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OPEN PEER REVIEW

Determination of Serum Calprotectin, Heparin-Binding Protein, and Some Inflammatory Factors in Patients with COVID-19

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About this report

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1 Original submission

2 comments · 8 Oct 2023 – 17 Oct 2023

Referee 1 Reviewer

MAJOR REVISION 8 Oct 2023

Dear authors,
I appreciate the time and effort you devoted to this study. The topic is novel, interesting, and within the scope of the journal. However, I have some concerns that should be resolved before the manuscript is considered for publication. Please address my comments:
Title and abstract
1-Aims: Since your study was not correlational research, I recommend the following wording for the objective: "This study aimed to investigate the serum levels of calprotectin, HBP, and other inflammatory markers in COVID-19 patients."
2-In the Result section, since the comparison of age was not the primary objective of the study, please omit the first sentence. The comparison of the serum levels of calprotectin and other inflammatory markers should be clarified using descriptive statistics (mean difference, confidence interval, or at least a P-value). Furthermore, the correlation between calprotectin and other markers should be described with a correlation coefficient and P-value.
Introduction
3-"COVID-19 disease/infection" is scientifically incorrect. Please replace it with "SARS-CoV-2 infection" or "COVID-19" throughout the text.
Method
4-Although the study was approved by the University of Hamedan, it was performed at Ayatollah Kashani Hospital in Tehran. Is there a typographical error, or is it written correctly? Please check it.
5-Please mention the study period and the inclusion and exclusion criteria in the method section.
6-Was data collection based on the census method or sampling? If it was based on sampling, please add how the sample size was estimated and describe the sampling method. If not, please mention that it was based on the census method.
7-Statistical method: Which type of regression was used for analyzing the data? Linear, logistic, …? It should be mentioned in the text.
Results
8-If other baseline characteristics of the patients (e.g., gender, underlying diseases, …) were collected, please report them.
9-The figures of the manuscript were well designed, which is appreciated, but this is not enough. The text under "Elevation of serum inflammatory biomarkers in two groups" and "Correlation of inflammatory factors in patients with COVID-19 with the control group" lacks descriptive and inferential statistics. Please report the data as mean differences, confidence intervals, and P-values. Furthermore, please add a table reporting the findings of the univariate and multivariate regression models. Findings of the regression models should be reported using β, confidence intervals, and P-values.
Discussion
10-Please mention the small sample size as a study limitation.
11-How did you adjust for confounding variables (e.g., gender, underlying diseases, …) in the study? Please clarify this in the discussion section. If confounders were not adjusted for in the study, this should be noted as a study limitation.
Figures
12-Is the correlation between calprotectin and inflammatory markers assessed by a Spearman logistic regression model? Please check it.

Language
13- The manuscript should be edited by a language editor to improve grammar, punctuation, fluency, and general style.
Best regards

Referee 2 Reviewer

REJECT 17 Oct 2023

dear author
Hello
I reviewed your article titled "Determination of serum calprotectin, heparin-binding protein, and some inflammatory factors in patients with COVID-19 ".
My comments are as follows:
In this study, several inflammatory markers were investigated in COVID-19 patients referred to the clinic as outpatients.
Since patients with COVID-19 whom specialists treat on an outpatient basis have a mild form of the disease, measuring inflammatory markers in the serum provides no additional benefit to the patients. It just imposes an additional cost on the system.
Therefore, the findings of this study do not play a role in managing patients with COVID-19 and are not suitable for publication in this journal.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Hello, dear reviewer

Thanks for your review of the article, the errors have been checked and corrected.

1-Aims, since your study was not a correlational research, I recommend the following style objective: “This study aimed to investigate the serum levels of calprotectin, HBP, and other inflammatory markers in COVID-19 patients.”

This study aimed to investigate the serum levels of calprotectin, HBP, and other inflammatory markers in COVID-19 patients.

2-Result section, since comparison of age was not the primary objective of the study, please omit the first sentence. Comparing the serum levels of calprotectin and other inflammatory markers should be clarified by descriptive statistics (mean difference, confidence interval, or at least P-value). Furthermore, correlation between calprotectin and other markers should be described with correlation coefficient and P-value.

SARS-CoV2 viral RNA was detected in the pharyngeal swab specimens of COVID-19 patients calprotectin, FERR, CRP levels, LDH activity, PLT, and NEU counts were found significantly higher in patients compared with controls ($p<0.05$) whereas no statistically significant difference was observed in HBP level ($p>0.05$). Serum calprotectin showed significant correlations with CRP and FERR levels, LDH activity, and NEU count($p<0.001$).

Introduction

3-“COVID-19 disease/infection” is scientifically incorrect. Please substitute it with “SARS-CoV-2 infection” or “COVID-19” throughout the text.

OK

Method

4-Although the study was approved by University of Hamedan, it was performed in Ayatollah Kashani Hospital in Tehran. Is there a typo error? Or it wrote correctly? Please check it.

Only samples were collected from Ayatollah Kashani Hospital, the text was corrected

5-Please mention time of the study, inclusion and exclusion criteria in the method section.

pharyngeal swab samples were collected from all participants from 5 Aug to 30 Sep 2022 for SARS-CoV-2 testing

6-Was data collection based on census method or sampling? If it was based on sampling, please add how to estimate sample size and sampling method. If not, please mention it was based on the census method.

The amount of sample volume using the formula $N= (Z^{1-\alpha/2}+ Z^{1-\beta})^2 \times (\delta_1^2+ \delta_2^2) / (\mu_1 - \mu_2)^2$ and considering the statistical power of 90% ($Z^{1-\beta}$) and The confidence level of 95% ($Z^{1-\alpha/2}$) was calculated, taking into account the possibility of patients leaving the plan and losing samples, 35 samples were selected for the patient group and 35 samples were selected for the control group

7-Statistical method, which type of regression was used for analyzing the data? Linear, logistic, …. It should be mentioned in the text.

Logistic regression analysis

Results

8-If other baseline characteristic features of the patients (e.g., gender, underlying diseases, …) were collected please report them.

OK,Done

9-The figures of the manuscript were well designed, which is appreciated, but is not enough. The text under the “Elevation of serum inflammatory biomarkers in two groups” and “Correlation of inflammatory factors in patients with COVID-19 with the control group” lacks descriptive and inferential statistics. Please report the data as mean difference, confidence interval, and P-value. Furthermore, please add a table reporting the findings of univariate and multivariate regression model. Findings of regression model should be reported using β, confidence interval, and P-value.

Elevation of serum inflammatory biomarkers in two groups

COVID-19 patients had significantly higher levels of calprotectin compared with control subjects ($p<0.05$) (Fig. 1A) whereas, an increasing trend in patients' HBP levels did not reach statistical significance ($p>0.05$) (Fig. 1B). Serum FERR and CRP levels were also found to be significantly higher in patients compared with healthy subjects ($p<0.05$) (Fig. 1C and Fig. 1D, respectively). COVID-19 patients also showed markedly higher ($p< 0.05$) LDH activity (Fig. 1E) together with a boost in NEU (Fig. 1F) and PLT count (Fig. 1G).

Correlation of inflammatory factors in patients with COVID-19 with the control group

Logistic regression analysis was performed to determine possible correlations between calprotectin and other variables. Statistical analysis showed a significant association between calprotectin and serum levels of CRP, FERR, percentage of neutrophils, and LDH activity ($p<0.001$) (Fig. 2A to Fig. 2D, respectively), but no correlation was observed between calprotectin with PLT count ($p>0.001$) (Fig. 2E), and HBP levels (Fig. 2F). In addition, serum HBP level was not correlated with CRP and FERR levels, LDH activity, NEU, and PLT count(table 1).

Table 1: Logistic regression analysis correlations between calprotectin and HBP with other variables

	B	**S.E**	**P value**	**OR (95% CI)**
Calprotectin × PLT	0.000019	0.000005	0.002	1.000019 (1.000007-1.000034)
HBP× PLT	0.000017	0.000006	0.005	1.000017 (1.000005-1.000028)
Calprotectin × LDH	0.000068	0.000018	<0.001	1.000068 (1.000032-1.00010)
HBP× LDH	0.00002	0.000007	0.003	1.00002 (1.000007-1.000033)

Calprotectin × HBP	0.000068	0.000021	0.001	1.000068 (1.000027-1.00011)
Calprotectin × Neu	0.0006	0.00015	<0.001	1.0006 (1.00034- 1.0009)
HBP× Neu	0.00010	0.00034	0.002	1.00010 (1.000037-0.00017)
Calprotectin × CRP	0.0057	0.002	<0.001	1.0057 (1.0025-1.0089)
HBP× CRP	0.0014	0.00043	0.001	1.0014 (1.00052-0.0022)
Calprotectin × FERR	0.00012	0.000034	<0.001	1.00012 (1.000054-1.00019)
HBP× FERR	0.000031	0.000011	0.004	1.000031 (1.000010-1.000052)

Discussion

10-Please mention low sample size as a study limitation.

Done,OK

11-How did you adjust confounding variables (e.g., gender, underlying diseases, …) in the study? Please clarify it in the discussion section. If confounder did not adjust in the study, it should be noted as a study limitation.

Although people with a history of multiple organ failure, as well as people with cardiovascular diseases, a history of organ transplants, diabetes, and a history of inflammatory diseases, were excluded from the study, the results of this study can be interpreted due to limitations such as (i) The possible effects of ethnicity and gender on biochemical parameters and (ii) infection of patients with different types of SARS-CoV-2 virus (iii) small sample size were considered

Figures

12-The correlation between calprotectin and inflammatory markers is assessed by Spearman logistic regression model?? Please check it.

Done,OK

Language

13- The manuscript should be edited by a language editor to improve grammar, punctuation, fluency, and general style.

Done,OK

2 Revision 1

0 comments

No public comments in this round.