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Evaluation of the relationship between viral load and biochemical parameters in Covid-19 patients

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Abstract

In this study, we aimed to investigate the relationship between the viral load detected by PCR and the biochemical and demographic data of patients who were admitted to our hospital and positive for SARS-CoV-2 infection. Data from 132 laboratory-confirmed adult patients were retrospectively analyzed. COVID-19 patients were classified in different groups as pneumonia-non pneumonia and symptomatic- asymptomatic patients. In all, 77.2% patients were symptomatic and 39.4% had pneumonia. The most common laboratory abnormalities of all patients were elevated C-reactive protein (CRP), D-dimer, Fibrinogen and Neutrophil/lymphocyte ratio (NLR). Statistically significant differences ($p < 0.05$) were found between the symptomatic and asymptomatic groups regarding CRP, NLR, Prothrombin Time (PT), international normalized ratio (INR), D-dimer and Fibrinogen. Additionally of these parameters significantly higher aspartate amino-transferase (AST), blood urea nitrogen (BUN), creatinine and lactate dehydrogenase (LDH) observed in pneumonia group compared to non-pneumonia group. The cycle threshold (Ct) values in all patients were 32.42 ± 6.03 and there were no significant differences in Ct values between the groups. There was a negatively significant correlation between Ct and blood urea nitrogen (BUN) (r=-0,205, P=0.019). Abnormalities of several hematologic and biochemical biomarkers were associated with SARSCoV-2 infection and disease severity. To investigate the association with disease severity and viral load, quantitative PCR results would be more accurate than semi-quantitative Ct results.

Keywords: COVID-19, cycle threshold, SARS-CoV-2, viral load

Introduction

The effects of severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), which started in the state of Hubai of China and spread rapidly to the other countries, still continues all over the World [1]. Coronavirus disease 2019 (Covid-19, an infectious disease caused by SARS-CoV-2), which causes a wide range of lung diseases from pneumonia to acute respiratory syndrome, can also cause coagulopathy and bleeding disorders [1,2].

As in the diagnosis and treatment of many diseases, the importance of laboratory findings in the treatment and follow-up cannot be denied in SARSCoV-2 infection [3].

Real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) is routinely used for SARS-CoV-2 detection in different types of samples oftenly collected with oropharyngeal or nasopharyngeal swabs and bronchoalveolar lavage. The cycle threshold (Ct) values of qRT-PCR represent the number of amplification cycles required for the target gene to exceed a threshold level. Ct values are inversely related to viral load and correlated with copy numbers of the viral RNA in the sample which means lower Ct values corresponding to higher viral copy numbers [4].

Although PCR is the gold standard method in the diagnosis of Covid-19, healthcare professionals also benefited from biochemical and hematological parameters [1-3,5].

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On the other hand, the accuracy of the PCR test was debatable in all countries [5].

Laboratory findings are important not only in diagnosis but also in treatment follow-up [6].

The most common laboratory abnormalities in Covid 19 disease are increased neutrophil decreased lymphocyte hypoalbuminemia increased d-dimer levels and increased CRP and LDH levels [1].

Studies in the field of Covid 19 since the beginning of 2020 have revealed that biochemical and hematological data correlate with the severity of the disease [1,7,8] .

In this study, we aimed to reveal the relationship between the viral load detected by PCR and the biochemical and demographic data of patients who were admitted to our hospital and positive for SARS-CoV-2.

Material and Methods

Study Design

A total of 132 adult patients admitted to the Pamukkale University Hospital or people who were close contact with the confirmed Covid-19 case between March 19 to May 13, 2020 were enrolled in this retrospective observational cohort study. Patients were diagnosed as COVID-19 according to guidelines for the diagnosis and treatment of COVID-19 infection from the Turkish Ministry of Health, Directorate General of Public Health created mainly in line with WHO recommendations. Only laboratory confirmed cases were included in this study with nucleic acid testing. Chest computed tomography scan was acquired to the patients upon admission to the hospital. Clinical, demographic and laboratory data were obtained from patients' medical records. We classified and evaluated COVID-19 patients in different groups as pneumonia-non pneumonia and symptomatic- asymptomatic patients. The study was approved by the Research Ethics Commission of Pamukkale University and Turkish Ministry of Health (Approval No. 60116787-020/31814, 2020-05-21T09_52_32)

Viral loads detection

Combined naso-oropharyngeal swabs were collected from the patients who met the criteria laid out in the guidelines and analysed with reverse-transcription polymerase chain reaction (RT-PCR) in Central Laboratory of our hospital. The RNA was isolated using vNAT solution (Bioeksen, Istanbul, Turkey). For all reactions, Rotor-Gene Q (Qiagen, Antwerp, Belgium) instrument and Biospeedy SARS-CoV-2 RT-qPCR kit (Bioeksen) were used. The kit is performed in one step with targeting the RdRp (RNA-dependent RNA polymerase) gene fragment reverse transcription (RT) and rt PCR (QPCR) (RT-QPCR). The data was analyzed using Rotor-Gene Q Software. A cycle threshold value less than 40 is interpreted as positive for SARS-CoV-2 RNA.

Biochemical measurements

The complete blood count analysis was carried out on Mindray BC-6800 system through the electrical impedance method, and Neutrophil/lymphocyte ratio (NLR) was calculated in accordance with the CBC results. Serum biochemical parameters were analyzed with electrochemilumisence method on Cobas 702 autoanalyser (Roche Diagnostics GmbH, Mannheim, Germany). Coagulation assays included D-dimer and Fibrinogen were performed with ACL TOP 700 laboratory analyzer (Instrumentation Laboratory, Werfen, USA).

Statistical analysis

All statistical analyses were performed using SPSS 25.0 software. Kolmogorov Smirnov and Shapiro Wilk tests were used for determination of normal distribution. Continuous variables were defined by the mean ± standard deviation and categorical variables were defined by number and percent. For independent groups comparisons, we used Independent samples t test when parametric test assumptions were provided, and Mann-Whitney U test were used when parametric test assumptions were not provided. We used spearman correlation analysis to analyze the relationships between continuous variables. Statistical significance was determined as $p<0,05$.

Results

A total of 132 (56.8 % male, 43.2 % female) patients were included in the study. In all, 77.2% were classified as in the symptomatic group (n=102), 22.7% in the asymptomatic group (n=30), and in the other classification 39.4% is in the pneumonia group (n=50), 60.6% in the non-pneumonia group.

Mean age of all patients were 45.64 ± 19.17 (18-85) years and mean age of patients with pnomonia group was higher (54.06 ± 18.27 vs 40.18 ± 17.81 years, $p < 0,001$). Demographic and clinical characteristics of the patients are summarized in Table 1.

The most observed frequent symptoms at the time of admission were cough (53%), fever (32.6%), and followed by dyspnea (19.7%) and myalgia (9.8%). But besides that important part of the patients were laboratory-confirmed fully asymptomatic cases (22.7%). Characteristics of the patients in terms of symptoms are given in Table 1.

The C-reactive protein (CRP) (29.18 ± 53.84 mg/L), NLR (3.81 ± 4.37), D-dimer (322.86 ± 557.99 ng/mL) and Fibrinogen (404.65 ± 198.3 mg/dL) levels were elevated and the other parameters were normal in all patients. Symptomatic group had significantly higher levels CRP, NLR, Prothrombin Time (PT), international normalized ratio (INR), D-dimer and Fibrinogen and significantly lower level of albumin compared to the asymptomatic group ($p < 0.05$). In pneumonia group compared to the non-pneumonia group same abnormal results were observed as symptomatic group and additionally aspartate amino-transferase (AST), blood urea nitrogen (BUN), creatinine and lactate dehydrogenase (LDH) were significantly higher and platelet count was significantly lower ($p < 0.05$). Laboratory findings of the all patients and the groups are given in Table 2.

The Ct values in all patients were 32.42 ± 6.03 (range, 10 to 40). There was no significant difference in Ct values between the groups ($p > 0.05$). When the relation between Ct values and biochemical and demografic parameters were analyzed with using the Spearman's rank correlation coefficient method in both patients and groups, there was a negatively significant correlation with BUN (r=-0,205, P=0.019).

Table 1. Demographic and clinical findings of patients with COVID-19

	All cases (n=132)	Pneumonia (n=52)	Non-Pneumonia (n=80)
Age, years	45.64 ± 19.17	54.06 ± 18.27	40.18 ± 17.81
Male, n (%)	75 (56.8)	33 (63.5)	42 (52.5)
Symptoms			
Asymptomatic, n (%)	30 (22.7)	3 (5.8)	28 (35)
Fever, n (%)	43 (32.6)	29 (55.7)	14 (17.5)
Cough, n (%)	70 (53)	38 (73.1)	32 (40)
Loss of smell or taste, n (%)	3 (2.3)	2 (3.8)	1 (1.25)
Headache, n (%)	8 (6.1)	3 (5.8)	5 (6.25)
Diarrhoea, n (%)	5 (3.8)	4 (7.7)	1 (1.25)
Dyspnea, n (%)	26 (19.7)	16 (30.8)	10 (12.5)
Fatigue, n (%)	8 (6.1)	4 (7.7)	4 (5)
Sore throat, n (%)	10 (7.6)	2 (3.8)	8 (10)
Myalgia, n (%)	13 (9.8)	5 (9.6)	8 (10)

Table 2. Demographic features and laboratory findings of the study population

	All cases (n=132)	Symptomatic cases (n=102)	Asymptomatic cases (n=30)	P value	Pneumonia (n=52)	Non-Pneumonia (n=80)	P value
Age, years	45.64 ± 19.17	46.75 ± 19.73	41.9 ± 16.91	0.283	54.06 ± 18.27	40.18 ± 17.81	0.0001
Male, n (%)	75 (56.8)	60 (58,8)	15 (50)	0,391	33 (63.5)	42 (52.5)	0,214
CRP, mg/L	29.18 ± 53.84	37.2 ± 58.92	1.9 ± 3.14	0.0001	44.17 ± 55.89	19.44 ± 50.46	0.0001
ALT, U/L	23.18 ± 19.94	24.44 ± 21.96	18.9 ± 9.57	0.273	24.71 ± 19.76	22.19 ± 20.11	0.526
AST, U/L	22.21 ± 12.32	23.4 ± 13.62	18.17 ± 4.16	0.19	25.35 ± 14.12	20.18 ± 10.59	0.006
GGT, U/L	38.45 ± 55.54	41.68 ± 59.94	21.56 ± 12.73	0.422	32.03 ± 22.7	46.4 ± 79.4	0.273
BUN, mg/dL	15.11 ± 10.86	16.09 ± 12.06	11.8 ± 3.44	0.431	17.92 ± 13.01	13.29 ± 8.83	0.023
Creatinin, mg/dL	0.89 ± 0.37	0.93 ± 0.4	0.77 ± 0.13	0.092	1 ± 0.44	0.83 ± 0.29	0.011
LDH, U/L	214.04 ± 88.67	217.76 ± 95.16	199 ± 54.18	0.74	242.48 ± 91.27	194.01 ± 81.63	0.0001
Troponin, ng/mL	21.97 ± 83.12	26.28 ± 92.41	4.48 ± 3.06	0.091	33.4 ± 111.46	8.38 ± 12.83	0.069
Albumin, g/L	41.24 ± 6.41	40.12 ± 6.22	46.39 ± 4.54	0.0001	39.64 ± 5.16	43.18 ± 7.25	0.003
Hb, g/dL	13.83 ± 1.99	13.64 ± 2.04	14.49 ± 1.7	0.071	13.47 ± 1.84	14.07 ± 2.06	0.092
NLR	3.81 ± 4.37	4.31 ± 4.81	2.08 ± 1.24	0.012	4.69 ± 5.67	3.23 ± 3.17	0.142
PLT, ×109 /L	241356.06 ± 68757.36	237068.63 ± 72422.61	255933.33 ± 52952.11	0.188	225711.54 ± 80156.55	251525 ± 58529.87	0.049
PT, s	13 ± 2.53	13.27 ± 2.79	12.1 ± 0.93	0.02	13.98 ± 3.32	12.37 ± 1.59	0.0001
PTINR	1.11 ± 0.21	1.13 ± 0.23	1.03 ± 0.07	0.016	1.18 ± 0.28	1.06 ± 0.13	0.0001
APTT, s	31.4 ± 4.43	31.32 ± 4.77	31.66 ± 3.16	0.305	31.51 ± 5.76	31.33 ± 3.36	0.729
DDimer, ng/mL	322.86 ± 557.99	380.86 ± 619.5	126.07 ± 136.8	0.038	496.73 ± 744.46	207.73 ± 349.56	0.0001
Fibrinogen, mg/dL	404.65 ± 198.3	436.71 ± 209.45	292.42 ± 91.04	0.02	441.15 ± 183.71	368.15 ± 208.91	0.028
Cycle threshold values (Ct)	32.42 ± 6.03	32.61 ± 5.94	31.8 ± 6.4	0.496	31.37 ± 6.46	33.11 ± 5.67	0.102

CRP, C-Reactive Protein; ALT, Alanine Transaminase;AST, Aspartate Transaminase; GGT, Gamma-Glutamyltransferase; BUN, Blood Urea Nitrogen; LDH, Lactate Dehydrogenase; Hb, Hemoglobin; NLR, Neutrophil To Lymphocyte Ratio; PTINR, International Normalized Ratio; PT, Prothrombin Time; APTT, Activated Partial Thromboplastin Time

Discussion

TheIn this retrospective study we aimed to evaluate the biochemical and demographical markers in patients with Covid-19 and also investigate the relationship between Ct values which are inversely related to viral RNA load. We hypothesized that lower Ct values may be associated with biochemical markers which were increased or decreased with Covid-19 disease and older ages which has worse outcomes.

Covid-19 patients have a wide range of symptoms can range from absence of symptoms to severe illness and fatal outcomes [9]. Among 616,541 persons with COVID-19 in United States, 4% of cases were asymptomatic, 70% had fever, cough, or shortness of breath, 36% reported muscle aches, and 34% reported headache, 8% patients experienced loss of smell or taste [10]. In a report among 72,314 cases in China published by the Chinese Center for Disease Control and Prevention, 81% of cases were reported as mild (no pneumonia or mild pneumonia), 14% were severe (defined as dyspnea, respiratory frequency ≥30/min, blood oxygen saturation ≤93%, PaO2/FiO2 <300 mmHg, and/or lung infiltrates >50% within 24–48 h), and 5% were critical (i.e. respiratory failure, septic shock, and/or multiple organ dysfunction or failure) [11].

In our study, 22.7 % of cases were asymptomatic, 53 % had cough, 32.6 % had fever, 19.7 had dyspne and the other less frequently symptoms were myalgia, fatigue, sore throat, headache, diarrhoea and loss of smell or taste (Table 1). The reason for the high rate of asymptomatic patients in our study may have been obtained by screening and testing people in contact with Covid-19 positive patients without waiting for symptoms.

The laboratory abnormalities in patients with COVID-19 has been published and summarised in several meta-analysises and observed in hematologic, biochemical, coagulation paremeters and inflammatory biomarkers [6,12]. The most common laboratory abnormalities were hypoalbuminemia, lymphopenia, increased liver enzymes, increased ratio of neutrophils (NEU) to lymphocytes (LYM), elevated C-reactive protein (CRP) and lactate dehydrogenase (LDH), and increased D dimer and PT [1, 2]. In our study, the mean levels of CRP, NLR, D-dimer and Fibrinogen were elevated in all patients. But in the meanwhile, laboratory findings of the asymptomatic patients were normal. Compared with symptomatic patients there were statistically significant difference between CRP, NLR, D-dimer, PT, INR, fibrinogen and albumin levels. Ma Y et al. showed that asymptomatic patients' most of the laboratory test results were not statistically significant compared with symptomatic patients and reported that whether some asymptomatic patients' had abnormal laboratory indicators, the damage was minor and soon returned to normal [13]. Hovewer, when we compared the pneumonia and nonpneumonia groups there were lots of significant differences in several parameters between groups (Table 2). The Covid19 patients with pneumonia were significantly older (54 years) than with non-pneumonia (40 years) ($p<0.0001$). Liu et al. reported that among the elderly group of Covid19 patients the Pneumonia Severity Index score which can predict the mortality in community acquired pneumonia was higher than young and middle-aged group [14,15]. Renal disease and acute kidney injury associated with severity and in-hospital mortality of Covid-19 patients at the time of admission or during hospitalization [16, 17]. In our study, pneumonia group had significantly higher levels of serum creatinine and BUN compared to non pneumonia group. It is important to follow-up kidney function tests of these patients and increase the awareness of kidney injury development.

Most of the Covid-19 patients have coagulopathy and characterized by elevations in fibrinogen and D-dimer levels and prolongation of the PT and INR. These abnormal coagulation parameters are associated with poor prognosis and could be used as the significant indicators in predicting the mortality of Covid-19 [18, 19, 20]. These reports are consistent with our research showing that pnemonia group had significantly abnormal coagulation test results in favor of coagulopathy compared to the nonpneumonia group.

Viral load is inversely related to the Ct value and it has been previously recommended that the viral load of Covid-19 have an impact on disease severity and likelihood of transmission [21-24]. A systematic review summarized the studies and concluded that lower Ct values may be associated with worse outcomes and useful in predicting the clinical course and prognosis of patients with covid-19 and reporting of Ct values may offer benefit to clinicians in making clinical and patient-management decisions [25]. Although lower Ct values generally correlate with high viral loads, Ct value itself cannot be directly interpreted as viral load without a standard curve using reference materials because Ct influenced by the instruments and assay protocols [21, 26,27]. In our study, as shown in Table 2, no statistical significance was found between the groups in Ct values. This finding may be explained by Ct values should not be investigate as viral load with qualitative RT-PCR test results.

We also examined correlation between Ct values and biochemical and demografic parameters with Spearman method, and BUN was negatively correlated with the Ct value. This is consistent with previous study showing that BUN was positively correlated with the SARS-CoV-2 viral load, study also reported NEU and CK-MB were negatively correlated with viral load [28]. A recent study has shown that CRP, ALB, LYM (%), LYM and NEU were highly correlated to the Ct value [1].

Limitations of this study

Our study has several limitations. Firstly, this study is a single center cohort study, and the sample size was insufficient to compare Ct in different subgroups. Secondly, Ct is semiquantitative method and influenced by many preanalytical factors such as the quality, source, transportation and sampling timing of collected samples.

Conclusion

Abnormalities of several hematologic and biochemical biomarkers were associated with SARSCoV-2 infection. In covid-19 confirmed and also suspected cases, the severity of the disease can also be evaluated with the help of these parameters. To investigate the association with disease severity and viral load, quantitative PCR results would be more accurate than semi-quantitative Ct results.

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Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
This study was approved by Research Ethics Commission of Pamukkale University with decision dated 27/5/2020, and numbered 60116787-020/31814.

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