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Dengue Prevalence and Diagnosis in Pakistan

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Dengue virus, a positive sense RNA virus, involve in spreading dengue infection worldwide, consists of four related but genetically different serotypes. The present study depicts the outbreak and prevalence of dengue infection in different parts of Pakistan from 1982 to 2013. In addition to this most commonly used diagnostic techniques in the world and in Pakistan have been also discussed. Since 1934, the availability of vector (*Aedes aegypti)* into various parts of Pakistan served as a risk factor for dengue infection and transmission to other parts of the country resulted into several mini and major outbreaks of different intensity. Later on, factors such as the geographical growth of the vector, seasonality of unknown reasons in transmission patterns and increased rainfall in area extended the degree of infection. After the first documented outbreak of dengue illness in 1994 in Karachi, the situation remained more or less unchecked therefore, dengue illness started to appear in the Northern part of the country in 2007. In 2013, a new trend has been observed regarding dengue prevalence in Pakistan, when cases were reported from areas other than endemic belt such as Balouchistan and Khyber Pakthunkhwa provinces. The analysis of available data showed the major threat of this viral infection that need to be considered and preventive measures should be taken accordingly.

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1. INTRODUCTION

Dengue is an important viral, mosquito-borne infection found in humans. According to estimation, 2.5 billion people are in danger of dengue illness, about 975 million of those live in built-up areas (towns) in different countries located tropically and sub-tropically in Southeast Asia, the Americas and the Pacific globally [1]. The dengue virus is the causative agent of the disease dengue fever; this virus consists of four related but genetically different serotypes. Dengue virus (DENV) is a positive sense RNA virus with 11.8 kb genome in length, consists of single open reading frame, encoding 3-structural (membrane -M, envelope- E and capsid –C) and seven non-structural (NS1, NS2, NS2, NS3, NS4A, NS4B and NS5) proteins [2]. A limited identity is shared by the four dengue virus serotypes and the variability observed at amino acid level between these serotypes is from 25 to 40%. Even viruses of the same serotypes show considerable variations (~3 to 6% at the nucleotide level), and are phylogenetically divided and subdivided into genotypes and clades respectively. This genetic variability is due to intra host diversity (accumulation of genetically distinct genomes in individual hosts) as a result of error-prone nature of the enzyme that is responsible for viral RNA replication, RdRp or RNA-dependent RNA polymerase [3]. Illness due to one serotype of DENV provides life-long immunity against the similar serotype but does not provide complete immunity against other serotypes [4].

This review article covered the history of vector emergence in Pakistan, year wise circulation of 1-4 serotypes and risk factors associated with disease prevalence in Pakistan. The commonly used diagnostic techniques for dengue detection in the world and in Pakistan were also discussed in this review article.

2. CLASSIFICATION

DENV (dengue virus) is a member of genus Flavivirus within Flaviviridae family. The evolution of four serotypes of dengue virus in non-human primates shared a common ancestory and the entrance and circulation of 1–4 DENV serotypes occurred about 500–1,000 years ago through the urban cycle [5].

3. HISTORY OF ISOLATION OF FOUR SEROTYPES

In 1944, two related but immunologically distinct viruses DEN-I and DEN-2 were isolated by Sabin from the blood of the patients diagnosed with dengue clinically [7]. Two new serotypes of dengue viruses, were isolated by Hammon and co-workers in 1956 named as DEN-3 and DEN-4, along with already known DEN-I and DEN-2 during an epidemic of severe dengue hemorrhagic illness in Philippinian children [8-9].

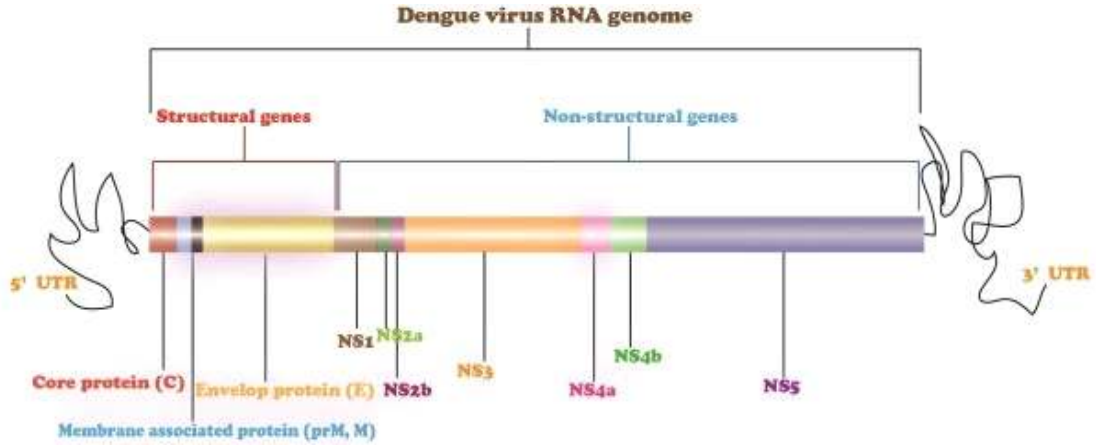

Fig. 1. Genome of dengue virus encoding proteins 3-structural (membrane (M), envelope (E) and capsid (C), glycoproteins and NS1, NS2, NS2, NS3, NS4A, NS4B and NS5 are among 7 non-structural proteins [6]

4. DENV EPEIDMIOLOGY, TRANSMISSION, PATHOGENESIS AND SYMPTOMS

Aedes aegypti and *Aedes albopictus*, found all over the world, are the vector mosquitoes, involved in transmission of dengue virus among people. From 4 to 7 day of mosquito-biting, the symptoms of dengue infection usually start and may last for three to ten days. For the transmissions of dengue virus, the mosquito has to feed on a person ill with dengue viral infection, through a period when the blood contains sufficient amounts of virus. This period expands on 5-days normally just when a person is about to show symptoms of illness. It is observed that some people don't show major symptoms but can pass infection to a vector (mosquito). After a blood meal, the mosquito needs an incubation period of 8 to 12 days before passing on the infection to other human in order to complete cycle. The infection remains in mosquito for the rest of its life that could be few days or week [10]. A definite seasonality has been observed in the transmission patterns of dengue in areas where dengue is endemic, but the reasons are not fully known. However, in some areas increased rainfall has been linked to an overall increase in dengue transmission [11,12].

The transmission rates may influence by cooler temperatures which affect adult mosquito survival, both the temperature and rainfall may control the physiological processes of mosquito such as reproduction and feeding [13,14]. The disease transmission pattern may also be influenced by Human activities, like storage methods of water in variety of containers, act as breeding site of mosquitoes [15]. The global circulation and spread of virus strains can be mapped and followed up by defining intra serotypic genetic variations [16] in addition to this the genetic differences that linked with disease severity can be recognized [16-18]. Furthermore, recent epidemiological comparisons suggest that the more virulent genotypes dengue virus are now displacing the lower epidemiological- impact [19] resulting in dengue outbreaks. In this situation, a methodology for real time, global surveillance is urgently required to track dengue strains and help anticipate the changes in the epidemiology of the illness.

Two types of infections caused by dengue virus are the DF and DHF also called as primary infection and secondary infection respectively. An acute febrile disease identified as dengue fever is caused by primary infection which is cleared in about 7- days in response to a compound immune reaction of the body. A complication resulted from secondary infection is called as dengue hemorrhagic fever or dengue shock syndrome [20]. 3-14 days of incubation period, multiple symptoms including fever confirm the presence of dengue virus in the blood [21].

The retro-orbital pain, headache, general body weakness, diffuse body pain including both muscle and bone, vomiting, altered taste feeling, centrifugal maculopapular rash, quick start of high fever, and sore throat along other symptoms characterize the classical dengue fever [22]. The term break bone fever describes the severity of the pain in disease caused by dengue. Previously infected smaller no. of people with one dengue serotype expands the degree of illness and result into endothelial seep out and bleeding when infected with other DEN-V serotype. This complex condition is called dengue hemorrhagic fever (DHF) also called as vasculopathy. A circulatory collapse may occur due to serous effusions and hemoconcentration produced as a result of vascular outflow in these patients. When this situation is combined with another complication of severe hemorrhagic fever, it results in DSS or dengue shock syndrome, which increases the fatality risk [23]. Symptoms produced by 1-4 dengue virus (DV) serotypes are similar. The severity of dengue fever range from a mild and self-limited DF to a more complicated life threatening DHF and DSS [21,15]. There is fever and a variety of symptoms after an incubation period which confirms the presence of dengue virus in the blood [15]. In order to detect dengue viremia, several techniques have been developed [24,25,15,26]. The two important factors responsible for increasing the disease complication to 30 folds are the geographical growth of the *Aedes Aegypti* and co-circulation of all four serotypes [27].

5. FACTORS PROMOTING DENGUE PREVALENCE IN PAKISTAN

The globalization has great effect on the re-emergence of several infectious diseases, an increased human trade and travel aids in the introduction of any diseases into new places and the re-emergence of diseases that had been eliminated in some regions [28]. In Pakistan there is a great threat of dengue endemics because the cities are crowded the sanitary conditions are poor. Impure water, low

vaccination coverage, huge number of refugees are among other important factors in this regard [6]. It is believed that Pakistan is endemic to all (1-4) dengue virus serotypes circulating the whole year with a climax during post-monsoon period between October and December [29,30]. The ideal conditions for the vectors exist in Southeast Asian region particularly for the period of monsoon. This period is characterized by the heavy rainfall, high relative humidity and fluctuations in daily temperatures reaching mid 30℃. These seasonal variations regulates the dengue fever, allow maximum growth and breeding opportunities for vector mosquitoes and increasing the chances of outbreak [17]. Like many other parts of the world, the dengue fever is prevailing as an endemic viral human infection in Pakistan. This shocking situation is very upsetting due to the factor that the focal point in disease management depends on the reduction of vector population till finding the specific vaccine or anti-viral remedy preparation [31]. Travellers from endemic areas are potential source for further spread of dengue infection [32, 33,22,34]. One of the important reason for persistence infection is in many tropical and sub tropical countries is the unplanned urbanization [35].

6. HISTORY OF VECTOR DETECTION AT VARIOUS TIMES

The principle vector of dengue, *A. aegypti* was collected in 1934 from various cities of Pakistan including Dera Ismail Khan, Peshawar, Lahore, Karachi and Larkana [36]. In 1949, the presence of *Aedes aegypti* was confirmed from a valley situated in kohat district (a Province of West Pakistan) named Kohat Hangu valley. It was collected on few occasions involved in day biting in the woody area of ChilliBagh [37]. Serological evidences of dengue virus infections dates back to 1968 [6]. Generally, there is a strong belief that the initial appearance of dengue virus in Pakistan at Karachi sea port was through tyre trading, and the tyres were contaminated with infected mosquito's eggs. Dengue was identified in Pakistan in province Punjab in 1982. Out of 174 suspected dengue cases, 12 were found positive for dengue virus [38]. In 1983, a survey was carried out in order to determine the role of different mosquitoes species as vector of various diseases that also confirmed the presence of *A. aegypti* [30]. In order to check the prevalence of dengue virus, Japanese encephalitis and West Nile in Karachi, a research was carried out in 1985. The results confirmed that 65% of the total population of Karachi found positive for haemagglutination inhibition (HI) antibodies against one or more of the three flaviviruses [39].

7. MAJOR OUTBREAKS OF DENV INFECTION IN PAKISTAN

7.1 First Outbreak (1994-1995)

The first confirmed outbreak of dengue infection with symptoms of dengue hemorrhagic fever was observed in Karachi in 1994 [40]. IgM identified in 15 patients out of 16 patients by using DENV-2 antigen. The circulation of DENV-1 and DENV-2 serotypes was also confirmed by the methods based on serological analysis during the 1994 outbreak [41]. During this outbreak the only study in which a molecular technique semi nested polymerase chain reaction (PCR) was used, carried out by Chan et al. [40]. From seras of infected patients the virus was isolated. Jamil et al. [42] described important morbidity and mortality witnessed by Pakistan due to several severe outbreaks of dengue viral illness in 1994. Reports of cases continued in the next year but in another city, located west to Karachi. Most of employees working at a power house in Hub complained about a large number of mosquitoes and pyrexia of unknown origin. By performing ELISA IgM and IgG antibodies, all serotypes of dengue virus detected. In this investigation detection of IgM antibodies for serotype 1 and 2 suggested that infection continued for 2 consecutive years in southern Pakistan. After this outbreak, the dengue infection prevailed in Pakistan as a major health threat [29]. Ten cases were confirmed at Haripur with four deaths in 2003 [43].

7.2 Second Outbreak (2005–2006)

In 2005, serotype-3 was reported in Pakistan after a break of ten years (Table-1). Dengue outbreak in 2005 involved the genotypic diversitification of DENV serotypes in Pakistan is not fully known and studied [2]. The 2005–2006 outbreaks in Karachi confirmed the circulation of DENV-3 for the first time [42,44]. In surveillance, the description and circulation of dengue virus serotypes is very important because there is a risk factor for DHF/DSS affected by pre-existing serotypes after the entry of a novel serotype to areas [45]. In 2005, according to estimation, 395 cases of dengue, all belonging to Karachi were confirmed by National Institute of Health Islamabad in their laboratory. By the use of ELISA and reverse transcription (RT- PCR), a

local hospital conducted a study including 106 patients, 9 of whom died. The cases tested positive were 42 for IgM antibody. According to ELISA presence of both dengue IgM and IgG was detected in six and of IgM only into three out of nine deaths [42]. During 2006 outbreak in Karachi DEN-2 and DEN-3 were found to be co-circulated [44]. In the year 2006, 52 deaths were observed out of 5800 suspected cases from all parts of Pakistan, the confirmed cases were 3000 in number [46]. In 2006 from May to November, most affected city was Karachi during this period of viral infection. Between August and October maximum number of cases was reported due to heavy rainfall in monsoon. 50 deaths occurred in Karachi out of 4500 suspected cases and confirmed cases were 1500 by IgM and IgG antibodies. The reason behind 2006 outbreak was the co-circulation of DEN-1 and DEN-2 [44].

Table 1. Serotypic prevalence of DENV in 1994-2006

Year	City	Dominant serotypes	Deaths	Reference
1994	Karachi	DEN-1, DEN-2	2	[40]
1995	Hub	DEN-1, DEN-2	0	[29]
2005	Karachi	DEN-3	9	[42]
2006	Karachi	DEN-2, DEN-3	50	[46]

7.3 Third Outbreak (2007-2011)

In Pakistan the worst years regarding dengue viral infection were 2007 and 2011 [47]. Fatima et al. [48] conducted a detailed study of major outbreaks in Lahore for a period of three years from 2007-2009. Dengue illness started to appear in the Northern part of the country in 2007 in Lahore, the dominant serotypes were DENV-2 and DENV-3 for the period of 2007 to 2009. After the analysis of 114 serum samples, total 20 patients were found positive for dengue virus. In 2007, DENV-2 was found circulating in 4 patients and DENV-3 in one patient [48]. According to private news channel ARY in November 2010, Out of the 5,050 patients, the patients reported from Sindh, Punjab and Khyber Pakhtunkhwa were 2,350, 1,885 and 158 respectively, DEN-1 and DEN-2 infection was found [49]. In 2008 from September to November 49 confirmed cases were observed, 26 at HFH and 22 at BBH [50]. In 2008, in the city of Lahore within Province of Punjab Pakistan DENV-4 was reported for the first time [51].

In year 2007 overall 2304 cases were reported from all parts of the country. Out of 1226 confirmed cases, 18 deaths reported. In the next year the situation remained more or less same with 2792 reported cases, 2469 lab confirmations and 17 deaths. The suspected cases in year 2009 were 1940, lead to 1085 lab confirmations and 13 deaths. A large number of dengue cases reported in 2010. Out of 15,901 suspected cases, 11,024 found positive for dengue illness and caused 40 deaths. The number of reported cases and deaths increased in 2011, as 219 deaths occurred out of 252935 suspected cases. The number of confirmed cases was in thousands (Table 2).

7.4 Fourth Outbreak (2012-2013)

Since 1994, dengue outbreaks have been reported in cyclic manner in Pakistan, but in 2013 a new trend have been observed regarding dengue prevalence in Pakistan , when cases reported from areas other than endemic belt such as Balochistan Khyber Pakthunkhwa provinces [52]. During Sep, 2013 an epidemiological study was conducted by Ahmed in Batkhela Malakand Agency, Khyber Pakhtunkhwa Pakistan. This study indicated the outbreak of dengue infection in Swat during August 2013 and its link in spreading dengue infections in nearby districts through travelers. In Swat emergency was declared due to report of 7000 infection and 26 deaths. Out of 7 admitted patients in the Ward No 1 District Head Quarter Hospital Batkhela, NSI detected in 4 so they confirmed for dengue fever and 3 were negative. 3 patients out of 4 confirmations showed traveling history to Swat as the Batkhela is adjacent to Swat [53]. Tire importation at Karachi sea port containing contaminated ggs of vector mosquitoes is considered as the first entry of dengue virus into Pakistan [6]. DEWS, the Disease Early Warning System of Pakistan. It is a sustainable and successful programme especially in the developing countries like Pakistan, where infectious diseases cause a major degree of mortality and morbidity. According to one of its recent news huge number of DF cases have been reported from all provinces of Pakistan. From 01 January to 11 September 2013, on the whole, 4,388 patients complained dengue fever from different parts of the country. A province Khyberpukhtunkhwa was on the top with 3,177 cases followed by 1098 cases in Sind province [52]. Random cases have been reported from the Punjab, Sindh, and Balochistan provinces apart from Khyberpukhtunkhaw.

Table 2. Number of reported cases and deaths into major cities of Pakistan from 2007-2011

Year	City	Cases	Deaths	All parts of country		
				Suspected cases	Laboratory confirmations	Deaths
2007	Karachi	950	20	2304	1226	18
	Lahore	258	0			
	Khyberpukhtunkhaw	0	0			
2008	Karachi	585	6	2792	2469	17
	Lahore	1358	9			
	Khyberpukhtunkhaw	30	4			
2009	Karachi	550	7	1940	1085	13
	Lahore	300	2			
	Khyberpukhtunkhaw	100	7			
2010	Karachi	4500	16	15,901	11,024	40
	Lahore	4000	3			
	Khyberpukhtunkhaw	0	0			
2011	Karachi	755	15	252935	17057	219
	Lahore	17,493	290			
	Khyberpukhtunkhaw	296	8			

[38]

Data downloaded from http://www.emro.who.int/surveillance-forecasting-response/outbreaks/ dengue-fever in-pakistan.html [52]

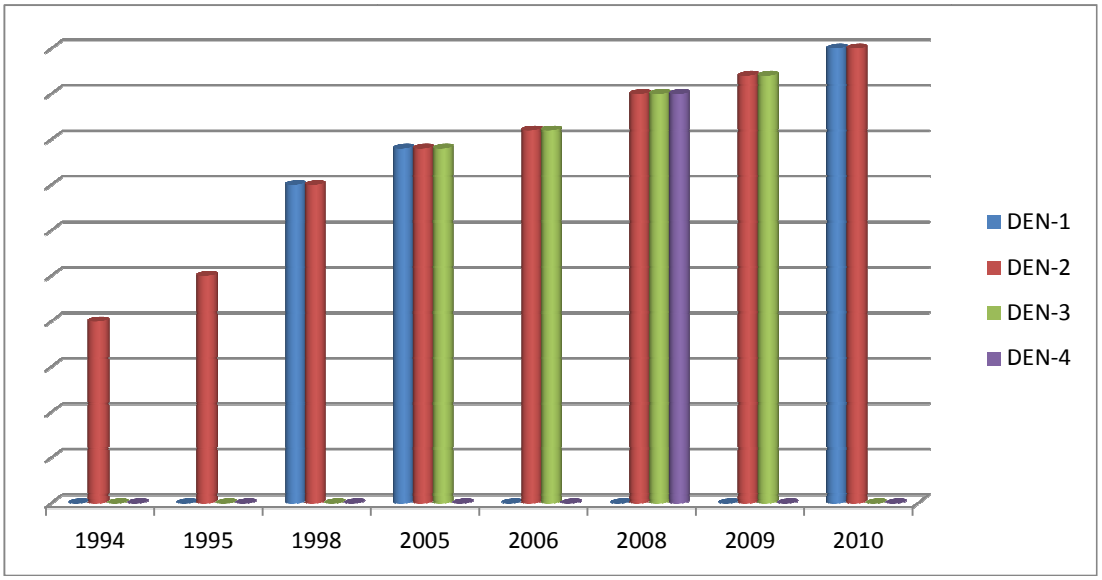

Fig. 2. Year-wise prevalence of the dominant dengue virus serotypes in Pakistan from 1994-2010

8. PREVALENCE OF DOMINANT DENV SEROTYPES IN PAKISTAN

As far as the year wise prevalence of dominant serotypes of DENV infection in Pakistan is concerned DEN-2 was the dominanting serotype from 1994 to 1995 [54,29]. In 1998 both DEN-1, DEN-2 were dominant [41]. All three serotypes DEN-1, DEN-2, DEN-3 in circulation dominated in 2005 [42]. Dominant serotypes of the year 2006 were DEN-2, DEN-3 [55]. A high prevalence of DEN-2, DEN-3, DEN-4 reported in the year 2008 [56]. DEN-2, DEN-3 was the dominant serotypes in 2009 [48]. Next year in 2010 DEN-1, DEN-2 again co-circulated in high ratios [55].

9. LABORATORY DIAGNOSTIC METHODS FOR DETECTION

In humans the dengue is among significant viral diseases, but its detection and laboratory

propagation of 1-4 dengue serotypes is very difficult. In most of the cases to initiate a supportive treatment for dengue fever the clinical suspicion is considered enough. A high conc. of thrombocytopenia, hematocrit, and leucopenia is shown by a complete blood picture. The serum albumin, chest X-Ray (if necessary) is among other laboratory tests. Dengue fever is not predicted by a normal blood count but platelets greater than 50,000 and leukocyte count less than 3 indicate a bad prognosis [5]. The molecular techniques involved in Laboratory diagnosis of dengue viral illness includes reverse transcription (RT)-PCR, for it different protocols are available which are <16 [57]. A seminested RT-PCR is one of these protocol developed by Lanciotti and coworkers. Its target areas are the C and pre M regions [24]. D1 and D2 are universal outer dengue primers used by it followed by combining primer D1 that is essentially serotype specific seminested PCR combination with only one of these TS1, TS2, TS3, or TS4 internal primers. This assay is commonly used for diagnosis and surveillance of dengue infection in different countries mainly in South-east Asian countries [58]. The sensitivity of virus isolation is known to exceed by using aliquots of the same sample by tis assay [21]. In use methods of dengue diagnosis include hemagglutination assays, ELISAs and immunofluorescence tests. Low count of white blood cells and platelets, test for abnormal liver function, for serologic diagnosis IgM ELISA test.

The initial acquired immune reaction of an individual's body against the DENV infection is characterized by the presence of antibodies IgM and IgG primarily produced in response to envelope proteins of virus. The clinical symptoms produced by 1-4 dengue virus serotypes are similar. The severity of infection rises gradually starting from mild DF to severe life-threatening DHF and DSS. The detection and quantification of dengue virus (DV) in plasma is carried out by using many reverse transcription PCR (RT-PCR) based methods [24-26,5].

9.1 Advantage of Using Serological Testing

For diagnosis of dengue infection serological assays are most commonly used because when they are compared with nucleic acid based methods or culture they are inexpensive and easy in performance. A secondary immune response occur when a person experience dengue infection for second time, followed by the production of higher levels of IgG because the memory B cells get stimulated from previous infection, and an IgM response to existing infection [59].

9.2 IgM Antibody-Capture Enzyme Linked Immunosorbent Assay

Mac-ELISA is one of Classic serological testing for dengue [60]. Mac-ELISA was developed by the AFRIMS (Armed force research institute of medical sciences) for the diagnosis of dengue in areas where co-circulation of Japanese encephalitis and dengue occurred [61]. At present many in-house Mac-ELISAs are developed by different groups. The detection of dengue specific IgM in test serum is carried out by the capture of all IgM and by the use of human specific IgM to a solid phase. Commercial kits showing variable sensitivity and specifity are available and are more than 50 [61-63]. A mixture of four dengue antigens used in this assay, generally derived from infected cell culture dengue virus or the brain preparations of infected suckling mouse [64]. The crossreactivity with other flaviviruses and dengue specific IgG are the factors responsible for false positive results and therefore, a limitation factor of Mac-ELISAs, especially in those areas where co-circulation of different flaviviruses occur. The sera of patients of malaria and leptospirosis indicate non-specific reactivity by some tests [65].

9.3 IgG ELISA

In paired seras, dengue infection confirmation takes place by the use of dengue specific IgGdetection, this type of ELISA is called IgG ELISA. Another use of this assay is in classification of primary and secondary Infections [66-68]. The detection of a past infection with dengue is carried out by the classic IgG ELISA; involve the use of same antigen as in the MAC-ELISA. Multiple dilutions of sera tested in order to find an end-point dilution. This assay has a correlation with previously used haemagglutination assay. After the infection, more robust response will obtained by performing higher end-point dilution. There is a correlation between this assay and previously used the haemagglutination assay [60].

9.4 IgM/IgG Ratio

For distinguishing the primary from secondary infection a dengue virus E and M protein-specific

IgM/IgGratio.canbe used. Most common assays for this purpose are IgM capture and IgG capture ELISAs. In this method if the IgM:IG OD ratio is greater than 1.2 then the dengue infection is called as primary infection (using sera at 1:100 dilution)or 1.4 (sera at 1:20 dilutions) or it is designated as secondary infection if its ratio is less than 1.2 or 1.4 [69,70].

9.5 Neutralizing Assays

Two tests, used in order to define the infecting serotypes after a primary infection are micro-neutralization assay and PRNT (plaque reduction neutralization technique).The main purpose of these tests is vaccine studies and research [71-75].

9.6 Nucleic Acid Amplification Tests

To diagnose the dengue infection several NAATs (nucleic acid amplification tests) have been developed. Among these techniques some are used for serotyping and other are quantitative [59].

9.7 PCR (RT-PCR) Reverse Transcriptase

During the last decade, several RT-PCR assays have been introduced. Different genes are targeted by these in-house assays by using different amplification procedures. NAATs which are most frequently used are based on a nested RT-PCR assay, one step multiplex RT-PCR assay, and a single RT-PCR assay [26].

9.8 Real-Time RT-PCR

A one step assay called the real-time RT-PCR assay allows the quantification of virus titre in about 1.5 hours. The need for post-amplification electrophoresis is replaced by the detection of the amplified target by fluorescent. There are several RT-PCR assays that may be singleplex (identifying single serotype in a reaction) or multiplex (detecting all serotypes from a single sample) [76-78].

9.9 Nucleic Acid-Sequence Based Amplification Assay (NASBA)

This is an amplification assay which is isothermal RNA-specific which is used for dengue virus. The NASBA assay is comparable to that of other NAATs in its performance [79].

9.10 Antigen Detection

The detection of dengue antigen from the tissues like spleen, liver, and lymph nodes as well as tissues from fatal cases (slides from paraffin-embedded, fresh or frozen tissues) is carried out by the use of an enzyme and a colorimetric substrate along with antibodies that targets antigens which are dengue specific [80-82].

9.11 NS1 Antigen and Antibody Detection

All flaviviruses produce NS1, a glycoprotein and is necessary for viability replication of viruses. Many tests developed to diagnose dengue infection are based on the use of NS1 because the protein is secreted into bloodstream. Among these tests are the lateral flow antigen detection and measurement of NS1-specific IgM and IgG responses, antigen-capture ELISA. Kits for the detection of NS1 antigen are now available commercially but, different dengue serotypes are not differentiated by these kits till now [83-85].

10. DENGUE DIAGNOSTIC TECHNIQUES IN PAKISTAN USED BY DIFFERENT RESEARCHERS

In Pakistan the laboratory diagnosis for dengue virus infection was performed abroad in collaborating centers due to unavailability of diagnostic tests locally. As a result of huge pressure of cases reported from Karachi during 2005-2006 epidemic ELISA-based commercial kits were imported at national and local hospital level. For the diagnosis of dengue Khan et al. [86] in 2006 evaluated two commercially available ELISA-based kits (PanBio versus C albiotech) against RT-PCR in a hospital setting in Karachi (AKUH). They concluded that for diagnosis of dengue virus detection RT-PCR is considered as gold standard but cost effective serological methods in dengue endemic countries are also important. Among these two commercially available ELISA-based kits PanBio ELISA was found to be more sensitive and specific. Although for a rapid dengue diagnosis a number of products are available in market, Naz et al. [87] in 2010 conducted a study in order to evaluate the performance of few products and compared with ELIZA. They concluded that device for timely dengue diagnosis could be IgA RDT (a cost effective and efficient rapid test) at all levels of healthcare settings. In resource limited countries RDTs may also be helpful in epidemiological studies as these are accurate

and inexpensive [87]. Sajid et al. [88] carried out a study during August–October 2011 in department of Pediatrics Faisalabad. The patients included in this study were confirmed serologically positive for IgM, Ig G anti bodies by ELISA and fulfilling the WHO diagnostic criteria. Chest X ray, blood counts, typhoid test, abdominal ultra-sonography, liver function tests were also performed. They suggested continuous surveillance due to variations found in clinical presentation of Dengue fever in children of Pakistan. In 2012 Hakim et al. [89] subjected 85 serum samples and 3 whole blood samples to Immuno fluorescent staining technique followed by RNA extraction from dengue serum samples and then exposed to one step multiplex Real Time RT PCR and finally to 2 percent agarose gel. Positive results obtained on one step multiplex Real Time RT PCR for all 85 serum samples also bands obtained for dengue serotype 2, 3 and 4 on 2% agarose. On the basis of results they explained that for the diagnosis further epidemiological studies and research purpose immunofluorescent technique will be very helpful in future. In order to ensure a cost effective, rapid early dengue diagnosis in Pakistan Hussain et al. [90] conducted a study with an aim to locally develop a lateral flow device by the use of nano-gold labeled anti-NS1 antibodies for the detection of NS1 Ag in the serum. For detection of NS1 this diagnostic device showed promising results. In another study for dengue serology the comparison of the efficacy of two commercially available (Acon and BIAS-3) rapid diagnostic test devices was made. On the basis of their results they found rapid test devices (based on immunochromatographic method) unreliable diagnostic tools for dengue detection [91].

Now it's the responsibility of Govt. of Pakistan in general and health authorities in particular to supply more reliable rapid diagnostic tests for dengue infection.

11. IMMUNIZATION AND TREATMENT OF DENGUE

Currently no specific treatment is available for dengue fever. Lives of people in severe dengue could be saved by the medical care provided by physicians and nurses. The experience of effects of the disease and its progression is essential for the physicians and nurses in order to save lives and to decrease mortality rates to less than 1% from more than 20%. At the same time no vaccine against dengue is there to protect against disease [92].

12. CURRENT DENGUE SITUATION IN PAKISTAN 2014

World Health organization (WHO) in collaboration with Government of Pakistan, compiled an epidemiological bulletin during the first eleven days of 2014. According to this bulletin out of 69 confirmed dengue illness cases, 68 were from Sind province and only one case was reported from the province of Punjab. A WHO health expert said that the major reason behind these cases was the rising temperatures. Day time temperature of Sind these days is above 20℃ that is suitable for the breeding of mosquitoes [93]. As dengue is now endemic to almost all Provinces of Pakistan so, it poses great health threat. In the absence of a suitable vaccine, currently available tools should be utilized. Better diagnostic techniques, should be adopted for early diagnosis in order to reduce mortality ratios.

13. CONCLUSION

The present study highlighted the importance of vector presence, re-emergence, disease spread and commonly used diagnostic methods in the world and Pakistan. As genetic diversification of dengue serotypes in Pakistan remained more or less un-checked and consequently led to many outbreaks and geographic expansion of vector population. Now dengue is both a health threat and endemic to almost all Provinces of Pakistan. Therefore, more scientific research in a developing country like Pakistan is needed in order to develop new or improved dengue control approaches and techniques. The factors like serotypic study of dengue infection, its transmission patterns, risk factors management and reasons behind major breakouts would help in reduction of vector population and outbreaks. Without these measures the coming years will be more critical in terms of vector transmission and mortalities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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