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Seroepidemiology and Gender Related Differences in Laboratory Characteristics of Dengue Virus Infection: A Hospital Based Study, Bhopal

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Abstract:
Introduction: *Data regarding gender related differences in serological and haematological findings of dengue infection is lacking in Indian settings especially in Central part of India where dengue is endemic.*
Objectives: *To study seroepidemiology and association of serological and haematological findings with gender in dengue seopositive patients.*
Methods: *1071 dengue suspected patients attending to hospital from 1st January 2014 to 31st December 2015 were included. Their serum samples were tested for NS1 antigen and IgM and IgG antibodies by immunochromatographic test. Total leucocytes count (TLC) and platelet counts of seropositive patients were noted. Association of laboratory findings were correlated with gender by using Chi-square test and P value was obtained.*
Results and Discussion: *16.43% samples were found to be seropositive with Male:Female ratio 1:0.76. Maximum seropositivity was detected in 21-30 years. 69.31% were primary and 17.04% seropositive cases were labelled as secondary dengue infection. 13.63% IgM+IgG positive samples couldn't be classified. Thrombocytopenia and and leucopoenia was detected in 75(42.61%) and 69(39.20 %) dengue seropositive cases respectively. No significant association was found in haematological findings with gender (P >0.05).*
Conclusions: *The current study found a consistent pattern of male predominance and young age. High index of suspicion of severe dengue fever should be maintained in all patients irrespective of gender.*
Keywords:*Dengue, Gender, Leucopoenia, Seroepidemiology, Thrombocytopenia*

I. Introduction

Dengue is caused by a positive stranded RNA virus of the flaviviridae family with four distinct serotypes (dengue 1 to 4) that are related antigenically.[1]It is estimated that approximately 2.5 billion people are at risk of dengue infection with 50 million infections occurring annually worldwide.[2] Dengue viral infection is endemic in the Indian subcontinent affecting 35 states/UTs including Madhya Pradesh.[3]

The spectrum of Dengue infection may range from asymptomatic infection to undifferentiated fever, Dengue fever (DF), Dengue Haemorrhagic fever (DHF) or Dengue Shock Syndrome (DSS).Recovery from infection provides lifelong immunity against that serotype but confers only partial and transient protection against subsequent infection by the other three serotypes. Secondary infection with a serotype different from that causing primary infection may lead to DHF and DSS which can be fatal. [1]

Diagnosis of primary dengue is made by the detection of IgM anti-DENV antibodies which appear 5-7 days after the onset of illness and persist for 2-3 months whereas a secondary infection is characterized by production of IgG antibodies and a weak IgM response.[2]The diagnostic methods currently available are viral isolation,viral RNA detection by reverse transcriptase PCR (RT-PCR) or detection of dengue virus specific IgM antibodies by enzyme linked immunosorbent assay (MAC-ELISA) and/or the rapid dengue immunochromatographic test(ICT).[2] Simple and rapid ICT with the detection of NS1 antigen, IgM/IgG antibodies provide opportunities for point-of-care diagnosis especially in many dengue endemic settings, where laboratory diagnostic resources are limited.[4]

Previous studies across the world have reported gender related differences in serological and haematological findings of dengue infection. [5-6] However, data regarding this aspect of dengue fever is lacking in Indian settings especially in Central part of India where dengue is endemic. In the absence of vaccines and specific treatment for the DF, prevention and control of the disease mainly depends upon effective vector control measures based on the epidemiological surveillance that provides reliable estimate of the disease. [7] In view of this, the present study was designed to study epidemiology of dengue infection in and around Bhopal

based on serological parameter and to study association of serological and haematological findings with gender in dengue seopositive cases.

II. Materials & Methods

2.1) Type of study: *Cross sectional study*

2.2) Study period: *1st January 2014 to 31st December 2015 (24 Months)*

2.3) Specimen collection: *Blood sample was collected from patients presenting to our hospital with dengue like illness for which dengue serology was requested. About 2-3 ml of blood was collected using strict aseptic precautions.*

2.4) Specimen processing: *Serum was separated by standard methods and tested simultaneously for NS1 antigen and IgM and IgG anti-dengue antibodies by ICT (SD Bioline Dengue Duo rapid test). The instructions of the manufacturers were meticulously followed while performing the tests and results were interpreteted.*

2.5) Haematological parameters*: Platelet count and Total Leucocyte Count (TLC) of seropositive patients were noted. Thrombocytopenia was defined as a platelet count of <100,000/µL and Leucopenia was defined as a leucocyte count of <5000/µL.* [2]

2.6) Serological classification of Primary and Secondary dengue infection: [2]

NS1,NS1+IgM,IgM positive patients were labelled as primary while NS1+IgM+IgG, NS1+IgG, IgG positive patients were labelled as secondary dengue infection. IgM+IgG positive patients couldn't be classified as ELISA was not done to assess the ratio of IgM/IgG.

2.7) Statistical analysis:

Associations of serological and haematological findings were correlated with gender by using Chi-square test. Statistical significance was defined as P value < 0.05.

III. Results

Total 1071 blood samples were received for dengue serology during study period. Out of which, 176 samples (16.43%) were found to be positive for one or more serological parameter. Among the dengue seropositive cases, the proportion of males was higher giving male: female ratio (M: F) ratio 1:0.76. However, this difference was not found to be statistically significant (P = 0.55) [Table 1].

Table 1: Genderwise distribution of Total Samples and Seropositive Samples

Gender	Total Samples n (%)	Positive samples n(%)
Male	634 (59.19)	100 (15.77)
Female	437 (40.80)	76 (17.39)
Total	1071 (100)	176 (16.43)
P value	-	0.5*

* Nonsignificant

Maximum seropositivity i.e 48 (27.27%) was detected in 21-30 years followed by 11-20 years of age group 46 (26.13%) [Fig.1].

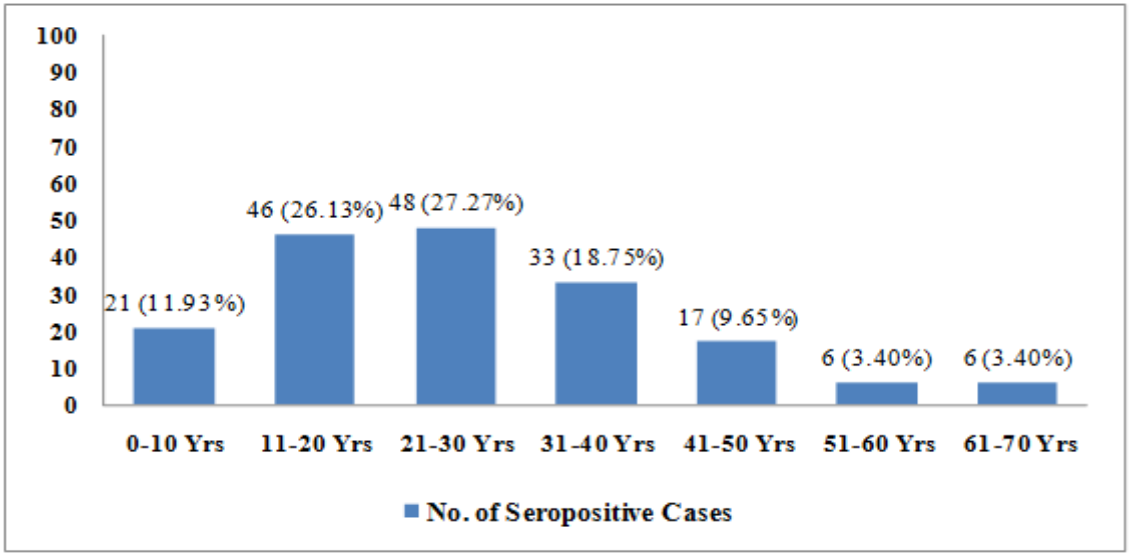

Figure 1: Agewise distribution of seropositive cases

NS1 Antigen was detected in majority of 102 (57.95%) dengue seropositive cases [Fig. 2].

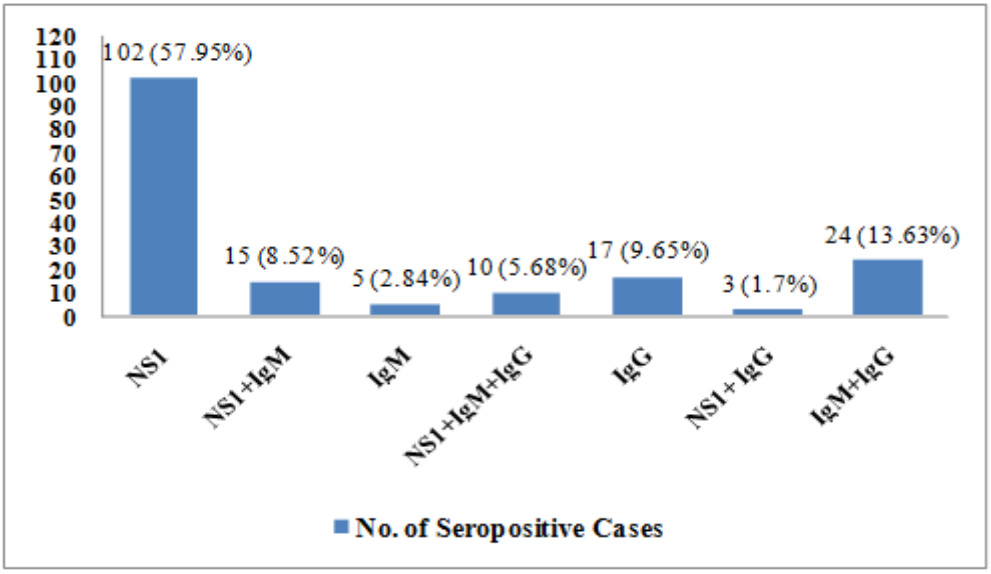

Figure 2: Distribution of serological parameters in dengue seropositive cases

122 (69.31%) were primary and 30(17.04%) seropositive cases were labelled as secondary dengue infection. 24 (13.63%) IgM+IgG positive samples couldn't be classified [Table 2]. No significant association was found in primary and secondary dengue infection with gender (P = 0.6) [Table 3].

Table 2: Distribution of Primary and Secondary dengue cases based on serology

	Primary Infection	**Secondary Infection**	**Not Classified**
Serological Parameters (n)	NS1 (102) NS1 +IgM (15) IgM (05)	NS1+IgM+IgG (10) IgG (17) NS1+IgG (03)	IgM+IgG (24)
Total Positivity n (%)	122 (69.13%)	30 (17.04%)	24(13.63%)

Thrombocytopenia was detected in 75(42.61%) while Leucopoenia was detected in 69(39.20 %) dengue seropositive cases. No significant association was found in haematological findings with gender (P >0.05) [Table 3].

Table 3: Genderwise distribution of laboratory findings

Gender	**Total Samples n (%)**	**Positive samples n(%)**	**Primary dengue Infection n (%)**	**Secondary dengue Infection n (%)**	**Platelet Count < 100,000/µl n (%)**	**Total leucocyte count < 5000//µl n (%)**
Male	634 (59.19)	100 (15.77)	67 (67)	15 (15)	42 (42)	40 (40)
Female	437 (40.80)	76 (17.39)	55 (72.36)	15 (19.73)	33 (43.4)	29 (38.15)
P value	-	0.5	0.6	0.6	0.4	0.3
Statistical Significance	-	Nonsignificant	Nonsignificant	Nonsignificant	Nonsignificant	Nonsignificant

IV. Discussion

Dengue is known for its serious life threatening complications. Early laboratory diagnosis of acute dengue virus infections is important to provide appropriate treatment of the patient and to prevent potential dengue outbreak.

In the present study, dengue seropositivity was found to be 16.43% [Table 1] which is comparable to the rate reported by Shaikh Khalida et al (18.5%).[8] In other similar Indian studies, Saini S et al[9] in Loni, Maharashtra found 30.6% positivity rate while N. Bhattacharya et al[10] in Kolkata, West Bengal reported 38.3% dengue seropositivity. The reason for variation in dengue positivity may be related to history of herd immunity, introduction of new serotype or geographical variations in a particular area.

Understanding male –female differences in infection rates and severity of disease is important for public health control programme. In the present study, the proportion of males was observed to be higher than females giving male: female ratio (M: F) ratio 1:0.76 [Table 1]. Three independent studies from epidemics in India done by Ray et al[11], Agarwal et al[12] and Wali et al[13] found nearly twice the number of male patients infected with dengue compared to females M:F being 1:0.57 , 1.9:1 and 2.5:1 respectively. Male predominance may be attributed to their more outdoor exposure to transmitting agent. An analytical study was conducted in Singapore from1998 to 2000.The study concluded that predominance of male cases were likely due to greater male exposures to dengue carrying mosquitoes during daytime hours either at the work place or while travelling to and from work. [14] However, Yew et al [15] did not find movement history as a contributing factor for male predominancy and suggested male – female differences in the use of health services. It has been established earlier that many Asian countries lower disease incidence in women may be a statistical artefact related to lower reporting and care seeking for women. In our study, difference in dengue seropositivity among males and females was not found to be statistical significant (P = 0.55) [Table 1]. Well designed studies considering both biological and social factors that influence the disease patterns in the community are required to determine the sex differences.

In Southeast Asia, dengue infection is predominantly a childhood disease and is an important cause of paediatric hospitalization. However, age shift from paediatric age group to young adults have been observed in different surveillance reports.[16] Hospital based studies have similarly reported increasing infection rates among adults mentioning that it is contrary to the popular belief that dengue is paediatric disease.[13,17] Results of the present study also confirmed this notion [Fig. 1]. The trend for increased incidence among young adults has important implications for control and prevention.

NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness. NS1 Ag level varies from 0.04-2 µm/ml in acute phase serum samples, to only 0.04µg/ml or even less in convalescent phase serum. This is the reason for its high detection rate in acute phase sera. [18] In this study, only NS1 antigen was positive in 57.95% of the cases [Fig. 2]. These cases would have been failed to be detected at that time if only antibody based assays are used for the laboratory diagnosis of dengue. Similar findings were observed in other studies[17,20] in which the sensitivity of detection of dengue infection increased when both antigen and antibody based assays were used as diagnosis

The differentiation between primary and secondary dengue infection is important as secondary dengue infection are more commonly associated with DHF and DSS. [1] Differentiation requires detection of IgM and/or IgG antibody. During acute phase of the disease, the presence of DENV IgM antibody alone suggest primary infection and the concomitant detection of DENV IgM and IgG antibodies is suggestive of secondary infection. [2] In the present study, using ICT assay, primary infection cases were found to be 122(69.31%) and secondary infection cases were 30 (17.04%).14 (13.63%) cases couldn't be classified as ELISA was not done to assess the ratio of IgM/IgG [Table 2]. Similar observations have been reported by Kidwai AA et al[19] and Rashmi KS et al[20]. Though incidence of primary and secondary dengue infection was observed to be higher in females, this difference was not found to be statistically significant (P value: 0.6) [Table 3].

Myelosuppression in DF leads to thrombocytopenia and leucopenia. 75(42.61%) dengue seropositive cases were detected to have thrombocytopenia in our study [Table 3]. Soumy K et al[17] and Kulkarni RD et al[21] proved a significant association between thrombocytopenia and dengue infection. In the present study, leucopenia was detected in 69 (39.20%) seropositive cases [Table 3]. In two independent hospital based studies [6, 17], leucopenia was found in approximately 60% of dengue seropositive cases. Thus, haematological findings can play a complementary role in prompting the suspicion and facilitating the timely diagnosis and management of dengue even before availability of serological test results. Chakravarti et al[6] reported significant association of thrombocytopenia and leucopenia with females as compared to males. However, in the present study no significant association was found in haematological findings with gender (P value >0.05) [Table 3].

There are certain limitations of our study. Since it is a hospital based study, it may represent only the tip of the iceberg in the overall pattern of dengue infection. In addition, ELISA and molecular method of higher sensitivity and specificity were not used.

V. Conclusions

The current study found a consistent pattern of male predominance and young age in the reported incident cases of dengue fever. This study re-emphasizes the need for the inclusion of NS1 antigen detection based assay for the early and accurate diagnosis of dengue virus infection. It is recommended to test dengue specific NS1 antigen and IgM and IgG anti dengue antibodies simultaneously for categorizing infection as primary or secondary for effecting monitoring and treatment. Reduced platelet count and TLC can be used as a cost–effective laboratory investigation prompting a high suspicion of dengue infection especially in resource poor setting where serological tests may not be available. High index of suspicion of DHF and DSS should be maintained in all patients irrespective of gender.

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