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ORIGINAL ARTICLE

Inflammatory Biomarkers as Prognostic and Predictive Factor in Patients with COVID-19

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ABSTRACT

Background: *COVID-19 severity is often linked to excessive inflammatory responses. Biomarkers such as C-reactive protein (CRP), IL-17, and IL-33 have potential roles in inflammation and disease progression. Identifying these biomarkers may help predict disease severity and guide clinical management.* ***Objective****: This study aimed to investigate the role of CRP, IL-17, and IL-33 as indicator factors for Covid-19 severity.* ***Methodology****: The study involved ninety-five Covid-19 patients divided into mild and severe, along with (50) healthy controls. Levels of CRP, IL-17 and IL-33 were measured by ELISA in patients and healthy controls.* ***Results****: The results of the study indicated highly significant relation between the Covid-19 groups and healthy controls regarding C-reactive protein levels, as well as Il-17 and IL-33.* ***Conclusion****: The biomarkers are highly associated with Covid-19. Increased levels of IL-17, IL-33 facilitates lung injury through production other pro-inflammatory cytokines and that explain the immunopathology of Covid-19 infection, so could be used as a predictive factor for intensity of disease in patients with COV1ID-19.*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome (SARS-CoV-2) virus that was identified in late 2019 in Wuhan city, China[1,2]. On March 2020, the World Health Organization (WHO) fixed COVID-19 as a global health emergency[3]. The features of COVID-19 clinically range from asymptomatic/mild symptoms like cough, headache, fever, sore throat and nasal congestion, to severe or critical symptoms like pneumonia, respiratory failure, disseminated intravascular coagulation (DIC) and multi-organ failure[4,5].

It is well-documented that the progression of COVID-19 severity due to role of the inflammatory responses[6,7]. The excessive inflammation and dysregulation of host immune defenses triggered by rapid replication of SARS-CoV-2, cells destruction leads to recruit macrophages and neutrophils into lung tissue which results in a cytokine storm (CS) that characterized by overproduction of cytokines and excessive immune cells activation, which leads to injuries in many organs such as spleen, liver, heart, kidney. Furthermore, can cause vascular injury and even death[8-11].

Acute lung injury, lung fibrosis and emphysema in severe cases COVID-19 is characterized by inflammation and respiratory tissue damage are highly correlated with T helper 17 cell responses[12]. The major cytokines released from T helper (Th) is IL-17 and considered one of the many cytokines involved in cytokine release syndrome, also it's produced by CD8+ T cells, invariant natural killer T cells, neutrophils, gamma delta T cells, and type 3 innate lymphoid cell. IL-17 triggers many signaling pathways to produces many other cytokines and chemokines by various alveolar cell types. The imbalance between T-helper-cell (TH) subsets and regulatory T-cells (Treg) can contribute pathogenesis of COVID-19[13,14].

Interleukine-33, is member of "alarmins" family that released rapidly by damaged alveolar epithelial cells, endothelial cells, and apoptosis that contributes to inflammation in airway diseases[15]. Interacting of IL-33 with chemotactic, and pattern recognition receptors (PRRs) to boost immune cells in the host's immune defiance, make the IL-33 play as intercellular signals[16,17]. It may enhance differentiation and expansion of Foxp3+ T regulatory cells (Treg), through up-regulate (abnormally) expression of its own receptor ST2 on Treg cells, which impairs the suppressive function of Treg cells by increased expression of GATA3. The GATA3+ Foxp3+ Treg cells dysregulation may due to impaired secretion of type 2 cytokines and tolerance, thus promoting auto-inflammatory lung disease. Recent studies provided a significant correlation between COVID-19 severity and IL-33 expression[18-20].

This study aimed to investigate the role of IL-17, IL-33 and CRP in patients with mild and severe COVID-19

to determine the levels of these parameters would changes by severity of disease and to use them as biomarkers to predicting COVID-19 prognosis.

METHODOLOGY

Study Design and Population

The study was carried out from March to July, 2023. One hundred forty-five (145) participants were included; (50) were healthy controls and (95) were positive COVID-19 patients, who were admitted to the General Teaching Hospital, Iraq and their diagnosis was confirmed by positive Reverse Transcriptase Polymerase Chain Reaction test (RT PCR).

The severity of the disease was classified into two groups mild (n=50) and severe group (n=45) based on peripheral oxygen saturation, respiratory rate and chest computed tomography (CT) scan findings. Severe cases were confirmed by the criteria: (1) Respiratory rate > 30 breaths/min, or (2) oxygen saturation < 90% on room air or (3) arterial blood oxygen partial pressure ≤ 300, according to the world health organization (WHO)[3]. While patients with moderately disease were with lower respiratory tract disease and the oxygen saturation ≥ 94%.

Inclusion Criteria

Inclusion criteria are non-vaccinated COVID-19-positive patients were enrolled in the study.

Sample Collection

Blood samples (10 ml) were collected from all participants, and then, the clotted blood samples were centrifuged at 1500 rpm for 10 minutes. The sera preserved at -20°C until use.

Immunological Marker Assessment

Serum levels of IL-17, IL-33 were performed according to the ELISA Kits (Eagle Biosciences, USA), whereas the commercial kits produced by Abbott Laboratories (Abbott, Architect, USA) were used to determine the level of CRP.

Statistical analysis

The data was analyzed by SPSS (version 26). The differences of cytokines evaluate by ANOVA test. The Chi-square test applied for categorical variables. Pearson's correlation coefficients used to evaluation the correlation.

RESULTS

As shown in Table 1 and Figure 1, we found a highly significant difference among patients' groups ($p<0.000$) regarding the age; the mean age of healthy controls was 34.21±12.55, while for mild and severely infected patients with COVID-19 were (42.01±16.35; 63.11±11.56, respectively). According to the gender, most of the severely Covid-19 group (60%) were females, while mild Covid-19 group (62%) and healthy individuals (70%) were males, with a highly significant difference ($p<0.000$).

The serum CRP level among the severe infected groups (59.45±40.15mg/dl) was found to be higher than mild and healthy controls (13.05±31.99 mg/dl; 0.69±1.31mg/dl, respectively) with a highly significant difference ($p<0.000$). The level of IL-17 in Covid-19 patient groups and healthy control group were (35.90±22.56 pg/ml, 35.16±7.91 pg/ml and 19.90±13.24 pg/ml, respectively) with a significant difference ($p<0.03$). Serum levels of IL-33 were notably higher in patients compared to healthy individuals. When comparing IL-33 concentrations between the mild and severe patient groups, it was (206.76±422.26 pg/ml and 180.41±414.66 pg/ml, respectively), both groups showed increased levels when contrasted with healthy controls (36.47±110.77 pg/ml), with significant differences noted between the groups ($p<0.02$) (Figure 1).

Our study revealed a positive correlation among IL-17 and IL-33 levels in both patient groups as illustrated in (Table.2).

Table 1: Clinical characteristics between healthy control and Covid-19 groups.

Parameters		Mild group N=50	Severe group N=45	Control group N=50	P-value
Age (Years)		42.01±16.35	63.11±11.56	34.21±12.55	<0.000
Gender	Female	38%	60%	30%	<0.00
	Male	62%	40%	70%	
CRP (mg/dl)		13.05±31.99	59.45±40.15	0.69±1.31	<0.000
IL-17 (pg/ml)		35.16±16	35.91±22.56	19.90±13.24	<0.03
IL-33 (pg/ml)		180.41±414.66	206.76±422.26	36.48±110.77	<0.02

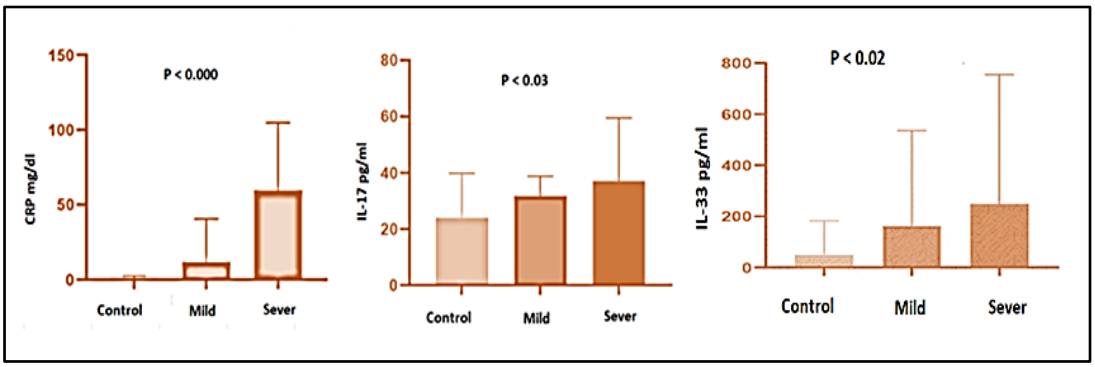

Fig. 1: Comparison of the serum level of the biomarkers among Covid-19 groups.

Table 2: Correlation between IL-17 and IL-33 levels in Covid-19 groups[a]

Group	Mild	Severe
Parameter	**Interleukin-33, r**	
IL-17	0.224*	0.371*

*a: Correlation was performed by the Pearson analysis. * p < 0.05.*

DISCUSSION

The COVID-19 infections impair the immune response and play a crucial role in the multi organ damage and mortality. The virus invades nearly all varieties of immune cells, resulting in a disruption of both innate and adaptive immune reactions, along with an overproduction of inflammatory agents[21,22]. Aging play a significant role in COVID-19 infection and considered an indicator of disease severity through its association with an increment in the expression of Angiotensin-Converting Enzyme-2 (ACE-2), in old people the receptor for SARS-CoV-2 spike protein precipitates in replication of the virus. On the other hand, immune dysregulation can contribute to the cytokine storm[15].

Immune senescence in older patients infected with COVID-19 contributes to increase the risk of severity, because older cells and tissues have a lower capacity to repair damage[23]. Our study demonstrated that patients with severe disease were in the age group of 63.11±11.56 years, which agrees with researchers who found that older individuals had a higher risk of developing severe disease[24-26].

The role of inflammatory parameters, reflect hyper inflammation and predicting prognosis in COVID-19 disease. C-reactive protein (CRP) is a biomarker of inflammation synthesized in response to overproduction of inflammatory cytokines[27]. This study revealed that the highest levels of CRP were increased significantly in severe group compared to mild and healthy controls (p<0.000). These findings were consistent other with researchers, who reported that elevated levels of CRP are related to the severity of viral infections including Covid-19[28,29]. So, higher CRP play a role in damage of lung tissue and closely related to higher mortality[30].

Uncontrolled levels of cytokines is a systemic inflammatory response that can be triggered by infections and contribute to lung tissue injuries, through exhibited high levels of inflammatory cytokines according to the severity of COVID-19[31]. Many cytokines involved in the cytokine storm, one of these is interleukin-17, which is a predominant mediator of pulmonary inflammation mainly produced by T helper17 (Th17) cells. Dysregulation of T helper17 cells and enhanced expression of IL-17 in the lungs promote the production of downstream pro-inflammatory molecules such as IL-1β, TNF-a , IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1/CCL2) involved in the pathogenesis of acute respiratory distress syndrome[32].

Our results showed that serum IL-17 levels in mild and severe COVID-19-group were significantly higher than in control group (p<0.03). The results are consistent with those who reported that the upregulation of IL-17 is the main mediator of immunopathology and subsequent respiratory distress in Covid-19 patients[17,33,34].

One member of the “alarmins” family is IL-33 which is released in response to immune activation or cellular damage, so act as "intercellular signals" that strengthen susceptible cells in the host's defense response through interacting with chemotactic and pattern recognition receptors (PRRs)[35]. Alarmins are harmful substances released by SARSCoV-2-damaged cells that increases the inflammatory response in endothelial and epithelial cells. This is in alignment with its pro-inflammatory role in respiratory disorders[36]. The current study revealed a higher significantly increase in IL-33 levels in both groups of COVID-19 compared to control, demonstrating its role in the progression of COVID-19. The results of other studies confirm this, indicating that IL-33 may contribute to the pathogenic mechanisms of COVID-19 severity. It might also be a good strategy for treating the COVID-19 progression[18,37,38]. Further, a positive correlation was obtained between serum level of cytokines (IL-17 and

IL-33) among mild and severe Covid-19 groups. These findings together suggest that they have a significant role in immune-pathogenesis of COVID-19 and could be related to disease severity and progression which consist with Muhammed, *et al.* [39].

CONCLUSIONS

Severe COVID-19 patients had higher CRP and IL-33 more than moderately group, but IL-17 level between both Covid-19 groups was at close levels higher than healthy control, and may be considered to predict the disease severity in COVID-19 patients, and provide more definitive approaches for therapy.

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Declaration

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Authors contribution:

WFH: contributed to the study design, data collection, statistical analysis, interpretation of results, and drafting of the manuscript. **RHF**: provided supervision, contributed to the study design, data interpretation, and critically revised the manuscript for important intellectual content. Both authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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