Original Article

Analysis Of Haematological And Biomedical Parameters During Dengue Infection From 2022 To 2023 Among North Indian Population: A Randomized Controlled Trial Study

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Abstract:

Background: Dengue fever, also known as Breakbone fever, results from mosquitoes transmitting the dengue virus and is prevalent in tropical areas worldwide. Humans serve as the primary host for the virus, with female mosquitoes acquiring the Dengue virus from viraemic humans during feeding.

Aims and objective: To estimate the haematological Parameters in dengue infection in 2022-2023 and correlate its co infection with malaria.

Material and Methods: The present Randomized controlled trial (RCT) was performed on 110 patients suffering from dengue of both sexes of North Indian population from Swati Path Lab, referred from Community health centre by clinical diagnosis Gularbhoj, Udham Singh Nagar, Uttrakhanad. Over a period of 1 year from June 2022 to May 2023. Dengue cases which were confirmed by serological tests with Dengue combi test (NS1, IgG, & IgM). If any single parameter i.e NS1, IgG, IgM antibody showed positivity , then in that condition we did more investigations and collected results like reports of Hb, TLC, Platelet count and Malaria Co- infection.

Results: Most patients infected with Dengue typically present with low platelet counts. Among the patients, 30 (27.3%) had a total leukocyte count of less than 4000 cells/mm^3, while 8 (7.3%) had a count exceeding 11,000 cells/mm^3. The majority, comprising 82 (74.5%) patients, fell within the

normal range for total leukocyte count. The NS1 test for dengue showed a higher positivity rate of 56 (50.1%) compared to IgG and IgM, which were 27 (24.5%) and 29 (26.4%) respectively.

Conclusion: Patients exhibiting prolonged fever, severe anemia, and respiratory distress alongside dengue should prompt consideration of potential malaria co-infection. Conversely, individuals with malaria presenting symptoms such as bleeding, headaches, arthralgia, or elevated hematocrit should raise suspicion of concurrent dengue infection. Healthcare professionals operating in endemic regions for both illnesses must maintain a high level of vigilance for co-infection, particularly in cases demonstrating atypical features, unexpected laboratory findings, or inadequate treatment responses, and should initiate simultaneous treatment for both infections.

Keywords: Aedes aegypti, Co infection, Dengue, Haemoglobin, Noth India, Malaria,

INTRODUCTION:

Dengue fever, also known as Breakbone fever, results from mosquitoes transmitting the dengue virus and is prevalent in tropical areas worldwide. Humans serve as the primary host for the virus, with female mosquitoes acquiring the Dengue virus from viraemic humans during feeding [1]. The virus infects the mosquito mid gut and spreads systematically over 10-12 days. Aedes aegypti, a highly anthropophilic mosquito, is an efficient arbovirus vector due to its multiple bites before egg-laying and close proximity to humans. Climate influences vector biology, affecting their abundance and distribution, crucial for vector-borne disease epidemics [2].

During the monsoon season, the prevalence of waterborne diseases tends to rise. Dengue, in particular, has emerged as a significant arthropod-borne viral illness, recognized by the World Health Organization as a crucial health concern. According to the data presented in the accompanying graph, the instances of Dengue infections have shown a marked increase in the 21st century [2, 3]. It is marked by severe pain resembling that of bone fractures. Annually, around 400 million people globally endure the effects of Dengue fever, with about 40% of the world's population at risk of exposure and infection. Since Dengue fever is a viral disease, it cannot be treated with antibiotics [3]. Dengue exhibits various clinical presentations, with unpredictable outcomes. While most patients recover, a subset progresses to severe disease, characterized by plasma leakage. Identifying those at risk is challenging but vital for timely intervention to prevent severe conditions [4].

The primary indicators of dengue fever encompass a sudden onset of high temperature (reaching up to 40 °C or 104 °F), accompanied by chills, intense headache (typically felt behind the eyes), muscle and joint pain, nausea, vomiting, flushed skin, and, in some instances, a rash resembling measles [5]. Initially, dengue fever symptoms may be mild, leading to potential misidentification as a flu, cold, or viral infection. In rare circumstances, dengue fever can progress into a more perilous manifestation known as dengue hemorrhagic fever. This severe form may entail bleeding, a reduction in blood platelet count (thrombocytopenia), blood plasma leakage, or the gravely threatening dengue shock syndrome, characterized by dangerously low blood pressure[5, 6, 7].

Approximately 1/12th of an adult male's body weight consists of blood, totaling 5-6 liters and comprised of 55% plasma and 45% formed elements. Blood performs vital roles in transporting oxygen, removing carbon dioxide, delivering nutrients, and managing waste. Additionally, it plays a crucial part in carrying hormones, enzymes, and vitamins, while safeguarding the body through leukocyte activity, serum bactericidal power, and immune response facilitated by lymphocytes [7, 8]. Erythrocytes, also called red cells, are numerous, lack a nucleus, and transport oxygen via haemoglobin. Conversely, leukocytes, or white cells, contribute to the body's defense, although they are less abundant than red cells. Platelets, or thrombocytes, primarily function to halt blood loss from wounds (hemostasis) [8, 9].

Dengue fever is primarily prevented by avoiding mosquito bites, as it is transmitted by the Aedes mosquitoes, mainly Aedes aegypti. This involves measures such as using insect repellent, wearing

long sleeves and pants, using mosquito nets while sleeping, and eliminating standing water where mosquitoes breed. While there's no specific antiviral treatment for dengue fever, supportive care is essential, especially in managing symptoms and preventing complications. Early detection and proper medical management can significantly reduce the risk of complications such as dengue shock syndrome or dengue hemorrhagic fever. This includes ensuring proper hydration, monitoring for signs of shock, and administering appropriate medical care as needed [4, 10, 11].

MATERIAL AND METHODS:

The present Randomized controlled trial (RCT) was performed on 110 patients suffering from dengue of both sexes of North Indian population from Swati Path Lab, referred from Community health centre by clinical diagnosis Gularbhoj, Udham Singh Nagar, Uttarakhand. Over a period of 1 year from June 2022 to May 2023. Dengue cases which were confirmed by serological tests with Dengue combi test (NS1, IgG, & IgM). If any single parameter i.e NS1, IgG, IgM antibody showed positivity , then in that condition we did more investigations and collected results like reports of Hb, TLC, Platelet count and Malaria Co- infection.

Type of study - Randomized controlled trial (RCT)
Sample size calculation: According to convenient sampling & taking the value as reference according to SINGH B et al. [12] the minimum number of sample size was calculated. The sampling formula is $N = z^2_{\alpha} \times p \times q / L^2$ where N is sample size; p is percentage; q = 1-p, Type of error $\alpha = 5\%$, Allowable error L= 12% of p. So, estimated sample size calculated was (N) 110.

Inclusion criteria:
- Age ≥ 5 years
- Fever or history of fever for ≤ 72 hours.
- Clinical symptom consistent with potential dengue infection, such as suspected dengue or undifferentiated fever, observed in a patient residing in an area endemic to dengue
- The CHC physician deemed the individual suitable for outpatient care at the time of study enrollment, indicating the absence of severe disease symptoms
- Written informed consent.

Exclusion criteria:
- Identifying features that indicate a potential alternative diagnosis, such as pneumonia or otitis
- The physician assesses that it is improbable for the patient to make daily follow-up visits, possibly due to the considerable distance between the clinic and the patient's location.

The samples were anonymized, randomly coded and de- linked from any identity sources (ICMR National guidelines for bio- medical & health research involving human participants, ICMR, 2017, sec 5, Box 5.2) [13].

Laboratory evaluation:
Nursing personnel, male orderlies, or pathology technician collected 4 to 6ml of blood from each patient, adhering to strict aseptic measures. Serum was then obtained using standardized techniques, with all procedures conducted after securing informed consent from the patients. Malaria diagnosis involved identifying the species and quantifying parasites through examination of Giemsa-stained thick and thin peripheral blood films under oil immersion. A slide was deemed negative if no parasites were observed in 100 high-power fields. Each blood film underwent scrutiny by two seasoned microscopists. For dengue diagnosis, serum samples were tested for IgM and IgG anti-dengue antibodies using the Dengue IGM capture enzyme-linked immunosorbent assay (MAC ELISA) and IgG MAC ELISA rapid diagnostic test (Panbio Pty Limited, Queensland, Australia) Following

diagnosis, patients were categorized into groups: dengue and malaria co-infection (Group A), malaria mono-infection (Group B), and dengue mono-infection (Group C) [6, 7].
Including cell count (e.g. TLC, DLC, Platelet count), requires the use of Leishman stain, known for its effectiveness in providing detailed blood cell morphology. The stain, containing methylene blue and eosin in a 1.5:1 ratio in methanol, is applied through a modified procedure. The smear on a glass slide is covered with Leishman stain, followed by the addition of buffer after 1 minute, and washing with de-ionized water. The observation under a microscope reveals distinct characteristics of various blood cells [6, 7].

RESULTS AND OBSERVATION:

In this present observational study, 110 patients diagnosed with Dengue infection and its co-infection with Malaria were analyzed to determine the total number of patients (both positive and negative) and the distribution of infected patients according to age, along with the corresponding percentages for both genders. These findings are presented in Tables/Figures 1.1 and 1.2.

Case Distribution	No of Patient	Percentage
DENGUE POSITIVE	110	78.6%
MALARIA POSITIVE	4	2.8%
NEGATIVE	26	18.6%

Table/figure 1.1: Case distribution according to negative and positive dengue malaria infection

Age group	No of patient	Percentage
1-10 YEARS	2	1.8%
11-20 YEARS	11	7.8%
21-30 YEARS	20	14.3%
31-40 YEARS	9	6.4%
41-50 YEARS	20	14.3%
51-60 YEARS	29	20.7%
61-70 YEARS	8	5.7%
71-80 YEARS	7	5%
81-90 YEARS	4	2.8%
TOTAL	110	100%

Table/figure 1.2: Distribution of patient according to age

Most patients infected with Dengue typically present with low platelet counts. Among the patients, 30 (27.3%) had a total leukocyte count of less than 4000 cells/mm^3, while 8 (7.3%) had a count exceeding 11,000 cells/mm^3. The majority, comprising 82 (74.5%) patients, fell within the normal range for total leukocyte count. Detailed percentages of platelet and total leukocyte counts among Dengue and Malaria-positive patients, as well as Haemoglobin Concentration & Coagulation Profile in Dengue Patients, are presented in Table/Figure 1.3 & 1.4, respectively.

Platelet count	No (%) of patient	TLC	No (%) of patient
<50000	42 (38.2%)	<4000	30 (27.3%)
50000-100000	15 (13.6%)	4000-11000	82 (74.5%)
100000-150,000	24 (21.8%)	>11000	8 (7.3%)
>150000	29 (26.4%)		

Table/figure 1.3: Platelet Count and Total leukocyte count (TLC) in Dengue and Malaria Positive Patient

Haemoglobin	No (%) of patient	Coagulation Profile	Who have done PT & APTT	PROLONGED PT & APTT
.<9	10 (9.1%)	PT	36 (32.7%)	21 (19.1%)
9-10.9	7 (6.4%)	INR	39 (35.5%)	11 (10%)
11-11.9	14 (12.7%)	APTT	21 (19.1%)	12 (10.9%)
12-16	79 (71.8%)			

Table/figure 1.4: Haemoglobin Concentration & Coagulation Profile in Dengue Patient
*PT: Prothrombin time, *INR: International normalised ratio, *APTT: Activated partial thromboplastin time

The NS1 test for dengue showed a higher positivity rate of 56 (50.1%) compared to IgG and IgM, which were 27 (24.5%) and 29 (26.4%) respectively. The distribution of patients with different types of dengue and malaria percentages is presented in tables/figures 1.5.

DENGUE TEST	No (%) of patient	MALARIA	No (%) of patient
IgG positive	27 (24.5%)	Malarial Antigen performed	110 (100%)
IgM positive	29 (26.4%)	Dengue malaria both are positive	00
NS1 positive	56 (50.1%)	Malaria positive	4 (2.9%)
		Malaria negative	136 (97.1%)

Table/figure 1.5: Showing numbers with percentage of dengue test by card method and co-infection of malaria in dengue patient.

DISCUSSION:

Dengue presents formidable hurdles with its varied and occasionally life-threatening symptoms impacting various bodily systems. It remains a prominent factor in mortality and morbidity rates across northern India. Staying alert, promptly recognizing symptoms, closely monitoring clinical and lab markers, and swift intervention play pivotal roles in reducing mortality rates. High-grade fever commonly accompanies all cases [14].
The majority of cases, 110 out of 140, tested positive for dengue according to the current study data. Among these cases, 4 were positive for malaria, while the remainder showed no infection of either dengue or malaria. Consequently, we selected the 110 cases with positive dengue infections for further study.Among the current study who tested positive for dengue and malaria, a platelet count exceeding 150,000 was observed in 42 cases (38.2%). This count was notably higher compared to the platelet counts observed in the studies conducted by Sudeb Mukharji et al. [15], Riffat Mehboob et al. [16], and Rashmi MV et al [17]. In these studies, platelet counts falling within the ranges of 50,000-99,999 and less than 50,000 were higher in Riffat Mehboob et al. [16] and Rashmi MV et al. [17] respectively exhibited in Table/figure 1.6.

PLATELET COUNT	Present study	Sudeb Mukharji et al	Riffat M. et al	Rashmi MV et al
>150000	42(38.2%)	8 (9.7%)	3 (6%)	20 (20%)
100000-1,49,999	15(13.6%)	18 (21.9%)	11 (22%)	12 (12%)
50000- 99,999	24(21.8%)	24(29.2%)	36 (72%)	11 (11%)
<50000	29(26.4%)	27 (32.9%)	-	57 (57%)

Table/figure 1.6: showing comparison of Platelet Count in Dengue and Malaria Positive Patient with present study.

The current study observed a higher haemoglobin concentration of 71.8% in the range of 12-16, contrasting with the findings of Patel MI et al. [18], Meena KC et al. [19], and Arhalina et al., who reported concentrations of 9%, 63%, and 63.8%, respectively. Concentrations below 9, within 9-10.9, and within 11-11.9 were lower compared to the aforementioned studies displayed in Table/figure 1.7.

Haemoglobin	**Present study**	Patel MI et al.	Meena KC et al	Arhalina et al.
<9	10(9.1%)	44 (18%)		35(16.6%)
9-10.9	7 (6.4%)	6 (25%)	4 (4%)	59 (28.0%)
11-11.9	14(12.7%)	120 (48%)	33 (33%)	100 (47.6%)
12-16	79(71.8%)	23 (9%)	63 (63%)	134 (63.8%)

Table/figure 1.7: showing comparison Hemoglobin Concentration in Dengue Malaria Patient with present study

In the current study, the observed Total Leukocyte Count in Dengue Malaria Infection revealed proportions of <4000 (27.3%), 4000-11000 (74.5%), and >11000 (7.3%). Notably, the occurrences of <4000 and >11000 were lower in the present investigation compared to the findings of Dongre T et al. [20], Meena kc et al.[19], and Dhobale RV et al. [21], while the range of 4000-11000 showed higher proportions.
Furthermore, among Dengue Malaria patients, the percentages of IgG, IgM, and NS1 positivity were 24.5%, 26.4%, and 50.1%, respectively. The prevalence of NS1 observed in this study was lower compared to the findings of Chetal SD et al. [22], Ganeshkumar P et al [23], and Rashmi mv et al. [17] conversely, the rates of IgG and IgM positivity were higher compared to the research conducted by CHHOTALA ET AL and Rashmi mv et al. [17].

CONCLUSION:

Patients exhibiting prolonged fever, severe anemia, and respiratory distress alongside dengue should prompt consideration of potential malaria co-infection. Conversely, individuals with malaria presenting symptoms such as bleeding, headaches, arthralgia, or elevated hematocrit should raise suspicion of concurrent dengue infection. Healthcare professionals operating in endemic regions for both illnesses must maintain a high level of vigilance for co-infection, particularly in cases demonstrating atypical features, unexpected laboratory findings, or inadequate treatment responses, and should initiate simultaneous treatment for both infections. Rapid diagnostic kits for dengue or malaria detection are accessible and aid in early diagnosis and treatment. However, there is a pressing need in our region for a single combined rapid diagnostic kit capable of detecting both dengue and malaria.

Declarations:
Consent: Written Informed consent was taken from all the patients included in the study.
Funding: No Funding Sources.
Competing interests: The authors declare no competing interests.

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