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DENGUE FEVER;

ROLE OF NON STRUCTURAL PROTIEN-1 (NS-1) POSITIVITY IN DENGUE CASES: AN EXPERIENCE FROM A QAZI HUSSAIN AHMED MEDICAL COMPLEX NOWSHERA.

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ABSTRACT… Objectives: To determine the frequency and characteristics of dengue patients. **Study Design:** Cross sectional observational study. **Setting:** Qazi Hussain Ahmed Medical Complex Nowshera. **Period:** 5th July to 25th Sept 2017. **Material and Methods:** A total of 72 cases were received for dengue serology. Relevant information's were collected on a pre-designed questionnaire prepared in accordance with the objectives of the study. **Results:** A total of 117 patients were referred from fever clinic and emergency OPD for dengue serology. 72(61.5%) were males and 45(38.5%) were females. 24(20.5%) cases were dengue positive. 14(12%) were NSL1 positive, 8(8.8%) were IgM positive and 2 (1.7%) were IgM&IgG positive. We received patient in the range of 4 years to 60 years, Mean with SD was 27 +3 years. Out of 14 NSL1 positive cases 8 were males and 6 females. 2 females were IgG positive. The spectrum of dengue in correlation with gender was significantly positive with p value .026. In two cases platelet at first visit were 58000/cmm^3 that were both IgM&IgG positive. Out of 24 positive dengue cases two cases were also positive for plasmodium vivax (ring tropozoites). 6 cases were managed in hospital and discharged home with an average stay of 3 days and 4 cases referred to Lady Reading Hospital Peshawar for repeated platelet transfusion. Mortality was zero in our cases. **Conclusion:** The suspicion rate of the clinician for dengue from fever clinic was 1:7. The cause of poor rate can be contributed to the patient insist for doing the dengue test before they are screened for MP and FBC etc. NSL1 was positive in 6 cases that shows that people reach the health care facility for screening well in time and patient are educated about the dengue. Females 50% positive cases were IGM and IGG positive that shows female receive the health care later than males as NSL1 positivity in female gender is less than males. The spectrum of dengue in correlation with gender was significantly positive with p value .025 that shows mosquito has some affinity for specific gender, or dengue virus has it for difference in gender or the inside immunity of the both gender is involved that causes different mode of presentation and activation of antibodies.

Key words: Dengue Fever, Nowshera, NS1 Positivity.

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INTRODUCTION

The clinical and demographic presentation of dengue disease has changed tremendously in our country in the last two decades as reported in the literature. Very little data so for published in the form of research studies have been reported from our country that highlights different aspects of individual outbreaks of dengue fever.[1] Regarding the structure of the Dengue Virus it is single-strand RNA virus with member of flaviviridae group, and genus flavivirus. It has four serologic subtypes as; DV-1, DV-2, DV-3 and DV-4. Worldwide 50-100 million cases of Dengue Fever (DF) are reported each year and 250,000 to 500,000 cases of Dengue Hemorrhagic Fever (DHF) are reported annually in literature.[2] In 1994 Pakistan the first outbreak due to serotype DV-2 was reported by Aga Khan University Hospital (AKUH) in Pakistan.[3] AKUH again reported in December 2005, from Karachi that three main hospitals of the city are loaded with a sudden outbreak of patients with Dengue hemorrhagic fever. This time the Genotyping of this outbreak revealed the presence of DV-3. This epidemic was

correlated with the introduction of DV-3 in patients previously sensitized to DV-1 and DV-2 resulting in a very severe form of disease. After that our country experienced its largest and most severe outbreak of DHF in 2006 and DV-2 and DV-3 were isolated as the main serotypes in these cases.[4,5] However, there is short of data reported in medical literature regarding the overall infectivity of DV in our country. The Clinical and pathological features in Dengue infection are not well understood due to its different characteristics that all the four serotypes represent invariably in different parts of the country. Primary infection caused by any type of the dengue virus leads to mild or moderate clinical disease that lasts for approximately 4- 6 months in the body and then body develops a lifelong immunity to that specific infecting type of DV.[6] In 2003, Dengue virus was reported from Haripur district of Khyber Pukhtoonkhwa where 1000 individuals were approximately infected and 7 deaths reported. There DV- 2 was the main culprit serotype for causing infectivity. The same year 2003 in khushab and Nowshera (our study district) a total of 2500 cases were reported and 11 deaths notified. DV- 2 was found in 7 patients out of 17 cases studied. In 2004 25 dengue cases were reported from Islamabad and Karachi. In 2005, Karachi experienced death of 13 patients out of total reported 500 cases. In 2006, dengue epidemic was reported in Karachi, Sukkar, Nawabshah, Rawalpindi and Islamabad where 5400 total cases were reported and 55 deaths notified.[7-8]

We tried to maintain a data and serum bank of the dengue patients presenting to Qazi Hussain Ahmed Medical Complex Nowshera, a teaching hospital, for the presence of dengue NS-1 Protein and IgM & IgG antibodies from 5th July to 25th Sept 2017. In present study, we evaluated clinical and laboratory features of dengue patients hospitalized with dengue fever during the said period.

MATERIAL AND METHODS

This cross sectional observational study was conducted in Qazi Hussain Ahmed Medical Complex Nowshera from 5th July to 25th Sept 2017. A total of 72 cases were received for dengue serology were enrolled irrespective of age and sex. 5 ml of blood was taken from patient, 3ml in gel tube for detecting IgM &IgG and 2ml in EDTA tube for NS-1(Non structural protein 1). Two drops of buffer was added. ICT method used for detection of NS-1 and IgM & IgG.

Result entered on the proforma and data entered in SPSS version 16 for analysis. The sampling was random and purposive only. The demographic variables were age, sex and address of the patients, while the research variables were types of antigenic protein and IgM & IgG, platelets count and co infection with malarial parasite. All the variables presented in percentages to find out the frequencies for each variable.

RESULTS

A total of 117 patients were referred from fever clinic and emergency OPD for dengue serology. 72(61.5%) were males and 45(38.5%) were females (Table-I). We received patient in the range of 4 years to 60 years, Mean with SD was 27 +3 years. (Table-II). 24(20.5%) cases were dengue positive. 14(12%) were NSL1 positive, 8(8.8%) were IgM positive and 2 (1.7%) were IgM &IgG positive. Out of 14 NSL1 positive cases 8 were males and 6 females. 2 females were IGG positive (Table-III). The spectrum of dengue in correlation with gender was significantly positive with p value .026. (Table-IV). In two cases platelet at first visit were 58000/cmm^3 that were both IgM&IgG positive. (Table-V). Out of 24 positive dengue cases two cases were also positive for plasmodium vivax (ring tropozoites). (Table-VI).

		Frequency	Percent
Valid	Male	72	61.5
	Female	45	38.5
	Total	117	100.0

Table-I. Gender wise distribution

	Valid	117
	Missing	0
Mean		27.58
Median		25.00
Mode		35.00
Std. Deviation		3.27
Range		56.00
Minimum		4.00
Maximum		60.00

Table-II. Age statistics

		Frequency	Percent
Valid	Negative	93	79.5
	NSL1	14	12.0
	IGM	8	6.8
	IGM+IGG	2	1.7
	Total	117	100.0

Table 3. Dengue spectrum

		Dengue Test				Total
		negative	NSL1	IgM	IgM + IgG	
sex	Male	58	8	4	2	72
	Female	35	6	4	0	45
Total		62	93	14	8	2
Pearson Chi-Square (P-Value)						.026

Table-IV. Sex vs Dengue test cross tabulation

		Dengue Test			Total
		NSL1	IgM	IgM + IgG	
Platlet	58000.00	0	1	1	2
	88000.00	1	2	0	3
	89000.00	1	0	0	1
	115000.00	1	2	0	3
	116000.00	1	0	1	2
	117000.00	1	0	0	1
	119000.00	2	1	0	3
	121000.00	0	1	0	1
	125000.00	1	1	0	2
	128000.00	6	0	0	6
Total		14	6	2	24
Chi square p-value					0.3

Table-V. Platelets in dengue positive cases

MP		Value
Negative	Number of Cases	22
Positive	Number of Cases	2
Total	Number of Cases	24

Table-VI. Malarial parasites positivity in Dengue cases.

DISCUSSION

Dengue disease incidence is commonly have been reported in the rainy and wet season in our part of the country. In Pakistan the it is commonly reported to occur from August to October months period in Pakistan.[9]

In our study 72(61.5%) were males and 45(38.5%) were females. We received patient in the range of 4 years to 60 years, Mean with SD was 27 +3 years. Similarly a study from Aga Khan University also reported that highest proportion of seropositivity was noted in patients with age ranging 26–40 years.[1] Median age in contrast in patients with dengue decreases in our study. Another study reported that most common age group affected by dengue was 11–25 years in that correlates with our findings.[2] We also observed a gradual increase in the number of children affected by DV in our population.

In our study 24(20.5%) cases were dengue positive. Out of 24 total positive cases, 14(58.33%) were NSL1 positive, 8(33.33%) were IgM positive and 2 (8.33%) were IgM &IgG positive. Out of 14 NSL1 positive cases 8 were males and 6 females. 2 females were IGG positive. In another study from Pakistan reported samples that were assayed for both IgM and IgG antibodies detection against the antigen of dengue viruses. They reported 90.8% of their cases were IgM positive and 35.5% were IgG antibody positive. They recorded 24% of their NSI protein positive cases that were consequently negative for both IgM and IgG on ELISAs that elaborates that NS1protein positivity alone does not confirm dengue infection.[10] The antigen non-structural protein-1 is immunogenic and is related to dengue viremia but should not be all alone contributed to the disease status. Assays including immunochromatographic tests (ICT) for DV and enzymatic immunoassays (ELISA) that detect NS1 in plasma, serum, and whole blood. These tests are useful for incidence studies. But ICT positivity for NS1 does not solely confirm the DV infection.[11] A Meta analysis report showed that NS1 assay has intermediate sensitivity (ranging 34% to 76%) and high specificity for dengue disease diagnosis.[12] But when it combines with IgM positivity then sensitivity increases in tens for dengue infection.[13]

A severe outbreak that was reported in Santos, Brazil in 2010. They did studies for diagnosis of dengue NS1 antigen detection along with the detection of IgM, IgG and RNA by PCR Techniques. They noted that false NS1 negative

results were noted in majority of the cases. They evaluated the 379 positive NS1 cases followed by PCR- RNA only 37.7% cases NS1 positive were positive for dengue diseases on PCR.[14]

In another study from Sri Lanka six assays were conducted on 259 positive NS1 patients, out of which 99 were confirmed dengue and 160 patients were only febrile illnesses and not positive dengue cases. As we also noted in two cases that were Malrial parasite positive. NS1 antigen sensitivity reported in another study was 49% to 59% while detection of IgM antibodies for DV showed a sensitivity of 71% to 80%. By combining bothe the ICT and assays of IgM antibody and NS1 antigen an increased in the sensitivity upto 93% was noted.[15]

Secondly high rate of false dengue infection by NS1-ICT should be discouraged as low importance of NS1 protein in diagnosis and need of more sensitive test for proper diagnosis specially IgM and IgG or by ELISA or PCR-RNA techniques must be given importance before labeling a patient as Dengue positive.

If Khyber Pukhtoon Khawa our state has to control dengue infection we need a multi disciplinary response starting from the individual response till the advocacy and social mobilization based on lessons learned from other countries, usage of the latest technologies keeping on board all the stake holders in dengue case management and need to be addressed as a national emergency policy with commitment of resources at all levels.

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Remember, the greatest failure is not to try.

– Unknown –

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Hamzullah Khan	Data collection, paper writing, editing, analysis of data, article formulation.	
2	Fazli Bari	Paper review, editing, supervision, analysis, article formulation.	