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A study on Hematological and Biochemical Parameters of Dengue Fever Patients in Janakpurdham, Madhesh Province, Nepal

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Abstract

Background: Over 350 million people are estimated to get the counter with flavivirus each year, causing dengue one of the most common arboviral diseases. Following the COVID-19 outbreak, there has been a recorded rise in the prevalence of dengue fever in the South Asian countries. In 2022, there was a significant dengue outbreak in Nepal. Data on haematological and biochemical parameters, which are crucial for the clinical management of dengue patients, are lacking in Nepal's Madhesh Province. The purpose of this study was to present the first baseline data on the haematological and biochemical parameters of dengue virus-infected patients.

Methods: The data was extracted from admitted patients at Janaki Medical College, Teaching Hospital (JMCTH) and Janaki Health Care and Teaching Hospital (JHCTH) diagnosed with dengue fever between August to November-

2023. Hematological and biochemical parameters were recorded from the medical records and analyzed.

Results: There were 74 serologically positive dengue cases in all, with 32.4% being female and 67.6% being male. Of the 74 patients, one-fourth had NS1 positive, 24.3% had IgM, and 21.6% had IgG. Leucopenia (87.2%) and thrombocytopenia (66.2%) were the most frequent haematological findings, whereas serum glutamic-oxaloacetate transaminase (76.6%) and Serum glutamic pyruvic transaminase (58.1%) were the biochemical markers with higher levels.

Conclusion: The study explores the most common haematological and biochemical parameters of the patients diagnosed with dengue that may alarm clinicians of the possibility of dengue virus infection in the study region.

Keywords: Dengue, Leucopenia, Madhesh Province, Thrombocytopenia

Introduction

Dengue is a most prevalent arboviral disease caused by the RNA virus named as Dengue virus (DENV), which is existed in four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. The dengue virus is a member of the *Flavivirus* genus within the *Flaviviridae* family^[1]. Dengue is one of the most significant viruses causing arthropod-borne disease and it continues to pose a serious threat to global public health, which is transmitted via the mosquito vectors *Aedes. Aegypti* and *Aedes albopictus*^[2]. The dengue fever (DF) is a self-limiting illness with no long-lasting health consequences, after the fever subsided. However, dengue hemorrhagic fever (DHF) is a potentially fatal illness which is characterized by increased permeability of the blood vessels. It can result in hemoconcentration, bleeding, thrombocytopenia, pleural effusion, and hypovolemic shock^[3]. According to the recent World Health Organization (WHO) assessment, DF is endemic to over 128 countries worldwide and threatens and provides a risk to approximately 3.6 billion people. Over 350 million people are thought to contract the flavivirus each year^[4]. There has been a rise in the prevalence of DF in South Asian countries after the COVID-19 outbreak. Compared to the cases recorded in 2020, the prevalence of DF in India has more than tripled (44,585 vs. 193245 cases). Incidences of DF have been reported to have increased seven times in Pakistan (6016 vs. 52894 cases) and 19 times in Bangladesh (1405 vs.

28429 cases) in 2021^[5].

Even though the first dengue case in Nepal was reported in 2004, the indigenous Nepali population's scientific record was not released until 2006^[6]. Since then, the country has been reporting yearly outbreaks and isolated clinical cases of dengue^[7]. Nepal is a landlocked Himalayan country, ecologically has divided in to three zones: Mountain, Hill and Terai region. Nearly half of the Nepal's population lives in tropical or subtropical climate of Terai, where most of the dengue episodes have occurred. According to the Ministry of Health and Population of Nepal, there were 54232 confirmed cases of dengue in 2022, and 67 deaths from the disease were recorded in that country^[8].

The bite of a female *Aedes aegypti* mosquito carrying one of the four serotypes of dengue virus results in human infection. It takes eight to twelve days for the virus to start replicating in the vector (extrinsic incubation). In humans, on the other hand, the incubation period lasts, on average, five days and can extend up to fifteen days^[9]. Clinical, epidemiological, and laboratory results are used to make the diagnosis of DF. Tests that are specific (viral isolation and serology for specific antibody identification) and non-specific (blood cell count, platelets count, prothrombin time, activated partial thromboplastin time, liver function tests) are used. The most notable haematological alteration is leucopenia, which has been documented to have decreased to less than 2000 cells/ μ l. Nonetheless, at the beginning of the illness, modest leucocytosis together with neutrophilia has been documented. Apart from leucopenia, the other most prominent hematological change associated with thrombocytopenia is an elevated hematocrit value^[10, 11]. The biochemical indicators that fluctuate most frequently are those related to liver function tests, such as serum albumin levels, alkaline phosphatase, and glutamic-pyruvic transaminase (SGPT) and glutamic-oxaloacetic transaminase (SGOT)^[12]. An elevated serum creatinine level has also been reported^[13].

For a nation with limited resources like Nepal, managing dengue cases during an epidemic is an enormous challenge. In 2009, the WHO updated the definition of a dengue case based on clinical and laboratory characteristics^[14]. This allowed for more convenient clinical management and early detection of severe dengue. In order to prioritize their management, it is critical to identify the cases that have a greater chance of developing severe dengue. A combined assessment of early symptoms and laboratory test data is crucial in order to identify the cases that are more likely to develop severe dengue^[15]. The Madhesh Province of Nepal, which occupies 6,126,288 people and accounts for over 6.5% of the nation's total geographical area, is the most densely populated province in the nation according to the senses from 2021. Although the Madhesh Province is thought to be dengue-free, the nation as a whole was impacted by the most recent outbreak. There are a few published researches on dengue, however the most of them are limited to Nepal's Bagmati Province. Despite this, there is insufficient data regarding the clinical and laboratory characteristics of dengue in patients in Madhesh Province in Nepal. This lack of the region has drawn our attention. So,

we aimed to highlight the hematological and biochemical characteristics of dengue patients who were diagnosed in the two tertiary care centers in the capital city of Madhesh Province, Nepal.

Materials and Methods

An observational study was conducted at the two tertiary health care centers: JMCTH (400 bedded) and JHCTH (100 bedded) of Janakpurdham, the capital city of Madhesh Province, Nepal, providing high-quality medical care for cases referred from the nearby districts. The study included all patients with a serological confirmed (dengue NS1 antigen or IgG or IgM antibody positive), whether they were admitted or from the outpatient department (OPD), during August to November-2023.

Data collection was done by reviewing medical records of individual patients obtained from the medical records section of the hospitals. Specific data was gathered about the biochemical and haematological parameters. Ethical approval towards this study was obtained (ref. no. 017/IRC-JMC/2024/011) by the Institutional Review Committee of Janaki Medical College (IRC-JMC).

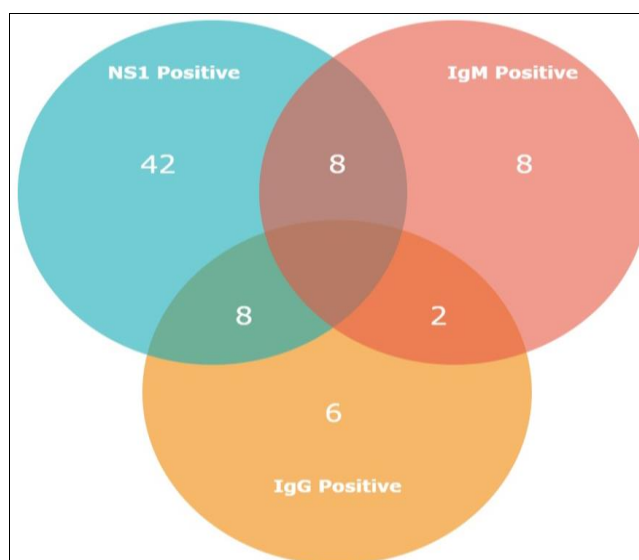
Results and Discussion

A total of 74 serologically positive patients were included in this study from the both tertiary care centers: JMCTH and JHCTH during 21 of August to 28 of November 2023. Of the 74 patients, one-fourth was NS1 positive, followed by IgM and IgG at 24.3% and 21.6%, respectively. An identical percentage (10.8%) of the patients presented NS1:IgM and NS1:IgG mixed positivity, while just 2 (2.7%) patients had shown IgM:IgG positivity, as reported on table 1. The mean \pm SD age of the patients was 28.43 \pm 14.25 years, with 50 (67.6%) being male and 24 (32.4%) being female. The total leucocyte count, neutrophils, lymphocytes, and platelets of the dengue-positive patients were determined to be 4678.45 \pm 2762.59 cells/ μ l, 64.94 \pm 13.8%, 27.25 \pm 12.60%, and 126799.75 \pm 63398.42 cells/ μ l, respectively, table 2. A substantial reduction in the total leucocyte and platelets count were observed, as compared to their normal reference level. Almost one-fifth i.e. 65 (87.8%) out of 74 of the patient had shown decreased total leucocyte count and more than half i.e. 49(66.2%) out of 74 had shown decreased platelets count. The biochemical results, on the other hand, were found significantly raised, as compared to their normal reference range. The mean \pm SD of creatinine, SGOT and SGPT were observed as 1.09 \pm 0.29 mg/d, 66.13 \pm 51.22 U/L and 86.16 \pm 120.46 U/L, respectively. Out of 74, one-fourth, or 50 (76.6%), had raised SGOT, and more than half, or 43 (58%), had increased SGPT, as presented on table 3 and 4. However, the patient's gender status had no discernible differences on either the hematological or biochemical parameters. Furthermore, a significant positive correlation between platelets and total leucocyte count ($r=0.474$), SGOT and creatinine ($r=0.370$) as well as SGOT and SGPT ($r=0.839$) were observed. In addition, a negative correlation of lymphocyte observed with total leucocyte ($r=-0.259$) and neutrophil count ($r=-0.951$).

Table 1: Distribution of Dengue antigen and antibody positivity

Antigen/ antibody	Number of Patients	%
NS1 positive	58	78.3
IgM positive	18	24.3
IgG positive	16	21.6
NS1 positive & IgM positive	8	10.8
NS1 positive & IgG positive	8	10.8
IgM positive & IgG positive	2	2.70
Total	74	

One fourth of patients had NS1 positive

**Fig 1:** Venn diagram showing dengue antibody positivity**Table 2:** Demographic and laboratory parameters among all Dengue patients

Parameters	Number	%	P-value
Sex			
Male	50	67.6	-
Female	24	32.4	-
	Mean± SD	Reference value	
Age (years)	28.43±14.25	-	-
Total leucocyte count (cells/μl)	4678.45±2762.59	5000-11000	<0.0001
Neutrophil (%)	64.94±13.18	40%-60%	0.002
Lymphocyte (%)	27.25±12.60	20%-40%	<0.0001
Eosinophil (%)	1.03±1.53	1%-4%	<0.0001
Monocyte (%)	6.77±4.05	2%-8%	0.011
Platelet count (cells/μl)	126788.75±63398.42	150000-400000	<0.0001
SGOT (U/L)	86.16±120.46	8-45	0.004
SGPT (U/L)	66.13±51.22	7-43	<0.0001
Creatinine (mg/dl)	1.09±0.29	0.6-1.2	0.003
Total			

Total leucocyte and platelet count were significantly reduced, however the biochemical markers, SGOT, SGPT, and creatinine, were significantly raised relative to their normal range.

Table 3: Laboratory parameters among Dengue patients by gender status [Mean± SD]

Parameters	Male	Female	p-value
Total leucocyte count (cells/μl)	4418.740±2354.10	5219.54±3458.97	0.246
Neutrophil (%)	64.98±13.86	64.87±11.94	0.975
Lymphocyte (%)	27.48±13.05	26.79±11.85	0.828
Eosinophil (%)	0.86±1.57	1.37±1.40	0.178
Monocyte (%)	6.76±4.33	6.79±3.50	0.975
Platelets (Cells/μl)	118007.36±63219.92	145083.33±61035.92	0.085
SGOT (U/L)	93.10±140.94	71.72±58.46	0.479
SGPT (U/L)	68.27±58.71	61.66±30.91	0.607
Creatinine (mg/dl)	1.12±0.34	1.03±0.16	0.223

All the hematological as well as biochemical parameters were not differs significantly with patient's gender status.

Table 4: Abnormal hematological and biochemical parameters among all dengue patients

Parameters	Number	%
Total leucocyte count		
Decreased: <5000 cells/ µl	65	87.8
Elevated: >11000 cells/ µl	4	5.4
Normal:5000-11000 cells/ µl	5	6.7
Neutrophil		
Decreased: <40%	2	2.7
Normal: 40-60%	24	32.4
(Elevated: >60)	48	64.8
Lymphocyte		
Elevated: >40%	12	16.2
Normal: 20-40%	62	63.8
Eosinophil		
Elevated: >4%	6	8.1
Normal:1-4%	68	91.8
Monocyte		
Elevated: >8%	27	36.5
Normal:2-8%	47	63.5
Platelet count		
Decreased: 150000 cells/ µl	49	66.2
Normal: 15000-400000 cells/µl	25	33.8
SGOT		
Elevated: >45 U/L	50	76.6
Normal:<45 U/L	24	32.4
SGPT		
Elevated,;>43U/L	43	58.1
Normal:<43U/L	31	41.9
Creatinine		
Elevated: >1.2mg/dl	26	35.1
Normal:<1.2mg/dl	48	64.9

One-third of the patients had lower platelet counts, while one-fourth of the patients had lower leucocyte counts. One-fourth of the patients showed elevated SGOT, and over half had increased SGPT

Table 5: Correlation of Laboratory parameters among Dengue patients by age

		Correlations									
		Age	Total leucocyte count	Neutrophil	Lymphocyte	Eosinophil	Monocyte	Platelets	SGOT	SGPT	Creatinine
Total leucocyte count (Cells/ µl)	r	-.153	1								
	p-value	.194									
Neutrophil (%)	r	-.041	.218	1							
	p-value	.731	.062								
Lymphocyte (%)	r	-.011	-.259*	-.951**	1						
	p-value	.928	.026	.000							
Eosinophil (%)	r	.113	.216	-.046	.008	1					
	p-value	.339	.065	.697	.945						
Monocyte (%)	r	.164	-.009	-.301**	.017	-.226	1				
	p-value	.163	.941	.009	.888	.053					
Platelets (cells/µl)	r	-.015	.474**	.069	-.128	.207	.070	1			
	p-value	.900	.000	.558	.275	.077	.552				
SGOT (U/L)	r	.006	-.066	.109	-.077	-.105	-.077	-.250*	1		
	p-value	.960	.575	.354	.516	.375	.517	.032			
SGPT (U/L)	r	.020	-.046	.062	-.027	-.117	-.077	-.177	.839**	1	
	p-value	.863	.697	.599	.819	.319	.516	.132	.0001		
Creatinine (mg/dl)	r	.003	-.102	.151	-.025	-.132	-.356**	-.172	.370**	.210	1
	p-value	.978	.388	.198	.834	.261	.002	.143	.001	.072	

*. Correlation is significant at the 0.05 level.
 **. Correlation is significant at the 0.01 level.

Platelets exhibited a significant positive association with leucocyte count, however, total leucocyte and neutrophil count showed a significantly negative correlation with lymphocytes. Significant negative correlations were found

between neutrophils, lymphocyte and monocytes. While SGOT showed a significant positive correlation with both creatinine and SGPT, whereas, platelets showed a significant negative correlation with SGOT.



Fig 2: Madhesh Province (red) of Nepal

Nepal is a small South Asian landlocked nation with a very diverse topography. Nepal has been classified into the Mountain, Hill, and Terai regions based on geographical ecology. Recently, Nepal has reorganized into seven Provinces and this study was conducted in the Madhesh Province, which is a Terai region and shares its southern border with India. The Madhesh Province, which also borders Koshi and Bagmati Province, is the most densely inhabited. The Bagmati Province is the most affected by dengue, however, the last endemic impacts the whole country, even the dengue-free Madhesh Province. In spite of published studies on dengue from the Bagmati Province, there is not enough information available for the Madhesh province. The limitations of currently available management strategies, such as vaccinations and pesticides, have resulted in a rapid increase in the prevalence of dengue worldwide in recent decades. Hence, for effective and timely treatment of dengue, a patient's clinical and laboratory findings are crucial. It has been observed that assessing early symptoms along with laboratory findings significantly reduce the risk of developing severe dengue. The aim of this study was to examine the pattern of haematological and biochemical characteristics of dengue patients who were diagnosed at JMCTH and JHCTH, the two specialized tertiary care centers in Madhesh Province, Nepal. The patient's enrolment in study was begun with the first dengue positive patient on 21 of August and end with the last patient on 28 of November 2023. Out of 74 with dengue, 50 patients (67.6%) were male and 24 (32.4%) were female. One-fourth (78.3%) of the samples had positive NS1, followed by IgM and IgG (24.3% and 21.6%, respectively). The early onset of infection may account for some of the greater percentage of NS1 positivity among the enrolled patients. IgM:IgG mixed positive findings were obtained in 2.7% of cases, however, mixed positive results for NS1: IgM and NS1:IgG were observed in an identical percentage (10.8%), table 1. A similar high positive incidence of NS1 was seen in a study involving acute patients who had symptoms for 0–5 days^[17].

Overall, DF patients showed a significant reduction in their platelet count (126788.75 ± 63398.42 cells/ μ l, $p < 0.0001$) and total leucocyte count (4678.45 ± 2762.59 cells/ μ l, $p < 0.0001$) compared to their normal range. Regarding the biochemical measures, they were likewise considerably higher than their normal range: SGOT (86.16 ± 120.46 U/L, $p = 0.004$), SGPT (66.13 ± 51.22 U/L, $p < 0.0001$), and creatinine (1.09 ± 0.29 mg/dl, $p = 0.003$). It has been observed that 65 (87.8%) of the patient had decreased leucocyte, 2 (2.7%) had decreased neutrophil, 49 (66.2%) had decreased lymphocytes. A similar study showed leucopenia ($OR_A = 0.0999$, $p < 0.001$) and thrombocytopenia

($OR_A = 1.000$, $p = 0.006$) related to dengue^[18]. Similar decreased leucocyte and platelet counts in 26.5% and 59.8%, respectively, were reported in another Ethiopian investigation^[19]. These results are consistent with previous observations in dengue patients. In this study, increased levels in reference to the normal values of SGOT in 76.6% and SGPT in 58.1% of the cases were observed, which showed the elevated levels of SGOT in a greater proportion of the cases than SGPT. An elevated level of SGOT, SGPT and creatinine had been observed in 50 (76.6%), 43 (58.1%) and 26 (35.1%) respectively. This is an agreement with the other studies elsewhere that reported SGOT and SGPT higher levels in 68.5% and 39.2% of the cases, respectively^[20]. In a different investigation, comparable findings of elevated SGOT 72.2% and SGPT 27.3% were also documented^[21]. Though dengue virus is known to be a hepatotropic, it has been found involved in other organs also. The increased level of SGOT in comparatively higher number of dengue patient could be explained due to excess release of SGOT from damaged muscles cells of nonhepatic source. The elevated creatinine levels was observed in 26 (35.1%) patients, which could be the direct effect on glomerular and tubular cells or as a result of tissue injury caused by deregulated host immune response against the viral antigens^[22]. A prevalence of acute kidney infection 27.5% was reported in a study conducted in DF patients^[23]. The study additionally showed a significant negative correlation between lymphocytes and total leucocyte and neutrophil counts, but a significant positive association with leucocyte counts ($p = 0.01$). A hospital-based correlation research included 99 dengue patients had similarly demonstrated a positive correlation ($p = 0.292$) between leucocyte and platelets count^[24].

SGOT was found to have a remarkable positive correlation with both creatinine and SGPT. This correlation between SGOT, creatinine, and SGPT may be explained by the dengue virus's involvement in both glomerular (nonhepatic cells) and hepatic cells.

Probably this is the first attempt to study hematological and biochemical parameter of dengue infection in Madhesh Province of Nepal, however it has several limitations. Being the cross-sectional study design, it limits the comparisons of those dengue cases with the control group and serial hematological and biochemical changes were not done. Furthermore, study could be done in future for better understanding of dengue viral infections. Despite of the associated limitations, this preliminary study finally provides the first baseline data on hematological and biochemical parameter of dengue infection in the Province.

Conclusion

A comprehensive evaluation of early signs and laboratory testing including haematological and biochemical markers, is critical for directing treatment and prognosis of dengue fever. In this study, most prevalent haematological results were leucopenia and thrombocytopenia, whereas the biochemical values were increased SGOT and SGPT. Thus, such common laboratory results could warn clinicians about the likelihood of dengue infection in the research area.

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