Evaluation of the peripheral blood T and B cell subsets and IRF-7 variants in adult patients with severe influenza virus infection

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Abstract

Background and Aims: Influenza virus is one of the leading infections causing death among human beings. Despite known risks, primary immune deficiency due to Interferon Regulatory Factor-7 (IRF7) gene defect was reported as a possible cause of the risk factors for complicated influenza. We aimed to investigate the changes in peripheral T and B cell subsets in adult patients with severe seasonal influenza virus infection and the investigation of variants of IRF7 gene.

Methods: In this study, 32 patients, hospitalized due to influenza infection-related acute respiratory failure were included.

Results: The median age of the patients was 76 years (26-96), and 13/32 (40.6%) were in the intensive care unit. Central memory Th, effector memory Th, TEMRA Th, cytotoxic T lymphocytes (CTL), central memory CTL of the patients were found to be increased, naive CTL were decreased. There was a significant increase in the percentage of effector memory Th, and a decrease in the percentage of naive CTL in patients ≥65 years-old compared to patients <65 years old (P = .039, and P = .017, respectively). IRF7 gene analysis revealed two different nucleotide changes in three patients; c.535 A > G; p.Lys179Glu (K179E) and c584A > T; p.His195Leu (H195L), located in the fourth exon of the IRF7 gene.

Discussion: The increases in central and effector memory Th, central memory CTL and decrease of naive CTLs may be secondary to the virus infection. K179E (rs1061502) and H195L (rs139709725) variants were not reported to be related with susceptibility to an infection yet. It is conceivable to investigate for novel variants in other genes related to antiviral immunity.

Keywords: interferon regulatory factor 7, respiratory failure, severe influenza virus infection
INTRODUCTION

Influenza virus infection will continue to be a problem today and in the future, as in the past, because a persistent and effective prevention method could not be developed due to the frequently changing nature of the virus. Seasonal influenza infection affects 3 to 5 million individuals, and responsible for up to 650 000 deaths annually worldwide.1 Clinical presentation ranges from mild to severe and even to death in patients with high risk. It is well defined in the literature that especially young children, elderly people, and patients with chronic diseases are susceptible to infection and severe complications.2 However, healthy adults may also experience severe influenza infections, complications, and mostly developed complication is pneumonia and related respiratory failure causing death.2

Influenza virus activates all components of the immune system. Upper respiratory tract epithelial cells and secretory immunoglobulin A are the first fighters of the innate immune system against influenza viruses. Pathogen associated molecular patterns (PAMPs) of the influenza virus were recognized by pattern recognition receptors (PRR) located on or in the innate immune system cells (retinoic acid inducible gene-1 protein, [RIG-1], Toll-like receptors [TLR3, TLR7, TLR8]) that activate transcription of genes encoding interferons type I (IFN-α, IFN-β), and III (IFN-λ), and proinflammatory cytokines (interleukin 6 and 1 β) were activated. Type I and III IFNs are important in antiviral immunity both in already influenza virus-infected and non-infected cells against the virus infection.6-8 After the overexpression of IFN genes, IFN-α, IFN-β, and IFN-λ were secreted outside the cells, and attached to the IFNα/β receptors and IFN ligand receptors on the cell surfaces. After the binding of the IFNs to the related receptors, Janus-kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway was activated. Phosphorylated STAT1 and STAT2 bind to IRF9, then interferon-stimulated gene factor 3 (ISGF3) complex is generated. ISGF3 translocates into the nucleus and binds to the IFN-stimulated response element (ISRE) resulting in transcription of various IFN-stimulated genes. Myxovirus resistance genes family (Mx), interferon-induced transmembrane protein family, cholesterol 25-hydroxylase and TRIM, and various other ISGs are important in the inhibition of viral attachment, entering the cells, viral transcription, replication, and shedding.

Ciancanelli et al defined a loss of function mutation in the interferon regulator factor 7 (IRF7) gene in a child and his family members, all experienced only severe influenza infection reminding susceptibility to influenza virus in 2015.9 They reported compound heterozygous mutations -p.Phe410Val(F410V) and p.Gln421X(Q421X)- and showed that in fibroblast type I IFN, in plasmacytoid dendritic cells (pDC) type I and III IFN generation were affected and resulted in increase of influenza A virus replication. They also showed that IFN generation diminished in airway epithelial cells causing faster and increased viral shedding.9 The defined IRF7 mutation was accepted as the first single monogenic disease among primary immunodeficiencies, which makes the patient susceptible to influenza virus and has taken its place in the classification of primary immunodeficiencies.10

After antigen presentation of antigen presenting cell (APC) to the CD4 and CD8+ T lymphocytes via major histocompatibility complex (MHC) I and II in the regional lymph nodes, mature T lymphocytes gain effector cell function and migrate to the infection site, and show antiviral activity. Mature CD4+ T lymphocytes help differentiation of antigen-specific cytotoxic CD8+ T lymphocytes, B cell activation, and production of virus-specific antibodies, activate alveolar macrophages via cytokines. Specific CD8+ lymphocyte response against virus peaks 7 days after the onset of infection, differentiates into cytotoxic effector cells in order to eliminate virus. After the initial proliferation of antigen specific CD8+ T lymphocytes to control the infection, a pool of effector antigen-specific memory T cells is generated. Then effector T lymphocytes start to die and memory T cells proliferate and migrate to the secondary lymph organs in order to fight against a second encounter with the virus, and long-term immunity. Naïve T cells express CD8 + CD45RA+ CCR7+, and with maturation CCR7 expression reduces, and the mature CD8 + CD45RA+ CCR7− cells migrate to the infection site. Thus, after the viral infection, subsets of effector memory cells (CD8+ CD45RA− CCR7−), central memory cells (CD8+, CD45RA−, CCR7+, CD62L+), and memory cells (CD8+ CD45RA+ CCR7−) can be simultaneously detected.11 Gonzalez et al reported decreased memory T lymphocytes, both effector and central memory T cells, and decreased effector CD8+ T lymphocytes in the peripheral blood in patients with severe influenza.12 Transient lymphopenia and the reduction of CD4+ and CD8+ cells in the blood of H1N1 influenza patients have been previously reported; this reduction is thought to have a role in influenza severity.

B lymphocytes are also important in viral control via specific antibody production. After viral infection, antibodies produced by B lymphocytes as a response to Type I IFN, resulting with clearance of the virus. During the course of infection, long standing plasma cells and memory B cells are generated in order to provide long-term immunity against re-infections.

Determining the susceptible individuals for influenza complications can prevent morbidity and mortality by preventive measures like vaccination or chemoprophylaxis against influenza infection. In this study, our aim was to evaluate the lymphocyte subsets of the patients during severe influenza infection, and to investigate IRF7 gene variants whether they have a role in the severe course of influenza virus infection.

METHODS

2.1 Patient selection

In this study, 32 patients with influenza virus infection and respiratory failure hospitalized in Hacettepe University Hospital, Internal Medicine Services, and Intensive Care Unit (ICU) during the flu season
between November 2016 to March 2017 and November 2017 to March 2018 were included. All patients were older than 18 years and agreed to participate in this study and signed informed consent. Patients with the diagnosis of any type of cancer and/or under chemotherapy were not included in the study. Influenza infection was diagnosed by the positive test results of the respiratory samples of the patients for Influenza A or B with multiplex polymerase chain reaction tests (influenza, parainfluenza, rhinovirus, respiratory syncytial virus, adenovirus, bocavirus, coronavirus, enterovirus, metapneumovirus). These tests were performed in the Central Microbiology Laboratory of Hacettepe University Hospital.

Demographic parameters and clinical characteristics (co-morbid diseases, presence of fever, cough, sputum, dyspnea, and other symptoms, type of respiratory support), and influenza test results were recorded. Presence of infiltration on chest X-ray or computerized chest tomography, and laboratory test results (white blood cell count, lymphocyte, neutrophil, platelet counts, hemoglobin, hematocrit, transaminases, blood-urea-nitrogen, serum creatinine, erythrocyte sedimentation rate, c-reactive protein, calcitonin) were also recorded.

The respiratory failure (type 1 or type 2) was defined as the presence of hypoxemia or hypercapnia in the arterial blood gases tests or the requirement of respiratory support during the hospitalization period of the patients with influenza infection. The study was carried out with the approval of the Non-interventional Clinical Research Ethics Board of Hacettepe University GO 17/114 decision number, and funded by Hacettepe University Scientific Research Coordination Unit (Project ID: 14742).

2.2 Collection of samples

For the detection of nucleotide changes in IRF7 gene and analysis of T and B lymphocyte subgroups, 3 mL peripheral blood was drawn to a tube with EDTA. Genomic DNA extraction, and lymphocyte subgroup analyzes with flow cytometry were performed on the same day. Isolated DNA samples were frozen and stored at −20°C for IRF7 gene analysis.

2.3 Methods

2.3.1 Flow cytometry

For B lymphocyte subgroups measurement, peripheral mononuclear cells were stained at optimal concentrations with a mixture of the following antibodies: anti-CD19 peridinin chlorophyll protein (PerCP), anti-CD27 allophycocyanin (APC), anti-CD21 (APC), anti-IgD fluorescein isothiocyanate (FITC), anti-CD38 (FITC), and anti-IgM phycoerythrin (PE) (Becton Dickinson, San Jose, California). All the isotype controls were purchased from Becton Dickinson, were embedded to detect unspecific staining. Using panel B1, B cells were subdivided into several subpopulations: naive B cells (CD19+/CD27− IgM+/IgD−), marginal zone B cells (CD19+/CD27+ IgM+/IgD−), switched memory B cells (CD19+/CD27− IgM−/IgD+), and IgM-only memory B cells (CD19+/CD27+ IgM+/IgD−). Staining the B cells with panel B2 distinguished transitional B cells (CD19+/CD21low CD38++ IgM+), CD21low expressing B-cell (CD19+/CD21low CD38low IgM+), and plasmablasts (CD19low CD21int CD38++; IgM+). For T lymphocyte subgroup measurements, peripheral mononuclear cells were stained with a mixture of the following antibodies at optimal concentrations: anti-CD4 (FITC), anti-CCR7 (FITC), anti-CR45RA (APC), anti-CD3 (PerCP), anti-CD4, anti-CD8 (PerCP). T lymphocytes were divided into subpopulations: helper T (Th) cells (CD4+), naive helper T cells (CD4+ CCR7+ CD45RA−), central memory helper T cells (CD4+ CCR7+ CD45RA+), effector memory helper T cells (CD4+ CCR7− CD45RA−), TEMRA helper T cells (CD4+ CCR7− CD45RA+), cytotoxic T cells (CD8+), naive cytotoxic T cells (CD8+ CCR7+ CD45RA−), central memory cytotoxic T cells (CD8+ CCR7+ CD45RA+), effector memory cytotoxic T cells (CD8+ CCR7− CD45RA+), and TEMRA cytotoxic T cells (CD8+ CCR7− CD45RA+). Finally, we analyzed the data by Applied Biosystems Attune Acoustic Focusing Cytometer Instrument, and Attune Cytometric Software (v 1.2.5.3891).

2.3.2 IRF7 mutation screening

DNA was extracted from blood samples of patients, and controls in EDTA tubes by using Qiagen BioRobot EZ1 device, and Qiagen EZ1 DNA Blood 200 μL Kit. IRF7 gene sequencing analysis was performed by Sanger sequence analysis method. Amplified gene fragments of IRF7, by ABI Prism (9700) Thermal Cycler, were applied to sequencing reaction according to the specified protocol. After sequencing reactions, samples were purified using the ZymoResearch commercial kit, and sequencing analyses was performed on ABI3130.

2.3.3 Statistical analysis

Data were analyzed by SPSS 20.0 program (SPSS Inc., Chicago, Illionis). Descriptive statistics were used, and numerical values are expressed as median (minimum-maximum), categorical values as numbers and percentages. Independent groups are compared with one way ANOVA test, and $P$ value <.05 is accepted as statistically significant.

3 RESULTS

3.1 Baseline characteristics

The median age was 76 years (26–96), and 21/32 (65%) were female. 13/32 (40.6%) were in ICU. Non-invasive mechanical ventilation (NIMV) applied to 13/32 (40.6%) of the patients and invasive
mechanical ventilation (IMV) was required in 5/32 (15.6%) of the patients. Median length of hospitalization was 11.5 (4-56) days, and 3/32 (9.3%) were died in the hospital.

The most prevalent underlying diseases were Chronic Obstructive Pulmonary Disease (COPD) which was present in 13/32 (40.6%), and chronic heart diseases were the second most prevalent one (12/32, 37.5%) in our patient population. The demographic and clinical characteristics of patients were given on Table 1.

The 31 patients had positive respiratory tract swab PCR for Influenza A virus infection (IAV), one was positive for Influenza B virus (IBV), and seven patients had at least one positive test for a second virus (Table 1).

The most frequent symptom was dyspnea in patients at the time of hospital admission, and was present in 31 (96.8%) patients. Cough with/without sputum were present in 19 (59.3%), and fever was present in 15/32 (46.8%) of the patients. 3/32 (9.3%) patients had cardiopulmonary arrest at the time of hospital admission. The presenting symptoms of the patients were summarized in Table 1.

The diagnosis was pneumonia in 21 patients (65.6%), decompensation of heart failure in six patients (18.7%), and COPD exacerbation in 5 (15.6%) patients. All the 32 patients received oseltamivir as antiviral treatment, and 28 (87.5%) received an extended-spectrum antibacterial treatment.

The laboratory results of the patients at the time of the hospital admission were given in Table 2. Leukopenia (<1500/mm³) was present in 18/32 (56%) of the patients, c-reactive protein was higher than 0.5 mg/dL in 28 (87.5%) of the patients. Lung infiltration was

| TABLE 1 | Demographic and clinical characteristics of the patients with influenza virus infection |
| --- | --- |
| Total number of cases | 32 (100) |
| Age (years), median (minimum-maximum) | 67 (26-96) years |
| Number of elderly patients, ≥65 years old | 19 (59%) |
| Gender |  |
| Female | 21 (65.6%) |
| Median duration of hospital, days | 11.5 (4-56) |
| Symptoms at the time of hospital admission |  |
| Dyspnea | 31 (96.8%) |
| Cough ± sputum | 19 (59.3%) |
| Fever | 15 (46.8%) |
| Cardio-pulmonary arrest | 3 (9.3%) |
| Diagnosis at the time of hospital admission |  |
| Pneumonia | 21 (65.6%) |
| COPD exacerbation | 5 (16.5%) |
| Decompensated heart failure | 6 (18.6%) |
| Asthma attack | 2 (6.2%) |
| Others* | 3 (9.3%) |
| Comorbid illnesses |  |
| At least one comorbid illness | 29 (90.7%) |
| COPD | 13 (40.6%) |
| Diabetes mellitus | 12 (37.5%) |
| Asthma | 7 (21.8%) |
| Heart diseases (coronary heart disease, heart failure) | 7 (21.8%) |
| Immunosuppressive treatment | 3 (9.3%) |
| Influenza infection type |  |
| IAV + IBV | 2 |
| IAV + RSV | 1 |
| IBV + RSV | 1 |
| IAV + Parainfluenza | 1 |
| IAV + Bocavirus | 1 |
| IAV + Rhinovirus | 1 |
| IAV, only | 25 |
| Type of oxygen support (respiratory support) |  |
| Nasal canula, oxygen mask | 14 (43.8%) |
| NIMV | 13 (40.6%) |
| IMV | 5 (15.6%) |
| Exitus | 3 (9.3%) |

| TABLE 2 | Laboratory parameters of the patients at the time of hospital admission |
| --- | --- |
| Laboratory parameters | Results* |
| Leukocyte count (/mm³) | 7000 (3200-26 000) |
| Leukocytosis (>10,000/mm³), n (%) | 5 (15.6) |
| Leukopenia (<1500/mm³), n (%) | 18 (56) |
| Neutrophil count, (/mm³) | 4700 (2200-17 800) |
| Lymphocyte count, (/mm³) | 1200 (92-3300) |
| Erythrocyte Sedimentation Rate (ESR, mm/h) | 18 (2-120) |
| Elevated ESR (>30 mm/h), n (%) | 13 (40.6) |
| C-reactive protein (CRP, mg/dL) | 2.6 (0-16) |
| Elevated CRP (>0.5 mg/dL), n (%) | 28 (87.5) |
| Blood-urea-nitrogen level, mg/dL | 20 (9-55) |
| Serum creatinine, mg/dL | 1 (0.3-6) |
| ALT, U/L | 17.5 (86-156) |
| AST, U/L | 34 (8-132) |
| Acute liver failure, n (%) | 2 (6.2) |
| Acute renal failure (GFR < 60 mL/min/1.73 m²), n (%) | 10 (31.2) |
| Lung infiltration on chest X-ray/CT, n (%) | 23 (71.8) |

Abbreviations: COPD, chronic obstructive pulmonary disease; IAV, influenza A virus; IBV, influenza B virus; IMV, invasive mechanical ventilation; NIMV, non-invasive mechanical ventilation; RSV, respiratory syncytial virus.  
*A patient had diarrhea, 1 patient deterioration of general condition, and 1 had acute hypoxia. 

Abbreviations: ALT, alanine transaminase; AST, Aspartate transaminase; CT, Computerized tomography.  
*Results are given in median (minimum-maximum), and number (percentage).
present in 23/32 (71.8%) of the patients in chest X-ray or computerized tomography (CT).

### 3.2 T and B lymphocyte subsets

Median WBC count was 7000/uL (3200-26000), and lymphocyte count was 988/uL (92-3078). B lymphocyte subsets were analyzed in 30 patients, and T lymphocyte subsets were analyzed in 16 patients. The median T and B lymphocyte subsets of the patients, and comparison with normal population were given in Table 3. The percentage of central memory Th, effector memory Th, TEMRA Th, cytotoxic lymphocytes (CTL), central memory CTL were increased, naive CTL were decreased when compared with healthy groups. Comparison of the elderly patients (≥65 years old) with younger patients showed a significant increase in the percentage of effector memory Th, and a decrease in the percentage of naive CTL in patients ≥65 years-old (P = .039, and P = .017, respectively).

CD19+ B lymphocytes, naive B lymphocytes (CD19+ CD27- IgM+ IgD+), marginal zone B lymphocyte (CD19+ CD27+ IgM+ IgD+) were in normal ranges, and no significant differences were detected between older and younger patients. Isotype switch memory B Lymphocyte (CD19+ CD27+ IgM- IgD-) number and percentage were higher in patients ≥65 years old compared to younger patients but were in normal limits when compared to healthy adult population.

| TABLE 3 | The median T, and B lymphocyte subsets of the patients, and comparison with normal population |
|----------|-------------------------------------------------------------------------------------|
|          | Total patients (N:32)                                                               | <65 years old (N:13) | ≥65 years old (N:19) | P value | Normal range, % |
| Lymphocyte | % | 19 (4-39) | 16 (6-39) | 21 (4-34) | .730 |
|            | n | 1200 (92-3000) | 1200 (92-3078) | 1200 (300-3300) | .715 |
| CD4+ T (Th) | % | 41 (9-49) | 43 (25-49) | 36 (9-46) | .193 |
|            | n | 417 (66-963) | 405 (66-947) | 365 (165-744) | .791 |
| Naive Th (CD4+ CCR7 + CD45RA+) | % | 31 (1-54) | 37.8 (17-54) | 24.7 (1-45) | .023 |
|            | n | 124 (7-137) | 157 (10-384) | 74 (7-194) | .064 |
| Central Memory Th (CD4+ CCR7 + CD45RA-) | % | 31 (0-50) | 31 (25-43) | 26 (0-50) | .75 |
|            | n | 101 (0-318) | 122 (28-293) | 70 (0-318) | .266 |
| Effector Memory Th (CD4+ CCR7-CD45RA-) | % | 23 (2-56) | 20.9 (2-30) | 33 (5-56) | .039 |
|            | n | 58 (4-497) | 50 (9-189) | 89 (21-417) | .168 |
| TEMRA Th (CD4+ CCR7-CD45RA+) | % | 9 (3-42) | 10 (3-22) | 8.6 (5-42) | .560 |
|            | n | 26.5 (6-312) | 16 (6-97) | 29 (11-312) | .525 |
| CD8+ T Lymphocyte (CTL) | % | 31 (12-56) | 29 (6-97) | 33 (17-56) | .559 |
|            | n | 302 (89-777) | 297 (89-602) | 307 (159-777) | .711 |
| Naive CTL (CD8+ CCR7 + CD45RA+) | % | 8 (1-37) | 12.7 (8-33) | 6.9 (1-37) | .017 |
|            | n | 30.9 (3-113) | 51 (9-113) | 15 (3-75) | .153 |
| Central Memory CTL (CD8+ CCR7 + CD45RA-) | % | 6.8 (1-36) | 9 (1-19) | 6.4 (1-36) | .711 |
|            | n | 18.9 (3-55) | 21 (3-114) | 16.9 (3-155) | .832 |
| Effector Memory CTL CD8+ CCR7-CD45RA- | % | 26.4 (8-68) | 23.4 (9-53) | 39 (16-416) | .315 |
|            | n | 99.4 (8-416) | 74.8 (8-178) | 124 (16-416) | .186 |
| TEMRA Tc | % | 49 (14-64.9) | 49.2 (14-64) | 36.8 (18-4-65.6) | .958 |
|            | n | 134 (37-435) | 143.7 (47-294) | 125 (37-435) | .715 |
| CD 19+ B Lymphocyte | % | 13 (5-33) | 12 (5-22) | 13 (6-33) | .842 |
|            | n | 227 (32-826) | 114 (57-430) | 153 (32-826) | .330 |
| Naive B Lymphocyte (CD19+ CD27+ IgM+ IgD+) | % | 55 (0-80) | 58.9 (15-80.6) | 48 (0-80) | .163 |
|            | n | 69 (0-461) | 62.2 (12-290) | 72 (0-461) | .982 |
| Isotype switch memory B Lymphocyte (CD19+ CD27+ IgM+ IgD+) | % | 22.4 (4-68) | 20.4 (7.5-53) | 31.3 (4-68) | .899 |
|            | n | 32 (4-255) | 23.3 (5-103) | 40 (4-255) | .144 |
| Marginal zone B Lymphocyte (CD19+ CD27+ IgM+ IgD+) | % | 10 (0-91) | 11 (2.5-72) | 7 (0-91) | .375 |
|            | n | 16 (0-75) | 17 (2-51) | 16 (0-751) | .756 |

Note: All values are given as median (minimum-maximum), and "n" presented as cells/micL. P values <0.05, which was considered statistically significant, was shown as bold.
Mutation screening for IRF7 gene by Sanger sequencing identified two different heterozygous variants in three patients: c.535 A > G; p.Lys179Glu and c584A > T; p.His195Leu. The K179E and N195L variants detected in our patients are located in the fourth exon of the IRF7 gene, encoding the constitutive activation domain (CAD) of the IRF7 protein (Figure 1). CAD domain forms in the refraction zones and defects leads non-functional IRF7 protein.14 The variants detected in our patients are shown in Figures 2.

4 | DISCUSSION

In this study, 32 adult patients hospitalized due to severe influenza virus infection with respiratory failure were evaluated in terms of T and B lymphocyte subgroups and mutation screening was performed for IRF7 gene. To the best of our knowledge, this is the first study conducted on T and B lymphocyte subsets and IRF7 gene analysis in adults with severe influenza infection. Central memory Th, effector memory Th, TEMRA Th, CTLs, central memory CTLs were found to be increased, naive CTLs were decreased. The increase in the effector memory Th, and a decrease in the naïve CTL percentages were significant in elderly patients compared with younger patients with severe influenza infection.

Age was one of the most important risk factors for severe influenza infection, complications, and mortality.2 In our study group, the median age of the patients was 67 years, and 19/32 patients were aged 65 and over. In a study, the average age of 221 patients hospitalized due to severe acute respiratory infection caused by influenza was reported to be 74.1 years old and 75.6% of patients were over 65 years old.15 Whereas, in another study, the mean age of 88 patients with H1N1 influenza-related severe respiratory infection was reported as 48 ± 15 years.16 The difference in the most affected age groups of the patients can be explained by distinct influenza strains, for example, H1N1 was associated with more severe infections in younger individuals, or difference in the study designs.16

At least one chronic medical disease was present in 90.7% of our patients. COPD, and chronic heart diseases were the leading comorbidities which was compatible with the literature.2,15

Lymphopenia was present in 62.5% of our patients. When compared to the healthy adult population, naïve Th, naïve CTL were decreased, and central memory Th, effector memory Th, TEMRA Th, CTL, and central memory CTLs were increased in patients with severe influenza infection. Naïve Th, and naïve CTL ratios were lower in patients with >65 years old compared to younger patients as expected. The decrease in naïve T cell population and increase in effector and central memory T lymphocytes can be considered as a response of the immune system to the viral infections.

Lymphocyte subgroup analysis during influenza infection generally addresses the respiratory tract, lungs and regional lymph nodes, and effector and memory T and B lymphocytes are increased in these infection sites, and effector memory CD4+ and CD8 + T lymphocytes
are located in the tissues.\textsuperscript{17,18} Severe influenza infections with H1N1 pdm09, resulted a decrease in circulating effector memory and central memory CTLs compared to patients with mild infection and healthy individuals.\textsuperscript{11} These changes can be interpreted as a result of the migration of these cells from the peripheral circulation to the infection area. Memory CD8+ and CD4+ T lymphocytes produced during the infection with influenza virus are responsible for rapid immune response when encountered with the same virus later. In the case of infection with influenza virus are responsible for rapid immune response when encountered with the same virus later. In the case of infection with influenza virus is reported to be involved in the inhibitor domain of IRF7 and to predispose to influenza virus infection, resulting in impaired IRF7 function and a significant decrease in IFN production.\textsuperscript{24} Also recently, it has been reported that mutations in IRF9, RIG-1, and TLR3 genes cause susceptibility to severe influenza virus infection.\textsuperscript{25-28} In the presence of an IRF9 homozygous “dysfunction” mutation, the formation of the ISGF3 complex cannot be achieved and therefore cannot respond appropriately to the type I IFN response due to virus infection.\textsuperscript{25}

Although there are multiple risk factors for complication of influenza infection, a genetic predisposition other than IRF7 in these patients may also cause susceptibility to severe influenza virus infections. Genetic variants in IRF7 or IRF9, RIG-1, and TLR3 affecting the immune response may cause predisposition to influenza virus in addition to the traditional risk factors.

The limitations of our study were the small sample size, and the cross-sectional design of the study. There is a lack of knowledge on the distribution of the lymphocyte subsets before or after the virus infections, so we cannot conclude the causality of these changes on complication of influenza infection.

5 | CONCLUSION

In conclusion, the effector memory Th and CTLs were increased and naive T cells were decreased in adults with severe influenza infection compared to healthy controls. We could not detect a previously identified variant of IRF7, but we cannot disregard other genetic defects for severe influenza infections in our patients. Genetic analysis especially in young patients with severe influenza infection without a known chronic disease will contribute to the literature.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.

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