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PREVALENCE OF DENGUE FEVER AND COMPARATIVE ANALYSIS OF PRIMARY AND SECONDARY DENGUE CASES IN A TERTIARY CARE HOSPITAL, AMRITSAR, PUNJAB.

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ABSTRACT

Background: The most challenging problem associated with patient management in Dengue infection is early diagnosis. Secondary infection with dengue virus is the most accepted risk factor for the development of dengue haemorrhagic fever. Confirmation of Dengue infection, this, is the most essential prerequisite in the management of its complications, hence reducing morbidity and mortality associated with it

Aim: To determine the prevalence of Dengue Fever cases in a tertiary care hospital, Amritsar, Punjab, by serological tests IgM ELISA and IgG ELISA, to differentiate between the primary and secondary dengue cases.

Methods: A cross sectional comparative study in patients with fever, testing positive for NS1 antigen and IgM ELISA designated as confirmed Dengue fever were included in the study. Detailed history was obtained and complete clinical examination was done. To distinguish between primary and secondary infections, IgG ELISA was performed in all the samples, which was markedly raised in patients with secondary infection.

Results: Out of 282 patients presenting with fever and associated constitutional symptoms, 100 Confirmed Dengue Fever cases, testing positive with NS1 Ag by ELISA, were included in the study. It was found that 50% had primary Dengue and other 50% had secondary Dengue infection after testing them with IgM and IgG ELISA. Amongst the 100 positive cases 41 had hemorrhagic manifestations, of which 33were secondary dengue infected patients.

Conclusion: As this study was conducted in an endemic area, a higher prevalence of secondary infection was seen with higher morbidity. All infected cases must be monitored for all parameters i.e NS1Ag, IgM, IgG to differentiate between primary and secondary infection as the possibility of patient landing into DHF/DSS is more in secondary infection, which is attributable to prevalence of all DEN virus serotypes in Indian subcontinent

KEYWORDS : Dengue, Primary and Secondary dengue, IgG and IgM Elisa, Amritsar.

INTRODUCTION

Dengue is emerging as the most prevalent mosquito borne viral infection affection tropical and subtropical countries. Around 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asian Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue[1].

Therefore, the emerging pattern and the increasing trend in the incidence of dengue infection are of great concern as there is no specific treatment of dengue, and most forms of therapy are supportive in nature. Furthermore, a licensed vaccine is not available yet.

Dengue virus belongs to family flaviviridae. All four serotypes viz. DEN(1-4), can cause illness ranging from the self-limiting,classical DF to the life-threatening denguehaemorrhagic fever and dengue shock syndrome DHF/DSS. Secondary infection with another serotype on multiple infection is a major factor for DHF/DSS. Severe plasma leakage can lead to shock, with the mortality rate for untreated patients varying between 10% and 20% but can reach as high as 40% with the involvement of shock[2].

Although secondary infection with dengue virus is the most widely accepted risk factor for the development of dengue hemorrhagic fever, there is no simple and reliable method that can routinely be used to discriminate between primary and secondary infections in the early days of an infection. The most challenging problem associated with patient management in Dengue infection is early diagnosis.[3-5]

Present study was conducted to determine the seroprevalence of primary and secondary dengue infection amongst clinically suspected patients attending a tertiary care hospital in Amritsar. The IgM capture ELISA available showed positivity for confirmed dengue cases with parallel IgG testing that further helped in diagnosing secondary infections. Hence, serologic diagnosis of dengue virus infection using a commercial capture ELISA of both IgM and IgG distinguishes primary and secondary infections.

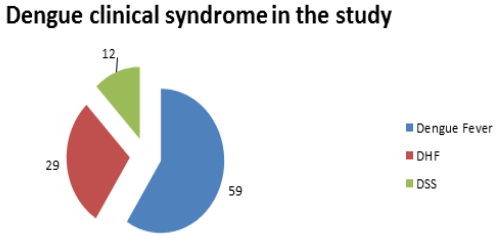
MATERIALS AND METHODS

This was a Cross-sectional comparative study performed from July 2017 to Oct 2017 in Government Medical College and Hospital, Amritsar. Clearance and permission of institutional ethical committee was taken. A total of 282 clinically suspected patients with fever were screened, of which 100 patients with confirmed dengue infection (Testing positive for NS1 dengue antigen) both out patients and inpatients, attending the hospital were included in this study. The subjects included in this study were patients with Fever and other associated symptoms like headache, retro-orbital pain, myalgia, arthralgia, itching/rashes, rise in haematocrit and reduction in platelet count. Patients with fever due to other causes like malignancy, autoimmune diseases, malaria, enteric fever, pharyngitis, tonsillitis, influenza and positive serology for other arboviral infections except dengue fever were excluded from this study. Detailed history was obtained from all the patients and complete clinical examination was done.

Qualitative detection of dengue IgM and IgG antibodies in serum samples of the patients was done using IgM antibody capture (MAC) ELISA kit and the Panbio Dengue IgG indirect ELISA kit as per manufacturer's instructions. Primary dengue infection is defined as those patient with positive NS1 Ag and Ig M test and negative IgG ELISA results. Secondary infection is defined for Ig G ELISA positive results by calculating the ratio of optical density values IgM/IgG. The IgM/IgG OD values ratio was used to classify if the patient was suffering from primary(>1.2) or secondary (<1.2) dengue virus infection (at 1:100 serum dilution).

RESULTS

A total of 282 serum samples of clinically suspected, both outpatients and inpatients were tested, of which 100 (35.4%) serologically confirmed of the Dengue infection,by testing positive for NS1 Ag using ELISA. The age of the patients included was from 5-60 years. Patients with fever and other associated symptoms like headache, retro-orbital pain, joint pains, purpuric rashes and thrombocytopenia were recorded. Majority patients presented with classical Dengue fever 59%, DHF was seen in 29% cases and 12% cases presented with DSS (Figure1).

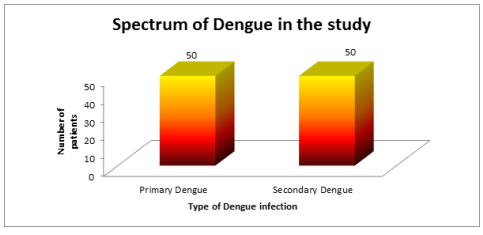


Patients testing positive for dengue belonged to the age group of 5-60 years and predominantly belonged to male sex. Overall male predominance was seen with a ratio of 1.56:1, with 64% male and 36% female population. Among the age group, positivity was significantly high in 10-40 year age group [Table 1].

Age wise distribution of cases in the study population

</= 10 years	2
>10-40 years	78
>40 years	20
Total	100

Out of 100 NS1 positive cases 68 tested positive for IgM, 28 for IgG and 22 for both IgM and IgG ELISA. The prevalence of cases with primary and secondary infection was found to be i.e. 50% each in the study population. The classification of primary vs secondary dengue infection was done by calculating the IgM/IgG antibody ratio in all the Ns1 antigen positive confirmed dengue cases as already explained in material and methods section (Figure 2).



By clinical evaluation, All the patients presented with the constitutional symptoms of Fever and majority had headache/retroorbital pain/myalgia/rash but of all 41(DHF and DSS) cases had haemorrhagic manifestations including petechiae, gum bleeding and epistaxis etc. A point to be noted is 33(80%) cases were found out to be secondary dengue infected patients and all the patients(12/12) in haemorrhagic shock were infected with secondary infection (Table 2).

Type of Fever	Primary infection	Secondary infection
DF	42	17
DHF	8	21
DSS	0	12
total	50	50

DISCUSSION

Dengue is now recognised as an important emerging public health problem. Global prevalence of Dengue has grown dramatically in recent decades with an estimated 50 million dengue infections occur annually and approximately 2.5 billion people at risk of acquiring infection because of their inhabitance in dengue endemic countries.This has made early diagnosis of dengue more important with the help of serological markers to reduce morbidity and mortality.

In the present study the percentage positivity of Dengue infection amongst the screened population of clinically suspected patients was 100/282 i.e. 35.4 by testing positive for NS1 Ag ELISA, Of the 100 confirmed dengue positive cases (64%) were males and (36%) females. Among the age group, positivity was significantly high in 10-40 year age group in our study [Table 1], attributable to the fact, that active adults are involved in more outdoor activities so have a higher chances of getting infected6,7. Male patients outnumbered females in the aforementioned study(1.56:1) in all the dengue infected patients, which is in concordance with the earlier studies undertaken in north india 7,8 and south Indian states. High prevalence amongst males is explained by their greater comparative number and more health seeking behaviour and also to their more exposure to daytime biting by the vector due to their outdoor activities 9 .

Of the 100 confirmed cases 68% tested positive for Ig M, 28% for Ig G, and 22% for both the antibodies. By calculating the ratio of IgM/IgG , the prevalence of primary and secondary infections came out to be equal i.e. 50% each.. Similar studies were done by Ratho et al10 2005, an outbreak of dengue fever in Chandigarh, India, during September to December, 2002. Of 218 acute phase sera tested, the presence of dengue specific IgM antibodies in acute phase serum samples comprised the total of 76 (35%; 76/218) cases and dengue specific IgG antibodies in 55patients (72.4). Detection of specifically elevated IgG antibodies to dengue virus by ELISA is a valuable diagnostic tool. According to WHO, secondary dengue infection is characterized by higher Ig G ,which may/may not be accompanied by elevated IgM levels. The Panbio Dengue IgG antibodies ELISA detects to Dengue infection above this threshold. The assay does not detect low level IgG antibodies from past exposure typically present in many individuals from endemic regions. According to Hawkes et al early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases. According to Pei- Yun Shu et al, capture IgM and IgG ELISA has become the most powerful assay for sero diagnosis due to its high sensitivity, specificity and simplicity. The results shown in this study of the comparison of the HI test with the capture IgM/IgG ratio support the current trend towards using capture IgM and IgG ELISA to differentiate primary and secondary infections .A total of 103 serum samples collected between days 3 and 30 after the onset of symptoms were analyzed for HI and capture IgM/IgG ratio. Good correlation was found, with a result concordance of 89.4%.11

In our study, 41/100 cases had haemorrhagic manifestations that included 29 DHF and 12 DSS, point to be noted is all the 12 cases were found out to be secondary infection.

Krishnasamy K et al12 showed in her study that Dengue fever was seen in 43 cases, eighteen cases had haemorrhagic manifestations. As our study was conducted in a an endemic region of the Indian subcontinent, an unusually high prevalence of secondary infection and thus the complications have been observed.

CONCLUSION

As this study was conducted in an endemic area of Indian subcontinent, so an unusually high prevalence of secondary dengue infection has been observed. This can also be attributed to the fact that the cases were carefully selected based on the clinical evaluation. This study indicates a slightly higher prevalence among the male section of the population. Early discrimination of primary and secondary dengue helps to reduce the morbidity and mortality.

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