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Research Article
Bioinformatics: Human Gene Expression Profile after Respiratory Syncytial Virus Infection

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Abstract

Background and Objective: Respiratory syncytial virus infects human (infant, young children, elderly and immunocompromised patients) all over the world and causes respiratory infections usually end up with severe complications such as asthma, bronchiolitis, pneumonia. The objective is to detect the rate of human immune gene expression after RSV infection. **Materials and Methods:** Real time PCR array analysis is a high sensitive and specific assay used to study the effect of RSV infection on the rate of gene expression of human immune-inflammatory genes in 24 healthy persons. About 5 mL blood samples were taken and messenger RNAs of white blood cells were extracted. **Results:** Eighteen immune inflammatory gene (CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCR1, CCR2, CCR3, CXCL10, CXCL11, CXCL2, CXCL5, IL-1α, IL-1β, IL5, IL-8 and TNF-α) were upregulated after RSV infection. The rate of upregulation ranged between 2.62 fold change (FC) for CCL2 (the lowest upregulated gene) and 63.76 FC for each of CXCL10, CXCL5 and IL-1α as the most upregulated genes. **Conclusion:** The RSV causes impact on gene expression of human cells through interfering with key immune pathways which may lead to severe complication such as asthma and cytokine storm.

Key words: RSV, RT-PCR array, gene expression, gene ontology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Respiratory syncytial virus (RSV) is a negative-sense single-stranded RNA genome, belongs to the pneumovirus of Paramyxoviridae family[1]. The RSV causes serious infections such as bronchiolitis, pneumonia, airway hyperactivity and asthma (in immunocompromised persons), infants and the elderly[2]. The RSV infects main immune cells represented by respiratory epithelium, myeloid dendritic cells, plasmacytoid dendritic cells, neutrophils and monocytes[3]. These targeted immune cells lead to modulate immune system through driving a number of actions such as changing the normal stimulation of immune cells[4]. Overcome the function of professional antigen-presenting cells. Activate immune-inflammatory signal transduction pathway which lead to stimulate the production of various cytokines usually end up with asthma or/and cytokine storm[5]. Studying the effect of RSV on rate of gene expression of human inflammatory cytokines is a new scientific aspect lead to detect the complete profile of affected immune genes after RSV infection[6]. In this study two new technique (Real time PCR and Microarray) were combined to be as RT-PCR-Array which used to detect the gene expression of biological genes line (immune-inflammatory gene line) in human cells.

RT-PCR-Array technique is a sensitive and specific technique used to study the immune cells genomic response to viral infections[7]. This technique provide accurate method to determine the pattern of changes in gene expression in immune cells after viral infections[7]. In addition it helps to gathered accurate data on immune cell's gene regulation[7]. The aims of this study is to determine the effect of RSV infections on the rate of transcripts (mRNA) expression of upregulated and downregulated inflammatory cytokines genes. Such study may lead to provide more interpretations of the impact of RSV on the gene level of human immune inflammatory gene expression especially those associated with asthma and cytokine storm.

MATERIALS AND METHODS

Bioinformatics data provide important information about any scientific aspect which may need correct using of it to get new results through connection between bioinformatics and experimental data inside research laboratories. This study concerns such aspect through gathering the results of most performed researches on RSV infections and its impact on human immune genes. Affected genes from bioinformatics data gathered and arranged to be as acute samples[8-12] (Table 1). Acute samples then will normalized with internal control genes represented with five housekeeping genes (Beta-2-microglobulin, Hypoxanthine phosphoribosyl transferase 1, Ribosomal protein L13a, Glyceraldehyde-3-phosphate dehydrogenase and Actin-beta) [13]. From the fold change of acute genes, Ct values were calculated from standard gene tables[13] (Table 1).

Control samples: This study was performed in microbiology laboratory, Department of Biology, College of Science, University of Mosul, Mosul, Iraq from September 15, 2018 to May 20, 2019. Acute samples also normalized with normal (control) genes taken from healthy parsons. From 24 healthy persons 5 mL blood sample is taken and the white blood cells

Table 1: Affected genes after infection with RSV as acute samples

Gene name	Gene symbol	Fold change	Ct control	Ct acute
Chemokine (C-C motif) ligand 1	CCL1	3.7	24.8	21.9
Chemokine (C-C motif) ligand 2	CCL2	2.0	24.9	23.6
Chemokine (C-C motif) ligand 3	CCL3	5.3	32.6	28.7
Chemokine (C-C motif) ligand 4	CCL4	4.0	27.0	21.3
Chemokine (C-C motif) ligand 5	CCL5	6.0	30.5	27.8
Chemokine (C-C motif) ligand 7	CCL7	2.0	28.1	22.5
Chemokine (C-C motif) receptor 1	CCR1	3.0	31.3	28.0
Chemokine (C-C motif) receptor 2	CCR2	3.0	29.1	24.6
Chemokine (C-C motif) receptor 3	CCR3	3.0	25.9	22.9
Chemokine (C-X-C motif) ligand 10	CXCL10	2.0	27.7	21.8
Chemokine (C-X-C motif) ligand 11	CXCL11	2.0	31.6	28.9
Chemokine (C-X-C motif) ligand 2	CXCL2	2.9	25.0	22.4
Chemokine (C-X-C motif) ligand 5	CXCL5	5.0	26.9	21.0
Interleukin 1, alpha	IL-1α	4.0	29.6	23.7
Interleukin 1, beta	IL-1β	1.8	28.8	24.4
Interleukin 5 (colony-stimulating factor, eosinophil)	IL5	4.2	32.6	28.8
Interleukin 8	IL-8	2.7	31.1	28.0
Tumor necrosis factor alpha	TNF-α	5.2	34.2	30.1

were isolated to extract their RNA using blood RNA kits (Invitrogen, USA). The amount of RNAs samples were determined using spectrophotometer analysis with optical density (O.D) at λ_{260}. Purity of RNA Samples to proteins ($O.D_{260/280}$ ratio) were checked (pure RNA is between 1.7 - 2.0)[13].

Gene expression data analysis: The Ct values of acute and control samples were analyzed using Real-time-reverse transcription PCR-array (RT-PCR-array) analysis (SABioscience) to get the upregulated and downregulated genes after infection with RSV. Between 0.5 or 1.0 µg of total RNA for 96 well plate formats was used for every sample[13]. The contents are mixed and followed by brief centrifugation. The mixture is incubated at 42°C for 5 min and immediately chilled on ice for at least 1 min. From RNAs samples The cDNAs were prepared and added to RT-qPCR Master Mix (Master mixes contain SYBER Green and reference dye). Then the master mixtures were used to prepare the experimental mixtures (cDNA and RT-qPCR Master Mix) as 25 µL to each well of the PCR-array) which is aliquot across the PCR-arrays (SAbioscince). Place the plate in real-time thermal cycler (Bio-Rad-iCycler) and programed (95°C, 1 min,1 cycle; 95°C, 15 sec, 40 cyc le; 60°C, 1 min)[13]. The threshold cycle (Ct-value) is calculated for each well using the instrument's software analysis (Blank Excel Spread-sheet SABioscience Data Analysis Template Excel File)[13].

RESULTS

The expression of eighteen immune inflammatory genes (CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCR1, CCR2, CCR3, CXCL10, CXCL11, CXCL2, CXCL5, IL-1α, IL-1β, IL5, IL-8 and TNF-α) were upregulated after RSV infection. The rate of upregulation ranged between 2.62 fold change (FC) for CCL2 (the lowest upregulated gene) and 63.76 FC for each of CXCL10, CXCL5 and IL-1α (the most upregulated genes). Each gene has its specific position on the RT-PCR- Array represented with abbreviated symbol, threshold value (Ct value) and new fold change after normalization (Table 2).

The upregulation in expression of all genes were scattered randomly and reflects the random effect of RSV against different immune genes(Fig. 1). There variations may belong to different factors such as; type and function of gene, ability of virus to control the gene and the location of gene. The affected genes (upregulated genes) after RSV infection are represented by 2 groups, 1st group include 9 genes classified as 5 chemokines genes (CXCL10, CXCL11, CXCL2, CXCL5, CXCL8), 3 Interleukins (IL1A, IL1B and IL5) and TNF (Fig. 2). The 2nd group (9 genes) includes 6 cytokines (CCL1, CCL2, CCL3, CCL4, CCL5 and CCL7) and three cytokine receptors (CCR1, CCR2 and CCR3). The control genes (housekeeping genes) upregulated with only 1 fold of gene expression (its Ct value = 1 fold) (Fig. 3).

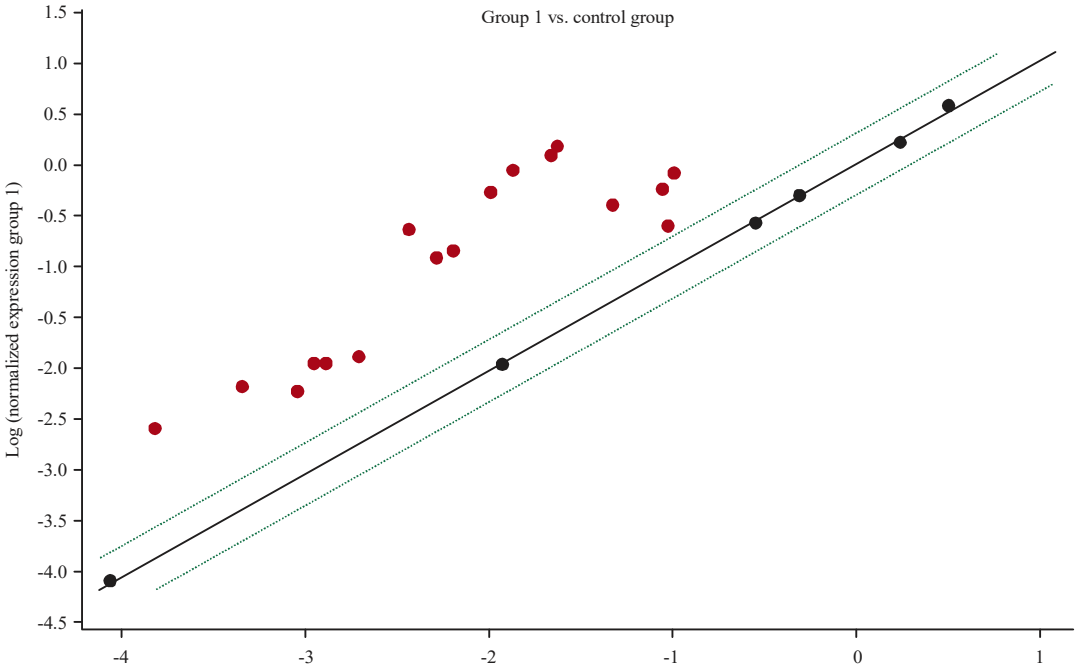

Fig. 1: Scatter plot of upregulated immune inflammatory genes

Log (normalization expression control group), Red color: Upregulated genes, Black color: Unchanged gene, Green color: Downregulated genes

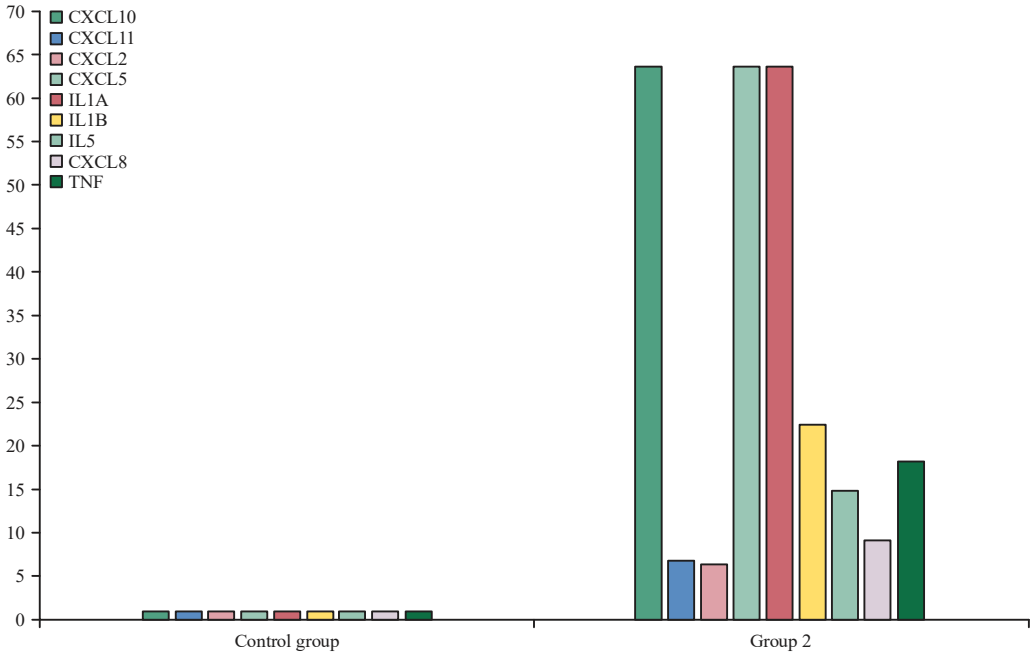

Fig. 2: Most high upregulated immune genes after RSV infection

Table 2: Position of genes on array, gene symbol, Ct values (control and acute), new fold change after normalization

Position on array	Gene symbol	Ct control	Ct acute	New fold change
A04	CCL1	24.8	21.9	7.97
A10	CCL2	24.9	23.6	2.62
B04	CCL3	32.6	28.7	15.94
B05	CCL4	27.0	21.3	55.50
B06	CCL5	30.5	27.8	6.93
B07	CCL7	28.1	22.5	51.78
B09	CCR1	31.3	28.0	10.51
B10	CCR2	29.1	24.6	24.16
B11	CCR3	25.9	22.9	8.54
C11	CXCL10	27.7	21.8	63.76
C12	CXCL11	31.6	28.9	6.93
D03	CXCL2	25.0	22.4	6.47
D05	CXCL5	26.9	21.0	63.76
E09	IL-1α	29.6	23.7	63.76
E10	IL-1β	28.8	24.4	22.54
F05	IL5	32.6	28.8	14.87
F08	IL-8	31.1	28.0	9.15
G05	TNF-α	34.2	30.1	18.31

DISCUSSION

The results showed high impact of RSV infection on human immune gene expression. The RSV stimulates expression of 18 immune genes (CXCL10, CXCL11, CXCL2, CXCL5, CXCL8, IL1A, IL1B , IL5, TNF, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCR1, CCR2 and CCR3) with different fold change (FC) (2.62 FC for CCL2 and 63.76 FC for CXCL10, CXCL5 and IL-1α) (Fig. 2 and 3).

The upregulated chemokines genes (CXCL10, CXCL5) and interleukin 1 alph (IL-1α) showed high expression rate (63.76 FC) for each one after infection with RSV (Fig. 2). These genes function as pleiotropic effects including stimulation of monocytes, natural killer and T-cell migration[14-15]. Resent study recorded increasing of CXCL5 after RSV infection and CXCL10 was secreted from the airway epithelium following RSV infection[16]. Other studies recorded low upregulation of these genes (between 2-6 FC) after RSV infection[1,9]. The CCL4 and CCL7 were upregulated with high rate (55.50 and 51.78 FC respectively) (Fig. 3). They function as chemokinetic and inflammatory genes against viral infections and attract macrophages during inflammations[5]. Another study showed

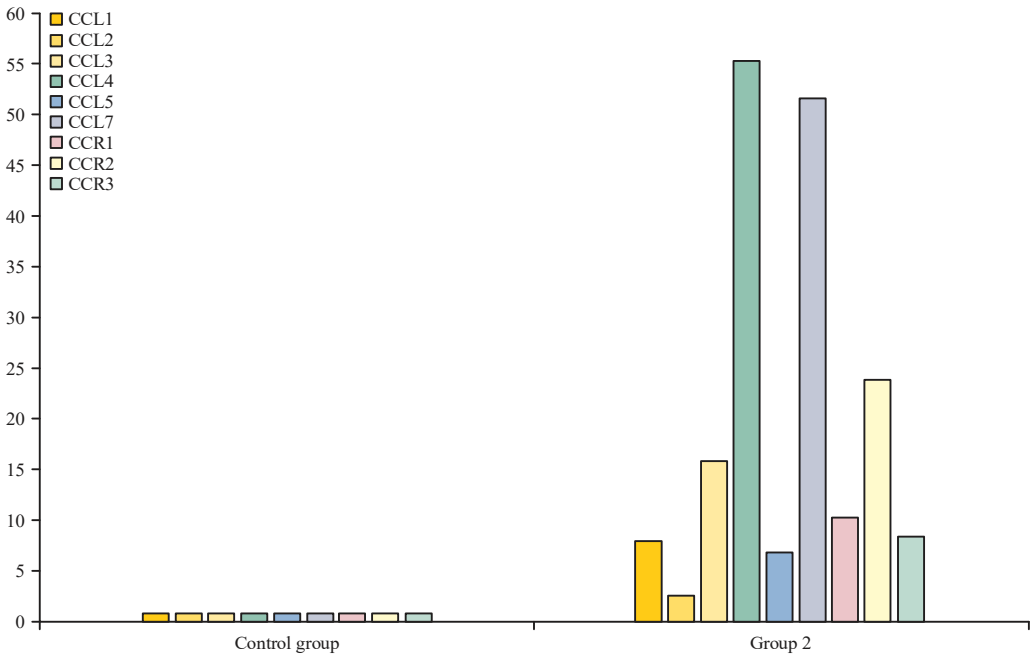

Fig. 3: Upregulated genes with low, moderate and high rate after infection with RSV

moderate increasing in gene expression of CCL4 after RSV infection in mast cells[1] whereas CCL7 increased in low rate after infection with RSV[9].

The others eight cytokines genes (CCR3, IL-8, CCR1, CCL3, IL5, TNF, IL-1β and CCR2) which upregulated with moderate rate of expression ranged between 8.54 FC for CCR3 and 24.16 FC for CCR2 (Fig. 2 and 3). Three genes (IL1, IL5, IL8) were recorded in previous study as upregulated genes that increased during RSV infection and lead to cause asthma complication[17]. In general their functions are mediating cellular process, biological adhesion, response to stimulus and molecular transducer activity[18-20]. Another study showed increasing in the expression of CCR1, CCR2 and CCR3 genes (upregulated) in epithelial cells monocytes after RSV infections[10]. Another study recorded that the infection with RSV stimulate increasing in mRNA of IL-8 in a bronchial epithelial cell[21]. For TNF and IL-1β previous study showed significant increasing in pulmonary epithelial cells when infected with RSV[11].

The lower upregulated genes (CCL2, CXCL2, CCL5, CXCL11 and CCL1) showed low rate of expression ranged between 2.62 FC for CCL2 and 7.97 FC for CCL1 (Fig. 2 and 3). The general functions of these genes are mediating immune system process, response to stimulus and biological regulation[22-24]. Previous study recorded similar results with increasing rate of gene expression as 5, 5, 47.3, 3.9 and 2.9 FC for each of CCL5, CCL1, CXCL2 and CCL2, respectively[8,25].

The results showed that each immune gene was upregulated (after infection with RSV) with various rate of expression. This variations in gene expression may belong to interactions with different factors such as variation in blood sampling time, the state of immunity in different patients, load of virus in blood, type of virus (virulence, template), route of infection and presence of other non-viral diseases. To overcome all these variations this study subjected all acute genes to two standard controls. First control was normalizing acute genes with internal housekeeping genes (genes that produce mRNA in fixed rate of fold change through host life). Second control was the normalizing of acute genes with normal genes (patients not infected with respiratory diseases). This double normalizations may reduce these variations and give more accurate and new results. Future recommendation include studying the correlation between the upregulated genes and the functions of their proteins (Proteomic study) which may be used directly as immune proteins against RSV.

Gene ontology: Through gene ontology analysis all 18 genes play important roles in key immune inflammatory pathways such as asthma pathway, Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway and NOD-like receptor signaling pathway.

CONCLUSION

Results showed that RSV infections stimulate upregulation in the rat of gene expression in different immune inflammatory human genes. The upregulated genes play key

roles in main immune pathways which may result in severe complications such as asthma and cytokine storm. These results may improve our knowledge about relationship between RSV infection and host cell at molecular level.

SIGNIFICANCE STATEMENT

This study discover the ability of RSV to cause significant impact on patients through stimulation (upregulation) of 18 key human inflammatory genes that can be beneficial to know the complete human immune gene expression profile through RSV infection. This study will help the researcher to uncover the profile of affected genes (18 immune inflammatory genes) and connect it with key immune pathways especially asthma pathway after RSV infection. In addition it will allow easy connection between RSV infection and genomic-proteomic field) that many researchers were not able to explore.

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