HEMATOLOGICAL AND BIOCHEMICAL PROFILES OF PATIENTS WITH DENGUE VIRUS INFECTION IN LALITPUR, NEPAL

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ABSTRACT

Dengue viral infection is a disease transmitted by mosquitoes of the Aedes genus, and it arises as a result of being bitten by mosquitoes that are carrying the virus. This infection induces a wide range of changes in the body's immune system. Thus serological evaluation together with hematological and biochemical parameters in cases of dengue viral infection make an accurate clinical diagnosis. The main objective of this study was to study the serological, hematological, and biochemical parameters among patients infected with Dengue virus. A total of 102 blood samples were collected at Asrik Diagnostic Lab, Lalitpur during the period of August to October 2022. Samples were analyzed by following methods: hematological tests were done by coulter counter, Biochemical tests were done by fully automated analyzer and serological tests were done by ELISA. Of the 102 blood samples NS1 antigen was detected in 62 (60.9%) whereas anti-dengue IgM antibody was detected in 10 (9.8%). Leukopenia and thrombocytopenia were present in 33 (53.1%) and 32 (51.6%) of the 62 NS1 positive samples, respectively. Of the 62 cases, only 16 samples were tested for the alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Out of 16 samples, ALT and AST level were significantly higher in 11 (68.8%) and 14 (87.5%) positive cases, respectively. The statistical analysis showed that there was no significant association between NS1 and IgM. It can be concluded that the investigation of leukocytes, thrombocytes, ALT, and AST helps to diagnose the dengue efficiently.

KEYWORDS

NS1, Leukocytes, Thrombocytes, ALT, AST, Dengue

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INTRODUCTION

Dengue is a mosquito-borne viral infection transmitted through the bite of infected female *Aedes aegypti* to a lesser extent *Ae. albopictus* causing a wide spectrum of clinical presentation.[1,2] Dengue virus (DENV) belongs to Flavivirus and family Flaviviridae, is spherical enveloped, single-stranded RNA virus, containing four distinct serotypes (DENV-1, DENV-2, DENV-3, DENV-4). They consist of three structural proteins (capsid C, membrane M and envelope E) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5).[3-5] The NS1 protein can be detected in the early stages of infection and mark virus replication as this protein appears in association with the infected cell on its surface.[4]

The virus circulates in the blood of an infected person for 2-7 days. Around the same time the person develops a fever. The severity of the disease will usually only be apparent around the transition of the febrile to an afebrile phase which often coincides with the onset of the critical phase.[6] During the critical phase, an increase in capillary permeability leads to plasma leakage, which may increase hematocrit levels.[7,8] Bone marrow suppressed in DENV infection affects all cell populations bone marrow including a complete ablation of mature megakaryocytes, platelet progenitor cells.[8] Recent studies indicate that megakaryocytes are important in the maintenance of the various homeostasis within the bone marrow including hematopoiesis.[9-12] Platelets are not only dysregulated hematopoietic population during DENV infection; thrombocytopenia, leukopenia (decrease white blood cell counts) are also observed to be severe particularly in dengue hemorrhagic fever.[11-13]

Liver enzymes aspartate amino-transferase (AST) and alanine aminotransferase (ALT) are elevated in DENV infection.[12] Various studies detailed the increase in AST more than ALT.[12-15] The first case of dengue reported in Nepal was from a Japanese traveler after he returned his country in 2004.[16] Whereas, the documented case report of dengue from the indigenous Nepali population was published only after 2006.[17,18] Since, then, sporadic clinical cases of dengue outbreak has been recorded.[19,20] As per the data obtained from Epidemiology and Disease Control Division (EDCD) as of 31st Dec, 2022 a total of 54,784 dengue cases have been identified with 88 deaths.[2,21] Out of the top districts reporting dengue cases, the highest numbers were from Kathmandu (14,375), Lalitpur (9,614) and Bhaktapur (6,145). Dengue cases has spiked in the year 2022 in comparison with the previous year 2021 with total of 540 cases.[21] The literature review indicates that there were few studies which obtained the seroprevalence of dengue IgM was 34.2 and dengue NS1 antigen was 24.1%, respectively performed by Shrestha *et al*.[22] Moreover, Habib *et al*[23] among 100 samples investigated through diagnostics test showed 62.0% positive patients with NS1, 10.0% positive cases with dengue IgM cases were detected. Complete blood count (CBC) showed remarkable reduction in platelets (32 cases) and leucopenia in 24 positive cases. Although this study does not successfully cover the present scenario of Nepal, it will help to access the dengue viral infection spreading rate and it also provides the association of dengue viral infection with the hematological and biochemical parameters.

MATERIALS AND METHODS

Study area and participants: A laboratory based cross sectional study was carried out in Asrik Diagnostic Laboratory, Lalitpur from August to October 2022 on a convenience sample of 102 patients. Patients of all ages visiting laboratory who were serologically confirmed with dengue specific IgM antibody and NS1 antigen were included. Patients with a history suggestive of hematological disorder in the past, patients with any confirmed chronic disease, and patients who refuse to participate in the study were excluded.

Sample collection: During the laboratory visit, a total of 102 blood samples were collected from the patients. Initially, these samples were analyzed to detect NS1 antigen as well as IgM antibody. Subsequently, the blood samples were tested for hematological parameters such as total leukocyte count (TLC), differential leukocyte count (DLC), platelet count, and hemoglobin (Hb) using an automated blood cell counter (Human Count 30 TS, Germany). In addition, biochemical tests were performed to measure the levels of ALT and AST using a (Mindray BS 230, China) analyzer. The laboratory reference ranges were used to determine the cutoff values for each of the test results.

Data collection and statistical analysis: Information regarding both the patients' demographic profiles and laboratory test results was gathered, and this data was inputted and analyzed using statistical software called SPSS 17.0. Descriptive statistics were utilized to determine the frequency and percentage of the data, which were then represented in tables. To assess the correlation between the NS1 antigen test and IgM, a Chi-square test was conducted.

RESULTS

Total 102 febrile patients were tested for dengue specific serology. Of these, 62 (60.8%) patients were positive with dengue NS-1 whereas 10 (9.8%) positive cases with IgM. As shown in Table 1, out of 62 positive cases 36 (58.1%) were male. Majority of cases were from adult age group. As depicted in Table 2, 33 (53.2%) patients had leukopenia and 32 (51.6%) had thrombocytopenia. In the majority of cases, the neutrophils and lymphocytes levels were within the normal range. However, in some cases, there was a variation in these levels, with neutrophils being higher and lymphocytes being lower than normal.

Table 3 depicts the biochemical (amino-transferases) parameters of dengue positive cases. On analysis 11 (68.8%) had high level of

Table 1: Distribution of patients on the basis of age and gender			
Age group in years	Sex		Total
	Male (%)	Female (%)	n (%)
< 20	5 (8.1)	2 (3.2)	7 (11.3)
21-40	13 (21.0)	12 (19.4)	25 (40.3)
41-60	14 (22.6)	8 (12.9)	22 (35.5)
>60	4 (6.5)	4 (6.5)	8 (12.9)
Total	**36 (38.1)**	**26 (41.9)**	**62 (100)**

Table 2: Hematological parameters of dengue cases		
Hematological parameters	Range	n (%)
Total leukocyte count	< 4000	33 (53.2)
	4000-11000	29 (46.8)
		62 (100)
Neutrophils	< 40	2 (3.2)
	40-75	35 (56.5)
	> 75	25 (40.3)
		62 (100)
Lymphocytes	< 20	28 (45.2)
	20-50	33 (53.2)
	>50	1 (1.6)
		62 (100)
Hemoglobin mg/dl	< 13	12 (19.4)
	13-18	47 (75.8)
	>18	3 (4.8)
		62 (100)
RBC count million/cumm	<4.5	12 (19.4)
	4.5-6.5	50 (80.6)
		62 (100)
Platelets count cells/cumm	<1500000	32 (51.6)
	1500000-400000	30 (48.4)
		62 (100)

Table 3: Liver Function Test of dengue cases		
Parameters	Range	n (%)
Alanine aminotransferase (ALT) Unit/litre	<45	5 (31.3)
	>45	11 (68.8)
Total		**16 (100)**
Aspartate aminotransferase (AST) Unit/litre	<35	2 (12.5)
	>35	14 (87.5)
Total		**16 (100)**

ALT and 14 (87.5%) had high level of AST. There was no significant association between NS1 and IgM.

DISCUSSION

Biochemical and hematological laboratory investigation reports were observed in a total of 62 dengue positive patients. High number of dengue positive findings were observed among the adult age groups (21-40 years) which consisted of 25 (40.2%) positive total cases. This finding was similar to the study done by Dinkar et al[24] and Yung et al[25] where age group of (20-41 years) were the most affected. In another study done by Habib et al,[23] a total of 73 dengue cases 32 (43.8%) showed remarkable thrombocytopenia and 24 (32.8%) showed leukopenia which was in agreement with present findings. However, Rao et al,[26] reported thrombocytopenia in 50 cases (~90%),

followed by leukopenia in 43 cases (~76%). The variation in percentage of leukopenia and thrombocytopenia in various studies could be because of the fact that the CBC (complete blood count) in dengue patient change by the day of fever.[27] The result of this study showed that in majority of cases IgM were negative but NS1 was positive; similar findings have been reported by Shrestha *et al*[22] and Habib *et al*.[23]

In our study, neutrophilia was observed in 25 patients (40.3%) with lymphocytopenia in 28 patients (45.2%). This finding was similar to the study done by Chaloemwong *et al*.[28] The neutrophil percentage were in negative correlation with the lymphocyte percentage. However, as the infection progresses, the adaptive immune response is activated, and lymphocytes start to produce antibodies against the virus which leads to a reversal of the earlier changes, with an increase in lymphocyte percentage and a decrease in neutrophil percentage.[29] Study done by Kalabamu and Maliki[30] also observed that lymphocytopenia was a significant predictor for dengue positive results.

Among the total of 62 dengue positive cases, only 16 had done AST test. Out of 16 cases, an increase in AST was observed in 14 (87.5%) whereas ALT was observed in 11 (68.8%) cases. These findings were similar to the study done by Ferede *et al*[13] and Kuo *et al*[31] where they also reported elevated levels of biochemical parameters [AST 46 (45.1%) and ALT 18 (17.6%)], respectively. This may be due to the enzyme ALT is originally present in the liver. Since the DENV infection also involves a multiple organs including liver, therefore, AST could be significant.[15,17] Additionally, during the early days of dengue fever, there is an initial inflammatory response that leads to the activation of the innate immune system, including neutrophils. Neutrophils release a protein called myeloperoxidase, which can also increase AST levels.[15] Therefore, the higher AST levels in dengue cases may also be due, in part, to the activation of the innate immune system.

In this study, it is concluded that the diagnosis of dengue cases were preliminary limited to CBC, AST tests or dengue NS1 or dengue serology tests. Despite the prevailing belief that IgM antibody testing is more effective than NS1 antigen detection, our research findings revealed a higher prevalence of NS1 positivity compared to IgM antibodies in the studied population. The higher prevalence of NS1 antigen positivity observed in our study population suggests that NS1 testing should be given due consideration as a valuable diagnostic tool. Further research is warranted to elucidate the factors contributing to this discrepancy and validate these findings in larger cohorts. It is important to note that the pattern of liver enzyme elevation in dengue fever can vary depending on the severity of the disease, and some cases may have higher ALT levels than AST levels. Therefore, the pattern of liver enzyme elevation should be evaluated in conjunction with other clinical and laboratory parameters to determine the severity of dengue fever and the appropriate management.

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