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EVALUATING THE EFFECT OF SEROLOGICAL MARKERS AND PATIENT DEMOGRAPHICS ON HEMATOLOGICAL PARAMETERS VARIATION IN DENGUE FEVER

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Abstract

Objective: To evaluate the association between Variations in Hematological Parameters (Platelets count, leucocyte count), Dengue serological markers (NS1, IgG, IgM) and Patient demographics (Age and Gender) in patients presenting with dengue fever. Methods: A cross-sectional comparative study was conducted across healthcare facilities in Mardan, Pakistan. Data from 250 serologically confirmed dengue patients were analyzed. Complete blood counts and rapid immunochromatographic serological results were correlated with demographic data using ANOVA and Chi-square analyses. Results: The cohort (M = 30.56 years; 67.6% male) predominantly presented with NS-1 only positivity (n=143), Patients with combined IgG and IgM positivity exhibited significantly higher WBC counts compared to NS1 only patients ($p = 0.010$). Age demonstrated a significant inverse correlation with both platelet ($p < 0.001$) and WBC counts ($p < 0.001$), with pediatric patients presetting the highest baseline levels. Female patients exhibited significantly higher platelet counts than males ($p = 0.010$). Conclusion: Hematological parameters in dengue infection are significantly modified by the patient's immunological stage and demographic profile. In resource-constrained settings, combining qualitative serological profiles with CBC data provides a necessary-based context for clinical interpretation.

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INTRODUCTION:

Dengue fever is a major global public health problem and a common vector-borne viral disease[1]. It is caused by the dengue virus, transmitted by the *Aedes aegypti* mosquito, and is the pathogen of dengue fever (DF), a tropical disease [2]. Mosquitoes that carry the dengue virus typically live at altitudes below 6,500 feet. The risk of contracting dengue from mosquitoes at higher altitudes is extremely low [3]. For most people, dengue fever is a self-limiting disease requiring only minimal supportive care. However, in less than 1% of patients, without treatment, severe dengue symptoms, including fluid retention, shock, and multiple organ failure, can develop and even become life-threatening [4]. After viral exposure, white blood cells produce various signaling proteins, such as interferons and other cytokines. This can lead to a variety of symptoms, such as headache, fever, muscle aches, and joint pain [5]. Sometimes dengue fever leads to a potentially fatal complication, also known as dengue shock syndrome (DSS), the clinical presentation varies in both adults and children and depends on the severity of the infection, immune status, age, and genetic background [6]. There are multiple methods for diagnosing dengue fever. The tourniquet test is the most important physical examination testy. Among other tests, we use various laboratory methods to diagnose dengue fever. These include complete blood count, cell culture, PCR-based nucleic acid amplification, and other serology tests. All of these can be used to confirm the diagnosis of dengue [7]. The dengue fever virus, also known as the dengue virus, belongs to the genus Flavivirus and the family Flaviviridae. The genome is a single-stranded, positive-sense ribonucleic acid with 10,700 bases [8]. It is spherical, with an envelope in which virus particle and other surface proteins are arranged in icosahedral symmetry [9]. Dengue virus has four main types and a fifth serotype was later reported in 2013 [10]. When a person is infected with one serotype of the dengue virus, re-exposure to that same serotype usually results in a milder infection due to immunity. However, if reinfection occurs with a different serotype, it can lead to a more severe form of dengue infection [11]. Approximately 50% of the global population lives in areas at risk of dengue transmission, with 100-400 million new cases reported annually, according to the World Health Organization (WHO) [12]. In 2024 with WHO reporting more than six million new cases by April, which includes three to four million confirmed cases, of which 16000 were severe, and more than 3000 casualties reported globally [13]. The present research has its focus on the Mardan district of Khyber Pakhtunkhwa (KP), to which the least focus has been given during the outbreaks [14]. The dengue mosquito usually breeds in stagnant water. This is a common mosquito that becomes a dengue carrier when it comes into contact with a dengue-carrying patient. Then, this mosquito can further spread the infection to other healthy subjects, and the cycle continues [15] Virus transmission occurs through saliva when the mosquito bites the host. Inside the mosquito, the virus first replicates in secondary tissues, i.e., the salivary glands. This replication ensures that infectious virus particles are present in the saliva, ready to be transmitted to a human during the mosquito's bite [10].

The purpose of this research is:

1. To find the link among the severity of dengue fever, patient demographics (gender, age), CBC parameters, specifically platelet count and WBC count, and serological markers, i.e., NS1, IgG, and IgM (general)
2. To correlate the hematological pattern with dengue serological markers (NS1, IgG, and IgM specific
3. To analyze the relationship between patient demographic characteristics and hematological parameters.
4. To determine the associations between CBC findings (platelets and WBC counts) and the severity of disease.

The complete blood picture of dengue viral infection shows leukopenia and thrombocytopenia as the most important abnormal findings. The laboratory results serve as the primary tool for clinicians to evaluate disease severity and decide on treatment approaches. The patient demographic characteristics of age and gender create a wide range of parameter changes, as these factors affect both the immune response and disease severity.

LITERATURE REVIEW:

FRFG Azin et al, conducted a study to examine variations in laboratory tests during dengue fever. They observed temporal variation in abnormal lab findings across different DENV serotypes. The goal was to determine whether certain lab tests could help doctors assess disease severity and make early treatment decisions. According to their research, leukopenia is the most common hematological abnormality in dengue infection, with the WBC count sometimes falling below $2 \times 10^3/\mu L$. At the early stage of the disease, patients may occasionally present with mild leukocytosis, which is frequently associated with neutrophilia. As the disease progresses, a shift towards lymphocytes is observed in this pattern, and atypical lymphocytes begin to appear on the peripheral smear. Throughout the course of illness, hematocrit levels require careful daily monitoring to detect any significant changes.

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In cases progressing to dengue hemorrhagic fever (DHF), a rise of 20% or more in hematocrit from baseline is typically observed, often accompanied by significant thrombocytopenia (platelet count < 100×10^9/L)[16].

A retrospective single-center study was conducted by **Juthatip Chaloemwong et al**. at Chiang Mai University Hospital. According to their research, routine blood parameters, including hemoglobin (Hb), hematocrit (Hct), white blood cell count, white blood cell differential percentage, and platelet count, change daily during dengue fever. From day 3 to day 10, the hematocrit and hemoglobin levels in dengue fever group were statistically higher as compared to the control group. On day 4, more than 50% of patients developed thrombocytopenia, reaching a peak of 80% on day 6. The total white blood cell count in the dengue fever group was lower than that in the control group. The average white blood cell count is lowest on the 4th day of fever [17].

SW Huang and colleagues conducted a study to build a machine-learning model to predict severe dengue from personal information and laboratory test results of dengue patients. To this end, factors associated with disease severity were first examined and later included in the predictive model. These factors included age, gender, DENV NS1 antigen, viral RNA levels in the blood, and anti-dengue IgG and IgM antibodies. The results showed that severe dengue patients were generally older. The median age of patients with severe dengue was 75 years, while the median age of patients with mild dengue was 55 years. In contrast, male or female patient status and viral load were not clearly linked with the severity of disease. In nutshell, factors such as anti-dengue antibody (IgG and IgM) levels, serum DENV NS1 antigen and age were associated with disease severity, especially in DENV-2 infected patients [18].

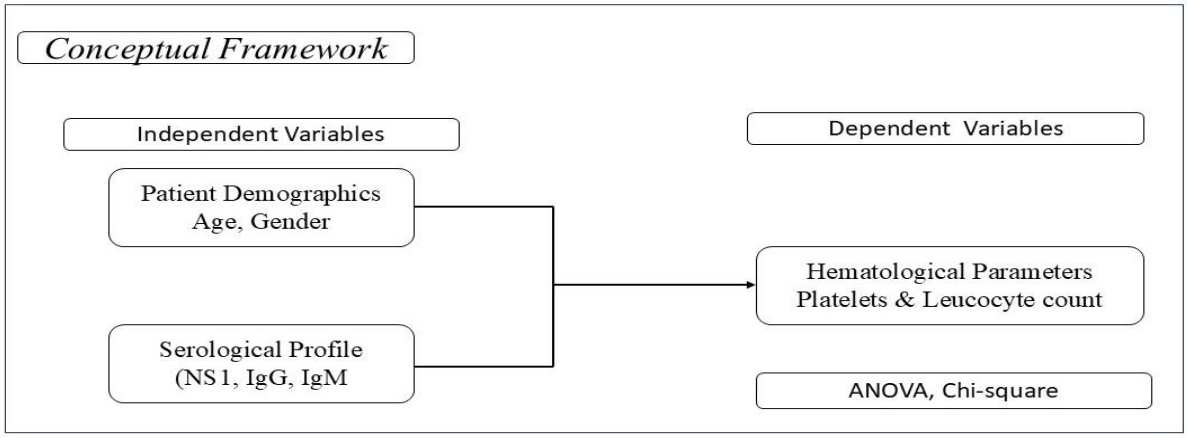

Figure 1 Conceptual framework of Demographic and Serological Associations with Hematological Variations

MATERIALS AND METHODS:

A retrospective, cross-sectional observational study was conducted over a six-month period utilizing secondary clinical data from public and private healthcare facilities in Mardan, Pakistan. Utilizing a non-probability convenience sampling technique, clinical records were extracted for 250 patients across all age demographics and genders who presented with clinical symptoms of dengue fever. Including criteria required preexisting, documented serological confirmation specifically the dengue detection of Dengue NS1 antigen, IgG and IgM antibodies and concurrent Complete Blood Count (CBC) profiles. Data were compiled and analyzed using SPSS to generate descriptive statistics and conduct comparative analyses (independent t-tests, One-way ANOVA and Pearson Chi-square tests) to determine statistical association between patient demographics, serological profiles and hematological variations with significance established at p value ≤ 0.05. Ethical approval for retrospective data analysis was granted by the institutional research committee and all patient records were fully de-identified to maintain strict confidentiality. A primary constraint of this retrospective chart review is the absence of documentation regarding the specific day of illness onset.

RESULTS:

Demographics

The study was conducted on Dengue patients from Mardan. Total of 250 participants with minimum age of 2 years child and oldest participant being 75 years of age, with mean 30.56, standard deviation 16.35. Total male participants were 169 out of 250 i.e. 67.6% and Female population 81 i.e. 32.4%. Among the participants, Total 24

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participants are among pediatrics (from 1 to 10, 9.6%), 57 are teens (from 11 to 19, 22.8%), 141 are adults (20 – 50, 56.4%) and 28 are among geriatrics (above 50, 11.2 %).

Diagnostics

According to International Guidelines for Dengue diagnostics, Dengue NS1 Antigen Detection test, IgG strip-based ICT test and IgM strip-based ICT test were conducted on all those participants. Among 250 patients, 143 were Only NS1 positive, 6 were NS1 and IgG positive, IgM Negative and similarly 6 participants were IgG and IgM positive while NS1 negative. 88 Participants were NS1 positive, IgG positive and IgM positive. 7 Participants were NS1 positive, IgM positive, IgG Negative.

Platelets Count

For all those 250 patients, the minimum platelets count reported were 13000 while maximum reported were 385000 with mean value 170584, Standard deviation 64035. Those platelets count are recorded and segregated according to Internationally recognized guidelines of thrombocytopenia severity and clinical management. 141 of those 250 participants were Normal (56.4%) with platelets above 150,000/µl, 86 patients had Mild thrombocytopenia (34.4%) with platelets ranging from 100,000 to 150,000/µl, 18 patients had Moderate thrombocytopenia (7.2%) platelets ranging from 50,000 to <100,000/µl and Only 4 patients had severe thrombocytopenia (1.6%) with platelets ranging from 20,000 to <50,000 /µl and Only 1 patient had platelets count 13000 being in the very severe category (0.4%).

Leucocytes Count

According to WHO disease classification, Patients are categorized into Low, Normal and high range of leucocytes. For all those 250 patients, the minimum leucocytes count reported were 2000 while maximum reported were 14700 with mean 5857.1 and Standard deviation 2097.7. 190 participants fall the normal range from 4000 to 11000 WBC count per /µl among them 149 (59.6%) falls in the below average category and only 41 (16.4%) falls in the above average category. 56 (22.4%) patients were reported to be in Low WBC count category while only 4 (1.6%) patients fall in High WBC count.

Table 1: ***Baseline Demographics and Clinical Characteristics***

Parameter	Category	Frequency (n)	Percentage (%)
Gender	Male	169	67.6
	Female	81	32.4
Age Group	Pediatric (1–10 years)	24	9.6
	Teens (11–19 years)	57	22.8
	Adults (20–50 years)	141	56.4
	Geriatrics (>50 years)	28	11.2
Serological Profile	NS1 Only	143	57.2
	NS1, IgG, IgM Positive	88	35.2
	NS1, IgM Positive	7	2.8
	NS1, IgG Positive	6	2.4
	IgG, IgM Positive	6	2.4

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Parameter	Category	Frequency (n)	Percentage (%)
Platelet Status	Normal (>150,000 /µL)	141	56.4
	Mild Thrombocytopenia (100,000–150,000 /µL)	86	34.4
	Moderate Thrombocytopenia (50,000–100,000 /µL)	18	7.2
	Severe Thrombocytopenia (20,000–50,000 /µL)	4	1.6
	Very Severe Thrombocytopenia (<20,000 /µL)	1	0.4
Leukocyte Status	Normal (4,000–11,000 /µL)	190	76.0
	Leukopenia (<4,000 /µL)	56	22.4
	Leukocytosis (>11,000 /µL)	4	1.6

Association of Hematological Patterns with Dengue Fever

The mean WBC count differed significantly among dengue test group. ANOVA test being performed on all the group to verify mean difference of WBC count among dengue fever patients with different serology. F value 4.007 with p value 0.004 (<0.05) seems statistically significant. Post hoc analysis Tukey HSD showed patients with IgG and IgM positive tests had significantly higher WBC count with mean value 8317 /µl compared with patients with only NS1 positive tests with mean 5511 /µl, p value 0.010 (<0.05). Other groups showed intermediate (NS1 & IgG positive), NS1, IgG & IgM positive) and (NS1 and IgM positive). They did not differ significantly from each other. Hence these results suggested that WBC may vary with dengue serology pattern, potentially reflecting differences in primary vs secondary infection or immune response intensity.

For Platelet count difference among dengue serology groups, although overall ANOVA suggested a significant difference in mean platelet counts across dengue test groups (F=2.845, p value 0.025), post hoc pairwise comparisons using Games-Howell did not detect statistically significant differences between any two groups. Mean platelet count was lowest in patients with only NS1 positivity (160,748/µl) and highest in patients with IgG and IgM positivity (216,167 /µl) reflecting the expected trend of early thrombocytopenia in acute dengue and recovery in later immune phases.

Association of Demographical variations with Hematological Patterns

Data Analysis shows significant Association between Age and Platelets with Phi equals 0.527, Cramer's V equals 0.305 with p value <0.001. this indicates that platelet patterns vary across age groups, with moderate strength of association.

ANOVA results show a statistically significant difference in mean platelet count across age categories with F value equals 45.408 p value <0.001, Post hoc Games Howell comparisons revealed that pediatrics had significantly higher platelets count than teens, adults and Geriatrics with p value <0.002. Adults had significantly higher platelets count than geriatrics with p value <0.001 and platelet counts in teens and geriatrics were not significantly different p value being 0.197. hence these results indicate that age significantly influences platelet levels with children showing highest counts and elderly lowest.

On the other hand, A statistically significant association was observed between age category and WBC count with Pearson chi square equals 21.546, p value equals 0.01, Cramer's v equals 0.169, indicating that WBC patterns vary across age groups. Most WBC counts were within the normal range of below average across all ages. WBC increasing with the age trend was not observed.

ANOVA demonstrates a significant difference in mean WBC count across age categories F value 6.249 p value <0.001. Post hoc Tukey comparisons revealed that peads had significantly higher WBC counts than teens with mean

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difference 1887 p value 0.001, adults' mean difference 1195 p value 0.041 and geriatrics mean difference 2060 p value 0.002, No other pairwise differences were statistically significant. These results indicate that age significantly influences WBC counts with children with exhibiting the highest values and elderly the lowest.

Variations in Hematological patterns based on gender

The mean leucocytes count was 5844/ µl in males with standard deviation 2038 and 5884 /µl in females with standard deviation 2230 hence no statistically significant difference is observed in WBC count between males and females with F value being 0.020 and p values 0.889 indicating that gender does not significantly influence WBC levels in this study.

On the other hand, the mean platelet count was 163373 / µl in males with Standard deviation 57593 and 185,630 / µl in females with standard deviation 73880. F value being 9.121 and p value 0.003 suggesting heterogeneity of variance. ANOVA revealed a statistically significant difference in mean platelet counts between males and females with F value 6.768 & p value 0.010, with females exhibiting higher platelet counts than males.

Table 2: ***Statistical Associations of Hematological parameters***

Variable 1	Variable 2	Statistical Test	Test Value	p-value	Significance
Serology	Leukocytes (WBC)	ANOVA (F)	4.01	0.004	Significant
	Platelets	ANOVA (F)	2.85	0.025	Significant
Age Category	Platelets	ANOVA (F)	45.41	< 0.001	Significant
	Leukocytes (WBC)	Pearson Chi-square	21.55	0.010	Significant
Gender	Platelets	ANOVA (F)	6.77	0.010	Significant
	Leukocytes (WBC)	ANOVA (F)	0.02	0.889	Not Significant

Discussion

This study explains that hematological variations in dengue fever specifically thrombocytopenia (Platelets count) and leukopenia (White blood cells count) are significantly correlated with the immunological phase of infection as shown by serological markers (NS1, IgG and IgM). The findings highlight that Complete blood count profiles are not the strong indicators of disease severity but rather dyna mic reflections of the patients' real time immune activation and demographic baseline.

The demographic analysis confirmed significant physiological variances, notably the inverse correlation between age and both platelet and white blood cells reserves. Pediatric cohorts maintained the highest baseline counts, a trend that steadily declined across adult and geriatric populations.

Furthermore, the higher platelet retention observed in female patients suggest baseline biological and hormonal differences that indicates serious consideration during clinical assessment. These demographic variations demonstrate that relying on universal reference ranges is in sufficient for effective patient triage in dengue endemic regions.

The serological profile provided vital context for hematological data. The significant elevation of WBC counts in patients presenting with combined IgG / IgM positivity, compared to those with NS-1 only positivity, reflects the physiological transition from early viral replication and bone marrow suppression to secondary immune activation. In many resource constrained settings, where advanced diagnostic monitoring is unavailable, the majority of patients present in the early NS-1 positive phase before antibody development.

While the retrospective nature of this study and the absence of illness day documentation preclude tracking day by day disease progression, the findings validate a pragmatic clinical approach.

Conclusion

Hematological parameters in dengue infection are highly dynamic and inextricably linked to patient demographic baseline and stage of immune activation. Relying on complete blood counts in isolation provides an incomplete diagnostic picture. In resource constrained healthcare

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environments, integrating rapid serological profiling with CBC analysis offers a critical, staged based context that significantly enhances clinical decision making. Further prospective studies should focus on longitudinal day by day hematological tracking to further refine these presentation profiles into predictive clinical tools.

Institutional Review Board Statement The study was approved by the Research and Ethics Committee (REC) of The Professional Institute of Health Sciences, Mardan, Reference number TPIHS/REC/2025/003 and Date of Approval: 30th October 2025

Author Contributions:

Muhammad Salman: Conceptualization, Investigation, Writing - Original Draft.

Abdul Ghani Khan: Investigation, Data Curation, Writing – Original Draft

Ali Khan: Writing - Review & Editing, Funding Acquisition, Resources.

Waqar Ahmad: Validation, Writing - Review & Editing.

Muhammad Asim: Investigation, Data Curation.

Haris Shoaib Khan: Conceptualization, Methodology, Formal Analysis, Supervision, Writing - Original Draft (Results).

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