Seprevalence and Epidemiology of Dengue NS-1 Antigen with Its Seasonal Variation Diagnosed At Tertiary Care Hospital, Jamnagar, Gujarat (India)

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Abstract: Dengue is one of the most serious mosquito-borne viral infections affecting tropical and subtropical countries in the world. Since there is no immune prophylactic or specific antiviral therapy available, timely and rapid diagnosis plays a vital role in patient management and implementation of control measures. The present study was planned to diagnose the dengue infection by detecting dengue NS-1 antigen & to study the seroprevalence of dengue NS-1 antigen. Dengue NS-1 antigen testing done by enzyme linked immunosorbent assay (ELISA) was performed during July-2017 to September-2018 and the data were analyzed retrospectively. A total 3397 serum samples received from Guru Gobindsingh Hospital (O.P.D. & Indoor) for the detection of Dengue NS-1 Ag. Out of 3397 samples, total 841 (24.48%) were Positive for Dengue NS1 Ag among this, 548 (65.16%) male and 293 (34.84%) female. And 307 (36.50%) were 0-20 years of age, 448 (53.27%) were 21-40 years of age and 86 (10.23%) were >40 years of age. Present study shows that dengue fever is seen more in 21-40 years of age group and more common in male compare to female. Effective implementation of vector control measures through efforts toward vector breeding source reduction and with the use of personal prophylactic measures against mosquito bites will help in reducing the dengue prevalence in the community.
Keywords: Dengue, seroprevalence, Aedes aegypti, NS1antigen, ELISA.

INTRODUCTION

Dengue virus infection is an important mosquito borne Arboviral infection of human. The global incidence of Dengue Fever (DF) and Dengue Hemorrhagic Fever has increased dramatically in recent decades [1]. It is a very rapidly growing public health problem currently faced by people living in tropical and sub-tropical countries. It is transmitted by Aedes aegyptii and Aedes albopticus which are day biting mosquitoes. It affects more than 2.5 billion people annually and 975 million people who reside in tropical and sub-tropical countries in South East Asia, the Pacific and the America with Africa bearing the major burden of the disease accounting to 900 million cases annually [2].

It is a flu like illness that affects all age groups. Epidemics are more frequently occur during monsoon and post monsoon period. It is maintained in nature through a biological transmission between susceptible vertebrate hosts by haematophagous arthropods [3].

Dengue fever is caused by dengue virus, a positive stranded RNA virus in the genus Flavivirus, belonging to family Flaviviridae [4]. Till now, dengue fever was believed to be caused by four different serotypes that share antigenic relationships (DENV-1, DENV-2, DENV-3 and DENV-4). The fifth variant DENV-5 has been isolated in October 2013 which follows the sylvatic cycle unlike the other four serotypes which follow the human cycle [5]. Principle vectors of transmission for Dengue infection are arthropods of the Aedes genre, especially Aedes aegypti and Aedes albopictus. In tropical areas, maximum transmission of disease occurs in the months of rainfall [6] owing to increased breeding of vectors in various water collection sites like old tyres, coolers, old earthenware pots, coconut shells etc [7]. Density of mosquito population will be high (3-4 female mosquitoes per house) during the rainy season as compared the dry season (1-2 female mosquitoes per house) [8].

Dengue viral infection in human causes a wide spectrum of illness from asymptomatic or mild febrile illness, i.e. Dengue Fever (DF), which may evolve to severe disease form like Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [4]. The characteristic symptoms of dengue are sudden onset of fever, severe headache, retro-orbital pain, muscles, joint and bone pain (the alternative name for dengue, "break bone fever" comes from associated muscle and joint pain), macular or maculopapular rash and minor haemorrhagic manifestation, including petechiae, ecchymosis, purpura, epistaxis, bleeding gums, haematuria or positive tourniquet test result [9, 10]. The dengue virus genome is about 11,000 base of positive-sense single stranded RNA (ssRNA) that coded for 3 structural proteins (capsid protein C, membrane protein M, envelope protein E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5), it also included short noncoding region on both the 5' and 3' end[11].

MATERIALS AND METHODS
Study design

A retrospective study was conducted at Microbiology Department of M. P. Shah Govt. Medical College and Guru Gobindsingh Hospital Jamnagar, Gujarat (India) from July 2017 to September 2018. Samples were received to Microbiology Department from indoor patient who admitted in hospital, outdoor patients, community and primary health center, of suspected cases of dengue fever is characterized by fever lasting for 0-5 days. A total 3397 samples were collected and processed for detection of dengue virus NS-1 antigen in patient's serum by Dengue NS-1 antigen enzyme linked immunosorbent assay (ELISA) method.

Specimen selection criteria

Sample collected within 5 days of onset of Fever.

Sample collection and storage

Patients suspected of Dengue fever were examined by hospital clinicians at either outpatient services or for inpatients, when attending the emergency unit or upon admission to a ward. A single blood sample (approximately 2-3 ml) was collected from each patient suspected of Dengue virus infection at the time of admission into hospital. Specimen collection and separation of serum were performed using strict aseptic precautions and following standard microbiological methods. Serum samples for ELISA test were prepared and stored at 2-8°C until tested.

Detection of dengue ns-1 antigen by elisa method

Serum samples were screened for dengue NS-1 antigen at Department of microbiology of M. P. Shah Govt. Medical College Jamnagar by PLATELIA[tm] DENGUE NS 1 ELISA kit manufactured by BIO-RAD was used. PLATELIA[tm] DENGUE NS -1 Ag is one step sandwich format micro-plate enzyme immunoassay for the qualitative detection of dengue virus NS 1 antigen in human serum. The test uses murine monoclonal antibodies (MAb) for capture and revelation. Samples directly incubated with conjugate in ELISA micro-plate wells sensitized with MAb. If antigen is present in sample, an immune-complex MAb-NS 1-MAb/peroxidase formed. After a washing step, the presence of immune-complex is demonstrated by distribution in well of a chromogenic solution initiating a color development reaction, them enzymatic reaction by an acid solution. And the optical density (OD) measured by ELISA reader at 450/620nm.

Interpretation

- If sample ratio (OD/cut off) is <0.50 considered non-reactive for Dengue NS-1 antigen.
- If sample ratio (OD/cut off) is ≥1.0 considered reactive for Dengue NS 1 antigen

RESULTS AND DISCUSSION

Out of the 3397 cases tested, 841(24.48%) were positive for Dengue NS-1 Antigen.

**Table-1: Sero-prevalence of Dengue NS-1 Antigen**

Sample Tested	Positive Sample	Sero-prevalence (%)
3397	841	24.48%

Out of these 841 positive samples, males were 548(65.16%) and females were 293(34.84%) (Table-2 & Figure-1).The chi-square statistic is 5.306 and P-value is 0.02125 this show male to female ratio was statistically significant (P-value <0.05)

Table-2: Sex wise Sero-positivity of Dengue NS-1 Antigen

|	Total Samples	Positive Samples (%)	Chi-square	P value
Male	2067	548 (65.16%)		
Female	1330	293 (34.84%)	5.306	<0.05
	3397	841 (100%)		
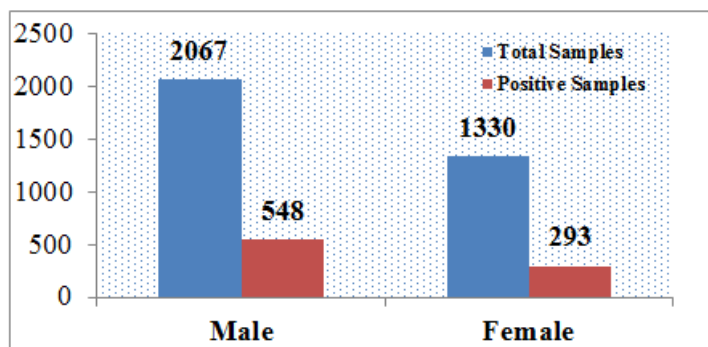
Fig-1: Sex wise Sero-positivity of Dengue NS-1 Antigen

Among the total positive case, 307(36.50%) were between 0 to 20 years of age groups, 448(53.27%) were between 21-40 years of age years of age groups, 86(10.23%) were from>40 years of age groups. The chi-square statistic is 33.826 and P-value is 0.00001, this show age groups wise distribution of dengue NS 1is statistically significant (P value <0.01) (Table-3).

Table-3: Age-group wise Sero-positivity of Dengue NS-1 Antigen

Age(Years)	Total Samples	Positive Samples (%)	Chi-square	P value
0-20	1174	307 (36.50%)	33.826	<0.01
21-40	1594	448 (53.27%)		
>40	629	86(10.23%)		
	3397	841		
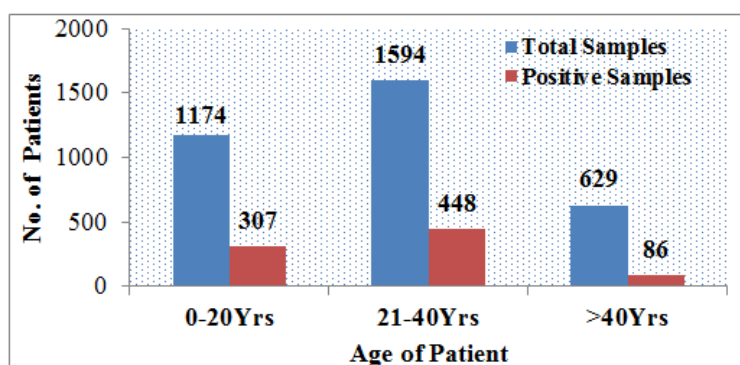
Fig-2: Age-group wise Sero-positivity of Dengue NS-1 Antigen

Table-4: Month wise distribution of Dengue NS-1 Antigen Cases

Month	No. of cases (n=3397)	Positive cases (n=841)
July-17	147	16(1.90%)
August-17	214	47(5.59%)
September-17	445	140(16.65%)
October-17	760	313(37.22%)
November-17	605	191(22.71%)
December-17	273	49(5.83%)
January-18	132	2(0.23%)
February-18	78	2(0.23%)
March-18	44	1(0.12%)
April-18	42	0(00)
May-18	69	8(0.95%)
June-18	73	5(0.59%)
July-18	79	7(0.83%)
August-18	134	17(2.21%)
September-18	302	43(5.11%)

Seasonal Variation of Positive Dengue NS-1 antigen Cases

In this study, out of 841 confirmed positive cases for dengue NS-1 antigen. Highest positive case (553 positive cases) was found in between October 2017 to December 2017 in post-monsoon season, followed by 203 positive cases was found in July 2017 to September 2017 and 67 positive cases was found in July 2018 to September 2018 in monsoon season (Table-4).
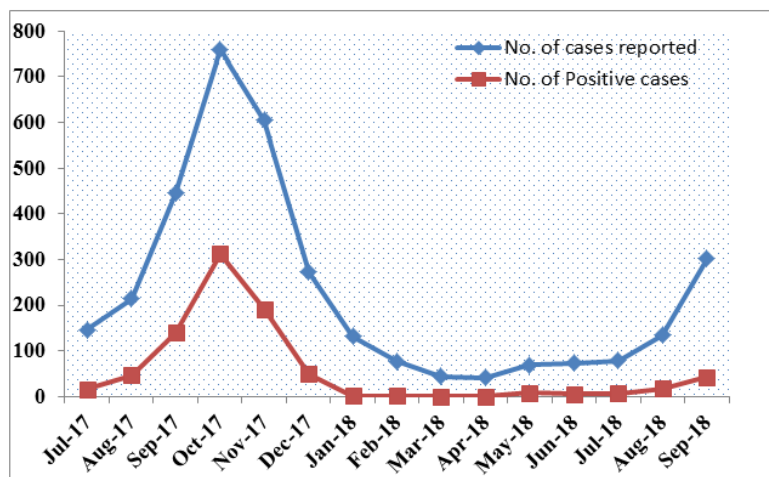

Fig-3: Month wise distribution of Dengue NS-1 Antigen cases

Dengue fever is a dangerous and depilating disease, and it's growing threat to global health. Dengue is the second most widespread in the world [4]. The world health organizations have estimated that between 50 to 100 million people suffer from dengue fever. The biggest issue is that dengue fever is spreading fast. In the present study, 24.48% patients were serologically positive for dengue NS-1 antigen infection. This higher prevalence rate may be due to endemic nature in the country. The age wise distribution of sero-positive cases was also statistically significant towards males over females. This difference is probably due to a gender related variance in lifestyle, farming, and other occupations.

Table-5: Comparison of Seroprevalence of Dengue NS1 Antigen

Study	Sero-prevalence	Male	Female	0-20 years	21-40 years	Monsoon (July-Sept-17)	Post-monsoon (Oct-Dec-17)
Pankti pargi *et al*., [12]	26.56%	72.04%	27.96%	35.33%	54.37%	25.04%	67.41%
Rohitash K *et al*., [13]	18.75%	66.70%	33.30%	-	-	-	-
Lata R patel *et al*., [14]	16.30%	66.47%	33.53%	-	-	-	-
Present study	24.48%	65.16%	34.84%	36.50%	53.27%	24.13%	65.75%

CONCLUSION

Study shows that Dengue fever is more seen in 21-40 years of age. In this present study Dengue NS 1 antigen is more common in male compare to female. Timely preventive and control measures are essential for early detection of an impending outbreak and to reduce the prevalence rate. Dengue cases were more during September to November in the post-monsoon season which is useful to plan special preventive strategies. The study draws attention toward the male, young adult age group. Dengue infection is no more an urban area infection but it has penetrated in rural areas also.

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