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Analysis of IL33 in SARS-CoV-2 patients in Hilla city, Iraq

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Abstract--In this study, 170 patients with COVID-19 and Apparent health control (AHC) were included. The value of the il33 and demographic data was collected and their correlation with disease severity was analyzed. Patients were 47.17±11.72 years old on average, while control participants were 31.77±8.84 years old. the patients' group consisted of 38 (54.3%) males and 32 (45.7%) females, whereas the control group consisted of 33 (33.3%) males and 67 (67.0%) females. The case (with Covid 19) group had significantly greater levels of (IL33) than the control group.

Keywords---IL 33, COVID-19, TNF.

Introduction

The outbreak of Coronavirus Disease 2019 (Covid-19) in Wuhan, China, whose spreading dynamics remain unknown [1,2]. Interleukin 33 (IL-33), a newly identified member of the IL-1 cytokine family and a key regulator of inflammatory and immunological responses, has its gene on chromosome 9 [3]. SARS-CoV-2 infection tends to affect people of various ages, with the median age of infection being about 50 years [4] IL-6, TNF-, IL-8, IL-1, IL-21, and monocyte chemo-attractant protein-1 (MCP-1) are increased in macrophages and/or monocytes in response to SARS-CoV-2 infection to enhance pathogen clearance and tissue regeneration [1]. IL-33 also plays a role in the development of renal, neurological, hepatic, pulmonary, and ocular disorders. It is a cytokine produced by cells that increases inflammatory responses and has an alarming property [5].

Materials and Methods

In this study, 170 subjects were divided into two case-control groups, with the case group consisting of 70 patients with covid-19 who were all admitted to the ICU and diagnosed by a specialist physician with severe acute respiratory syndrome due to covid-19 documented by RT-PCR along with other clinical and laboratory criteria in Marjan Teaching Hospital in Babylon province. One hundred seemingly healthy persons were included in the study's control group, all of whom were found to be free of covid-19 using the Rapid Test (covid-19 antibody test negative).

Specimens Collection

Patients' blood specimens and AHC 3 ml venous blood were collected aseptically from all individuals using gel tubes and EDTA tubes for gating blood serum and then stored at (-20°C) at Babylon University's Virology Research Unit. The remaining 3ml of blood was maintained in the jelly tube without anticoagulant and allowed to coagulate on the laboratory table at 37°C for up to 1 hour. To prevent loss of bioactive human IL-33, the serum was centrifuged at 2500 rpm for 15 minutes before being collected and stored at -20°C until use.

Methodology of Research

After a thorough description of the study's purpose and methods, all participants were asked to agree to participate in the study. A complete questionnaire was completed, which included information such as name, age, gender. Serological testing for IL-33 was performed on all patients. Control subjects were chosen based on their medical and family history.

Human IL-33 (Interleukin 33) ELISA Kit

To quantify total IL33 in the blood, an enzyme linked immunosorbent assay (ELISA) was used to measure the concentration of IL33 in the serum of patients with respiratory illnesses. The Elabscience ELISA kit, which includes the solutions and components listed in the table below.

ELISA Reagents Preparation

- Buffer solution for washing:
This solution was made by diluting the standard wash buffer supplied with the kit in a ratio of 1:25, with the total volume of 30ml of wash buffer diluted in 750 ml of deionized or distilled water D.W and gently mixed. The wells in the microtiter plates were washed with wash buffer.
- Preparation of a standard solution:
These solutions were utilized to create the standard curve, which was then used to compute the concentration of the above-mentioned factors. These solutions must be made in 15 minutes. The preparation was completed by adding one ml of the standard and sample diluent solution provided with the kits, which resulted in a high concentration, and then transferring 0.5 ml of it into tube number 2, which contained 0.5 ml of standard and sample

diluent, to obtain a concentration half that of the previous concentration, and so on to obtain seven serial dilutions in the wells from (B-H), while zero concentration was placed in well A. (diluted only).

Statistical analysis

Antibody with biotinylation for detection:

- This solution is included in the kit in a 120 L quantity. It was made by diluting it 1:100 using a specific solution
- HRP (horseradish peroxidase conjugated):
- It was prepared by diluted in a ratio 1:100 with solution that provide with kit

Assaying Principles

The enzyme-linked immunosorbent assay (ELISA) is a medical diagnostic technique that employs antibodies/antigens and color change to identify a drug. The Sandwich-ELISA principle is used in the IL33 test, according to the Elabscience® made corporation in the United States. An antibody specific for Human IL33 has been pre-coated on the micro-ELISA plate. Standards or samples are mixed with the specific antibody in the micro-ELISA plate wells. After that, each microplate well is incubated with a Biotinylated detection antibody specific for Human. IL33 and an Avidin-Horseradish Peroxidase (HRP) conjugate. The addition of stop solution stops the enzyme-substrate reaction, and the color changes to yellow. At a wavelength of 450 nm the optical density (OD) is measured spectrophotometrically.

Elabscience ELISA technique for the assay

Procedures manual

- To the first two columns of wells, 100 µl of standard working solution was added. Each concentration of the solution was added to one well side by side in duplicate.
- In the remaining wells, 100 µl of sample serum was added.
- The plate was sealed with the kit's sealer and incubated at 37°C for 90 minutes.
- Without washing, the liquid was removed from each well, and 100 µl of Biotinylated Detection Ab working solution was immediately added to each well.
- The plates were covered with the plate sealer and gently mixed before being incubated at 37°C for 1 hour.
- After removing the solution from each well, 350 µl of wash buffer was added to each well. The solution from each well was removed and the plate dried against the clean absorbent paper after being soaked for 12 minutes. This step was repeated three times
- Each well was filled with 100 µl of HRP Conjugate working solution.

- The plate was sealed and incubated for 30 minutes at 37° C using the plate sealer.
- The solution was withdrawn from each well and washed five times with 350µl of wash buffer, as in the previous step.
- Substrate Reagent was added to each well in a volume of 90 µl.
- To shield the plate from light, it was immediately sealed with a new plate sealer and incubated for about 15 minutes at 37°C.
- Each well was filled with 50 liters of Stop Solution.
- Using a microplate reader set to 450 nm, measure the optical density (OD value) of each well at the same time, and compute the results using the Excel Microsoft Office 2016 program. ($y = 0.5653x - 1.1904$)

Table 1
In the plate, IL33 standards are arranged

| Tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-------|------|-------|-------|-------|-------|-------|-------|
| Pg/ml | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.63 |
| O.D | 2.78 | 2.203 | 1.779 | 1.005 | 0.691 | 0.39 | 0.312 |

Results

Patients with covid-19 and control participants had similar demographics.

Table 2
The demographic features of covid-19 patients and control groups

| | | | Control (N=100) | Patients (N=70) |
|------------------|------------|------|-------------------------|--------------------------|
| Age groups | <30 year | No | 47 | 2 |
| | | % | 47.0% | 2.9% |
| | 30-39 year | No | 32 | 23 |
| | | % | 32.0% | 32.9% |
| | 40-49 year | No | 14 | 16 |
| | | % | 14.0% | 22.9% |
| ≥ 50 year | No | 7 | 29 | |
| | % | 7.0% | 41.4% | |
| Mean± SD (Range) | | | 31.77±8.84 (21-53 year) | 47.17±11.72 (28-69 year) |
| Gender | Male | No | 33 | 38 |
| | | % | 33.0% | 54.3% |
| | Female | No | 67 | 32 |
| | | % | 67.0% | 45.7% |

N: number of cases; SD: standard deviation

Table 3
Comparison of the mean values of IL33 between case and control groups

| | | Median (IQR) | Mean Rank | Sum of Ranks | Mann-Whitney U | P. value |
|------|----------|----------------|-----------|--------------|----------------|----------|
| IL33 | Control | 3.09 (2.47) | 50.89 | 5089.00 | 39.000 | <0.001* |
| | Patients | 67.04 (158.40) | 134.94 | 9446.00 | | |

Discussion

The participants in this study were 170 people: 70 covid-19 patients who were admitted to the intensive care units in Merjan and Emam Al-sadiq hospitals in Babylon province, Iraq, between February and December 2020, and 100 seemingly healthy people. The demographic features of patients and control participants are depicted in (Table 2). The mean age of patients was 47.17 ± 11.72 and that of control subjects was 31.77 ± 8.84 years. These results correspond with the previous studies [5]. which found that the mean age of the patients with COVID-19 was 41.17 ± 15.11 years, while [6] revealed the mean age of the 101 hospitalized patients with confirmed COVID-19 was 45 ± 18.01 years (range, 11 months to 80 years). The frequency distribution of patients and control subjects according to age was also shown in (Table 2). These results found that the highest percentage of 29 (41.4%) of patients with COVID 19 fall in the age group (≥ 50 years), while the lowest percentage was 2 (2.9%) of them those aged less than 30 years. Regarding control' group included 47 (47.0%) of the age group <30 year, 32 (32.0%) for 30-39 year, 14(14.0%) for 40-49 year, and only 7(7.0%) for ≥ 50 year. These results agreed with the study findings done by [7].which revealed that the highest percentage 34.6% of the patients with COVID 19 fall in the age group (50–69 years).

Again, the patients' group consisted of 38 (54.3%) males and 32 (45.7%) females, whereas the control group consisted of 33 (33.3%) males and 67 (67.0%) females. These findings were consistent with previous study in Italy conducted by [8] found that the majority of COVID 19 patients (59.2%) were men, while[9]. found that 58.7% of COVID 19 patients were males and 41.3 percent were girls. Furthermore, the findings of this study corroborated those of [10].who found that the majority of Covid 19 patients were men. Males were shown to be more likely than females to be at risk (OR =0.54, CI=0.37-0.79, P=0.001). Females are less affected by COVID-19 than males, according to [11]. COVID-19 is more prevalent in older age groups, according to numerous research [12]. According to [13].there is a link between age and natural immunity, which has been examined elsewhere, and older people are more likely to develop infections as natural immunity drops with age.

In terms of gender, these findings corroborated previous research suggesting that male patients may have higher levels of angiotensin-converting enzyme 2 (ACE2), which is influenced by male sex hormones, putting them at increased risk of SARS-CoV-2 infection and poor clinical outcomes[14]. Furthermore, because the ACE2 gene encodes ACE2 expression, it is located on the X-chromosome, allowing

females to be theoretically heterozygous while males are absolutely homozygous, allowing males to be potentially high ACE2 expressors [15]. Females with X-linked heterozygous alleles, often known as sex dimorphism, may be able to halt the progression of SARS-CoV-2 infection and catastrophic clinical consequences by triggering a mosaic advantage[15].

Table 4 shows that the case (with Covid 19) group had significantly greater levels of (IL33) than the control group. The higher IL-33 levels in severe infection could occur from epithelial damage caused by intense contacts between the airway epithelium and activated immune cells, according to several interpretations. The SARSCoV-2-derived papain-like protease (PLpro), which is a potent inducer of IL-33 in epithelial cells [16], could also cause epithelium-derived IL-33 to activate inflammatory responses in the lungs. To see if SARSCoV-2 infection causes epithelial cells to produce IL-33[17]. IL-33, in particular, stimulates fast neutrophil migration via macrophage-derived CXCL1 and CXCL2, whereas neutrophil elastase and cathepsin G also contribute to IL-33 processing and maturation, hence exacerbating inflammatory reactions. It's possible that pathogenic 17 T cells can speed up neutrophil migration to the lungs by producing IL-17. Furthermore, in severe COVID-19 cases, immature neutrophils have been identified[18]. Increased IL-33/ILC2 responses may be to blame for neutrophil dysregulation, as IL-33 can educate neutrophils towards a specific immunosuppressive phenotype via ILC2s, dampening the appropriate antiviral T cell immune response [17].

Conclusion

This study concludes that those in who age group (≥ 50 year) were significantly more likely to be hospitalized for COVID-19 compared with those in who age group less than < 30 years while males are significantly more likely to be hospitalized for COVID-19 compared with females. The immunological parameter (IL33) levels for the case (with Covid 19) group was significantly higher than that for the control group.

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