PATHOLOGICAL PARAMETERS HELPFUL IN SCREENING AND STRATIFICATION OF COVID-19 PATIENTS

Mir Attaullah Khan[1], Mohammad Sajid[1], Subhan Ud Din[1], Anam Attaullah[2]

ABSTRACT

Objective: To evaluate the effectiveness of various screening tests in stratification and triage of patients suffering from Covid-19.

Methods: It was a retrospective study conducted at Departments of Pathology and Medicine Gajju Khan Medical College Swabi from 1st January 2021 to 31st August 2021.where was this study undertaken and from? To ?. After ethical approval was obtained from the Institutional ethical/review board of GKMC/BKMC,. 08 ml of blood from each patient was collected. 05 ml blood was put in a tube containing anti-coagulant while remaining blood was put in a tube without anti-coagulant. Both tubes were sent to laboratory for various patholological parameters like complete blood count (CBC), Hb level, prothrombin time (PT), blood sugar, urea, C-reactive protein (CRP) and lactic dehydrogenase (LDH). For hematological parameters hematology analyzers were used while biochemical parameters were performed by automated chemistry analyzer. D-dimer was determined semi quantitively by Latex agglutination test.

Results: A total of 200 Covid-19 patients diagnosed on PCR, were included in the study. Out of 200 patients 30 (15%) were anemic, 80 patients (40%) had leukocytosis; 74% (148) having lymphocytosis, 03% (06) had leukocytopenia and 02 % (04) patients having absolute neutropenia. Regarding D-dimer level, 160 (80 %) patients had level of more than 250-500 ng/ml while 20 % (40) patients had 500-1000ng/ml. There was a significant difference in the level of D-dimer of mild to moderate versus severely ill patients (p=0.0002). Biochemical parameters were mostly deranged in critically ill patients showing great significance in terms of p-value (p=0.003).

Conclusions: Significant correlation was found in the severity of the disease and values of pathological screening parameters. Moreover, these parameters were successfully utilized for stratification of Covid-19 patients.

Keywords: D-dimer; COVID-19, SARS-COV2

INTRODUCTION

Corona viruses are important human and animal pathogens. They are further subdivided into four generations, alpha, beta, gamma and delta corona viruses[1].The corona viruses that cause diseases in human include HCOV-229, HCVNL63, middle east respiratory syndrome corona viruses (MERS-COV) and severe respiratory syndrome corona viruses (SARS-COV and SARS-COV2). Corona viruses are medium sized RNA viruses whose names derive from their characteristic crown like appearance under electron microscope. Length of genome of these viruses is 27 to 32kb[2,-6] Corona viruses are widely distributed in birds, mammals and bats with a large variety in their genotypes[2, 3].

Most human corona virus infections are mild but the previous epidemic of two beta corona viruses (MERS-COV, SARS-COV) have caused more than 37 % and 10% mortality in the past[3, 4].

Corona viruses are enveloped non-segmented positive sense RNA viruses belonging to the family coronaviridae, rinoviridae, nidovirales and arterviridae [5]. They are distributed vastly in humans and mammals[2].

The corona viridae family is subdivided into torovirinae and coronavirinae subfamilies. Coronavirinae is further classified into alpha, beta, gamma and delta corona viruses (COVs)[6].

They can be isolated from different animal species including birds and mammals such as camels, dogs, bats, mice and cats. First case of corona infection was detected in Wuhan city of China in December 2019 and was thus named as Covid-19. From Wuhan it spread very rapidly to other countries of the world and the World Health Organization (WHO)

[1] Gajju Khan Medical College, Swabi
[2] Peshawar Dental College, Peshawar

Address for Correspondence
Dr. Mir Attaullah Khan
Professor of Histopathology, Gajju Khan Medical College Swabi, Pakistan
mirattullah9@gmail.com
+92 333 9152904

announced the disease as a global pandemic on 11th March 2020. It (SARS-COV2) caused more than 6 million deaths globally [7].

Disease caused by the SARS-COV2 is classified into mild, moderate sever and critical. Most common symptoms of Covid-19 include fever (98.6%), fatigue (69.6%), dry cough and diarrhea.[8] Clinical presentation may range from asymptomatic patient to septic shock and multi-organ dysfunction and failure[9],[10,11.] Various Pathological tests are used for diagnosis, stratification and triage of Covid-19 patients[10]

MATERIAL & METHODS

It was a record based retrospective study. It was conducted in the Pathology Department of Gajju Khan Medical College (GKMC) and Department of Medicine Bacha khan Medical Complex (BKMC) Swabi from 1st January 2021 to 31st August 2021. A total of 200 patients during this period were included in the study. After initial evaluation of the patients nasal or pharyngeal swabs were taken by rubbing both the nostrils or pharynx of them respectively in an isolated room for this purpose taking all the protective measures. These swabs were properly placed in sterilized and labeled tubes and sent to pathology Department for diagnosis by PCR. Diagnosed patients were admitted in special medical units based on their clinical findings. After admission 08 ml blood sample of each Covid-19 patient was sent for hematological and biochemical tests in two special tubes (05 ml in EDTA tube and 03 ml in non EDTA tube) specified for this purpose. EDTA mixed blood was used for full blood count and Hb estimation by hematology analyzer while non EDTA blood was used for separation of serum and then estimation of RBS,CRP,LDH,fibrinogen, urea and D.dimer in it.

Complete blood counts were performed on blood samples of all these patients by hematology analyzer. Values of TLC, platelets count and Hb levels were entered in a proforma prepared specifically for this study.

Latex agglutination test was used for semi quantitative estimation of D-dimer fragments in the plasma. In this assay monoclonal antibodies react with fibrin D-dimer fragments. For this assay we took 20 ul of plasma and mixed it with 70 ul of D-dimer reagent and observed for agglutination for 3 minutes. If agglutination did not take place the level of D-dimers is less than 250 ng per/ml to be considered as negative. If agglutination was positive, it meant that level of D-dimer was more than 250 ng/ml. In positive cases serial dilution of the sample was also performed. For this purpose, we mixed 100 ul of sample with 100 ul of saline in one tube labeling it A. We also put 100 ul saline in other two tubes labeled as B and C. We made a serial dilution of 1:2, 1:4 and 1:8 by taking 100 ul from the tube A and putting it in tube B. Similarly we took 100ul from tube B and put it in the tube C. Again, we performed the same procedure for agglutination, by taking 70 ul from reagent and 20 ul from serial dilution mixture present in tube A, B and C. Normal level of D-dimer is less than 250 ng/ml in an undiluted sample. When positive in undiluted sample, its level was from 250 to 500 ng/ml. When positive in serial dilution of 1:2, its level was 500 to 1000 ng/ml. If positive agglutination was seen in 1:4 dilution, D- dimer level was 1000 - 2000 ng/ml and if positive in 1:8 dilution D-dimer level was more than 2,000 ng/ml. A raised level of D.dimer characterized a thromboembolic condition in the body and is thus a useful marker for health care provider to know within three minutes about the hemostatic and coagulation defect of the patient. Its negative value excludes a thromboembolic condition while positive value guides the clinician for other supportive investigations. All biochemical parameters i.e. RBS, CRP, LDH, fibrinogen and urea were performed by automatic chemistry analyzer and entered in the proforma already used for hematological parameters.

Statistical analysis:

All the data were subjected to SPSS Version 20. Simple descriptive statistics like bivariate statistical analysis test and chi square test were applied in the analysis for P-value estimation. P value of <0.05 was considered as significant.

RESULTS

A total of 200 Patients of covid-19 were included in the study. There were 120 males and 80 females. Most of the patients were in the age group of 41-60 years comprising 50% of the total patients.(table-1)

Table-1 Gender and age wise distribution of patients

Gender	Upto 20 years	21-40 years	41-60 years	> 60 years	Total
Male	20	25	60	15	120
Female	10	20	40	10	80
Total	30	45	100	25	200

They were diagnosed by PCR. Samples of these patients were subjected to hematology analyzer for determination of Hb, TLC and platelet count. PT and D-dimer levels were also performed from the samples of the patients. For PT estimation special tubes were used. Blood hemoglobin (Hb), random blood sugar (RBS), serum ionic calcium (Ca), C-reactive protein (CRP), prothrombin (PT), lactate dehydrogenase (LDH) and fibrinogen levels were also estimated. Thus, complete hematological, chemical and hemostatic pictures of the Covid-19 patients were determined.

In critically ill patients the TLC was significantly raised (13,500 ± 6,799.2) compared to mildly and moderately ill patients (12,000 ± 6,400.41 & 9,150.20 ± 4,154.32, $p = 0.001$). Similarly there was significant mean difference of TLC, neutrophils% (N%), lymphocytes% (L%) and monocytes% (M%) between cases having mild and those having moderate symptoms ($p = 0.020$, $p = 0.003$, $p = 0.003$ & $p = 0.004$ respectively). There was a significant difference in TLC ($p = 0.001$), N% ($p = 0.000$), L% (0.001), and L/N ratio ($p = 0.001$) between moderately ill and severely ill patients. 30 out of 200 (15%) patients were anemic, 80 patients (40%) had leukocytosis with most of them (74%) having lymphocytosis. 03% (06) patients had leukopenia with 02 % having absolute neutropenia. (table-2)

Table-2 Pathological parameters in different severity based groups of Covid-19 patients

Pathological parameter	Normal/Increased/ decreased	Mild n=90	Moderate n=70	Severe n=40	Sub toatal	Total cases n=200
TLC	Normal	15	3	0	18	200
	Increased	85	66	38	179	
	Decreased	0	01	02	03	
Hb	Normal	84	56	29	169	200
	Increased	01	0	0	01	
	Decreased	05	14	11	30	
RBS	Normal	75	50	25	150	200
	Increased	09	16	15	40	
	Decreased	06	04	0	10	
CRP	increased	84	63	37	184	200
	Normal/decreased	06	07	03	16	
LDH	Normal range	25	07	02	32	200
	Increased	63	60	36	159	
	Decreased	02	03	02	07	
Fibrinogen	increased	85	65	38	188	200
	Normal/decreased	05	05	02	12	
Urea	Normal	68	55	25	148	200
	Increased	20	15	15	50	
	Decreased	02	0	0	02	

Regarding D-dimer level, 160 (80 %) patients had D dimer level of more than 250-500 ng/ml while 20 % (40) patients had 500-1000ng/ml. There was a significant difference in the level of D-dimer levels of mild to moderate versus severely ill patients (p-0.0002) (table-3)

Table-3, Severity wise distribution of patient on the basis of serum D.dimer level.

Severity of disease	D.Dimer level	Number of patients	percentage
Mild	< 250 ng/ml	0	45
	250-500 ng/ml 90	90	
	>500 ng/ml	0	
Moderate	< 250 ng/ml	0	35
	250-500 ng/ml 90	70	
	>500 ng/ml	0	
Severe	< 250 ng/ml	0	20
	250-500 ng/ml 90	40	
	>500 ng/ml	0	
Total		200	100%

In group comparison of moderate versus severely ill cases, there was found a significant difference in RBS, CRP, PT, LDH and fibrinogen levels (p = 0.001, p = 0006, p=<00.1, p=<0.001 and p=<0.001) respectively. Comparison of the mild to severely ill cases showed a significant difference in fibrinogen and urea level (p = 0.001 & p = 0.045) respectively. These results show direct relationship with severity of the disease.

DISCUSSION

Covid-19 is a respiratory infection with significant impact on the hematologic system, hemostasis and biochemical status of the body. Corona virus (covid-19) has a very large pattern of respiratory manifestations including dry cough, dyspnea, pneumonia, pulmonary edema etc. Coagulopathies including elevated D-dimer and increased prothrombin time (PT) have also been described in covid-19 patient. Critically ill patients have complications like septic shock, strokes, ischemic limbs, venous thromboembolism, increased D-dimer and decreased platelets counts with high mortality rates.[12-13]

Severe complications of Covid-19 include acute respiratory distress syndrome (ARDS), arthritis, acute cardiomyopathy and shock. Thromboembolic complications, Guillain-Barre syndrome, conjunctivitis and Lymphopenia have also been reported.[14-16]. Other Hematological complications like leucopenia, thrombocytopenia and thromboembolism are also commonly found in critically ill Covid-19 patients [17, 18]. Patients in our study showed almost similar results.

Coronavirus can infect bone marrow cells[19] causing growth inhibition and apoptosis. It can also cause immune damage to blood cells by inducing autoantibodies. This way it can cause low grade disseminated intra vascular coagulation (DIC) which results in decreased number of platelets and elevated levels of D-dimer in blood[19]. Thrombocytopenia, coagulation disorders, increased D-dimer levels and elevated PT and APTT levels are common in Covid-19 and are associated with increased risk of ARDS and death[20-22]. D-dimer estimation and PT can therefore serve

as diagnostic indicators for disease progression[23].All these pathological derangements are due to immunological platelet destruction, inappropriate platelet activation, increased consumption of platelets and impaired megakariopoiesis[24].Lymphopenia has also been reported in covid-19 patients in Wuhan, Singapore and Washington in critically ill patients[25, 26]. Several factors like expression of ACE2 receptors on the surface of lymphocytes may contribute to covid-19 associated lymphopenia.. The SARS- COV-2 may directly infect these cells and cause their lysis[27]. Moreover, several cytokines and interleukins like IL-6, IL-2, IL-7, interferons, MECP-1 and TIVF may promote lymphocyte apoptosis and thus decrease in their number[28]. Cytokine activation also causes atrophy of lymphoid organs i.e. spleen which impair lymphocyte turnover. Lactic acidosis occurring in Covid-19 patients also contributes in inhibition of lymphocyte proleferations[29]. All the above-mentioned parameters in these various studies showed close similarity to those in our study.

Covid-19 is associated with pro-thrombotic state causing microvascular thrombosis and pulmonary embolism[30]. Similarly, D-dimer and PT are significantly elevated in patients having severe covid-19. D-dimers are elevated due to fibrinolysis occurring in DIC secondary to Covid-19 infection[31]. The study at hand showed slightly different results compared to these. Reason might be small size of the sample, demographic factors or nature of the study as being single centered.

CONCLUSION

All findings of this study suggest that the above mentioned hematological and biochemical parameters can be used for screening purpose as well as clinical stratification and triage of the patients at the time of presentation. Similarly, these can be used for identification of critically ill patients for subjecting them to intensive care in health care facilities at the earliest.

LIMITATIONS

Limitations of this study are its retrospective nature, single center basis and its small sample size.

RECOMMENDATIONS

This study, if conducted on a large sample size involving many centers, its results can be made more authentic and applicable to a large population.

REFERENCES

1. Fehr AR, Perlman S. corona Viruses, an overview of their replication and pathogenesis. Method Mol Biol 2015; 1282:1-23
2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223):497-506.
3. Al-Tawfiq JA, Zumla A, Memish ZA. Travel implications of emerging coronaviruses: SARS and MERS-CoV. Travel medicine and infectious disease 2014;12(5):422-8.
4. Fehr, A.R., Perlman, S. Coronaviruses: An Overview of Their Replication and Pathogenesis. In: Maier, H., Bickerton, E., Britton, P. (eds) Coronaviruses. Methods in Molecular Biology, vol 1282. Humana Press, New York.
5. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet 2020;395 (10224):565-74.
6. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, evaluation and treatment of coronavirus (COVID-19). Statpearls [internet]. 2022; Feb 5.
7. Hu Q, Guan H, Sun Z, Huang L, Chen C, Ai T et al. Early CT features and temporal lung changes in COVID-19 pneumonia in Wuhan, China. European journal of radiology 2020; 128:1901-7.
8. Hassan SA, Sheikh FN, Jamal S, Ezeh JK, Akhtar A. Coronavirus (COVID-19): a review of clinical features, diagnosis, and treatment. Cureus 2020;12(3).
9. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The lancet 2020; 15(39): 514-23.
10. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The lancet respiratory medicine 2020; 1;8(5):475-81.

11. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. Jama 2020; 17;323(11):1061-9.
12. Yang M, Ng MH, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome. Hematology 2005; 1;10(2):101-5.
13. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive care medicine 2020 ;46(6):1089-98.
14. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. Jama 2020;28;323(16):1612-4.
15. Cao J, Tu WJ, Cheng W, Yu L, Liu YK, Hu X et al. Clinical features and short-term outcomes of 102 patients with coronavirus disease 2019 in Wuhan, China. Clinical Infectious Diseases. 2020; 28;71(15):748-55.
16. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. Journal of thrombosis and hemostasis 2020;1;18(6):1324-9.
17. Jolicoeur P, Lamontagne L. Impairment of bone marrow pre-B and B cells in MHV3 chronically-infected mice. Corona- and Related Viruses: Current Concepts in Molecular Biology and Pathogenesis1995;193-5.
18. Fan BE. Hematologic parameters in patients with COVID-19 infection: a reply. American journal of hematology. 2020;95(8): E215.
19. Amgalan A, Othman M. Exploring possible mechanisms for COVID-19 induced thrombocytopenia: Unanswered questions. J Thromb Haemost 2020;18(6):1514-6.
20. Giannitsis E, Mair J, Christersson C, Siegbahn A, Huber K, Jaffe AS. How to use D-dimer in acute cardiovascular care. European Heart Journal: Acute Cardiovascular Care. 2017;6(1):69-80
21. Cohen J. The immunopathogenesis of sepsis. Nature. 2002;420(6917):885-91. 28-21
22. Chen T, Wu DI, Chen H, Yan W, Yang D, Chen G. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020; 26(368): 232-8.
23. Xiong M, Liang X, Wei YD. Changes in blood coagulation in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. British journal of hematology 2020;189(6):1050-55.
24. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. Jama 2020;323(16):1612-4.
25. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. International journal of oral science 2020;12(1):1-5.
26. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M. Inhibitory effect of tumor cell–derived lactic acid on human T cells. Blood. 2007;109(9):3812-9.
27. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet 2020;395(10223):507-13.
28. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA cardiology 2020 Jul 1;5(7):811-8.
29. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine 2020 ;180(7):934-43.
30. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The lancet respiratory medicine 2020 ;8(5):475-81.
31. Ibañez C, Perdomo J, Calvo A, Ferrando C, Reverter JC, Tassies D. High D dimers and low global fibrinolysis coexist in COVID19 patients: what is going on in there? J Thromb Thrombolysis 2021;51(2):308-12.