https://doi.org/10.29309/TPMJ/2025.32.06.9334

The Professional Medical Journal
www.theprofesional.com
2025, Volume, 32 Issue, 06

ORIGINAL ARTICLE

Heamatological and biochemical parameters with different serological status in dengue patients.

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Article Citation: Amin H, Majeed S, Omer W, Najeeb S, Wali N, Fatima tuz Zuhra. Heamatological and biochemical parameters with different serological status in dengue patients. Professional Med J 2025; 32(06):694-701. https://doi.org/10.29309/TPMJ/2025.32.06.9334

ABSTRACT… Objective: To compare the hematological and biochemical parameters of dengue patients within different serological markers. **Study Design:** Cross Sectional study. **Setting:** Carried out in a Tertiary Care Hospital at Rawalpindi. **Period:** August 2023 to December 2023. **Methods:** Of 250 seropositive dengue cases Hematological analysis was done by Mindray 3000 plus. For biochemical analysis Beckman &coulter, AU480 was used. Subjects were distributed into seven serogroups (NS1 positive, NS1+IgG, NS1+IgM positive, IgM positive, IgG positive, IgG+IgM, NS1, IgM+IgG and triple positive). Results were analyzed using SPSS software, ANOVA test was used, and means were compared in different groups. P value of <0.05 was considered as significant. **Results:** A total of 178 (71%) among study group were male. Mean age was found to be 37±13.8 years. NS1 antigen either in isolation or in combination was positive in 80%of patient at the time of presentation. The most affected parameters among the whole study cohort were low mean platelet count (81.90±49.2) and higher mean value of ALT (92.81±75.07). The mean value of haematological and biochemical parameters were studied in different serological groups. No significant difference of mean haemoglobin and platelet was observed in between different serological groups however Mean TLC differ significantly within these subgroups (p value = <0.05). The Mean values of various biochemical parameters did not exhibit any significant difference within the different serological groups. **Conclusion:** Dengue is more prevalent in young population and in males. Thrombocytopenia and elevated ALT levels were most common laboratory finding observed in these patients. NS1 was the most frequent marker at the time of presentation. Only Mean TLC (p value = <0.05) was observed to be significantly different in between different serological groups.

Key words: Biochemical Parameter, Dengue Fever, Haematological Parameter, IgM, IgG, NS1.

INTRODUCTION

Dengue Fever (DF) is one of the main health problem affecting tropical and sub-tropical regions.[1] World Health Organization (WHO) has reported that annually almost 50 million people are affected from dengue infection, with nearly half of the cases from endemic countries.[2] Frequent outbreaks of dengue are observed in the monsoon season in regions of endemicity.[3]

Dengue fever is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4).[4] These serotypes are closely related but distinct antigenically.[3] The Dengue virus infection may be asymptomatic or it can present as any of the three categories: Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Dengue is self-limiting disease but can prove lethal if not properly managed.[5] Mortality rates can go as high as 40% if serious complication is not treated, however with treatment; the mortality rate is 1-2%.[6]

Biomarkers used for diagnosis of the disease include either direct detection of dengue viral RNA or products secreted from virus as NS1 protein. Host immune response to infection such as virus-specific immunoglobulin M [IgM] and immunoglobulin G [IgG] can also be used as diagnostic biomarker.[7] Virus detection methods are used for an early and serotype-specific identification.During first five days of the illness,NS1 antigen detection is fairly rapid and

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Article received on: 21/02/2025
Accepted for publication: 30/04/2025

low cost test, compared to methods such as reverse transcriptase-polymerase chain reaction.[8] However beyond 5 to 6 days after onset of fever and at the termination of the acute phase of infection, serological assays for anti-DENV antibodies is the method of choice.[9] There is no specific single, perfect diagnostic biomarker which exist during whole period of illness, especially in patients suffering a secondary dengue infection.[7]

Series of changes in biochemical and haematological parameters are commonly reported in dengue infection.[10] The blood counts also supplement serology and used in diagnosis and prognosis of the disease.[11] Liver function tests (LFTS) are usually deranged in this disease, characterized by mild elevation in serum total bilirubin, alanine transaminase (ALT) and aspartate transaminase (AST) however, serum albumin levels are commonly decreased in dengue infection and used as good prognostic markers. These changes can also prove to be valuable in identifying the complications and planning proficient management approach thus dropping morbidity and mortality.[12] CBC parameters including hemoglobin (Hb), hematocrit (Hct), platelet count and WBC count along with differentials alter each day of the illness in patients of dengue fever.[13]

The changing patterns of biochemical and haematological parameters in the initial stage of disease and their association with different serological results are not well known in our part of world.[12] Different patterns are also observed in the antibody response depending on the primary or secondary nature of dengue infection.[7] Hematological abnormalities are most commonly detected in dengue infection, association of the blood count and biochemical changes with serology was studied in these patients which may predict several clinical outcomes. As these tests are commonly available and performed as routine investigations, these association may support clinicians in resource-limited settings to predict course of patient illness and to prevent life threatening consequences of dengue fever.[14]

METHODS

This is a comparative study using cross sectional approach. Total of 250 patients' record was obtained. Data comprised of Lab test result of serologically confirmed (ELISA) Dengue patients from the period August 2023-December 2023. Patient's Laboratory test result were collected at the time of presentation to hospital. Data was collected after obtaining informed consent and permission from ethical review committee of the institute. Only adult patient diagnosed with dengue infection (NS-1 and/or IgM and/or IgG positive) were included in this study. Patients with chronic disease were excluded from the study. DENV-infected patients were divided into seven groups ie, NS1-only, IgM-only, IgG only and dual positive (NS1 + IgM-positive, NS1+IgG positive and IgG +IgM postive) and triple positive with (NS1, IgM and IgG positive) groups.

Specimen Collection and Processing

A total of 6ml Venous blood sample was collected from each patient. Whole blood was collected in a K_3 EDTA vacuum tube and a gel and/or clot activator tube. A complete blood profile was performed in all cases with a hematology analyzer (Mindray 3000 plus). Following hematological parameters were analysed-Hemoglobin (Hb), total leucocyte count (TLC) and paltelets (PLT). A chemistry analyzer (Beckman & coulter AU480 was used to perform biochemical analyses on enzymes (ALP, ALT), bilirubin (total), urea and creatinine from a serum sample.

The data was entered and analyzed on SPSS 22. Descriptive variables were analyzed as Mean±Standard deviation and Categorical variable were described in frequency and percentage. Anova test was used to calculate the p value to compare means of haematological, biochemical parameters in different serological parameters. p value of ≤ 0.05 as considered significant.

RESULTS

Characteristics and Demographics of the Study Population

Total of 250 patients were studied. Among the

study group there was predominately male population (71%). (Figure1). The demographic detail of the study cohort is summarized in Table-I.

Male	178 (71%)
Female	72(29%)
Male: female ratio	2.4:1
Mean Age	37±13.8 years
Age range	16-73 years

Table-I. Demographics of the study population

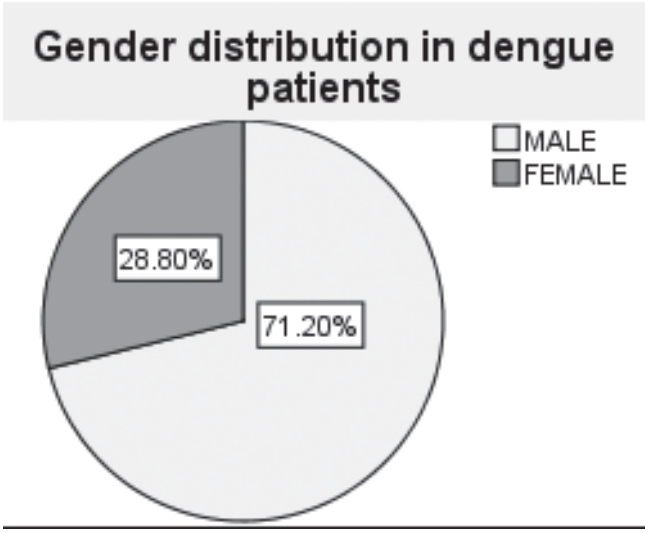

Figure-1. Frequency of male and female in study population

Among 250 dengue-positive subjects, 11% were found to be less than 20years of age and only 6% were above 60 years of age. Furthermore, the age group 20–40 years was found to have almost half of patients (49%) followed by 34% patients between age group of 41-60 years. The percentage of patient and their gender distribution in each group is summarised in Figure-2.

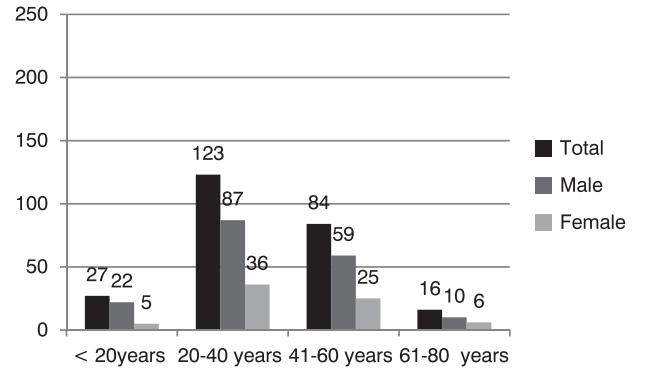

Figure-2. Frequency and gender distribution of dengue patients in different Age groups

Table-II represent the mean values of some haematological and biochemical parameters in whole study group. Most of the mean values calculated are within normal reference range except the low mean platelet count (81.90±49.2) and higher mean value of ALT (92.81±75.07). (Table-II)

Variable	Mean Values±SD	Range
Hb g/dl	13.05±2.2	3.7-17.7
TLC count X10^9/L	4.71±2.23	0.80-16.20
PLT count X10^9/L	81.90±49.2	2-291
Total bilirubin (mg/dL)	0.80±2.69	0.03-28
ALT (U/L)	92.81±75.07	12-397
ALP(U/L)	93.41±46.1	34-288
Urea (mg/dL)	20.08±13.52	7-126
Creatinine (mg/dL)	0.69±0.23	0.2-1.5

Table-II. Mean values of Important Hematological and biochemical findings in study participants

Hb haemoglobin, TLC, total leukocyte count; PLT, Platelet count; ALP, alkaline phosphatase; ALT, alanine aminotransferase

Out of all the 250 cases, 200(80%) were positive for NS1 antigen either in isolation or in combination, making NS1 most common serological marker for the diagnosis in majority of dengue cases (Table-III). This group is followed by the subset with positive IgG antibodies n=122(49%) and positive IgM antibodies n=66(26%) (Table-II). The study population was divided into seven serological subsets according to their serum markers positivity. Figure-3 represents the frequency of patients in different serological sub sets.

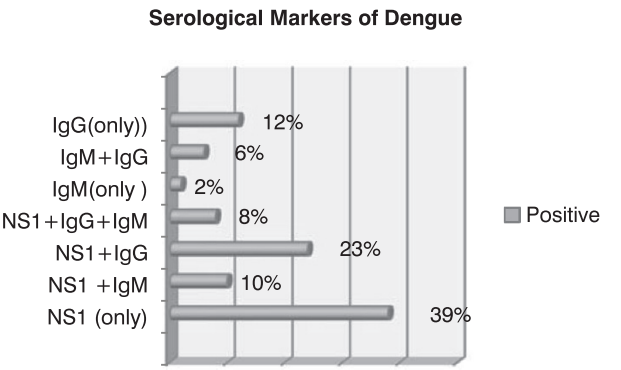

Figure-3: Frequency of study patients in different serological groups

Serological Marker	Positive	Negative
NS1 (Total) (NS1only)+(NS1+IgM)+ (NS1+IgG) (NS1+IgM+IgG)	200 (80%)	50 (20%)
IgM (Total) (IgM only)+(IgM +IgG) + (NS1+IgM) (NS1+IgM+IgG)	66 (26%)	184 (74%)
IgG (Total) (IgG only)+(IgM +IgG) + (NS1+IgG) (NS1+IgM+IgG)	124 (49%)	126 (51%)

Table-III. Status of major serological markers in Study population:

The mean value of haematological and biochemical parameters were also calculated in different serological groups of study population (Table-IV and Table-V respectively)

No significant difference of mean haemoglobin was observed in between different groups. Whereas lowest mean platelet count (68.33±42.63) was observed in IgM seropositive group but the difference was not found to be statistically significant. Mean TLC differ significantly within different serological groups (p value = <0.05) with lowest mean TLC being observed in patients who were exclusively NS1 positive. (Table-IV).

The Mean values of various biochemical parameters observed in this study did not exhibit any significant difference within the different serological groups (Table-V).

Table-VI represent the frequency of deranged haematological and biochemical parameters in different serological groups of dengue patients. The most affected haematogical parameter were low platelet count (72%) reduced TLC (46%) of cases. The biochemical parameter that was significantly affected was raised ALT in 25% of patients with dengue followed by ALP in 10 % of cases. The derangement in both haematological and biochemical parameters were observed in much higher frequency in subgroup of patients with NS1 positivity.

Variable	Serological Group	N	Mean ± SD	Range	P-Value
Hb g/dL	NS1	97	13.18±2.1	3.7-17.2	0.120
	IgM	9	10.35±4.27	4.6-16.9	
	IgG	29	13.07±2.4	3.90-16.10	
	NS1+IgM	24	13.11±2.08	7.0-17.0	
	NS1+IgG	56	13.34±1.81	9.0-17.1	
	IgM+IgG	15	11.97±2.50	5.80-15.2	
	NS1+IgM+IgG	20	13.1±2.1	8.9-17.7	
TLC count X10^9/L	NS1	97	4.00±1.77	0.80-12.1	0.000*
	IgM	9	4.45±2.03	1.30-6.9	
	IgG	29	6.167±2.16	1.40-10.40	
	NS1+IgM	24	4.43±1.72	2.5-9.70	
	NS1+IgG	56	4.74±2.64	1.5-16.2	
	IgM+IgG	15	5.64±2.3	1.9-9.8	
	NS1+IgM+IgG	20	5.58±2.2	2.4-11.2	
PLT count X10^9/L	NS1	97	76.59±47.12	22-291	0.582
	IgM	9	68.33±42.63	10-119	
	IgG	29	88.70±53.0	18-190	
	NS1+IgM	24	84.08±40.74	2-167	
	NS1+IgG	56	83.91±48.04	16-254	
	IgM+IgG	15	101.93±73.38	24-281	
	NS1+IgM+IgG	20	82.0±50.0	18-230	

Hb haemoglobin, TLC, total leukocyte count; PLT, Platelet count
*signififcant

Variable	Serological Group	N	Mean ± SD	Range	P-Value
Total bilirubin (mg/dL)	NS1	97	0.59±0.31	0.10-1.63	0.774
	IgM	9	0.39±0.20	0.25-0.54	
	IgG.0	29	0.70±0.54	0.10-2.16	
	NS1+IgM	24	0.41±.014	0.20-0.63	
	NS1+IgG	56	1.74±2.01	0.3-2.8	
	IgM+IgG	15	0.39±0.27	0.20-0.59	
	NS1+IgM+IgG	20	0.46±0.24	0.20-1.1	
Alanine aminotransferase ALT (U/L)	NS1	97	98.66±79.7	12-382	0.642
	IgM	9	75.0±49.49	40-110	
	IgG	29	94.17±75.39	22-245	
	NS1+IgM	24	58.25±25.9	25-100	
	NS1+IgG	56	78.04±47.33	23-188	
	IgM+IgG	15	87.50±74.24	35-140	
	NS1+IgM+IgG	20	111.75±102.34	36-397	
Alkaline phosphatase;; ALP(U/L)	NS1	97	96.21±56.0	34-288	0.626
	IgM	9	140±63.63	95-185	
	IgG	29	99.29±38.37	44-178	
	NS1+IgM	24	68.50±31.14	39-141	
	NS1+IgG	56	91.85±32.26	47-178	
	IgM+IgG	15	69.50±13.43	60-79	
	NS1+IgM+IgG	20	88.33±32.8	37-142	
Urea (mg/dL)	NS1	97	21.4±18.6	7-126	0.937
	IgM	9	23.0±7.07	18-28	
	IgG	29	17.82±8.31	9-44	
	NS1+IgM	24	16.1±3.68	12-24	
	NS1+IgG	56	21.90±11.04	10-50	
	IgM+IgG	15	20.50±14.84	10-31	
	NS1+IgM+IgG	20	18.5±7.6	12-40	
Creatinine (mg/dL)	NS1	97	0.73±0.23	0.20-1.3	0.303
	IgM	9	0.75±0.21	0.60-0.90	
	IgG	29	0.617±0.16	0.4-1.0	
	NS1+IgM	24	0.56±0.27	0.20-0.90	
	NS1+IgG	56	0.75±0.26	0.50-1.5	
	IgM+IgG	15	0.75±0.21	0.60-0.90	
	NS1+IgM+IgG	20	0.62±0.19	0.30-0.90	

Table-V. Mean Biochemical parameters in different serological groups

Variable	Patients N (%)							
	NS1	IgM	IgG	IgM+IgG	NS1+IgM	NS1+IgG	NS1+IgM +IgG	Total
Haemoglobin ≤ 7g	7 (35%)	3 (15%)	3 (15%)	2 (10%)	2 (10%)	2 (10%)	1 (5%)	20 (8%)
TLC 4.0 ≤ X10^9/L	55(48%)	4 (3.5%)	4(3.5%)	4(3.5%)	14 (12%)	28 (24%)	6 (5.2%)	115(46%)
PLT count ≤ 100X 10^9/L	75 (41%)	6 (3.3%)	20 (11%)	10 (5.5%)	17 (9.4%)	40 (22%)	13 (7.2%)	181(72%)
Alanine aminotransferase (ALT) >56 U/L	28 (44%)	2 (3.2%)	8 (13%)	1 (1.6%)	5 (2%)	10 (16%)	9 (14%)	63(25%)
Alkaline phosphatase (ALP) >120 U/L	13 (50%)	1(4%)	4 (15%)	_	1(4%)	5 (19%)	2 (8%)	26 (10%)
Urea > 30mg/dL	4 (36%)	1 (9%)	1 (9%)	1 (9%)	_	4 (36%)	1 (9%)	11(4%)
Creatinine >1.2mg/dL	1(33%)	_	_	_	_	2 (66%)	_	03 (1%)

Table-VI. Deranged Haematological and biochemical parameters in Dengue patients with different serological status

DISCUSSION

Patient of Dengue fever presents with alteration in haematological and biochemical parameters. For management of dengue disease, it is crucial to identify the various hematological, biochemical abnormalities and serological markers that alters during the course of illness.[1]

Our study showed that dengue infection was more commonly observed in young adults. About 49% of our patients were between 20 to 40 years of age. This is also supported by studies from other part of the world.[15,16] Male predominance (71%) was observed in the current study. The reasons for it could be larger proportion of male population reporting to hospital as compared to female.[17] One of the other reason could be that men have greater exposures to dengue-carrying mosquitoes throughout daytime at their workplace or travelling to and from work.[18]

All serotypes of dengue virus have high level of NS1 Ag circulating in during the initial phase of illness. With progressive decrease in NS1, Antibody detection becomes prominent in subsequent days.[18] Most of the patient included in this study were in acute phase and ordering a dengue NS1 antigen assay is one of the initial investigations carried out in our set up for dengue infections, This could be the likely basis for very high rate of identifying NS1Ag (80%) in our study population, similar finding has also been endorsed from other studies conducted previously[1,19], however in the later stages of illness, dengue serology is more useful.[8] In primary and in most secondary infections dengue-specific IgM and IgG begin to appear only around fifth day of fever, both the IgM and IgG type antibodies do not appear before third day of illness.[20]

In this study, routine biochemical parameters were analyzed seeking their association with dengue infection. The most common haematological findings were thrombocytopenia and leukopenia observed in 72% and 46% patients respectively. Among the biochemical parameters analyzed, main findings were elevated ALT during course of disease. These findings of our study are in agreement with the published studies worldwide.[1,15,21,22] The ALT is mostly linked with hepatocytes, minute activity is also observed in cardiac and skeletal muscle. ALT levels are usually raised because of damage to these two sources and hepatic damage.[21] The study also reported that serum creatinine and blood urea were raised in 4 % and 1%of the cases in our study, which is much less as compare to study carried out in Ethiopia reporting 19.6% and 14.7% of the cases with deranged blood urea and creatinine respectively.[21]

In this study we also compared the haematological parameter in different dengue specific serological groups. Out of the 118 cases of thrombocytopenia 75 (41%) were NS1 positive. This is in agreement with previous studies showing association of thrombocytopenia with NS1 antigen.[20,23] A total of 35% and 48% cases with NS1 positivity exhibited low haemoglobin and TLCs respectively. It is evident from the results that among all the serological groups of the study NS1Ag positive cases were more commonly affected as far as haematological and biochemical parameters are concerned. A study conducted earlier also recognised a strong correlation of pancytopenia with NS1 positive patients along with deranged SGPT values.[24] During initial few days of illness NS1 Ag is present at high level in blood. In acute stage the level of Antigen can be as high as 2 to 10 mcg/ml in the sera, while in the convalescent stage it may drop to 0.4 mcg/ml or less, this could be `the likely explanation for the higher detection rate of NS1 with haematological derangement.[25,26] A study carried out in Thailand observed that lowest TLC was observed from day 2 to day 10 (lowest on day 4) of illness and lowest platelet count was seen from day 3 to day 10 (lowest on day 6) in dengue patients. There are few experimental studies also available on NS1 that have discussed contribution of NS1 in dengue pathogenesis.[26]

Laboratory parameters can play be helpful in prompting the suspicion of dengue infections thus facilitating its timely diagnosis, even before the serological tests results are available.[18]

LIMITATION

Clinical features and disease severity of the patients were not evaluated in this study.

CONCLUSION

Maximum prevalence of dengue was found in young males. Thrombocytopenia and elevated ALT levels were observed in dengue patients. NS1 was the most common serological marker in dengue patients at the time of presentation. No significant difference of mean haematological and biochemical parameters were observed in between different serological groups with exception of Mean TLC, which differ significantly within these subgroups (p value = <0.05).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Huma Amin:** Manuscript writing, data collection, statistical analysis.
2	**Shameela Majeed:** Revision of manuscript, result, compilation.
3	**Wafa Omer:** Revision of manuscript, proof reading, final draft.
4	**Shumaila Najeeb:** Data collection.
5	**Nadia Wali:** Revision of the manuscript.
6	**Fatima tuz Zuhra:** Data collection ethical approval.