HIF-1α is Associated with Improved Survival in ARDS due to COVID-19: A Prospective Study

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Abstract

Background

Acute respiratory distress syndrome (ARDS) due to COVID-19 is accompanied by severe hypoxemia and hyperinflammation. Hypoxia-inducible factor (HIF) pathway plays a fundamental role in detecting hypoxia and developing appropriate responses. The epidemiological report claimed a lower rate of disease in the population living at high altitudes and hypothesized that adaptation to hypoxia might be advantageous for SARS-CoV-2 infection. This study was designed to examine the frequency of polymorphisms in the HIF-1α and PHD2 (prolyl hydroxylase domain 2) genes, which are involved in the adaptation to hypoxia, and the relationship of existing polymorphisms with survival in the ARDS clinic developed due to COVID-19.

Methods

The study included 297 patients who developed ARDS due to COVID-19 infection and were admitted to the tertiary intensive care unit. Age, gender, hospitalization diagnosis, arterial blood pressure, heart rate, APACHE II score, SOFA laboratory parameters during hospitalization, vasopressor, dialysis and mechanical ventilation need during treatment, length of hospital stay, and 30-day mortality status were recorded. DNA was isolated from the blood samples by spin colon method with the QIAamp DNA MiniKit (Cat.No.51106, QIAGEN, Germany).

Results

Patients were divided into 3 groups according to their HIF Inducible Factor-1α (C/T SNP [11549465]) genotypes. Frequencies were 71.13% for the homozygous CC genotype, 26.4% heterozygous CT genotype, and 2.36% for the homozygous TT genotype. Median age (p=0.631), APACHE II (p=0.205), and SOFA (p=0.077) scores were similar in all three groups. However, the need for dialysis, mechanical ventilation, and vasopressor was less in the homozygous TT-genotype group than in the other groups (p<0.05). The mortality rate was also lower in this group compared to other groups (p<0.05). PHD2 (C/T SNP [480902] and [516651]) polymorphism, clinical and laboratory features were similar in all groups. Moreover, 30-day mortality did not differ between the groups.

Conclusion

In conclusion, we revealed polymorphism in HIF-1α and PHD2 genes in ARDS patients due to COVID-19. The rate of HIF-1α polymorphism was 26.4% heterozygous CT-genotype and 2.36% for homozygous TT-genotype. 30-day mortality and adverse outcome (dialysis, vasopressor use, MV need) were significantly lower in TT homozygous. However, none of the polymorphisms in the PHD2 genes affected mortality and adverse outcome.

Background

ARDS is a life-threatening disease with acute onset, progressive respiratory dysfunction, and hypoxemia with bilateral lung infiltration. ARDS is characterized by a strong inflammatory response and impaired oxygenation due to ventilation-perfusion mismatch.1

The hypoxia signaling pathway is involved in the perception of hypoxia. The hypoxia-inducible factor (HIF) in this pathway is a heterodimeric transcription factor, with alpha and beta subunits. Beta is a structural protein and exists in a single form. Alpha protein has 3 different isoforms; these are HIF-1α, HIF-2α, and HIF-3α. HIF is named by the alpha structure in its content (HIF-1, HIF-2, and HIF-3). Under normal oxygen conditions, HIF-α is hydroxylated and subsequently degraded by prolyl hydroxylase (prolyl hydroxylase domain, PHD). PHD activity is reduced in hypoxic conditions. Accordingly, the increased level of HIF-α migrates to the nucleus and binds with HIF-β, and the resulting complex initiates the transcription of many genes that play a role in response to the hypoxic state.2 Genes controlled by HIF cause a very broad biological output. Responses at the cellular level include differentiation, migration, cytoprotection, apoptosis, and alteration in the function of organelles such as mitochondria.3 Organ or organism-level responses are changes in the inflammatory, immune-regulatory system, and energy metabolism.4 Although there are three different types of PHD, which are activated by sensing the presence of oxygen and initiate the degradation of HIF-α, the most frequently expressed form in tissues is PHD2, which acts as the main regulator of HIF activity, and the gene encoding it is known as EgnR1.5

Differences in HIF and PHD activity, which is involved in the hypoxic signaling pathway, may affect the responses to hypoxemia and increased inflammation. Single nucleotide polymorphism is the most common type of polymorphism, and variation in genetic structure can affect the function of the related protein. In this study, the effects of polymorphisms frequently encountered in HIF-α and PHD2 genes on ARDS clinical presentation secondary to COVID-19 infection were investigated (for HIF-α; C/T, for SNP rs 11549465 and PHD2; C / T; SNP rs516651 and T / C; SNP rs480902).

In the research conducted, functionally active genetic variants were detected in HIF-1α (C/T; SNP rs 11549465) and PHD2 (C / T; SNP rs516651 ve T / C; SNP rs480902). These genetic variants are associated with erythrocytosis, pulmonary hypertension, and chronic mountain sickness.5–7 There is limited information about the effect of polymorphic forms resulting from single nucleotide polymorphism of proteins in the HIF pathway on the ARDS clinic. The PHD2 rs516651 TT-genotype was indicated to be associated with mortality in patients with ARDS.8 This study examined the effects of previously determined point mutations in the HIF-1α gene and PHD on the ARDS clinic developing secondary to COVID-19 infection.

Material and Methods

The study was approved by the Erciyess University Clinical Research Ethics Committee (No:2021/142). Patients aged 18 years and older who developed ARDS due to COVID-19 infection were included in this study. Informed consent forms were obtained from the patients and/or their relatives included in the study. Since the study was conducted in the early period, the patients did not have the COVID-19 vaccine. Previously defined Berlin diagnostic criteria were considered for the diagnosis of ARDS.1
Age, gender, hospitalization diagnosis, arterial blood pressure, heart rate, APACHE (acute physiological and chronic health assessment) II score, laboratory parameters during hospitalization, vasopressor, dialysis and mechanical ventilation need during treatment, length of hospital stay, and 30-day mortality status were recorded.

Peripheral blood samples were taken into tubes containing EDTA and stored at -20°C until the day of analysis. Obtaining DNA from peripheral blood samples was performed using the QIAamp DNA mini Kit (Cat. No. 51106, QIAGEN, Germany) by the spin column method. All samples were measured for purity and concentration using the Nanodrop Thermo Scientific Spectrophotometer. Total reaction (10 µL) was monitored using a specific primary probe (Light Snip HIF1α, rs 11549465 (lot#: 14262101 TIB MOLBIOL GmbH) and rs 11549467 (lot#: 28422101 TIB MOLBIOL GmbH) FastStart DNA Master HybProbe master mix (cat#: 03 003 248 001-Roche) and DNA. Quantitative Real-Time PCR Light Cycler 480 Instrument II (Roche Diagnostics, Germany) was used to detect HIF 1α gene (rs11549465) and PHD2 gene (rs 516651 and rs 480902) polymorphisms. Denaturation was performed at 95°C in 10 minutes. Quantification was achieved with 45 cycles of 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 15 seconds. Melting was performed at 95°C for 20 seconds, 40°C for 20 seconds, and 85°C in 0.2 continuation mode, and cooling at 40°C. C and T alleles for rs 11549465, C and T alleles for rs 516651, and T and C alleles for rs 480902 were analyzed by melting curve analysis in channel 530 after using RT PCR. The study was carried out in Erciyes University Medical Faculty Medical Genetics Laboratory.

**Statistics**

Statistical analysis was performed with the SPSS program. Demographic information of the patients, APACHE II score at admission to the intensive care unit, P/F, creatinine, BUN, white blood cell, hemoglobin, CRP, procalcitonin values, length of hospital stay, and mortality were recorded. Mean (standard deviation) and median (Q1, Q3) values were calculated as descriptive statistics for continuous variables, and frequencies and percentages were determined for categorical variables. Kruskal-Wallis and Mann-Whitney tests were used for intergroup comparisons of continuous variables. The correlation between the variables was examined using the Spearman rank correlation coefficient. Kaplan-Meier, log-rank test, and Cox regression analyses (dependent variable mortality status) were used for survivor analysis. The statistical significance level was accepted as p<0.05.

**Measurements and Main Results**

This prospective observational study included 291 patients who developed ARDS secondary to COVID-19 infection, and 164 died during the 30-day follow-up period.

**HIF-1α (C/T SNP [rs11549465]) Polymorphism**

71.13% of the patients were homozygous CC-genotype, 26.4% were heterozygous CT-genotype, and 2.36% were homozygous for TT-genotype. Demographic and clinical characteristics of the patients according to the groups are presented in Table 1. Median age (p=0.631), APACHE II (p=0.205), and SOFA (p=0.077) scores were similar in the three genotype groups. However, the need for dialysis, mechanical ventilation, and vasopressor was less in the homozygous TT-genotype group than in the other groups (p<0.05). Mortality rate was also lower in this group compared to others (p<0.05). The laboratory values of the patients at admission were similar in all three groups (Table 2).

When multivariate Cox analysis was performed, HIF-1α rs 11549465 CT (HR=0.689 (0.479-0.991), p=0.044) and TT (HR=0.158 (0.019-0.992, p=0.049)) caused a decrease in 30-day mortality compared to CC. Other independent risk factors for thirty-day mortality were patient age (HR=1.014(1.001-1.026) p=0.032) and APACHE II value (HR=1.029 (1.012-1.067) p=0.005) and need for dialysis (HR=1.974 (1.441-2.703 p=0.001) (Table 3), Kaplan-Meier analysis revealed significantly lower mortality rate for the homozygous TT- genotypet (p=0.041)(Figure 1.).

**Prolyl hydroxylase 2 gene (C/T SNP [480902] and [516651])**

They were divided into 3 groups according to the prolyl hydroxylase 2 (PHD2) gene (C/T SNP [516651] genotypes. 78.3% of the patients showed homozygous CC-genotype, 21.3% heterozygous CT-genotype, and 0.4% homozygous TT-genotype.

The patients’ demographic, clinical, and laboratory characteristics are presented in Table 1.

**Clinical and laboratory features were similar in groups formed according to prolyl hydroxylase 2 gene (C/T SNP [480902] and [516651]) polymorphism status. Furthermore, 30-day mortality did not differ between the groups.**

Table 1. Clinical properties in patients with ARDS due to COVID-19 by HIF-1α (rs 11549465) and PHD2 gene (EGLN1 rs 516651 and EGLN1rs 480902) polymorphism status ((mean±sd) (median;Q1,Q3), n(%)).

They were divided into 3 groups according to the prolyl hydroxylase 2 (PHD2) gene (C/T SNP [480902]) polymorphism. Homozygous CC-genotype was detected in 54.2% of the patients, heterozygous CT-genotype in 39.2%, and homozygous TT-genotype in 6%.

**Discussion**

In this prospective study, the effect of X and Y single nucleotide polymorphisms in HIF-1α (C/T SNP [11549465] ve PHD2 genes (C/T SNP [480902] ve [516651]) on 30-day mortality and morbidity in patients with ARDS due to COVID 19 was investigated. Considering the HIF-1α HIF-1α (C/T SNP [11549465] polymorphism, it was revealed that 30-day mortality rates were lower in homozygous TT and heterozygous CT genotypes compared to the wild-type CC
genotype. Besides, mechanical ventilator support and vasopressor drug use were also less in variants containing the T allele. It was observed that the studied polymorphisms (rs 480902 and rs 516651) in the PHD2 gene did not affect clinical parameters and mortality.

Acute lung injury, ARDS, and severe coronavirus disease 2019 are examples of inflammatory lung conditions characterized by pulmonary hypoxia at the tissue and cell levels. Damage to the lung vascular structure in ARDS patients causes hypoxia and defects in vascular permeability. Apoptosis occurs in alveolar epithelial cells and lung endothelial cells in ARDS. Weakening of the connections between lung endothelial cells and leukocyte infiltration causes vascular leakage and pulmonary edema. The HIF signaling pathway controls key processes that govern inflammatory lung injury and vascular repair, including maintenance of lung endothelial cell viability, proliferation of alveolar epithelial cells, repair of damaged areas, and establishment of intercellular connections. Different results have been obtained in studies investigating the effects of the HIF-1α signaling pathway in acute lung injury/ARDS. A study of ventilation-induced lung injury in rats demonstrated a role for HIF-1α in the lung protection conferred by adenosine. However, studies have demonstrated that HIF-1α can promote acute lung injury and inflammation. Use of YC-1, a HIF inhibitor, reduced lung injury in a rat model of trauma-hemogenic shock. Another study using the hypoxic ischemia/reperfusion model reported that increased pulmonary HIF-1α level was associated with higher VEGF level and more vascular damage. In an endotoxin-induced lung inflammation model with endothelial cell-specific PHD2 knockout rats, reduction of PHD2 reduced lung vascular permeability, edema, and inflammatory cell infiltration. Furthermore, it was indicated that endothelial barrier function and intercellular junction integrity are improved. However, in a model created by incubation with a septic lymph node, the HIF-1α gene deleted (HIF-1α gene silencing) increased cell viability and decreased inflammatory cytokine expression. These results have revealed that the HIF-1α signaling pathway can produce different effects according to the stage of acute lung injury/ARDS disease (injury or recovery period) and the cell type studied (alveolar epithelium, lung endothelium, or leukocytes).

Although studies have reported how the HIF-1α signaling pathway affects the ARDS symptoms, there is not enough information about how the variations seen in the HIF-1α gene affect these symptoms. Single nucleotide polymorphism is the most common genetic variation. The HIF-1α rs 11549465 single nucleotide polymorphism examined in this study is located in the coding region of the gene and results in the replacement of the amino acid proline at position 582 of HIF-1α with serine. It has been reported that this variation causes various cancers, such as prostate, breast, and colon cancer, to progress more aggressively. It is claimed that HIF-1α expression is increased in cells with HIF-1α rs747465 mutation, that increased HIF-1α level increases vascularization in tumor tissue under hypoxic conditions, resulting in accelerated tumor growth and spread. The HIF-1α rs 11549465 polymorphism may be a risk factor for the development of diabetic complications, chronic obstructive pulmonary disease, and skin disease other than cancer. It is considered that single nucleotide polymorphism may cause disease by affecting the promoter and enhancer activity in the gene, the stability of messenger RNA, and the subcellular localization of messenger RNA and/or proteins. In a prospective observational study involving 241 patients hospitalized for acute kidney injury, the HIF-1α rs 11549465 polymorphism was studied, and individuals with the T allele had higher adverse outcomes such as dialysis, mechanical ventilation need, and in-hospital mortality. However, this result contrasts with the results obtained from experimental studies in which the kidney was exposed to hypoxia. In experimental studies, it was determined that increased HIF-1α activation has a protective effect against ischemic damage.

In this study, the effect of rs 11549465 single nucleotide polymorphism seen in the HIF-1α gene on ARDS clinic was investigated, and as a result, it was demonstrated that patients with the polymorphic form had better survival compared to the wild type. The cause-effect relationship underlying this survival advantage remains to be determined and may be the subject of further studies. During ARDS, epithelial cells, primarily alveolar Type I, die and are shed. During healing, Type II alveolar cells proliferate and spread to fill the spaces emptied by Type I cells on the basement membrane. Repair of the alveolar epithelium is critical to clinical recovery. An experimental study by McClendon et al. reported that HIF-1α was activated in ATII cells after lung injury, and increased HIF-1α level supported epithelial cell proliferation and migration during the repair period. In summary, the HIF-1α signaling pathway accelerates alveolar epithelial repair after acute lung injury. The fact that the level of HIF-1α was not determined (due to cost) is a critical limitation of our study. We know from previous studies that HIF-1α levels are increased in groups containing the T allele. From this point of view, if we accept the high level of HIF-1α in groups containing the T allele in our study, it may be possible to explain the better survival rates observed in T allele groups.

Declarations

Ethics approval and consent to participate: The study was approved by the Erciyes University Clinical Research Ethics Committee (No:2021/142). Informed consent was obtained based on the Declaration of Helsinki.

Consent for publication: Not Applicable.

Availability of data and materials:

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request (Because we do not want our database data to be used in other studies).

Competing interests: The authors declare no competing interests.

Funding: This work was supported by the Erciyes University (TSA-2021-11160)

Authors’ contributions:

HS: Conceptualization, Methodology, Writing- Original draft preparation, Software
SK: Writing- Original draft preparation and software
References


### Tables

**Table 1.** Clinical properties in patients with ARDS due to COVID-19 by HIF-1α (rs 11549465) and PHD2 gene (EGLN1 rs 516651 and EGLN1rs 480902) polymorphism status ((mean±sd) (median;Q1,Q3), n(%)).

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIF-1α rs 11549465</th>
<th>HIF-1α rs 11549465</th>
<th>HIF-1α rs 11549465</th>
<th>p</th>
<th>Prolylhydroxylase 2 rs 480902</th>
<th>Prolylhydroxylase 2 rs 480902</th>
<th>Prolylhydroxylase 2 rs 480902</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td></td>
<td>CC</td>
<td>TC</td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>207(71.1)</td>
<td>77(26.4)</td>
<td>7(2.5)</td>
<td></td>
<td>158(54.2)</td>
<td>116(39.8)</td>
<td>17(6)</td>
<td></td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>98/109b (47.3/52.7)</td>
<td>22/55a (28.6/71.4)</td>
<td>4/3b (57.1/42.9)</td>
<td>P&lt;0.05</td>
<td>64/94 (40.5/59.5)</td>
<td>54/62 (46/53)</td>
<td>6/11(35/64)</td>
<td>0.498</td>
</tr>
<tr>
<td>Age, years</td>
<td>64 (55-73)</td>
<td>62 (52-71)</td>
<td>65 (51-69)</td>
<td>0.631 (0.396)</td>
<td>64(53-72)</td>
<td>64(55-73)</td>
<td>60(52-68)</td>
<td>0.261</td>
</tr>
<tr>
<td>BMI</td>
<td>27 (24-31)</td>
<td>27 (24-30)</td>
<td>31 (26-34)</td>
<td>0.803</td>
<td>27(24-30)</td>
<td>27(24-31)</td>
<td>26(23-29)</td>
<td>0.661</td>
</tr>
<tr>
<td>APACHE II on ICU admission</td>
<td>17 (13-20)</td>
<td>16 (14-20)</td>
<td>14 (12-16)</td>
<td>0.205</td>
<td>16(13-20)</td>
<td>17(14-20)</td>
<td>15(10-21)</td>
<td>0.377</td>
</tr>
<tr>
<td>SOFA on day one</td>
<td>3 (3-4)</td>
<td>3 (3-3,5)</td>
<td>3 (3-3)</td>
<td>0.077</td>
<td>3(3-4)</td>
<td>3(3-4)</td>
<td>3(3-4)</td>
<td>0.910</td>
</tr>
<tr>
<td>Dialysis n</td>
<td>47 (23)</td>
<td>18 (23)</td>
<td>1 (14)</td>
<td>0.860</td>
<td>30(19)</td>
<td>33(28)</td>
<td>3(17)</td>
<td>0.159</td>
</tr>
<tr>
<td>Mechanical Ventilation</td>
<td>145 (70)a</td>
<td>38 (49)b</td>
<td>2 (29)b</td>
<td>&lt;0.001</td>
<td>81(51.3)</td>
<td>78(67)</td>
<td>11(64)</td>
<td>0.672</td>
</tr>
<tr>
<td>Vasopressor use</td>
<td>117(56.5)a</td>
<td>33 (43)b</td>
<td>1 (14,3)b</td>
<td>0.016</td>
<td>79(50)</td>
<td>62(53)</td>
<td>9(52)</td>
<td>0.892</td>
</tr>
<tr>
<td>Hospital stay days</td>
<td>17 (12-26)</td>
<td>17 (12-27)</td>
<td>28 (16-30)</td>
<td>0.273</td>
<td>19(12-27)</td>
<td>16(10-22.7)</td>
<td>22(12-32)</td>
<td>0.012</td>
</tr>
<tr>
<td>Mortality at 30 days</td>
<td>128 (60.8)a</td>
<td>36 (47)b</td>
<td>2 (29)ab</td>
<td>0.023</td>
<td>98(62)</td>
<td>76(65)</td>
<td>9(52)</td>
<td>0.059</td>
</tr>
</tbody>
</table>

**Table 2.** Laboratory properties in patients with ARDS due to COVID-19 by HIF-1α (rs 11549465) and PHD2 gene (EGLN1 rs 516651 and EGLN1rs 480902) polymorphism status.
### Table 3. Cox regression analysis results in ARDS patients due to COVID-19 grouped by HIF-1α gene (rs 11549465) polymorphism

<table>
<thead>
<tr>
<th>Covariables</th>
<th>Univariate HR (95% CI)</th>
<th>p-value</th>
<th>Multivariate HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α rs 11549465; CT vs. CC</td>
<td>0.648(0.452-0.930)</td>
<td>0.018</td>
<td>0.689(0.479-0.991)</td>
<td>0.044</td>
</tr>
<tr>
<td>HIF-1α rs 11549465; TT vs. CC</td>
<td>0.124(0.017-0.888)</td>
<td>0.038</td>
<td>0.158(0.019-0.992)</td>
<td>0.049</td>
</tr>
<tr>
<td>Gender; male vs. female</td>
<td>1.17(0.873-1.569)</td>
<td>0.292</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.020(1.008-1.032)</td>
<td>0.002</td>
<td>1.014(1.001-1.026)</td>
<td>0.032</td>
</tr>
<tr>
<td>APACHE II</td>
<td>1.059(1.032-1.087)</td>
<td>&lt;0.001</td>
<td>1.029(1.012-1.067)</td>
<td>0.005</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.001(1.000-1.003)</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>0.999(0.979-1.022)</td>
<td>0.908</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt;/FiO&lt;sub&gt;2&lt;/sub&gt; ratio mmHg</td>
<td>0.995(0.985-1.005)</td>
<td>0.297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical Ventilation</td>
<td>5.999(3.640-9.886)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis</td>
<td>2.155(1.580-2.939)</td>
<td>&lt;0.001</td>
<td>1.974(1.441-2.703)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vasopressor</td>
<td>0.405(0.056-2.924)</td>
<td>0.370</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

30-day Kaplan Meier plot of ARDS patients due to COVID-19 by HIF-1α (rs 11549465) genotypes. CC = homozygous CC genotype (n=207), CT = Heterozygous CT genotype (n=77), TT = homozygous TT genotype (n=7) p=0.