

Covid-19 vs Dengue - Role of WBC counts in segregation of cases at an early stage with special reference to NLR as a diagnostic and prognostic factor

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Abstract

Both SARS-CoV and dengue virus can present as mild diseases which subsides by itself or can get severe resulting in a patient’s death. In tropical and subtropical countries, where dengue epidemics are commonly seen, COVID-19 can be easily misdiagnosed with dengue, because they present with common symptoms in the initial stages but have different clinical management which makes it challenging for the health care professionals to diagnose in the early stages. Moreover, there have been few cases reported where there was co infection by the viruses’ making the picture still more confusing and challenging for the doctors. In poor and developing countries, access to specific diagnostic tests, the funding for them is limited and complete blood count (CBC) is the single most common test performed in patients. This study aimed to evaluate, analyse and compare the WBC count, differential leucocyte count and

Neutrophil lymphocyte ratio (NLR) among COVID-19 and dengue cases. An attempt was made in diagnosing these two conditions with the above parameters at an early stage and to determine if further investigations or referrals would be required. Inflammatory marker NLR was also analyzed with series of samples of the patients during their hospital stay in predicting the outcome of cases. This study was a retrospective one done for duration of 1 year between Jan 2021 and Jan 2022 and included two different groups of patients, COVID-19 and dengue patients. A total of 106 cases of confirmed COVID-19 and 100 cases of dengue were included in the study. We propose utilizing the WBC count, differential count and NLR in early diagnosis and segregation of COVID 19 and dengue cases so that patients with COVID 19 can be isolated at the earliest, prompt treatment be given, decreasing the lead time, trying to combat the pandemic and the patients with dengue can be

treated accordingly avoiding the complications. RT-PCR and dengue serology can be done on selected cases when required decreasing the burden on the health system and its resources. Patients with COVID-19 present with leucopenia or Leucocytosis, with Lymphopenia as the most significant finding, which is a useful and reliable indicator of the severity of the disease. Neutrophil lymphocyte ratio (NLR) was increased in majority of these cases and is described as an independent biomarker for indicating poor clinical outcomes in these patients. In contrast to COVID 19, the WBC count in dengue is characterized by progressive leucopenia with lymphocytic predominance and is associated with decreased NLR. Integrating the NLR in the diagnosis and prognosis of patients with COVID-19 and dengue would help in identification and prediction of cases with increased severity so that prompt treatment can be done decreasing the mortality and for better outcome of these patients.

Key words: CBC, WBC, NLR, SARS Co V, Dengue, Leucocytosis, Lymphocytosis, Lymphopenia

Introduction

SARS-CoV-2 (Severe acute respiratory syndrome corona virus 2) has been creating a havoc in medical history for causing one of the worst pandemics humanity has witnessed since December 2019, where it first started in Wuhan city of China ⁽¹⁾. On January 30, 2020, the World Health Organization (WHO) announced Covid-19 as a Public Health Emergency of International Concern (PHEIC), and 2 months later, on March 11, it was declared as a global pandemic ^(2,3). As per WHO Corona virus (COVID-19) Dashboard, on 13th May 2022, this disease affected almost 51.7 Cr people worldwide and claimed lives of more than 62.6 lakhs.

Dengue is an arthropod borne disease caused by dengue virus and is a major public health problem in most of the countries ⁽⁴⁾. DENV belongs to the family Flaviviridae, and it is transmitted to humans by the Aedes aegypti mosquitoes ⁽⁵⁾. Its caused by four antigenically distinct serotypes DENV-1, DENV-2, DENV-3, and DENV-4) ⁽⁶⁾ Majority of the cases of DENV infections may remain asymptomatic, but few may present with a wide range of clinical symptoms, ranging from mild dengue fever to a severe and sometimes fatal form of the disease known as DHF and DSS⁽⁷⁾. It affects up to 100 million people annually, with 5, 00,000 cases of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths, mostly among children ⁽⁸⁾.

Both SARS- Co-V2 and Dengue viruses are enveloped positively single stranded RNA viruses which belong to different families namely Coronaviridae and Flaviviridae respectively and they differ in their basic structure. Though both of them are viruses, their effects on haemopoietic cells are different, reason might be due to the difference in their basic structure.

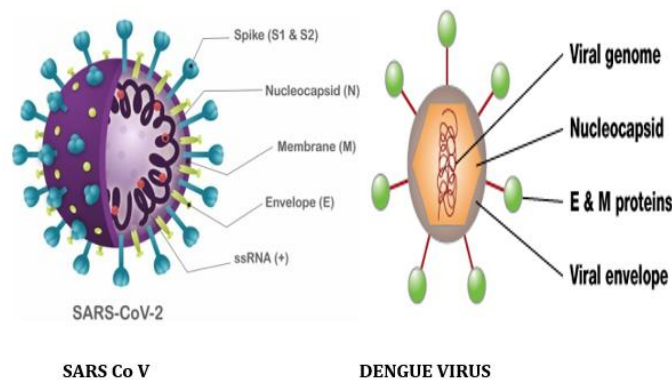


Table 1: Structural differences among SARS CoV and Dengue Virus

Species	sars-co-2	Dengue virus
Family	Coronaviridae	Flaviviridae
Diameter	65-25nm	50nm
Gene material	ssRNA	SsRNA

Structural protein	Spike (S) glycoprotein, Envelop (E) glycoprotein, Membrane(M) glycoprotein and Nucleocapsid(N) glycoprotein	Nucleocapsid(N) or core protein, Membrane(M) glycoprotein, Envelop (E) glycoprotein,
Characteristic findings	Crown like spikes (corona) on the outer surface	Non-structural protein-1(NS 1)

SARS-CoV-2 is primarily transmitted from person-to-person through droplets while coughing and sneezing, enabling the virus to disperse up to 1 to 2 m^(9,10) The transmission occurs not only from symptomatic and pre-symptomatic patients but also probably from those who are asymptomatic⁽¹¹⁾ with the incubation period ranging from 2 to 14 days⁽⁹⁾ Dengue is a major public health problem throughout tropical and sub-tropical regions⁽¹²⁾ This disease is caused by any of the four serotypes of dengue virus (DENV), transmitted by mosquitoes, primarily the *Aedes aegypti* species and the development of a efficacious vaccine still remains a challenging task^(13,14)

Both SARS-CoV and dengue virus can present as mild diseases which subsides by itself or can get severe resulting in a patient's death. In tropical and subtropical countries, where dengue epidemics are commonly seen, COVID-19 can be easily misdiagnosed with dengue, because they present with common symptoms of fever, muscle aches, fatigue, nausea and vomiting in the initial stages which makes it challenging for the health care professionals to diagnose in the early stages^(15,16) Moreover there have been few cases reported where there was co infection by the viruses⁽¹⁷⁾, making the picture

still more confusing and challenging for the doctors. This is a medical concern because both diseases have similar clinical symptoms in the early stages but have different clinical management. Major challenges in diagnosis often occur because of the limited access to specific diagnostic tests for each disease^(18,19) and a misdiagnosis in the acute stage of these diseases can lead to an incorrect therapeutic approach and consequently to unfavorable clinical outcomes for patients.

In poor and developing countries, access to specific diagnostic tests, the funding for them is limited and complete blood count (CBC) is the single most common test performed in patients⁽²⁰⁾

A complete blood count serves as the most accessible, potent, and readily available test as most routine laboratories are equipped with hematology analyzers. Various hematological parameters help in diagnosing the diseases at an early stage; few of them show changes as the disease worsens.

This study aimed to evaluate and analyze the WBC count, differential leucocyte count and Neutrophil lymphocyte ratio (NLR) from COVID-19 patients and comparing them with those of dengue cases. An attempt was made in diagnosing these two conditions with the above parameters at an early stage and to determine if further investigations or referrals would be required. Corrective precautionary steps can be taken and definitive treatment started accordingly. Inflammatory marker NLR was also analyzed with series of samples of the patients during their hospital stay in predicting the outcome of cases.

Material and methods

This study was a retrospective one done for the past 1 year between Jan 2021 and Jan 2022. We included two different groups of patients: COVID-19 and dengue

patients. Suspected COVID-19 patients were diagnosed with nasopharyngeal swabs using the Real-Time RT-PCR. Dengue cases were diagnosed through blood samples for detection of Dengue NS1 Ag or IgM antibodies. About 3 ml of venous blood was collected from each positive individual in an EDTA vacutainer tube on the day of admission to the hospital. A complete blood count (CBC) was performed using the Automatic Hematology Analyzer Sysmex XN 350. The WBC counts, Differential leucocyte count and NLR of the samples processed were noted to study their role in segregating COVID-19 cases from dengue and then the cases were followed up for Neutrophil lymphocyte ratio to note their importance in prognosis and severity of both the diseases.

Inclusion criteria

First samples of patients arriving at the ER and outpatient departments at Owaisi group of hospitals, Hyderabad, India with fever and other symptoms (cough, shortness of breath, myalgias, fatigue, rashes, petechiae, nausea and vomiting) who were eventually diagnosed with SARS-CoV-2 or dengue.

Exclusion criteria

All other causes of fever and History of any other hematologic abnormality.

Results and discussion

A total of 106 cases of confirmed COVID-19 and 100 cases of dengue were included in the study. It was noted in our study that both COVID-19 and dengue affected the males predominantly with 65.1% and 58% respectively (Table 2). The reason could be that percentage of males staying outdoors is more than the females thereby exposing them to the causative agents /patients/vectors. Dengue patients were younger than those with COVID-19 with 59 % cases seen in 11-30 years of age. In

contrast, COVID-19 tends to affect adults and older adults, with 44.3 % cases seen in 41-60 years of age group (Table 2). Most severe cases affected are those over 60 years of age with other co morbidities.

WBC counts showed considerable variations in both the sets of cases. Leucocytosis was seen in 44.3% of the cases in COVID 19 patients, with predominant Neutrophilia seen in 65.1 % and marked Lymphopenia in 62.3% of the cases (Table 3). Probable cause of Leucocytosis can be correlated to co-infection or due to medications by steroids and was seen more predominantly in severe and critical cases, most of the cases showed increased counts as the severity increased.

Lymphopenia was found to be a sensitive and reliable parameter for severity and outcome ⁽²¹⁾. The explanation for it might be that lymphocytes also express ACE-2 receptors, the virus causes their lysis. After one to two weeks, there is a surge in cytokines producing ‘cytokine storm’, Lymphopenia then becomes more prominent due to atrophy of lymphoid organs and hence their decreased production and turnover ^[24]. Frater et al and Li et al have identified Lymphopenia as the most common finding in COVID-19 affected patients corresponding to 63% and 64.5% ^(22, 23) which coincided with the results of the present study.

Results of this study was found to be consistent with study conducted by Barger et al and Khalid et al in which Neutrophilia was reported most commonly in serious COVID-19 patients requiring hospital admission ^(25,26) The possible cause of Neutrophilia may be related to the ‘cytokine storm’ along with superimposed bacterial infection and drugs used in the treatment.

In contrast dengue patients had leucopenia in 33% of the cases with relative lymphocytosis in 66% of the cases. Leucopenia is caused by bone marrow suppression with

decrease in polymorphs and increase in lymphocytes especially atypical lymphocytes due to stimulation by viral antigens. Similar values were seen in studies done by Agrawal et al and Malathesa et al ^(27, 28) with 64% and 66% respectively. Neutrophilic Leucocytosis was seen in cases where there were secondary bacterial infections.

Neutrophil to lymphocyte ratio (NLR) is a simple parameter used to assess the inflammatory status of a subject. The increase in NLR could indicate a poor clinical prognosis and be an independent indicator of mortality. ^(29, 30) This study revealed higher NLR in 77.4% of the cases being followed up by daily CBCs, progressing NLR indicate increased severity thus indicating a bad prognosis, reason being explained by the increase in the Neutrophil count and a lower limit of lymphocyte count in COVID-19 patients. Since, there is a strong systemic inflammatory response in COVID-19 infection, virus-induced inflammatory markers IFN- γ , IL8, IL-6, GCSF, TNF- α activates neutrophils. Conversely, helper T-lymphocytes and other immune cells are considerably declined, causing an overall increase in NLR. The present study had similar results with studies done by Usul et al, Waris et al and sit et al ^(31, 32, 33) found a significantly lower NLR at the time of admission in mild cases with positive test results which increased as the severity increased, with reason being cited was that viral infections have increased lymphocytes which over a period of time would decrease causing Lymphopenia and increased NLR.

NLR was decreased in patients with dengue infections in 68% of the cases on admission, reason being due to lymphocytic predominance and normal or reduced neutrophils counts. This was on contrary to the study done by Chaloe Wong et al ⁽³⁴⁾ who noticed that during first 5 days there is Neutrophil predominance and

decreased lymphocytes but later on, after 5 days the lymphocyte count increase and neutrophils decrease thereby decreasing the NLR. The possibility of this varied result could be due to superimposed bacterial infections early in the course of the disease.

Table 2: Demographic characteristics of COVID-19 and dengue patients.

Demographic characteristics	COVID-19 (N %)	DENGUE (N %)
Median age	41-60 years (44.3 %)	11-30 years (59 %)
Male sex	65.1%	58%

Table 3: Comparison of WBC parameter and NLR among COVID-19 patients and dengue patients

Laboratory findings	COVID-19(%)	Dengue (%)
Total WBC Count	Leucocytosis (44.3%)	Leucocytosis (09%)
	Leucopenia (3.8%)	Leucopenia (33%)
Differential cell count	Neutrophilia (65.1%)	Neutrophilia (06%)
	Relative Lymphocytosis (-)	Relative Lymphocytosis (66%)
	Lymphopenia (62.3%)	Lymphopenia (--)
Neutrophil lymphocyte ratio (NLR)	Increased (77.4%)	Decreased (68 %)

Limitations of the study

Small study population is a limitation of the present study. Moreover, the study cohort included only those patients who presented to our hospital.

Conclusions

Lastly, we would like to conclude that early diagnosis of COVID 19 and dengue is important as it would help the clinicians to plan the further course of action. Patients with COVID 19 would be advised for isolation/quarantine so as to limit the disease and immediate treatment given to decrease the severity, its complications and mortality due to the disease. Whereas patients with dengue need not be isolated but monitored and treatment given so as to avoid complications and ensure complete recovery.

Complete blood count (CBC) is the single most common and inexpensive test performed in patients. It has the capacity to diagnose, predict prognosis & complications and have usefulness in monitoring treatment response. We propose utilizing the WBC count in early diagnosis and segregation of COVID 19 and dengue cases so that patients with COVID 19 can be isolated at the earliest, prompt treatment be given, decreasing the lead time trying to combat the pandemic and the patients with dengue can be treated accordingly avoiding the complications. RT-PCR and dengue serology can be done on selected cases when required decreasing the burden on the health system and its resources.

Patients with COVID-19 present with leucopenia or Leucocytosis, with Lymphopenia as the most significant finding, which is a useful and reliable indicator of the severity of the disease. Neutrophil lymphocyte ratio (NLR) was increased in majority of these cases and is described as an independent biomarker for indicating poor clinical outcomes in these patients. In contrast to COVID 19, the WBC count in dengue is characterized by progressive leucopenia with lymphocytic predominance and is associated with decreased NLR. It can also be used in diagnosing these two conditions along with WBC

count and the differential leucocyte count. Integrating the NLR in the diagnosis and prognosis of patients with COVID-19 and dengue would help in identification and prediction of cases with increased severity so that prompt treatment can be done decreasing the mortality and for better outcome of these patients.

Abbreviations

Complete blood count (CBC), White blood cells (WBC), Neutrophil lymphocyte ratio (NLR)

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