Cytokine profile in COVID-19 infection: focus on interleukin-13, interleukin-33, and tumor necrosis factor-α as immunological markers

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ABSTRACT

COVID-19 is a pandemic disease that has a wide spectrum of symptoms from asymptomatic to severe fatal cases due to hyperactivation of the immune system and secretion of pro-inflammatory cytokines. This study aimed to assess the level and impact of interleukin (IL)-13, IL-33, and tumor necrosis factor (TNF)-α cytokines on immune responses in mild and moderate COVID-19-infected Iraqi patients. A prospective case-control study was conducted from January 2023 to January 2024; it included 80 patients infected with moderate COVID-19 infection who consulted in different private clinics and 40 healthy controls. The serum of both groups was tested for quantification of serum IL-13, IL-33, and TNF-α using the human enzyme-linked immunosorbent assay method. The mean age of the moderate COVID-19 patient group was 43.67±1.85 years, while the mean age of the healthy control group was 34.45±3.12 years with a statistically significant (p=0.0081), but there was no statistically significant difference in IL-13, IL-33, and TNF-α levels between the patients and control groups. This study highlights the importance of age, gender, and body mass index as risk factors associated with COVID-19 infection. There were no significant differences in IL-13, IL-33, and TNF-α levels between moderate COVID-19 patients and healthy controls. The receiver operating characteristic curve analysis of IL-13, IL-33, and TNF-α shows moderate potential (non-significant) as a biomarker for predicting mild and moderate COVID-19. Pearson correlation analysis showed a strong potential correlation between IL-13, IL-33, and TNF-α.

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Introduction

COVID-19 is the most contagious respiratory disease, and it is caused by severe acute respiratory syndrome coronavirus 2, causing more than six million deaths all over the world until now.[1] The disease can be either asymptomatic, or patients may complain of fever, cough, headache, confusion, weakness, diarrhea, nausea, vomiting, and dyspnea.[2,3] The disease can be classified as mild, moderate, severe, and critical.[4] Genetic factors like the variant of rs2106809 ACE2 are important in severe clinical cases in male patients, and AGT rs699 polymorphism had an important role in COVID-19 infection.[5] The immunopathology of this disease includes invasion, replication, activation of the immune response, cytokine storm, respiratory distress syndrome, and finally, multiple organ dysfunction syndrome.[6] Cytokine secretion is an important cause of morbidity and mortality.[7] Immune response generates a release of different cytokines like interleukin (IL)-13, IL-33, and tumor necrosis factor (TNF)-

α and other cytokines from vascular endothelial cells, alveolar epithelial cells, and different immune cells that contribute to immune response and can lead to severe disease.[8] IL-13 is a proinflammatory cytokine involved in tissue and pulmonary inflammation and fibrosis, and increased levels are seen in severe cases of COVID-19 infection.[9] Another key cytokine that has an important role in the regulation of inflammation is IL-33, which acts as an alarmin cellular signal released during injury, amplifying inflammation and tissue injury.[10] It is expressed on epithelial and endothelial cells and plays an important role in the activation of Th2 cells; it contributes to the regulation of inflammation in COVID-19 infection, with increased levels seen in cases with severe tissue damage. It may also be used as an indicator for prognosis.[11] Additionally, TNF-α is another proinflammatory cytokine that regulates immune reaction, and in severe cases, its secretion is increased, causing hyperinflammation and tissue damage like cytokine storm ending with acute respiratory distress syndrome (ARDS) and multi-organ failure.[12] This study shed light on the level and effect of these cytokines (IL-13, IL-33, and TNF-α) on immune response in mild and moderate COVID-19-infected Iraqi patients.

Materials and Methods

This is a prospective case-control study conducted from January 2023 to January 2024, including 80 patients infected with a moderate state of COVID-19 who consulted at different private clinics and 40 healthy controls. The study was approved by the Ethical and Scientific Committee of Al-Kindy College of Medicine, University of Baghdad. The inclusion criteria were patients with COVID-19 confirmed by positive laboratory test [real-time polymerase chain reaction (RT-PCR)] from nasopharyngeal swabs complaining of fever, cough, dyspnea, and weakness, while the exclusion criteria were other respiratory diseases and negative COVID-19 RT-PCR.

Blood samples were collected from both groups and analyzed for complete blood count using an automated analyzer (China), biochemical C-reactive protein tests using turbidimetry, and coagulation D-Dimer tests using enzyme-linked fluorescence assay according to the instructions of the manufacturers. Serums of both groups were tested for quantitation of serum IL-13, IL-33, and TNF-α using a human enzyme-linked immunoassay kit (Catalog No. YLA0676HU, YLA1505HU, and YLA1337HU, respectively) (Biont, China), depending on the sandwich enzyme-linked immune sorbent assay. The standard curve was drawn by plotting the optical density *vs*. the concentration.

Statistical analysis

The data of this study was analyzed using Excel (Microsoft, Redmond, WA, USA) and a statistical package for the social sciences (SPSS version 25, IBM, Chicago, IL, USA). Descriptive studies and categorical analysis for different variables were done using the Chi-square test and student's *t*-test. The Mann-Whitney test is a non-parametric test used to detect the significant effect of different variables. The receiver operating characteristic (ROC) curve was used to detect the cut-off, sensitivity, and specificity of different markers. A $p \leq 0.05$ was regarded as significant.

Results

The mean age of moderate COVID-19 patient groups was (mean±standard error mean) 43.67±1.85 years, while the mean age of the healthy control group was 34.45±3.12 years, which was statistically significant (p=0.0081). There was a statistical significance among the sexes in the study groups (p=0.026) (*Supplementary Table 1*). All patients were from the Baghdad province, and they were engaged in different occupations like students, housewives, and retired. There was also a significant difference regarding weight and body mass index (BMI) (p=0.04, and p=0.00, respectively).

The distribution of different cytokines in *Supplementary Table 2* shows the p-values for IL-13, IL-33, and TNF-α that were 0.158 and 0.264, and 0.264, respectively, which are greater than 0.05. It suggests that there is no statistically significant difference in IL-13, IL-33, and TNF-α levels between the patients and control groups.

Regarding the ROC curve of IL-13 in predicting COVID-19, Figure 1 demonstrates the sloping of the curve upwards, which indicates it had a discriminatory power. The area under the curve (AUC) of IL-13 was 0.579, which showed a moderate power of predicting COVID-19, but p=0.173 indicates no significant difference between AUC and 0.5, which means it cannot be reliable in predicting COVID-19. The 95% confidence interval (CI) of 0.486 to 0.669 indicates a wide range of possible values for the true AUC. At a cut-off value of <1.665 ng/mL, sensitivity was 62.5%, specificity was 70%, the positive predictive value was 80.6, the negative predictive value was 48.3, and accuracy was 0.3250.

The ROC curve of IL-33 for predicting COVID-19 had an upward sloping, which indicates that IL-33 had some discriminatory power (AUC=0.524; 95% CI=0.431 to 0.616; p=0.693). At a cut-off value of ≤56.409ng/mL, sensitivity was 90%, and specificity was 30% (Figure 2). The positive predictive value was 72, the negative predictive value was 60, and accuracy was 0.200. Additionally, Figure 3 illustrates the

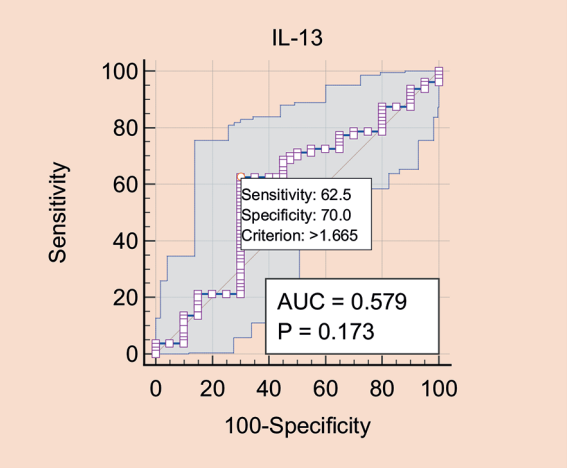

Figure 1. Receiver operating characteristic curve plot of interleukin (IL)-13 for predicting COVID-19 [area under the curve (AUC)=0.579; 95% confidence interval of 0.486 to 0.669; p=0.173]. At a cut-off value of <1.665 ng/mL, the sensitivity was 62.5 %, and specificity was 70%.

TNF-α ROC curve plot for predicting COVID-19 (AUC=0.549; 95% CI=0.456 to 0.640; p=0.372). At a cut-off value of ≤7.997 ng/mL, sensitivity was 65%, and specificity was 45%. The positive predictive value was 70.3, the negative predictive value was 9.1, and accuracy was 0.1625.

The Pearson correlation analysis was conducted to assess the relationships between cytokine levels (IL-13, IL-33, and TNF-α) and demographic variables (age, weight, height, and BMI), there were strong positive correlations between IL-13 and IL-33 (Figure 4) (r=0.844, p<0.01), IL-33 and TNF-α (Figure 5) (r=0.932, p<0.01), IL-13 and TNF-α (r=0.823, p<0.01), weight and IL-33 (r=0.453, p<0.01), weight and TNF-α (r=0.319, p<0.05), age and weight (r=0.447, p<0.01), and weight and BMI (r=0.799, p<0.01) as documented in *Supplementary Table 3*. Moreover, there was a weak positive correlation between TNF-α and erythrocyte sedimentation rate (r=0.062, p>0.05), suggesting a possible not significant association, while a negative correlation was observed between lymphocyte level and TNF-α (r=-0.176, p<0.05), indicating a probable inverse relationship (*Supplementary Table 4*). Spearman's correlation analysis was used to assess the relationship between cytokine levels (IL-13, IL-33, and TNF-α) and demographic variables (gender, smoking, and vaccination status). Figure 6 showed a positive correlation between IL-13 and the weight of COVID-19-infected patients (R^2linear=0.062). *Supplementary Table 5* shows a strong positive correlation between gender and IL-33 (r=0.766, p<0.01) and neither correlations between IL-13, IL-33, and TNF-α with smoking and vaccination status nor gender with TNF-α and smoking.

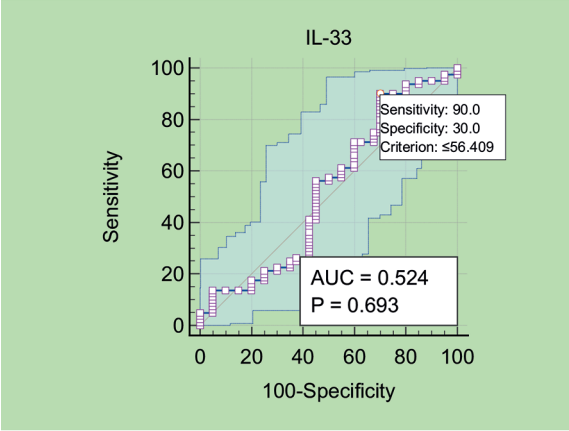

Figure 2. Receiver operating characteristic curve plot of interleukin (IL)-33 for predicting COVID-19 [area under the curve (AUC)=0.524; 95% confidence interval of 0.431 to 0.616; p=0.693]. At a cut-off value of ≤56.409ng/mL, sensitivity was 90 %, and specificity was 30%.

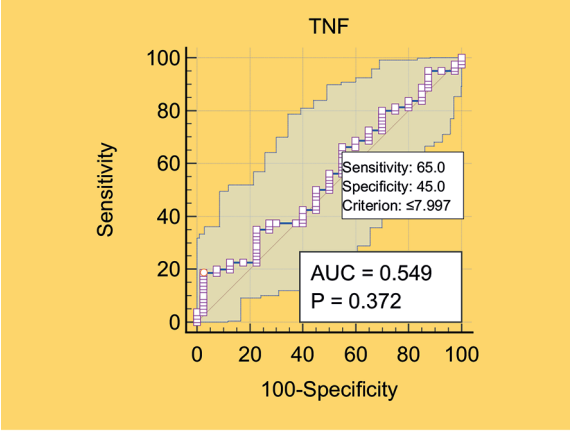

Figure 3. Receiver operating characteristic curve plot of tumor necrosis factor (TNF)-α for predicting COVID-19 [area under the curve (AUC)=0.549; 95% confidence interval of 0.456 to 0.640; p=0.372]. At a cut-off value of ≤7.997 ng/mL, sensitivity was 65 %, and specificity was 45%.

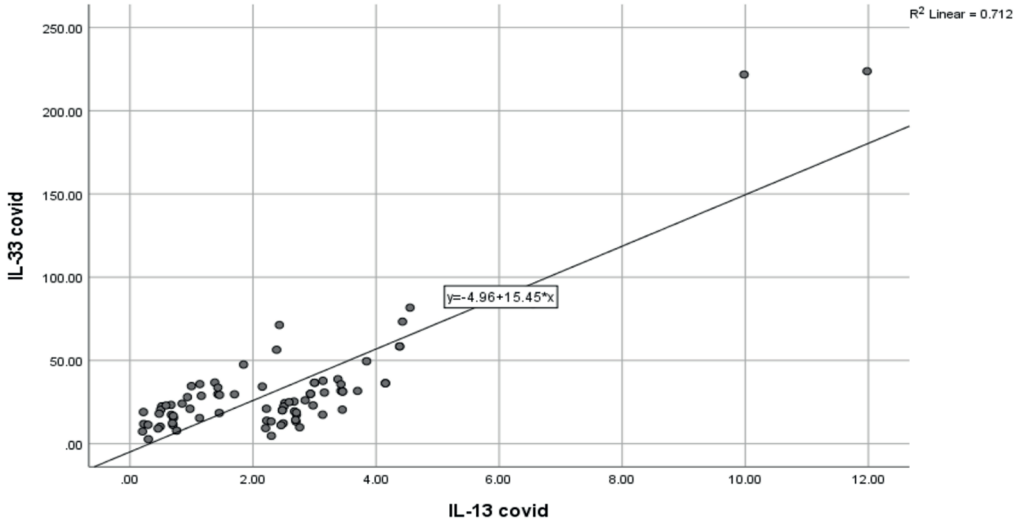

Figure 4. Positive correlation between interleukin (IL)-13 and IL-33(R2linear=0.712).

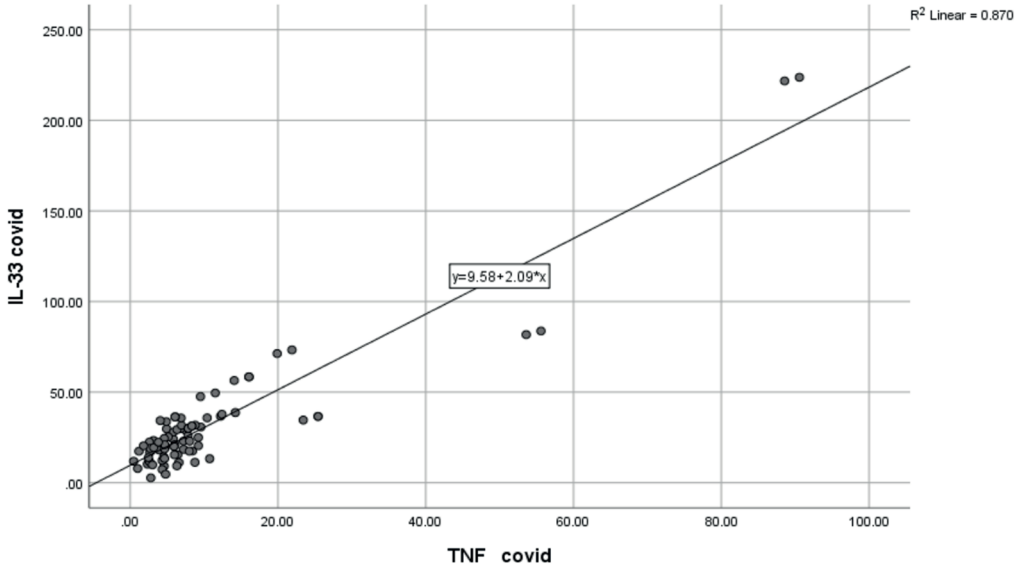

Figure 5. Positive correlation between interleukin (IL)-33 and tumor necrosis factor (TNF)-α (R2linear=0.870).

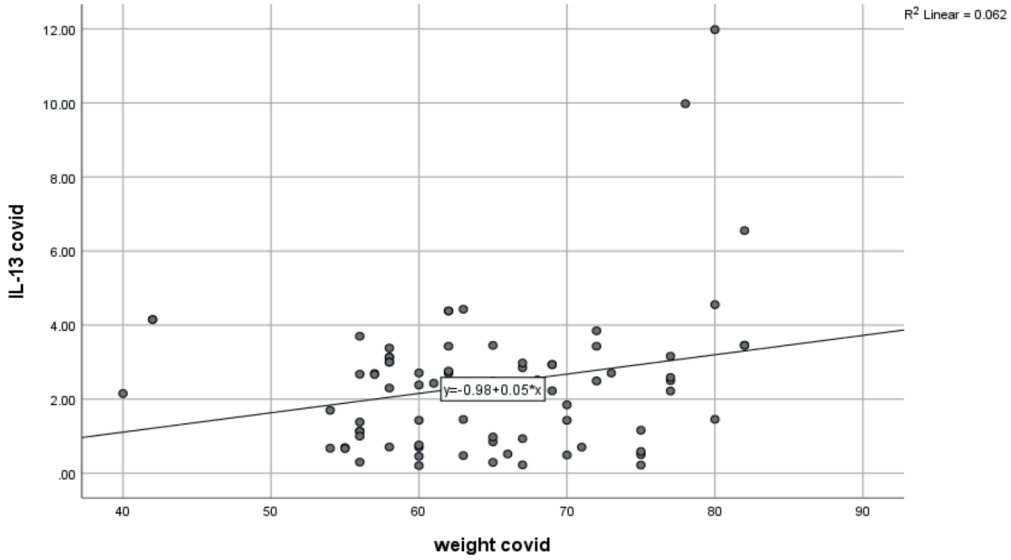

Figure 6. Positive correlation between interleukin (IL)-13 and weight of COVID-19 infected patients (R2linear=0.062).

Discussion

The study suggests female sex (57.5% *vs*. 36.3%, p=0.026) and older age group patients (43.67±1.85 years *vs*. 34.45±3.12 years, p=0.0081) have increased risk for COVID-19 infection. These findings align with other studies illustrating age and sex as risk factors for severe COVID-19.[13,14] COVID-19 patients had a significantly increased weight and BMI (p=0.0445 and p=0.0081, respectively), well-documented risk factors for infection in the literature.[15,16] Other risk factors (smoking, chronic diseases) lack significant differences, which is not in line with other studies.[17] This may be due to the small sample size and other characteristics of patients' selection. The findings of this study illustrate the role of some cytokines (IL-13, IL-33, and TNF-α) in the mild and moderate state of COVID-19 infection because these cytokines are important in the regulation of the immune response. Results showed no significant difference between patients and the control group (p=0.158, p=0.264, and p=0.264, respectively). These findings may indicate that these cytokines might not play a primary role in the pathogenesis of mild and moderate COVID-19-infected patients. These findings disagree with other findings that reported increased IL-13 was associated with risk factors for severe COVID-19 infection that needs mechanical ventilation.[18] Other studies

reported that alarmin cytokine (IL-33), which regulates immune cells with strong interaction among neutrophils, macrophages, dendritic cells, innate lymphocytes, Th cells (CD4+), Tc (CD8+), Th17, Treg in COVID-19 phagocytose, is elevated and upregulated in severe COVID-19 infection cytokine storm, and confers poor disease outcomes.[19] The last cytokine was TNF-α, which is elevated in severe advanced stages of COVID-19 infection with ARDS in association with other elevated proinflammatory cytokines like IL-1, IL-6, CXC motif chemokine ligand 10, macrophage inflammatory protein 1 α, and chemokine ligand 2.[20] Moreover, increased levels of IL-10, IL-23, and TNF-α levels were associated with severe and critical cases of COVID-19 linked to the hospital mortality rate of the disease.[21] The differences with this study may be due to mild and moderate state of COVID-19 infection, small sample size, and outpatient treated patients that did not need hospital admission or mechanical ventilation.

A possible biomarker for expecting COVID-19 infection is IL-33, which had an AUC of 0.579, representing a moderate biased power in predicting COVID-19. This value is not statistically significant (p=0.173). IL-33 and TNF-α also had poor discriminatory power in detecting COVID-19. Based on the ROC curve analysis of cytokines, the AUC moderate values with a lack of statistical significance mean that they cannot be used as a biomarker for predicting COVID-19 and discriminated it from the control group. This needs further validation with larger sample sizes in combination with other clinical and laboratory parameters.

Pearson correlation coefficients demonstrated in *Supplementary Table 3* reported that a strong positive correlation exists between IL-13, IL-33, and TNF-α, suggesting a possible common regulatory mechanism involved in disease pathogenesis influenced by a positive correlation with weight and that obesity was correlated with increased chronic inflammation. This is in accordance with other results that show these cytokines are involved in the inflammatory response against COVID-19 infection.[22] This study also showed a strong positive correlation between IL-33 and anti-COVID-19 immunoglobulin G antibodies, suggesting an important role of IL-33 in stimulating humoral immune response. *Supplementary Table 4* reports the absence of correlation between these cytokines and inflammatory parameters, indicating the absence or a limited role of these cytokines in acute phase response. Additionally, Spearman's correlation coefficients between cytokine levels (IL-13, IL-33, and TNF-α) and variables such as gender, smoking, and vaccination status exemplified a significant positive correlation between females and IL-33 levels. This may be due to possible hormonal or immunological factors specific to females that may contribute to higher IL-33 levels. Other confounding factors like smoking and vaccination status may have a limited effect on cytokine levels. This was in agreement with other studies that showed risk factors like age, gender, obesity, and smoking contribute to the severity and mortality of COVID-19 due to alteration in the expression of receptors on the surface of epithelial cells.[23] This needs further studies to prove this by studying the immune mechanisms that affect the clinical outcome of the patients.

Limitations of the study

The small sample size of this prospective cross-sectional study limits some findings that might not be representative of the general population.

Conclusions

This study highlights the importance of age, gender, and BMI as risk factors associated with COVID-19 infection. There are no significant differences in IL-13, IL-33, and TNF-α levels between mild and moderate COVID-19 patients and healthy controls. ROC curve analysis of IL-13, IL-33, and TNF-α shows moderate potential as a biomarker for predicting COVID-19. Pearson correlation analysis showed a strong potential correlation between IL-13, IL-33, and TNF-α.

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Online supplementary material:

Supplementary Table 1. Demographic variables of COVID-19 infected patients and healthy control group.

Supplementary Table 2. Distribution of median level of interleukin (IL)-13, IL-33, and tumor necrosis factor-α in patients compared with control healthy group.

Supplementary Table 3. Pearson correlation between cytokines [interleukin (IL)-13, IL-33, and tumor necrosis factor-α] and other demographic variables.

Supplementary Table 4. Pearson correlation between interleukin (IL)-13, IL-33, and tumor necrosis factor-α levels and laboratory variables.

Supplementary Table 5. Spearman's correlation between interleukin (IL)-13, IL-33, and tumore necrosis factor-α levels and other variables.