



ASSOCIATION OF BIOMARKERS DERIVED FROM HEMATOLOGICAL PARAMETERS IN COVID-19 POSITIVE PATIENTS AS COMPARED TO COVID-19 NEGATIVE PATIENTS ADMITTED IN TERTIARY CARE HOSPITAL, KISHANGANJ

Internal Medicine

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ABSTRACT

Background and objective: In COVID-19 positive patients presents with fever, throat pain, cough, respiratory distress, myalgia and fatigue are the initial symptoms which may later on progress to metabolic acidosis, adult respiratory distress syndrome, multiorgan failure and ultimately shock leading to death. Multiple morbid factors like hypertension, diabetes mellitus etc. increase the mortality in these patients. This present study aimed to demonstrate the associations of biomarkers derived from hematological parameters with the COVID-19 patients as compared to COVID-19 negative patients in the emergency room at the first visit. **Materials and methods:** Total 169 patients of more than 18 years were included who have been considered as possible Covid-19 positive according to following criteria of Scientific Committee of Ministry of Health. From all the patients after getting clinical symptoms suggestive of COVID-19, throat swab and nasopharyngeal swab were taken for detection of COVID-19 virus by real time PCR, blood was drawn for rapid antigen test, hematological profile, no other test were performed initially. **Results:** Males were significantly affected as compared to females both in COVID-19 positive and negative cases. But as compared to ages there was no significant difference between both sexes. There was no significant difference in symptoms between COVID-19 positive and negative patients except "loss of taste" where COVID-19 patients were significantly affected as compared to COVID-19 positive patients. In case of hematological parameters, platelet count, leukocyte count, absolute neutrophil count, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio and systemic inflammatory index were significantly high in COVID-19 patients as compared to COVID-19 negative patients, but there was no significant difference in absolute lymphocyte count in both the groups. **Conclusions:** Due to less accessibility as well as more time consuming of RT-PCR test leukopenia, neutropenia, thrombocytopenia were more easily diagnosed at the first visit in very short time and it was found valuable in the initial diagnosis of COVID-19. Low NLR and SII were also found in COVID-19 patients.

KEYWORDS

Covid-19 positive and negative patients, RTPCR tests, Biomarkers from hematological parameters. Tertiary care hospital.

INTRODUCTION:

Covid-19 was first epicentered in Hubei province, Wuhan, China, then was spreaded throughout the World within a span of few days to months^{1,2}. World Health Organization (WHO) declared it as pandemic in the World on 12/03/2020 as it produced numerous challenges in the global health care system³. Though the main target of COVID-19 is lung but as the disease becomes serious, it will involve other systems in the body. Nearly half of the patients are either asymptomatic carriers or pre-symptomatic. Patients presents with fever, throat pain, cough, respiratory distress, myalgia and fatigue are the initial symptoms which may later on progress to metabolic acidosis, adult respiratory distress syndrome, multiorgan failure and ultimately shock leading to death^{4,5}. Though the exact mode of transmission is unknown, but circumstantial evidences supports person-to-person transmission, and the most possible routes are contact and droplet-based⁶. In early 2020, by using next generation sequencing of the throat swab from infected patients in Chinese Centre for Disease Control a novel coronavirus was identified which was previously named as Acute Respiratory syndrome coronavirus 2^{7,8}. Covid-19 plays an important role in the pathogenesis of disease manifestations by triggering antiviral immune system and thus produces uncontrolled inflammatory response that leads to hematologic abnormalities⁹. Different types of hematological abnormalities facilitate the secondary microbial infection leading to multiorgan failure, shock and death. Different types of comorbidities like hypertension, obesity, and diabetes mellitus are associated with increased risk of coronavirus infection. Obesity is the most important factor that alters metabolic, hormonal and inflammatory function^{10,11}.

Hypertension may be an isolated predictor of severe coronavirus infection at extremes of ages¹². Diabetic patients are very prone to develop pneumonia due to coronavirus or secondary bacterial infections, sepsis and may be associated increased morbidity and mortality^{13,14,15}. Again, these co-morbid survived patients have increased chance of post-Covid complications. Blood tests play an important role in the early diagnosis of coronavirus disease like leukocyte count, lymphocytic or neutrophil predominance, markers of inflammation, markers of collateral organ damage in the form of acute renal failure or acute hepatic failure, biomarkers providing information of the nature of pneumonia whether it is due to secondary bacterial infection or not¹⁶. In complete blood count total white blood cell count, neutrophil, lymphocyte and platelet count and its volume and certain ratios are included^{17,18,19}. Neutrophil is the most important

cell responsible for initiating and continuing inflammatory process guided by mast cells, macrophages and epithelial cells. Stress increases neutrophils and reduces lymphocytes, hence the ration of these two parameters are used as inflammatory marker²⁰. Till now extensive elaborative studies regarding the correlation between the hematological abnormalities and severity of COVID-19 patients are limited worldwide. Again, due to local mutation of coronavirus in different Geographical locations, mortality related to coronavirus disease vary in those areas²¹. West Bengal is a densely populated areas with high co-morbid factors. Hence, this present study aimed to demonstrate the associations of biomarkers derived from hematological parameters with the COVID-19 patients as compared to COVID-19 negative patients in the emergency room at the first visit.

MATERIALS AND METHODS:

This was a retrospective cross-sectional study carried out jointly in two Tertiary care Hospitals in the line with research regulations after getting the approval from the Institutional Ethical Committee of MGM Medical College & LSK Hospital, Kishanganj and Bihar. Total 169 patients of more than 18 years were included who have been considered as possible Covid-19 positive according to following criteria of Scientific Committee of Ministry of Health. The criteria include patient with either at least one sign of symptom of either fever or acute respiratory disease like cough or respiratory distress, or the clinical features which is unexplainable by any other disease or history of travel to another country in the previous 14 days before the of symptoms of the patient or his relative or close contact with COVID-19 patient positive by Real time PCR²². From all the patients after getting clinical symptoms suggestive of COVID-19, throat swab and nasopharyngeal swab were taken for detection of COVID-19 virus by real time PCR, blood was drawn for rapid antigen test, hematological profile, no other test were performed initially. Swab samples were tested as urgent basis as also rapid antigen tests. Complete blood count was done in hematological laboratory.

Systemic inflammatory index (SII) = Thrombocyte count X Neutrophil count/Lymphocyte count.

Neutrophil-lymphocyte ratio (NLR) = Absolute neutrophil count/Absolute lymphocyte count.

Platelet-lymphocyte ratio = Absolute count of platelet / Absolute lymphocyte count.

Statistical Analysis:

Data was analysed by IBM SPSS 25.0. Shapiro-Wilk test, histogram and Q-Q plots. Chi-square test was utilized to categorical variables expressed in number and percentage. The independent samples were analysed by t-test and were presented as mean and standard deviation in case of parametric variables and in case of non-parametric variables by Mann-Whitney U test to derive median and interquartile range.

Binary Logistic regression test was done to assess diagnostic utility of biomarkers and systemic inflammatory index.

Receiver operating characteristic (ROC) curve was generated, Youden's index was used to detect cut-off values in the diagnosis of COVID-19. Area under the curve was calculated. 95% confidence limit was calculated, two-tailed $p < 0.05$ was considered as significant.

RESULTS:

Males were significantly affected as compared to females both in COVID-19 positive and negative cases. But as compared to ages there was no significant difference between both sexes. There was no significant difference in symptoms between COVID-19 positive and negative patients except "loss of taste" where COVID-19 patients were significantly affected as compared to COVID-19 positive patients [Table 1].

Table 1: Comparison of patients between RTPCR positive and negative patients:

Demography	Item	Total (n = 169)	Positive (n = 116)	Negative (n = 53)	P value
Gender	Female	49 (28.99%)	35 (30.17%)	14 (26.41%)	0.05**
	Male	120 (71%)	81 (69.82%)	39 (73.58%)	
Age	General	47.55 ± 13.20	47.35 ± 13.22	48 ± 13.15	0.766*
	Female	46.91 ± 14.50	46.12 ± 14.42	48.92 ± 14.49	0.274*
	Male	47.81 ± 12.62	47.88 ± 12.62	47.66 ± 12.61	0.464*
Symptoms	Fever		102 (87.93%)	44 (83.01%)	<0.389**
	Cough		91 (78.45%)	35 (66.03%)	<0.085**
	Diarrhea		39 (33.62%)	14 (26.41%)	<0.347**
	Dyspnea		62 (53.45%)	21 (39.62%)	<0.094**
	Headache		49 (42.24%)	24 (45.28%)	<0.711**
	Loss of taste		31 (26.72%)	27 (50.94%)	0.002**
	Sore throat		10 (8.62%)	8 (15.09%)	0.204**
	Chest pain		2 (1.72%)	0	0.337**

*Independent sample t-test

**Chi-square test

***Two tailed test with proportion related p value calculator

In case of hematological parameters, platelet count, leukocyte count, absolute neutrophil count, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio and systemic inflammatory index were significantly high in COVID-19 patients as compared to COVID-19 negative patients, but there was no significant difference in absolute lymphocyte count in both the groups [Table 2].

Table 2: Comparison of hematological parameters between RTPCR positive and negative patients:

Parameters	RTPCR positive (n = 116)	RTPCR negative (n=53)	P value
Platelet	205.24 ± 87.9	235.67 ± 82.7	0.03*
Leukocytes	5087.84 ± 3539.04	9983.77 ± 2741.75	0.04*
Neutrophils	3715.05 ± 2695.97	6834.56 ± 2038.96	0.03*
Lymphocytes	2394.91 ± 707.99	2273.77 ± 625.75	0.2866*
NLR	2.406 ± 1.78	3.134 ± 1.18	0.003*

PLR	115.56 ± 45.5	130.7 ± 45.7	0.04*
SII	425.45 ± 243.6	855.6 ± 614	0.000*

*Independent sample t-test

DISCUSSION:

COVID-19 infection is very serious infection with high mortality, hence urgent diagnosis is required, and definite presence of virus can be diagnosed by Real time Polymerase Chain Reaction (RT-PCR). But in the COVID era, due to exponentially increasing number of patient in the face limited number of staffs for doing the tests and limited number of laboratories approved for performing these tests results of these tests take longer period of time to come to the patients. So during visit in the emergency inexpensive complete blood count is very essential to detect possible COVID-19 infection. In the present study 69.82% males (81 out of 116) were affected by COVID-19 with average age of 47.88 ± 12.62 years which was nearly similar to the study of Usul E et al, where 69.3% males were COVID-19 with average age of 46.2 ± 15.5 years²³. Average age of males were higher in males as compared to females (46.12 ± 14.42). But in the study of Guan et al, average age of males were 47 and 52% were affected²⁴. Again the study done by Li et al demonstrated high median age of males (59 years), but 56% males were affected²⁵. So from all the studies it has been demonstrated the middle group males were affected more frequently as compared to females.

In the present study COVID-19 positive patients fever, cough and diarrhea were more common, whereas, in the study of Usul E et al along with fever and cough sore throat was also very common²³. Again in the study of Yang et al, same findings were demonstrated like fever and cough were the predominant symptoms²⁶. But contrary to the present study diarrhea was the less common features in the study of Guan et al²⁴. Another study done by Huang et al, demonstrated 98% and 76% patients presented with fever and cough respectively out of 41 patients²⁷.

The present study showed low platelet, leukocyte and neutrophil count in COVID-19 positive patients which was consistent with study of Usul E et al and Guan et al^{23,24}. Again thrombocytopenia and leukopenia were found in the study done by Assiri et al and Xu et al respectively^{28,29}. In case of viral pneumonias leukocyte level is less than 10000/cc whereas, in bacterial pneumonias it may be as high as 40000/cc³⁰. In some study platelet count was disproportionately low in respect of the clinical status of the patient³¹. So, in case viral pneumonias, immune system may play a significant role to decrease the leukocyte, platelet and neutrophil count. In one study there was evidence of mild thrombocytopenia in COVID-19 positive patients at first visit³².

Although unproven, there is a relationship between neutrophilia and bacterial infection and lymphocytosis and viral infection and this can be differentiated by neutrophil/lymphocyte ratio (NLR)^{33,34}. In a retrospective study in patients suffering from pyrexia of unknown origin, NLR was higher in case of bacterial infection as compared with viral infection³⁵. Early diagnostic marker of aiv-H7N9 patients was NLR as demonstrated in the study done by Zhang et al³⁶.

Systemic inflammatory index (SII) acts as prognostic marker for the follow-up of the patients with sepsis and predict the prognosis of small cell lung cancer and hepatocellular carcinoma^{37,38,39}. In the same study it was demonstrated that in COVID-19 patients this index was significantly low.

CONCLUSIONS:

Due to less accessibility as well as more time consuming of RT-PCR test, leukopenia, neutropenia and thrombocytopenia were more easily diagnosed at the first visit in very short time and it was found valuable in the initial diagnosis of COVID-19. Low NLR and SII were also found in COVID-19 patients.

REFERENCES:

- Daria S, Asaduzzaman M, Shahriar M, Islam MR. The massive attack of COVID-19 in India is a big concern for Bangladesh. The key focus should be given on the interconnection between the countries. Int J Health Plann Manage. 2021; <http://doi.org/10.1002/htm.3245>.
- Daria S, Islam MR. The second wave of COVID-19 Pandemic in Bangladesh. An Urgent call o save lives. Asia Pac J Public Health. 2021; <http://doi.org/10.1177/10105395211021686>.
- World Health Organization. <http://covid19.who.int/>.
- Bajgain KT, Badal S, Bajgain BB, Santana MJ. Prevalence of comorbidities among individuals with COVID-19: a rapid review of current literature. Am J Infect Control 2020; 49:238-246.

5. Salehi-Abari I, Khazaeli S, Salehi-Abari F, Salehi-Abari A. Practical guideline for screening the patients with SARS-CoV-2 infection and Persian Gulf criteria for diagnosis of CoVID-19. *Adv Infect Dis.* 2020;10:67.
6. Centre of Disease Control and Prevention. First travel-related case of 2019 Novel coronavirus Detected in United States. US department of health & Human Services (2020) www.cdc.gov/media/releases/2020/p0121-novel-coronavirus-travel-case.html.
7. Lu R, Zhao X, Li J, Nlu P, Yang B, Wu H et al. Genomic characterization and epidemiology of 2019 novel coronavirus: Implications for viral origins and receptor binding. *Lancet* 2020;395:565-574.
8. Gorbelenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA et al. The species severe acute respiratory distress syndrome related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol.* 2020;5:536-544.
9. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo O et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis.* 2020;94:91-95.
10. Foidi M, Farkas N, Kiss S et al. Obesity is a risk factor for developing critical condition in COVID-19 patients: a systematic review and meta-analysis. *Obes Rev.* 2020;21:e13095.
11. Zhou Y, Chi J, Lv W, Wang Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19). *Diabetes Metab Res Rev.* 2021:e3377.
12. Clark CE, McDonagh STJ, McManus RJ, Martin U. COVID-19 and hypertension: risks and management. A scientific statement on behalf of the British and Irish Hypertension Society. *J Hu Hypertens.* 2021;35:304-307.
13. Corrao S, Pinelli K, Vacca M, Raspanti M, Argano C. Type 2 Diabetes Mellitus and COVID-19: A Narrative review. *Front Endocrinol (Lausanne).* 2021;12:609470.
14. Abu-Farha M, Al-Mulla F, Thanaraj TA et al. Impact of diabetes in patients diagnosed with COVID-19. *Front Immunol.* 2020;11:576818.
15. Li H, Tian S, Chen T, et al. Newly diagnosed diabetes is associated with a higher risk of mortality than known diabetes in hospitalized patients with COVID-19. *Diabetes Obes Metab.* 2020;22:1897-1906.
16. Bekdas M, Goksugur SB, Sarac EG, Erkocoglu M, Demircirglu F. Neutrophil/lymphocyte and C-reactive protein/mean platelet volume ratios in differentiating between viral and bacterial pneumonias and diagnosing early complications in children. *Saudi Med J.* 2014;53(5):442-447.
17. Ilhan M, Ilhan G, Gak AF, Bademler S, Atmaca F, Ertekin C. Evaluation of neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and red blood cell distribution width-platelet ratio as early predictor of acute pancreatitis in pregnancy. *J Matern. Fetal Neonatal Med.* 2016;29(9):1476-1480.
18. Yazar FM, Bakacak M, Emre A et al. Predictive role of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios for diagnosis of acute appendicitis during pregnancy. *Kaobsiung J. Med. Sci.* 2015;31(11):591-596.
19. Liu J, Li S, Zhang S et al. Systemic immune inflammatory index neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio can predict clinical outcomes in patients with metastatic non-small cell lung cancer treated with nivolumab. *J Clin. Lab. Anal.* 2019;33(8):e22964.
20. Xiang N, Havers F, Chen T et al. Use of National Pneumonia surveillance to describe Influenza A(H7N9) virus epidemiology, China. *Emerg. Infect. Dis.* 2013;19(11):17841790.
21. Hasan MM, Das R, Rasheduzzaman M et al. Global and local mutations in Bangladeshi SARS-CoV-2 genomes. *Virus Res.* 2021;297:198390.
22. Bakanligi TCS. "Olasi Vaka Tanimi" COVID-19 Yeni Koronavirus Hastaligi. 2020; <http://covid19bilgi.saglik.gov.tr/depo/tedavi/COVID19>
23. Usul E, San I, Bekgoz B, Sahin A. Role of hematological parameters in COVID-19 patients in emergency room. *Biomark Med.* 2020;14(13):1207-1215.
24. Guan WJ, Ni ZY, Hu Y et al. Clinical characteristics in coronavirus disease 2019 in China. *N Engl J Med.* 2020;382(18):1708-1720.
25. Li Q, Guan X, Wu P et al. Early transmission dynamics in Wuhan, China, of novel corona virus-infected pneumonia. *N Engl J Med.* 2020;382:1199-1207.
26. Yang AP, Liu J, Tao W, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int. Immunopharmacol.* 2020;84:106504.
27. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
28. Assiri A, Al-Tawfiq JA, Al-Rabeah AA et al. Epidemiological, demographic and clinical characteristics of 47 cases of Middle-East Respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet.* 2013;13(9):752-761.
29. Xu XW, Wu XX, Jiang XG et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China, a retrospective study. *BMJ.* 2020;368:m606.
30. Azap A. "Viral pneumonias: clinical diagnosis and treatment". *Turk Klinik Mikrobiyoloji ve enfeksiyon Hastaliklari Dernegi.* www.klimik.org.tr/wp-content/uploads/2013/10/viral-pn/C3/B6monilar-tan/C4/B1-vetedavi-1-DrAlpayAzap.pdf
31. Xu Y, Zhang Y, Jiang F et al. Comparison of relevant indicators of coagulation and fibrinolysis in patients with varying severity community acquired pneumonia. *Zhonghua Yi Xue Za zhi.* 2015;95(24):1925-1929.
32. Fan BE, Chong VC, Chan SS et al. Hematologic parameters in patients with COVID-19 infection. *Am. J. Hematol.* 2020;95(6):E131-134.
33. Loonen AJ, de Jager CP, Tisserams J et al. Biomarkers and molecular analysis to improve blood stream infection diagnostics in an emergency care unit. *PLoS ONE.* 2014;9(1):e87315.
34. Yoon B, Son C, Um SJ. Role of neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community acquired pneumonia. *Ann. Lab Med.* 2013;33(2):105-110.
35. Naess A, Mo R, Nilssen SS, Eide GE, Sjrursen H. Infections in patients hospitalized for fever as related duration and other predictors at admittance. *Infection.* 2014;42(3):485492.
36. Zhang Y, Zou P, Gao H et al. Neutrophil-lymphocyte ratio as an early new marker in AIV-H7N9-infected patients: a retrospective study. *Ther. Clin. Risk. Manag.* 2019;15:911919.
37. Lagunas-Alvarado M, Mijangos-Huesca FJ, Teran-Gonzalez JO et al. Systemic inflammatory index in sepsis. *Med. Int. Mex.* 2017;33(3):303-309.
38. Hu B, Yang XR, Xu Y et al. Systemic inflammatory index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin. Cancer. Res.* 2014;20(23):6212-6222.
39. Hong X, Cui B, Wang M, Yang Z, Wang L, Xu Q. Systemic inflammatory index, based on platelet counts and neutrophil-lymphocyte ratio, is useful for predicting prognosis in small cell lung cancer. *Tohoku J Exp. Med.* 2015;236(4):297-304.