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EVALUATION OF HEMATOLOGICAL PARAMETERS IN CONFIRMED NON-STRUCTURAL-1 ANTIGEN (NS1AG) DENGUE INFECTED PATIENTS.

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Abstract

Introduction: The most prevalent virus spread by mosquitoes in Pakistan is dengue virus. The high ratio of illness to mortality caused by dengue virus infection may include clinical features ranging from mild dengue fever to dengue shock syndrome, which may even be lethal. **Objective:** This study aimed to investigate the haematological parameters in patients with confirmed Non-Structural 1 Antigen (Ns1Ag) dengue infection. **Study Design:** Descriptive cross-sectional study. **Place of Study:** Govt. Naseerullah Khan Babar Memorial Hospital, Peshawar. **Duration of study:** From September to December 2022 (Four Months). **Methods:** Participants were enrolled in the current study regardless of gender after being recruited and clinically examined by medical professionals. Immunochromatography was used to identify Non-Structural 1 Antigen (NS1Ag) of dengue virus in all cases. Blood was drawn and processed to get a complete blood count to study haematological parameters. The obtained data was analyzed via the statistical programme for social sciences version 22. **Results:** In this study, 102 dengue-infected individuals were recruited, with 61.8% males and 38.2% females. Haemoglobin levels were normal in 90.2% of dengue patients and decreased in 9.8%. The majority of female patients had low haemoglobin levels. White blood cell counts were low in 26.5% of patients. Neutrophil levels were high in 67.6% of the patients. Male patients (n=47) had more abnormal neutrophils than female patients (n=22). Low lymphocyte levels were reported in 51% of patients, whereas high lymphocyte levels were found in 13.7% of dengue-infected patients. Low platelet levels were identified in 30.4% of individuals, with males suffering from thrombocytopenia more than female infected with dengue virus. **Conclusion:** Based on the results obtained in the current study the dengue-infected individuals had higher levels of neutrophils, lower levels of lymphocytes, and lower levels of platelets. Also, males were seen to have suffered more than females. It is critical to developing diagnostic criteria based on clinical signs and symptoms for more accurate and prompt diagnosis.

Keywords: Complete Blood Count, Dengue virus, Non-Structural-1 Antigen, Ns1Ag, Platelets, RBC, WBC.

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INTRODUCTION

Dengue was reported in China in 1992 AD, whereas the French and West Indies experienced a dengue epidemic in 1635[1]. Over the past three centuries, this epidemic spread to the world's tropical, subtropical, and temperate regions. from Prompt diagnosis early in infection can be helpful in preventing it becoming deadly. Dengue can be diagnosed early with the NSI antigen and reverse transcription polymerase chain reaction, but these tests are expensive and widely unavailable[2]. Similarly, many laboratories use the IgM antibody ELISA method[3]. In 2009, the WHO classified dengue as either having symptoms or not having symptoms of severe dengue. Numerous studies have demonstrated that the most crucial haematological factor involved in the diagnosis of dengue is the platelet count[4,5]. Since mosquitoes are the vectors involved in the spread of the dengue virus, arthropod-born viral diagnoses was also possible[6]. This virus has four serotypes and is a member of the Flaviviridae family. The primary dengue vector, Aedes aegypti, is active during the day[7]. Extrinsic incubation starts in the vector when a female mosquito bites a person during the viremia phase. It can last up to twelve days, while intrinsic incubation lasts between three and five days in humans[8].

The genetic material of the dengue virus is made up of 11,000 nucleotides and bases[9]. It has a cubic shape and comprises a lipoprotein envelope-encased nucleocapsid. Three structural protein genes and seven non-structural protein genes are present along its length[10]. Fever, headache, myalgia, arthralgia, and minor haemorrhagic manifestation are among the signs and symptoms of dengue virus[11]. In a dengue patient, several biochemical and haematological changes take place[12]. Dengue fever debuted in Pakistan in 1994, and it has since spread throughout the nation[13]. The dengue epidemic in Pakistan[14] is being fought against a backdrop of limited economic resources, a lack of awareness and education, and a government with limited sustainability. Pakistan Emergency Plan of Action launched a project in which they provided long-lasting insecticidal nets and mosquito repellent sprayed and raised public awareness[15] to control the dengue virus epidemic in Pakistan. According to a WHO report released on Oct 3 2022, KP patients comprised 25% of all dengue patients in the previous year[16]. Haematological and biochemical parameters are crucial for diagnosing and treating dengue fever[17]. There have been reports of various haematological and biochemical findings, including thrombocytopenia, anaemia, leukopenia, elevated AST and ALT levels, lymphocytosis and neutrophilia, raised bilirubin, albumin, and cholesterol[18].

Children under 15 years of age are more likely than adults to experience severe dengue fever[19]. There is no specific treatment for dengue fever, but patients should get plenty of rest, drink plenty of water, and consult a doctor. Muscle aches and pains can be managed with supportive care such as antipyretics and painkillers[20]. Our study aims to assess the haematological characteristics of dengue fever patients to improve the sensitivity of early diagnosis.

MATERIALS AND METHODS

The Naseerullah Khan Babar Memorial Hospital Peshawar, served as the site of this descriptive cross-sectional study. From September to December 2022, four months were spent conducting the survey. One hundred-two patients of both sexes were recruited for the study. Following a thorough examination by a medical specialist, all patients were recruited. Dengue Non-Structural-1 Antigen testing was performed on all enrolled patients (NS1Ag). Patients who tested positive had their blood samples further processed for CBCs, while those who tested negative were disqualified from the study. Patients or their guardians were asked for written informed consent after being informed of the project's goals. Stakeholders gave their ethical approval for the study.

All patients with NS1 serological confirmation of dengue were included in this study. Three (03) millilitres (ml) of blood was collected for serological testing and put in a gel tube (yellow top). Serological testing required centrifuging blood for five (05) minutes. A complete blood count (CBC) was performed on blood samples from dengue patients with serological NS1 confirmation to determine various haematological parameters. The automated haematology analyzer Sysmec 2100 was used to obtain the haematological parameters. After processing the sample, the analyzer automatically generated a report that included parameters like Hemoglobin (Hb), White Blood Cells (WBC), Platelets (Plts), and Differential Leucocyte Counts (DLC).

The blood smear was made according to the protocol so that it could be examined under a microscope. A drop of blood was spread on a glass slide and left to dry. To fix the blood cells, methanol was added to the glass slide. After that, the prepared Giemsa stain was added to the slide and staining was allowed for five (05) minutes after using a slide to remove the Giemsa stain. Before performing a microscopic examination, the slide was dried. After adding the cedar oil, the slide was examined under a microscope. The hematological parameters were also counted through microscopy to confirm the heamatological analyzer findings. After data analysis using the statistical package for social sciences version 22.0, descriptive analysis, including frequencies, proportions, and percentages, was obtained (SPSS-22). Additionally, SPSS 22 was used to generate the tables and graphs.

RESULTS

One hundred-two patients were included in this study, with 63 (61.8%) men and 39 (38.2%) women. Numerous haematological variables were investigated, including haemoglobin (Hb), white blood cells (WBCs), neutrophils, lymphocytes, and platelets. 90.2% of patients (92/102) had haemoglobin levels greater than 12 g/dl, while only 2.9% of patients (3/102) had <9 g/dl. The percentage of patients (7/102) with haemoglobin between 9.1 and 12.0 g/dl was only 6.9%. Haemoglobin had a mean value of 2.873. According to tables 1 and 2, only three male patients had low haemoglobin levels (less than 9g/dl).

Table 1: Hemoglobin in different categories.

Groups	Frequency	Percent
<9	3	2.9
9.1-12.0	7	6.9
>12.0	92	90.2
Total	102	100.0

Table 2: Gender-wise distribution of Hemoglobin in different categories

Parameters		Haemoglobin			
		< 9	9.1 - 12.0	>12.0	Total
Gender	Male	3	2	58	63
	Female	0	5	34	39
Total		3	7	92	102

Twenty-seven patients (26.5%) had leukopenia (less than 4000 WBCs) or White Blood Cells (less than 4000) count. Both male and female dengue patients showed leukopenia. As shown in tables 3 and 4, 71.6% of patients (73/102) had normal leukocyte counts between 4000 and 11000, and only two male patients had leukocytosis with counts greater than 11000. WBC count had a mean value of 1.755.

Table 3: White blood cells in different categories.

Groups	Frequency	Percent
<4000	27	26.5
4000-11000	73	71.6
>11000	2	2.0
Total	102	100.0

Table 4: Gender-wise distribution of WBC in different categories

Parameters		WBC			
		<4000	4000-11000	>11000	Total
Gender	Male	15	46	2	63
	Female	12	27	0	39
Total		27	73	2	102

5.8% of patients (6/102) with neutrophil counts of 1.9% of men and 3.9% of women had levels below 50%. 27/102 patients, or 26.5%, had between 51 and 71% neutrophil counts. It was found that 69/102 patients, or 67.6%, had neutrophilia—a neutrophil count greater than 70%. According to tables 5 and 6, 46% of male patients had neutrophilia, compared to 21.5% of female patients. Neutrophils had a mean value of 2.618.

Table 5: Neutrophils in different categories.

Groups	Frequency	Percent
<50	6	5.9
51-70	27	26.5
>70	69	67.6
Total	102	100.0

Table 6: Gender-wise distribution of Neutrophils in different categories

Parameters		Neutrophils			
		<50	51-70	>70	Total
Gender	Male	2	14	47	63
	Female	4	13	22	39
Total		6	27	69	102

While 35.7% (36/102) of patients had average lymphocyte counts of 20 to 40%, 51% (52/102) had lymphocytopenia with a less than 20% lymphocyte count. In contrast to 16.6% of female dengue patients, 34.3% of male dengue patients had lymphocytopenia. Only 14 out of 102 patients (13.7%) had lymphocytosis, with a lymphocyte count more significant than 40%, with more female patients than male patients (Tables 7 and 8). In our study, the mean lymphocyte value was 1.627.

Table 7: Lymphocytes in different categories.

Groups	Frequency	Percent
<20	52	51.0
20-40	36	35.3
>40	14	13.7
Total	102	100.0

Table 8: Gender-wise distribution of Lymphocytes in different categories

Parameters		Lymphocytes			
		<20	20-40	>40	Total
Gender	Male	35	22	6	63
	Female	17	14	8	39
Total		52	36	14	102

Seventy-one patients (69.6%) had an average platelet count between 150000 and 450000, while 31 patients (30.4%) had thrombocytopenia with less than 150000. Male patients (20.5%) were more likely than female patients (9.8%) to have thrombocytopenia (Tables 9 and 10). This study found that the mean platelet value was 1.696.

Table 9: Platelets in different categories.

Groups	Frequency	Percent
<150000	31	30.4
150000-450000	71	69.6
Total	102	100.0

Table 10: Gender-wise distribution of Platelets in different categories

Parameters		Platelets		
		<150000	150000-450000	Total
Gender	Male	21	42	63
	Female	10	29	39
Total		31	71	102

DISCUSSION

In this study, 102 patients were examined for various haematological parameters. It was discovered that 90.2% of the patients had normal haemoglobin levels, with only 9.8% having anaemia (haemoglobin less than 10 mg/dl). Only 2% of patients had leukocytosis, while 26.5% had leukopenia. 5.9% of patients had neutropenia, 67.6% had neutrophilia (neutrophil count greater than 70%), 51% had lymphocytopenia, and 13.7% had lymphocytes. 34.4% of the patients showed thrombocytopenia[21].

In contrast to the study by Saara N. et al., where 28% of the patients had anemia[22], only 10% of the patients in our study had anaemia. Lalita Shreshtha et al.[23] reported similar results. According to other studies, haemoglobin was not significantly impacted by a dengue virus infection, with 62% of patients having levels within the normal range of 10 to 15 g/dl[24]. In this study, we discovered 25.6% of patients had leucopenia, compared to 35% who had the condition, and 71.6% of patients had average leucocyte counts, versus 65% who had such counts, according to Raima Kalhoro et al. [25]. In contrast to our findings, another study revealed 31%[26] of the patients to have leucopenia. Leucopenia was found in a high percentage of patients in other studies[27,28], consistent with our results.

Compared to the 30% and 6% values found by Naeem, A. et al. [29], neutropenia and neutrophilia percentages in this study were 5.9% and 67.6%, respectively. Compared to the current study[30], a survey conducted in Bangladesh found neutropenia to be 13.7% and neutrophilia to be 27.6%. 13.7% of patients had lymphocytosis, consistent with a few other studies[29,30,31], and 51% of cases in the current study had lymphocytopenia compared to other studies. In another study, 74.3% of patients had lymphocytopenia, which is much higher than our findings and may be related to the fact that these patients were in a critical phase[32].

According to Jayadas et al., 30.4% of the patients in this study had thrombocytopenia. Low platelet count is a biomarker and a significant clinical feature of dengue [32]. Compared to Debatosh Paul et al., where 60.3% of male and 39.7% of female patients had thrombocytopenia, 20% of the male patients in this study had the condition.

Platelets play a role in tissue repair and hemostasis[33].

Conclusion

According to the current study, dengue-infected people exhibited greater neutrophil levels and lower lymphocyte and platelet levels. Male patients also experienced more pain than female ones did. For a more precise and speedier diagnosis, it is essential to define diagnostic criteria based on symptoms of dengue infection.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

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