Original Research Article

Association between Clinical Severity, Inflammatory Markers, and Autonomic Function in COVID-19 Patients: A Hospital-Based Analytical Study

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Abstract:

Introduction: COVID-19 pandemic primarily affected the respiratory system, but cardiovascular complications including autonomic dysfunction were increasingly recognized. Heart rate variability (HRV), a non-invasive measure of autonomic function, reflects the sympathovagal balance and provides an early warning of cytokine storms and impending cardiovascular complications. **Aim:** This study aimed to evaluate the relationship between COVID-19 clinical severity and HRV, and to examine the correlation between HRV and inflammatory markers. **Methods:** In this hospital-based case-control study 164 RT-PCR-confirmed COVID-19 patients and 43 age and gender-matched healthy controls participated in the study .Their clinical profiles, inflammatory markers were noted on admission and their HRV parameters (time and frequency domains) were evaluated using 5-minute ambulatory ECG recordings. Logistic regression was used to assess associations between HRV and clinical severity; ANOVA was used for post hoc comparisons across groups. **Results:** Among the 164 COVID-19 patients, 44% had mild or no symptoms, while 56% had moderate to severe infection. Co morbid conditions were present in 62.8%, with diabetes being the most common (43.9%). HRV parameters—including HF, LF, LF/HF ratio, and PNN50 were significantly associated with disease severity in both logistic regression models ($p < 0.05$). Weak correlations were observed between HRV (SDNN) and inflammatory markers such as D-Dimer, LDH, IL-6, and CRP. **Conclusion:** This study confirmed the presence of autonomic dysfunction in COVID-19 patients, with more pronounced dysfunction observed in those with greater clinical severity. Heart rate variability serve as a valuable early indicator of COVID-19 severity and progression

Key words: heart rate variability, inflammatory markers, COVID-19, SARS COV-2

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Introduction :

The COVID 19 pandemic was primarily associated with severe pulmonary disease. But it was known to affect multiple organ systems, including the cardiovascular system and can lead to complications such as myocarditis, high blood pressure, irregular heart rhythms and Autonomic Nervous System (ANS) disturbances. [1,2,3] The manifestations of autonomic imbalance in COVID 19 patients provide a clue to the impending adverse cardiovascular outcomes.[4] The cause for autonomic dysfunction in COVID -19 patients is not clear and might be multifactorial.[5] One possible factor is lung injury, which can lead to hypoxemia and subsequently disrupt autonomic nerve function.[6] Additionally, the virus may directly damage myocardial cells or exacerbate pre-existing cardiac conditions, resulting in conduction abnormalities.[7]

Additionally, changes in inflammatory cytokine levels can influence the functioning of the autonomic nervous system.[8] The cytokine storm is one of the key factor contributing to the severity of COVID-19.[9] Neurotransmitters released by autonomic nerves interact with immune cells such as neutrophils, monocytes and T cells play a crucial role in modulating immune responses and inflammation. Thus the imbalance in the production of both systemic and local cytokines has been linked to the intensity of symptoms and overall disease severity.[10] Therefore, there is a need for a readily accessible, noninvasive tool that can offer early warning signs of an impending cytokine storm.

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Heart rate variability (HRV) is a physiological metric, regulated by the autonomic nervous system that has been used for decades to evaluate ANS in various clinical settings.[11] The autonomic nervous system (ANS) reacts rapidly to changes in physiological states, providing signals such as increase or decrease in HRV that may indicate an impending cytokine storm earlier than conventional laboratory tests currently available.[12] Autonomic balance should be taken into consideration while evaluating diagnostic and therapeutic approaches of COVID-19,so we aimed to find out the association between COVID-19 severity and HRV, as well as the correlation between inflammatory marker levels and HRV.

Methods:

This observational cross-sectional study was carried out in October 2020 among patients admitted to a tertiary care center for COVID-19 treatment. The study was initiated after receiving approval from the institutional human ethics committee. Individuals aged between 18 and 60 years were enrolled after providing informed consent. The study included patients with mild to severe COVID-19 (including those with hypoxia) who tested positive by RT-PCR. For correlation analysis, assuming a small-to-moderate expected effect size ($r = 0.25$), with a two-tailed significance level (α) of 0.05 and power ($1 - \beta$) of 0.80, the required sample size is approximately 123 subjects. Information regarding their presenting symptoms, oxygen saturation at admission, and existing comorbidities was recorded. Inflammatory markers, including C-reactive protein (CRP), D-dimer, interleukin-6 (IL-6), and lactate dehydrogenase (LDH), were noted at the time of admission. Patients with abnormal ECG findings at admission, a history of coronary artery disease, or those taking beta blockers or beta agonists were excluded. The control group consisted of 43 healthy individuals belonging to the age group of 18-60 years, who tested negative for SARS-CoV-2 via RT-PCR from nasopharyngeal and oropharyngeal swabs participated in the study. All participants underwent a general and systemic clinical evaluation.

Assessment of HRV:

The prerequisite for a patient to undergo HRV assessment are they should avoid smoking, caffeine intake for 2 h and alcohol intake for 36 h prior to the assessment. They should have had enough rest, at least 8 h of uninterrupted sleep on the night prior to the assessment of HRV and normal breakfast on the day of assessment. Patients were instructed to lie down quietly in a couch in supine position for 5 min to alleviate their anxiety in a sound proof room with dim lighting and the ambient temperature of the room was about 20 to 25 °C. The procedure was explained to the patient. An ambulatory ECG system (INCO digital NIVIQURE, Bangalore, India) was used to assess the autonomic activity in lead II for 5 min. It is a multichannel digital data acquisition system which enables to acquire, analyze and store ECG data. ECG data was obtained at a sampling rate of 1,024 Hz in standard lead II configuration. The data was transferred with the help of interface RS232C-compatible module from the recording unit to the

computer. The transferred data was analyzed using inbuilt software system.

The series of RR intervals obtained were further subjected to frequency and time domain analysis. Time domain indices of HRV indicate the amount of variability in measurements of interbeat interval (IBI), which represent time period between successive heartbeats. Time domain measures include standard deviation of NN intervals (SDNN), root mean square of successive RR interval differences (rMSSD) and percentage of successive RR intervals (pNN50). Frequency domain measurements assess the absolute or relative power distribution across different frequency bands. Frequency domain measures include Low Frequency (LF) and High Frequency (HF).[13] HF component indicates the parasympathetic activity, whereas LF represents both sympathetic and vagal activity and SDNN, rMSSD and pNN50 measure parasympathetic activity.[14]

Statistical analysis: After examining for normality of distribution and homogeneity of variance, all data were expressed in terms of mean ± standard deviation and percentage. Study groups were compared using t test and chi-square test. Nonparametric tests were employed for variables with skewed distribution.

Association between clinical severity of COVID19 and the measures of Heart rate variability was explored using logistic regression analysis after adjusting for common clinical covariates. Post hoc comparison of HRV between Covid19 patients with varying severity and healthy controls was performed using analysis of variance. A p value of < 0.05 was considered statistically significant. All data were analyzed using SPSS version 17.

Results:

A total of 164 eligible subjects with COVID-19 infection confirmed with RTPCR test were recruited. This included 72 (44%) patients with asymptomatic or mild COVID-19 infection and 92 (56%) subjects with moderate to severe infection. 43 healthy controls, matched for age and gender, were recruited for post hoc analysis.

The mean age of COVID-19 infected subjects was 54.5 ± 15.6 years, with 116 (70.9%) males and 48 (29.3%) females. Demographic, clinical and laboratory data of COVID 19 patients were summarized in Table.1

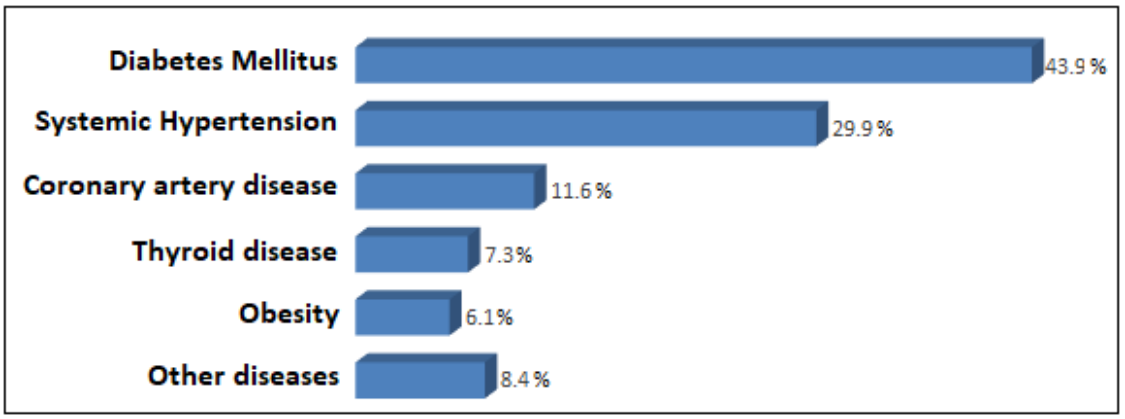
Table 1: Demographic, clinical and laboratory characteristics of the study population with Covid19 confirmed by RT-PCR method.

Variables	**Total (N = 164)**	**Asymptomatic to mild infection (N = 72)**	**Moderate to severe infection (N = 92)**	**P value**
Age (in years)	54.5± 15.6	50.9 ± 17.5	57.3 ± 13.4	0.009
Gender Male Female	 116 (70.9%) 48 (29.3%)	 49 (68.1%) 23 (31.9%)	 25 (27.2%) 67 (72.8%)	0.505
Duration of illness (median days and range)	4 (0 – 20)	2 (0 – 10)	4 (0 – 10)	0.004

Common symptoms				
Fever	97(59.1%)	33 (46.5%)	64 (71.1%)	0.002
Cough	93 (56.9%)	34 (47.9%)	59 (65.6%)	0.024
Breathlessness	75 (45.7%)	17 (24.3%)	58 (63.7%)	0.0001
Diarrhea				
Other symptoms				
Presence of ≥ 1 comorbidities	103 (62.8%)	40 (56.3%)	63 (69.2%)	0.091
HRV measures				
Heart rate	96.3 ± 50	87.0 ± 35	103.7 ± 58.3	0.025
High frequency (HF)	72.3± 19.3	68.5 ± 20.8	75.4 ± 17.6	0.025
Low frequency (LF)	27.6 ± 19.3	31.5 ± 20.8	24.6 ± 17.6	0.026
HF/LF	7.1 ± 11.4	5.4 ± 7.1	8.5 ± 13.7	0.061
LF/HF	0.5± 0.6	0.66 ± 0.71	0.43 ± 0.5	0.022
PNN50	5.4 ± 8.0	3.67 ± 6.1	6.7 ± 8.9	0.012
rMSSD	106 ± 188	72.2 ± 125.9	131.9 ± 221.3	0.031
SDNN	125.7 ± 147	100.0 ± 96.4	144.9 ± 175	0.040
Inflammatory markers				
Interleukin – 6	85.1 ± 190.8	58.3 ± 71.6	98.8 ± 228.2	0.258
Ferritin	612.9 ± 479.2	474.9 ± 375.4	682.8 ± 512.0	0.012
Lactate dehydrogenase	423 ± 197.6	359.4 ± 133.8	451.9 ± 215.2	0.007
D-dimer	7.33 ± 53.5	1.46 ± 3.13	10.4 ± 65.8	0.389
Adverse outcomes				
Need for Oxygen therapy	106 (68.8%)	24 (37.9%)	82 (91.1%)	0.0001
ICU admission	50 (32.5%)	5 (7.8%)	45 (48.9%)	0.0001
Need for ventilator support				
Mortality	4 (2.6%)	0 (0.0%)	4 (4.6%)	0.073

The majority, 103 (62.8%), had at least one comorbidity, with diabetes mellitus being the most common (43.9%), followed by systemic hypertension (29.9%) and coronary artery disease (11.6%). Figure.1

Figure 1: Baseline morbidity profile of the study population.



Other diseases include malignancy, kidney disease and liver disease. Neuropsychiatric disorders known to significantly affect heart rate variability were excluded from the study protocol.

Subgroups of SARSCOV 2 infected subjects were matched for gender and comorbidity profile. Moderate to severely ill COVID-19 subjects were elderly and had delayed admission from the onset of illness compared to those with no / mild symptoms. As expected symptomatology, laboratory markers and clinical outcomes were significantly worser among moderate to severe COVID19 subgroup. Almost all measures of heart rate variability were significantly different between the subgroups of COVID 19 subjects.

We explored for association between clinical severity of COVID19 and the measures of heart rate variability using two adjustment models in multivariate logistic regression analysis (Table 2). In model 1, after adjusting for age, gender and comorbidities, we found that heart rate, high and low frequency domains and their ratios and PNN50 were significantly associated with clinical severity of covid19. In adjustment model 2 involving age, gender and duration of illness, we found that frequency domains and their ratios were significantly associated with clinical severity of COVID19.

Table 2: Multiple logistic regression analysis of association between measures of heart rate variability and clinical severity of Covid19 infection.

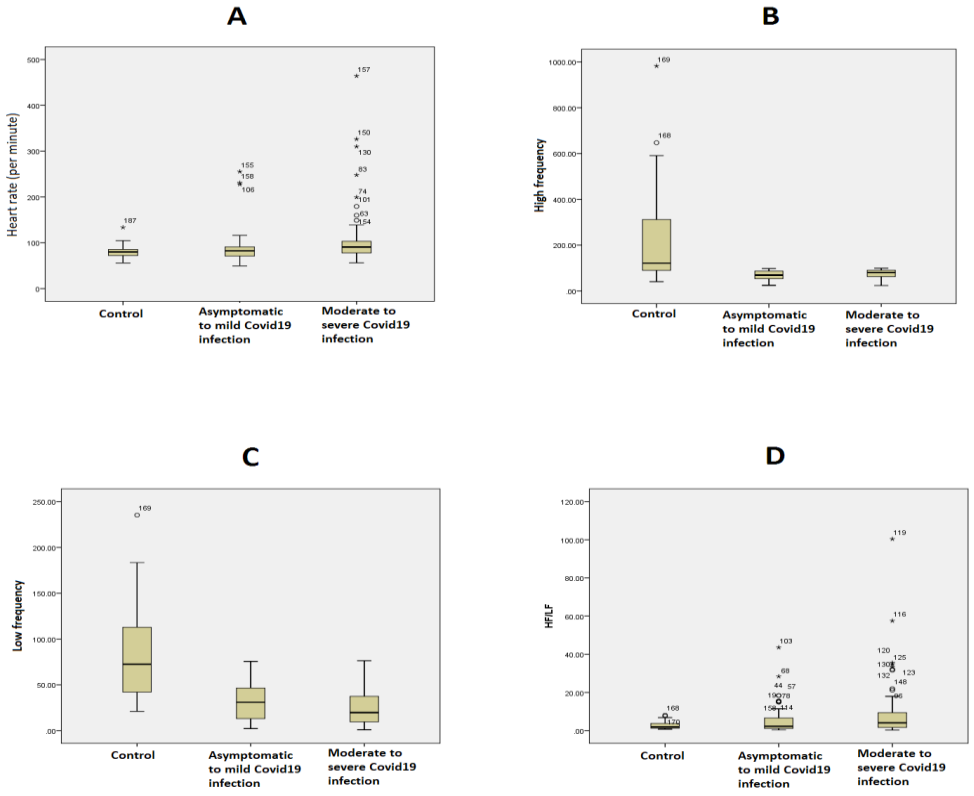
Measures of Heart Rate Variability	Adjustment model 1£		Adjustment model 2¥	
	Adjusted Odds ratio (95% confidence interval)	P value	Adjusted Odds ratio (95% confidence interval)	P value
HR	1.01 (1.0 – 1.02)	0.049	1.008 (1.00 – 1.017)	0.063
HF	1.02 (1.002 – 1.037)	0.031	1.024 (1.003 – 1.045)	0.023
LF	0.981 (0.965 – 0.998)	0.032	0.977 (0.957 – 0.997)	0.023
HF/LF	1.024 (0.983 – 1.067)	0.256	1.036 (0.978 – 1.097)	0.235
LF/HF	0.517 (0.29 – 0.92)	0.025	0.506 (0.275 – 0.994)	0.048
PNN50	1.057 (1.008 – 1.11)	0.021	1.04 (0.989 – 1.097)	0.123
rMSSD	1.002 (1.00 – 1.004)	0.056	1.001 (0.999 – 1.004)	0.246
SDNN	1.003 (1.00 – 1.005)	0.06	1.002 (0.999 – 1.004)	0.254

£ Adjustment model 1: Age, Gender and presence of at least one comorbidities.
¥ Adjustment model 2: Age, Gender and duration of illness.

- Figure 2 shows graphically represented post-hoc analysis, comparing heart rate variability between covid19 patients and healthy controls.
- All measures of HRV were significantly different between healthy controls and subgroups of covid19 subjects.

Figure 2: Graphical Representation of association between severity of COVID19 and measures of heart rate variability.

Figure 2A shows higher heart rate among patients with moderate to severe Covid19 infection compared to mild or asymptomatic COVID19 and control subjects. Among frequency domain, High (HF) and low (LF) frequencies were more pronounced in control subjects compared to COVID19 subjects (2B, 2C). Ratio of high to low frequency (HF/LF) was significantly more in moderate to severe Covid19 infection compared to mild or asymptomatic COVID19 and control subjects (2D).



Correlations of inflammatory markers and HRV

There was a weak positive correlation between D-Dimer (on Admission) and SDNN (rho - 0.01, p - 0.943). There was a weak negative correlation between IL-6 (on Admission) and SDNN (rho- 0.09, p - 0.390). There was a weak positive correlation between LDH (Admission) and SDNN (rho = 0.04, p = 0.705). There was a weak negative correlation between CRP (Admission) and SDNN (rho = -0.14, p = 0.231). Table.3.

Table 3: Correlations of inflammatory markers and HRV

Correlation	Spearman Correlation Coefficient	P Value
CRP (Admission) vs SDNN	-0.1	0.231
D-Dimer (Admission) vs SDNN	0.0	0.943
LDH (Admission) vs SDNN	0.0	0.705

Discussion :

Elevated inflammatory marker levels are widely used to assess the progression and severity of COVID-19, while a reduction in Heart Rate Variability (HRV) is also associated with worsening disease. Hence, monitoring the relationship between variations in heart rate variability (HRV) and changes in inflammatory marker levels is vital for effectively managing SARS-CoV infection and predicting possible complications.

In our study, among the time domain parameters, the mean rMSSD was 106 ± 188, while the mean SDNN value was 125.7 ± 147. These values suggested a parasympathetic predominance. Previous research had indicated that a parasympathetic overtone was present when rMSSD exceeded 40 and SDNN was greater than 60.[14] Based on these criteria, the study confirmed that COVID-19 patients exhibited signs of parasympathetic dominance.

Traditionally, the LF/HF ratio has been regarded as an indicator of sympathovagal balance in the cardiovascular system.In our study there was statistically significant weak negative correlation between LF/HF and Hear rate. Our findings indicated the presence of an increased parasympathetic activity in COVID-19 patients, independent of confounders such as age, gender and comorbidities including diabetes mellitus, hypertension & obesity. In a study conducted among 2,745 participants with COVID 19 illness Natarajan and colleagues found a decrease in HRV.[15] In an another retrospective cohort study of 271 COVID-19 patients authors found that higher HRV was associated with better survival outcomes in older patients, independent of major prognostic indicators, while lower HRV was linked to more severe disease and complications.[16] In COVID 19, SARS CoV 2 infection, activates the sympathetic nervous system and inflammatory pathway, whereas vagal stimulation brings about anti-inflammatory regulatory response and also limits the viral infection. When there is decreased vagus activity, there occurs uncontrolled inflammatory response, leading to hyper-inflammation which results in cytokine storm.[17] Heart rate variability is a measure of vagal activity. A recent meta-analysis of over 51 studies, with 2,238 patients demonstrated an inverse relationship between HRV and inflammation.[18] A common measure of HRV which indicates the vagal activity is the standard deviation of the interval between heartbeats (SDNN). This time domain measure was observed to correlate inversely with the inflammatory marker CRP.[19]

In our study, we found a weak positive correlation between SDNN and D-Dimer and LDH levels at admission. We also observed a weak negative correlation between SDNN and IL-6 and CRP levels at admission. However, none of these correlations were found to be statistically significant.

A recent meta-analysis by Williams et al. highlighted strong associations between both high-frequency (HF) and low-frequency (LF) components of heart rate variability (HRV) and circulating inflammatory markers. However, certain HRV indices showed stronger correlations with specific inflammatory markers like resting HF-HRV was negatively correlated with interleukin-6 (IL-6), C-reactive protein (CRP), fibrinogen, and white blood cell (WBC) count, but no significant association was observed with interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF-α). Similarly, resting LF-HRV was inversely related to IL-6, CRP, and WBC count, though it showed no such correlation with fibrinogen, IL-1, or TNF-α.[20]

A decline in HRV can serve as an early indicator of clinical deterioration, even when a patient appears to be improving. Regular monitoring of HRV may serve as an early indicator of an upcoming inflammatory response. Currently, clinicians rely on rising biomarker levels and clinical scoring systems to assess the severity of COVID-19. Early initiation of appropriate therapy could help prevent or lessen the impact of a cytokine storm. Since predicting which patients will require anti-inflammatory treatment is challenging, short-term HRV monitoring may serve as a useful, noninvasive tool to assist in triage and guide treatment decisions throughout the course of the illness.

Limitations of the study:

Several factors contributed to the limitations of this prospective observational study. Frequencies of laboratory testing and therapeutic interventions were not standardized because they were ordered at the discretion of the primary care team. Incorporating critical illness severity scores, such as the Multiple Organ Dysfunction Score or the Sequential Organ Failure Assessment (SOFA) Score, would have helped in better assessing the clinical relevance of changes in HRV and inflammatory markers. Additionally, the small sample size restricts the generalisability and applicability of the findings to a broader population Larger, prospective, randomized, multisite-controlled trials are warranted to evaluate the potential value of HRV in the management of COVID-19 patients.

Conclusion:

The current study has demonstrated that COVID-19 is associated with autonomic dysfunction.COVID-19 patients with autonomic dysfunction are more likely to experience increased severity. This association can have implications for triage, predictor of severity, for monitoring disease progression and subsequent treatment modalities to be followed throughout the disease course

Acknowledgements: Nil

Conflict of interest: Nil

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