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IL-6 Gene (174G/C) Single Nucleotide Polymorphism as an Indicator of COVID-19 Severity in Egyptian Patients

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The viruses' direct damage plays a part in the disease's beginning, the cytokine storm brought on by COVID-19 is crucial to the emergence of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). This study aimed to investigate the correlation between IL-6 and its IL- 6 gene (174G/C) single nucleotide polymorphism with the pathogenesis of COVID-19 severity in Egyptian patients.
Materials & Methods: Egyptian patients proved to be COVID-19 positive were classified into three groups; Group 1 (mild cases), Group 2 (moderate cases) and Group 3 (severe and critical cases). Complete blood count, liver function tests, renal function tests, CRP, and coagulation profile, serum ferritin, D-dimer, procalcitonin, lactate dehydrogenase and detection of serum interleukin 6 levels, and IL-6 gene polymorphism were investigated for all enrolled 135 patients.
Results: There was a significant decrease of platelets count of group II & III as compared to group I. There was a significant increase of total leucocytic count, CRP, ferritin, D – dimer, procalcitonin,

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blood urea and IL6 of group III as compared to group I & II. There was significant decrease of relative lymphocyte counts % of group III as compared to group I & II. There was significant increase of LDH of group II & III as compared to group I. There were no statistically significant differences were found as regards hemoglobin, SGPT, SGOT, serum creatinine and I.N.R & interleukin-6 174G/C distribution of the different studied groups.
There were positive correlations between serum interleukin 6 levels and CORAD scores, ferritin count, LDH, and D- dimer, and blood urea, and only a negative correlation with relative lymphocyte count %.
Conclusion: interleukin-6 plays an important role in the pathogenesis of COVID-19. In addition, it reveals that serum IL6 can be used as a predictor of disease severity in COVID-19 patients, but interleukin-6 174G/C genotype distribution cannot be used as a predictor of disease severity in COVID-19 Egyptian patients.

Keywords: COVID-19 severity; COVID-19 disease's; cytokine release syndrome.

1. INTRODUCTION

The coronavirus disease of 2019 (COVID-19), which the World Health Organisation (WHO) has classified as a public health emergency of international significance, has severely taxed public health systems in both developed and underdeveloped nations. When compared to SARS-CoV and MERS-CoV, SARS-CoV-2 exhibits a more effective transmission pattern [1].

After attaching to the lung's type II alveolar epithelial cells, SARS-CoV-2 stimulates the innate and adaptive immune systems; this overstimulation of the immunity may harm the host cells more than the SARS-CoV-2 as a foreign invader [2].

A systemic inflammatory reaction, also known as "cytokine release syndrome" (CRS) or "cytokine storm syndrome" (CSS), can be carried on by a number of issues, including infections in addition to certain pharmaceuticals [3].

Although the viruses' direct damage plays a part in the disease's beginning, the cytokine storm brought on by COVID-19 is crucial to the emergence of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [4]. ARDS with hypoxemic respiratory failure and macrophage activation syndrome (MAS) are the symptoms of severe COVID-19 that occur most commonly. Overexpression of pro-inflammatory cytokines damages important organs, particularly the lungs, and induces endothelial dysfunction in both of these clinical disorders [5].

A pleiotropic cytokine known as IL-6 is produced by immune cells such dendritic cells (DC), monocytes, macrophages, B cells, and certain subsets of activated T-cells as well as by other cells that are not immune like fibroblasts, epithelial cells, and keratinocytes [6]. It aids in the pathogenesis of autoimmune and inflammatory disorders [7].

There have been multiple documented polymorphisms of the IL-6 gene, which is found on chromosome 7. The single nucleotide polymorphism (SNP) 174C and -174G in the promoter region, which has been linked to IL6 transcription rates, is the polymorphism that has been the topic of the most research. IL-6-174C alleles can be found in the general population at an average prevalence of about 40% [8].

It has been demonstrated that the 174G/C (rs1800795) and 572C/G (rs1800797) IL-6 gene promoter polymorphisms have a direct effect on the two different transcription and secretion levels of IL-6 [9].

IL-6 -174C allele carrier status is linked to higher levels of IL-6 production and more severe kinds of pneumonia typically. The G allele 174 SNP is coupled with the enhanced transcription upon endotoxin and IL-1 activation. This data supports the idea that IL-6 is essential for novel coronavirus pneumonia (NCP) development because it was the IL-6-174 C allele, not the G allele, that increased the risk of sepsis carried on by pneumonia [10].

The researchers hypothesized that IL-6 and its IL- 6 gene (174G/C) single nucleotide variants was responsible for differentiation of COVID-19 severity in Egyptian patients.

This study aimed to investigate the correlation between IL-6 and its IL- 6 gene (174G/C) single nucleotide polymorphism with the pathogenesis of COVID-19 severity in Egyptian patients.

2. MATERIALS AND METHODS

This prospective observational study was conducted in Tanta University Hospitals over a duration of 18 months from October 2020 to June 2022, on Egyptian patients proved to be COVID-19 positive aged more than 18 years old of both genders by clinical correlation combined with positive PCR or rapid antigen detection test.

The Tanta University ethics review board granted the study their approval. The Tanta University Faculty of Medicine's Research Ethics Committee issued the acceptance code, which was 34033/08/20. ClinicalTrials.gov recorded the trial and assigned it a special registration identification number (ID: NCT04544033).

Following a thorough explanation of the study's objectives, each patient signed a written informed permission. A secret code number was used to maintain the confidentiality of the data and the privacy of the participating patients.

Patients suffering from long-term illnesses, such as HIV infections and hepatitis B or C. Any malignancy or chronic inflammation, as well as any other chest disorders (such as tuberculosis), were prohibited from participation in this study. Autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis were also not included.

Egyptian patients proved to be COVID-19 positive were classified according to Ministry of health and Population COVID-19 Protocol (MOHP), EGYPT [11].

Mild criteria were defined as patients with mild clinical symptoms & clinically stable with CT changes including no changes or just subpleural nodule or subpleural line.

Moderate criteria were presented as patients with non-specific and specific respiratory infections (pneumonia). CT changes in both lungs included ground glass opacities (GGO), crazy paving, consolidation, and multiple interlobular thickening.

Severe criteria were defined as patients with respiratory distress: respiratory rate (RR) > 30/min, peripheral oxygen saturation (SPO2) < 92 at room air. Chest radiology showed more than 50% lesion or progressive lesion within 24 to 48 hours. CT changes in both lungs included extensive (GGO), crazy paving, consolidation, multiple interlobular thickening, and fan-shaped distribution of peri-bronchial thickening.

Critical criteria were demonstrated as patients meet the criteria of the respiratory failure requiring mechanical ventilation or multi-organ failure.

Critical case with any of the following included:

1. Moderate hypoxemia, which indicated by: Peripheral oxygen saturation (SPO2) / Fractional inspired oxygen (FIO2) ≤ 235, SPO2 < 95% despite the use of ≥ 4 L of oxygen by nasal cannula, partial arterial oxygen tension (PaO2)/ Fractional inspired oxygen (FIO2) ≤ 200 mmHg.
2. Moderate to severe hypoxemia with PaO2 /FIO2 < 150 mmHg.
3. Sepsis, diagnosed by quick Sequential Organ Failure Assessment score (q SOFA) i.e. by the presence of 2 out of 3:

- Change of sensorium
- Tachypnea > 22 breath/min
- Hypotension i.e. systolic blood pressure < 100 mmHg.

4. Septic shock diagnosed by the presence of sepsis that needs norepinephrine to maintain mean arterial pressure (MAP) ≥ 65 mmHg and/or associated with blood lactate > 4mmol/L.
5. Multi-organ failure i.e., ≥ 2 organ failure, diagnosed by SOFA score of ≥ 6 6. Coagulopathy i.e., platelets count < 100,000/mm^3, PT > 13 seconds and/or fibrinogen < 1 g/L.

Cytokine storm syndrome is uncommon hyper-inflammatory syndromes that may be triggered by severe viral pneumonias. The syndrome is associated with multi-organ failure, an elevated cytokine profile, and may be associated with thrombosis. Features included severe COVID-19 patient with adult respiratory distress syndrome (ARDS) showing: Unremitting fever, cytopenia, elevated ferritin, increased inflammatory markers e.g., CRP, LDH, IL-6 increases.

COVID-19 patients were divided into three groups:

- Group I (Mild): 45 COVID-19 patients with mild criteria.
- Group II (Moderate): 45 COVID-19 patients with moderate criteria.

- Group III (Severe & Critical): 45 COVID-19 patients with severe and critical criteria.

The following procedures were applied to all patients:

In addition to demographic factors like age and gender, clinical evaluation also involved taking a patient's medical history. The CO-RADS Categorical CT Assessment Scheme for COVID 19 Patients was classified.

2.1 Specimens

Blood collections were performed under sterile conditions. A 5.0 ml blood was collected from each patient, about 3.0 ml of them were collected in plain tube then centrifuged, and serum was taken for Interleukin 6 level assay. The remaining 2.0 ml of blood were collected in EDTA vacutainer tube & stored at -20°C for molecular investigation.

Routine laboratory investigations including complete blood count (CBC), liver function tests, renal function tests, CRP, coagulation profile were performed. Other investigations such as serum ferritin, D-dimer, procalcitonin, and lactate dehydrogenase (LDH) were investigated also.

Specific laboratory investigations including detection of serum interleukin 6 (IL-6) level using Enzyme-linked Immunosorbent Assay (ELISA) Kit for human Interleukin 6 provided by Bioneovan Company, (catalogue no: In-Hu2192) and detection of IL-6 gene polymorphism using restriction fragment length polymorphism (RFLP) method.

2.1.1 Measurement of serum Interleukin 6 level

Using Enzyme-linked Immunosorbent Assay (ELISA) Kit for human Interleukin 6 provided by Bioneovan Company, (catalogue no: In-Hu2192).

Preparation of the wash: The 30X washing concentrate was diluted 30 times with distilled water.

Procedure:

1. Dilution of Standards: Five wells were set for standards in a micro ELISA strip plate. In well 1, 100 µl standard solution was mixed well with 50µl standard dilution buffer. 50 µl of standard dilution buffer was added to well 2, 50 µl of the well 1 solution was discarded, and 100 µl of the well 1 solution was put into well 2.

Well, 3 received 50 µl of the well 2 solution, followed by 50 µl of standard dilution buffer, which was thoroughly mixed. 50 µl of the solution from well 3 was added to well 4, and 50 µl of the standard dilution buffer was then added and thoroughly mixed. 50 µl of the solution from well 4 was put into well 5, 50 µl of the standard dilution buffer was added and thoroughly mixed, and 50 µl of the solution from well 5 was discarded.

After dilution, the total volume in all the wells was 50µl with different concentrations. These concentrations were 60 pg/ml, 40 pg/ml, 20 pg/ml, 10pg/ml, and 5pg/ml, respectively.

2. In the micro-ELISA strip plate, as a blank control, a well was left unfilled. 40 ml of sample dilution buffer and 10 ml of sample were introduced to sample wells (dilution factor is 5). Without hitting the well wall, samples were put onto the bottom and gently mixed.
3. Incubation was performed for a duration of about 30 min at a temperature of 37℃ being sealed with closing plate membrane.
4. Distilled water was used to dilute the concentrated washing buffer. (30 times for 96 T).
5. It was aspirated and refilled with the wash solution. After 30 seconds of relaxing, the wash solution was dumped. Five times, the washing process was carried out.
6. Except for the blank control well, 50 l of HRP-conjugate reagent was applied to each well.
7. Incubation was well demonstrated in Step 3.
8. Washing was well demonstrated in Step 5
9. Coloring: Each well received 50 l of Chromogen Solution A and 50 l of Chromogen Solution B, which were then gently mixed together and incubated at 37 °C for 15 minutes.
10. Termination: To stop the reaction, 50 l of stop solution was added to each well. The well's blue color was altered to yellow.
11. The micro titer plate reader was used to read the absorbance O.D. at 450 nm. The blank control well's OD value was set to zero.

2.1.2 Detection of IL-6 gene polymorphism using RFLP method

Principle:

1. Extraction of DNA from the sample
2. Amplification of DNA by PCR using specific primer.
3. Usage of restriction enzymes to cut PCR product.
4. Electrophoresis of DNA fragments on agarose gel to separate them by size for detection of polymorphism.

DNA Extraction Protocol: Genomic DNA Mini Kit (Blood) from Biovision Company was used to extract DNA from peripheral whole blood utilising DNA extraction kits.

Measurement of DNA Concentration and Purity: Using a De Novix DS-11 spectrophotometer, the absorbance (A) at 260 nm was used to calculate the DNA concentration. A260/A280 ratio of pure DNA was (1.7-2.0).

Conventional Polymerase Chain Reaction (PCR): Amplification of the DNA extracts using conventional PCR followed by gel electrophoresis.

DNA amplification: DNA extracts were amplified for RS1800795 polymorphism using the primers provided by Clinilab company:

Forward: 5′-CATGCCAAAGTGCTGAGTCA -3'
Reverse:
5'- TCTTTGTTGGAGGGTGAGGG -3'

Individual PCR reactions were performed in a total volume of 20 µl using 2 µl of DNA extract, 1 µl of each primer, 7 µl nuclease free water and 10 µl master mix (Roche Molecular Biochemicals) to make up the volume. DNA amplification was carried out using Thermo cycler instrument.

The amplification protocol was as follows: 35 cycles of denaturation at 95 ^{o}C for 3min, annealing at 61 ^{o}C for 30 seconds, 72 ^{o}C for 30 sec followed by final extension at 72 ^{o}C for 10 min and hold at 4^{o} C.

Visualization of amplified material by gel electrophoresis: The samples were demonstrated as a clear, sharp, distinct band at the specific molecular weight (211 bp).

Detection of rs1800795 polymorphism using restriction enzyme fragment length polymorphism:

1. Vials containing amplified DNA samples, restriction enzyme, and assay buffers were put on ice before the experiment began.
2. The restriction enzyme Hin1II (NlaIII) (provided by Thermo scientific® lot no: 01169397) was used to digest the test samples. NlaIII detects the palindromic and complementary DNA sequence of CATG/GTAC and cuts outside of the G-C base pairs. This cutting method results in sticky ends with GTAC overhangs at the 3' end. The reaction mixture contained 2 µl Hin1II (NlaIII) enzyme, 10 µl DNA sample (PCR product), 2 µl 10X Buffer Tango, and 18 µl Nuclease free water
3. After preparing the reaction tubes, the components were mixed by gentle pipetting and tapping.
4. The tubes were incubated at 37^{o}C for 16 hours (overnight).
5. After incubation, A sterile tube was used containing a mixture of 1 µl of the loading buffer and 5µl of the reaction mixture
6. Then samples were electrophoresed in agarose gel.

2.2 Statistical Analysis

Categorical data were presented as numbers and frequencies (%) after analysis using Chi-square test, while parametric data were analyzed by One-way ANOVA test and post-hoc Tukey's HSD Test and expressed as mean ± standard deviation. Kruskal-Wallis Test was used for the statistical evaluation of non-parametric data, which are expressed as median and interquartile range with intergroup comparison carried out using Mann–Whitney test. The results were statistically significant when the P-value was less than 0.05.

3. RESULTS

In this study, 135 patients who had been clinically correlated with a positive PCR or quick antigen detection test for COVID-19 were examined. The patients were distributed as follows: 45 patients in Group I (mild cases), 45patients in Group II (moderate cases), and 45 patients in Group III (severe and critical cases).

Regarding demographic data including age and gender, there were no statistically significant

differences in the comparison of mean values of the different groups (p value = 0.15, 0.256 respectively).

The CORAD scores presented in different groups as follows:

Group I: included seven patient (15.5 %) had score 1, 15 patients (33.3 %) had score 2, 19 patients (42.2%) had score 3, 4 patients (8.9%) had score 4, and no patients (0 %) had score 5.
Group II: included 2 patients (4.4%) had score 3, 10 patients (22.2%) had score 4, and 33 patients (73.3%) had score 5.

Group III: included only one patient (2.2%) had score 3, 4 patients (8.88%) had score 4, and 40 patients (88.88%) had score 5.

The comparison of the distribution of CORAD scores in the three groups there was statistically significant difference (p value < 0.0001) Table 1.

Table 1. Demographic and clinical data of the studied groups

		Group I (n=45)	Group II (n=45)	Group III (n=45)	P value
Age (years)		45.22 ± 12.4	48.13 ± 13.5	50.68 ± 16.84	0.15
Gender (F/M)	**F**	15 (33.3%)	13 (28.8%)	19 (42.3%)	0.256
	M	30 (66.6%)	32 (71.2%)	26 (57.7%)	
CORAD score (1-5)	**1**	7 (15.5 %)	0 (0%)	0 (0%)	< 0.0001*
	2	15 (33.3 %)	0 (0%)	0 (0%)	
	3	19 (42.2%)	2 (4.4%)	1 (2.2%)	
	4	4 (8.9 %)	10 (22.2%)	4 (8.88%)	
	5	0 (0%)	33 (73.3%)	40 (88.88%)	

Data are presented as mean ± SD or number (%)

Regarding Complete blood pictures characteristics of the studied groups, there were no statistically significant differences in the comparison of mean values of hemoglobin of the different groups (p value = 0.222). There was significant decrease of mean value of platelets count of group II & III as compared to group I (p value = 0.001, and 0.025 respectively).

While there was no significant difference between mean values of platelets count of group II & III (p value = 0.334). There was significant increase of mean value of total leucocytic count of group III as compared to group I & II (p value = 0.014, and 0.001 respectively). While there was statistically insignificant difference between mean values of total leucocytic count of group I & II (p value = 0.316). There was significant decrease of mean value of relative lymphocyte counts % of group III as compared to group I & II (p value < 0.0001, and < 0.0001 respectively). While there was no significant difference between mean values of relative lymphocyte counts % of group I & II (p value = 0.95) Table 2.

Table 2. Blood picture parameters of the studied groups

	Group I (n=45)	Group II (n=45)	Group III (n=45)	P value
Hemoglobin (gm/dl)	11.65 ± 1.27	12.21 ± 1.87	11.86 ± 1.78	0.222 P1= 0.084 P 2= 0.52 P 3= 0.299
Platelets count ($\times10^3$/mm^3)	278.66 ± 100.67	213 ± 95.38	233.98 ± 105.86	0.04* P1= 0.001* P 2= 0.025* P 3= 0.334
Total leucocytic count ($\times10^3$/mm^3)	7.8 ± 5.73	6.79 ± 4.11	10.27 ± 5.056	0.04* P1= 0.316 P 2= 0.014* P 3= 0.001*
Lymphocyte (Relative count %)	20.22 ± 4.41	20.28 ± 5.49	15.89 ± 5.38	< 0.0001* P1= 0.95 P 2= < 0.0001* P 3= < 0.0001*

*Data are presented as mean ± SD. * Denoted significant difference between groups (P < 0.05). P value presented the comparison between the three groups. P1 value presented the comparison between Group I and Group II. P2 value presented the comparison between Group I and Group III. P3 value presented the comparison between Group II and Group III.*

Regarding Inflammatory markers of the studied groups, there was significant increase of mean value of CRP of group III as compared to group I & II (p value < 0.0001, and < 0.0001 respectively). There was no significant difference between mean values of CRP of group I & II (p value = 0.914). There was significant increase of mean value of ferritin of group III as compared to group I & II (p value < 0.0001, and 0.048 respectively). Also, there was significant increase of mean value of ferritin of group II as compared to group I (p value = 0.008).

There was significant increase of mean value of D - dimer of group III as compared to group II & I (p value < 0.001, and < 0.0001 respectively). Also, there was significant increase of mean value of D - dimer of group II compared to group I (p value = 0.0001). There was significant increase of mean value of procalcitonin of group III as compared to group I & II (p value = 0.003, and = 0.001 respectively). There was no significant difference between mean values of procalcitonin of group I & II (p value = 0.573). There was significant increase of mean value of IL6 of group III as compared to group I & II (p value < 0.0001, and < 0.0001 respectively). While there was no significant difference between mean values of IL6 of group I & II (p value = 0.235) Table 3.

Regarding Biochemical and coagulation parameters of the studied groups, there was significant increase of mean values of LDH of group II & III as compared to group I (p value < 0.0001), while there was no significant difference between mean values of LDH of group II & III (p value = 0.753). There were no statistically significant differences were found between the studied groups as regard mean values of SGPT, SGOT, serum creatinine and I.N.R of the different studied groups (p value > 0.05).

Table 3. Inflammatory markers of the studied groups

	Group I (n=45)	Group II (n=45)	Group III (n=45)	P value
CRP (mg/dl)	32.31 ± 25.56	31.38 ± 32.84	66.26 ± 67.75	< 0.0001* P 1= 0.914 P 2= < 0.0001* P 3 = < 0.0001*
Ferritin (ng/ml)	272.68 ± 164.84	405.56 ± 242.3	508.36 ± 327.64	< 0.0001* P1= 0.008* P2 = < 0.0001* P 3= 0.048*
D dimer (ng/ml)	0.257 ± 0.36	0.652 ± 0.49	0.95 ± 0.42	< 0.0001* P1= < 0.0001* P2 = < 0.0001* P 3= 0.001*
Procalcitonin (ng/ml)	1.11 ± 1.78	1.33 ± 1.18	4.39 ± 8.45	0.002* P1= 0.573 P2 = 0.003* P 3= 0.001*
Interleukin 6 (pg/ml)	72.5 ± 19.81	81.73 ± 19.79	127.85 ± 63.86	< 0.0001* P1= 0.235 P2 = < 0.0001* P 3= < 0.0001*

*Data are presented as mean ± SD. * Denoted significant difference between groups (P < 0.05). P value presented the comparison between the three groups. P1 value presented the comparison between Group I and Group II. P2 value presented the comparison between Group I and Group III. P3 value presented the comparison between Group II and Group III*

Table (4): Biochemical and coagulation parameters of the studied groups

	Group I (n=58)	Group II (n=46)	Group III (n=47)	P value
LDH (U/L)	202.24 ± 139.76	343.87 ± 152.81	354.46 ± 194.23	< 0.0001* P 1= < 0.0001* P 2= < 0.0001* P 3= 0.753
SGPT (U/l)	48.79 ± 28.43	42.45 ± 22.83	51.86 ± 43.98	0.377 P 1= 0.335 P 2= 0.634 P 3= 0.172
SGOT (U/l)	46.69 ± 24.39	45.67 ± 22.88	56.82 ± 71.91	0.403 P 1= 0.908 P 2= 0.25 P 3= 0.231
Serum creatinine (mg/dl)	1.04 ± 0.54	1.07 ± 0.404	1.44 ± 1.46	0.051 P 1= 0.84 P 2= 0.24 P 3= 0.055
Blood urea (mg/dl)	34.95 ± 9.19	32.09 ± 13.18	51.11 ± 30.82	< 0.0001* P 1= 0.458 P 2= < 0.0001* P 3= < 0.0001*
I.N.R	1.22 ± 0.135	1.35 ± 0.15	1.53 ± 0.29	0.18 P 1= 0.085 P 2= 0.863 P 3= 0.138

*Data are presented as mean ± SD. * Denoted significant difference between groups (P < 0.05). P value presented the comparison between the three groups. P1 value presented the comparison between Group I and Group II. P2 value presented the comparison between Group I and Group III. P3 value presented the comparison between Group II and Group III.*

There was significant increase of mean value of blood urea of group III as compared to group I & II (p value < 0.0001, and < 0.0001 respectively). While there was no significant difference between mean values of blood urea of group I & II (p value = 0.458) (Table 4).

Interleukin-6 174G/C genotypes distribution of the studied groups was presented as follows:

- 44 patients (97.8%) had GC genotype, one patient (2.2%) had GG genotype, and none had CC genotype in Group I.
- 42 patients (93.4%) had GC genotype, three patients (6.6%) had GG genotype, and none had CC genotype in Group II.
- 41 patients (91.2%) had GC genotype, three patients (6.6%) had GG genotype, and only one patient (2.2%) had CC genotype in Group III.

Table 5. Interleukin-6 174G/C genotypes distribution of the studied groups

		Group I (n=45)	Group II (n=45)	Group III (n=45)	P value
Interleukin-6 174G/C	GG	1 (2.2%)	3 (6.6%)	3 (6.6%)	0.258
	GC	44 (97.8%)	42 (93.4%)	41 (91.2%)	
	CC	0 (0%)	0 (0%)	1 (2.2%)	
	G allele	46	48	47	0.216
	C allele	44	42	43	

** Denoted significant difference between groups (P < 0.05)*

Table 6. Correlation between Interleukin 6 174G/C genotypes distribution, serum IL6 levels with different parameters of COVID – 19 patients

	Interleukin6 174G/C genotypes	Serum IL6
	P value	P value
Age	0.184	0.295
Gender	0.154	0.643
CORAD score	0.365	0.001*
HB	0.06	0.081
Platelets count	0.335	0.123
Total leucocytes count	0.072	0.27
Relative lymphocyte count %	- 0.651	0.002*
CRP	0.249	0.058
Ferritin	0.537	0.01*
LDH	0.583	0.001*
Procalcitonin	0.329	0.104
D – dimer	0.585	0.001*
SGPT	0.485	0.245
SGOT	0.556	0.079
Urea	0.722	0.004*
Creatinine	0.189	0.597
I.N.R	0.445	0.606
Patients' concentration	0.493	0.733
Interleukin-6 174G/C genotypes		0.174

** Denoted significant difference between groups (P < 0.05)*

There was no significant difference between the studied groups as regard interleukin-6 174G/C distribution (p value = 0.258). As regard Interleukin-6 174G/C allele distribution, Group I showed 46 G allele and 44 C allele, Group II showed 48 G allele and 42 C allele, while Group III showed 47 G allele and 43 C allele. There was no significant difference between the studied groups as regard interleukin-6 174G/C allele distribution (p-value = 0.216) (Table 5).

There were positive correlations between serum interleukin 6 levels and CORAD scores, ferritin count, LDH, and D- dimer, blood urea, and only a negative correlation with relative lymphocyte count % (P value < 0.05). There was no significant correlation between Interleukin-6 174G/C genotypes distribution and other different recorded parameters of the studied groups (p-value> 0.05) (Table 6).

4. DISCUSSION

Since the start of the novel coronavirus disease 2019 (COVID-19) in Wuhan, China in December 2019, there have been over 70 million infections that have been officially confirmed around the world, and this number is still rising. The majority of infected people exhibit no symptoms or have minor symptoms including anosmia, sore throat, malaise, and arthralgia. However, the clinical course may be more severe in older individuals, those with diabetes and hypertension, those with HIV or who are on long-term immunosuppressive therapy, and those who are pregnant [12].

After adhering to the lung's type II alveolar epithelial cells, SARS-CoV-2 stimulates the innate and adaptive immune systems; this overstimulated immune response may harm the host cells more than the SARS-CoV-2 as a foreign invader [13].

The present study showed that 47 (34.8%) were females while 88 (65.1%) were males. This was in accordance with Li et al., who included that 56% of all patients were male, and 44% were female patients [14].

The Yang et al., study found that high protein expression of ACE2 receptors in certain organs was linked to organ failures. The increased circulating ACE2 levels are higher in men than in women and increased also in diabetic or cardiovascular patients. This helped to explain the increased prevalence of the male gender [15].

The present study showed that regarding age and gender, there were no statistically significant differences in the comparison of mean values of the different groups. This was in accordance with Huang et al., who reported that there were no significant differences in the disease severity of

COVID-19 patients regarding age and gender [16].

In contrast, Eljilany and Elzouki., suggested that the severity of COVID-19 increased with age. They also revealed that older patients with associated comorbidities have a poor prognosis with an increased rate of complications of COVID-19. This may be attributed to the chronic disorders and declined immune function in elderly people that increased their liability to infections and their complications [17].

As regard the distribution of CORAD scores in the three groups there was a statistically significant difference.

In the present study, there were no statistically significant differences in the comparison of mean values of hemoglobin of the different groups. This was in agreement with Bergamaschi et al., who found that Anaemia mainly affects old, fragile people and is caused by inflammation; it is occasionally linked to iron and/or vitamin deficits. Anaemia does not directly affect mortality. As opposed to, According to Henry et al.'s report from, anaemia in severe cases of COVID-19 is not solely caused by inflammation but also involves a number of other factors, such as direct cytopathic injury brought on by infection of circulating erythrocytes or their bone marrow precursors, indirect erythrocyte damage brought on by hemolytic anaemia, or thrombotic microangiopathy. The present study measured hemoglobin levels once COVID diagnosis and not followed the Hb level through the prognosis of the COVID patients [18,19].

In the current study, there was a significant increase in mean values of the total leucocytic count of severe groups. This was in accordance with Anurag et al., who found that severe COVID-19 patients demonstrated increased total leukocytic count and differential neutrophil count. In contrast, Sun et al., suggested that lower counts of leucocytes, lymphocytes, eosinophils, platelets, and hemoglobin were presented in COVID-19 patients [20,21].

In the current study, there was significant decrease of mean values of relative lymphocyte counts % of severe cases as compared to mild & moderate This was in accordance with Azab et al., who explained that reduced lymphocyte count in sever COVID-19 infection was due to lymphocyte sequestration in specific target organs [22].

This was in accordance with Tan et al., who concluded that the majority of COVID-19 patients exhibit lymphopenia; this indicates that SARS-CoV-2, like SARS-CoV, may primarily impact lymphocytes, particularly T cells [23].

This was in accordance with Vaninov., suggested that A cytokine storm is brought on by viral particles that invade other cells after spreading from the respiratory mucosa. It is thought that cytokine storm development is significantly influenced by damage to T cells. As a result, lymphopenia might be a reference parameter that is applied in the COVID-19 diagnostic [24].

This was in accordance with Tan et al., who suggested that the significant reduction of human leukocyte D antigen expression (HLA-DR) with the depletion of natural killer (NK) cells, CD4 + lymphocytes, and CD19 + lymphocytes lead to significant affection of the function of lymphocyte. Lymphocyte affection was closely correlated with high levels of IL6. The development of lymphopenia in COVID-19 patients because of inducing lymphocytic apoptosis might be due to high IL6 levels [23].

As regards platelets count, the mean values of group moderate and severe were statistically significant decrease as compared to group mild This was in agreement with Thachil., that found SARS-CoV-2 could hold megakaryocytes and block the release of platelets as another reason for thrombocytopenia [25].

In the present study, there was significant increase of mean values of inflammatory markers such as CRP, d-dimer and lactate dehydrogenase of severe cases as compared to mild & moderate cases, this was in agreement with Wölfel et al., who concluded that A dysfunctional immune response causes unregulated inflammation and end-organ damage, and the severe/critical phase of the disease is characterized by severe interstitial pneumonia and elevations in inflammatory markers that seem to be primarily immune-mediated rather than due to direct viral cytotoxicity [26].

The current results revealed that IL-6 levels were higher in COVID-19 patients with severe symptoms than those with mild and moderate symptoms and there were positive correlations between serum interleukin 6 levels and CORAD scores, ferritin count, LDH, and D- dimer, blood urea, and only negative correlation with relative

lymphocyte count %. Liu et al., reported that IL-6 increased in 67.9 % of COVID-19 patients on admission and that it was significantly higher in patients with severe symptoms. Also, Sayah et al., reported that the values of IL-6 were significantly higher in patients with severe COVID-19 patients than in non-severe patients [27,28].

Zhang et al., and Michot et al., explained that, By increasing arterial permeability, IL-6 can impair tissue perfusion, harm endothelial cells, and result in the production of microthrombi. Fluid builds up in the interstitial spaces and lung tissue as a result of this increase in vascular permeability, which clinically shows as acute respiratory failure [29,30].

Also, Verma et al., reported that in multiple investigations, the cytokine profiles of COVID-19 patients were examined, and it was discovered that the cytokine storm is associated with a poor prognosis in severe COVID-19 cases. Viral infections activate innate and adaptive immunity, which causes the production of several cytokines, including IL6 [31].

Additionally, Falahi et al., suggested that, in predicting and advancing Covid-19, IL-6 is a significant inflammatory cytokine that exceeds C-reactive protein (CRP) and other prognostic factors like leukopenia, fibrinogen, ferritin, prothrombin time, and D-dimer [32].

This study showed significant increase of mean values of procalcitonin of severe cases as compared to mild &moderate cases, this was in accordance with Huang et al., who found that Viral infections reduce PCT production due to interferon-, whereas bacterial infections promote extrathyroidal synthesis of PCT, which is actively maintained by high levels of IL-6, IL-1, and TNF. This explains why high serum PCT levels were linked to severe COVID-19 and mortality, but serum PCT concentrations were normal in COVID-19 cases that were not complex. Patients with severe COVID-19 may be more susceptible to bacterial infection because to the immune system's malfunction brought on by low lymphocyte levels and decreased immune cell function [33].

In the present study, there was significant increase of mean values of serum ferritin of severe cases as compared to mild &moderate cases, this was in accordance with Siddiqi and Mehra., who found that during the systemic hyperinflammation stage of COVID-19, inflammatory cytokines and biomarkers such ferritin, PCT, and D-dimer are markedly elevated. In this stage, known as the cytokine storm, severe hyperinflammation can cause multi-organ failure and cardiac collapse [34].

The current study showed that there were no statistically significant differences between the studied groups as regard mean values of SGPT and SGOT. This is in accordance with Zhang et al., who found that SARS-CoV-2 infection usually did not harm the liver. In contrast, Bertolini et al., reported that COVID-19 patients had elevated liver enzymes on admission and that those elevations were associated with severe disease [35,36].

In the present study, no statistically significant differences were found between the studied groups as regard mean values of serum creatinine of the different studied groups This was in accordance with Ok et al., who found that serum creatinine levels were similar in the severe and non-severe patients in contrast with Nogueira et al., who found that COVID-19 can not only affect the lungs but also, it can affect the functions of kidney leading to an increase in serum creatinine and blood urea nitrogen in patients with severe COVID-19. The impairment of the kidney can be explained by high expression of ACE2 in proximal convoluted tubules of kidney [37,38].

In the present study, there was a significant increase in mean values of blood urea of group III as compared to groups I & II, this was in accordance with Ismail et al., who found that an increase in CORAD score was correlated with a significant increase in LDH, liver enzymes, and urea levels [39].

In the present study, there was no significant difference between the studied groups as regard interleukin-6 174G/C distribution, as the most frequent genotype is GC. This was in agreement with Falahi et al., who reported There is no statistically significant difference between patients with severe COVID-19 and those with mild COVID-19. They discovered that both patient groups had higher frequencies of G allele and GG genotype SNPs 174 G / C (rs1800795) than the C allele and CC genotype [32].

Falahi et al., reported that, in contrast to this study, a recent investigation found the IL-6 polymorphism at position 174 G/C (rs1800795) was closely correlated with the risk of developing COVID-19 in the Turkish population [32].

In contrast, Fishchuk et al., showed the rate of the CC genotype and C allele of the IL-6 gene polymorphism in all patients of the study were significantly increased compared with population frequencies. Also, Dhabaan et al., reported that the frequency of IL-6 -174 G/C genotyping and GG genotype in COVID-19 patients were significantly increased when compared with the control group [40,41].

Additionally, Hassan., reported that the CC genotype showed a higher rate in healthy pregnant women compared to pregnant women with COVID-19. In contrast, the G allele demonstrated significant frequency in pregnant women with COVID-19 and a higher rate compared with healthy pregnant women. The G allele appeared as a causative allele associated with infection risk with COVID-19, while the C allele appeared as a preventive allele against COVID-19 infection [42].

Additionally, an Ulhaq and Soraya., 2020 meta-analysis reported a link between increased IL-6 production and pneumonia severity and the C allele of the 174 G/C (rs1800795) SNP [43].

This discrepancy in findings between studies could be attributable to variations in sample size, patient inclusion and exclusion criteria, the pathophysiology of diverse inflammatory illnesses, geographic location, ethnic diversity, and racial heterogeneity.

It is important to be aware of the present study's potential shortcomings. First of all, RNA expression was not measured in this work to evaluate the effects of various SNP genotypes on IL-6 expression levels. Second, there was only one Egyptian community from whom to choose the patients, making the sample size somewhat limited. Third, other SNPs in other IL-6 areas were not examined in this investigation.

This study’s recommendations included the need for further studies on a larger number of patients for more comprehensive statistical analysis to clarify the distribution of Interleukin-6 174G/C polymorphism between COVID-19 patients and correlate with disease severity. Further studies may be needed to clarify the clinical usefulness of the combination of IL6 with other inflammatory biomarkers like IL-10 in critically ill patients. The need for further studies on other SNPs of the IL-6 gene.

5. CONCLUSION

The present study provides at the time of admission evidence for the differentiation of severe and non-severe cases of COVID-19 based on the laboratory test results. It suggests that interleukin-6 plays an important role in the pathogenesis of COVID-19. In addition, it reveals that serum IL6 can be used as a predictor of disease severity in COVID-19 patients, but interleukin-6 174G/C genotype distribution cannot be used as a predictor of disease severity in COVID-19 Egyptian patients.

ETHICAL APPROVAL

The Tanta University Faculty of Medicine's Research Ethics Committee issued the acceptance code, which was 34033/08/20. ClinicalTrials.gov recorded the trial and assigned it a special registration identification number (ID: NCT04544033).

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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