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Severe Lymphocytopenia in COVID 19 Positive Patients Admitted in Intensive Care Unit in India: An Observational study

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Abstract

Introduction: lymphocytopenia is very significant finding in COVID 19 disease. It is main reason for pathogenesis of acute respiratory distress syndrome. Since decrease lymphocytes leads to increased cytokines to fight against the virus and thus more injury to the tissues.

Objective: To look for proportion of patients having severe lymphocytopenia in moderate to severe cases of COVID 19 disease

Material and methods: COVID 19 Patients admitted in Intensive care unit of tertiary care center were enrolled in the study. Required investigations were performed according to the protocol of given by ministry of health and family welfare. Lymphocyte count <20 % on DLC (Differentail leucocyte count) or <1000cells/mm3 were taken as severe lymphocytopenia.

Results: almost all patients in ICU had severe lymphocytopenia in DLC and mean absolute lymphocyte count of patients in these patients was 399.8±712.3 cells / mm^3 . Around 2/3rd of patients had lymphocyte count <1000cells /mm^3. Mean neutrophil count was >10,584.8±6,948.2 cells /mm^3 with almost all patients having high (>3:1) Neutrophil to Lymphocyte (N/L) ratio.

Conclusion: Severe lymphocytopenia and high Neutrophil to Lymphocyte ratio is marker of severe disease and hospitalization in ICU in COVID 19 disease. One should be vigilant and keep monitoring the patient for rapid progression of the disease in such cases.

Keywords: COVID 19, Lymphocytopenia, Severe, Intensive care Unit

INTRODUCTION

With the current ongoing COVID-19 pandemic, we have been facing problems in understanding the disease. Various studies have shown that immune dysregulation and cytokine storm are the main pathogenesis of severe acute respiratory distress syndrome (ARDS) in this disease [1]. SARS CoV-2 virus impairs the immune system by decreasing the activated T cell (specially CD 4) and reducing the lymphocyte count and increasing the expression of several cytokines and inflammatory markers [1,2]. Some studies have found that there is no difference between the cytokine's levels between mild and severe cases, but T cells were significantly reduced in severe cases [2]. While, some studies have found that although IL6 levels were marker of severity in covid 19 disease but it was significantly low in severe and critical COVID 19 diseases as compared to severe and critical cases of sepsis [2,3]. This indicated that lymphocytopenia and cytokine induced injury could be the main cause of pathogenesis in severe COVID

19 diseases. Lymphocytopenia was found consistent with severe disease in many previous studies [4,5]. The cause of lymphocytopenia could be virus induced rapid exhaustion of T cells, down regulation of T cell expansion and cytokines storm itself [5]. So we aimed to study the prevalence of lymphocytopenia in patients admitted in Intensive care unit.

METHODS

Patients admitted in Intensive care unit (ICU) of COVID 19 positive ward in a tertiary care center from 15 September to 15 October 2020 were enrolled in this study. It was an observational prospective study. The patients were labeled COVID-19 positive either by positive RTPCR test, rapid antigen test or TRUNAAT test (sample taken from nose and throat). Patients with COVID 19 positive reports, who were moderate {SpO_2: 94-90% in Room air (RA) and respiratory rate(RR) 24-30/minute}, severe (SpO_2 <90% on RA and RR>30/minute)or critically ill {Acute respiratory distress syndrome (ARDS) i.e. SpO_2/ FiO_2 <315} according to the ministry of health and family welfare guidelines were admitted in COVID 19 ICU ward[6]. Patients with mild symptoms with co-morbidity were admitted in isolation ward and were not included in our study.

The investigation which were sent on admission were Complete blood count (CBC), liver function test (LFT), kidney function test (KFT), glucosylated hemoglobin (Hb_{A1C}), random blood sugar (RBS), serum electrolytes (sodium, potassium, calcium), c-reactive protein (CRP), serum Lactic dehydrogenase (LDH), serum ferritin, D- Dimer, prothrombin time (PT), international normalized ration(INR), activated partial thromboplastin time (aPTT), Arterial Blood Gas analysis (ABG), Contrast enhanced computed tomography (CECT) scan of chest if patient condition allowed to go to radiology department or bed side Chest X ray. ECG (electric cardiography) was done where ever it was necessary. When secondary bacterial infection was suspected serum procalcitonin was sent. Necessary tests were repeated every 48-72 hourly or more frequently when required. Random blood sugars were done in all diabetic patients every 6 hourly for strict glucose control. Vital parameter including pulse, blood pressure, respiratory rate, hydration, SpO_2 monitoring were monitored every 2 hourlies.

Lymphocytopenia was defined if lymphocyte count fell below 20 % or absolute lymphocyte count <1000cells/mm^3. Neutrophil/lymphocyte (N/L) ratio >3.5, CRP>30mg/L, serum ferritin >350mcg/L, LDH>450 and D –Dimer >1000ng/ml were taken as high risk and was observed for in these sick patients.

The treatment was given according to the guidelines of ministry of health and family welfare. All patients were started on oxygen by face mask (FM) when SPO_2 was below 94%. If SPO_2 was still not maintaining on FM, then patient was shifted to non-rebreathing bag mask (NRBM), then to non-invasive mode of ventilation i.e. high flow nasal canula (HFNC) and then BIPAP (bi level positive airway pressure) ventilator and finally to invasive ventilation mode. Some patients were directly taken to non-invasive or invasive ventilation if the condition of patients was very not good. Patients were started on injection Dexamethasone 0.1-0.2 mg/kg in moderate and 0.2-0.4 mg/kg in severe/critical cases. Low molecular weight heparin (enoxaparin) was started (0.5mg/kg once daily in moderate and 1-2mg/kg in twice daily in severe/ critical cases). Injection ceftriaxone was started as empirical antibiotic in all sick patients. Injection Ramedesivir was given in only early cases with duration of illness less than 10 days. Some patients were also given Plasma therapy. Tablet zinc 50 mg once daily for 7 days, tablet vitamin C 500 mg thrice a day for 7 days and tablet N Acetylcystein one tablet thrice a day for coughing were also given. Awake prone position was practiced for improving the oxygenation status. Other supportive management was given as and when required.

Statistical analysis: data was entered in Microsoft Excel and Mean and standard deviation was calculated for continuous variable and proportions were found in discrete variables.

RESULTS:

Total 120 patients were enrolled in this study. The mean age of patients was 58.8± 13.7years with male to female ratio of 5:1. The mean hemoglobin was 11.3 ± 2.5 g/dL. Mean {Standard Deviation (SD)} of Total Leukocyte count (TLC) was 13,335.5± 6,927cells/mm^3. Mean (SD) Differential count were: Neutrophil - 85±8.2 %, Lymphocyte- 10±6.9 %, Mid cells (including eosinophil and basophil)-5±2.5 %. Mean (SD) Absolute Neutrophil count (ANC) 10,584.8±6,948.2 cells /mm^3. Mean (SD) absolute

Volume 4, Issue 4; July-August 2021; Page No 1222-1226

Lymphocyte count (ALC) was 399.8±712.3 cells / $mm^{3.}$ Mean (SD) platelet count was 1.94 ± 0.9 lakhs /mm. Mean neutrophil to lymphocyte (N/L) ratio were 11.8±6.8. Proportion of patients with abnormal blood counts is shown in *Table 1*.

Among the liver function test, mean Serum Bilirubin, Alanine Transaminase, Aspartate Transaminase, Alkaline phosphatase, Protein and Albumin were 0.66±0.78mg/dL, 72.24± 82.42 U/L, 64.21±73.82 U/L, 267.1± 242.6U/L, 5.8±0.83 g/dL, 3.5±0.49 g/dL respectively. Percentage of patients with abnormal LFT is given in *Table 2.*

Among the kidney function test, mean blood Urea and serum creatinine were 77.8±56.9mg/dL and 2±2.8 mg/dL respectively. Percentage of patients with abnormal KFT is given in *Table 3.*

The common co-morbid conditions associated were: diabetes in 76 (64 %), hypertension was seen in 38 (32%) and chronic kidney disease in 6(7.2 %). There were 26 (21.6 %), 20 (15.6 %), 58 (48.3 %), 11(9.1 %) and 5 (4.1 %) patients on bi-level positive airway pressure (BIPAP), invasive SIMV mode, non-rebreathing mask (NRBM), flexi mask (FM) and High flow nasal Canula (HFNC) mode of ventilation respectively. The patients were transferred to medical ICU once they became COVID- 19 negative by RTPCR test.

DISCUSSION

Lymphocytopenia was almost ominous sign of COVID 19 disease and severe lymphocytopenia (<10 %) is seen sign of severe disease in patient with COVID 19 disease.

In our study we found males were more affected with moderate to severe disease than females which was also seen previous other studies [7,8]. Studies have shown that there is higher risk of males to die from the disease. This could be because males have higher expression of ACE2 receptors, smoking is more found in males in our country, there might be some immunological difference because of X chromosome and may be females are usually homemakers and stay at home and thus less exposed to virus. COVID 19 disease mainly effects older age groups very severely as seen in several previous studies and ours [9,10]. This could be because of older people usually ha ve co-morbid illness and COVID 19 disease is seen to be more severe in patients with comorbid illness. This could also be cause of immuno-senescence in older age which means inadequate pathogen recognition and thymic atrophy etc.

Studies have found that increased leucocyte count is common in non- survivors and in patients with underlying complications like myocardial involvement [11,12]. Luecocytosis was seen in three fourth of the ICU admissions in our study. Neutrophilia is common in COVID19 disease as SARS CoV-2 induced inflammatory mediators like Interleukin-6 and interleukin 8, causes neutrophil count to rise which itself is a pro-inflammatory. We found mean ANC of >10,000 cells /mm^3. Such a high number of neutrophils will further increase the inflammation in order to destroy the virus infected cells and may further cause injury to tissues. Almost all the patients had lymphocyte count less than 20 %, out of which two third of the patients had lymphocyte count <10 % in their DLC. Although absolute lymphopenia (<1000cells/mm3) was seen in about $2/3^{rd}$ of the patients similar to several other studies also [4,11,13]. The reasons for lymphocytopenia could be direct destruction of cells by the virus as these cells possess ACE 2 receptors, cytokine storm and also coexisting lactic acidosis due to hypoxia. SARS CoV-2 also directly suppresses the cellular immunity and decreases CD4+ and CD8+ T lymphocytes. Thus high neutrophil and low lymphocytes were common finding in severe disease. So, almost all the patients admitted in ICU had N/L ratio >3.5 which indicates that it is strong predictor for hospitalization in ICU as seen in previous other studies [14,15]. A meta-analysis done by Xiaolong Zong et al showed thrombocytopenia in patients whose hospital stay was >10 days as well in severe disease [16]. The cause could be either viral induced destruction or immune mediated by cytokine storm. We found thrombocytopenia in one quarter of the patients.

Altered liver function test with hyprotenemia was seen in almost $2/3^{rd}$ of the patients in our study. Metanalysis done by Wu Z H and Yang D L showed that liver dysfunction was found in severe disease and is associated with increased mortality [17]. Liver dysfunction could be because of cytokines produced injury, hypoxic injury due to ARDS or due to oxidative or drug induced injury. Deranged kidney function test was found in around $2/3^{rd}$ of the patients with severe disease. A systemic analysis done by Wang M et al also showed that there is higher chance

Volume 4, Issue 4; July-August 2021; Page No 1222-1226

of kidney dysfunction in COVID 19 disease. This is due to increased ACE2 (Acetylcholinesterase 2) receptors in kidneys which cause direct viral injury as well as immune mediated injury by increased production of cytokine to clear the virus [17]. Studies have shown the average incidence of AKI (Acute kindney injury) to be 3.7 % and degree of AKI is associated with poor prognosis [17].

CONCLUSION: Lymphocytopenia along with high N/L ratio is important prognostic marker of disease severity in COVID 19 disease and one should remain alert in these patients with proper monitoring. Males are more prone to develop severe disease. Liver and kidney dysfunction are common in patients with severe COVID 19 disease and should be closely monitored.

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Volume 4, Issue 4; July-August 2021; Page No 1222-1226

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Volume 4, Issue 4; July-August 2021; Page No 1222-1226