

Seroepidemiology of Dengue Viruses in Basrah, Southern Iraq

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Abstract

Background: Dengue virus infection has been an important and serious public health concern in southeast Asia, where local outbreaks of dengue fever occurred almost every year. To our knowledge, no nationwide investigation has been carried out to determine the actual extent of infection in the general population.

Methods: A total of 282 random serum samples were collected from the general population in Basrah in 2019-2020. The antibody-captured enzyme-linked immunosorbent assays were used to detect DENV-specific IgM and IgG. Demographics data were used for risk analysis.

Results: The weighted overall seroprevalence was 30.9% for anti-DENV IgM, and 64.9% for anti-DENV IgG, respectively. A significant rise of DENV IgG seropositive rate had been noted since early adulthood stage, from 49.4% at the age group of 10–30 years to 68.8% at the age group of 31–50 years. For people aged over 50 years, the seropositive rate reached 81.4%. Age, and regions of residency were associated with the IgG seropositivity. There was no statistically significant difference in seroprevalence of anti-Dengue IgM, indicating recent infection, among predictors including gender, and residency.

Conclusions: Our results indicated that the majority of population in Basrah exposed to dengue virus and the prevalence of IgG antibody against dengue virus rises with age. and regions of residency are associated with the exposure of population to infection by dengue viruses. Further studies are needed to realize the current situation of seroprevalence of dengue fever in southern Iraq.

Keywords: Seroepidemiology, Dengue fever, southern Iraq

INTRODUCTION

Dengue, one of the most threatening mosquito-borne diseases of humans, is caused by any of the four-serotypes (DEN-1 to 4) of dengue virus, a positive-strand RNA virus. Two clinical forms of dengue infection have been recognized: dengue fever, a relatively mild, self-limiting febrile illness and dengue hemorrhagic fever/dengue shock-syndrome (DHF/ DSS), a severe infection with vascular and haemostatic abnormalities that can lead to death¹. Dengue virus infection constitutes a major public health concern and is estimated to cause about 300 million cases in a year across the globe. About 3.97 billion people, who are living in the epidemic areas in the tropical and subtropical regions, are at risk of acquiring the viral infection^{2,3}.

Environmental changes such as rising temperatures, increased rainfall, accelerated urbanization and industrialization, population growth, and poor waste and water management, which lead to the proliferation of mosquito species (*Aedes aegypti* and *A. albopictus*) that transmit DENV, are all possible reasons for the upsurge of dengue cases^{3,4}. Dengue transmission has increased worldwide, particularly in Asia and Latin America since the 1970s⁵, but limited information on the disease is available from the Middle East. Saudi Arabia⁶ and Yemen^{7,8} have reported a few epidemics of dengue. Climate conditions in the Middle East not all are favourable for the disease vector, but all other risk factors for dengue are potentially increasing⁹. The existence of a large immigrant work force from dengue-endemic countries, increased travel from and to dengue-endemic countries and increased

urbanization are expected to increase the likelihood of the emergence of dengue in the Middle East.

Seroprevalence studies help identify populations previously infected by dengue. This is important because the lifelong immunity developed after infection with one of the four DENV serotypes is type-specific; secondary infection by heterologous serotype is frequently, not associated with severe dengue manifestations^{2,10}. Seroprevalence studies reveal subclinical DENV infections, as the majority of the infections are subclinical and case reporting underestimates the true rate². Patients with subclinical infections can contribute to the overall DENV transmission cycle¹¹. Additionally, estimating both clinical and subclinical infections provides reliable information to support modeling for future vaccine demand and delivery strategies².

The aim of this study is to determine the seroprevalence of DENV infections in symptomatic and healthy adult population in districts of Southern Iraq, and to identify factors associated with seropositivity.

Materials and methods:

The study site, Basrah is located in southern Iraq, has an estimated population of ~3.796.000. This study was conducted in four sites namely; A=north (served by Al-Mawanaa teaching hospital, Al-Faihaa teaching hospital), B=south (Al-sader teaching hospital), C=west (Al-basrah teaching hospital) D=East, (Paediatric and Oncology center) these sites were selected to represent almost all population in Basrah, there is a likelihood of transmission to humans, from lakes and river in these areas may act as breeding sites for the vectors and a habitat for arboviruses reservoirs.

Serum samples of 282 patients that live in Basrah region were collected in the years 2019 and 2020. The samples were collected from patients during their visit to hospitals in Basrah city. The mean age of the study population was 45 years (range = 10–80). Male to female ratio was 1:1.6 (39.3% males and 60.6% females) of the total study population.

The study utilizes those samples from a hospital-based cross-sectional descriptive survey and retrospective data from an ongoing study that started in December 2019. To augment the collection samples of febrile illness patients from identified hospitals were collected with the assistance of clinical officers in the four respective study districts.

A total volume of approximately 5 mL of blood was collected from the participants. The blood samples were incubated in room temperature for 1 hour, followed by 15 minutes centrifugation at 3,000 rpm and collection of the serum fraction. The serum samples were stored at -70°C until use.

Known as IgM Antibody Capture Enzyme-Linked Immunosorbent assay. All the Serum samples brought to the laboratory were screened for exposure to DENV. This was done using commercial kits namely; MAC-ELISA Kit of Arboviruses (DENV IgM Capture (DxSelect™, FOCUS Diagnostic, USA).

An enzyme-linked immunosorbent assay (ELISA) specific for detection of Anti human IgG Immunoglobulin to DENV was used and the procedure followed according to the manufacturer's instructions (DxSelect™, FOCUS Diagnostic, USA).

Chi-squared test was used to compare the seroprevalence between genders and between age groups.

Results:

Antibodies against dengue virus (*Flaviviridae*,) as specified below, were detected in 87 of 282 (30.9%) samples positive for IgM antibody and 183 of 282 (64.9%) samples were positive for IgG antibody. There was a gradual increase in IgM and IgG seropositivity as the age increased where the highest IgG seropositivity was detected among age group of greater than 50 years of age (81.4%) and the least among early age group (49.4%). The difference was statistically significant ($P < 0.05$). However, there were no differences related to gender (Table-1). Recent infections were more among rural community (35.8%) compared to urban group (20.6%) while old infections (IgG) was dominant among urban community (80.4%) compared to (57.4%) in rural group. The differences in terms of the rates of exposure between urban and rural community was statistically significant ($P < 0.05$). Areas D and A represent the focuses of highly exposed peoples in these areas which was 73.3% and 68.6% respectively, compared to areas B and C (Table-1 and Table-2).

Table-1: The demographic characteristics of study population

Character	No. tested (%)	No. IgM+ve (%)	No. IgG+ve (%)
Age group			
10 -30	93	11(11.8)	46(49.4)
31- 50	135	50(37.0)	93(68.8)
51-80	54	26(48.1)	44(81.4)
Gender			
Male	108	30(27.7)	73(67.5)
Female	174	57(32.7)	110(63.2)
Residence			
Rural	190	68(35.8%)	109(57.4%)
Urban	92	19(20.6%)	74(80.4%)
Study sites			
A	150	54(36.0)	103(68.6%)
B	66	18(27.2%)	36(54.5%)
C	51	9(17.6%)	33(64.7%)
D	15	6 (40%)	11(73.3%)
Total	282	87(30.9%)	183 (64.9%)

Table-2: Distribution of dengue virus IgM and IgG antibody according to ELISA test of different regions of Basra province.

Area	Area of samples collection	Examined	IgM+ve N(%)	IgG+ve N(%)
A	North(Served by Al-Mawanae and Al-FaihaTeachinh hospital)	150	54 (36.0)	103 (68.6%)

B	South(Served by AlSader Teaching Hospital)	66	18(27.2%)	36(54.5%)
C	West(Basrah General Hospital)	51	9(17.6%)	33(64.7%)
D	East (Pediatic & Oncology hospital)	15	6 (40%)	11(73.3%)
	Total	282	87(30.9%)	183(64.9%)

Table -3: Symptomatic status of study population in relation to DENV seropositivity

Symptoms	No. tested	DENV-IgM N(%) +ve	DENV-IgG N(%) +ve
Symptomatic	174(61.7%)	49/174(28)	81/174(46.5)
Asymptomatic	108(38.3%)	38/108 (35)	102/108 (94.4)
Total	282	87/282(30.8)	183/282(64.8)

Dengue virus seropositivity was higher among asymptomatic individual collectively for IgM (35%) and IgG (94.4%) compared to patient with febrile illness (symptomatic) which was 28% and 46.5% for IgM and IgG respectively (Table-3).

Table-4: - Clinical presentation of DENV symptomatic patients

Symptoms	No.	Percentage	DENV -IgM	DENV -IgG
Fever/ Headache	139	80 %	39(28%)	64(46%)
Intense abdominal pain & tenderness	104	60 %	29(28%)	48(46%)
Persistent vomiting & GIT	87	50 %	24(28%)	40(46%)
Bleeding of mucosal membranes	2	1.2 %	0	1(50%)
Joint pain	104	60 %	29(28%)	48(46%)
Postural hypotension (lipothymia)	17	10 %	5(29%)	8(47%)
Thrombocytopenia & atypical lymphocyte	17	10 %	5(29%)	8(47%)
Progressive increase in hematocrit	9	5 %	0	0
Total	174/282 (61.7%)			

Table -4 show the clinical presentation of symptomatic patient . There were no significant differences between patients at various clinical presentation almost the same rates of seropositivity to IgM (28%) and IgG (46%) antibodies for dengue virus. Fever and headache was presented in 70% and 80% respectively. However, 50% of patients complain of joint pain and vertigo, severe malaise, chills, cough and abdominal pain presented in 30%, 28%, 18%, 16% and 14% respectively (Table-4). The major complain of seropositive to DFV was fever (70%), headache (80%), joint pain (50%) and vertigo (30%).

Table- 5: Seasonal distribution of DENV infections in relation to seropositivity

Season	No. tested (%)	DENV –IgM	DENV –IgG
Winter	45	7(15%)	29(64.4%)
Spring	59	11(18.6%)	33(55.9%)
Summer	98	37(37.7%)	63(64.3%)
Autumn	80	32(40%)	58(72.5%)
Total	282	87(30.9%)	183(64.9%)

The majority of cases of DENV was detected during hot season (Summer and autumn which was 64.3% and 72.5% respectively) although the rates of exposure to infections occur all the year around in our areas(Table-5).However, recent infections presented with high rate of IgM seropositivity during summer (37.7%) and autumn (40%) compared to winter (15%) and spring (18.6%).The difference between rates at hot seasons and cold seasons were statistically significant ($p < 0.05$).

Table-6:DENV antibody levels: the proportion of susceptible

Type of responses to DENV	No. +ve (%)
Recent : IgM	87/282(30.9%)
High titer of IgG	119/282(42.2%)
Low titer of IgG	64/282(22.7%)
Total	270/282(95.7%)

The majority of individuals in the community has been exposed at a time to DEV (95.7%) although the presence of antibody could be not protective (Table-6). Suppose that individuals posses IgM and/or IgM and high titer of anti-DENV IgG which was 30.9% for IgM and 42.2% for high IgG titer could be protected leaving the rest of > 50% susceptible to DENV infection.

Table-7: The main hematological investigation in DENV infections

Investigation (normal values)	No. Total +ve	Normal	High	Low
Hb% (Male 13-17, Female 12-15)		170/270(63%)	65/270(24%)	35/270(13%)

PCV (Male 40-52, Female 37-47)	270/282 (95.7%)	170/270(63%)	65/270(24%)	35/270(13%)
Platelets (150-450)		155/270(57%)	75/270(28%)	40/270(15%)
Total WBCs (4500-11000)		140/270(52%)	105/270(39%)	25/270(9%)

The main hematologic investigations for the responders of participants(95.7%) is presented in table-7. The majority of DENV infected are presented with almost normal hematological findings except for platelet count and total WBCs which showed marginal increase among 28% and 39% respectively. On the other hand, small proportion of patients presented with low hematologic parameters.

DISCUSSION

Despite having relatively low incidence of reported dengue cases in Basrah district, an overwhelming 64.9% of participants had serological evidence of previous DENV exposure, out of which 30.8% were recent exposures. This discrepancy could be attributed to the dengue notification system which is based on passive reporting and may not reflect the actual disease burden due to underreporting, misreporting^{5,12}, and the failure to capture subclinical infections². They were not diagnosed clinically by a healthcare professional, as they were either asymptomatic or had minimal symptoms not necessitating medical attention or hospitalization. The nonspecific nature of symptoms misattributing it to other febrile illnesses may partly account for this¹². Our findings suggest a possibility that a high number of subclinical dengue cases are present in this community. This corroborates to that most dengue infections are clinically unapparent and are consistent with high rates reported in countries such as Singapore¹⁴, South India¹², and Saudi Arabia¹⁵ in contrast to that reported in Iraq by Raddle et al(2008)¹⁶.

The prevalence of IgG antibodies increased with increasing age, with >81% of the population having been exposed to dengue after the age of 55 years. Several studies have shown an association between age and IgG dengue seropositivity^{12,15,17,18}, consistent with the long-term persistence of anti-DENV IgG once a person is infected. The increased seroprevalence with age suggests that the longer a person resides in an endemic area, the higher the chance of being infected by DENV^{15,17}.

Dengue has now become hyperendemic in many countries including Middle eastern area with all four DENV serotypes co-circulating, with fluctuations of the dominant serotypes over time and location^{6,7}. In recent years, there has been a shift observed in the age distribution of dengue cases toward older age groups, involving adults in the productive age group^{5,17}. Another notable shift is the rise in dengue incidence in rural areas, which is gradually approaching the high levels observed in urban communities¹⁷. With the changing epidemiology of DENV infections world-wide, it has become increasingly important to obtain up-to-date seroprevalence data to further understand the distribution and burden of DENV infections. An improved understanding of dengue epidemiology, burden and its dynamic characteristics are important for public health planning. Seroprevalence studies in healthy volunteers provide information on infection history in the population, from which inferences about disease burden may be drawn. Since age reflects duration of exposure, age-stratified data provide insights into transmission dynamics^{18,19}. There is a lack of dengue

seroepidemiological data from Iraq and no previous study has used a population representative sample of urban population.

Clinical manifestations of dengue virus infection range from asymptomatic, mild flu-like symptoms, to severe life-threatening dengue complications such as dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) ²¹. DHF/DSS cases are associated with a secondary-type dengue antibody response, which makes the second dengue infection worse than the first due to

“antibody-dependent enhancement of infection” ²¹. Those asymptomatic infection cases, which induce an antibody response but lack clinical symptoms requiring a medical consultation, pose challenges to disease prevention

programs. The incidence of dengue has grown dramatically worldwide

in recent years. ^{22,23}. The majority of outbreaks were reported in northern of Basrah. Notably, due to the special geographic and habitat nature of the area.

In recent years, arboviruses that cause dengue, chikungunya, and Zika illnesses have rapidly expanded across the globe, of which the immunopathogenesis enhanced in the setting of high seroprevalence of dengue antibodies as the case in the present study due to structural similarities between Zika and dengue virus ^{24,25}. It is important to study dengue virus seroprevalence to project future epidemic patterns in Basrah. There have been scanty studies of seroprevalence of dengue virus from Iraq, and few results were published in the literature ¹⁶. Considering the massive population morbidity and the high rates of asymptomatic cases, existing studies were not enough for determining the current immunological status of dengue in the community ^{26,27}. This study was the first study to investigate the seroprevalence, measured by the presence of IgG and IgM antibodies of dengue virus in general populations from different geographical areas in Basrah to figure out the whole picture of dengue disease prevalence in southern Iraq.

In countries with the presence of dengue vectors, with dengue virus circulation, WHO suggested the preparedness and response plans should focus on strategies to reduce risk of transmission. Rapid investigation of all clinically suspected cases should be carried out as well as laboratory testing for confirming the presence of dengue virus ²⁸

The present study and the other studies conducted in Brazil ²⁰, Singapore ¹⁴, and Saudi Arabia ¹⁵ found that dengue mostly affected adults in the age group of 21–

70 years old. This may be because adult groups (>20 years old) engage in more outdoor activities, giving them more chances of being exposed to infected mosquitoes than the younger age group (<20 years old) ²⁹. In addition, mild or mainly asymptomatic DV infection was

normally found in children ³⁰. Previous studies from south Asian countries ²¹ found that males are more prone to DF than females, suggesting that the more common outdoor work habit of males gave them more chances than females to be bitten by mosquitoes ^{29,31}. However, an equal dengue infection rate was observed in male and female populations in Basrah in this study, revealing no differences in vector exposure frequency or health care-seeking behavior between males and females. More studies are needed to verify this point.

Dengue infection has a wide clinical spectrum that varies with different regions and age groups ^{5,12}. Early recognition and understanding of the clinical problems in the febrile phase in a particular region lead to early diagnosis, notification and control of a

dengue outbreak. In previous studies, investigators observed that the most frequent symptoms were fever, vomiting, thrombocytopenia and leucopenia

in Saudi Arabia¹⁵, while fever, myalgia, arthralgia and headache were the most frequent in Malaysia²²; however, in the present study, there were no significant differences in most clinical symptoms associated with DF. The study has shown that the clinical manifestations in dengue patients varied with different DV serotypes, probably due to differences in their pathogenesis, replication ability and infection activity¹². In addition, some chronic diseases, such as hypertension, diabetes, renal diseases, hepatic diseases, cardiovascular diseases, thyroid disease and arthritis, were established to be risk factors for DHF in previous study by Figueiredo et al³².

Conclusions: Our results indicated that the majority of population in Basrah exposed to dengue virus and the prevalence of IgG antibody against dengue virus rises with age and regions of residency are associated with the exposure of population to infection by dengue viruses. Further studies are needed to realize the current situation of seroprevalence of dengue fever in southern Iraq

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