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IL-6 Predict Disease Severity and Potential Therapeutic Target in COVID-19 Patient.

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ABSTRACT

Background: Abnormal immunological features were well-known in patients with COVID-19 is higher serum concentrations of inflammatory mediators. In deceased patients, serum level of interleukin 2, IL-6, IL-8, and TNF a show significant higher concentration than in improved patients. specifying the need for IL-6 detection for initial expectation of severity. Therapeutic management aimed at correcting excessive and aberrant host immune responses are postulated to be successful.

Thus, the aim of present works is to find out the distribution of IL-6 serum concentration among mild, and sever cases of patients with COVID-19 infection in Iraqi populations, and to find out whether SNP of IL-6 **rs1800795 174 (C/G)** genotype could effect on disease severity.

Patients and Methods: The case control study was established on the basis of three groups. 1st group was include 15 healthy volunteers which considered as a control groups, 2nd group include 20 mild cases of patients with SARS-CoV-2 infection, and 3rd group include 20 sever ill patients with SARS-CoV-2 infection, 5 ml of Blood samples were collected by venipuncture from 40 patients and 15 healthy controls, by disposable syringe under aseptic technique, about 2 ml of venous blood were drawn by in ethylinditetracitic acid (EDTA) tube and stored at 4°C for DNA extraction for detection of *IL-6* gene polymorphism by ARMS-PCR. And Three milliliters of blood were put in gel tube and allowed to clot then the serum was separated by centrifugation 1500 rpm for 5 minute. Then serum has been separated in Eppendorf tube for IL6 detection by ELISA test.

Results: Present study demonstrated that, IL-6 CC genotype was more frequent in severely ill patients with COVID-19 group than in healthy control group, in a significant manner (p = 0.014). Therefore, C/C genotype can be regarded as a risk factor. Regarding allele analysis, allele C was more frequent in severe group than in healthy control group, in a significant manner (p = 0.018). Therefore, C allele can be regarded as a risk factor.

Conclusions: In conclusion, We identified a strong correlation between the case severity of COVID-19 and frequency C/C genotype of the **rs1800795174** SNP gene, in addition present data recommended to consider IL6 cytokines as akey factor to realize the therapy response in COVID-19 infected peoples.

KEYWORDS: IL-6; Disease severity; potential therapeutic; COVID-19 Patient.

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1. INTRODUCTION

The outbreak of extreme acute respiratory infection with coronavirus-2 (SARS-CoV-2) continues to show new globally confirmed cases on a regular basis[1]. According to the WHO study, about 14% of patients established serious diseases needful oxygen treatment and 6% go throughunfavorably ill patients necessitating intensive care unit[2]. Thoseseverely ill patients have a bad prognosis, with a rise in death rate [3].No particular anti-viral approach has proved effective to date, and the strength of clinical administration is mainly depending of management of symptoms to a large extend. Early identification and care of serious and critically ill patients are key concerns that require urgent screening. Abnormal immunological features were well-knownin patients with COVID-19 is higher serum concentrations of inflammatory mediators, according to the clinical characteristics [4]. In deceased patients, serum level of interleukin 2, interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and tumor necrosis factor a (TNF a) show significant higher concentration than in improved patients[5]. Moreover, the levels of these inflammatory mediators in thrilling cases were significantly higher than in mild cases[6], specifying the need for IL-6 detection for initial expectation of disease severity [7].Therapeutic management aimed at correcting unnecessary and unusual host immune reactions are postulated to be successful [8]. tocilizumab, considered a possible therapeutic alternative which is a monoclonal antibody directed against IL-6 receptor [9].

The aim of the currentwork is to find out the serum concentrations of IL-6 among mild, and sever cases of patients with COVID-19 infection in Iraq, and to find out whether SNP of IL-6 **rs1800795 174 (C/G)** genotype couldeffect on disease severity and whether have a prognostic value. INTRO-3.

2.MATERIALS AND METHODS

2.1 Patient Group and Sample Collection

The case control study was established on the basis of three groups. 1st group was include15 healthy volunteers which considered as a control groups, 2nd group include 20mild cases of patients with SARS-CoV-2 infection, and 3rd group include 20 sever ill patients with SARS-CoV-2 infection,patients were hospitalized at Alshifaa Hospital, the nominated transitory hospitals for COVID-19. The diagnostic requirements for COVID-19 have been adopted by the National Health Commission's Interim or 7th Edition Guidelines, which include, history, clinical symptoms, Chest CT examination, and SARS-CoV-2 RNAdiscovery results were primarily included as a diagnostic criteria [10].

5 ml of Blood samples were collected by venipuncture from 40 patients and 15 healthy controls, blood samples were collected by disposable syringe under aseptic technique, about 2 ml of venous blood were drawn by in ethylinditetracitic acid (EDTA) tube and stored at 4℃ for DNA extraction for detection of *IL-6* gene polymorphism. Three milliliters of blood were put in gel tube and allowed to clot then the serum was separated by centrifugation 1500 rpm for 5 minute. Then serum has been separated in Eppendorf tube for IL6 detection by ELISA test by using monoclonal anti IL6 Ab ELISA kit, supplied from ELAB.Bioscince (Korea) and procedure done according to manufactural instruction. Optical density, Which is measured spectrophotometrically at a wave length of 450nm, OD value is proportional to the concentration of IL-6 and then calculate the concentration of IL-6 in the sample by comparing the OD of the samples to the standard curve.

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2.2.Genomic DNA Extraction

Geneaid's (Frozen Blood) Genomic DNA miniextraction kit (USA) was used to extract the genomic DNA from samples of blood conferring to the instructionof company. Moreover, the Nano-drop spectrophotometer (THERMO, USA) was utilized to check purity, and concentration of extracted genomic blood DNAthrough absorbance reading at 260/280nm and calculated the concentration of DNA in (ng/μL). The genotypes of the IL-6 (**rs1800795174**) gene were determined by ARMS-PCR.

2.3.ARMS-PCR Primers

ARMS-PCR Primers for detection of IL-6 gene polymorphism **rs1800795174 (C/G)** were designed in this study by using (Web-Based Allele-specific primersof SNPs- Genome Institute, National Center for Genetic Engineering and Biotechnology (BIOTEC) (PathumThani, Thailand). Likewise, the primers weredelivered from Macrogen Company, Korea as below:
Wild type Forward Primer 5': CCCTAGTTGTGTCTTGTC
Mutant Forward Primer 5': CCCTAGTTGTGTCTTGTG
Common Reverse Primer 5': ACTTGTGGAGAAGGAGTTCA 7

2.4.Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) software (IBM, Chicago, USA, version 16) and Microsoft office Excel 2007. Quantitative variables were presented as mean, standard deviation and range whereas qualitative variables were expressed as number and percentage. Chi-square test and Yates correction for continuity were used to compare differences in frequencies of genotypes and alleles among groups. Kruskall Wallis test was used to compare mean rank of serum IL-6 levels among all groups or among all genotypes within a single group. Mann Whitney U test was used to compare mean rank of serum IL-6 levels between any two groups or between any two genotypes within a single group. The level of significance was considered at $p \leq 0.05$ and the level of high significance was considered at $p \leq 0.01$.

3. RESULTS

The Comparison of Interleukin -6 (IL-6) genotypes between patients with severe clinical presentation and healthy control group is shown in table 1. In the dominant model, CC genotype was more frequent in severe group than in healthy control group, 15 (75 %) versus 5 (33.3 %), respectively, in a significant manner ($p = 0.014$). Therefore, C/C genotype can be regarded as a risk factor with an odds ratio (OR) of 6.00 (95% confidence interval of 1.37 -26.24) and an etiologic fraction (EF) of 0.63.

Regarding allele analysis, allele C was more frequent in severe group than in healthy control group, 33 (82.5 %) versus 17 (56.7 %), respectively, in a significant manner ($p = 0.018$). Therefore, C allele can be regarded as a risk factor with an odds ratio (OR) of 3.61 (95% confidence interval of 1.21 -10.72) and an etiologic fraction (EF) of 0.48.

In addition to that, when Interleukin -6 (IL-6) genotypes between patients with mild clinical presentation and healthy control group is shown in table 2. none of genotypes showed significant difference in its frequency distribution between mild group and healthy control group

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(p> 0.05). Moreover, none of genotypes showed significant difference in its frequency distribution between mild group and healthy control group (p> 0.05).

Table 1: Comparison of Interleukin -6 (IL-6) genotypes between patients with severe clinical presentation and healthy control group

Model	**IL-6 rs1800795 174 (C/G)**	**Severe *n* = 20**	**Control *n* = 15**	***p*-value**	**OR**	**95 % CI**	**EF**	**PF**
Dominance	CC	15	5	0.014 C S	6.00	1.37 - 26.24	0.63	---
	C/G+GG	5	10	Reference				
Alleles	C	33	17	0.018 C S	3.61	1.21 - 10.72	0.48	---
	G	7	13	Reference				

n: number of cases; **IL-6**: interleukin-6; **OR**: odds ratio; **CI**: confidence interval; **EF**: etiologic fraction; **PF**: preventive fraction; **Y**: Yates correction for continuity; **C**: Chi-square test; **S**: significant at $p \leq 0.05$; **NS**: not significant at $p > 0.05$

Table 2: Comparison of Interleukin -6 (IL-6) genotypes between patients with mild clinical presentation and healthy control group

Model	**IL-6 rs1800795 174 (C/G)**	**Mild *n* = 20**	**Control *n* = 15**	***p*-value**	**OR**	**95 % CI**	**EF**	**PF**
Dominance	CC	11	5	0.203 C NS	2.44	0.61 -9.80	0.41	---
	C/G+GG	9	10	Reference				
Alleles	C	27	17	0.353 C NS	1.59	0.60 -4.23	0.23	---
	G	13	13	Reference				

n: number of cases; **IL-6**: interleukin-6; **OR**: odds ratio; **CI**: confidence interval; **EF**: etiologic fraction; **PF**: preventive fraction; **Y**: Yates correction for continuity; **C**: Chi-square test; **NS**: not significant at $p > 0.05$

Table 3: Comparison of Interleukin -6 (IL-6) genotypes between patients with severe clinical presentation and patients with mild clinical presentation

Model	**IL6 rs1800795 174 (C/G)**	**Severe *n* = 20**	**Mild *n* = 20**	***p*-value**	**OR**	**95 % CI**	**EF**	**PF**
Dominance	CC	15	11	0.185 C NS	2.45	0.64 -9.39	0.34	---
	C/G+GG	5	9	Reference				
Alleles	C	33	27	0.121 C	2.27	0.79 -6.49	0.31	---

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				NS				
	G	7	13	Reference				

n: number of cases; **IL-6**: interleukin-6; **OR**: odds ratio; **CI**: confidence interval; **EF**: etiologic fraction; **PF**: preventive fraction; **Y**: Yates correction for continuity; **C**: Chi-square test; **NS**: not significant at $p > 0.05$

The serum interleukin -6 (IL-6) levels according to clinical severity of the disease is shown in figure 1. There was highly significant variation in the serum IL-6 level among severe group, mild group and healthy control group ($p< 0.001$), the level being highest in the severe group followed by mild group and finally by control group.

4. DISCUSSION

According to result of present study, IL-6, CC genotype was found to be more frequent in severe group than in healthy control group, 15 (75 %) versus 5 (33.3 %), respectively, in a significant manner ($p = 0.014$). Therefore, C/C genotype can be regarded as a risk factor for prediction development of SARS-CoV-2 infected patient in to sever grade with cytokine storm. And such impact of SNP on disease severity also has been studded previously on other diseases,

In the presentpresentation of Severe respiratory disorder coronavirus 2 infection, a few is decoded about IL-6 association and its SNP in relation to pathogenesis idiopathic pulmonary damage in SARSCoV- 2 infection, excluding its important role to recruit the cytokine (IL-6) storm. A meta-analysis study that was done previously to demonstrate the IL6 gene polymorphism with tendency as well as disease severity of pneumonia, proposed the carrier status of IL6 174 C allele with higher IL-6 production and pneumonia severity (11). Thus, it came in agreement with present work, since we found that severe ill patients with IL-6 CC genotype was found to have significant ($p< 0.001$) higher concentration of serum IL-6 as shown in figure 1.

Jin and Wang concise the worth of single nucleotide polymorphisms (SNPs) and its impactonimmune response, so-called “immunogenetic profiling” (12). Likewise, some lung disease like chronic obstructive pulmonary disease (COPD) and asthma, degree of such disease severity was differentially detectedthroughSNP analysis (13). IL6-174 C/C genotype also associated with respiratory syncytial virus (RSV) infection extent (14).

According toknownfunction of IL-6 as anessentialorganizer for the CD4 T cell destiny, IL6 SNPcouldexplain moreabout COVID-19 pandemic. Therefore, present studyproposedthat, IL6 SNPact as biomarker to disease severity or to distinguish the pathological changesresulting from COVID-19 viral infections, which comprisingliability of certainpopulationsto have asymptomatic infection or other having insusceptible to the virus as identified earlier in different infectious diseases (15, 16).

Results of IL-6 blood concentrationwas assessed and examined in allcases that was included in this study. It is progressivelydocumented that extreme, malfunctionedbody immune system may playvital role in the progress and upkeep of seriousstages of illness. Manyresearchers had stated that elevated IL-6 and other inflammatory Mediatorsconcentrationsassociated with degree of disease illness [17, 18]. Though present data was in reliable with that of these researches.

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Nevertheless, association does not assurancecausativeness. Extremelyhigher IL-6 levels (> 107 pg/ml), whichstrictlyaccompanying with noticeable SARS-CoV-2 critically ill patients [19]. Additionalmeta-analysis studythat was done by Muhammad et al. included nine researches (1426 cases), and it proven that increases inbloodconcentration of IL-6 was concomitantwith augmented risk of COVID-19 complications and bad prognosis [20]. Thus,Hypothetically, consuming IL-6 receptor blockers in initial stage of COVID-19 might be furtheruseful to counter the consequence. Since IL-6 consider as a pyrogenic proinflammatory cytokine. Thus,by using receptor Blockers, it is expectable that body temperature might decline, and respiratory distress might be relieved to some point.

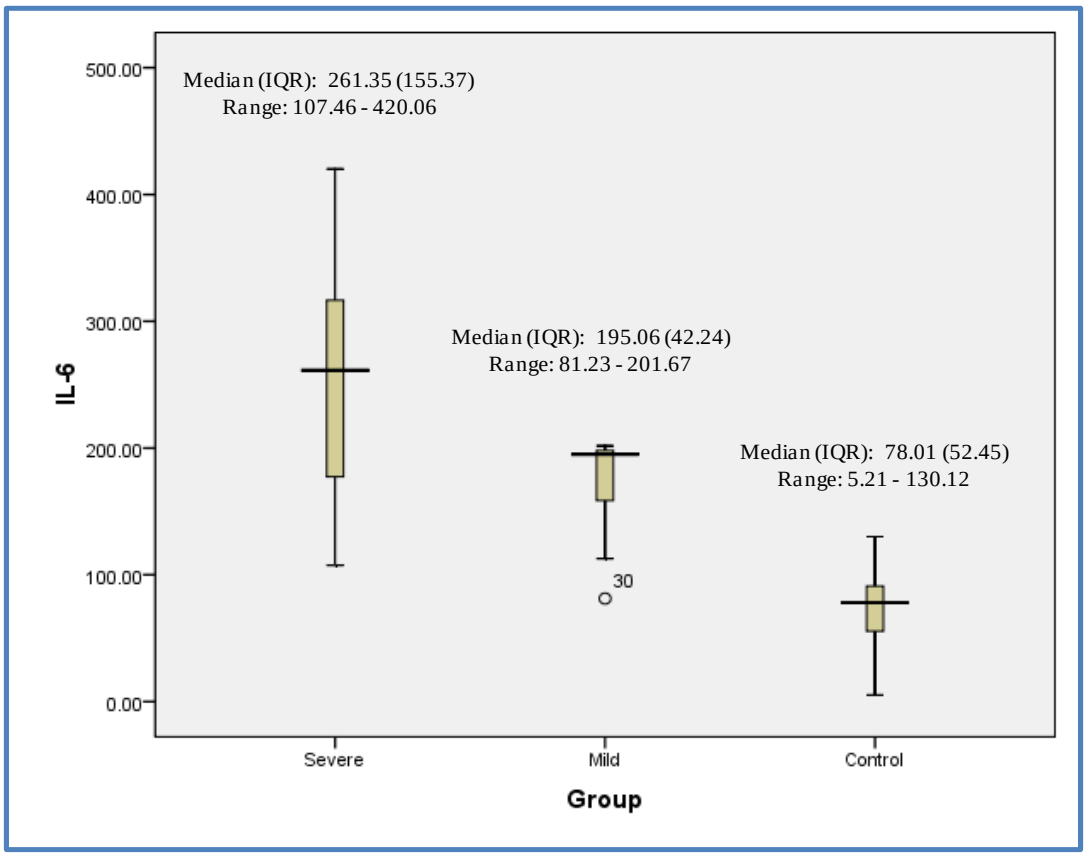

Figure 1: The serum interleukin -6 (IL-6) levels according to clinical severity of the disease

CONCLUSION

In conclusion, this case control study was conducting regarding the between the case severity of COVID-19 and SNP of the IL6 genes in addition to serum level of IL-6in Iraqi population. IL6 genes. We identified a strong correlation between the case severity of COVID-19 and frequency C/C genotype of the **rs1800795174** SNP gene, in addition present data recommended to think throughproinflammatory IL6 cytokine as akeyplayer to comprehend the therapeutic response for COVID-19 viral infection, due to strong association between its serum concentration and disease severity.

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