International Journal of Advances in Medicine
Shenoy GC et al. Int J Adv Med. 2021 Aug;8(8):1120-1126
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: https://dx.doi.org/10.18203/2349-3933.ijam20212756

Original Research Article

Comparison of neutrophil-lymphocyte ratio among severe acute respiratory illness COVID-19 positive and negative patients and to correlate with disease severity

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Received: 13 June 2021
Revised: 27 June 2021
Accepted: 28 June 2021

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ABSTRACT

Background: COVID-19, a pandemic caused by SARS-CoV-2, has caused a wide impact globally. Clinical spectrum of COVID-19 ranges widely including asymptomatic infection, mild upper respiratory tract infection, severe pneumonia, ARDS, MODS and even death. Neutrophil-lymphocyte (NL) ratio is an indicator of systemic inflammatory response. Many previous studies have shown NL ratio to be good prognostic marker for COVID-19 pneumonia.
Methods: A study was conducted on 265 SARI (severe acute respiratory illness) patients who were admitted in hospitals attached to BMCRI between May 2020 and September 2020. History was taken, general and systemic examination was done. Patients were categorized into moderate and severe illness. RT-PCR for all patients was done using throat and nasal swab. Total WBC counts and differential counts were estimated. NL ratio was correlated with pneumonia severity and compared between COVID-19 positive and negative SARI.
Results: The study included 265 SARI patients, of which 135 were COVID-19 positive patients and 130 were COVID-19 negative. The patients were further sub-categorised into moderate and severe SARI. Mean age for COVID-19 positive severe SARI was higher than the moderate SARI. Males were majority of the subjects. Out of 265, 99 patients (37.4%) had diabetes. Out of this, 34 (42%) were COVID-19 positive (severe) followed by 28 (51.9%) COVID-19 positive (moderate). Mean NL ratio was higher in COVID-19 positive (severe) subjects 15.95±10.31 followed by COVID-19 positive (moderate) subjects 8.99±6.22. There was significant difference with respect to NL ratio between COVID-19 positive and negative subjects. NL ratio levels were high in subjects having diabetes in COVID-19 positive severe sub group and COVID-19 positive severe hypertension sub group as compared to other sub groups.
Conclusions: NL ratio was higher in patients with COVID-19 positive SARI as compared to COVID-19 negative SARI. There was significant correlation between increase in NL ratio and disease severity of COVID-19 positive pneumonia. We found that increase in NL ratio was associated with co-morbidities like diabetes mellitus and hypertension.

Keywords: COVID-19, SARI, Neutrophil-lymphocyte ratio

INTRODUCTION

In December 2019, Wuhan city in China experienced an outbreak of pneumonia cases of unknown cause. Later the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously known as 2019-nCoV), was isolated from these viral pneumonia patients in January 2020. WHO designated this pneumonia as coronavirus disease 2019, that is, COVID-19, in February 2020.[1]

The Wuhan city was locked down on 23 January 2020 whereas, WHO declared a public health emergency of international concern on 30 January 2020.[2]

Initially the disease started with zoonotic transmission but soon it occurred to spread through human to human transmission. Transmission of the virus causing COVID-19, i.e. SARS-CoV-2 was considered to be via droplets rather than aerosols but strong directional airflow may support the spread by droplet upto more than 2 meters.[3]

Clinical spectrum of COVID-19 ranges widely including asymptomatic infection, self-limiting mild upper respiratory tract infection, severe pneumonia, ARDS, MODS and even death.[1] The WHO has officially declared COVID-19 as a pandemic and all the countries are facing difficulties in controlling and treating the disease.[2] There is a need for reliable indicator to assess the severity of the disease.

Inflammation plays an important role in disease development and also a reliable prognostic factor. As an immune response to respiratory infection, neutrophils influx into the alveoli, causes tissue damage, vascular stasis and cytotoxicity. Earlier studies have demonstrated lymphopenia due to sustained release of anti-inflammatory cytokines leading to widespread apoptosis of lymphocytes.[2] NL ratio is shown to be good predictor of mortality in hospitalized patients with community acquired pneumonia. It is cheaper and easier to perform the test.[4] NL ratio is an indicator of systemic inflammatory response. Many previous studies have shown NL ratio to be good prognostic marker for COVID-19 pneumonia. However, correlation of NL ratio with severity of COVID-19 pneumonia is less studied and this study intents on comparison of NL ratio in COVID-19 SARI and non-COVID-19 SARI and correlation of the same with severity of the disease.

Objectives

The objectives were to estimate NL ratio in patients with SARI, to correlate between SARI disease severity and NL ratio and to compare NL ratio in COVID-19 positive and negative SARI patients.

METHODS

The present study was conducted on patients visiting hospital affiliated to Bangalore Medical College and Research Institute, Bangalore (BMCRI).

This was a cross-sectional type of study. The study was conducted during the period from May 2020 to September 2020.

The study was conducted in hospitals affiliated to Bangalore Medical College and Research Institute, Bangalore.

Inclusion criteria

Patients/attenders willing to give informed consent, patients of either sex with age more than 18 years and patients admitted in COVID-19 suspect hospital and diagnosed with SARI were included in the study.

Exclusion criteria

Patient not willing to give informed consent and patients with age less than 18 years were excluded from the study.

Methodology of data collection

After obtaining institutional ethics committee clearance, the patients getting admitted to hospitals affiliated to Bangalore Medical College and Research Institute, during the period from May 2020 to September 2020 were taken up for the study.

A total 265 patients diagnosed with SARI who were admitted in hospitals attached to BMCRI were included in the study. History was taken, general physical examination and a detailed systemic examination was done. Patients were categorized into moderate and severe illness according to WHO clinical criteria. RT-PCR for all patients was done using throat and nasal swab. Total WBC counts and differential counts, platelet counts were estimated. NL ratio were correlated with pneumonia severity and compared between COVID-19 positive and negative SARI and also correlation with co-morbidities was done.

WHO criteria for categorizing the patients[5]

Mild disease

Symptomatic patients with ILI symptoms without evidence of viral pneumonia or hypoxia were categorized as mild.

Moderate disease

Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including SpO2 ≥90% on room air were categorized as moderate.

Severe disease

Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following like respiratory rate >30 breaths/min, severe respiratory distress or SpO2 <90% on room air were categorized as severe.

Statistical analysis

Data was analyzed by descriptive statistics. Chi square test used to see association between qualitative variables and

correlation co-efficient was used to see relation between quantitative variables. P<0.05 was considered statistically significant. Independent sample test was applied for quantitative variables between COVID-19 positive and COVID-19 negative subjects.

RESULTS

The study was conducted on 265 patients diagnosed with SARI, of which 135 were COVID-19 positive patients and 130 were COVID-19 negative. Mean age of subjects with COIVD-19 positive (severe) was higher 58.70±15.598 followed by COVID-19 positive (moderate) 55.13±14.480 (Table 1).

Number of patients above 65 years 80 (30.2%) was higher followed by age group of 36 to 45 years 51 (19.2%). Chi square test was applied to associate the age with severity. Chi square test showed statistically significant association with respect to age (χ^2=35.15; p=0.002) (Table 2). In our study males were higher 174 (65.7%) as compared to females 91 (34.3%). Chi square test was applied to associate the gender with severity. Chi square test showed no statistical significant association with respect to gender (χ^2=3.16; p=0.78) (Figure 1).

Table 1: Distribution of mean age.

COVID-19	Severity	N	Minimum	Maximum	Mean	SD
Negative	Moderate	69	20	86	46.90	17.132
	Severe	61	19	91	52.56	15.988
Positive	Moderate	54	25	80	55.13	14.480
	Severe	81	24	96	58.70	15.598

Table 2: Distribution of age among the groups.

Age (in years)		COVID-19 negative		COVID-19 positive		Total
		Moderate	Severe	Moderate	Severe	
Less than 25	Count	9	4	1	1	15
	%	13.0%	6.6%	1.9%	1.2%	5.7%
26 to 35	Count	9	5	5	7	26
	%	13.0%	8.2%	9.3%	8.6%	9.8%
36 to 45	Count	22	12	8	9	51
	%	31.9%	19.7%	14.8%	11.1%	19.2%
46 to 55	Count	7	13	13	11	44
	%	10.1%	21.3%	24.1%	13.6%	16.6%
56 to 65	Count	9	10	9	21	49
	%	13.0%	16.4%	16.7%	25.9%	18.5%
Above 65	Count	13	17	18	32	80
	%	18.8%	27.9%	33.3%	39.5%	30.2%
Total	Count	69	61	54	81	265
	%	100.0%	100.0%	100.0%	100.0%	100.0%
Chi square value=35.15						
P=0.002*						

*significant.

Table 3: Distribution of mean NL ratio.

COVID 19	Severity	N	Minimum	Maximum	Mean	SD
Negative	Moderate	69	0.69	46.00	5.93	7.87
	Severe	61	1.51	46.32	7.23	7.17
Positive	Moderate	54	0.89	25.47	8.99	6.22
	Severe	81	1.93	45.85	15.95	10.31

In the study, out of 265 (100%) subjects, 99 (37.4%) had diabetes, of which 34 (42%) were COVID-19 positive (severe) followed by 28 (51.9%) were COVID-19 positive (moderate) (Figure 2). Chi square test was applied to associate the diabetes with severity. Chi square test showed statistically significant association with respect to

diabetes (χ^2=15.79; p=0.001) and out of 265 (100%) subjects, 102 (38.5%) had hypertension, of which 33 (40.7%) were COVID-19 positive (severe) followed by 31 (50.8%) were COVID-19 negative (severe) (Figure 2). Chi square test was applied to associate the hypertension with severity. Chi square test showed no statistically significant association with respect to hypertension (χ^2=7.15; p=0.067).

Table 4: Comparison between COVID-19 negative and COVID-19 positive groups using independent sample t test.

Comparison	COVID-19 negative versus COVID-19 positive	
	Mean difference	P value
NL ratio	-6.62	0.00*

*significant.

Table 5: Comparison between moderate and severe subgroups using independent sample t test.

Comparison		Moderate versus severe	
		Mean difference	P value
COVID 19 negative	NL ratio	-1.29	0.33
COVID 19 positive	NL ratio	-6.96	0.00*

*significant.

Table 6: Comparison of NL ratio based on diabetes in COVID-19 and non-COVID-19 subjects.

Parameters	COVID-19	Severity	Diabetes	N	Minimum	Maximum	Mean	SD
NL ratio	COVID-19 negative	Moderate	NIL	56	.69	46.00	5.82	7.22
			Present	13	1.33	41.05	6.42	10.55
		Severe	NIL	37	1.51	46.32	7.87	8.27
			Present	24	2.33	28.96	6.24	5.05
	COVID-19 positive	Moderate	NIL	26	.89	21.72	9.19	5.25
			Present	28	1.46	25.47	8.80	7.10
		Severe	NIL	47	1.93	33.61	14.78	8.69
			Present	34	2.00	45.85	17.57	12.16

Table 7: Comparison of NL ratio based on hypertension in COVID-19 and non-COVID-19 subjects.

Comparison		Severity	Diabetes	N	Minimum	Maximum	Mean	SD
NL ratio	COVID-19 negative	Moderate	NIL	47	.69	46.00	6.04	7.82
			Present	22	1.45	41.05	5.70	8.15
		Severe	NIL	30	2.59	46.32	8.14	8.93
			Present	31	1.51	23.75	6.34	4.91
	COVID-19 positive	Moderate	NIL	38	1.24	25.00	9.17	5.81
			Present	16	.89	25.47	8.56	7.28
		Severe	NIL	48	2.29	45.71	15.18	10.06
			Present	33	1.93	45.85	17.07	10.72

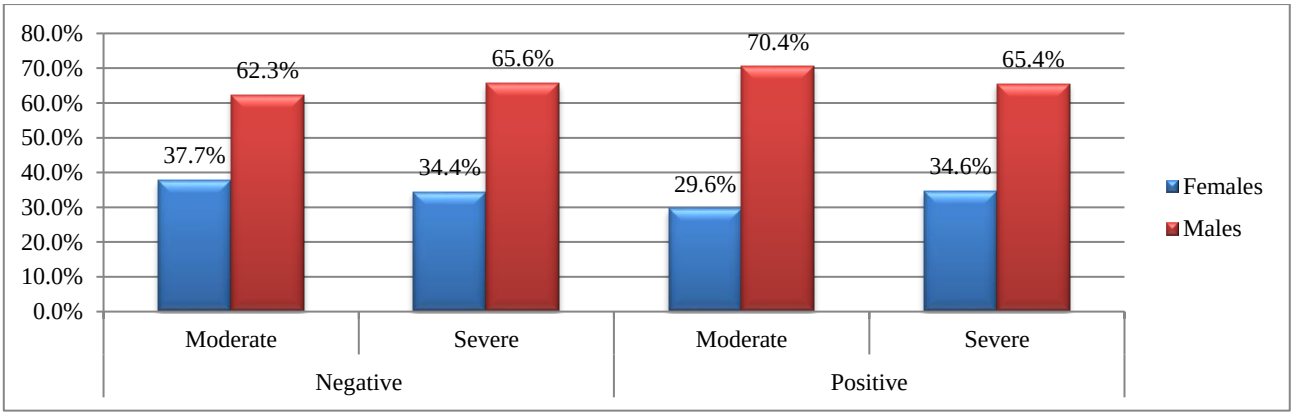

Figure 1: Distribution of gender among the groups.

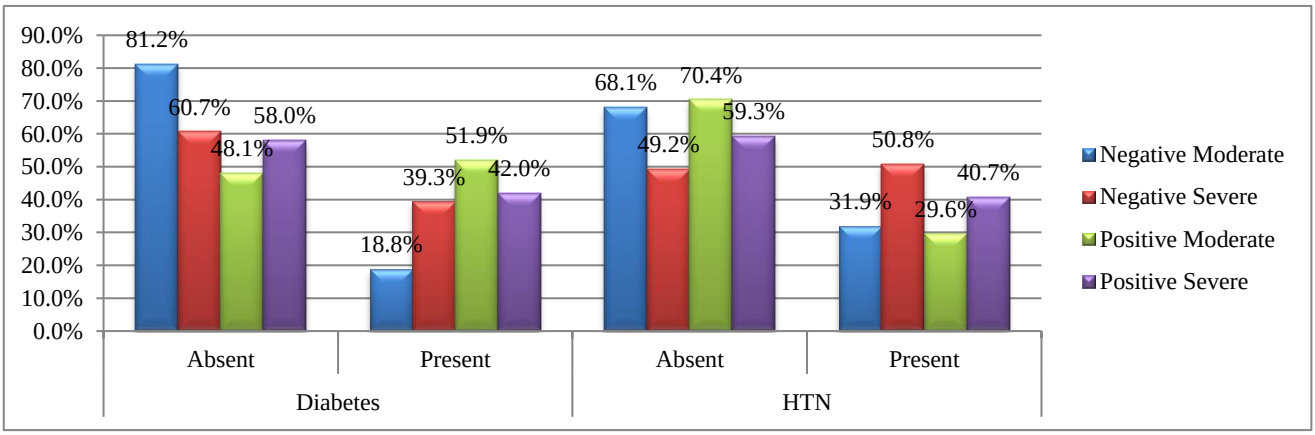

Figure 2: Distribution of co-morbidities.

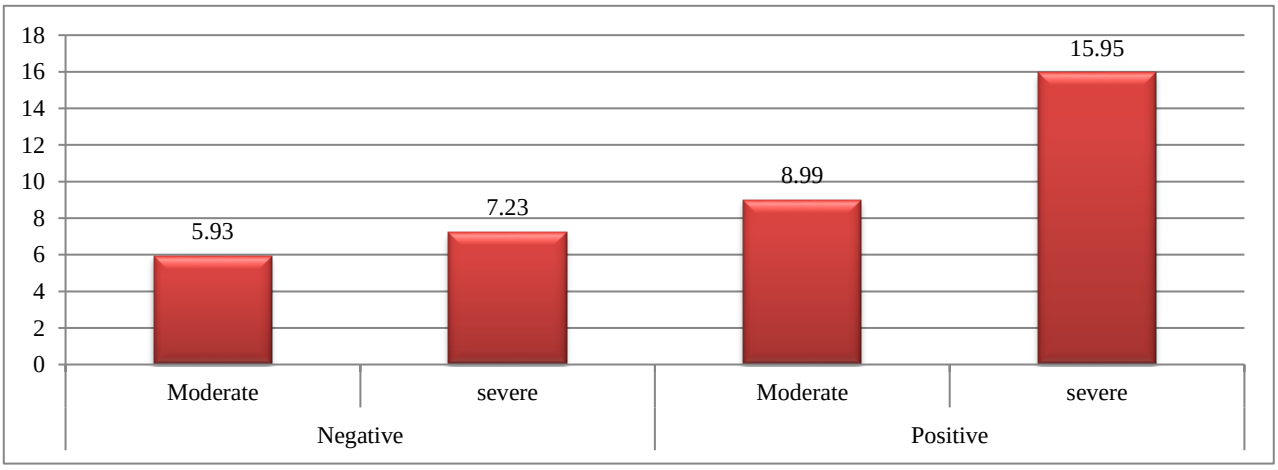

Figure 3: Distribution of mean NL ratio.

In our study mean NL ratio was higher in COVID-19 positive (severe) subjects 15.95±10.31 followed by COVID-19 positive (moderate) subjects 8.99±6.22 (Table 3) (Figure 3).

Independent sample t test showed statistically significant difference with respect to NL ratio (p=0.00) between COVID-19 positive and negative subjects (Table 4).

In COVID-19 positive subjects, NL ratio showed statistically significant difference (p=0.00) between moderate and severe sub groups whereas there was no statistically significant difference seen in COVID-19 negative sub groups (Table 5).

In the study NL ratio (17.57±12.16) levels were high in subjects having diabetes in COVID-19 positive severe sub group as compared to other sub groups (Table 6) and NL

ratio (17.07±10.72) was high in subjects having hypertension in COVID-19 positive severe sub group as compared to other sub groups (Table 6 and 7).

DISCUSSION

In our study, demographic characteristics of the study subjects showed males were higher, 174 (65.7%) as compared to females, 91 (34.3%) which was similar to Zhou et al having majority male subjects and Mousavi-Nasab et al where 57.1% were males.[6] Tatum et al cited females to be majority in their study.[2] Turk Thorac J studied 204 patients of which 115 were COVID-19 positive SARI with majority male (67.8%) patients and of 99 COVID-19 negative patients also male (62%) patients were more than female.[7]

In the present study mean age of subjects with COIVD-19 positive (severe) was higher, 58.70±15.598 followed by COVID-19 positive (moderate) 55.13±14.480, distribution of subjects based on age showed subjects above 65 years, 80 (30.2%) was higher followed by age group of 36 to 45 years, 51 (19.2%). According to Nalbant et al study, the mean age (SD) was 53 (18) years for COVID-19 positive patients and 60 (14) for COVID-19 negative patients, with difference being not statistically significant, also no significant difference between gender distribution.[8] In the study by Mousavi-Nasab et al they studied patients with the mean age of 42.7±12.4 (ranging from 19 to 78) years of them 40.2% being in the age range of 30 to 49 years.[6] They also cited that 20% cases had severe disease where as 80% were non severe cases. In the study by Turk Thorac J et al mean age for COVID-19 positive was 51.8 years and that of COVID-19 negative was 51.3 years. In the study by Mon et al age for severe COVID-19 was higher as compared to mild cases (67.9±12.3 versus 53.2±15.6).[9]

We also studied distribution of diabetes and hypertension among the groups and out of 265 (100%) subjects, 99 (37.4%) had diabetes of which majority were severe COVID-19 positive followed by moderate COVID-19 positive SARI with statistical significant association with respect to diabetes. 102 patients had hypertension, which was more common with severe COVID-19 negative patients and then with severe COVID-19 positive patients, however association with hypertension was not statistically significant. Similarly, in the study by Zhou et al majority of COVID-19 patients had associated co-morbidities most common being hypertension and diabetes.[1] Mon et al when compared between severe and mild COVID-19 patients, found significant difference in diabetes, hypertension, chronic kidney disease and tumour, however no significant difference was found for cardiovascular disease and COPD.[9]

NL ratio is major inflammatory marker and previous studies have shown NL ratio to be good prognostic indicator. In the present study, when we studied distribution of mean NL ratio, we found that it was higher in severe COVID-19 positive SARI patients followed by moderate COVID-19 positive SARI patients indicating that NL ratio is elevated in COVID-19 positive patients significantly as compared to negative patients and associated with disease severity. Similar results were found by Mousavi-Nasab et al where NL ratio was higher in severe COVID-19 patients compared to non-severe COVID-19 patients.[6] Eslamijouybari et al also found that NL ratio was significantly higher in COVID-19 patients as compared to control group.[10]

Yang et al also showed similar results stating that elevated NL ratio was significantly associated with severity of illness.[11] A meta-analysis by Chan stated that higher levels of NL ratio were present in severe disease as compared to non-severe disease which is supporting the results of our study.[12] A metanalysis done by Xiaoming et al to evaluate the predictive value of NL ratio suggested that NL ratio has good predictive in terms of severity and mortality in COVID-19 hence helping clinicians in predicting severity early and treating adequately.[13] In contrast to our study, Turk Thorac J et al showed that NL ratio was lower (average NL=5.89) in COVID-19 positive SARI and was higher (avg NL=11.41) in non-COVID-19 SARI.[9]

Regarding the association with co-morbidities, our study found that NL ratio was significantly higher in severe COVID-19 patients with diabetes and hypertension. Tatum et al stated that patients who had NL ratio higher than the determined cutoff, had more number of mean associated co-morbidities.[2] Similarly in the study by Yang et al significantly high frequency of occurrence of co-morbid conditions were shown in severe patients in the order of diabetes followed by hypertension and renal dysfunction.[11]

In our study we found that higher value of NL ratio was associated with more severe COVID-19 disease, similarly Yan et al study showed that NL ratio was higher in non-survivor group of COVID-19 patients which was significantly associated with all cause in hospital mortality.[14] Liu et al found that NL ratio more than 3.13 was independent risk factor for progression to critical illness in patients with COVID-19.[15]

Limitation

In our study, the limitation was that we had not considered the effects of steroid treatment of the disease on the NL ratio.

CONCLUSION

NL ratio was higher in patients with COVID-19 positive SARI as compared to COVID-19 negative SARI. There was significant correlation between increase in NL ratio and disease severity of COVID-19 positive pneumonia. We found that increase in NL ratio was associated with co-morbidities like diabetes mellitus and hypertension.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
2. Tatum D, Taghavi S, Houghton A, Stover J, Toraih E, Duchesne J. Neutrophil-to-Lymphocyte Ratio and Outcomes in Louisiana COVID-19 Patients. Shock. 2020;54(5):652-8.
3. Sommerstein R, Fux CA, Vuichard-Gysin D, Abbas M, Marschall J, Balmelli C, et al. Risk of SARS-CoV-2 transmission by aerosols, the rational use of masks, and protection of healthcare workers from COVID-19. Antimicrob Resist Infect Control. 2020;9(1):100.
4. Curbelo J, Bueno SL, Galván-Román JM, Ortega-Gómez M, Rajas O, Fernández-Jiménez G, et al. Inflammation biomarkers in blood as mortality predictors in community-acquired pneumonia admitted patients: Importance of comparison with neutrophil count percentage or neutrophil-lymphocyte ratio. PLoS One. 2017;12(3):0173947.
5. WHO. Fact sheet: Clinical management of COVID-19 patients: living guidance, 2021. Available at: https://app.magicapp.org/#/guideline/j1WBYn/section/j2xMZn. Accessed on 29 May 2021.
6. Mousavi-Nasab SD, Mardani R, Nasr Azadani H, Zali F, Vasmehjani A, Sabeti S, et al. Neutrophil to lymphocyte ratio and C-reactive protein level as prognostic markers in mild versus severe COVID-19 patients. Gastroenterol Hepatol Bed Bench. 2020;13(4):361-6.
7. Garg PK, Khera PS, Saxena S. Chest-X-ray-Based Scoring, Total Leukocyte Count, and Neutrophil-to-Lymphocyte Ratio for Prediction of COVID-19 in Patients with Severe Acute Respiratory Illness. Turk Thorac J. 2021;22(2):130-6.
8. Nalbant A, Kaya T, Varim C, Yaylaci S, Tamer A, Cinemre H. Can the neutrophil/lymphocyte ratio (NLR) have a role in the diagnosis of coronavirus 2019 disease (COVID-19)? Rev Assoc Médica Bras. 2020;66(6):746-51.
9. Kong M, Zhang H, Cao X, Mao X, Lu Z. Higher level of neutrophil-to-lymphocyte is associated with severe COVID-19. Epidemiol Infect. 2020;148:139.
10. Eslamijouybari M, Heydari K, Maleki I, Moosazadeh M, Hedayatizadeh-Omran A, Vahedi L, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in COVID-19 patients and control group and relationship with disease prognosis. Casp J Intern Med. 2020;11(1):531-5.
11. Yang AP, Liu J, Tao W, Li H. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020;84:106504.
12. Chan AS, Rout A. Use of Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in COVID-19. J Clin Med Res. 2020;12(7):448-53.
13. Li X, Liu C, Mao Z, Xiao M, Wang L, Qi S, et al. Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. Crit Care. 2020;24(1):647.
14. Yan X, Li F, Wang X, Yan J, Zhu F, Tang S, et al. Neutrophil to lymphocyte ratio as prognostic and predictive factor in patients with coronavirus disease 2019: A retrospective cross-sectional study. J Med Virol. 2020:10.
15. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med. 2020;18:206.

Cite this article as: Shenoy GC, Ramaiah M, Kamaraddi S, Somashekar P, Subramanya SK. Comparison of neutrophil-lymphocyte ratio among severe acute respiratory illness COVID-19 positive and negative patients and to correlate with disease severity. Int J Adv Med 2021;8:1120-6.