Original Research Article

Inflammatory biomarkers and severity of COVID-19: Cross sectional study among Egyptian patients

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Abstract

The newly emerging coronavirus disease 2019 (COVID-19) is characterized by multisystem inflammatory syndrome. The development of SARS-CoV-2 complications usually starts within few days following infection, and the severity of the disease determines its outcome. Vitamin D insufficiency is associated with risk of lung infections, also cell-based studies reported the ability of vitamin D to control enveloped virus growth. We aimed to investigate the relationship between the most eminent inflammatory biomarkers and the level of vitamin D aiming to provide a tool for early diagnosis and prediction of disease progression. The current study was approved by Research Ethics Committee (REC), Kasr Al-Ainy. After confirmation of being COVID-19 by PCR, the admitted patients were categorized as mild-moderate, and severe-critically ill based on clinical and radiologic data. The total levels of serum 25(OH)D, as well as other pro-inflammatory biomarkers were measured and were analyzed by receiver operating characteristic curve (ROC) analysis for detection of their association with COVID-19 disease severity and to determine their sensitivity and specificity at optimum cutoff points. The area under the curve (AUC) ROC for predicting COVID-19 disease severity was the highest (of 0.97) for vitamin D, inflammatory cytokines, liver enzymes, ferritin, and D-Dimer. In addition, high serum levels of creatinine, and elevated liver enzymes associated with severe-critical COVID-19. The low 25(OH)D was associated with the disease severity.

Keywords: Pro-inflammatory cytokines, vitamin D, COVID-19, multisystem inflammatory syndrome.

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Introduction

In early December 2019, it was owed to being a several pneumonia cases had aroused which were identified later as a novel coronavirus (SARS-CoV-2) which was renamed later by WHO as (COVID-19).[1]

At first, zoonotic disease with human-to-human transmission mainly by droplet mode of infection through respiratory secretions but later, some cases were found to be asymptomatic yet contagious where they remain a silent cause of the wide spread of infection.[1]

Studying human response to COVID-19 revealed a limited inflammatory response with major cytokine changes. [2] Disease severity and the possible outcome was indicated by different serum markers like anemia, deranged leukopenia and leukocytosis, were associated with bad clinical and prognostic outcome in hospitalized patients. Similarly, serum biomarkers like tumor necrosis factor-alpha (TNF-α), interleukin (IL-6), D dimer, ferritin, C-reactive protein (CRP), vitamin D showed the importance in detecting COVID-019 pathogenesis.[3,4] Cytokines play a major role in host inflammatory response. IL-6, produced by various cell types, including lymphocytes, monocytes, and fibroblasts, specifically is one of the most important cytokines due to its multicellular functions. Elevated IL-6 levels had been reported obviously in respiratory failure patients, which may be an underlying mechanism of severe lung fibrosis observed in COVID-19 patients. In addition, SARS-CoV-2 is manifested by high viral replication rate with relevant lower respiratory tract infection resulting in high Il-6 level in respiratory failure patients.[5,6]

Not only cytokines do play a role but also CRP serves as a serum marker for inflammatory response. CRP mainly binds to damaged cell surface activating complement system cascade followed by clearance of microbe infected cells by phagocytosis. [7]

Upon measuring CRP level in hospitalized patients, it was recorded to reach 80-170 mg/L in COVID-19 cases and to reach 111–234g/dl on intensive care unit (ICU) admission. Survived cases showed a lower CRP peak with a rapid declination rate than severe cases or cases that died. Far elevated levels of CRP were recorded in two-thirds of severe COVID-19 patients. [8] These CRP serum level fluctuations associated with the infection process make it a reliable prognostic marker for the detection of disease progression.[7-9]

Some studies had found a relation between severe COVID-19 and low levels of vitamin D (vitamin D deficiency). Vitamin D, which has an immunomodulatory effect, can reduce the risk of infections and concentrations of pro-inflammatory cytokines. Some assumed that vitamin D deficiency could be an underlying cause for catching COVID-19 infection especially in chronic diseases, hepatic, or renal patients rather than an outcome of infection.[10]

Recently, 25-hydroxy vitamin D (25-OHD) a state of vitamin D has been used as a suggestive therapeutic agent that can be used in the management of COVID-19, also had been questionable for its role in prophylaxis against infection.[10]

Our study aimed at investigating the relationship between the most potential serum pro-inflammatory biomarkers (TNF-α, IL-6, D-dimer, ferritin, and CRP) and vitamin D level and their correlations with clinical severity which may provide a tool for better prediction of disease progression and prognosis.

Patients and Methods

The study protocol was reviewed and official approval by the Research Ethics Committee (REC), Kasr Al Ainy (NO; N-41-2020). Data confidentiality were maintained throughout the research as being anonymous. No identified risk was reported as all blood samples were taken under complete aseptic conditions. They were collected as a part of patients' routine laboratory investigations with extra 5 ml from PCR positive cases then screened for IL-6, TNF-α, D-dimer, ferritin, and CRP for detecting their levels, and estimating AST, ALT, and vitamin D levels.

Study design

This is a cross sectional study conducted at Kasr Al Ainy Hospital, Cairo, Egypt from 2020-2021.

Study populations

Patients above 18 confirmed to be infected with COVID-19, categorized as mild and moderate on one side and severe and critically ill on the other side according to clinical and radiological findings were included in the study. They were categorized as follow; mild (no or mild pneumonia), moderate (pneumonia without hypoxia), severe disease (with dyspnea, hypoxia, or >50 percent lung involvement on imaging within 24 to 48 hours) and critical disease (with respiratory failure, shock, or multiorgan dysfunction). [11] Data were collected from hospital records of patients which included age, sex, smoking, history of chronic diseases.

Sample size was calculated based on assumptions from previous research[12] on proportions of cases with high IL-6 in mild to moderate (59%) and severe to critical (97%) COVID-19 groups, using STATA program for sample size on proportions estimation test, study parameters were: two-sided alpha of 0.05, power of 0.80, delta 0.3800 (difference), $P1 = 0.59$ and $P2 = 0.97$.

Inclusion criteria

The patients included in current study were COVID-19 positive as confirmed by PCR for filing eligibility criteria, all patients were above 18 years from both sexes and we did not exclude patients with disease conditions such as DM, HTN, chronic heart diseases   etc. All participants in this study were assessed at time of admission.

Laboratory estimated parameters

The collected blood samples from all patients were centrifuged, and supernatant serum samples were collected and used for estimation of all parameters according to manufacturer instructions. The transaminases (AST and ALT) were measured using human AST ELISA Kit (ab263881, Abcam co, UK) and human ALT ELISA Kit (ab234578, Abcam co, UK). Quantification of D-dimer levels was done using human D-dimer ELISA Kit (ab260076, Abcam co, UK).

Using Abcam's human C Reactive Protein ELISA Kit (CRP) (ab99995, Abcam co, UK), and human ferritin ELISA Kit (ab108698, Abcam co, UK), quantitative measurement of CRP and serum ferritin, respectively were determined using calorimetric methods.

Sandwich (quantitative) assay of IL-6 and TNF-α was done using human IL-6 ELISA Kit (ab46027, Abcam co, UK) and human TNF alpha ELISA Kit (ab181421, Abcam co, UK), respectively. Finally, quantitative estimation of the total 25(OH) vitamin D was done using colorimetric method vitamin D (ab213966, Abcam co, UK).

Statistical analysis

All collected data were revised for completeness and accuracy. Pre coded data were entered on the computer using the statistical package of social science software program, version 21 (SPSS) and statistically analyzed. Data were summarized using mean, SD, median and IQR for quantitative variables, number, and percent for qualitative variable. Comparison between qualitative variables was done using chi square test for qualitative variables. Fisher exact test was used when one expected cell or more are less than 5. Checking normality distribution of quantitative variables was done then comparison between quantitative variables using independent T test for quantitative variable which where normally distributed and nonparametric Kruskal-Wallis and Mann-Whitney tests for quantitative variables which were not normally distributed. Receiver operating characteristic (ROC) curve was used to assess the discriminate power of the clinical biomarkers in predicting the disease severity. Correlations between levels of vitamin D from one side and TNF-α, IL-6, CRP, D-dimer and serum ferritin on the other side were done using Spearman test.[13] The probability (*P*)-values equal to or less than 0.05 were considered as statistically significant.

Results

A total of 56 patients were included in the analysis. Their age ranged between 37 and 85 years and the mean ±SD was 60.52 ±11.54 years. 64.3% of them were in the severe-critical disease category (n=36) while 35.7% (n=20) were mild-moderate. As shown in Table 1, there was no statistically significant difference between the mild-moderate and severe-critical group regarding gender, smoking, chronic disease history. However, for the severe-critical group the age (mean ±SD=63.64±11.85 years) was larger than the mild-moderate group (mean ±SD =54.90 ±8.68 years). This difference was statistically significant (P=0.006).

Table 1. Characteristics of the 56 study subjects as arranged by COVID-19 disease severity.

	COVID-19 disease severity		
	Mild-Moderate	Severe-Critical	*P* Value
Age mean ± SD	54.90 ± 8.68	63.64 ± 11.85	0.006
Gender N (%)			
Male	10 (29.4)	24 (70.6)	NS
Female	10 (45.5)	12 (54.5)	
Chronic disease N (%)			
No	7 (21.2)	26 (78.8)	0.011
Yes	12 (54.5)	10 (45.5)	
Smoking N (%)			
Non-smoker	14 (34.1)	27 (65.9)	NS
Smoker	6 (40.0)	9 (60.0)	

P value >0.05 is. Not significant (NS).

Table 2 shows the bivariate association between clinical and laboratory parameters and COVID-19 disease severity categories. In the clinical assessment parameters, fever, rapid heart rate, high respiratory rate and oxygen saturation were associated with disease severity. Severe-critical cases had significantly higher respiratory rate and lower oxygen saturation means ±SD, 24 (20:26) and 87 (81:90), respectively compared to mild-moderate cases means ±SD of 16 (16:18) and 95 (95:96), respectively. In the laboratory parameters, platelet count, ALT, AST, D-dimer, CRP, ferritin, IL-6, TNF-α and vitamin D levels were significantly associated with COVID-19 disease severity, ($p < 0.001$). While no bivariate association detected between hemoglobin level, total leucocytes count, LDH and creatinine and disease severity.

Table 2. Clinical and biochemical data of the 56 study subjects.

	COVID-19 disease severity		
	Mild-Moderate	Severe-Critical	*P* value
Vital Signs			
SBP	126.0 ±8.21	127.58 ±11.37	NS
DBP	75.75±7.48	80.61±12.23	NS
Temp	37.59 ±0.83	37.36± 0.78	NS
RR*	16.0 (16:18)	24.0 (20:26)	<0.001
HR	84.5 ±8.41	102.24± 13.69	<0.001
Oxygen saturation			
SO_2* (%)	95 (95:96)	87(81:90)	<0.001
Complete blood count			
TLC	8.31±3.79	10.66±4.98	NS
HB (g/dl)	12.93±1.74	12.17±2.12	NS
Platelet	317.85±122.66	220.79±90.36	0.003

Table 2. Continued.

	COVID-19 disease severity		
	Mild-Moderate	Severe-Critical	*P* value
Kidney profile			
CREAT* (mg/dl)	1.10(0.8:1.1)	1.14(1:1.5)	0.028
Liver enzymes			
AST (U/L)	40.1± 4.3	70.1±15.1	<0.001
ALT (U/L)	36.0±4.1	67.8± 13.6	<0.001
Coagulation markers			
D-DIMER* (ng/ml)	504.7±110.5	944±170.7	<0.001
Inflammatory markers			
CRP* (mg/l)	46.0 (25.9:56.2)	98.6 (91.5:114.9)	<0.001
Ferritin (ng/ml)	619.9±103.62	944.0 ±170.7	<0.001
Interleukins and tumor necrosis factors			
IL6 (pg/ml)	144.7±15.7	228.6±34.1	<0.001
TNF* (pg/ml)	60.8 (59.7:62.2)	89.4 (83.05:93.75)	<0.001
Vitamin D level			
Vitamin D* (ng/ml)	19.12(17.97:23.85)	13(12.25:14.75)	<0.001

* Median and interquartile range. *P* value >0.05 is. Not significant (NS).

ROC analysis for the 8 factors associated with COVID disease severity is shown in Figure 1. They all have excellent discriminatory power to differentiate mild-moderate from severe-critical COVID-19 disease with ROC curve AUC of 0.97 for vitamin D, IL-6, TNF, AST, ALT, Ferritin, D-Dimer and CRP.
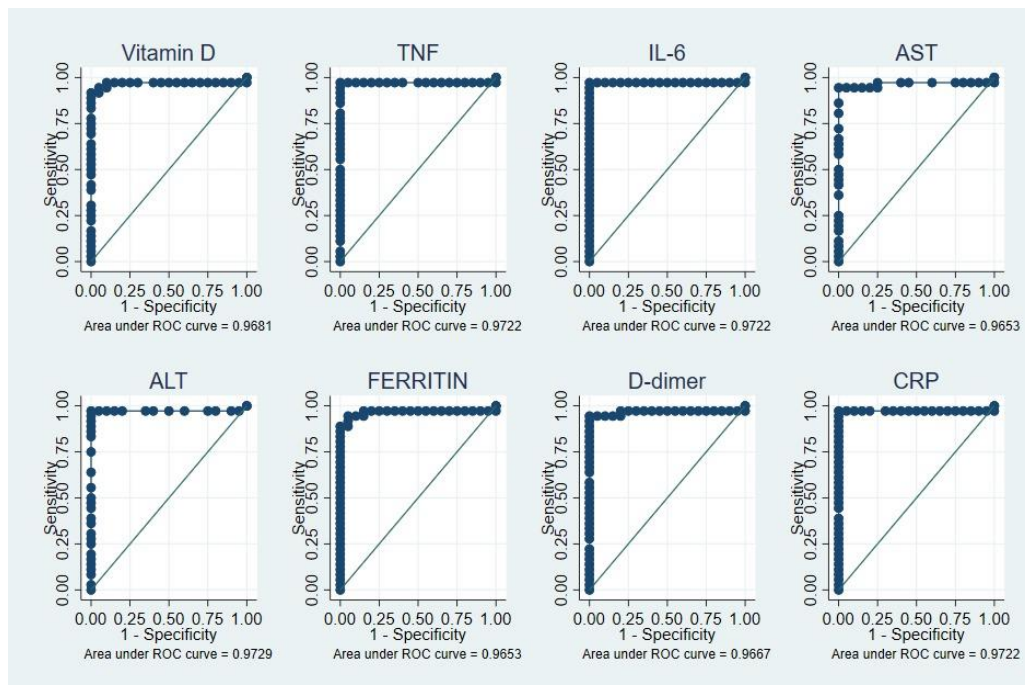

Figure 1. ROC analysis for the 8 parameters: vitamin D, TNF, IL-6, AST, ALT, Ferritin, D-Dimer and CRP. They all have excellent discriminatory power to differentiate mild-moderate from severe-critical COVID-19 disease with the area under ROC curve (AUC) of 0.97.

Table 3 shows the detailed AUC and confidence interval (CI) of the eight factors and their sensitivity and specificity at optimum cutoff points. Vitamin D level at 16.1 ng/dl cut off correctly classified 94.6% of cases with sensitivity and specificity of 91.7% and 100% respectively. Both IL-6 and TNF at cut-of 201.4 and 75.2, respectively correctly classified 98.2% of cases and had sensitivity of 97.2% and specificity of 100%. ALT and AST had sensitivity of 97.2 and 94.4 at cut-off value of 51 and 56, respectively, both showed sensitivity of 100%. CRP at >75.8 and ferritin at >793) had 97.2 and 91.7 sensitivity, respectively, and 100% specificity for both.

Table 3. Diagnostic performance of biomarkers in differentiating disease severity

Marker	AUC*	95% CI	Recommended cut off	Correctly classified %	Sensitivity	Specificity
Vitamin D	0.968	0.913 - 1.000	≤16.1	94.6	91.7	100.0
IL-6	0.972	0.918 - 1.000	> =201.4	98.2	97.2	100.0
TNF	0.972	0.918 - 1.000	>=75.2	98.2	97.2	100.0
Ferritin	0.965	0.910 - 1.000	>= 793.4	92.8	91.7	95.0
CRP	0.972	0.911 - 1.000	>= 75.8	98.2	97.2	100.0
AST	0.965	0.910 - 1.000	>= 56	96.4	94.4	100.0
ALT	0.973	0.910 - 1.000	>= 51	98.2	97.2	100.0
D-Dimer	0.968	0.911- 1.000	>= 763.2	96.4	94.4	100.0

* Area Under the ROC curve

Correlation study

The correlation between level of vitamin D from one side and TNF-α, IL-6, CRP, D-dimer and serum ferritin on the other side were done. Data showed a negative association between vitamin D and TNF-α (r = -0.0669; P <0.001), IL-6 (r = -0.755; P<0.001) and CRP (r=-0.796; P<0.001), D-dimer (r=−0.704; P<0.001), and serum ferritin (r=−0.704; P<0.001) (Figure 2).
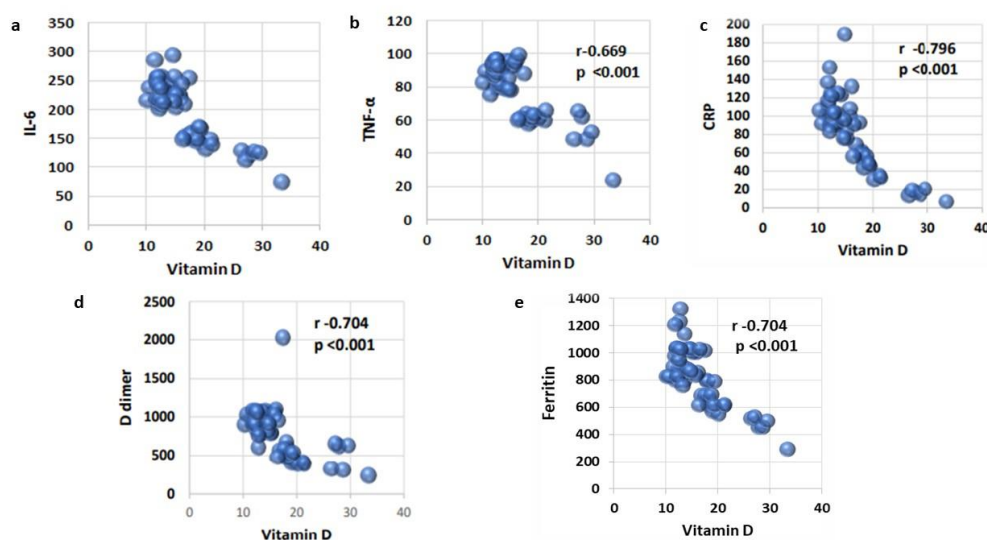

Figure 2. Correlation between Vitamin D levels in all studied groups and estimated inflammatory biomarkers. Vitamin D levels was negatively correlated with (a) IL-6 (r= -0.755; P<0.001), (b) TNF-α (r=-0.0669; P<0.001), (c) CRP (r=-0.796; P<0.001), (d) D-dimer (r=−0.704; P<0.001), and (e) serum ferritin (r=−0.704; P<0.001).

Discussion

It was previously reported that the worse outcome of COVID-19 is highly correlated with the degree of respiratory system affection, in addition to level of pro-inflammatory cytokines that underline severity of symptoms and pathophysiology of the disease.[14]

The degree of viral load is the contributory factor for development of cytokine storm syndrome (CSS) and secondary hemophagocytic lymphohistiocytosis (sHLH) in addition to cytopenia, increased ferritin, and IL-6. This viral load drives the occurrence of hyper-inflammation and augments immune response.[15, 16]

In our work, the results showed increased levels of IL-6, TNF-α, CRP and serum ferritin in all PCR positive cases and the levels of these cytokines were positively correlated with severity of the cases that was confirmed clinically.

During progression of the disease particularly in severe cases of COVID-19, the pro-inflammatory cytokines and D-dimer increase. In this context, using anti-inflammatory treatment was approved during the acute phase of lung affection aiming to inhibit cytokine storm especially anti-IL-6.[17] Thus, one of the most important challenges is to early identify which patient will develop a more severe form of COVID-19 infection and which one will be in need for specific line of treatment.

Currently, our results showed high serum levels of creatinine, AST, and ALT in patients with severe-critical COVID-19 compared to mild-moderate patients that may indicate that liver, and kidney dysfunctions were involved. Indeed, it may highlight the occurrence of hepatic and renal damage secondary to viral infection.[18] Meanwhile, the intervention should be taken in time aiming to prevent irreversible organ damage.

LDH is a cytoplasmic enzyme, which is not supposed to be present in the extracellular space, consequently detection of this enzyme in serum samples may suggest disruption of the cell membrane and loss of cellular integrity.[19] Previously, the association between several pulmonary diseases and serum levels of LDH was reported. In pulmonary diseases, alveolar macrophages and polymorphonucleocytes may be a potential source of increased serum LDH, in addition LDH was detected previously in bronchoalveolar lavage samples, hence it could be used to follow up pulmonary inflammation.[20]

D-dimer and fibrin degradation products were estimated at the time of admission for COVID patients, and their results showed significant increase in these parameters among the non-surviving patients compared with survivors.[21] In the present cross-sectional study, increased levels of D-dimer at hospital admission of COVID patients were estimated and data confirmed significant increase in D-dimer level in sever-critical patients who required ICU admission compared with mild-moderate cases.

In our study, we confirmed increased serum ferritin; the acute protein that increases in response to systemic inflammation and infections, or secondary to malignancy.[22] In a meta-analysis included 40 studies (containing 9,542 COVID patients), the authors highlighted the role of ferritin in addition to IL-6 level as a prognostic factor for detecting the poor outcome of COVID infection.[23]

Away from its major contribution in bone and mineral metabolism, vitamin D plays a crucial role in regulation of immune response, especially during viral infection. The risk of respiratory-tract infections becomes higher in case of vitamin D insufficiency. Furthermore, studies carried on cell-culture have shown that vitamin D controls enveloped viruses' growth and had direct antiviral effects knowing that coronavirus is an enveloped virus, vitamin D may prove as having role in its pathogenesis.[24] This fact may be due to upregulating human beta β-defensin2 and antimicrobial peptide in response to vitamin D. Not only antiviral effect but also it guards against tuberculosis by increasing secretion of cathelicidin the antimicrobial peptide, decrease secretion of chemokines, downregulating dendritic cell activation, and consequently activation of T-cells.[24]

Vitamin D levels were reported to be associated with higher levels of the anti-inflammatory cytokines and lower levels of the proinflammatory cytokines such as interleukin (IL-6). [25] Moreover, vitamin D could regulate the innate immunity and its deficiency has been associated with chronic inflammatory states. [26]

Hundreds of studies were conducted on COVID patient since its arousal in Wuhan 2019. Some studies correlated levels of pro-inflammatory cytokines with the severity of diseases, others showed associations between vitamin D levels and number of COVID-19-positive cases.[27,28] In a pilot randomized clinical study performed in Reina Sofia University Hospital, Cordoba Spain, all COVID patients received standard lines of treatment according to the guide line of this hospital then those patients were divided into 2 groups, one group supplemented with vitamin D and the other with placebo. Thereby, intake of vitamin D at a high dose could significantly reduce the needs for ICU admission for the hospitalized cases. [29]

The present study reported association between vitamin D levels measured at time of admission with the severity of COVID-19 symptoms and levels of other pro-inflammatory factors (IL-6, TNF-α, and CRP), D-dimer and serum ferritin. Our results showed that serums vitamin D level was significantly decreased in sever-critical patients compared to mild-moderate patients ($P<0.001$) and these findings were consistent with other previous studies. [28,29] Moreover, the AUC ROC for predicting adverse outcome of COVID severity was the highest of 0.97 for vitamin D, IL-6, TNF-α, AST, ALT, ferritin, D-Dimer and CRP.

In conclusion, our study demonstrated that high levels of IL-6 and TNF-α were associated with pulmonary inflammation and extensive lung damage, and it is recommended to start early treatment with anti-cytokines and immunosuppressive therapy in certain hospitalized patients who are exhibiting rapid respiratory decompensation detected by our cut off values. Also, keeping appropriate blood levels of vitamin D could be a good preventive measure against catching the infection.

Author Contributions

AMS, AF, AAA, conceptualization. SS, EAH, AAA, methodology. LR, HH, SS, validation. LR, AYA, investigation. AMS, AAA, SS, data curation. All authors shared in writing original draft preparation, writing, review and editing. AMS, project administration and final revision.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and official approval by the Research Ethics Committee (REC), Kasr Al Ainy (NO; N-41-2020).

Informed consent

A signed consent form was obtained from each study participant.

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