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Scenario of dengue infection & its control in Pakistan: An up–date and way forward

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ABSTRACT

Dengue fever is one of the major health problems in tropical and subtropical areas throughout the world. The causative agent of dengue fever is the dengue virus which is an enveloped single stranded RNA virus belongs to the family Flaviviridae and has five distinct serotypes (DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5). Dengue virus is transmitted to human via bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. The clinical symptoms of dengue fever ranging from mild to severe form as dengue hemorrhagic fever and dengue shock syndrome. Pakistan is dengue endemic since 1994 but from 2006, Pakistan faced the worst condition regarding dengue in which thousands of people affected by the disease and hundreds of people lost their lives. DENV-2, DENV-3 and DENV-1 are the prevalent serotypes in Pakistan. Common diagnostic techniques are being used in Pakistan such as enzyme-linked immunosorbent assay, polymerase chain reaction and rapid diagnostic tests, while differential diagnosis, limitations of diagnostic methods and poor health care system are the real challenges in dengue diagnosis. Favorable climatic conditions, unplanned urbanization, travelling *etc.*, are major factors responsible for dengue epidemics in Pakistan. This presentation provides update about dengue circumstances in Pakistan and also describes the way how to improve dengue situation in Pakistan.

1. Introduction

Dengue fever is the most frequently spreading mosquito borne disease and it is the major health problem in tropical and subtropical areas throughout the world[1]. Dengue is a Spanish word which means “fastidious”, it is derived from “dinga” which means an evil of spirit and break bone fever was first described by Benjamin Rush in 1789[2]. The causative agent of dengue fever is the dengue virus which is an enveloped single stranded RNA virus belongs to the genus Flavivirus and family Flaviviridae. Dengue virus is transmitted to human via bite of infected female mosquito of the genus *Aedes*, mostly *Aedes aegypti* (*Ae. aegypti*) and rarely *Aedes albopictus*. The *Ae. aegypti* is a day biting mosquito, was found

in tropical and subtropical areas and breeds in collected stagnant water[3]. *Ae. aegypti* becomes infected by the dengue infected person and can transmit virus to non-infected person after an incubation period which is 8–10 days[3]. Dengue fever represents broad range of clinical symptoms including mild fever to sever forms[4]. Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the severe forms of dengue which are responsible for high morbidity and mortality rate in the world[5]. Severe headache, muscles and joints pain, rash, nausea, vomiting are the symptoms of dengue fever while DHF are characterized by high fever, haemorrhagic phenomena, hepatomegaly, and often circulatory disturbance and shock[6]. Dengue is the major cause of hospitalization, and it is

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estimated that 500 000 people infected with severe dengue require hospitalization in which children are dominant while about 2.5% affected people die annually[7].

2. Genomic organization

Dengue virus is a 50 nm enveloped virus with positive sense single stranded RNA that directly translated into protein. Dengue has a genome of about 11 kb that encodes a single large poly protein, and is consequently divided into three structural proteins: C, PrM, E, seven non-structural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5, and short non coding regions on both 5' and 3' end Figure 1[8,9].

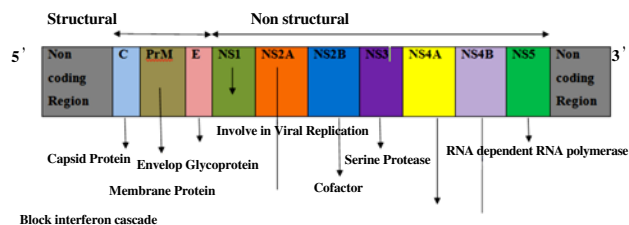


Figure 1. Genome organization of dengue virus.

3. Dengue serotypes

Serotype is a group of viruses grouped together based on their antigens on the surface of virus. The evidence for strain differences among dengue viruses was first detected by Albert[10]. Dengue viruses are classified into four serotypes: DEN-1, DEN-2, DEN 3 and DEN-4. The antigenically intent but closely related serotypes of dengue show 65%-70% sequence homology[11]. Each serotype has got different genotype exhibiting comprehensive genetic variability which causes difficulty in vaccine development against all four dengue serotypes. Recovery of dengue infection by one serotype provides long life immunity against selective serotype while cross immunity to the other dengue serotype is temporary[4]. Re-infection of dengue with new serotype can show great risk of developing dengue hemorrhagic due to antibody dependent enhancement, a phenomenon in which virus-specific antibodies strengthen the entry of virus, and in some cases the replication of virus within monocytes/macrophages and granulocytic cells[12,13]. Infection caused by all four serotype of dengue is similar but several studies showed that DEN-2 and DEN-3 after related to severe form of dengue while DEN-3 causes mild illness[14-16]. Recently policy of the Centre for Infection Diseases and Research reported a fifth serotype which follows sylvatic cycle unlike the human cycle which is followed by other dengue serotypes[17,18].

4. Relationship of dengue vector with serotypes

Mosquito's vectorial capacity influences the dengue transmission and epidemiology[19]. The impact of mosquito-dengue interaction on

epidemiologically significant characters is critical in understanding of dengue epidemiology. Several reports showed the relation of dengue with mosquito. Platt *et al.* demonstrated that in dengue infected mosquitoes the preliminary time for meal and time spent probing are elongated as compared to uninfected mosquitoes[20]. Another study reported that dengue infection slows down the survival and fertility of female mosquito *Ae. aegypti*[21].

Dengue virus can modify the life-history traits of mosquito which are exactly involved in mosquito's vectorial capacity[20,22]. In *Ae. aegypti* infected female mosquitoes, DEN-2 probably extending their biting rate by increase their locomotor action up to 50%[23]. Another study showed that the biting mode of *Ae. aegypti* is influenced by dengue serotype 2. Study suggested that DEN-2 increases the encouragement of infected female *Ae. aegypti* to blood feeding and increases the motivation to blood re-feeding[24]. Dengue virus may also exhibit differences in transmission potential. Some dengue virus serotypes may cause high viremia in human host by infecting and replicating efficiently in target cells while some stains are transmitted more conveniently by vector mosquitoes as compared to other strains. For instance, DEN-2 of South East Asian genotype infected *Ae. aegypti* mosquito more efficiently as compared to American genotype[25].

5. Dengue serotype-specific clinical manifestations

Dengue infection can range from mild to severe forms as DHF and DSS. Infection with different dengue serotypes causes identical clinical symptoms, while different clinical manifestations with respect to dengue serotypes have been reported[26,27]. A study in Hong Kong found minimum lymphocyte count in DEN-3 cases as compared to other serotypes[28]. Higher pleural effusion index was found in DEN-2 compared with DEN-1 in a study from Thailand[29]. The prevalence of respiratory end point (combining cough, rhinorrhea, nasal stuffiness, or sore throat) was more in DENV-3 than DENV-2 in Taiwan[26]. Another study from Singapore presented that red eyes was common in patients infected with DEN-1 while joint pain and low platelets count was associated with DEN-2 cases. The study also documented that the relative risk of developing DHF and SD in DEN-1 was higher compared with DEN-2 and DEN-3[30]. Hasley reported higher frequency of headache and prostration with DEN-3 and high prevalence of malaise with DEN-2 and DEN-3 were also demonstrated by Hasley *et al*[31].

6. Dengue serotypes in Pakistan

Dengue was first introduced in Pakistan at Karachi sea port through the importation of tyres containing eggs of infected mosquitoes[32]. Dengue is endemic in Pakistan in the post monsoon period for many years and several outbreaks have been reported from Pakistan. Dengue virus infection was first detected in 1982 from serum samples that were collected in 1968 and 1978 Punjab province[33]. In 1985, a study conducted in Pakistan showed 50%-60% positive haemagglutination test for West Nile, Japanese encephalitis and DEN-2 Flavivirus in which Karachi citizens were

dominant[34]. Chan and colleagues documented the first DHF outbreak in 1994 and observed DEN-1 and DEN-2 in dengue patients[35]. In the following year, DEN-2 infection was reported from Balochistan[36,37]. Prevalence of DEN-2 was reported from Karachi in 1997[36,38]. In 1998, DEN-1 and DEN-2 identified in patients using ELISA study[39]. In 2003, dengue occurred in Haripur, Khushab and Nowshehra where DEN-2 was dominant serotype[40]. In 2005, DEN-3 infection was reported in Karachi by Jamil and colleagues[41]. Co-circulation of DEN-2 and DEN-3 was identified by Khan and colleagues in 2006[42]. Fatima *et al.* showed the co-circulation of DEN-2 and DEN-3 in dengue positive patients during 2007 to 2009[43] while Hamayoun and colleagues also reported DEN-3 infection in 2008[37]. Mahmood and colleagues identified DEN-1 and DEN-2 in dengue patients from Lahore, Saikhpura and Gujranwala cities in 2010[44]. A study conducted in Pakistan showed that the causative agent of dengue epidemic in 2011 was DEN-2[8] while in 2013 another study performed in Pakistan identified DEN-2 and DEN-3 in dengue patients from Swat[45].

Table 1

Prevalence of dengue serotypes in Pakistan.

Year	Dengue serotype	Region	References
1985	DEN-2	Karachi	[34]
1994	DEN-2	Karachi	[35]
1995	DEN-2	Baluchistan	[36,37]
1997	DEN-2	Karachi	[36,38]
1998	DEN-1 & DEN-2	Karachi	[39]
2003	DEN-2	Haripur, Khushab, Nowshehra	[40]
2005	DEN-3	Karachi	[41]
2006	DEN-2 & DEN-3	Karachi	[42]
2007	DEN-2 & DEN-3	Lahore	[43]
2008	DEN-2 & DEN-3	Lahore	[37,43]
2009	DEN-2 & DEN-3	Lahore	[43]
2010	DEN-1 & DEN-2	Lahore, Saikhpura, Gujranwala	[44]
2011	DEN-2	Punjab	[8]
2013	DEN-2 & DEN-3	Swat	[45]

7. Co-circulation and co-infection by dengue virus serotypes in Pakistan

The presence of all four dengue serotypes is common in dengue epidemic region and co-circulation of multiple dengue serotypes has been identified in Pakistan in last few years. Co-circulation of DEN-2 and DEN-3 was identified in 2006 while DEN-2, DEN-3 and DEN-4 co-circulation was determined during 2008 dengue outbreak in Lahore[37,42]. A study by Muhammad *et al.* showed 13.3% co-infection of DEN-2 and DEN-3 in 60 positive dengue samples[46]. Another published report represented DEN-2 and DEN-3 as predominant dengue serotypes circulating in 2007 to 2009 dengue outbreaks[43]. The co-circulation of multiple dengue serotypes promotes the incidence of co-infection with the frequency minimum (5%-30%) to maximum (40%-50%)[47]. The first co-infection by multiple dengue serotypes was identified from Puerto Rico in 1982[48]. Different percentage regarding dengue co-infections have been reported by several countries[49]. A study in Pakistan showed high percentage of dengue co-infection (27%) with DEN-1, DEN-2

and DEN-3 during 2011 dengue outbreak[50]. Co-infection in human and mosquito provides a chance of viral genome recombination and new strain production which could make the disease more severe and it is the primary element in development of severe forms of dengue as DHF/DSS[51]. According to published report co-infected patients are at high risk with respect to clinical symptoms as compared to mono infected patients[47]. Repeated mosquito feeding and movement from area in which one serotype is prevalent to the area where another serotype is common may be the causes of co-infection (Figure 2)[51].

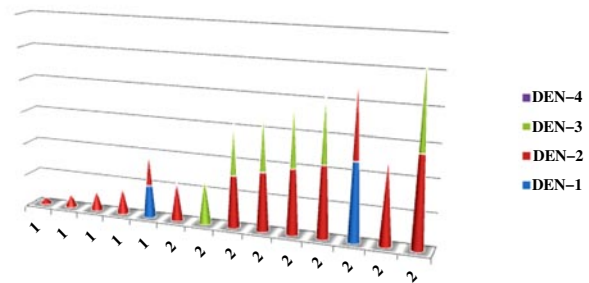


Figure 2. Prevalence of dengue serotypes in Pakistan.

8. Phylogenetic analysis dengue serotypes in Pakistan

Evolutionary studies are important to predict the origin and spread of viruses, to understand the viral pathogenesis, disease epidemics and virulence on genetic basis. Dengue as RNA virus evolves rapidly, positive mutations can cause phenotypic change in virus results in evolution of severe disease[52,53]. To approach phylogenetic analysis among dengue virus strains envelop (E) gene sequence generally used in recent years while in Pakistan NS3 gene sequence and C-prM junction has been used[41,54,55]. A study in Pakistan by Fatima *et al.* reported that circulating genotypes in the period 2007 to 2009 was subtype IV of DEN-2 and subtype III of DEN-3. The genotypes were determined in the study by phylogenetic analysis of C-prM junction sequence[43]. Khan and co-workers identified DEN-2 as prevalent serotype in the 2011 dengue outbreak in Pakistan. The study also characterized the serotype genetically as cosmopolitan genotype (IV) of DEN-2 and closely related to viruses isolated in India and Sri Lanka in the past two decades[56]. Another study from Pakistan showed that DEN-2 is the dominant serotype in years 2010, 2011 and 2013. The study also demonstrated that circulating DEN-2 in these years was introduced in Pakistan from two specific time events, first recorded from India to southern Pakistan in late 1980 and next from Sri Lanka in 2000[57]. Koo *et al.* reported that in Pakistan DEN-2, DEN-3 and DEN-4 were circulating from 2008 to 2011[58]. These serotypes share an Indian subcontinent ancestry and possibly were first introduced into southern Pakistan. In the same period, DEN-2 and DEN-3 evolve in situ as divergent population and both were circulating in southern Pakistan until 2009, after that DEN-2 moved to northern Pakistan and caused the worst condition regarding dengue in Punjab province of Pakistan in 2011(Table 1)[58].

9. Prevalence of dengue serotypes in geographical areas of Pakistan

9.1. Karachi

Karachi is the capital of Sindh province which is located in southern Pakistan. Karachi is also a victim of dengue since 1994[35]. After that many dengue cases have been reported from Karachi. Among four serotypes DEN-2 and DEN-3 are common in Karachi[39,41,42,59]. Sequence analysis explained that the genotype III of circulating serotype DEN-3 during 2005 in Karachi was similar to Indian strains[41]. A study by Koo *et al.* showed 99.1%-99.4% sequence similarity between DEN-2 strains isolated from Karachi during 2008-2009 and Indian/Sri Lankan strains identified during 2003 to 2004[58]. Furthermore, it is reported that DEN-3 isolates from Karachi and Hyderabad are genetically identical with Chinese and Indian strains[58].

9.2. Lahore

Lahore is the capital city of Pakistani province Punjab. Punjab is located in the north of Pakistan and is endemic to three serotypes of dengue (DEN-2, DEN-3 and DEN-4). Fatima *et al.* reported that DEN-2 and DEN-3 are the causative agents during three mini dengue outbreaks in Lahore[43] while another study showed DEN-2, DEN-3 and DEN-4 as prevalent serotypes in Lahore[37]. The phylogenetic analysis reveals that circulated genotype are subtype IV of DEN-2 along with dengue strains from northern India, China, and Indonesia while subtype III of DEN-3. Genotype III of DEN-3 is 99% homologous with Sri Lankan DEN-3 (III) strain[43].

10. Why Pakistan is dengue endemic

Pakistan is dengue endemic since 1994, and now dengue becomes a public health concern in Pakistan. From 1994 dengue cases have been reported but from 2006, Pakistan faced the worst condition regarding dengue in which thousands of people affected by the disease and hundreds of people lost their lives (Table 2). In Pakistan there are many factors involved in spread of dengue epidemics. The most important factor is the favorable climate, as the climate of Pakistan is most favorable to the mosquito especially in the post monsoon period in which hot and humid both conditions available. The increase in temperature is supportive for the mosquito (*Ae. aegypti* as it is dominant in Pakistan) and it also important in vector replication and maturation[59,60]. Another component is the unregulated and unplanned urbanization which provide intense breeding sites to *Ae. aegypti* due to incapability of environmental sanitation to cope up with the plethora of waste containers[61]. Other factors as improper sanitation facilities, over population, lack of fresh drinking water, inadequate mosquito control, air traveling, poor socioeconomic conditions, absence of public health support and awareness related to health effects are all play crucial role in dengue outbreaks[45,50,62].

Table 2

Dengue cases and deaths from 1994 to 2016 in Pakistan.

Year	No. of cases	No. of deaths	References
1994	145	1	[35]
1995	75	57	[40]
2005	106	9	[60]
2006	3 000	52	[63]
2007	1 208	22	[60]
2008	2 065	30	[60]
2009	1 085	13	[64]
2010	11 024	40	[64]
2011	18 000	350	[65]
2012	712	N/A	[66]
2013	2 180 (estimated)	72	[67–69]
2014	2 999	15	[68,70,71]
2015	5 237	6	[72]

11. Dengue diagnosis

Correct diagnosis of dengue infection is very important to distinguish dengue from other diseases and to successfully treat dengue illness. Symptoms based diagnosis of dengue is unreliable due to broad spectrum of symptoms produced by dengue[1]. Conventional methods to diagnose dengue infection are virus isolation, viral genome detection and serological diagnosis[4]. Confirmation of dengue by virus isolation method generally requires 1-2 wk[1] and its sensitivity is low therefore it has been gradually replaced by reverse transcription (RT)-PCR[73].

11.1. Viral genome detection

Molecular techniques for genomic sequence detection are RT-PCR, nested PCR, real-time PCR, nucleic acid sequence-based amplification[74].

11.1.1. RT-PCR

RT-PCR is highly sensitive technique for the detection of mRNA in which complementary DNA synthesise from RNA by reverse transcriptase which is later amplify by polymerase[75]. Several RT-PCR methods have been developed since 1990. The sensitivity of RT-PCR is better as compared to virus isolation and it varies from 80% to 100% depending on various factors[1].

11.1.2. Real-time PCR

Real-time PCR is a technique which is used to quantitative analysis of nucleic acid by fluorescent detection of labeled PCR product. The use of fluorescent probes in real-time PCR enables the detection of amplified product in real time. The two step nested PCR technique was developed by Lanciotti *et al.*[76], and the modification in nested PCR to one step multiplex PCR was developed by Harris *et al.*[77]. Viral titer determination and rapidity are the advantages of real-time PCR and therefore it can be used to study viral pathogenesis[1,74].

11.1.3. Nucleic acid sequence-based amplification

Nucleic acid sequence-based amplification is an isothermal RNA-specific amplification assay in which there is no requirement of thermal cycling instrument. RT is the initial stage in this method and the amplified product is detected by either by electrochemiluminescence or with fluorescent labeled probes. This technique is useful to study dengue infection[1].

11.2. Serological diagnosis

Serological tests are widely used for the detection of dengue infection. These tests include hemagglutination inhibition (HI) assay, rapid diagnostic test and enzyme-linked immunosorbent assays (ELISA) for the detection of IgG and IgM antibodies.

11.2.1. ELISA

ELISA is a common and laboratory technique for detecting proteins by antibodies and represents a significant addition to existing serological tools. Eva Engvall and Peter Perlmann published first paper on ELISA protocol in 1971[78]. ELISA has become an invaluable tool for dengue diagnosis especially IgM-capture immunoenzymatic technique (MAC-ELISA) which corresponds to the most important advances for IgM detection (G. G. M., 2004), (J, 2015). MAC-ELISA was developed by Kuno *et al.*[19,79]. MAC-ELISA is efficient technique than other serological methods[1] and after serological conversion the sensitivity and specificity of this method is 90% to 98% in clinical samples[4]. Another format is IgG capture ELISA (GAC-ELISA) for IgG antibodies detection. GAC-ELISA is easy to perform and its sensitivity is high as compared to HI test[4].

11.2.2. HI Test

HI test has been the standard dengue diagnosis method for many years (BA, 2004). The principle of HI test is based on the capability of dengue envelope protein to agglutinate red blood cells. The extent of agglutination inhibition by anti-dengue antibodies is measured in HI test[1]. Due to simplicity and sensitivity of HI test, it is possible to differentiate between primary and secondary dengue infection[4].

11.2.3. Rapid diagnostic test

Lateral flow based immuno chromatographic test or rapid diagnostic test have developed to screen several diseases. This simple and cost-effective immuno chromatographic assay was developed by Singer and Plotz in 1956[80]. A number of commercial rapid test kits for anti-dengue antibodies (IgM and IgG) are available in which some give results in 15 min[81]. It is suitable for point of care tests step test and can be used in dengue epidemic regions especially in areas with limited laboratory resources[82]. Unfortunately, the accuracy of most of these tests is variable and false positive results due to cross reactivity with other flavivirus associated with rapid strips.

12. Challenges in dengue diagnosis

Dengue diagnosis is really a challenging matter for any physician because most of its symptoms are similar to other disease such as malaria, rubella, leptospirosis *etc.* Incorrect and missed diagnosis is the major problem in dengue diagnosis. Atypical clinical appearance of dengue, use of tourniquet test by physicians and false positive results by serological test all are lead to incorrect dengue diagnosis. Similar conditions to dengue infection are generally the reason of missed diagnosis and a report by Lahiri *et al.* showed that the 2/3 of dengue patient died because of missed diagnosis. There are several factors that constitute difficulty in dengue diagnosis such as concomitant infection with dengue, incorrect history by patient,

restriction of diagnostic resources and laboratory errors[83].

There are certain limitations of diagnostic methods. Confirmation of dengue by virus isolation method requires weeks[1]. The sensitivity of virus isolation method is low[73], expensive lab requirements to maintain and develop, not possible to differentiate between primary and secondary infection all are the limitations of virus isolation method. PCR is an expensive technique, useful only in early phase of illness[74]. False negatives and positives due to cross contamination are also an issue in PCR[50].

Serological method as hemagglutination exhibit cross reactivity with other flavivirus infections, and this method requires chemical pretreatment for removal of nonspecific inhibitors[4]. Cross reactivity is also associated with MAC-ELISA[30]. GAC-ELISA enables to detect dengue serotypes[4]& another limitation of ELISA is the secondary confirmatory test which is necessary to differentiate between past and recent infection[84].

13. Non vector transmission of dengue virus

Dengue virus generally transmitted through mosquito biting, but rarely dengue virus transmission without a vector has been documented. Other modes of transmission include needle-stick-related transmission, transfusion related transmission, grafting related transmission and vertical transmission.

13.1. Needle-stick-related transmission

Needle-stick injury or cut with a sharp object is one of the most common accidents in routine medical practice. Similar to several infectious diseases (HIV, HBV) dengue transmission through needle stick has been confirmed but rarely and only in medical personnel. In this mode of transmission, the level of virus in the contaminated donor must be high as compared to usual rout of transmission[85].

13.2. Transfusion-related transmission

Dengue provides an excellent model of transfusion-transmitted disease. Although there is a large distribution of the disease worldwide, only three transfusions mediated dengue cases reported. The first case regarding transfusion transmission of dengue was documented in Hong Kong in 2002, the second case reported in Singapore and the third in Puerto-Rico in 2007. Clinical selection of donors, implementation screening test for dengue, and inactivation of pathogens are the available measures for reducing the transfusion related transmission of dengue[84].

13.3. Grafting-related transmission

Organ grafting is often the only treatment for end state organ failure, such as liver and heart failure. The transmission of dengue through renal grafting and bone marrow grafting has been documented previously with two reports one case with renal transplantation and other with bone marrow transplantation. In grafting-related transmission viraemic stage of the infected donor is necessary that's why it is uncommon mode and only few cases have been reported. Transplantation-related transmission may be avoided by donor screening system[85].

13.4. Vertical transmission

Vertical transmission is the transfer of disease which causing agent from mother to baby. Dengue virus can be transmitting by this mode due to small molecular size (about 40-60 nm). Pregnant women can acquire dengue infection like other diseases. However, vertical transmission of dengue reported only in late pregnancy which can result in congenital infection due to immuno pathogenesis, while incomplete development of fetus immune system might be the reason of vertical transmission failure in early pregnancy^[85].

14. Treatment of dengue according to risk groups

According to WHO, clinical guide uses three categories for case management (A, B, C). It based on the model of case classification that follows after a patient has fulfilled the criteria for probable dengue.

14.1. Group A

The person who is being diagnosed at the early stage belongs to this group. Patients in this group do not have warning signs. Bed rest and the oral fluid therapy is the primary achievement in dengue outpatient management. There should be oral fluid intake to replace fluid from fever or vomiting and to stop electrolyte imbalance. Paracetamol recommended if patient is uncomfortable with specific dose (10 mg/kg/dose, less than 3 - 4 times in children and less than 3 g/d in adults). Patients must be hospitalized if any of the indication appear like severe abdominal pain, persistent vomiting, cold and wet troubles, anxiety, bleeding and shortness of breathing. Commercial carbonated drinks, aspirin, ibuprofen or other non-steroidal anti-inflammatory agents (NSAIDs) should be avoided.

14.2. Group B

These are patients who are near to critical phase. In this group patients include with warning sign, with co-existing condition, with social crisis (living in remote area and unable to reach medical facility). Rapid fluid replacement is vital to prevent shock state development and the severity of disease can be modified by intravenous fluid therapy. Isotonic solution of 0.9% saline, Ringer's lactate or Hartmann's solution should be given. Start solution with 5-7 mL/kg/h for 1-2 h, then lower to 3-5 mL/kg/h for 2-4 h and then reduce to 2-3 mL/kg/h or less according to clinical counter. Continue volume 2-3 mL/kg/h for 2-4 h if haematocrit remains same or rises minimally and if it is rising rapidly increase the rate to 5-10 mL/kg/h for 1-2 h. Patients should be guided by the health care worker until the risk is over.

14.3. Group C

These are patients who are in the critical phase stage of the viral infection and require emergency treatment are categorized in this group. Patients in this group have severe plasma leakage, severe haemorrhage and severe organ impairment. All patients should be hospitalized with approach of blood transfusion facility. Accurate intravenous fluid recovery is crucial for patients. Plasma loss should

be recovered with isotonic crystalloid solution and to maintain effective circulation. Blood transfuse only in permanent severe bleeding^[56].

15. Way forward

Control of vector borne diseases including dengue is very challenging in Pakistan due to lack of trained entomologist, quality assurance, adequate monitoring and assessment system, and absence of designated vector authority department for dengue vector control interventions. By implementing following steps given below, dengue prevention can be better in future.

15.1. Health care system up gradation

In Pakistan, poor health care system may be responsible for high mortality rate from dengue in previous years. The rate of mortality can be decreased by implementing timely and appropriate clinical management, which involves early clinical and laboratory diagnosis, intravenous rehydration, staff training and hospital reorganization.

Health Ministry of Pakistan should plan health promotion campaigns for spreading awareness in community to eliminate the breeding sites of mosquitoes. Improved hygienic practices, use of personal protective measures, encourage using of larvicides, surrounding cleanliness and treatment of undesired stagnant water should be the objectives of on spot awareness campaigns.

15.2. Strengthening of surveillance system

Surveillance is a critical important of any dengue prevention and control program because it provides the information necessary for risk assessment and program guidance, including epidemic response and program evaluation. Poor dengue surveillance system of Pakistan is also the reason of unsatisfactory dengue situation. Presence of functional and continuous dengue surveillance at all levels is the primary requirement of dengue in Pakistan and it should be a part of national health care system. Passive surveillance, active surveillance and event-based surveillance should be the effective component of our surveillance system to determine dengue transmission, circulating serotypes and investigating unknown health events, namely fevers of unknown aetiology and clustering of cases.

15.3. Personal protection

Preventive measures by individuals may contribute some safety. Protective clothing may reduce the risk of mosquito biting and therefore it is recommended to wear full sleeves shirts, socks and trousers. Mosquito mats, coils, aerosols and repellents are largely used for personal protection. Plant extract such as neem oil and chemical such as DEET (N, N-diethyl-m-toluamide) are natural and chemical repellents which provide protection against mosquitoes. Insecticides treated material available to protect people who rest by day and cloths can be treated with insecticides to avoid mosquito biting through skin tight clothing. Precautions must be taken while using repellents either natural or chemical and use of mats, coils should be avoided in closed rooms. Clothes must be treated with insecticides at recommended dose to avoid irritation on skin.

15.4. Environmental management

The crucial part of dengue prevention is the environmental management and it remains applicable where dengue is endemic. Environmental management refers to the modifications in environment to reduce man-vector contact and consequent transmission hazard. Solid waste management, source reduction, improved and proper water supply system, proper drainage system, covering of domestic water-storage containers, cleaning of flower vases and recycling of old tyres all are included in environmental management. In Pakistan, environmental management should be improved by government to control dengue. Sanitation is the major issue in Pakistan and government should focus on street cleaning, better drainage system and removal of stagnant water. Home level environment management is very effective and it should be the main right of way. Prior to dengue transmission and during outbreak, cleaning and other public hygienic campaigns should be organized on regular basis in all community settings.

15.5. Biological control

Biological control agents may be used in dengue prevention. Larvivorous fish have been widely used to control *Ae. aegypti* in large water containers, and its performance depends on the type of container being used (WHO) while copepods also have played an obvious role in controlling *Ae. aegypti*[86]. Fungi as *Beauveria bassiana* and *Metarhizium anisopliae* have also been proposed as potential biocontrol agent of *Ae. aegypti*. A bacterium such as *Bacillus thuringiensis* subsp *israelensis* was found to be adequate to control mosquitoes. The use of endosymbionts as *Wolbachia pipientis* (gram negative bacterium) has also been reported by Bordenstein and Rosengaus and Thomas *et al.* for control of mosquito borne transmission of pathogens[62]. According to national guidelines for dengue control in Pakistan, mosquito fish should only be used in small clean water resources and it ought to be released after rigorous mosquito breeding sites evaluation surveys. Other biological agents such as *Bacillus thuringiensis* subsp *israelensis* should be applied only during first three instars of larval stage. *Wolbachia* based research is being carried out in Pakistan to control dengue virus transmission[62], and it should be effective in future.

15.6. Chemical control

Chemical control is one of the key components in dengue prevention. Dengue vector can be controlled chemically by using larvicides and space sprays. Larviciding control of *Ae. aegypti* is mostly limited to domestic use containers and it is used in short term basis. Larvicides that can be used for dengue control are pyriproxyfen, *Bacillus thuringiensis* (H-14) and temephos. Space spraying can kill mosquito by small droplets of insecticides in air and it has been the primary method used by maximum countries to control dengue fever/DHF. Larvicides are occasionally applied to mosquito's breeding sites to control vector population in Pakistan, but precautions must be taken while using larvicides, and it must be applied after proper and careful breeding sites assessment assay. Space spraying should be considered as epidemic contingency measure during dengue outbreak.

15.7. Research and development

Basic and operational research regarding dengue is also important in dengue control in Pakistan. Research should address the cost effective enhancement of new control methods. Pakistan's research and development organizations should focus on developing specific and sensitive rapid test devices for dengue diagnosis, an effective vaccine against all serotypes and non-insecticidal methods to control dengue.

15.8. Dengue vaccines

Vaccines are the most effective tool to control infectious diseases. A secure, efficient and inexpensive dengue vaccine against all dengue serotypes would characterize a major advancement in dengue control. The first dengue vaccine Dengvaxia (CYD-TDV) by Sanofi Pasteur was registered in late 2015 and early 2016. CYD-TDV is a live attenuated tetravalent chimeric vaccine. Other tetravalent live-attenuated vaccines such as TV003 by NIAID, DENVax by Takeda are undergoing in phase II clinical trials. Other vaccine candidates (based on subunit, DNA and purified inactivated virus platforms) are V180 by Merck and TDENV by PIV are at earlier stages of clinical development[87]. Pakistan has neither vaccine development program nor any international vaccine get authorization yet.

Conflict of interest statement

We declare that we have no conflict of interest.

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