

Correlation between NEWS, CT Severity Score and RT-PCR Cycle Threshold Value among Mild and Moderate COVID-19 Patients

SRICHARAN VIJAYAKUMAR¹, SANDEEP GARG², ANJU GARG³, SUNITA AGGARWAL⁴,
VIKAS MANCHANDA⁵, RANVIJAY SINGH⁶, SANJIT KUMAR⁷



ABSTRACT

Introduction: The clinical diagnosis of COVID-19 is supplemented by clinical severity indices. These indices are the National Early Warning Score (NEWS, which aids in risk stratification), CT severity score (radiological severity score), and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) cycle threshold (Ct value, which provides a semi-quantitative measure of viral load).

Aim: To assess the correlation between NEWS at admission, RT-PCR Ct value and CT severity score in mild and moderate COVID-19 patients.

Methods and Materials: This prospective cohort study was conducted in Maulana Azad Medical College and Lok Nayak hospital, New Delhi, from January to June 2021. The study included 50 subjects (25 with mild COVID-19 and 25 with moderate COVID-19). NEWS was calculated at admission and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Ct value was estimated using real-time RT-PCR. CT severity score was calculated based on High Resolution Computed Tomography (HRCT) chest findings. The correlation among the parameters was determined using Pearson correlation formula.

Results: The mean age of subjects in the mild and moderate COVID-19 groups were 49.52 years and 51.84 years, respectively. The mean RT-PCR Ct value of E gene was 24.48 and Rdrp gene was 24.56 in the mild COVID-19 group; while in the moderate group it was 23.72 for both E gene and Rdrp genes. The correlation between NEWS and Ct value of E gene (r -value=-0.06, p -value=0.68), Ct value of Rdrp gene (r -value=-0.03, p -value=0.79) and the correlation between CT severity score and Ct value of E gene (r -value=-0.05, p -value=0.73), Ct value of Rdrp gene (r -value=-0.06, p -value=0.68) was negative and insignificant. The mean CT severity score in mild COVID-19 group was 3.92, and in moderate COVID-19 group was 9.88. A significant positive correlation was found between the CT severity score and NEWS at admission.

Conclusion: The clinical severity of COVID-19 as estimated by NEWS corroborates with CT severity score while the relationship between RT-PCR Ct value and clinicoradiological severity needs to be ascertained by further research.

Keywords: Coronavirus, Ct value, National early warning score, Reverse transcription-polymerase chain reaction

INTRODUCTION

The Coronavirus Disease 2019 (COVID-19) pandemic has resulted in widespread mortality in India and worldwide [1]. Patients with comorbidities such as diabetes, hypertension, chronic kidney, liver and lung diseases have been found to be at high-risk for progression to severe COVID-19. Hence, there is a need for rapid, reliable, efficient and accurate methods to diagnose COVID-19 timely in order to prevent transmission, minimise complications and mortality associated with it. The utility of microbiological, immunological, biochemical and radiological techniques has come to the forefront in supplementing the clinical diagnosis of COVID-19. While the microbiological methods are used to confirm the diagnosis by definitively detecting the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral genome, the radiological methods are often used for differentiating COVID-19 from other infections and estimating the severity of disease based on the quantification of lung involvement. In this regard, National Early Warning Score (NEWS), Reverse Transcription-Polymerase Chain Reaction cycle threshold (RT-PCR Ct) value and CT Severity Score (CT-SS) have been useful adjuncts in establishing the diagnosis.

The clinical severity of COVID-19 is the most important tool for triaging and risk stratification. The NEWS is an early warning score which was developed by the Royal College of Physicians in 2012. It utilizes six bedside vital parameters (heart rate, systolic blood pressure, respiratory rate, temperature, level of consciousness and oxygen saturation at room air or the use of supplemental oxygen) [2]. Each of

these parameters is stratified based on the degree of derangement and assigned a score. A higher score signifies that the patient's vital parameters are deranged to a greater extent and clinical monitoring is needed more frequently. The aggregate NEWS is obtained by adding the individual parametric scores. The maximum NEWS that can be obtained is 20. Thus, NEWS serves as a proxy for the clinical severity to facilitate rapid triaging and employment of prompt and appropriate clinical responses. NEWS has been correlated with clinical severity and outcome of COVID-19 in some studies [3].

Computed Tomography (CT) of chest has been a valuable tool especially in suspected patients having typical symptoms of COVID-19 whose RT-PCR reports are pending or inconclusive as it establishes the diagnosis and aids in quick isolation. The sensitivity of chest CT is more than 90% according to some studies. On the basis of typical findings of COVID-19 pneumonia on chest CT, Li K et al., proposed a semi-quantitative scoring system used to quantify the extent of damaged lung tissue depending on the percentage area of the lung involved [4]. In this scoring system, each lobe was assigned a score from zero to five according to the estimated percentage area of lung involvement. The composite score, known as the CT Severity Score (CT-SS), is obtained by the sum of the individual lobar scores and ranges from 0 to 25 [Table/Fig-1]. CT-SS is an indicator of the burden of lung damage due to COVID-19 pneumonia and has been shown to be correlated with numerous factors such as age, time since symptom onset, biomarkers of clinical severity and clinical outcomes.

Percentage area of lobar involvement	Score
0%	0
<5%	1
5-25%	2
26-50%	3
51-75%	4
>75%	5
Total CT-SS	0-25

[Table/Fig-1]: Calculation of CT severity score (CT-SS).

RT-PCR is a highly sensitive and specific molecular method employed for the laboratory diagnosis of COVID-19. Quantitative RT-PCR yields Cycle threshold (Ct) values which provide an estimate of the viral load in the sample. The Cycle threshold or Ct value is defined as the number of cycles of amplification at which the fluorescence of a PCR product crosses the threshold, exceeding the background signal. The greater the amount of target nucleic acid in the sample, the sooner the fluorescence crosses the threshold and lower the Ct value [5]. Ct value is semiquantitative, with a 3-fold increase in Ct value corresponding to a 10-fold decrease in target nucleic acid. The Ct value is inversely proportional to the viral load of SARS-CoV-2 in a given sample. The Ct value also depends on a number of factors such as site of sample collection, day since onset of symptoms, age of the patient, type of RT-PCR kit used. Few studies have indicated that the RT-PCR Ct value of SARS-CoV-2 is correlated with clinical outcome and severity of COVID-19 [6].

This study aimed to evaluate the role of NEWS, Ct value and CT-SS together in the clinical context of COVID-19 and the correlation between them. It is of critical importance to decipher the correlation between clinical severity indices and radiological and microbiological indices ascertaining severity as it facilitates in quicker diagnosis and management of COVID-19.

MATERIALS AND METHODS

This prospective cohort study was conducted in Maulana Azad Medical College and Lok Nayak hospital, New Delhi, from January to June 2021. The Institutional Ethics Committee of Maulana Azad Medical College had approved the study (letter No. 244 dated 14/01/2021). The study was conducted in the general wards when the hospital was converted into an exclusively COVID-19 dedicated centre. The study enrolled 50 subjects, as a sample size of convenience.

Inclusion criteria: Adult (aged >18 years) COVID-19 positive patients, confirmed by either RT-PCR or Rapid Antigen Test (RAT) who presented with an oxygen saturation (SpO₂) of more than 90% on room air were included as subjects after taking informed consent.

Exclusion criteria: Patients with shock, Multiorgan Dysfunction Syndrome (MODS) or Acute Respiratory Distress Syndrome (ARDS) and patients admitted in Intensive Care Unit (ICU). Severe COVID-19 patients (who had an SpO₂ of less than 90% on room air) were not included in this study as it was assumed that their radiological severity (CT severity score) would corroborate easily with clinical severity.

The subjects were divided into two groups of 25 each:

- Mild COVID-19 patients whose SpO₂ was ≥94% on room air
- Moderate COVID-19 patients whose SpO₂ was 90-93% on room air.

Procedure

In both the groups, the NEWS was calculated based on the vital parameters on the day of admission. Baseline blood investigations including Complete Blood Count (CBC), Kidney Function Tests (KFT), Liver Function Tests (LFT), Prothrombin Time and International Normalized Ratio (PT/INR), D-dimer was done. The RT-PCR sample was sent for SARS-CoV-2 and the Ct values of E gene and Rdrp gene were obtained. High Resolution Computed Tomography (HRCT) chest was done and the CT-Severity Score (CT-SS) was

calculated based on the severity of lung involvement. The subjects were monitored regularly and in case of any evidence of clinical deterioration, appropriate blood markers were sent for corroboration. The following outcomes were assessed: discharge, transfer to ICU, first evidence of respiratory failure, coagulopathy, acute kidney injury, acute liver injury, acute myocardial injury or death.

STATISTICAL ANALYSIS

The data obtained was processed by Statistical Package for Social Sciences (SPSS) version 25.0, tabulated and represented graphically for interpretation. Continuous variables were presented as mean and standard deviation and were compared by Student's t-test. Categorical variables were presented as frequencies or percent values and compared by Chi-square test. The p-value less than 0.05 was considered significant for all tests. The correlation between NEWS, Ct value and CT severity score was done using Pearson correlation formula. The NEWS at admission was correlated with Ct value and CT-SS. The Ct value was also correlated with CT-SS.

RESULTS

The mean age in the mild COVID-19 group was 49.52 years and in the moderate COVID-19 group was 51.84 years [Table/Fig-2]. The gender distribution showed that 68% (34/50) of the subjects were males while 32% (16/50) of them were females.

Age group (years)	Mild COVID-19 (n,%)	Moderate COVID-19 (n,%)	Total (n,%)
21-30	6 (24%)	3 (12%)	9 (18%)
31-40	1 (4%)	2 (8%)	3 (6%)
41-50	4 (16%)	8 (32%)	12 (24%)
51-60	7 (28%)	4 (16%)	11 (22%)
61-70	6 (24%)	5 (20%)	11 (22%)
>70	1 (4%)	3 (12%)	4 (8%)
Mean±SD	49.52±17.45	51.84±15.19	

[Table/Fig-2]: Age-wise distribution of study subjects (N=50).

On comparison of the hematological parameters, there was a statistically significant difference in the mean platelet count between the mild and moderate COVID-19 group (3.04 L versus 2.19 L, p-value=0.01). Among the biochemical parameters, a statistically significant difference between the mild and moderate COVID-19 groups was found in the following parameters- mean AST, mean ALT, mean D-dimer and mean serum ferritin [Table/Fig-3].

Parameter	Mild COVID-19	Moderate COVID-19	p-value (Student's t-test)
Serum creatinine (NRR: 0.6-1.2 mg/dL)	0.9	0.9	0.79
AST (NRR: 5-40 U/L)	38.72	58.76	<0.01
ALT (NRR: 5-40U/L)	44.28	72.44	<0.01
ALP (NRR: 50-150 U/L)	92.28	120.16	0.05
CPK (NRR: 55-170U/L)	65	70	0.57
D-dimer (NRR: 0-500 ng/mL)	500	1200	0.03
Serum ferritin (NRR: 17-465 mg/dL)	467	900	0.01

[Table/Fig-3]: Mean biochemical parameters in study subjects.

NRR: Normal reference range; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; CPK: Creatine kinase

NEWS was used as the marker of clinical severity of COVID-19. The mean NEWS at admission was significantly greater in the moderate COVID-19 group (3.04) than in the mild COVID-19 group (0.36).

Analysis of the RT-PCR Ct values revealed that in mild COVID-19 patients, the mean Ct value of E gene was 24.48 and that of Rdrp gene was 24.56. In moderate COVID-19 patients, the mean Ct value of both E gene and Rdrp genes were 23.72. There was no statistically significant difference in the Ct values of both E gene (p-value=0.57) and Rdrp gene (p-value=0.54) between the groups [Table/Fig-4].

Parameter	COVID-19 severity		p-value
	Mild COVID-19 (n=25)	Moderate COVID-19 (n=25)	
Ct value E gene			
Mean ± SD	24.48±7.22	23.72±5.88	0.57
Range	10-33	12-34	
Ct value Rdrp gene			
Mean ± SD	24.56±6.85	23.72±5.82	0.54
Range	12-33	12-35	
NEWS at admission			
Mean ± SD	0.36±0.63	3.04±1.31	<0.001
Range	0-2	1-5	
CT severity score			
Mean ± SD	3.92±5.29	9.88±7.21	<0.01
Range	0-16	0-20	

[Table/Fig-4]: Mean RT-PCR Ct value, NEWS and CT severity score in study subjects.

The mean CT severity score in mild COVID-19 patients was significantly lower (3.92) than in the moderate COVID-19 patients (9.88) [Table/Fig-4].

The assessment of outcomes showed that overall, 76% (38/50) patients were discharged, 12% (6/50) patients developed sepsis, and 12% (6/50) patients developed respiratory failure and were transferred to ICU [Table/Fig-5]. There were no deaths among the study subjects. There were no incidences of acute kidney injury (serum creatinine more than two times of upper limit normal), acute liver injury (serum AST/ALT more than two times of upper limit normal), acute myocardial injury (serum CPK-MB>10% of serum total CPK) or coagulopathy (INR>1.5 times of normal reference) among the study subjects. The mean duration between admission and outcome was significantly higher in the mild COVID-19 group compared to moderate COVID-19 group (9.04 days vs 7.44 days, p-value=0.10) [Table/Fig-6].

Clinical Outcome	Mild COVID-19 (n,%)	Moderate COVID-19 (n,%)	Total (n,%)	p-value (Chi-square test)
Discharged	23(92%)	15(60%)	38 (76%)	0.01
Sepsis (TLC>11000/mm ³)	0	6(24%)	6(12%)	0.02
Respiratory failure (PaO ₂ <60 mmHg on ABG) and ICU transfer	2(8%)	4(16%)	6(12%)	0.66

[Table/Fig-5]: Clinical outcome variables in study subjects.

TLC: Total leucocyte count PaO₂: Partial pressure of arterial oxygen ABG: Arterial blood gas

Duration between admission and outcome (days)	COVID-19 severity		p-value
	Mild COVID-19 (n=25)	Moderate COVID-19 (n=25)	
Mean±SD	9.04±4.08	7.44±2.88	0.10
Range	2-20	3-15	

[Table/Fig-6]: Mean duration between admission and outcome in study subjects.

There was a statistically significant positive correlation between CT-SS and NEWS at admission in both the mild and moderate COVID-19 groups [Table/Fig-7]. There was a negative and insignificant correlation between CT-SS and Ct values of E gene and Rdrp gene. The correlation between NEWS at admission and Ct values of E gene and Rdrp gene was also negative and insignificant [Table/Fig-8,9].

CT Severity Score	NEWS at admission
r-value	0.55
p-value	<0.001

[Table/Fig-7]: Correlation between CT Severity Score and NEWS at admission.

Ct value	NEWS score at admission	
E gene	r-value	-0.06
	p-value	0.68
Rdrp gene	r-value	-0.03
	p-value	0.79

[Table/Fig-8]: Correlation between RT-PCR Ct value and NEWS at admission.

Ct value	CT severity score	
E gene	r-value	-0.05
	p-value	0.73
Rdrp gene	r-value	-0.06
	p-value	0.68

[Table/Fig-9]: Correlation between RT-PCR Ct value and CT severity score.

DISCUSSION

The study aimed to assess the correlation between NEWS which is a marker of clinical severity, RT-PCR Ct value which is a marker of viral load and CT-SS which is a marker of radiological severity of COVID-19. This study is probably the first study till date to incorporate all the three parameters together in the assessment of COVID-19 severity.

The radiological severity as determined by CT severity score corroborated with the clinical severity as determined by NEWS. The mean CT-SS in the moderate COVID-19 group was significantly greater than that of the mild COVID-19 group (p-value<0.01). Similar findings were reported in other studies; in a study by Abbasi B et al., a positive correlation between CT severity score and mortality and clinical severity (as determined by time to ICU admission, time to intubation and time to death) was found [7].

The analysis of correlation between the parameters provided useful insights. The correlation between the RT-PCR Ct value of E gene with NEWS (r-value=-0.06, p-value=0.68) and the Ct value of Rdrp gene with NEWS (r-value=-0.03, p-value=0.79) was negative but statistically insignificant. Other studies utilizing various markers as proxy for clinical severity have yielded different results. A study by Romero-Alvarez D et al found a negative but significant correlation between the Ct value of RT-PCR and ICU admission [8]. In this study, patients were taken irrespective of COVID-19 clinical severity. Another study by Tanner AR et al., revealed that the RT-PCR Ct value at admission is independently associated with the risk of mortality in addition to other factors such as age, NEWS2 and cardiovascular co-morbidities [9]. In the present study only patients belonging to mild and moderate COVID-19 clinical severity were taken and hence, this study provides an insight into this correlation in a limited subset of patients.

There was a significant positive correlation between CT-SS and NEWS at admission (r-value=0.55, p-value<0.001) in both the mild and moderate COVID-19 groups. A study by Akdur G et al., yielded similar findings; a higher CT severity score was associated with time-to-death within 14 and 90 days and the combined use of NEWS at admission and CT severity score yielded greater accuracy as NEWS≥7 and CT-SS>11 were associated with the highest hazard ratio [10]. Since a higher clinical severity is associated with a higher NEWS, the correlation between the NEWS at admission and CT severity score can therefore be extrapolated and the results of our study concurs with this finding.

The correlation between RT-PCR Ct value of E gene and CT severity score (r-value=-0.05, p-value=0.73) and that of Ct value of Rdrp gene and CT severity score (r-value=-0.06, p-value=0.68) was also negative but insignificant. A similar study conducted by Bakir A et al., analysed the relationship between the chest CT score and Ct value as a proxy for viral load; the results showed a positive correlation between the Ct value and chest CT score (r-value=0.197, p-value=0.01) [11]. Since chest CT score and CT-

SS are similar measures used for quantification of lung involvement in COVID-19, the results of our study can be said to concur with this study. Another study by Liu Z et al., examined the correlation between Lung Severity Score (LSS) which is similar to CT-SS and RT-PCR Ct value and found that LSS was inversely related to Ct value (r -value=-0.588, p -value=0.003). However, this study categorized patients into severe and non-severe COVID-19 and this negative correlation between LSS and Ct value was found only in severe COVID-19 [12]. Hence, there is paucity of data in the literature describing the relationship between the RT-PCR Ct value and CT Severity Score. Hence, the present study is one of the first studies which describes the correlation between these parameters in a specific subset of COVID-19 patients.

Limitation(s)

Firstly, at the time of this study, there were no published studies that measured the correlation between RT-PCR Ct value, NEWS and CT severity score, hence it was difficult to corroborate with a benchmark and get guidance from reliable resources. Secondly, due to the limited sample size of 50 and the inclusion criteria which permitted to enrol only patients of mild and moderate severity, estimation of data accuracy and extrapolation to the larger population was not possible. Thirdly, since this study was conducted around the time of the second wave of COVID-19 pandemic in India, resource constraints did not permit a follow-up CT chest or RT-PCR Ct value to look for any temporal variation in those parameters.

CONCLUSION(S)

The utility of NEWS, CT severity score, RT-PCR Ct value and other various clinical, radiological and immunological indices for supplementing the clinical diagnosis of COVID-19 cannot be overemphasized especially in a pandemic situation. Further studies delving into the correlation between clinical severity and other markers of disease severity should be actively encouraged as they can pave way for novel and effective interventions to combat this deadly COVID-19 pandemic.

REFERENCES

- [1] WHO Coronavirus (Covid-19) dashboard. [Internet] [updated 2021 Dec 13, cited 2021 Dec 13]. Available from: <https://covid19.who.int/>.
- [2] National Early Warning Score [Internet] [updated 2022 May 10, cited 2022 May 10]. Available from: <https://www.england.nhs.uk/ourwork/clinical-policy/sepsis/nationalearlywarningscore/>.
- [3] Covino M, Sandroni C, Santoro M, Sabia L, Simeoni B, Bocci MG, et al. Predicting intensive care unit admission and death for COVID-19 patients in the emergency department using early warning scores. *Resuscitation*. 2020;156:84-91. Doi: 10.1016/j.resuscitation.2020.08.124. PMID: 32918985.
- [4] Li K, Wu J, Wu F, Guo D, Chen L, Fang Z, et al. The clinical and chest CT features associated with severe and critical COVID-19 pneumonia. *Invest Radiol*. 2020;55:327-31. Doi: 10.1097/RLI.0000000000000672. PMID: 32118615.
- [5] Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. *BioTechniques*. 2005;39:75. Doi: 10.2144/05391RV01. PMID: 16060372.
- [6] Trunfio M, Venuti F, Alladio F, Longo BM, Burdino E, Cerutti F, et al. Diagnostic SARS-CoV-2 cycle threshold value predicts disease severity, survival, and six-month sequelae in COVID-19 symptomatic patients. *Viruses*. 2021;13:281. Doi: 10.3390/v13020281. PMID: 33670360.
- [7] Abbasi B, Akhavan R, Ghamari Khameneh A, Zandi B, Farrok D, Pezeshki Rad M, et al. Evaluation of the relationship between inpatient COVID-19 mortality and chest CT severity score. *Am J Emerg Med*. 2020;S0735-6757:30851-2.
- [8] Romero-Alvarez D, Garzon-Chavez D, Espinosa F, Ligñá E, Teran E, Mora F, et al. Cycle threshold values in the context of multiple rt-pcr testing for SARS-CoV-2. *Risk Manag Healthc Policy*. 2021;14:1311-17. Doi: <https://doi.org/10.2147/RMHP.S282962>. PMID:33824608.
- [9] Tanner AR, Phan H, Brendish NJ, Borca F, Beard KR, Poole S, et al. SARS-CoV-2 viral load at presentation to hospital is independently associated with the risk of death. *J Infect*. 2021;83:458-66. Doi: <https://doi.org/10.1016/j.jinf.2021.08.003>. PMID:34363885.
- [10] Akdur G, Daş M, Bardakci O, Akman C, Siddikoğlu D, Akdur O, et al. Prediction of mortality in COVID-19 through combining CT severity score with NEWS, qSOFA, or peripheral perfusion index. *Am J Emerg Med*. 2021;50:546-52. Doi: <https://doi.org/10.1016/j.ajem.2021.08.079>. PMID:34547696.
- [11] Bakir A, Hosbul T, Cuce F, Artuk C, Taskin G, Caglayan M, et al. Investigation of viral load cycle threshold values in patients with SARS-CoV-2 associated pneumonia with real-time pcr method. *J Infect Dev Ctries*. 2021;15:1408-14. Doi: <https://doi.org/10.3855/jidc.14281>. PMID:34780363.
- [12] Liu Z, Wang Q, Li J, Liu J, Wang H, Jia C, et al. The correlation between severity scores in computed tomography lung scans and viral load in the severity of novel coronavirus 2019 progression. *J Clin Ultrasound*. 2022;50:375-82. Doi: <https://doi.org/10.1002/jcu.23159>. PMID:35253226.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Resident, Department of Medicine, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India.
2. Director Professor, Department of Medicine, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India.
3. Director Professor and Head, Department of Radiodiagnosis, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India.
4. Director Professor, Department of Medicine, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India.
5. Professor, Department of Microbiology, Maulana Azad Medical College, New Delhi, India.
6. Senior Resident, Department of Medicine, Lok Nayak Hospital, New Delhi, India.
7. Postgraduate Resident, Department of Medicine, Lok Nayak Hospital, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Sricharan Vijayakumar,
4/3, Guru Kewal Residency, 12th Cross, 8th Main, Malleswaram,
Bengaluru-560003, Karnataka, India.
E-mail: sricharanembar@gmail.com

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Apr 06, 2022
- Manual Googling: Jun 08, 2022
- iThenticate Software: Aug 16, 2022 (8%)

ETYMOLOGY: Author Origin

Date of Submission: **Mar 31, 2022**

Date of Peer Review: **Apr 26, 2022**

Date of Acceptance: **Jun 10, 2022**

Date of Publishing: **Sep 01, 2022**