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Original Article

Clinical Correlation of Dengue Strains on the Basis of Seroprevalence in a Tertiary Care Hospital

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ABSTRACT

Dengue viruses are icosahedral in structure and contain a single-stranded positive- sense RNA sequence of 11kb inside their capsid protein, which belongs to *Flaviviridae* family, genera *Flavivirus*. *DENV* a vector dependent viral virus which presents a severe health danger worldwide. **Objective: T**o study the different strains of dengue on the basis of serotypes **Methods:** A cross sectional study was conducted at a tertiary care hospital Lahore, Primary and Secondary Health Department Lahore. The blood samples of 103 patients were collected from non-random sampling technique to check out the data of different parameters such as WBC's & Platelets through Complete Blood Count (CBC), NS1 from ELISA and nature of dengue strains through RT- PCR. **Results:** Of 103 positive dengue patients, there were n= 58 (33.63 ± 16.54) males and n=45 (40.64± 16.00) females. Data for the total patients is subjected to statistical differences by Paired *t-test* (*p<0.05). The overall percentage of dengue strains within the sample population was *DENV-2* (96%), *DENV-3* (2%), *DENV-1* & *DENV-2* (2% in females, nil in males) *DENV-2 & DENV-3* (2% both in males and females). According to clinical parameters the correlation of ELISA results with WBC's was significant (<0.0001) as well as ELISA and platelets of patients had no correlation with each other. **Conclusions:** Leukopenia and Thrombocytopenia is found particularly in *DENV-2* strain as well as we found two different strains in two patients. So, our research work is helpful for the identification in genetic similarity of dengue strains..

INTRODUCTION

The dengue virus (DENV) that causes dengue fever is a tropical viral infection. DENV is indeed a single- stranded RNA virus with a positive strand belonging to Flaviviridae family, genera Flavivirus. DENV a vector dependent virus which presents a severe health danger worldwide [1]. Dengue is an illness-causing repeating epidemic in Southeast Asia, the Pacific Rim, North America, Europe, and the Eastern Mediterranean [2]. According to the World Health Organization (WHO), annually 50 million cases of dengue virus infection were detected, putting over 2.5 billion people in danger [3]. The four main dengue virus serogroups that transmit dengue fever are DENV-1, DENV-2, DENV-3, and DENV-4. All four have the potential to cause serious illness [4]. Based on nucleotide mutations, DENV serotypes are further split into subtypes. Viral genetic variations were associated with variations in virulence [5]. Dengue viruses are icosahedral in structure and contain a single-stranded positive-sense RNA sequence of 11 kb inside their capsid protein, which is encased in a lipid bilayer generated by the host. The capsid protein (C), membrane protein (M), and envelope protein (E) make up mature DENV virions, whereas seven non-structural (NS)

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proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are encoded at the C-terminus [6,7]. The non-structural proteins of DENV were required for the virus's survival. NS1 also concerned in viral RNA replication or signaling. The protein NS2A was required in order to facilitate viral proliferation and bundling. NS2B is a serine protease that works with NS3 as a cofactor. NS3 is a group of proteins that acts as serine proteases [8,9]. Pathogens eventually travel from the area of inflammation to lymph nodes, where they engage monocytes and macrophages, that become invasion in sites. As a consequence, the inflammation grows and the pathogen travels through the lymph system. As a response of this initial viral load, numerous leukocyte cells, especially capillary monocytes, myeloid DC, and splenic as well as liver macrophages, get attacked [10]. Dengue fever is diagnosed in the laboratory using viral isolation, serological, and molecular Approaches [11]. As Viral isolation takes a long time, and molecular approaches are costly, the enzyme-linked immunosorbent test (ELISA) is the most often used method for diagnosing dengue fever. These tests look for antibodies against dengue, such as immunoglobulins Ig-M, IgG, and IgA, or antigens, such as non-structural NS-1 glycoproteins [12,13]. Our study's major goal was to figure out that which strain of dengue mostly effects on the parameters of complete blood count (CBC) specifically white blood cells (WBCs) and Platelets as well as also compare the sensitivity and specificity of different diagnostic techniques which were used in the diagnosis of dengue.

METHODS

A cross sectional study was done on non-random sampling technique of 103 dengue patients at a tertiary care hospital and the range of their age was from 25-65 years. The blood samples have confirmed results of CBC and ELISA, So then these blood samples was peruse for the RT-PCR at Primary and Secondary Health Department Lahore. The duration of the study was six months from September 2021 to February 2022. We took the sample of suspected dengue patients in two vials, 3ml whole blood in EDTA vial for CBC & RT-PCR and 3ml blood in coagulated vial for the separation of serum for ELISA. Patients with fever and body aches were included in this research work but the patients who have normal CBC parameters were excluded from this work. SYSMEX XN-550 was used for the CBC to check out the variation in WBC's and Platelets parameters as well as the serum samples of the concerned patients was used to done the ELISA through DENV Detect™ NS1 ELISA Kit method for the detection of NS1 antigen [14,15]. RNA Isolation and RT-PCR: We collected 3ml of blood samples from each EDTA vial and were centrifuge for 1 minute at 3500 rpm and then serum was separated from the whole blood for further procedure. Samples were directly undergone nucleic acid purification on automation through QIAGEN kit. We used Bosphore Dengue Virus Genotyping Kit v1 for RT-PCR which detects and discriminates RNA from Serotypes 1-2-3 and 4 of the Dengue Virus in human biological samples such as serum, plasma, saliva, and urine. In Bosphore Dengue Virus Genotyping Kit v1 the thermal treatment begins with initial denaturation for Taq DNA Polymerase activation (with hot-start capability) by following steps: Reverse transcription at 50ºC for 30 min, Initial denaturation at 95ºC for 14:30 min, Denaturation at 97ºC for 30 min, Annealing at 58ºC for 01:20 min and Hold at 32ºC for 05:00 min [16]. We analyzed the data on the basis of Mean and Standard Deviation ± SD by using two software the one was Statistical Package for the Social Sciences (SPSS) version 18 and other one is Graphpad prism version 8.4.3 [17].

RESULTS

Our study was conducted on both male and female dengue patients. Total 103 dengue patients were included out of which 45 were females and 58 were males. The mean age of males was 33.63 ± 16.54 years and for the females were 40.64 ± 16.00 years. Sample of dengue patients was collected from different locations. Mostly the patients were from Model Town Lahore. But there was considerable difference in location of both male and female from where sample was collected. Different strains of dengue virus have been known since now. The most common strain found in overall patients is DENV-2. Other than DENV-2, three more strains were seen in dengue patients i.e., DENV-3, DENV-1 & DENV-2, and DENV-2, DENV-3. The percentage of all strains in both males and females was seen in figure 1.

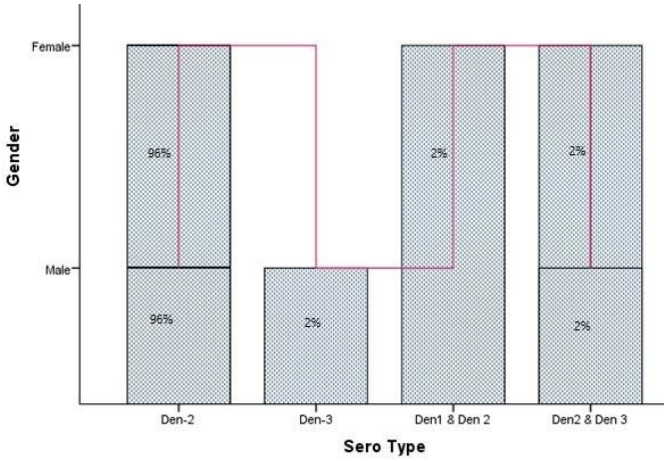

Figure 1: Comparison of serotypes between males and females

ELISA and RT-PCR, both were performed to detect the dengue virus in patients. Data is presented as mean and SD. Data for the patients is subjected to statistical variations in Paired sample t- test (*p<0.05). It is proposed from the results that all the patients had positive ELISA result. Out of which 90% patients' PCR result was positive (detected) while 10% patients' PCR result was negative (not detected)

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as seen in figure 2.

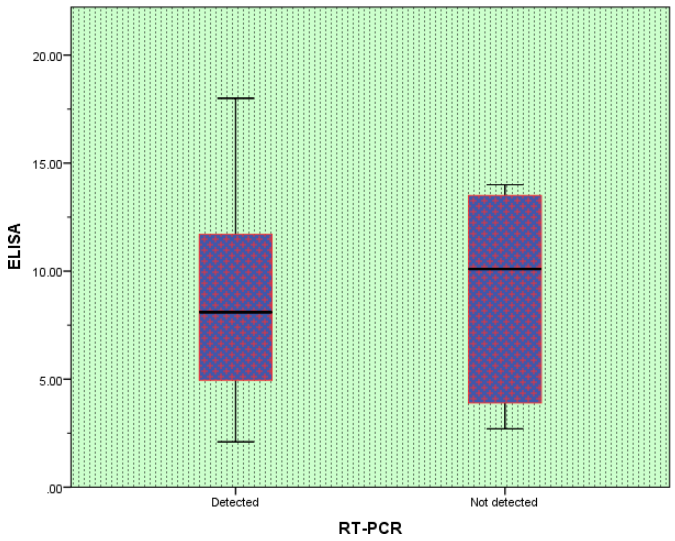

Figure 2: Comparison of ELISA and RT-PCR

Dengue affects the count of the white blood cells (WBCs). WBCs count drastically drops in the dengue patients. WBCs count in dengue patients (4432 ± 2286) in this study was lower than the normal range of WBCs count and the correlation between ELISA and WBCs results show significant correlation in figure 3 as well as ELISA and platelets of patients had no correlation with each other as the straight line shown in figure 4.

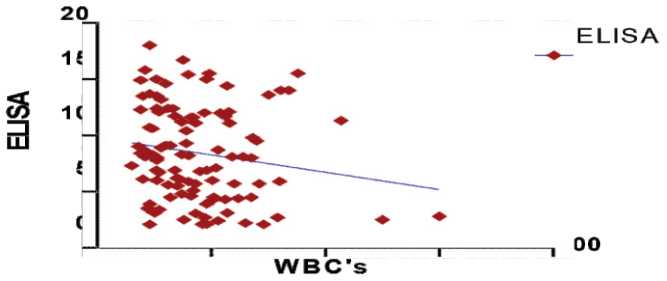

Figure 3: Correlation between ELISA and WBCs

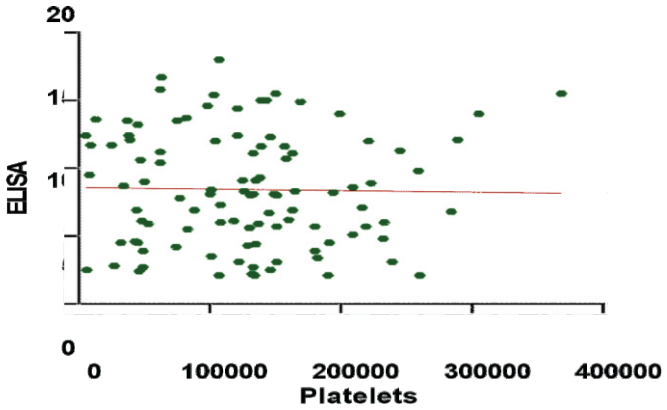

Figure 4: Comparison of ELISA and platelets

There was a change in the number of WBCs & platelets and the serotypes (p=0.001). The patients of our study had mostly DENV-2 strain in which WBCs count was in range of 4400 and the platelets were 127 x 109. While other strains had lower number of WBCs & platelets than the DENV-2. As, there are only two strains other than DENV-2, so it is difficult to compare them with DENV-2 as shown in figure 5 and 6.

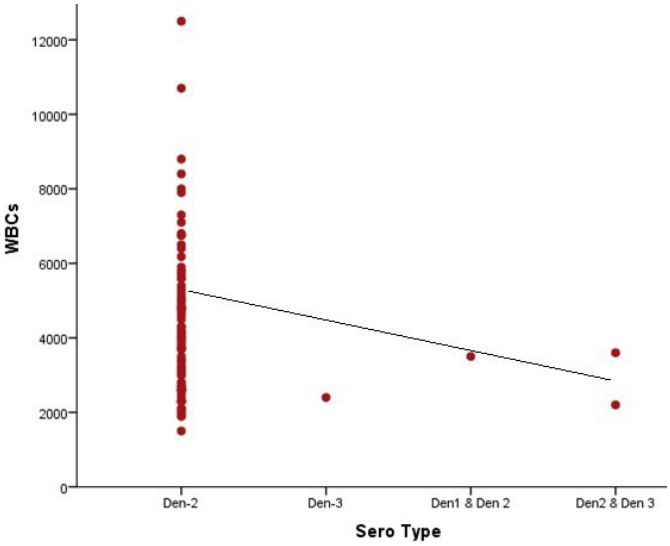

Figure 5: Comparison of dengue strains and WBCs count

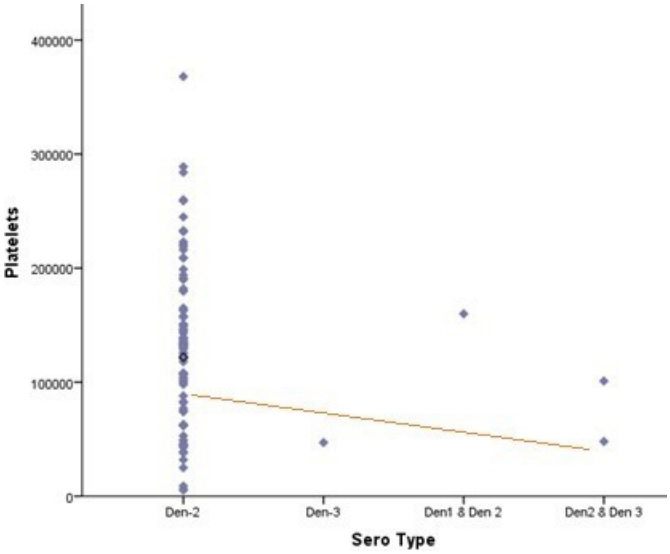

Figure 6: Comparison of serotypes and platelets

DISCUSSION

The spread of various DENV serogroups in a comparable geographical region creates an environment for the emergence of co-infections, a characteristic that is most visible during crises. Kim [18] recorded the impact of a combined infection of two DENV serotypes, DENV-2 and DENV-4, while in our investigation, the most frequent strain detected in overall patients was DENV-2 as it can damage the bone marrow and make antibodies which damage and kill the WBC's and platelets. Aside from

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DENV-2, three more strains have been identified in dengue patients: DENV-3, DENV-1 & DENV-2, and DENV-2 & DENV-3. DENV-2 accounted for 96% of the total, with DENV-3 accounting for just 3% of the male population. DENV-1 and DENV-2 were detected in 2% of females but not in males, whereas DENV-2 and DENV-3 were found in 2% of males and females. Ahmed and his colleague in 2014 recorded from 55 test samples for dengue virus, 34 were found to be positive; 33 were DENV-1 dengue virus and one was DENV-2 dengue virus [19]. However, we noted that all of the patients with sign and symptoms of dengue received a positive ELISA results in which 90% of patients had a positive PCR result, whereas 10% had a negative PCR results, with a mean and SD of 8.435±4.190. Ageep and his colleagues took two blood samples from each patient in his research work, one for routine hematological inquiry, commonly for white blood cell and platelet count, and the other for identification of dengue virus antibodies by using the ELISA method. Leucopenia (90%) and thrombocytopenia (88%) were the most common laboratory results [20]. However, in our analysis, WBC counts decreased in dengue patients, and the connection between ELISA and WBCs was negligible, with the mean and standard deviation of ELISA being 8.435±4.190 and 4432±2286 for WBCs and patients ELISA and platelets exhibited no association, with the mean and standard deviation of ELISA being 8.435 ±4.190 and 127 x10^9±73 x 10^9 for Platelets. We also determined in dengue fever that it may be triggered by virus-induced apoptosis or suppression of hematopoietic progenitor cells. Thrombocytopenia may be caused by the collapse of proximal platelets or bone marrow granulocytes by viruses, which subsequently limit platelet production. Dhanoa [21] and his colleagues observed that extreme thrombocytopenia was considerably greater in the DENV-2 cross group. DENV-2 cross individuals even had considerably lower baseline platelet counts than the stereo group. But there was a significant shift in the number of platelets and serotypes in our study work. Our study's participants were largely DENV-2 strains, with platelets counts ranging from 127 x 10^9 to 160 x 10^9, whereas other strains had less platelets than the DENV-2. In our research work, we found 2 different strains of dengue (DENV-2, DENV-3) in one patient and both (DENV-1, DENV-2) exhibit in another patient that was not reported previously in Pakistan. The overall effect on WBC's and Platelets of these patients with mean of 4434 for WBC and 127 x 10^9 for platelets.

CONCLUSIONS

The main purpose of our research work was to study about the different strains of dengue on the basis of serotype. The results of positive dengue patients are interpreted on the basis of WBC's, Platelets, ELISA and RT-PCR first time in Pakistan. Leukopenia and Thrombocytopenia is found particularly in DENV-2 strain as well as we found two different strains in same patients. It would be a leading mark for the researchers that there should be some genetic and functional resemblance in DENV-2, DENV-3 strains and also in DENV-1, DENV-2 strains. So, our research work is helpful for the identification in genetic similarity of dengue strains. Because we detected two different types of dengue strains in two different patients in our investigation, so it is recommended that the researchers should be moving forward with gene purification and sequencing.

REFERENCES

[1] 1. Haroon M, Jan H, Faisal S, Ali N, Kamran M, et al. Dengue outbreak in Peshawar: clinical features and laboratory markers of dengue virus infection. Journal of infection and public health. 2019,12(2):258-62. doi.org/10.1016/j.jiph.2018.10.138

[2] 2. Viennet E, Ritchie SA, Williams CR, Faddy HM, Harley D. Public health responses to and challenges for the control of dengue transmission in high-income countries: four case studies. PLoS neglected tropical diseases. 2016,10(9): e0004943. doi.org/10.1371/journal.pntd.0004943

[3] 3. Nathan M, Dayal-Drager R. Recent epidemiological trends, the global strategy and public health advances in dengue. Report on Dengue. 2006:30-4.

[4] 4. Organization WH. Comprehensive guideline for prevention and control of dengue and dengue hemorrhagic fever. 2011, Revised and expanded edition.

[5] 5. Ubol S, Chareonsirisuthigul T, Kasisith J, Klungthong C. Clinical isolates of dengue virus with distinctive susceptibility to nitric oxide radical induce differential gene responses in THP-1 cells. Virology. 2008,376(2): 290-6.doi.org/10.1016/j.virol.2008.03.030

[6] 6. Norazharuddin H, Lai NS. Roles and prospects of dengue virus non-structural proteins as antiviral targets: an easy digest. The Malaysian Journal of Medical Sciences: MJMS. 2018,25(5):6. doi.org/10.21315/mjms2018.25.5.2

[7] 7. Perera R, Kuhn RJ. Structural proteomics of dengue virus. Current opinion in microbiology. 2008,11(4):369-77. doi.org/10.1016/j.mib.2008.06.004

[8] 8. Halim SA, Khan S, Khan A, Wadood A, Mabood F, et al. Targeting dengue virus NS-3 helicase by ligand-based pharmacophore modeling and structure based virtual screening. Frontiers in Chemistry. 2017; 5: 88.doi.org/10.3389/fchem.2017.00088

[9] 9. Limon-Flores AY, Perez-Tapia M, Estrada-Garcia I,

DOI: https://doi.org/10.54393/pbmj.v5i4.341

Vaughan G, Escobar-Gutierrez A, et al. Dengue virus inoculation to human skin explants: an effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells. International journal of experimental pathology. 2005,86(5): 323-34.doi.org/10.1111/j.0959-9673.2005. 00445.x

[10] Durbin AP, Vargas MJ, Wanionek K, Hammond SN, Gordon A, et al. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. Virology. 2008,376(2):429-35. doi.org/10.1016/j.virol.2008.03.028

[11] Organization WH, Research SPf, Diseases TiT, Diseases WHODoCoNT, Epidemic WHO, Alert P. Dengue: guidelines for diagnosis, treatment, prevention and control. Switzerland: World Health Organization; 2009.

[12] Wang SM, Sekaran SD. Evaluation of a commercial SD dengue virus NS1 antigen capture enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. Journal of clinical microbiology. 2010,48(8): 2793-7.doi.org/10.1128/JCM.02142-09

[13] Hue KDT, Tuan TV, Thi HTN, Bich CTN, Le Anh HH, et al. Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. Journal of virological methods. 2011,177(2):168-73. doi.org/10.1016/j.jviromet.2011.08.002

[14] Rao AA, Gosavi S, Menon S. Dengue Fever: Prognostic Insights from a Complete Blood Count. Cureus. 2020,12(11).

[15] Nascimento EJ, George JK, Velasco M, Bonaparte MI, Zheng L, et al. Development of an anti-dengue NS1 IgG ELISA to evaluate exposure to dengue virus. Journal of virological methods. 2018, 257:48-57. doi.org/10.1016/j.jviromet.2018.03.007

[16] Angelo M, Ramalho WM, Gurgel H, Belle N, Pilot E. Dengue surveillance system in Brazil: A qualitative study in the federal district. International Journal of Environmental Research and Public Health. 2020,17(6):2062. doi.org/10.3390/ijerph17062062

[17] Sun J, Lu L, Wu H, Yang J, Xu L, et al. Epidemiological trends of dengue in mainland China, 2005–2015. International Journal of Infectious Diseases. 2017,57:86-91. doi.org/10.1016/j.ijid.2017.02.007

Pepin KM, Lambeth K, Hanley KA. Asymmetric competitive suppression between strains of dengue virus. Bmc Microbiology. 2008,8(1):1-10. doi.org/10.1186/1471-2180-8-28

[19] Wiwanitkit S, Wiwanitkit V, Broor S. Rapid diagnosis of dengue infection in acute phase/Author's Reply. Journal of Vector Borne Diseases. 2015,52(1):110.

[20] Ageep AK, Malik AA, Elkarsani MS. Clinical presentations and laboratory findings in suspected cases of dengue virus. Saudi medical journal. 2006,27(11):1711-3.

[21] Dhanoa A, Hassan SS, Ngim CF, Lau CF, Chan TS, et al. Impact of dengue virus (DENV) co-infection on clinical manifestations, disease severity and laboratory parameters. BMC infectious diseases. 2016,16(1):1-14. doi.org/10.1186/s12879-016-1731-8