Correlation between TARC and MDC gene expression and respiratory syncytial viruses in children.

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Abstract:

Background: Human respiratory syncytial virus(RSV) is the major etiological agent of respiratory tract illness particularly in children, and it provokes allergic and asthma exacerbation, so, the respiratory tract epithelial cell are stimulated and produce several chemokines such as MDC and TARC that play a major role in asthma attack.

Objective: To identify human genetic groups of RSV from children with respiratory tract infection and establish the relationship between RSV and allergy exaggeration through determination of the host gene expression (TARC and MDC) which are induced by RSV infection.

Methods: Patients suffering from respiratory tract illness (RTI), from several days to fourteen years old of both sexes were enrolled in this study. A nasopharyngeal swabs (NP) were taken from patients and subjected to molecular detection of RSV and gene expression of TARC and MDC were done for positive samples with RSV.

Results: Out of 230 children suffering from respiratory tract infection, 8 (8%) and 14 (14%) were detected with RSV type A and B respectively, and all epithelial cells of patients who have infected with RSV express TARC and MDC which are known to have an essential role in severity and hyper-responsiveness of allergy.

Conclusion: Measurement of host gene expression such as TARC and MDC revealed increased level of these genes in asthmatic patients, in children infected by RSV. Both genes may play an essential role in severity and hyper-responsiveness of allergy.

Key words: RSV, TARC, MDC, asthma

Introduction

Human respiratory syncytial virus (RSV) belongs pneumovirus genus, pneumovirinea subfamily, with paramyxoviridae and order of Mononegavirales, the

virion of RSV is made up of enveloped lipid with asymmetrical spherical shaped, 150 to 300 nm in measurement. Other morphology of virion that are 60-100 nm and up to 10 nm in length, filament-like, could be seen in both infected cultures and preparation of the virus(Brown, 2004). The viral envelope is a lipid bilayer obtained from the host plasmatic layer, Respiratory syncytial virus (RSV) is a ubiquitous infection that causes airway route aggravation, for example, laryngitis, croup and it likewise causes bronchitis.(1)(2). The major cause of bronchiolitis in worldwide is RSV, which form up to 70-80% of all other lower respiratory tract illness(3). Most children experienced RSV infection are symptomatic or have a notable clinical picture, comparable with other upper respiratory tract viral infections. The lower respiratory tract infection is more frequent, and the predominant sign is wheezing, the infants in the first three months of life, have relatively severe clinical signs(4).

RSV infection in early life is associated with the succeeding initial allergic airway illness (5). Animal experimental practice have added additional inspect to this relation, whether the infection with RSV, previously, during, or after taking allergen trial being displayed to modify allergic signs (6).

Chemokines can be categorized into four distinguishable categories: three types of essential (heparin-binding) proteins, termed as C, CC, and CXC. (the number and spacing of NH_2-terminal cysteine residues are the main determinants), and a fourth group, the CX3C chemokines, built of a substantial, glycoproteins linked through a COOH-mucin-like area bound to the membrane. Those receptors for the chemokines are express Edina cell sort limited design has permitted specificity in chemokine activity—for instance, individuals from the C group essentially stimulate lymphocyte chemotaxis, individuals from the CXC cluster affect neutrophil chemotaxis, and the CC group revitalizes monocyte, lymphocyte, and eosinophil chemotaxis(7)(8).

There are several CC chemokines network expression can be induced RSV infection, for instance, thymus and activation regulated chemokine (TARC), RANTES, MCP-1, macrophage-derived chemokine (MDC) and (MIP-1a and 1b), CXC, CX3C. However, TARC belongs to CC type of chemokines which shows chemotaxis for immature dendritic cells and naïve CD4 T cells (9). The receptor CCR4 can be allowed to bind with CCL17/TARC and CCL22/ MDC. The CCR4 is expressed on several a subpopulation of peripheral blood such as mature dendritic cells, lymphocytes, thymocytes and on blood platelets(10). The CCR4 expression

associated with TH2 responses. Raised CCR4 levels can proceed up two days after induced by T-cell receptor(11). Macrophage-derived chemokine (MDC/ CCL22) made up of 69 amino acid residues, synthesized by macrophage cells linage(12). other cells are consider as a source of MDC production including monocyte-derived dendritic cells, natural killer (NK) cells and bronchial epithelial cells(13), the MDC is clustered on chromosome 16q13, it contain of the 4 cysteine motif and highly conserved residues characteristic of CC chemokines, it is identity with other human chemokines such as TARC by less than 35%, and the later, TARC, is closed human comparative(14)(15).

Thymus-and activation-regulated chemokines (TARC) is a highly specific ligand of CCR4, and it is synthesized and produced by several cells including monocytes, dendritic cells and it as a serve assist recruitment, activation and development of Th2-polarized cells for CCR4 expression(16)(17).

The aim of present study was to identify human genetic groups of RSV from children with respiratory tract infection and establish the relationship between RSV and allergy exaggeration through determination of the host gene expression (TARC and MDC) which are induced by RSV infection.

Materials and methods

Data sets

Two Hundred and thirty children patients were randomly chosen, clinically, all patients were suffering from respiratory tract illness (RTI), the database of them were registered in this study, which encompass, name of the patient , age; gender and the major clinical symptoms of RTI, such as fever, cough, sneezing, nasal discharge (rhinorrhea),and asthma attach which evaluated, principally, by the consultant pediatricians via take the major clinical features of asthma which involved (wheezing and dyspnea), the age of elected patients was from several days to fourteen years old of both sexes.

Primers and Probes designing

The primers and probe were designed in this study by using the complete sequence of Nucleoprotein gene RSV-A (GenBank: KF973340.1), RSV-B (GenBank:

KF893260.1), and hMPV (GenBank: KF891365.1) from NCBI-GenBank and Primer3 plus design. The primers were provided by (Bioneer/Korea) (table 1.)

Table 1: Primers and probes used in this study

Primer	Sequence		Amplicon
RSV-A primer	F	5′-TGCAGGGCAAGTGATGTTAC-3′	86bp
	R	5′-TTTCTGCTTGCACACTAGCG-3′	
RSV-A probe	5′-VIC-GGTGGGGAGTCTTAGCAAAATCAGTT-BHQ-1		
RSV-B primer	F	5′-TGTGCACTTTGGCATTGCAC-3′	101bp
	R	5′-TTACTTGCCCTGAACCATAGGC-3′	
RSV-B probe	NED-TCCACAAGAGGGGGTAGTAGAGTTGA-BHQ-1		
hMPV primer	F	5′-AGAAACTCAGGCAGTGAAGTCC-3′	130bp
	R	5′-TCTCTTCCACCCAGCTTTTCTC-3′	
hMPV probe	FAM-ACCAGAACGTACTCCTTGGGGAA-BHQ1		

F: Forward, **R**: Reverse

Gene expression study primers

The gene expression primers of chemokines gene, which were measured from the airway epithelial cells that shaded with the NP swab, and hMPV genes were designed in this study by using NCBI-Genbank and Primer3 plus design Genbank codes: reference Actin gene (NM-001101.3), chemokine MDC gene (U83171.1), chemokine TARC gene (XM-011523256.1), M2-2 protein gene hMPV (AY530095.1), and G-protein gene hMPV (JQ309682.1), and these primers were provided by (Bioneer/ Korea), as in table 2.

Table 2: **The MDC, TARC G glycoprotein, M2-2, and Actin primers**

Primer	Sequence		Amplicon
Actin	F	5′-TCGTGCGTGACATTAAGGAG-3′	133bp
	R	5′-TTGCCAATGGTGATGACCTG-3′	
MDC	F	5′-TGTGAAGCCCCAAATTTGCC-3′	124bp
	R	5′-AAGCCAAGACCACACCATTG-3′	
TARC	F	5′-TGGGGCAATGTCAATGTTGG-3′	125bp
	R	5′-AGTTCTGTGTACCCAGCCAAG-3′	

F: Forward**, R:** Reverse

Viral RNA Extraction

Viral RNA was extracted from frozen nasopharyngeal swabs samples by using AccuZol™ Total RNA extraction kit (Bioneer, Korea). Manufacturer instructions were followed.

The total extracted RNA was assessed by a Nanodrop spectrophotometer Two measurements were carried out; namely, Purity and quantity of the extracted RNA by spectrophotometer at 260 nm and by Nanodrop at 280 nm (THERMO. USA).

Reverse Transcription cDNA synthesis step

Complimentary DNA was synthesized from extracred RNA samples by using the commercial kit "AccuPower® RocketScript RT PreMix" from Bioneer, Korea according to manufacturer instructions .

Real-Time PCR (qPCR)

The qPCR was performed for detection of (RSV-A) and (RSV-B) and Human metapneumovirus (hMPV) based on nucleoprotein gene and this technique was

carried out according to method described by Dola *et al*., (18) as in the following steps:

Reverse Transcription Real-Time PCR (RT-PCR)

One step RT-qPCR was performed for detection of respiratory syncytial virus type A (RSV-A), respiratory syncytial virus type B (RSV-B) and Human metapneumonia virus (hMPV) based on nucleoprotein gene and this technique was carried out according to method pronounced inGoTaq® 1-Step RT-qPCR manual technique System.

Real-Time PCR master mix:

The master mix was prepared by using one step Reverse Transcription and Real-Time PCR detection kit (AccuPower RocketScriptRT-qPCR PreMix, Bioneer. Korea), and done according to the company instructions .

The RT-PCR master mix reaction components were added into RT-PCR tube containing (8 wells strips tubes which containing RocketScript reverse transcriptase and TaqMan probe premix). Then all strips tubes vortexed and centrifuged for 3000rpm for 3 minutes in vortex centrifuge and transferred into the thermocycler.

Conditions of Real-Time PCR Thermocycler:

Real-Time PCR thermocycler conditions were set based on Instructions of the RT-PCR TaqMan kit and primer toughening temperature .

The RT-PCR thermocycler conditions were 50 °C, 15 min for one cycle in Reverse transcriptase step and for Pre-Denaturation step were 95 °C, 5 min for one cycle.

Real-Time PCR Thermocycler conditions

Real-Time PCR thermocycler condition was set according to primer annealing temperature and qPCR TaqMan kit instructions. The qPCR thermocycler conditions were 95 °C 5 min, 1 cycle for Pre-Denaturation step; 95 °C 20 sec for denaturation; 60 °C 30 sec for Annealing/Extension step and Detection (Scan) for 50 cycles.

Data analysis of qRT-PCR

The relative quantification method described by Livak and Schmittgen (19) were used to analyze the data results of qRT-PCR for housekeeping and target genes. The process of normalization of the obtained quantities from the test is necessary for the data to become biologically meaningful. The control sample is considered the calibrator to define the relative expression level.

Analysis of data The Real-Time PCR:

The threshold number of cycles (CT value) presented the positive amplification in Real-Time PCR cycles number was calculated.

Results and discussion:

The following data involved the display certain chemokines (CC) which induced by RSV and their role in pulmonary diseases such as allergy and asthma exaggeration, these CC are represented with thymus-and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), each those CC is expressed on bronchial epithelial cells and alveolar macrophage, they have common receptor which is chemokines receptors-4 (CCR4) that selectively expressed on Th2 cells which play a crucial role in asthma development. Whatever, the TRAC and MDC are also elevated in other chronic diseases such as atherosclerosis, Crohn's disease, and cigarette smoke-induced acute and chronic airway inflammation(20).

Interpretation of results of RSV-A&B TARC and MDC gene expression

In the below tables show the expression level of TRAC and MDC gene, which induced by both types of RSV (A and B), all these tables of positive RSV (A and B) have shown a high level fold change expression, and compared to the negative-RSV-A patients which represented in figure (1), there is a significantly different, the p-value is (0.0001), it is detonating RSV-A, B have induced the infected bronchial epithelial cells and alveolar macrophage to produce TRAC and MDC that ligand the specific receptor on the Th2, CCR4,. Th2 cells have an essential role in allergic diseases like asthma and allergic rhinitis(21)(22). Several studies have convinced the association between RSV and asthma development, as the study that worked by Zhang *et al.*, (23), they have concluded through their experiment, that RSV has a potent inducer to produce several chemokines such as TARC, RANTES, MCP-1, and MDC, and then they explained their result which encompassed the importance

of TARC and MDC in asthma development and exaggeration, TARC an effective chemotactic agent for CD_4^+ T lymphocytes, RSV-infected A549 cells can be induced for TARC production which has implicated in atopic asthma in human. In addition, other studies such as that achieved by(24), they proved RSV induce Th2 cytokines such as IL-4 and IL-13 that improve production of TRAC; also they observed the IL-4 alone did produce very small amount of TRAC mRNA. In one study that done by Takeuchi *et al*., (25), they have determined that TARC is a considering target for immunotherapy (IT) which has been observed the reduction amount of TRAC in patients who have suffered from allergic rhinitis after IT demonstration. MDC is another potent chemotactic, it has the capability to recruit Th2 lymphocytes, monocytes, immature DC, and IL-2-activated NK cells(26) . MDC is, as well, recognized in normal bronchial epithelial cells in human atopic asthma (27). The study like that done by Gonzalo *et al*., (28) they have reported role of MDC which is essential in allergic pulmonary inflammation experimentally induced by in mice, MDC is neutralized by antibodies that lead to block acidophil recruitment. Furthermore, in original article that published by Egypt Journal Pediatric Allergy in 2005 which have been done by Mohammad H. Ezzat and Kareem Y., they have measured MDC (CCL22) and its receptor CCR4 on peripheral blood T lymphocytes of asthmatic children, they founded the level of MDC was significantly higher in asthmatic children(29). However, the MDC might recruit Th2 lymphocytes into airway of asthmatic case, Th2 lymphocytes intensify the allergic inflammatory reaction through IL-4, IL-5 and IL-13 production which provoke the B lymphocytes into plasma cells which responsible for IgE production, moreover the IL-5 stimulate eosinophils for activation and differentiation(30)(31).

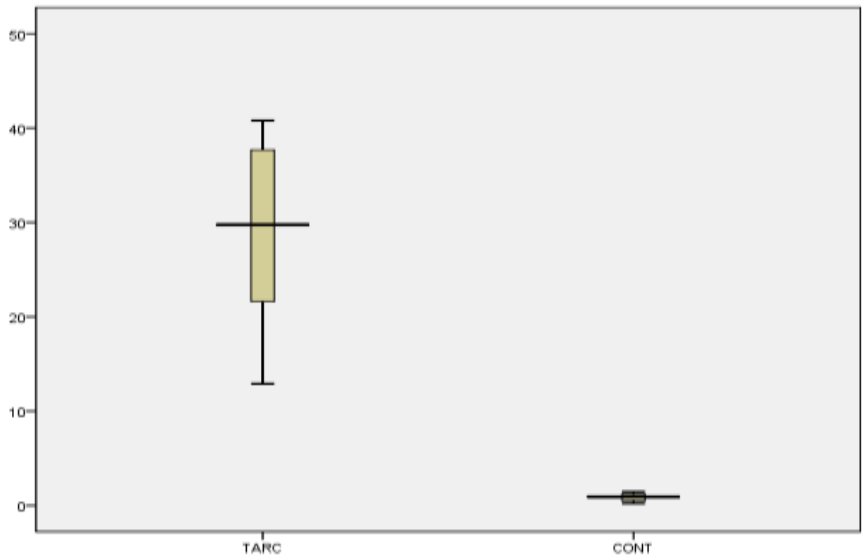


Figure 1: Boxplot shows comparison between RSV-A-induced TARC with control (RSV-A negative).

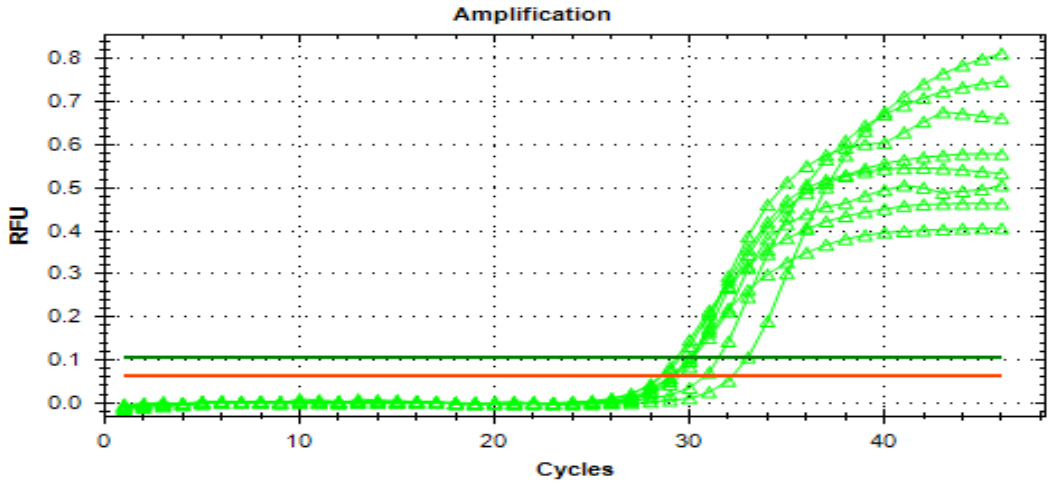


Figure 2: Amplification plot of RT-PCR for TARC gene in (RSV-A) (positive samples).

Figure (2) shows the expression level of TRAC gene, which induced by RSV-A that ranging from the high level 40.786 to lower level 19.160 folds.

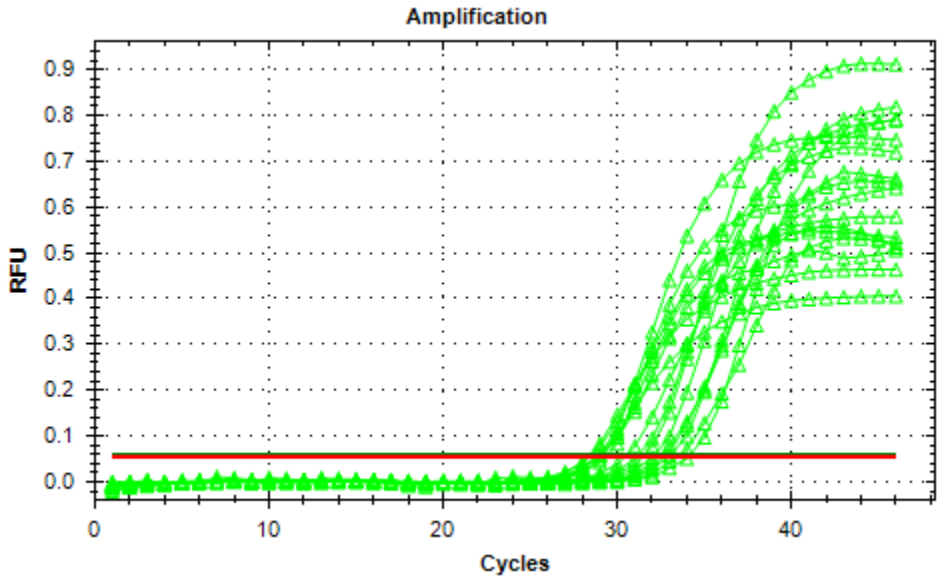


Figure 3: Real-Time PCR amplification plot for TARC gene in (RSV-B) positive patient sample

Figure (3) shows the expression level of TRAC gene, which induced by RSV-B that ranging from the high level 64.000 to lower level 9.646 folds.

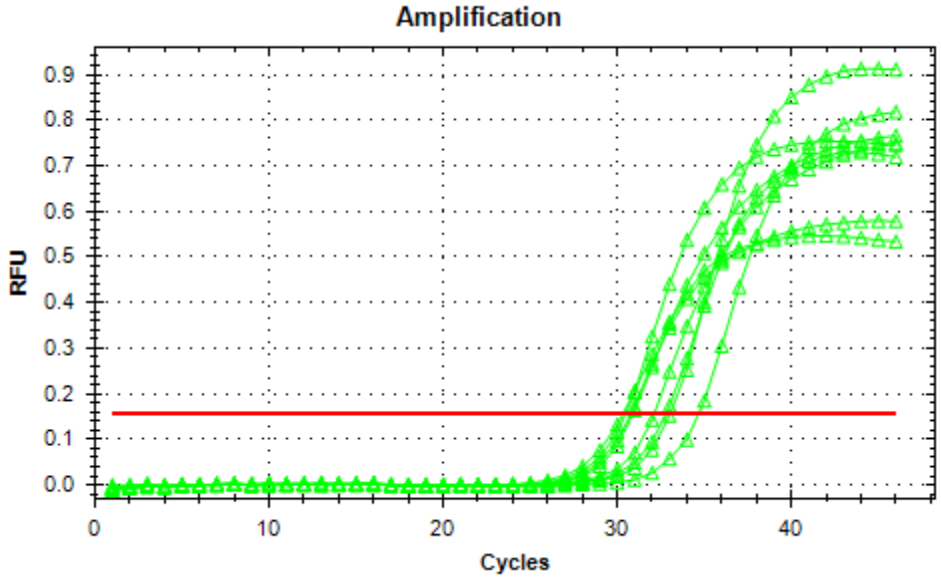
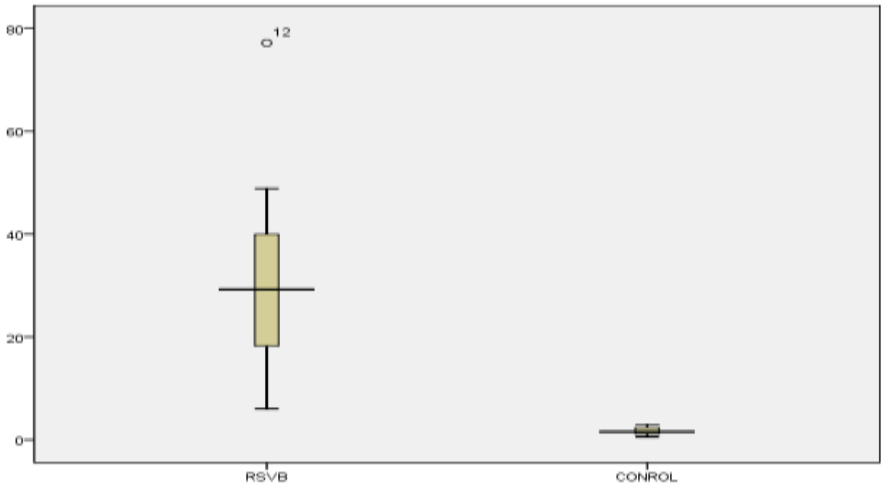


Figure 4: Real-Time PCR amplification plot for MDC gene in (RSV-A) positive samples.

Figure 4 shows the expression level of MDC gene, which induced by RSV-A that ranging from the high level 37.792 to lower level 7.727folds.



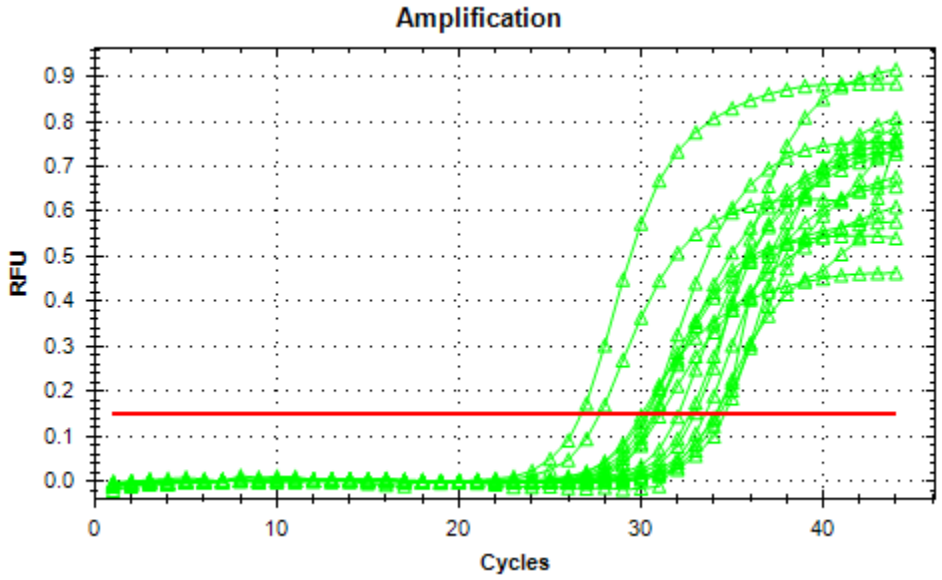


Figure 5: Real-Time PCR amplification plot for MDC gene in (RSV-B) positive samples

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