International Journal of Innovative Research in Medical Science (IJIRMS)
Volume 08, Issue 02, February 2023,
https://doi.org/10.23958/ijirms/vol08-i02/1620

Original article

Biochemical Markers of COVID -19: Role in Diagnosis, Management and Predicting Severity of the Disease

Dr Kiran Bhat [1], Dr Sohaib Ahmad [2]

[1]Professor, Department of Biochemistry, Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun, Uttarakhand, India; *drkiranbhat@gmail.com; kirsad@yahoo.co.in*

[2]Professor, Department of General Medicine, Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun, Uttarakhand. India; *sohadia@hotmail.com; sohaibahmad@srhu.edu.in*

*Corresponding author: Dr Kiran Bhat; *drkiranbhat@gmail.com; kirsad@yahoo.co.in*

Received 24 January 2023; Accepted 08 February 2023; Published 12 February 2023

Abstract

The emergence of variants of corona which are getting transmitted easily and are more virulent and fatal has made things more difficult and the need to know about the prognostic markers has taken a priority besides vaccination. All COVID-19 RT PCR positive patients were categorized into mild, moderate and severe category any time during hospital stay based on the WHO guidelines 2021. Comparison of all clinical and selected biochemical parameters (Ferritin, LDH, IL-6, TLC, NLR, albumin, D-dimer and procalcitonin) was done.

The Aim of the study was to establish a cut off level of the biomarkers of COVID -19 which could determine the severity of the disease and act as prognostic factors too. These biochemical markers include NLR, serum D-dimer, CRP, IL-6, ferritin, LDH, albumin, procalcitonin, AST and ALT($p<0.001$). A total of 240 patients who were RT-PCR positive were included in the study. Compared to patients with mild disease, those with moderate to severe disease had a significant higher levels of the markers except serum albumin($p<0.0001$). Albumin was significantly lower in these patients. A significant association was also seen between these markers and the outcome of the disease. Those who expired or left against medical advice (LAMA) had a significant higher levels of D-dimer, IL-6, CRP, procalcitonin and LDH as compared to those who were cured. IL-6, D-dimer, LDH, CRP, Procalcitonin and albumin are really helpful in knowing the severity of the disease and in disease surveillance.

Keywords: ***Albumin; cytokine storm; CRP; D-dimer; IL-6; lymphocytes; neutrophils; procalcitonin.***

Introduction

Ever since its identification in Wuhan district of the Chinese province of Hubei in December 2019, the SARS-Cov-2 has spread to almost 170 countries [1]. The emergence of variants of corona virus with more efficient transmissibility and/or virulence and fatality has made search for the prognostic markers imperative [2]. The SARS Cov 2 variant of concern (VOC) evolves in areas with low vaccine coverage and a high rate of virus in circulation. VOC is defined by WHO as virus variant with evidence of increase in transmissibility causing severe disease, significant neutralization by antibodies, reduced effectiveness of treatment or vaccines and diagnostic detection failure. The Delta variant (B.1.617.2) emerged in India at a time of low vaccine coverage and high transmission rate leading to outbreaks [3,4]. This variant of interest became variant of concern. It was dominant globally and was classified by WHO on May 2021[5].

During the second wave, many laboratory parameters were deemed for prognosis of COVID 19. Validating their potential as relevant prognostic markers, determining their cut-offs and ensuring their availability everywhere is challenging. Choosing a readily available and right test for the right geographical region is a better strategy but not bereft of potential errors [6]. The aim of our study was to establish cut off levels of some biochemical markers which are associated with the disease severity and the prognosis of a patient with COVID 19 at presentation.

Material and Methods

This cross-sectional study was conducted on all patients presenting with influenza like illness and positive for COVID -19 by RT- PCR. The COVID positive cases were confirmed by RT- PCR (ICMR approved Covipath kit) in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens.

All COVID-19 RT PCR positive patients were categorized into mild, moderate and severe category any time during hospital stay based on the WHO guidelines January 25th 2021 and guidelines of ICMR (Indian council of medical research) 2020 [1]. Patients with pre-existing chronic lung disease like chronic bronchitis and emphysema and those who were RT-PCR negative were excluded from this study. They were subjected to biochemical tests (viz. serum IL-6, D-dimer, procalcitonin, albumin, ferritin, AST, ALT, LDH and TLC) after taking a written consent from them and these clinical parameters were compared between the three categories of severity. Comparison of all clinical and some biochemical parameters was done. The serum biochemical parameters were assayed by ELFA

(enzyme linked fluorescent assay), chemiluminescence assay, nephlometry and colorimetric assay on auto analyzers in biochemistry section and microbiology section of Himalayan Central Diagnostic and Research Laboratory (HCD&RL). An attempt was made to estimate the cut off value of parameters to predict the transition to severe category from mild and moderate disease level. The final outcome was classified as either recovery (with or without residual disability) or death due to COVID-related complications.

Approval from both - research and ethical - committees was obtained before start of the study.

Results

A total of 240 patients (Males 163; 67.9%) with COVID-19 infection were included in the study and were admitted in this tertiary care hospital during March 2021 to July 2021. These patients were categorized into three clinical categories - mild (n=66; 27.5%), moderate (n=108; 45%) and severe (n=66;27.5 %) - based on WHO/ICMR guidelines 2021 for COVID -19. The major co-morbidities were diabetes (n=41;17.1%), hypertension (n=34;14.16%) and cardiovascular disease (n=26; 10.83%). Fever (n=158,65.8 %), breathlessness (n=173,72.1%) fatigue (n=201;83.75 %), dry cough, myalgia, loss of taste, nausea, vomiting, diarrhea and chills and loss of smell sensation and headache were some common symptoms. Compared with mild group, the moderate and severe groups were significantly older (median age 53.28±16.29) and had underlying comorbidities. The symptoms of shortness of breath, dyspnea and myalgia was more in severe group. Most patients had moderate type of illness progressing to unfavorable outcome. Even milder ones had a number of post COVID complications. Persisting headache, nausea, vomiting, diarrhea, weakness, slow cerebration and memory loss were seen in a number of cured patients. The results of the comparison of the biochemical parameters between the categories are given in table 1. Significant difference in TLC, neutrophils and lymphocytic ratio (NLR), AST, ALT, LDH, D-dimer. IL-6 procalcitonin, C-reactive proteins and Ferritin was observed.

Mortality was seen in 91 patients (n=91; 37.9%) and 149 patients were cured and were followed up later on OPD basis. The mean age was 51.40±14.79 years in those who were cured and 55.28±13.97 in those who expired. Significantly the disease severity was not associated with age although mean age of those who expired was 55.28±13.9 (p=0.39). Table 2 compares the two outcome groups. The groups were significantly different in terms of mean NLR, LDH, D-dimer. IL-6, procalcitonin, c-reactive proteins (CRP), ferritin and albumin. Mean troponin levels were comparable. The mortality rate was higher among patients with severe or critical levels of biochemical parameters.

The moderate and severe categories of patients were clubbed together and an attempt was made to calculate the cutoff value from mild and moderate plus severe using the receiver-operated curved (ROC) technique of the above-mentioned parameters (table 3). Multi-logistic regression analysis- predictive value was applied to all the parameters to detect severity of illness.

The area under curve was largest for albumin and area under curve for lymphocytes ranked second. Even troponin I (0.471) and ALT (0.450) showed great area under the curve emphasizing their significance in predicting the progression to severity.

Similarly, we calculated the cut-offs for patients in moderate and severe categories of disease (table 4) The area under curve is highest for serum albumin in transition from moderate to severe cases followed by ALT, IL-6 and AST. IL-6 had greater AUC in transition from moderate to severe as compared to mild to moderate.

Table 5 shows area under curve for ferritin, CRP and IL-6 is maximum (.72) followed by that of procalcitonin with credible sensitivity and specificity in the outcome groups.

Table 1: Comparison of biochemical parameters of the 3 clinical subgroups

Variable	Severity			p-value
	Mild	moderate	Severe	
NE	70.98 (62.72 - 79.03)	73.05 (62.23 - 81.30)	78.49 (68.28 - 84.85)	0.001
Lymphocytes	17.76 (10.28 - 22.26)	16.40 (7.74 - 24.8)	11.60 (3.13 - 16.87)	0.01
NLR	4.36 (2.96 - 7.95)	4.38 (2.54 - 10.49)	6.78 (3.89 - 30.16)	0.004
Albumin	3.53 ± 0.61	3.41 ± 0.52	3.41 ± 0.36	0.313
D-dimer	0.88 (0.47 - 2.11)	1.13 (0.67 - 2.15)	1.27 (0.91 - 3.48)	0.013
IL-6	13.07 (4.35 - 51.50)	37.80 (19.04 - 165.08)	56.34 (30.05 - 206.49)	< 0.0001
LDH	335 (251 - 430.25)	426 (263.75 - 568.25)	498 (355 - 688)	< 0.0001
AST	48 (32 - 74)	56 (35 - 87)	60 (40 - 112)	0.104
ALT	38.5 (21-75.75)	40.50 (29.0-72)	40 (29-77.5)	0.44
Ferritin	359.77 (201.26-689.28)	579.59 (226.83-1139)	953.04(267.7-1200)	<0.0001
CRP	39.75 (10.13 - 63.49)	53.41 (16.75-95.61)	100.93 (51.79 - 172.34)	<0.0001
Procalcitonin	0.13 (0.05 - 0.45)	0.31 (0.07 - 2.52)	0.58 (0.29-2.48)	<0.0001
Troponin	5.5 (1.5-23.03)	5.4(1.5-12.9)	16.20(5.4-39.8)	0.053

Table 2: Comparison of biochemical parameters of the outcome groups of COVID infection

Variable	Outcome		p-value
	expired	cured	
NE (N=240)	79.8 (73.65-87.56)	70.9 (62.35-79.15)	<0.001
Lymphocytes (N=240)	11.22(4.5-16.9)	17.3(9.28-23.3)	0.003
NLR (N=234)	6.78 (4.28-16.18)	4.13 (2.74-8.53)	0.002
Albumin (N=227)	3.35±0.52	3.51±0.51	0.023
D-dimer (N=219)	1.37(0.88-2.70)	0.90(062-1.97)	0.001
IL-6 (N=184)	84.06(34.94-242.77)	19.29(6.83-66.55)	<0.001
LDH (N=220)	467(337.35-654.50)	363.50(251.50-486)	<0.001
AST (N=226)	56(36-113)	50(34-78)	0.16
ALT (N=228)	40(27.5-69)	41(26-78)	0.97
Ferritin (N=229)	1072.48(459.09-1200)	359.77(197.56-720.48)	<0.001
CRP (N=206)	81.25(52.47-172.33)	37.36(14.66-78.30)	<0.001

Procalcitonin (N=176)	0.58(0.22-2.55)	0.14(0.05- 0.78)	<0.001
Troponin (N=96)	12.40(2.10-26.50)	5.50(1.50-16.95)	0.16

Table 3: Sensitivity and specificity and cut-off values of laboratory parameters in mild and moderate plus severe cases

Variable	Sensitivity	Specificity	AUC	Cut-off Value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
NE	41%	45%	.435	74.15	.357	.512
Lymphocytes	55%	56%	.573	15.5	.496	.650
NLR	45%	45%	.423	4.77	.347	.500
Albumin	60%	60%	.594	3.49	.507	.680
D-dimer	42%	40%	.372	0.96	.287	.457
IL-6	33%	33%	.296	27.85	.203	.389
LDH	38%	38%	.339	378.5	.263	.415
AST	43%	44%	.426	52.5	.345	.507
ALT	47%	48%	.450	39.5	.364	.536
Ferritin	39%	39%	.356	473.83	.281	.430
CRP	37%	37%	.356	47.19	.280	.433
Procalcitonin	37%	38%	.323	0.23	.238	.408
Troponin	46%	46%	.471	6.2	.342	.601

Table 4: Sensitivity and specificity and cut-off values of laboratory parameters in moderate and severe cases

Variable	Sensitivity	Specificity	AUC	Cut-off Value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
NE	38%	42%	0.39	77.69	0.30	0.47
Lymphocytes	57%	58%	0.61	13.45	0.52	0.70
NLR	42%	42%	0.39	5.78	0.30	0.47
Albumin	53%	55%	0.52	3.39	0.43	0.61
D-dimer	46%	47%	0.39	1.21	0.30	0.48
IL-6	44%	45%	0.44	49.39	0.34	0.55
LDH	44%	43%	0.40	445.00	0.31	0.49
AST	48%	49%	0.44	58.00	0.35	0.54
ALT	50%	52%	0.48	40.50	0.39	0.57
Ferritin	38%	37%	0.40	713.80	0.31	0.49
CRP	39%	39%	0.31	63.15	0.22	0.40
Procalcitonin	45%	46%	0.40	0.44	0.30	0.49
Troponin	38%	39%	0.32	8.80	0.19	0.45

Table 5: Sensitivity and specificity and cut-off values of laboratory parameters in outcome groups

Variable	Sensitivity	Specificity	AUC	Cut-off Value	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
NE (N=240)	67%	68%	0.68	76.25	.604	.751
Lymphocytes (N=240)	42%	42%	.385	14.27	.311	.459
NLR (N=234)	61%	61%	.621	5.45	.546	.697
Albumin (N=227)	44%	44%	.407	3.43	.331	.482
D-dimer (N=219)	62%	61%	.636	1.15	.561	.710
IL-6 (N=184)	69%	70%	.738	43.74	.665	.811
LDH (N=220)	59%	60%	.642	416.5	.567	.716
AST (N=226)	53%	53%	.556	55.5	.477	.634
ALT (N=228)	51%	49%	.502	39.5	.425	.578
Ferritin (N=229)	70%	70%	.724	557.93	.655	.792
CRP (N=206)	63%	63%	.721	55.83	.649	.793
Procalcitonin (N=176)	63%	63%	.708	0.39	.631	.784
Troponin (N=96)	57%	56%	.585	6.95	.465	.706

Discussion

The laboratory parameters namely procalcitonin, LDH, NLR, D-dimer, ferritin, IL-6, TLC and CRP are indicative of severity of the COVID infection and a bad prognosis. The identification of these critical patients at an earlier stage shall help in reducing the mortality due to this disease.

In our study the age did not show significant association with severity of the disease(p=0.39) although those with moderate and severe disease were slightly older (mean age 53±14.38 yrs). Yuan X et al showed mean age of 66yrs and47.9% were male whereas in our study 67.9%were male [7]. In study by Wang D et al the mean age of patients was 58 yrs and 51 % were male [8].

The TLC (Total leucocyte count) showed a direct relationship with severity of the disease. Raised TLC was significantly associated with severity of the disease and this is in accordance with study by Wang D [8]. Most viral infections are associated with lymphocytosis but infection with corona virus

family SARS -CoV, SARS CoV 2 cause lymphocytic depletion in infected people [7,9]. Elevated NLR (Neutrophil lymphocytic ratio) resulting in increased neutrophil count and decreased lymphocytic count are associated with high risk and bad prognosis. Thirumalaisamy P et al found lymphocytopenia clearly associated with disease severity [10]. NLR varied significantly in our study between mild, moderate and severe cases (p=0.004).

Cytokine release storm is a systemic inflammatory response with multi organ failure [11-14] and is seen associated with COVID -19 cases with ARDS. Amongst all the parameters excess of Interleukin 6 release is a key contributing factor to cytokine release syndrome [7,15]. In our study too we could find Interleukin significant in determining the severity and outcome of the disease(p<0.0001). The levels were much raised in those who expired (98.64 pg/mL) as compared to in those who survived (23.11 pg/mL). Lucia G et al suggested prompt use of tocilizumab before overwhelming secretions of cytokines have occurred for better prognosis and is possible by knowing the levels of IL-6, D-dimer and ferritin [16].

Yuan X et al Identified IL-6 as the most robust predictor of hypoxemia and a cut off level of IL-6 >24pg/mL predicted the development of hypoxia [7,14,15,17]. IL-6 levels proved to be a good indicator for the progression and outcome of the disease. The cut off value in our study for IL-6 was 43.74 pg/mL

The mortality and morbidity associated with COVID -19 is due primarily to the excessive immune activation [9,11].

The severity of the disease was also related to CRP levels and D-dimer levels by Yuan X et al [7]. This was also observed by Tang et al [6]. Ruan Q et al found CRP levels increased in COVID 19 patients [18]. They established a serum CRP median level of 40mg/L in survivors and 125mg/L in non- survivors whereas Huan Ie et al showed cut off of CRP level >10mg/ [6].

A significant difference was observed in the serum ferritin levels between those who had mild, moderate and severe disease(p<0.0001) and also between those who were cured and those who expired(p<0.0001).

Serum procalcitonin levels too showed significant difference between those who were cured and those who expired (p<0.0001) and also a significant difference was observed between mild moderate and severe cases (p<0.0001) [20-23].

Albumin levels showed an inverse relation to severity of the disease although not much significant(p=0.313) in our study. Thirumalaisamy P et al also found significant liver dysfunctions in those with severe COVID 19 disease than those with milder disease [7,10,24]. The study by Dan Wang et al in 171 patients showed sensitivity of 85.9% and specificity of 83.3% for albumin [8].

The LDH levels showed significant(p=<0.0001) direct relation to the severity of the disease and mortality due to disease(p<0.0001). Those who expired showed a mean value of 548 IU/L whereas those who were cured had a serum value of 386 IU/L.

AST and ALT did not show a significant difference in those who were cured and those who expired (P=.16 and.97 respectively) and not much significant difference was seen between mild and severe cases (p=0.104 and 0.44 respectively) [25].

In our study the concentration of IL-6, D-dimer and ferritin was much higher in severe cases than mild and moderate cases. This is a reflection of coagulation alterations [6,7]. This was also seen in the study by Wang D et al. They also observed a greater AUC for D-dimer, LDH and CRP. Even the sensitivity and specificity was higher. They had divided the patients into two main categories mild /moderate and severe /critical. The AUC is greater and sensitivity and specificity is good if we consider the two categories of patients [8].

Troponin I was also related with severity of the disease (p<0.053) and showed injury to myocardium. Mean value in severe cases=16.20 ng/L and in those who expired was 19.9 ng/L [21-23,26].

The strength of the study was that all patients were managed according to a diagnostic and treatment protocol to avoid any individual bias. Moreover, we were able to provide the cut-off values along with the sensitivity and specificity of these values for individual parameters that are readily available in most of the laboratories at a nominal cost. The limitation of the study was that all the patients were from a single tertiary care hospital and the overall numbers were not high. Due to the low numbers, the results of the study may not be extrapolatable to the general population. Nevertheless, this study may prove to be the benchmark of further studies on the subject in future.

Bibliography

[1] WHO. Clinical management of COVID 19 Patients. Living Guidance. January 25,2021. P14.

[2] Peeling RW, Heymann DL, Teo YY, Gracia PJ. Diagnostic for COVID 19: Moving from Pandemic Response to Control. Lancet. 2022; 399(10326): 757-768.

[3] Liu Y, Rocklöv J. The Reproductive Number of the Delta Variant of SARS-CoV-2 is Far Higher Compared to the Ancestral SARS-CoV-2 Virus. Journal of Travel Medicine. 2021; 28(7) taab124.

[4] Rocklov J, Sjodin, H, Wilder-Smith A. COVID-19 Outbreak on the Diamond Princess Cruise ship: Estimating the Epidemic Potential and Effectiveness of Public Health Countermeasures. Journal of Travel Medicine. 2020: 27(3) taaa030.

[5] WHO. Coronavirus disease (COVID-19): Variants of SARS-COV-2.4 December 2021. Q&A

[6] Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. Journal of Thrombosis and Haemostasis. 2020;18(4):844-7.

[7] Yuan X, Huang W, Ye B, Chen C, Huang R, Wu F et al. Changes of hematological and immunological parameters in COVID-19 Patients. International Journal of Hematology. 2020; 112: 553-559.

[8] Wang D, Li R, Wang J, Jiang Q, Gau C, Yang J et al. Correlation analysis between disease severity and clinical biochemical characteristics of 143 cases of COVID -19 in Wuhan, China; A descriptive study. Biomed Central Infectious Diseases. 2020; 20: 519.

[9] Huang I and Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. Journal of Intensive Care. 2020; 8: 36.

[10] Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. International Journal of Infectious Diseases. 2020; 95: 304-307.

[11] Sabaka P, Koskalova A, Straka I, Hodosy J, Liptak R, Kmotorkova B et al. Role of interleukin 6 as a predictive factor for a severe course of COVID-19: retrospective data analysis of patients from a long-term care facility during COVID-19 outbreak. Biomedical Central Infectious Diseases. 2021; 21: 308.

[12] Magro G. SARS-CoV-2 and COVID-19: is interleukin-6 (IL-6) the 'culprit lesion'of ARDS onset? What is there besides Tocilizumab? SGP130Fc. Cytokine X. 2020; 2(2): 100029.

[13] Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. Cell Host Microbe.2020; 27(6): 992-1000.e3.

[14] Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: A meta-analysis. Journal of Medical Virology. 2020 ;92(11): 2283-2285.

[15] Shimabukuro-Vornhagen A, Godel P, Subklewe M, Stemmler HJ, Schlößer HA, Schlaak M, et al. Cytokine

release syndrome. Journal of Immunotherapy and Cancer. 2018 ;6(1): 56.
[16] Guillen L, Padilla S, Fernandez M, Agullo V, Garcia JA, Telenti G et al. Preemptive interleukin-6 blockade in patients with COVID-19. Scientific Reports. 2020; 10:16826.
[17] Winthrop KL. Who needs a Corona? Arthritis and Rheumatology. 2020;72(6):874-876.
[18] Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan. China. Intensive Care Medicine. 2020; 46(5): 846-848.
[19] Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer,and ferritin in severe coronavirus disease-2019: a meta-analysis. Therapeutic Advances in Respiratory Disease. 2020; 14: 1-14.
[20] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet; 2020; 395(10229):1033-1034.
[21] Ghayda RA, Lee J, Lee JY, Kim DK, Lee KH, Hong SH et al. Correlations of Clinical and Laboratory Characteristics of COVID-19: A Systematic Review and Meta-Analysis. International Journal of Environmental Research and Public Health. 2020; 17: 5026.
[22] Rodriguez-Morales A.J, Cardona-Ospina JA, Gutierrez-Ocampo E, Villamizar-Pena R, Holguin-Rivera Y, Escalera-Antezana JP et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Medicine and Infectious Disease. 2020, 34, 101623.
[23] Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. International Journal of Infectious Diseases. 2020; 96: 467-474.
[24] Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan. China: Allergy. 2020; 75(7): 1730-1741.
[25] Singhai A, Sai P, Smritimayee P. Evaluation of liver functions in symptomatic Covid -19 patients. Journal of Family Medicine and Primary Care. Sep 2021;10(9):3252-3256.
[26] Azar MM, Shin JJ, Kang I, Landry M. Diagnosis of SARS-CoV-2 infection in the setting of the cytokine release syndrome. Expert Review of Molecular Diagnostics. 2020; 20(11): 1087-1097.
[27] Director General of Health Services (EMR Division). Revised guidelines on clinical management of COVID - 19. Ministry of health and family welfare. 31st March 2020.