β in bronchoalveolar lavage (BAL) of human metapneumovirus (HMPV) - or RSV-infected mice [24]. A higher level of TGF were reported among children patients suffered from either post-infectious bronchiolitis obliterans (PIBO) or acute bronchiolitis (848.9 (IQR 564.1–1210.3) pg/mL) when compared to the level of TGF among the control healthy group (601.3 (IQR 462.7–745.9) pg/mL) [26].

At most, the comparisons regarding the immune response parameters may be not justified yet as long as there are a discrepancy in the methods used to estimate the levels of IL-4, IL-10, TGF-β, and INF-y. Here in, these parameters were estimated on the transcriptional levels using quantitative real time –PCR, however, in most studies these parameters were estimated in the blood patients. Hence, method uniformity might result in other feedbacks.

5. Conclusion

The infection with human metapneumovirus (HMPV) triggered a distinct immune response in terms of Il-10, Il-4, TGF beta and INF-y. Degress gene expression of IL-10 was observed among positive cases infected with human metapneumovirus (HMPV), differenly, the genes expression of INF-y, TGF beta and IL-4 were significantly higher among positive patients.

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