MOLECULAR STUDY OF INTERFERON GAMMA IFN-ᵞ IN COVID-19 PATIENT IN BABYLON, PROVINCE

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ABSTRACT

Coronaviruses (CoVs) are respiratory illness-causing viruses that belong to the Coronaviridae family. These infect humans as well as some other animals. The first encounter with the CoV, identified as HCoV-OC43 and HCoVs229E, originated in the 1960s.(1)

They are enveloped viruses with a helical symmetry nucleocapsid and a single-stranded positive-sense RNA genome. Coronaviruses had some of the largest genome sizes among RNA viruses, ranging from 26 to 32 kilobases. They have distinctive club-shaped spikes that protrude from their surface, creating an image reminiscent of the solar corona in electron micrographs.(2)

Interferon (IFN) are a group of cytokines that were discovered to have antiviral properties nearly half a century ago. In the inflammatory response to infections, interferons are proteins released by a variety of cells. The immune system activates their development in response to pathogens or cytokines (3). Interferon-gamma (IFN-gamma) regulates immune and inflammatory responses in humans; there is only one form of IFNgamm. It's made by T-cells that have been programmed and natural killer cell. IFN- has antiviral and antitumor properties(3)

From a group of probable cases infected with the SARS-COV-2 virus (Covid-19), blood samples were taken from 100 patients and all blood samples were chickened using traditional PCR and 10 samples were taken for sequencing polymorphism detection., in order to assess the presence of IFN- in SARS-COV-2 patients,

The presence of IFN- with a high level is most common two weeks after infection, whereas a high level is associated with mild symptoms and is more common in men than in women.

The ten sequenced samples had 700bp multiple alignment results that were successful. In comparison to the referring reference IFN-ᵞ gene sequences, all ten analyzed samples revealed 100 percent identity with reference strain "NG 015840.1" no variable positions in all ten analyzed samples revealed 100 percent identity with reference strain "NG 015840.1".

I. INTRODUCTION

SARS-CoV-2 zoonosis was first recorded in China in late 2019, In contrast to the previous two outbreaks, the virus has quickly spread across the world, causing a destructive pandemic and a major health concern worldwide (4,5). Photographs of electron micrographs, Their name derives from their resemblance to the solar corona In the Wuhan (China) area, a new coronavirus, 2019-nCoV (of the family Coronaviridae and subfamily Orthocoronavirinae), has recently emerged, causing serious respiratory tract infections in humans.(6; 7; 8).

COVID-19 is a respiratory condition that mostly affects the upper respiratory tract, including the lungs. COVID-19 can cause a variety of breathing issues, ranging from moderate to severe. Older adults and people with other health problems, such as heart disease, cancer, or diabetes, can have more severe symptoms.(9)
COVID-19 causes mild to severe signs in around 80% of patients. You may be suffering from a dry cough or a sore throat. Pneumonia is a lung infection in which the alveoli become inflamed. (10)

In the host genome, the pathogenic in vivo appearance of SARS-CoVs falls in different stages. The binding of the virus to the host receptors, entry of the virus into the host cells by membrane fusion or endocytosis, the release of virus particles within the host cell followed by replication and biosynthesis of viral proteins, and budding/release of viral particles are sequential steps. According to the virologists, the pathophysiology and virulence mechanism of human SARS-CoV and SARS-CoV-2 are possibly similar (82% identical) to the work of non-structural proteins (nsps) and structural proteins (sps) (11)

Around 14% of COVID-19 cases are serious, resulting in inflammation of both lungs and lungs overflow with fluid and waste as the swelling worsens. Mucus, fluid, and other cells continue to combat the virus by filling the air sacs. This will make it more difficult for the body to absorb oxygen. It's possible that having trouble breathing. (9)

Novel coronavirus pneumonia is a new infectious disease that humans are not immune to. This means people are generally susceptible to infection. Presently, the main source of infection occurs in patients with viral infections; however, asymptomatic infected individuals may also become the source of infection (12)

SARS-CoV-2 has been shown to have novel glycosylation sites in the spike glycoprotein of 2019-nCoV, meaning that the virus can use various glycosylation sites to interact with its receptors. (13) According to research, SARS-CoV-2 spike protein has a greater affinity for the ACE2 receptor than SARS-CoV-1 spike protein. (14).

The infection will damage the walls and linings of the air sacs of the lungs in about 5% of severe COVID-19 cases. The lungs get inflamed and fill with fluid as the body continues to resist it. It could be more difficult for them to exchange oxygen and carbon dioxide as a result of this. (9)

There is evidence that 20-30% of seriously ill patients will form clots in their lungs, heart, brain, or legs, any of which can be fatal. (9)

Interferons are among the first cytokines produced during a viral infection and promote both innate and adaptive immunity. IFNs play a part in antiviral, antitumor, and immunomodulatory responses in addition to their antiviral properties (15). Interferons specifically target membrane receptors on healthy cells. Interferons are cytokines released by virus-infected cells that bind to membrane receptors on healthy neighboring cells and stimulate development of antiviral proteins (16). By inhibiting the development of viral nucleic acids and proteins, these antiviral proteins prevent neighboring cells from being infected (16).

Interferon-gamma (IFN-gamma) regulates immune and inflammatory responses in humans; there is only one form of IFN-gamma. It's created by T-cells that have been activated and natural killer cells. COVID-19 has a number of outcomes following infection with the SARS-CoV-2 coronavirus. Older males are more likely to develop the disease, which is more serious and fatal (18,19). COVID-19 can, however, be serious in some young people (20,21).

II. MATERIALS AND METHODS

Blood samples were taken from 100 Persons (75 patients with covid-19 and 25 health as control). After that, DNA extracted using Biocomma (Espaın)–RNA/DNA nucleic acid extraction Kits and G-spin™ Total DNA Extraction Mini Kit in order to assess the presence of IFN-G gene in SARS-COV-2 patients by using Conventional PCR. Ten samples for PCR product were taken for sequencing polymorphism detection.

IFN-G gene genotyping was performed with PCR using specific designer primers (Forward 5’-CGTTTCCGAGAATTAAGC-3’; Reverse 5’-CTGTGTGGCTTGTATTGTA-3’). In a 25 µl mixture of 4 µl DNA, 1.5 µl from each forward and reverse of the primer, 12.5 µl master mix and 5.5 µl nuclease-free water. The DNA amplification for IFN-G gene includes an initial denaturation of 2 min in 95 °C, 1 cycles of amplification (every cycle consists of denaturation of 30 s in 95°C, annealing of primers during 30 s in 59.3°C, and an extension of 40s in 72°C) and a final extension of 5 min in 72°C, then the PCR products were separated in 3% agarose electrophoresis system using ethidium bromide then visualized with the gel documentation, with 100 bp-ladder and photographed. The sequences of primers used for the amplification of the genes are presented in Figure 2 to detected gene polymorphisms and ELISA to evaluated the concentration of interferon-Gamma.
SPSS, ANOVA, and Chi–Square were used in the statistical analysis.

III. RESULTS AND DISCUSSION
In this study, all(100) participants,(75) patients with COVID-19 as the study group, and(25) healthy controls.

Using conventional PCR and gel electrophoresis to detected the presence INF gene with 700bp of IFN-G in positive patients of COVID-19. The PCR of targeted region of IFN-G gene was successfully shown monomorphic band 700bp as in figure (1).

Figure (1): Profile of gel electrophoresis of partial sequence of 700bp of IFN-G gene M1 molecular marker, 1-22 patient samples.

The PCR product of IFN-G gene were found to be in significant association with COVID-19 patients (P ≤ 0.493), revealed that the IFNG gene had a significant between alleles of the COVID-19 patient and control, the IFNG was 21.0% in control, whereas it was 62.0% in patients, as shown in the table (1-1) and Figure (2.)

Table (1-1) INF -Gene distribution and odd ratio of INF -Gene patient vs control groups

<table>
<thead>
<tr>
<th>INF-Gene</th>
<th>Positive</th>
<th>Control</th>
<th>Total</th>
<th>Odd Ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-Gene Positive</td>
<td>62</td>
<td>21</td>
<td>83</td>
<td>1.083</td>
<td>.838</td>
<td>1.400</td>
</tr>
<tr>
<td>INF-Gene Negative</td>
<td>13</td>
<td>4</td>
<td>17</td>
<td>0.640</td>
<td>.180</td>
<td>2.271</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>25</td>
<td>100</td>
<td>1.692</td>
<td>.371</td>
<td>7.720</td>
</tr>
</tbody>
</table>

Results of the present study shows that IFN-G level concentration estimated by ELISA. Both patients and controls have their concentrations tested and recorded in the Table (1.3).

Table (1-3) distribution of Concentration of INF-G in COVID-19 patient and control group
the result show there were significant between patient and controls with p value (0.165) These results suggest that INF- G level is related to COVID-19 disease activity, suggesting that INF- G may play some role in the pathogenesis of COVID-19 as shown in table (1-3). Persistent high levels of IFN-γ worsens the systemic inflammation, and increasing tissue injury and organ failure (22). Considering this ambiguous role of IFN-γ in the outcome, it is important to understand the serum pattern of this and other cytokines in patients with COVID-19.

this results were obtained using the Mean standard Deviation and P Value test using ELISA(ELISA) forevaluated INF- G. IFN-is the major cytokine produced by cells in response to viral infection and represents the central platform of the organism’s innate immune response to viral pathogens. (23, 24). This cytokine plays a role in every stage of the immune response (25). Antiviral security requires the IFN-system. Inhibits virus replication and stimulates T cell cytokine formation, enhancing the killing ability of cytotoxic T lymphocytes. (26).

Previous research has also shown that the level of IFN-Gamma was significantly higher in patients with new COVID-19 infection as compared to safe controls (27), however, Higher IFN-G levels were associated to a less prognosis, and higherIFN-levels were observed in early COVID-19 infection relative to healthy populations. Nevertheless, After ten days of symptoms, these levels were not maintained. In high - risk patients, IFN-G increased over time, but decreased in moderate patients, contradicting the findings reported here. In comparison to a control group, IFN-G(without disease) (28), Not only is IFN-G disruption related to COVID-19 and the immune system, but there is also a connection between micro biome change, cell transcriptome alteration, and gene overexpression, all of which can be related to the cytokine storm. (29).

Table (1-2) Concentration of INF- G gene in COVID-19 patient according age group

<table>
<thead>
<tr>
<th>Descriptives</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF- G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>64</td>
<td>167.82</td>
<td>36.96</td>
<td>0.165</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>151.28</td>
<td>51.59</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>165.37</td>
<td>39.55</td>
<td></td>
</tr>
</tbody>
</table>

The patients ranged in age from 20 to 65 years old. The Concentration of INF-Gin COVID-19 patient according age Furthermore, serum INF- G concentrations correlate with individual and composite measures of COVID-19 disease activity.

Serum INF- G concentrations are higher in patients with 20-39 y COVID-19 and low concentration are in > 60 y. INF- G concentrations are higher in patients with old age COVID-19 than in young patients with treated COVID-19 because more of patient has weak immune system, their concentration decrease in serum, INF- G at COVID-19 patients

The ten sequenced samples were successfully multiple alignment results of the 700bp samples revealed the 100% identity with reference strain "NG_015840.1" no variable positions in all ten analyzed samples in comparison with the referring reference IFNG gene sequences, Figure (2).
Figure (2): Multiple alignment sequences of ten patients samples with their corresponding reference sequences of the 700bp approximately amplicons of the IFNG gene sequences (selective sequence regions). The symbol “NG_015840.1” refers to the NCBI referring sequence, “IFNG:1-10” refer to the samples 1 to 10, respectively.

IV. CONCLUSION

The level of IFN-G rises in patient with COVID-19 The presence of IFN-G with a high level is most common two weeks after infection, especially those 20-39 year of age. no variable positions in all ten analyzed samples revealed 100 percent identity with reference strain “NG 015840.1”. which makes IFN-G are reliable mark in the diagnosis of the disease.

REFERENCES

9. 9. WebMD Medical Reference Reviewed by Neha Pathak, MD on September 23, 2020 Sources © 2020 WebMD, LLC. All rights reserved.
27. Mazin Jabbar Kathim1; Tamara Amer Taha2, Suzan Saadi Hussain3, Nihad Khalawe Tektook4 (2021) "IL-6, IL-0, IFN Gamma and CRP in Newly Diagnosed COVID 19 Patients” 1Department of Orthopaedic Surgery, Fallujah Technical Hospital, Inbar, 2Lecturer, College of Basic Education, University of Diyala, 3Prof, College of Science, Mustanisiriyah University, 4Assist. Prof. Middle Technical University, College of Medical and Health Technology, Medical Laboratory Techniques Dep., Baghdad-Iraq