Research Paper

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Sero Prevalence of Dengue Ns-1 Antigen in S.P.Medical.College, Bikaner (Raj)

Medical Science

KEYWORDS : Dengue infection, NS - 1 protein, rapid diagnostic test

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ABSTRACT

Introduction: 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

Material and Methods: Dengue NS-1 testing by immunochromatography was performed during sept and the data were analyzed retrospectively. A total 928 serum samples sent from Department of Medicine, Sardar Patel Medical College, Bikaner for the detection of Dengue NS-1Ag.

Results: Total samples tested were 928 out of which NS-1 seropositive were 174 (18.75%). All positive were confirmed by NS 1 ELISA test. Male:female ratio was 2:1 .More nos of cases were seen in age group 20- 30 years that is 92 (52.87%) .Urban:Rural ratio was 4:1 . Fever was the commonest presentation in all suspected patients 928 (100%) associated with headache in 898, associated with muscle pain in 814 then fever with headache with muscle pain in 847. Fever with rash in 32, fever with retroorbital pain in 8 and fever with haemorrhagic manifestation in 7 patients were observed. Patients with platelet count less than 50,000 were 39 (22.41%), 50,000 to < 1,00,000 were 58 (33.33%) and > 1,00,000 were 77(44.25%). According to day of fever, highest nos of seropatients pts were seen in 4th day that is 112 (64.36%) that followed by 3rd day that is 35 (20.11%) and from that more nos of seropositive male 68 (39.08%) and seropositive female 47(27.01%) were seen in 4th day of fever that is followed by on 3rd day 20 (11.49%) seropositive male and 4 (2.29%) seropositive female.

Conclusion: New dengue virus strains and serotypes will likely continue to be introduced into urban areas where the densities of Aedes aegypti are at high levels. So, for the early and rapid diagnosis NS 1 immunochromatography are very helpful in dengue infection.

INTRODUCTION:

Dengue fever is an important mosquito – borne viral disease of humans. This has been a recurrent phenomenon throughtout the tropics in the past decade. Annually, there are an estimated 100 million dengue virus infections worldwide[1].

Increasingly, cases of the more severe and potentially lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are reported with children bearing much of the disease burden[2]. The mortality rate of DHF in most countries is 5%, primarily among young children and adults [3]. Dengue virus is an enveloped positive sense RNA virus. The genomic RNA is approximately 11 Kb in length and is composed of three structural protein genes that encode for nucleocapsid or core protein(C), a membrane – associated protein (M) , an envelope protein (E), and seven non structural (NS) protein genes including NS 1 protein [4]. Among the non-structural proteins, NS 1 is highly conserved glycoprotein which appears essential for virus replication; no precise function has yet been assigned to it. During acute dengue virus infection, NS 1 is found associated with intracellular organelles or is transported through the cellular secretary pathway to the cell surfac[5-7]. Now days, detection of NS-1 Ag on rapid tests offer an even faster route to a presumptive dengue diagnosis. NS-1 (Non structural protein) is a highly conserved glycoprotein that is essential for the viability of dengue virus & is produced both in membrane associated & secretary forms by the virus. The detection of secretary NS-1 protein represents a new approach to the diagnosis of dengue infection[8]. It possesses not only group – specific but also type specific determinants and has been recognized as an impotant antigen in dengue infection[9].

There are four serotypes of dengue designated dengue 1 through dengue 4 (DEN 1 to DEN 4) that is antigenically related (Monath and Heinz, 1990). Recovery from infection by one serotype can confer life-long protection against that serotype; however, it provides only partial, transient immunity against subsequent infection by the other three dengue serotypes. All four serotypes cause disease, usually asymptomatic or mild dengue fever (Hal-stead, 1988). Progression from DF to DHF and DSS usually occurs after a second infection with a different serotype, which is due to immune-mediated enhancement of infection, known as antibody-dependent enhancement (ADE) (Halstead, 1988)[10]. The present study aim to diagnose the dengue infection by detecting dengue NS-1 antigen**,** also to study the seroprevalence of dengue NS-1 antigen and to establish their role for early diagnosis.

MATERIAL & METHODS:

Inclusion Criteria:

Clinically suspected Patients experiencing febrile illness consistent with dengue fever with two more of the following manifestations:

- Headache
- Muscle pain
- Haemorrhagic manifestation
- Retro-orbital pain
- Rash

Patients & study design:

Human blood samples from clinically suspected patients of dengue infection were collected in sufficient quantity from september 2013 to Oct. 2014 of the OPD & indoor patients of Department of Medicine, Sardar Patel Medical College, Bikaner. A total 928 serum samples were tested for the detection of NS 1 Ag.

The dengue NS 1 antigen rapid test is an in vitro immunochromatographic , one-step assay designed for the qualitative determination of dengue virus NS 1 antigen in human serum or plasma for the diagnosis early acute dengue infection. The test device contains a membrane strip, which is pre coated with anti dengue NS 1 Ag capture on the test band region. The anti dengue NS 1Ag – colloid gold conjugate and serum move along the membrane chromatographically to the test region "T" and forms a visible line as the antibody-antigen-antibody gold particle complex forms. This test also can detect all 4 dengue serotypes by using a mixture of recombinant dengue envelope proteins.

Remove the test device from the foil pouch and place it on a flat surface. With a disposable dropper, add 3 drops (100µl) of patient's serum was added into the sample well marked "S" and the test result was interpreted in 15-20 min[11].

Interpretation of the SD Bioline Dengue duo rapid test:
The presence of only one colour line within the result window indicated negative result and the presence of two colour lines ("T" band and "C" line) indicated a positive result. When no control line (C) was found the test was considered as invalid[11].

OBSERVATIONS & RESULTS:
Total 928 human samples were subjected to Dengue NS 1 Ag by immunochromatpgraphy based rapid testing. 174 (18.75%) were seropositive for dengue NS 1 Ag. All seropositive samples were confirmed by dengue NS 1 ELISA test. Highest no. of seropositive cases were noted in September and October and november months of the year 2013 and 2014. We observed maximum number of patients coming from urban region 743 (80.06%) of urban area while 185 (19.94%) were from rural area, so, urban: rural ratio is 4:1. More seropositive cases were noted in urban region. Male: female ratio of 2 : 1 with maximum seropositive cases were noted in age group 20 – 30 yrs that is 92 (52.87%).

The most common symptoms apart from fever were headache, muscle pain and rashes and less common retroorbital pain & haemorrhagic manifestations were observed. Patients with platelet count less than 50,000 were 39 (22.41%), 50,000 to < 1,00,000 were 58 (33.33%) and > 1,00,000 were 77(44.25%).

According to day of fever, highest nos of seropatients pts were seen in 4th day that is 112 (64.36%) that followed by 3rd day that is 35 (20.11%) and from that more nos of seropositive male 68 (39.08%) and seropositive female 47(27.01%) were seen in 4th day of fever that is followed by on 3rd day 20 (11.49%) seropositive male and 4 (2.29%) seropositive female.

DISCUSSION:
Dengue infection presents with nonspecific fever that mimics other viral illnesses. The availability of commercial ELISA assays to detect the DEN virus NS 1 protein in acute plasma provides an additional dengue diagnostic tool to the existing approaches of PCR , antibody capture ELISA and less frequently virus isolation [7,8,9] . The assessment of NS 1 antigen detection assays as diagnostic tool to the existing dengue diagnostic algorithms. In order to provide timely information for the management of the patients and early public health control of dengue outbreak it is important to establish the diagnosis of acute dengue virus infection during the first few days after manifestation of clinical symptoms.

The seasonality of transmission of dengue with increased activity in the post – monsoon season was seen in the present study ; in accordance with the reported patterns of dengue transmission. Even in the post epidemic period increased dengue virus activity was seen in post monsoon period september to November months of the year 2013 and 2014.These findings indicate that dengue infections are mostly seen in post – monsoon season hence preventive measures should be in full swing at the very onset of the monsoon. Another explanation can be the heavy rains of monsoon season, which usually start in July, August, resulting in stagnant water that serves as breeding ground for vectors of this virus and lead to increased activity in post monsoon period. That is compared with study of Ekta Gupta, Lalit Dar et al[12] in2006. The reasons may due to on these months large stores of water. Also the breeding habit of *Aedes aegypti* is highest during pre and post monsoon period. But sporadic cases extend up to December which indicates endemicity of the infection up to December.

Female aedes mosquito, the vector of the virus is peridomestic in nature. The tropical zones of the world having monsoon rains are the usual habitat of this vector[9]. The breeding of *Aedes aegepti* is highest during pre and post-monsoon period [10]. We found the epidemic in post monsoon period. Major contributory factors to this increased activity may be due to changes in weather pattern such as El-Nino phenomenon[2].

In present study , higher nos of males were affected than females and maximum number of cases were between 20 – 30 years of age group observed that is correlated with study done by Halstead [19] had pointed out as early as 1970 that males predominate among those with milder disease but females account for more severe illness. He suggested that either immune response in females are more competent than in males, resulting in greater production of cytokines, or the capillary bed of females is prone to increased permeability. Kaplan[17] in Mexico suggests that an incidence bias in favour of females is related to the timing of the survey interviews, while Goh[20] puts forward that low incidence among women occurs because they stay at home and are less exposed to infection[17,18] Gupta *et al*[12] from India showed a maximum number of cases between the ages of 21 to 30 years.

In present study, urban populations were highly affected that is 80.06% than rural population having 19.94%. Historically, DF/DHF has been reported as occurring predominantly among urban populations where density of dwellings and short flying distance of the vector create the right conditions for transmission[21]. Increased transport contact, mobility and spread of periurbanisation have been the most frequently cited reasons for spread of dengue to rural areas [22].

Data obtained in this study show DEN virus antigens were detected from as early as Day 1 (5.8% of samples) up to Day 9 (0.4% of samples) of fever. These findings are comparable to a study by Alcon *et al* [23] in 2006 who recovered NS1 antigen until day 9 of symptoms. Antigen detection was highest between Days 3 and 4 with a detection rate ranging from 12% to 38%.

CONCLUSION:
High prevalence rate in our region particularly in pre monsoon and monsoon season gives an alarm to the doctors regarding early and accurate diagnosis of dengue virus infection and its complications. Prompt diagnosis of evaluation of rapid dengue NS1 antigen test showed that this test

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is highly appropriate for diagnosis of dengue infection as it has proven to be a rapid, easily applicable, sensitive and specific method. NS1 antigen detection has potential value for screening patient samples during the early acute phase. It is rapid, easily be performed, interpreted early and has a extended shelf life. We conclude that rapid test is an effective tool, if when used in combination with NS 1 MAC ELISA in single sample of suspected cases, has the ability to improve the diagnostic algorithm contributing significantly to clinical treatment and to control dengue viral infection.

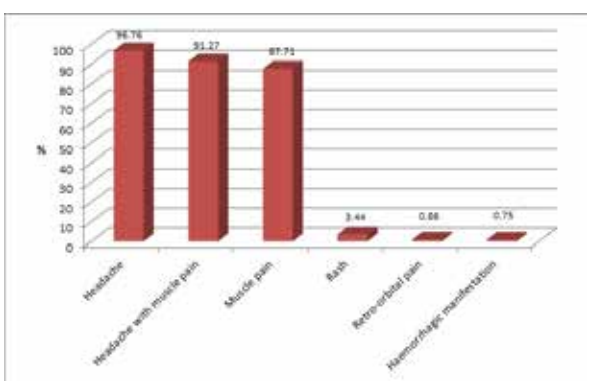
TABLE 1: Cases according to sex distribution.

	No. of Patients	%
Male	619	66.70
Female	309	33.30
Total	928	100

TABLE 2: Distribution of patients according to presenting symptom.

Commonest Presentation with fever	No. of Patients	%
Headache	898	96.76
Headache with muscle pain	847	91.27
Muscle pain	814	87.71
Rash	32	3.44
Retro-orbital pain	8	0.86
Haemorrhagic manifestation	7	0.75

FIGURE 1: DISTRIBUTION GRAPH OF PATIENTS ACCORDING TO PRESENTING SYMPTOMS.



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