ORIGINAL ARTICLE

High fluorescent lymphocytes in dengue infection; A navigator for hematopathologists.

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ABSTRACT… Objective: The aim of this research is the early detection of dengue infection on the basis of presence of high fluorescent lymphocyte (HFL) which enables the clinicians to tailor further investigations and early commencement of treatment to prevent complications and thus can reduce mortality and morbidity in dengue patients. **Study Design:** Prospective Research. **Setting:** Fauji Foundation Hospital in Rawalpindi. **Period:** February 2022 to July 2022. **Methods:** Samples of Full blood counts (FBC) received in Pathology laboratory were processed on automated Hematology analyzer Sysmex XN-series (XN-1000). All the FBCs that were of known dengue patients were excluded from the study. The FBCs with High Fluorescent Lymphocytes on White blood cell differential fluorescence (WDF) scattergram were selected and evaluated. Detection of dengue infection was done by using Dengue Virus ELISA (IgM and IgG) test. **Results:** Among one hundred samples with High Fluorescence Lymphocyte there were 77% were Dengue Positive. Binomial test did give a significant association of Dengue with High Fluorescence Lymphocyte (p<0.01). **Conclusion:** We draw the conclusion that presence of high fluorescent lymphocytes helps in the early detection of dengue positive cases.

Key words: High Fluorescent Lymphocytes, Dengue Virus, Sysmex XN-series.

INTRODUCTION

A five part differential with cluster resolution and a clear separation of some of the atypical cells circulating in peripheral blood is offered by the Sysmex XN series automated hematology analyzer (AHA).[1] White blood cells are distinguished by this AHA based on their size, internal complexity, and overall nucleic acid composition. These analyzer produce scattergrams and cell population statistics based on the three parameters forward scatter, side scatter (SSC), and side fluorescence (SFL), which aid in the suspicion of various illness states. Additionally, the XN 1000 AHA contains an automated flagging system that detects possible atypical lymphocytes.[2]

High fluorescence lymphocyte also known as atypical lymphocytes. It has increase content of ribonucleic acid (RNA). In the white blood cell differential channel, these can be seen as a region of high fluorescence above the lymphocyte and monocyte area. This distinctive high fluorescence property of these cells is due to high RNA content.[1]

The Aedes mosquito carrying the dengue virus bites people to spread the disease, which results in dengue infection. Around 2.5 billion individuals lived in dengue endemic regions and 50 million people globally were affected by this illness each year.[1,3] Clinical severity of this infection ranges from minor illness to dengue shock syndrome (DSS). Dengue infected patients most frequently have acute febrile illness (AFI) without localizing sign and symptoms that can mirror other viral diseases.[4] Various screening and diagnostic lab tests like full blood count (FBC) and serological tests are used to differentiate it from other viral infections and for final diagnosis. CBC shows variation after the onset of the fever, most likely start with leukopenia on 3rd or 4th day of fever and subsequently thrombocytopenia and increase hematocrit due to plasma leakage.[5,6] According

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to WHO, it is divided into four phases: mild undifferentiated febrile illness, Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome.[6]

Among all these stages, most severe is dengue hemorrhagic fever presented with hypotension, coagulopathy, decreased platelet count, and increased vascular leakage. Few of them may present with shock due to vascular collapse which results in dengue shock syndrome that can be life threatening if not treated timely.[7]

At present there are two serological tests are available by using ELISA method for the detection of dengue infection. First is NS1 antigen which reported 76% sensitivity and 98% specificity. Another one is dengue IgM antibody having 90% sensitivity and 93% specificity.[8] However, these serological tests might not be available in certain small local hospitals or in developing nations, so the clinical evidences from the physical examination, history taking, and standard laboratory tests are still significant.

It is evident from literature that Dengue infection triggers changes in the cellular and humoral immune system. Formation of IgM and IgG antibodies can detect alterations in the humoral arm of the immune system that can be identified through serological investigations. Literature review revealed that these specific changes in cellular and humoral immune system can be identify by the presence of activated lymphocytes that are typical for many viral infections including dengue, also called blue plasma lymphocytes. The presence of these atypical lymphocytes help in the early diagnosis and management of Dengue viral infection with high specificity and sensitivity.[9] Presence of atypical lymphocytes can be seen in other viral infections, like herpes, infectious mononucleosis, influenza, rubella, and viral hepatitis. These atypical lymphocytes gives high fluorescence activity, so they are known as high fluorescent lymphocytes (HFL).[10]

Currently dengue infection is prevalent all over the year with a highest frequency in the post-rainy season, it is now endemic in Pakistan. Younger people may be more vulnerable because the average age of patients has been reduced. Patients who are at risk of hemorrhage may be identified by total and differential leukocyte counts.[11]

The purpose of this research is the early detection of dengue on the basis of presence of HFLC. It enables the clinicians to tailor further investigations and treatment commenced earlier. It can prevent complications and thus can reduce mortality and morbidity in dengue patients. This study is also helpful for early detection of dengue viral infection in under resourced laboratories in developing countries.

METHODS
A prospective research was carried out at Fauji Foundation Hospital in Rawalpindi from February 2022 to July 2022. Ethical approval has been taken from ethical review committee (Ref. No. 412/ERC/FFH/Rwp, dated: 1/01/2022). 126,000 Samples of Full blood counts (FBC) received in pathology laboratory from both outpatient departments (OPD) as well as wards were processed on automated Hematology analyzer Sysmex XN-series (XN-1000). All the FBCs that were of known dengue patients were excluded from the study. The FBCs with High Fluorescent Lymphocytes on White blood cell differential fluorescence (WDF) scattergram were selected and evaluated. Detection of dengue infection was done by using Dengue Virus ELISA (IgM and IgG) test. All cases that confirmed for dengue infection were recorded in the software system and reported to the respective wards. Patients from OPD and walk-ins were contacted on their cell phones to report right away to the hospital.

In order to save and analyse the data, IBM-SPSS version 23.0 was used. Descriptive data includes age, gender, and type / place of sample. Association of High Fluorescence Lymphocyte was tested using binomial test. P-value less than 0.05 were considered statistically significant. Pie diagram and bar chart were also reported on studied parameters of samples.

RESULTS

Table-I reports that among one hundred samples with High Fluorescence Lymphocyte there were 77% were Dengue Positive. Binomial test did give a significant association of Dengue with High fluorescence Lymphocyte (p<0.01).

Parameter	Dengue	N	P-Value
High fluorescence Lymphocyte	Negative	23	<0.01*
	Positive	77	
*p<0.05 was considered statistically significant using Binomial Test			

Table-I. Association of high fluorescence lymphocyte with dengue

Figure-1 showed the characteristics of studied samples, among dengue 77 positive dengue samples 35.1% were age 5 – 25 years old, 45.5% were age 26 – 55 years old, 19.5% were 56 – 80 years old, 32.5% were male, 67.5% were female, 49.4% were from OPD, 16.9% from Walk in and 33.8% from ward.
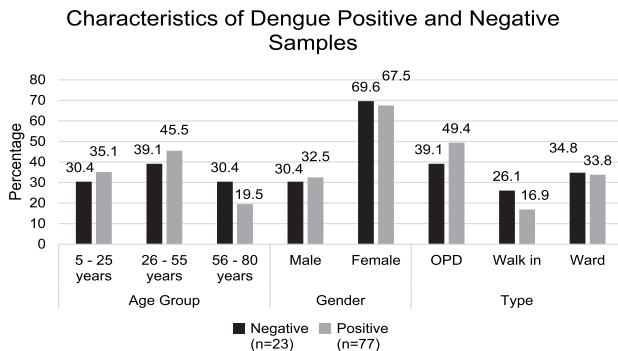

Figure-1. Characteristics of dengue positive and negative samples

DISCUSSION

Dengue virus is responsible for dengue hemorrhagic fever. It belongs to the family Flaviviridae. It spreads to people when female Aedes mosquitoes, especially Aedes aegypti bite them. DENV-1, DENV-2, DENV-3, and DENV-4 are its four serotypes.[12]

Dengue has been an issue in Pakistan since 2005, with the terrible outbreak occurring in 2011. Due to country's geographic location, changing climate, growing population, unplanned urbanization, and lack of effective vector control programs, dengue has become endemic in Pakistan.[13,14]

According to our study, females are more prone to dengue as compared to male. Most of the studies reported that male are more affected. This difference may be due to our hospital that caters more female patients.[13,14] Another study conducted at Lahore and reported mean age of patients was 45 years which is consistent with our study.[14] Maximum number of cases were referred from OPD.

Whenever a case of acute febrile illness is suspected, the first baseline investigation which is usually requested is complete blood count. In the underdeveloped countries like Pakistan where majority of the patients are not able to afford series of investigations for accurate diagnosis, we need a lab test for the early detection of the disease with limited resources. Therefore, we can utilize the usefulness of HFL in the rapid and accurate detection of dengue patients. where the initial sample submitted for cell counts might indicate a likely diagnosis, accelerate the start of supportive care, and enhance the result.[15]

One of the study conducted in India in 2021 and reported 87.3% of cases has high fluorescence lymphocytes which is in accordance to our study, which showed 77% of cases has high fluorescence lymphocytes.[16]

Another study from India compared the dengue positive cases with controls and stated increase high fluorescent lymphocytes in known dengue cases.[17]

The study conducted by Raharjo B & Hadi S showed that out of the 47 samples of Dengue Hemorrhagic Fever (DHF) patients, the HFLC results were between 2.0-32.3%, whereas the average range of normal HFLC values was between 0.0-1.4% and was 0.3%. The results are in accordance with our study which suggested that increased HFL is more likely to be attributed to atypical lymphocyte increase in dengue hemorrhagic fever.[18]

CONCLUSION

We draw the conclusion that presence of high

fluorescent lymphocytes helps in the early detection of dengue positive cases.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

SOURCE OF FUNDING

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Fariha Nasreen	Conception and design of study, Literature research, Data collection, Data analysis and manuscript writing.	
2	Attika Khalid	Collection and design of study, Final review of manuscript, Designed research methodology.	
3	Madeeha Rehan	Manuscript final reading and approval, Literature research, Designed research methodology.	
4	Asma Shaikh	Literature research, Data analysis and manuscript writing, Literature review.	
5	Humaira Rehman	Literature research, Data analysis and manuscript writing, Literature review.	