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Original Research Article

Evaluation and comparison of Neutrophil-To-Lymphocyte Ratio (NLR) as a diagnostic marker for dengue infection

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Abstract

Introduction: Bacterial and Viral infections are often hard to be distinguished in daily clinical practice. Biological markers obtained from a routine examination play an important role to minimize time in providing diagnose and giving therapy. Recently, the use of Neutrophil-lymphocyte ratio (NLR)and platelet-lymphocyte ratio (PLR) is greatly considered to differentiate types of infection found in the patients presenting with fever.

Material and Method: This study uses prospective cohort study design and involves patients presenting with fever who are admitted in the hospital. The initial NLRand PLR is examined and categorized into types of infection foundi.e. grade I DHF and grade II DHF. The ANOVA test and t-test are performed to find out the difference among study groups.

Result: In the present study, a total of 104 patients were clinically suspected on the admission as dengue fever, 80 of these were serologically confirmed dengue positive. A 44 of these were grade IDHF infections. The average age was 42.1 years, the range being 23-65 years. Based on 2009 WHO clinical criteria, 34 were identified as Dengue with warning Sign, 27 as Dengue Without warning Signs while 19 were identified as Severe Dengue. Some of the frequently observed clinical features were fever, headache, thrombocytopenia, leukopenia and rashBoth NLR and PLR correlated severity of DHF.NLR and PLR had significantly higher values in grade II DHF patients.

Conclusion: Neutrophil-lymphocyte ratioand PLR have benefit to predict diagnosis for the patients presenting with a fever. Bacterial infection is associated with the high value of NLRand PLR generally has a lower value in viral infection cases.

Keywords: Dengue, NLR, PLR, haematological, biochemical

Introduction

Dengue, an acute fever sickness spread by mosquitoes, is a serious global public health issue in both the subtropics and the tropics. Until 1970, there were just nine countries globally where severe dengue epidemics were reported. Nonetheless, the WHO reports that dengue is currently prevalent in over a hundred nations (World Health Organisation) [1]. The majority of people on earth-nearly 40%-live in dengue-endemic regions. Every year, the dengue virus infects over 400 million individuals, of which 100 million develop symptoms and 2000 people pass away as a result. The epidemiology of dengue fever in India was first described in Chennai (1780), whereas the country's first outbreak took place in Kolkata (1963). Later outbreaks have been reported in other locations of India. The epidemiology of dengue fever

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in India was first described in Chennai (1780), whereas the country's first outbreak took place in Kolkata (1963). Later outbreaks have been reported in other locations of India [2-4].
Unplanned urbanisation, changes in environmental conditions, host-pathogen interactions, and population immunological characteristics are all factors contributing to the spread of dengue in India. Over the previous fifty years, the number of cases has increased 30 times worldwide [3-5].
Dengue is a widespread viral infection, particularly in tropical regions. In actuality, Dengue is the most prevalent arbo-viral disease spread by mosquitoes, endangering half of the world's population. The symptoms of the illness range from a self-contained fever to death. Patients with profound and persistent shock brought on by plasma leakage and worsened by haemorrhage are most often fatalities from dengue. In individuals with dengue infection, thrombocytopenia is a key indicator of plasma leakage and third space loss [6, 7]. Patients with dengue may experience platelet destruction and VEGF secretion (vascular endothelial growth factor). Pleural effusion and/or ascites, which are both types of third space fluid loss, are caused by VEGF. Third space loss is typically accompanied by thrombocytopenia, increasing haemoglobin levels, and declining albumin levels. Any test or tests that serve as a predictor for these severity characteristics enable the surveillance of specific individuals who are at risk for plasma leak, haemorrhage, or shock, lowering morbidity and death [8].
In this study, we aimed to compare the clinical and laboratory findings that differentiate dengue fever from non-dengue febrile illnesses and further analyse the clinical and laboratory variables associated with severe dengue.

Materials and Method

Subject selection: Our present study was a single centre prospective study of 80 patients with laboratory-confirmed Dengue admitted to hospital in Mamata medical college, were enrolled for the study. Also, 40 healthy age and gender, matched, volunteers were also recruited in this study.

Sample analysis: According to the Ministry of Health and Family Welfare's (MOHFW) and Government of India's (GOI) guidelines, dengue was identified and verified by an ELISA test on the patient's respiratory samples.

Processing of the sample: After the patient's routine informed permission, 2 ml of venous blood was taken under aseptic conditions from the antecubital vein and placed in an EDTA vacutainer. The Sysmex automatic 5-part differential cell counter processed samples in one hour. Leishman's stain was used to colour peripheral streaks. Pathologists analysed the smears, and hematologic parameters with a focus on platelet count and WBC differentials were validated on microscopy. Quick test immunochromatographic testing and ELISA were used to perform dengue serology.

Baseline data collection: The demographic information, clinical status at the time of admission, follow-up information, and haematological results were all noted. On the day of admission, peripheral smears for a microscopy examination and samples for a complete blood count (CBC) were taken. Prior to blood sample, no treatment was given to any of the patients. On a 5-part haematology analyzer by Abbott Cell Dyn Ruby, the samples were evaluated for total blood count and results were recorded. Romanowsky stains were used to colour peripheral blood smears, and results were recorded.

Follow up: The patients whose clinical status remained unchanged were re-examined for laboratory indexes. The composite endpoint of the study was a clinical improvement, stability, or deterioration.
Cut-off for Neutrophil to lymphocytes ratio was defined as 4 and patient with NLR >4 would be considered as significant of severity.

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Cut-off for Albumin to Globulin ratio was defined as 1, and value less than 1 would be considered as significant of severity.

Statistical Analysis: Data on means and standard deviation are supplied (SD). Calculated values between tested confidence intervals differed from each other. Simple linear correlations were employed in correlation analysis. Using SPSS 15.0, all statistical analyses were performed (SPSS Inc., Chicago, USA).

Results

In this study, a total of 80 patients with dengue hemorrhagic fever and 40 healthy participants participated (DHF). Table 1 displays the demographic details of these study participants. 44 patients with grade I DHF and 36 individuals with grade II DHF made up the study's participants. Males made up the majority of the study's participants.
Out of the 80d engue patients, 42 were males and 28 were females. The average age was 42.1 years, the range being 23-65 years. The study had 77 patients who were discharged and 3 patients who succumbed either due to dengue fever or due to its complications.

Table 1: Comparison of demographics characteristics of among control and dengue haemorrhagic fever (DHF) infected patients

Characteristics		Control (n=40)	DHF Grade I (n=44)	DHF Grade II (n=36)	p Value
Age (Years)		44 ± 16	42 ± 11	38 ± 19	0.56
Gender	Male	23	22	20	1.08
	Female	21	20	18	0.56
Smoking		9	6	4	0.84
Alcohol Drinking		4	3	2	0.61
Co-morbidities	Diabetes	0	1	1	-
	Hypertension	4	2	3	0.94
	CardiovascularDisease	0	0	1	-
	Respiratory Disease	0	1	1	-

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.01).

A total of 80 patients diagnosed with dengue fever were enrolled in the study and followed up during hospitalization. The average hospitalization duration was 6.25days. The overall number of in-hospital deaths was 3. Compared to patients in the survivor cohort, in-hospital dead patients were significantly older, but no significant difference was observed in terms of gender. The most common comorbidity was hypertension, followed by diabetes. Except for hypertension, there was no statistically significant difference in the comorbidity frequency between surviving and dead patients. The clinical features presented of all patients on admission are shown in Table 2. Every admitted patient had a fever and both in fever duration before admission and overall fever duration. There was no significant difference between grade I DHF and grade II DHF.

Table 2: Comparison of sign and symptoms of among dengue haemorrhagic fever (DHF) infected patients

Sign and Symptoms	DHF Grade I (n=44)	DHF Grade II (n=36)	p Value
Retroorbital pain	31 ±6	36 ± 2	0.42
Headache	33 ± 4	35 ± 3	0.62
Arthralgia	28 ±2	27 ± 4	0.31
Back pain	16 ±2	17 ± 2	0.51
Vomiting	9 ±2	7 ± 1	0.92

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Diarrhoea	3 ±0.2	2 ± 0.7	0.36
Hematemesis	2 ±0.5	1 ± 0.2	0.24
Melena	2 ±0.8	1 ± 0.3	0.49
Other bleeding manifestations	29 ±3	21 ± 2	0.56
Rash	24 ±2	14 ± 2	0.27
Respiratory symptoms	34 ±3	28 ± 1	0.43
Oedema	0 ±0	1 ± 0.4	0.14

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.01).

Table 3: presents the laboratory finding in the grade I DHF and grade II DHF patients on admission. Hemoconcentration and thrombocytopenia were more common in grade II DHF patients than in grade I DHF.

Table 3: Comparison of haematological parameters among dengue haemorrhagic fever (DHF)infected patients

Haematological Parameters	**DHF Grade I (n=44)**	**DHF Grade II (n=36)**	**p Value**
Haemoglobin (g/dL)	11.7 ± 2.18	12.4 ± 1.41	0.64
Leukocyte ($\times10^9$/L)	4.3 ± 1.28	4.7 ± 1.52	1.09
Neutrophil ($\times10^9$/L)	2.4 ± 0.26	2.3 ± 0.48	0.83
Lymphocyte ($\times10^9$/L)	1.22 ± 0.26	1.43 ± 0.51	0.52
Monocyte ($\times10^9$/L)	0.27 ± 0.03	0.29 ± 0.04	0.26
Platelets ($\times10^9$/L)	1.19 ± 0.19	1.04 ± 0.26	0.61
Prothrombin time (Second)	15 ± 2.8	16 ± 3.7	0.72
NLR	2.8 ± 1.63	3.0 ± 1.94	0.34
PLR	0.94 ± 0.04	0.98 ± 0.06	0.59

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.01).

The role of certain circulating inflammatory biomarkers like Neutrophil-Lymphocyte Ratio (NLR), Platelet-Lymphocyte Ratio (PLR) and in discriminating between grade I DHF and grade II DHF. As shown in [Table 3], a comparatively lower mean NLR and PLR was recorded in grade I DHF with grade II DHF.

Table 4: Comparison of clinical findings with different value of NLR

Clinical Parameters	**DHF Grade I (n=44)**	**DHF Grade II (n=36)**	**p Value**
Albumin (g/L)	42.9 ± 5.21	45.2 ± 2.17	0.31
Sodium (mmol/L)	146 ± 9.21	153 ± 6.28	0.39
Potassium (mmol/L)	5.1 ± 1.84	5.9 ± 1.60	0.62
Serum Chlorine (mmol/L)	112 ± 6.37	114 ± 9.46	0.34
Serum urea nitrogen (mmol/L)	5.6 ± 1.06	6.3 ± 1.41	0.31
Serum glucose (mmol/L)	6.7 ± 1.46	7.61 ± 2.04	0.21
Creatinine (μmol/L)	101.49 ± 9.24	116.32 ± 6.84	1.34
Alanine aminotransferase (U/L)	19.64 ± 4.21	28.61 ± 5.37	0.64

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.01).

Association of biochemical markers with the risk of complications of grade I DHF and grade II DHF to find out the disease progression, we obtained the crude odds ratio (OR) after conducting the logistic regression analysis (Table 4). Given that the blood test results were influenced by age and gender, we excluded the possible effects of age and gender and

obtained the adjusted OR after the adjustment of gender and age. The results showed that significant elevations of different clinical biochemical markers were positively correlated with the risk of severe complications of grade II DHF (Table 4).

Discussion

Dengue fever is an important arboviral disease of global concern causing major outbreaks with mortality and morbidity in endemic 6 countries. Dengue patients had higher neutrophil percentage in first few days of fever, then this was reversed and percentage of lymphocytes increased. Neutrophil to lymphocyte ratio in the dengue group was >1 on the first 5-13 days then reversed on day 6 to day 9. In the present study, a significant higher proportion of dengue cases demonstrated NLR 2 was seen as the platelets improved and disease got better suggesting that NLR may be a prognostic indicator of dengue disease [5-7]. A higher NLR indicates a higher level of inflammation. Therefore, NLR can be used to predict the severity of inflammation and also itsHowever, in the present study, contrasting findings of higher NLR in relation with higher platelet counts were observed. This was probably as the lymphocytosis and neutropenia seen in viral conditions was ameliorated with improvement of platelet counts. The study's population comprised mostly of younger patients with 58% of people under twenty-eight years of age. Patients above 58 years of age comprises 5 % of the study population. This reflects the trend in vector borne illness, especially mosquito borne illness across the world and one postulated reason behind this is increased exposure of these younger people to the vectors. Dengue fever occurred twice more common in men than in women as in the study by Ing-kit Karoli R *et al*.[8]. Nearly both percent of patients got admitted within the first five days of fever as reported by Dutta *et al*.[9]. Of the symptoms discussed, the majority in our study were fever(all), myalgia (8 %), headache (5.9%) and G.I symptoms(8%) in contrast to myalgia occurring in more than one fifth of patients as reported by Ratageri VH *et al*. [10]. Viral typing may shed some light on the differences in presentation. Around 6% of patients in our study have comorbidities reflecting again the fact that relatively large number of people were young as reported by ing-kit Karoli R *et al*. [8]. And only conscious patients (during admission) have been taken for the study.

The levels of platelets and hematocrit also are usually used as indicators of the disease besides of the clinical condition of the hospitalized DHF patients. In this research, the median level of platelets in grade I DHF patients was 113.50 (103/μL), higher than that in grade II DHF patients only about 76.50 (103/μL) with a p value of 0.009. It means that there was a statistically significant difference. Thrombocytopenia, moreover, also has an important role in the pathogenesis of dengue virus infection. In patients with dengue virus infection, thrombocytopenia begins on the third day until on the seventh day, while platelet count will return to normal one on the eighth day or on the ninth day [11-13]. Thrombocytopenia in dengue virus infection occurs through destruction and shortening of platelet lifetime as well as suppression mechanism of bone marrow [14-16]. According to WHO, thrombocytopenia and plasma leak presence characterized by hemococcinent are important indicators in diagnosing DHF [1, 17].

Patients with DHF and hepatitis B have lower PLR than those with other diagnosis. It happens because platelets count in patients with DHF decreases. Platelet of the dengue virus-infected patients experiences mitochondrial dysfunction leading to the activation of apoptosis cascade which results in the cell death. Prolonged thrombocytopenia is more frequently noticeable in DHF case than in dengue fever [18, 19]. Our study presents a similar result as a retrospective study from Barde PV*et al*.[20], which reports that all patients with DHF have low platelet count and normal lymphocytes. It implies that PLR in patients with DHF is lower than in healthy adults.

This study has some weaknesses. First, it has smaller size of study samples.Therefore, it is not adequate to present broader description on some final diagnosis, especially in hepatitis B case. Further, the result has a bias in the interpretation on data used for the study. Second, this study is not able to rule out the co-infection influence in NLRand PLR values which

accompany the main diagnosis. Thus, the values of those parameters do not represent the real value of a diagnosis.

Conclusion

The study concludes that NLR may be used as prognostic marker in management of dengue fever as lower NLR correlates with lower platelet counts and increasing NLR is observed with improving platelet counts suggesting the normalisation of differential count. The platelet count of 60,000 with reference of NLR with cut off of 2 may be used to prognosticate the disease and take corrective therapeutic actions.

In our study we highlight the importance of NLR in dengue patients in predicting disease severity and mortality. The early application of NLR will be beneficial to patient classification, management and relief of medical resource shortage. In a developing country like ours where there are resource limited settings, NLR can be used as an effective marker to predict and stratify Dengue patients, which in turn would lead to efficient resource utilization. Studying the trends of NLR may also help in predicting the risk for mortality in severely ill patients.

It can be concluded that the neutrophil/lymphocyte count ratio in grade I DHF patients is higher than that in grade II DHF patients. It is also known that the higher the neutrophil/lymphocyte count ratio is, the lighter the degree of DHF severity is. Therefore, further researches are necessary to focus more on other factors that may affect dengue virus infection as well as to perform with more stringent inclusion criteria. Further researches are also essential to focus on the neutrophil/lymphocyte count ratio in children with DHF.

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