PREVALENCE AND CLINICO-LABORATORY CHARACTERISTICS OF MONO DENGUE AND DENGUE-MALARIA DUAL INFECTION AND THEIR ASSOCIATION WITH ACUTE KIDNEY INJURY AMONG PATIENTS IN PAKISTAN TERTIARY CARE HOSPITALS

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by

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ACE	Angiotensin converting enzymes inhibitors
ACS	Abdominal compartment syndrome
ADQI	Acute dialysis quality initiative
AKI	Acute kidney injury
AKIN	Acute kidney injury network
AST	Alanine aminoaspartame
ALP	Alanine aminotransferase
ALT	Alanine aminotransferase
aPTT	Activated thromboplastin time
ARB	Angiotensin receptors blockers
ARDS	Acute respiratory syndrome
ARF	Acute renal failure
AKD	Acute kidney disease
BMI	Body mass index
CRP	C reactive protein
C3	Compliment factors 3
COPD	Chronic obstructive pulmonary disease
C5	Compliment factor 5
CDC	Centre of disease control
CHF	Congestive heart failure
CKD	Chronic kidney disease
CPG	Clinical practice guideline
DAKI	Dengue induced AKI
DuAKI	Dual infection induced AKI
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
DIC	Disseminated intravascular coagulation
DM	Diabetes mellitus
DVI	Dengue viral infection
IHD	Ischemic heart disease
EDS	Expanded dengue syndrome
EIP	Extrinsic incubation period
GFR	Glomerular filtration rate
HCT	Hematocrit
HCV	Hepatitis C virus
Hb	Hemoglobin
Ig	Immunoglobulin
HTN	Hypertension
HUS	Hemolytic uremic syndrome
ICU	Intensive care unit
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IVF	Intravenous fluid
KDIGO	Kidney disease improving global outcomes
LFTs	Liver function test

LDH	Lactate dehydrogenase
MOD	Multi-organ dysfunction
MOI	Multi-organ involvement
MR	Mortality rate
MP	Malarial parasite
NSAIDs	Non-steroidal anti-inflammatory disease
PLT	Platelets
PT	Prothrombin time
PPI	Proton pump inhibitors
R.B.C	Red blood cells
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
RIFLE	Risk, Injury, Failure, Lethal, End stage renal disease
SCr	Serum creatinine
SEARO	Regional office for the South East Asia
SLE	Systemic lupus erythematosus
W.B.C	White blood cells
WHO	World health organization
INR	International normalized ratio
NR	Not reported

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PREVALENS DAN CIRI-CIRI MAKMAL KLINIK BAGI JANGKITAN DENGGI MONO DAN DWI DENGGI-MALARIA DAN KAITANNYA DENGAN KECEDERAAN BUAH PINGGANG AKUT DALAM KALANGAN PESAKIT DI HOSPITAL PENJAGAAN TERTIER DI PAKISTAN

ABSTRAK

Denggi dan malaria mempunyai manifestasi klinik dan ciri makmal yang serupa, menjadikannya sukar untuk membezakan antara kedua-dua jangkitan semasa tempoh endemik. Jangkitan virus denggi ialah penyakit bawaan vektor yang melemahkan, melumpuhkan dan berbahaya yang telah merebak dengan cepat di banyak bahagian dunia sejak beberapa tahun kebelakangan ini. Menurut tinjauan Pertuban Kesihatan Sedunia (WHO) di Pakistan *Plasmodium vivax* prevalens adalah 84% dan 14.9% dan 1.1% kes adalah disebabkan oleh *Plasmodium falciparum* dan jangkitan campuran. Demam denggi adalah punca utama penyakit dan kematian secara geografi. Walaupun langkah agresif diambil oleh pihak berkuasa yang berkaitan, Pakistan masih menghadapi krisis denggi yang semakin teruk sejak beberapa tahun lalu. Ia memerlukan keperluan mendesak untuk menilai kes denggi dan kes dwi jangkitan untuk pemahaman yang lebih baik tentang spektrum makmal klinikal untuk memerangi penyakit berjangkit ini. Selain itu, data terkini menunjukkan peningkatan laporan mengenai kecederaan buah pinggang akut (AKI) dalam kalangan pesakit denggi. Walaupun terdapat bukti ketara bahawa peningkatan sementara dalam kreatinin serum (SCr) dikaitkan dengan peningkatan morbiditi dan mortaliti, AKI masih merupakan komplikasi jangkitan mono denggi dan malaria denggi yang diabaikan di Pakistan. Terdapat kekurangan penyiasatan mengenai epidemiologi AKI teraruh denggi (DAKI) dan AKI akibat dwi jangkitan (DuAKI).

Malangnya, wujudnya sedikit kajian tentang DAKI malah tiada kajian tentang DuAKI di Pakistan. Majoriti penduduk yang terjejas oleh denggi tinggal di kawasan tropika dan subtropika disebabkan oleh turun naik yang tidak menentu dalam faktor risiko, iklim, kekurangan langkah kebersihan terutamanya pengemasan yang lemah dan kekurangan langkah kawalan nyamuk di seluruh dunia. Pakistan adalah endemik kepada denggi dan sederhana endemik kepada malaria oleh itu, jangkitan dwi dengan pelbagai patogen tidak jarang berlaku. Insiden keseluruhan AKI dalam kajian kami ialah 29.7%. Insiden DuAKI ialah 33.9% dan 27.9% dalam DAKI. Insiden DuAKI dalam fasa prospektif ialah 59.6% dan DAKI ialah 40.4%. Kesemua kes AKI yang tidak pulih dalam fasa prospektif tergolong dalam jangkitan dwi yang menunjukkan bahawa AKI yang teruk lebih dikaitkan dengan jangkitan dwi berbanding jangkitan mono. Pengecaman dan diagnosis lewat akan mengakibatkan hasil yang buruk semasa pengurusan dan pemulihan daripada penyakit. Walaupun terdapat pertindihan yang meluas di kawasan endemik malaria dan denggi, masih terdapat data yang terhad dalam kesusasteraan mengenai dua jangkitan penyakit berjangkit ini. Orang yang mempunyai simptom penyakit demam yang mesti disyaki sebagai pesakit. Di samping itu, data berkaitan pemulihan buah pinggang selepas AKI kini kurang. Oleh itu, kajian berbilang pusat telah dijalankan di wilayah Pakistan yang berbeza yang terdiri daripada kajian retrospektif dan prospektif dua fasa untuk menilai ciri-ciri makmal klinikal dan peramal demam denggi berdarah dan untuk menilai epidemiologi permulihan dalam kalangan pesakit yang dijangkiti jangkitan denggi mono dan denggi dan malaria dwi jangkitan, yang menghadiri hospital penjagan tertiari di Pakistan. Saringan data telah dilakukan dan mengesahkan pesakit denggi mono dan malaria dwi denggi dinilai untuk analisis. Semasa Fasa-I (retrospektif), sejumlah 512 pesakit denggi telah disemak. Demam denggi diperhatikan dalam 129

(25.2%) kes. Analisis regresi logistik menunjukkan bahawa penangguhan kemasukan ke hospital (aOR: 2.30, P-value 0.001: 95%CI: 1.14-2.78), diabetes mellitus (aOR: 2.71, P-value 0.009: 95%CI: 1.29-5.69), sesak nafas (aOR: 2.21, P-value 0.010: 95%CI: 1.20-4.05), kaitan dengan kumpulan risiko i-e. air bertakung, perjalanan ke kawasan endemik, tinggal di kawasan endemik (aOR:1.95, P-value 0.007: 95%CI: 1.19-3.17), tanda amaran (aOR:0.21, P-value <0.001: 95%CI: 0.13-0.35) lebih berkemungkinan dikaitkan dengan faktor risiko DHF berbanding DF. Kematian keseluruhan adalah 1.6% dan kira-kira separuh daripada pesakit telah tinggal di hospital berpanjangan (≥3 hari). faktor yang dikaitkan dengan kemasukan ke hospital yang berpanjangan dan kematian juga telah dinilai dalam fasa retrospektif. Semasa kajian fasa prospektif, 15.9% daripada 557 pesakit mempunyai AKI. Pemulihan buah pinggang dinilai dalam kalangan pemandiri AKI (n=89) selepas dua belas minggu dengan menggunakan beberapa kriteria pemulihan. Pemulihan buah pinggang dinilai oleh biopenanda fungsi buah pinggang (Kadar penapisan glomerular anggaran dan Kereatinin serum) dan proteinuria. Pesakit warga emas dengan komorbiditi, pelbagai disfungsi organ dan penggunaan ubat nefrotoksik semasa dimasukkan ke hospital mempunyai hasil buah pinggang yang lemah dengan sama ada kriteria pemulihan buah pinggang. Hasil buah pinggang kerana lebih mudah terdedah kepada faktor risiko dan penurunan dalam fungsi buah pinggang dalam bentuk penurunan kadar penapisan glomerular anggaran dan peningkatan kreatinin serum pada usia awal.Kajian semasa menunjukkan bahawa DF dan DHF menunjukkan profil makmal klinik yang berbeza dengan ketara. Sebaliknya, AKI terdapat dalam sebahagian besar pesakit denggi dan mereka yang mempunyai AKI meramalkan morbiditi, kematian dan kemasukan ke hospital yang berpanjangan. Pengenalpastian awal pesakit berisiko tinggi akan mempunyai kelebihan yang jelas dari segi keputusan yang sesuai

tentang rawatan dan pengurusan dalam unit pergantungan tinggi. Dalam kajian kami, AKI yang disebabkan oleh jangkitan dua lebih teruk berbanding dengan jangkitan AKI bertambah teruk dari segi penghinaan buah pinggang sinergistik dan kemasukan ke hospital yang berpanjangan berbanding dengan mono. Perkaitan antara AKI teruk dan jangkitan dwi saling berkait dengan hasil yang buruk disebabkan oleh kesan sinergistik yang merosakkan buah pinggang bagi kedua-dua patogen. Oleh itu, AKI dalam jangkitan mono denggi dan dwi jangkitan malaria denggi menunjukkan hasil buah pinggang yang tidak memuaskan dan memerlukan rawatan susulan yang rapid an lebih lama, terutamanya di bawah penjagaan nefrologi.

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ABSTRACT

Dengue and malaria have similar clinical manifestations and laboratory characteristics, thus making it difficult to distinguish between the two infections during the endemic period. Dengue viral infection is a debilitating and dangerous vector-borne disease that has spread rapidly and becoming endemic in many parts of the world in recent years. According to World Health organization (WHO) survey in Pakistan *Plasmodium vivax* prevalence is 84%. While, 14.9% and 1.1% cases were due to *P. falciparum* and mixed infection respectively. Dengue fever is the major cause of illness and death geographically. Despite of aggressive precautionary measures, Pakistan is still facing worsening dengue crisis over the past few years. It warrants an urgent need to evaluate dengue cases and dual infection cases to understand its clinico-laboratory spectrum in order to combat with these infectious diseases. Moreover, recent data indicated the rapid but transient rise in serum creatinine associated with the dengue induced acute kidney injury (AKI) among dengue patients. This is associated with increased morbidity and mortality in dengue patients. AKI is still a neglected complication of dengue mono infection and dengue malaria dual infection in Pakistan. There has been a dearth of investigation on epidemiology of dengue-induced AKI (DAKI) and dual infection induced AKI (DuAKI). Unfortunately, there are few studies on DAKI and there is even no research on DuAKI in Pakistan. Pakistan is endemic to dengue and moderately

endemic to malaria hence, dual infection with the multiple pathogens is not rare. In our study overall incidence of AKI is 29.7%, the incidence of DuAKI is 33.9% and 27.9% in DAKI in retrospective phase. The incidence of DuAKI in prospective phase is 59.6% and DAKI is 40.4%. All the non-recovery cases of AKI in prospective phase belongs to dual infection which indicated that the severe AKI was more associated with dual infection as compared to mono infection. Late identification and diagnosis will result in poor outcomes during disease management and recovery. Although, wide overlapping in symptomology of both infections exist, still limited data available in literature globally on dual infection of both pathogens. Person with the symptoms of febrile illness must be suspected patient in disease endemic areas. In addition, the data on post-AKI renal recovery is currently lacking. Therefore, a multi-center study was conducted in different provinces of Pakistan to evaluate the AKI in mono dengue infected patients and dual dengue malaria infected patients which comprised of two-phase retrospective and prospective study to evaluate the clinico-laboratory characteristics, predictors of dengue hemorrhagic fever (DHF) and to assess epidemiology of AKI and post-AKI renal recovery among mono dengue infected patients and dual dengue malaria infected patients attending tertiary care hospitals in Pakistan. Screening of data was done and confirmed mono dengue and dual dengue malaria infection patients evaluated for analysis. During Phase-I (retrospective), a total 512 dengue patients were reviewed. DHF was observed in 129 (25.2 %) cases. Logistic regression analysis indicated that delayed hospitalization (aOR:2.30, P-value 0.001: 95%CI: 1.14-2.78), diabetes mellitus (aOR:2.71 P-value 0.009: 95%CI: 1.29-5.69), shortness of breath (aOR:2.21 P-value 0.010: 95%CI: 1.20-4.05), association with risk group i-e. stagnant water, travelling to endemic areas, living in endemic areas (aOR:1.95 P-value 0.007: 95%CI: 1.19-3.17), warning

signs (aOR:0.21 P-value <0.001: 95%CI: 0.13-0.35) were more likely to be associated risk factors with DHF as compared to DF. The adjusted multivariable odds ratio indicated that these factors are more prone to cause the disease as compared to the crude odds ratio in the univariable analysis. Overall mortality was 1.6 % and approximately half of the patients had prolonged hospital stay (≥3 days). Several factors associated with prolonged hospitalization and mortality have also evaluated in retrospective phase. During prospective phase study, 15.9% of 557 patients had AKI. Renal recovery was assessed among the AKI survivors (n=89) after twelve weeks by using the several criteria of recovery assessed by renal function biomarkers, estimated glomerular filtration rate (eGFR), serum creatinine (Scr) and proteinuria. Elderly patients with comorbidities, multiple organ dysfunctions and use of nephrotoxic drugs during hospitalization had poor renal outcomes due to their more susceptibility with risk factors and decreased in renal function in the form of decreased eGFR and increased in serum creatinine in advance ages. Current study demonstrated that DF and DHF present significantly different clinico laboratory profile. On the other hand, AKI is present in substantial proportion of dengue patients and those with AKI portended significant morbidity, mortality and prolonged hospitalization. Early identification of high-risk patients will have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units. In our study dual infection induced AKI is worsened in term of synergistic renal insult and prolonged hospitalization as compared to the mono-infection. The association between severe AKI and dual infection is interlinked with poor outcomes due to the renal damaging synergistic effects of both pathogens. Hence, AKI in mono dengue infection and dual dengue malaria infection indicates

unsatisfactory renal outcomes and deserve careful and longer follow-up, especially under nephrology care.

CHAPTER 1

INTRODUCTION

1.1 Background

Dual infection is the simultaneous infection of a person with multiple pathogens. Mono and dual infections are becoming a worldwide issue in both developing and developed countries. Concomitant infection, double infection, concurrent infection, simultaneous infection, multiple infection, polymicrobial, polyparasitism, parasitemia, and multiple infection are all terms used to describe dual infection (Griffiths, Pedersen, Fenton, & Petchey, 2011). It can affect a single cell or multiple cells at the same time.

Dengue and malaria are the most common mosquito borne diseases globally. The concurrent infection of both these species is common in disease endemic area. Hence dengue malaria coinfection refers to the simultaneous coexistence of both pathogens in a single host. The actual burden of both diseases in endemic area could not be estimated. Literature reported the estimated burden of dengue infection 390 million per year (Assir, Masood, & Ahmad, 2014). This burden is three times higher than the reported burden of World Health Organization (WHO). Malaria is common and prevalent infectious disease in tropical and subtropical region like dengue infection (Kolawole et al., 2023). The global burden of malaria is 3.3 billion per year (Z. Iqbal et al., 2022). The prevalence of malaria in Pakistan is 64% (Mushtaq, Qadri, & Rashid, 2013).

Pakistan is endemic to dengue and malaria infection. The prevalence and incidence of dual infection became higher during the epidemic break especially before and after monsoon season. The incidence of dengue malaria dual infection in

Pakistan is 23.2 % (A. Abbasi et al., 2009). Considering the endemicity of dengue and malaria in the Pakistan, it is reasonable to envisage that the occurrence of concurrent infections would not be rare. However, due to non-systematic investigation of both diseases, only a few cases of malaria and dengue coinfection have been reported in Pakistan. The reported dengue and malaria dual infection based study demonstrated clinical manifestations ranging from low hematocrit (HCT), thrombocytopenia to particular hepatic injury (A. Abbasi et al., 2009). Another study in Pakistan exhibited severe thrombocytopenia considered to be criterion of disease severity, bad prognostic factor and its presence is associated with increase probability of malaria (Shazia, Owais, Moin, & Owais, 2014).

During dengue outbreak patients may acquire malaria. Multiple infections in a single case would drastically change the spectrum of clinical manifestations, which would complicate the diagnosis process. There are a number of reports from Asian countries describing coinfections of malaria and dengue with other agents (Magalhães et al., 2014; Santana et al., 2010). Multiple concurrent infections with overlapping clinical manifestations can pose a serious diagnostic challenge as well as a management dilemma. The many overlapping features and similarity of symptoms seen in patients presenting with acute febrile illness, such as high fever, headache, nausea and myalgia may complicate the diagnosis of acute fever (Sahu, Sahu, & Ambu, 2016). Prolonged fever, low hematocrit and thrombocytopenia are typical signs of dengue and malaria dual infection (A. Abbasi et al., 2009; Gesesew et al., 2016). Concomitantly renal injury induce by dengue and malaria dual infection could be worsened and associated with multiple organ involvement due to both species. Hence, dengue malaria dual infection had lethal effects on human health (A. Abbasi et al., 2009).

The objective of the present study was to understand the interplay of dengue malaria dual infection in mono dengue infection patients and the impact on the severity of clinical manifestations and renal physiology. This study also aimed to evaluate the clinical features, their comparison in DF, DHF, predictors of DF, DHF, their association with the length of hospital stay and risk factors associated with hospitalization, acute kidney injury and predictors of AKI.

1.2 Dengue fever

Dengue viral infection (DVI) is a debilitating and disabling vector-borne disease that has spread rapidly in many regions of the world in recent years. Dengue infection is the major cause of illness and death geographically (Aswi, Cramb, Moraga, & Mengersen, 2019). DVI is widely distributed in tropical and subtropical regions with variations in risk factors, fluctuations in climate, increased in international travel, lack of mosquito control measures and rapid globalization. In recent decades, world health organization (WHO) database designated dengue as an international problem and global burden. The majority of the population affected by dengue virus lives in tropical and subtropical regions because of the unpredictable fluctuations in risk factors, climate, lack of hygienic measures, particularly poor housekeeping and lack of mosquito control measures, rapid globalization and increased international travel. The disease's hue cycle shifts from asymptomatic illness to symptomatic dengue hemorrhagic fever, dengue shock syndrome. On the basis of hue cycle DVI could be classified into classical dengue fever (DF), non-classical DF, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Ajlan et al., 2019; Nedjadi et al., 2015; W.-H. Wang et al., 2020; Whiteman et al., 2020).

1.3 Dengue Virus, Vector and Host

Dengue infection is caused by dengue virus which is single strand positive sense RNA virus belongs to family Flaviviridae and genus Falvivirus subgenus stegomyia. After translation, the viral RNA forms the capsid (C), pre-membrane (prM) envelope, and seven non-structural proteins, which are NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.The pathogenicity is due to the prM, which is responsible for the fusion of genomic structure, while the envelope facilitates binding after pathogen recognition with host cell (Khetarpal & Khanna, 2016).

The most identified typical serotypes of dengue vector include DENV-1, DENV-2, DENV-3 and DENV-4 and DENV-5. The mosquitoes/vectors of major concern of pathogenicity are *Aedes aegypti*, *Aedes albopictus*, *Aedes scutellarin*. The major vectors affecting humans and causing dengue viral infection are *Aedes aegypti* and *Aedes albopictus*. Infection with one serotype results in long term immunity for that serotype but does not provide permanent protection for the others. It completes its life cycle in two phases i.e., epidemic and sylvatic (W.-H. Wang et al., 2020; Whiteman et al., 2020). The epidemic phase includes completion in human communities, while the sylvatic phase involves primates in jungles (Abduljalil & Abd AlGalil, 2022).

The dengue virus thrives in human hosts. After being bitten by an infected mosquito, the virus goes through an intrinsic incubation period (IIP) of 7-14 days in humans. The victim may experience early signs of fever after completing IIP. Dengue virus may circulate in the bloodstream during an acute febrile illness. In humans, this is known as the febrile viremic phase. This phase serves as a source of viruses for other mosquitos, which consume the dengue virus while feeding. The virus then infects the mid-gut of the mosquito and spreads systemically over an 8–

12-day period to complete its extrinsic period (EIP-its time taken by virus to complete its life cycle in mid-gut of mosquito) then EIP mosquito can infect other host during feeding/probing. The EIP of the virus in the gut was influenced by ambient temperature, whereas the mosquito remained infectious throughout its entire life (De et al., 2022).

1.3.1 Aedes Mosquito

Aedes aegypti is a small, dark mosquito with white bands on its legs and white lyre shaped markings on its body. It is highly resilient with the ability to rapidly bounce back to initial numbers after disturbances caused by the natural disaster or human interventions. *Aedes albopictus*: commonly known as Asian tiger is also a small, dark mosquito with a white dorsal stripes and banded legs. It is slightly hardier than *Aedes aegypti* and feeds both on human and animals (Jansen & Beebe, 2010).

1.3.2 Transmission of Dengue Virus

Dengue virus (DENV) transmission is determined by complex interactions between the host, vector and virus, which can be influenced by environmental factors such as temperature, rainfall, and humidity (Janaki et al., 2022). Dengue fever is a mosquito-borne arboviral infection that is hyperendemic in tropical and subtropical climates (S. A. Kularatne & Dalugama, 2022). In humans' transmission comprises of three phases includes enzootic (monkeys, aedes monkeys) epizootic (non-human primates from adjoining human epidemic cycles and epidemic cycle (human aedes human). Susceptible virus acquire infection after ingestion of blood from viremic person. After getting blood meal virus bind to undefined receptors inside the epithelium lining of mid-gut where it replicates and then infects the secondary tissues including the salivary glands. Virus will be transmitted to new host via salivary gland

upon the next probing event. The susceptible climate for the virus transmission is when temperature and humidity are favorable for the vector breeding especially during rainfall season. During the limited rainfall and dry season dengue vectors breed in water storage containers. In the absence of rain *Aedes aegypti*'s hasten its life cycle quickly by producing small size eggs. These small sized females require more amount of blood to get protein for the production of eggs. Hence, increasing the number of bites and consequently, infected persons.

1.4 Classification of Dengue Fever

Dengue fever is classified into several types, ranging from undifferentiated DF to differentiated DF. Patients with undifferentiated DF are asymptomatic. Sub-clinically, patients with undifferentiated DF exhibit flu-like symptoms. Differentiated DF is comprising of DF, DHF, and DSS. The three well-known phases of DF are acute febrile illness, critical illness and recovery. The characteristics, clinical manifestation with laboratory findings and complications have been described below in figure 1.1.

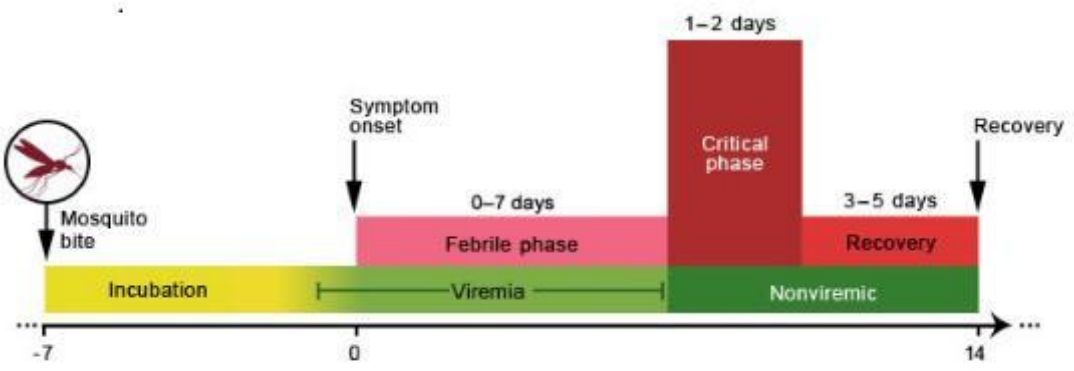

Figure 1.1 Three Phases during clinical course of dengue infection (URL: https://www.cdc.gov/dengue/training/cme/ccm/page47443.html)

1.4.1 Acute Febrile Illness

All three phases of DF share the initiative of acute/benign febrile illness. It starts from the high degree fever of 40ºC and lasts for 4-7 days. Symptoms includes retro-orbital pain, myalgia, arthralgia, headache, nausea, vomiting and sore throat (figure 1.2). Because of the pain and lethargy, DF is also known as breakbone fever in many parts of the world. A rash, either maculopapular or macular, petechiae, purpura on the skin, epistaxis, and intestinal bleeding are all common during a febrile illness. Flu like symptoms like runny nose, diarrhea sometime also accompany in acute febrile phase. Young children and teenagers usually presented with the dehydration, pain, body ache and febrile seizures which may become the complication of febrile illness. Factors affecting the acute febrile illness still undefined. Laboratory finding includes complete blood count i.e. mild to moderate thrombocytopenia, leucopenia, elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Mangold & Reynolds, 2013; Organization, 2011; Ruberto, Marques, Burke, & Van Panhuis, 2015; Sangkaew et al., 2021; Shauri, Ngadaya, Senkoro, Buza, & Mfinanga, 2021; Tanner et al., 2008).

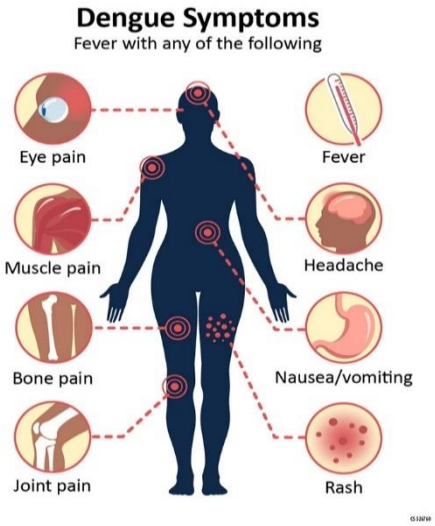

Figure 1.2 Sign and symptoms of dengue fever last from 1-7 days (URL: https://www.cdc.gov/dengue/symptoms/index.html)

1.4.2 Critical Phase

The critical phase begins within 4-7 days of illness. At this time patient's fever may improve. Some of the patients have improved health but those who exhibit plasma leakage due to the increased capillary permeability undergo the severity of infection DHF or DSS which causes sudden decrease in plate count, increase in hematocrit and decrease in leucopenia. Hypovolemia is due to increased plasma leakage. To maintain hemostasis, compensatory mechanism activated which reduces pulse pressure and increases the diastolic pressure. Pleural effusion, ascites and rarely pericardial effusion in critical phase due to plasma leakage was observed.

Patients may appear well despite early signs of shock. However, once hypotension develops, systolic blood pressure rapidly declines and irreversible shock and death may ensue despite resuscitation efforts. Patients can also develop severe hemorrhagic manifestations including hematemesis, bloody stool, melena, or menorrhagia, especially if they have prolonged shock. Uncommon manifestations include hepatitis, myocarditis, pancreatitis, and encephalitis.

1.4.3 Convalescence Phase

As the patient's plasma leakage decreases, he or she enters the convalescent phase (figure 1.3) and begins to reabsorb extravasated intravenous fluids, pleural and abdominal effusions. As a patient’s well-being improves, hemodynamic status stabilizes (although he or she may manifest bradycardia), and diuresis ensues. Because of the dilutional effect of the reabsorbed fluid, the patient's hematocrit stabilizes or falls and the white cell count usually begins to rise, followed by a recovery of the platelet count. The rash may desquamate and become pruritic during the convalescent phase.

Death usually occurs within 14 days of transmission of dengue virus in severe infection cases which is supported by literature having history of severe afebrile illness, multiple organ involvement, expanded dengue syndrome (Assir, Ahmad, Masood, Kamran, & Yusuf, 2014). Death after 14 days considered to be due to any other cause.

Data are limited on health outcomes of dengue in pregnancy and effects of maternal infection on the developing fetus (Pouliot et al., 2010). Perinatal transmission can occur and peripartum maternal infection may increase the likelihood of symptomatic infection in the newborn. All perinatal transmission cases described in the literature developed thrombocytopenia, most had evidence of plasma leakage in the form of ascites or pleural effusions. Nearly 40% had a hemorrhagic manifestation, and one-fourth had hypotension. Symptoms in perinatal infected neonates typically present during the first week of life. Placental transfer of maternal IgG against dengue virus (from a previous maternal infection) may increase the risk

for severe dengue among infants infected at 6–12 months of age when the protective effect of these antibodies' wanes.

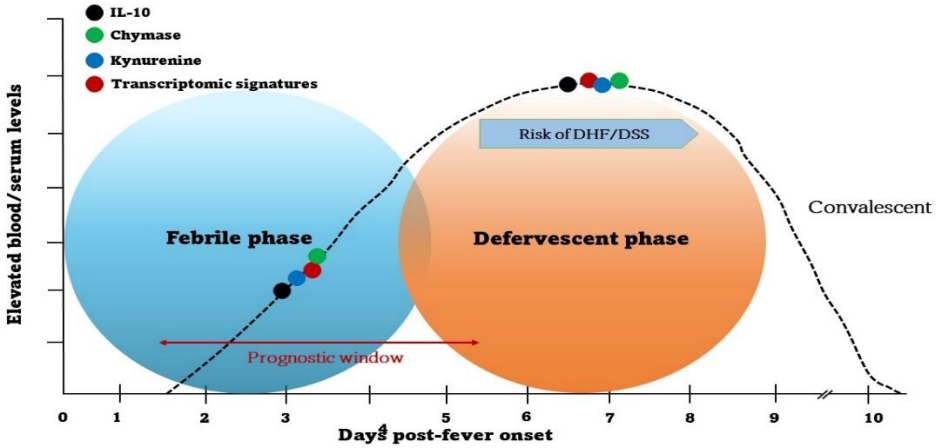

Figure 1.3 Biomarkers during prognostic window in dengue viral infection (Figure is self-constructed)

1.5 Diagnosis of Dengue Fever

Generally, diagnosis is based on the evidence of clinical symptoms during the acute febrile illness. However, there is necessity of accurate diagnosis of dengue. Accurate diagnosis is helpful in surveillance, management, intervention development and outbreak investigation in any endemic area. WHO described the tourniquet test for the diagnosis of dengue especially in endemic areas. DVI test must be accurate, inexpensive, easy to use, provide rapid results during the acute phase and distinguish between dengue and other infection. Diagnostic test for DVI includes virus detection, viral RNA detection, antigen detection and serological study described in table 1.1 and 1.2. Optimal time frame and different merits for the differential diagnosis of dengue has been summarized in figure 1.4 and 1.5. Direct method for the diagnosis involves the virus isolation, detection of viral genome while indirect method involves the antibody identification (IgM, IgG) in dengue patients (figure 1.4).

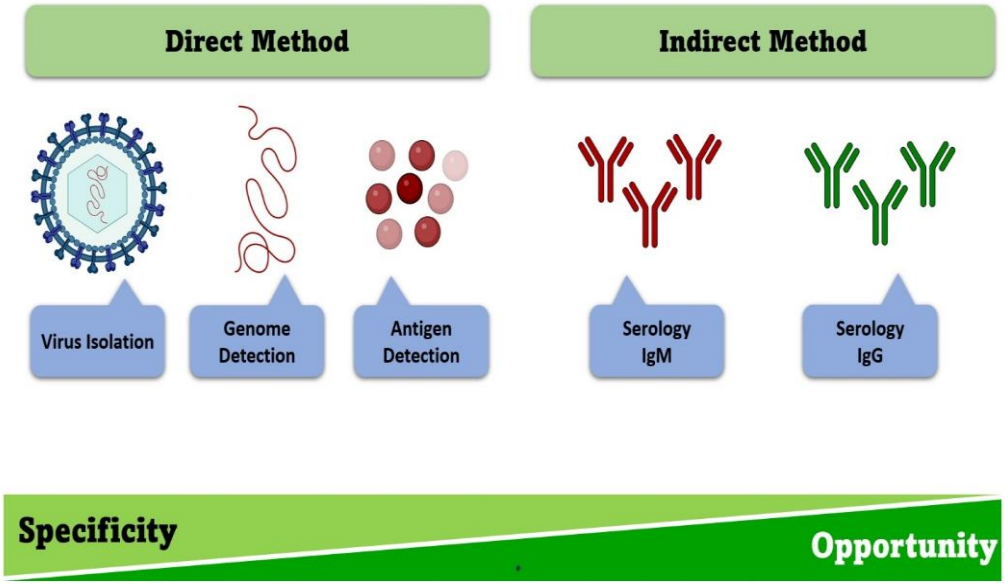

Figure 1.4 Direct and indirect laboratory methods for diagnosis of dengue viral infection (Figure is self-constructed)

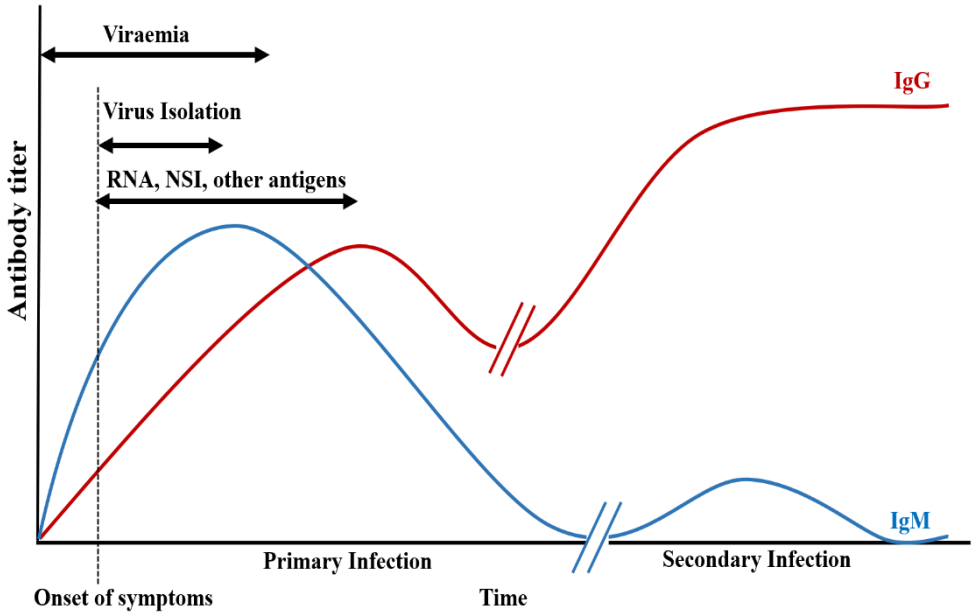

Figure 1.5 Markers for the diagnosis of dengue viral infection and changes in antibodies (IgM & IgG) response according to primary and secondary infection (Figure is self-constructed)

Diagnosis of dengue virus involves the three types of basic test antibody detection, antigen antibody detection and viral isolation. Advantages, disadvantages with the different types of available kits for each of test is described in table 4.4.

Table 1.1 Different diagnostic test for dengue viral infection

Technique	Recommended time duration	Specificity and sensitivity	Advantage	Limitations
Antibody detection				
IgM detection	3-5 days after the onset of symptoms and undetectable 2-3 months in primary dengue. 3 days after onset of symptoms and sometimes hindered by large scale IgG production in secondary dengue	Sensitivity: (61.5-100%) Specificity: (52-100%)	Confirmed infection Easy to perform and economical	Level of IgM usually low in secondary infection
IgG detection	One week after onset of symptom in primary dengue and 3 days after onset of symptoms in secondary dengue They remain for many years (three years)	Sensitivity: (46.4-99%) Specificity: (80-100%)	Same as above	
Rapid IgM detection using Strips	5 days after onset of symptoms and up to 2 months	Sensitivity: (20.5-97.7%) Specificity: (76.6-96.6%)		Level may fluctuate in secondary infection
Antigen/ Antibody Combined Detection				
NS1 and IgM Combo Kit	This combo test, useful in early infection stage from day 3 to onwards and up to sero-conversion period	Sensitivity: (89.9-92.9%) Specificity: (75-100%)	Confirm infection Easy to perform and less expensive than other methods	Not too sensitive as RNA detection or virus isolation
NS1 and IgM/IgG Combo kit	This test is useful in early stage of infection from day 3 to onwards and up to sero-conversion period (up to two weeks onwards). IgG is reactive in	Sensitivity: (93%) Specificity: (100%)	IgM level low in secondary infection Requiring more than one sample	

	secondary infection if both IgM and NS1 are non-reactive.			
Viral Detection				
Virus Isolation using cell culture	1-5 days of onset of symptoms in Primary Dengue and 1-4 days after onset of symptoms in secondary dengue	Sensitivity: (40.5%) Specificity: (100%)	Confirmed infection Inexpensive Specific Identifies serotype	Require sampling within 0-5 days of onset Results take one week Expertise needed
Virus Isolation mosquitoes	Same as above	Sensitivity: (71.5-84.2%) Specificity: (100%)		
Viral RNA RT PCR (Conventional)	Same as above	Sensitivity: (48.4-100%) Specificity: (100%)	Confirmed infection Sensitive and specific Identifies serotype and genotype Results in 24–48 hours	False-positive due to contamination Requires acute sampling at 0–5 days of post onset Expertise and expensive laboratory equipment needed no differentiation between primary and secondary infection
Viral RNA PCR (Real Time)	Same as above	Sensitivity: (58.9-100%) Specificity: (100%)		
Viral Antigen (NS1)	1 day after onset of symptoms in primary dengue and 6 days after onset of symptoms in case of secondary dengue	Sensitivity: (54.2-93.4%) Specificity: (92.5-100%)		

Abbreviations: IgM; immunoglobulin M, IgG; immunoglobulin G, NS1; Nonstructural protein 1, RNA; ribonucleic acid, RT-PCR; reverse transcriptase polymerase chain reaction. Reference redirected from (C.-H. Chen, Huang, Kuo, & Li, 2018; Kao, King, Chao, Wu, & Chang, 2005; Organization, 2011; Peeling et al., 2010).

Table 1.2 Recommended Dengue test on the basis of clinical manifestations

Clinical History	**Diagnostic test**	**Results**	**Interpretations**
Fever <5 days	NS1 or RCT	Positive Negative	Acute dengue infection DVI still cannot rule out. Repeat for Dengue IgM after day 5 of fever
Persistent fever from more than 5 days	IgM detection	Positive Intermediate Negative	Probability of DVI Repeat the test Repeat the sample for the antibody after 7 days of fever and/or IgG detection
Persistent fever from more than 5 days	IgG detection	Positive Intermediate Negative	IgG level elevation in acute or past infections. Repeat according to clinical manifestations IgG level elevation predicting secondary viral infection

Abbreviations: IgM; immunoglobulin M, IgG; immunoglobulin G, NS1; Nonstructural protein 1, RNA; ribonucleic acid, RT-PCR; reverse transcriptase polymerase chain reaction
Reference**:** Pakistan clinical practice guidelines on dengue viral infection (2020)

1.6 Dengue Case Classification

Dengue viral infection is classified into three types by the World Health Organization: undifferentiated dengue fever (UDF), dengue fever (DF), and dengue hemorrhagic fever (DHF). DHF is further categorized into four distinct grades (grade I-IV). These grades are assigned to patients based on their signs and symptoms as well as the severity of their hemorrhagic manifestations. The WHO classification system was revised in 2009 because the previous classification system was complex. Although the older classification remains popular, the new classification is based on the severity of the signs and symptoms. WHO's regional office of South-East Asia (SEARO) has included the previous classification (1997) of DVI in expanded and revised guidelines (WHO/SEARO,2011) (Figure 1.7). Now 2009 based classification system is used due to its ease of use and understanding. Both classification systems are still favor by WHO. Center of disease control and prevention (CDC) divided DVI

in three types: dengue, dengue like illness and severe dengue. Dengue like illness is characterized by fever and may be due to the other type of infections. Dengue patient labelling is according to dengue fever guidelines and treating physician (Organization, 2011). WHO and SEARO (regional office for the South East Asia) dengue classification of 1997 and 2009 elaborated in below Figure 1.6 and Table 1.3.

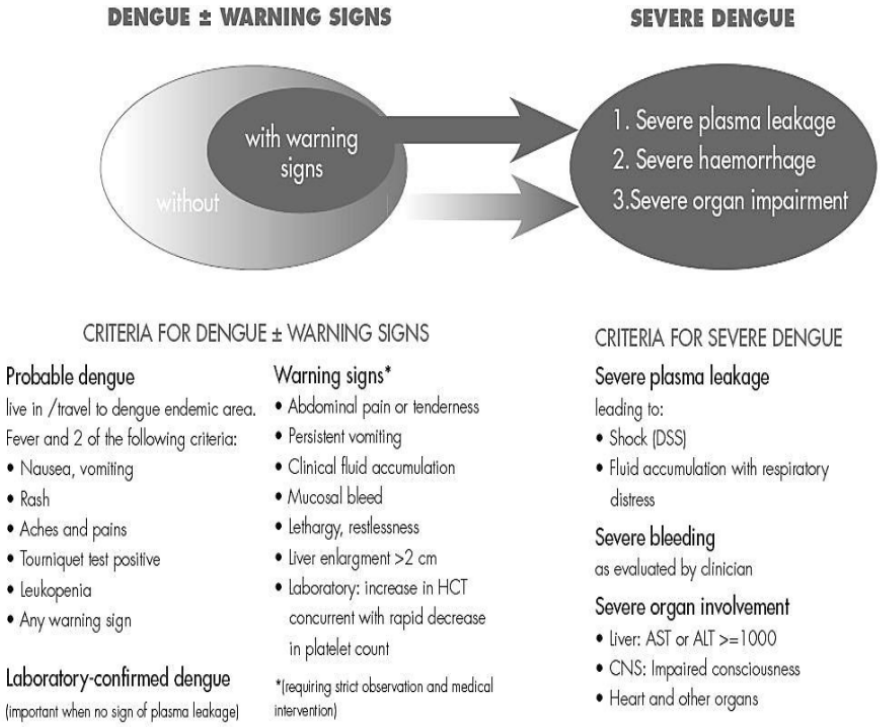

Figure 1.6 Dengue case classification according to criteria of WHO 2009(reprinted from WHO, 2009- World Health Organization)

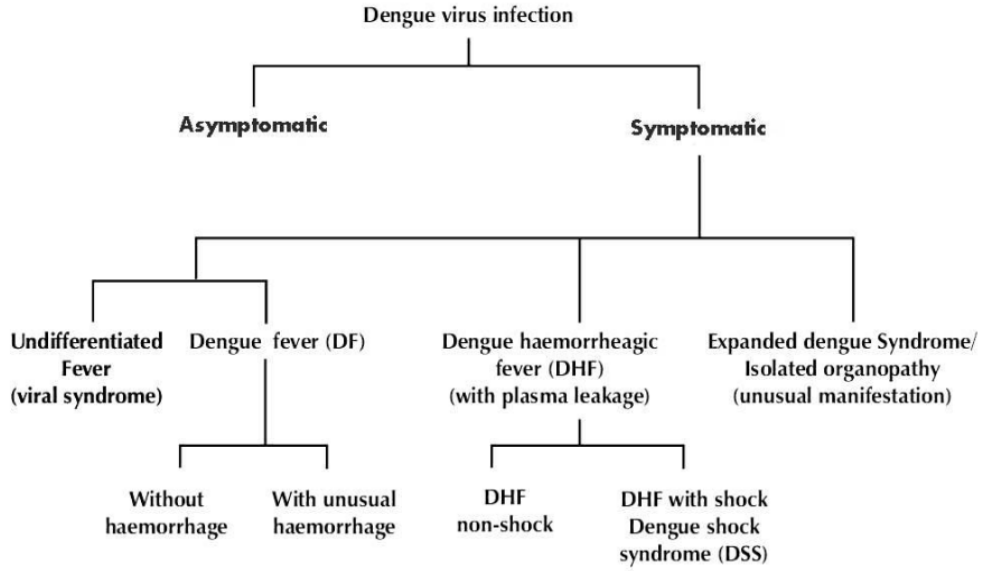

Figure 1.7 WHO/SEARO 2011 classification of dengue viral infection (reprinted from WHO/SERAO, 2011- Guidelines for prevention and control of dengue hemorrhagic fever)

Table 1.3 WHO classification of dengue infection and grading of severity of Dengue hemorrhagic fever

DF/DHF	Grade	Sign and symptoms	Laboratory
DF		Fever with two of the following: Headache,Retro-orbital pain, Myalgia, arthralgia, Rash,Hemorrhagic manifestations No evidence of plasma leakage.	Leucopenia (WBC ≤5000cells/mm3), Thrombocytopenia (Platelets<150000 cells/mm3) Increasing hematocrit (5%-10%) No evidence of plasma loss
DHF	I	Fever and hemorrhagic manifestation (positive tourniquet test) and evidence of plasma leakage	Thrombocytopenia <100000 cells/mm3 HCT rise ≥20%
DHF	II	As in Grade I plus spontaneous bleeding	Thrombocytopenia (Platelets <100 000 cells/mm3) HCT rise ≥ 20%
DHF*	III	As in Grade I or II plus circulatory failure, weak pulse, pulse pressure(≤20mmHg), hypotension, restlessness	Thrombocytopenia (Platelets <100 000 cells/mm3) HCT rise ≥ 20%
DHF*	IV	As in Grade III plus profound shock with undetectable BP and pulse	Thrombocytopenia <100 000 cells/mm3 HCT rise ≥20%

Abbreviations: DF; dengue fever, DHF; dengue hemorrhagic fever, WBC; white blood cells, HCT; hematocrit * DHF III and IV is dengue shock syndrome (DSS)
http://www.who.int/csr/resources/publications/dengue/Denguepublication/en

1.7 Epidemiology of Dengue

Dengue is global problem and spreading geographically. The targeted host for dengue is adults. About 2.5 to 3 billion peoples are inhabitant of dengue transmitted zone according to the WHO survey. About 390 million peoples are exposed to dengue per year and 96 million are symptomatic for DVI. According to WHO average number of dengue cases are rising day by day in last 50 years. Recently WHO described that 100 million cases of dengue infection were recorded and half of the world population is at risk of DVI. The persons of tropical and subtropical regions are ideal victim of dengue virus but now there is an expansion in the involved countries. The worldwide distribution of DVI is becoming global burden. The ideal season for DVI is post rainy season and summer when the mosquito vector population is at its peak ("Drug Bank," 2019; Fritzell et al., 2018; Koh, Bachtiar, & Hariman, 2019; S. K. Roy & Bhattacharjee, 2021).

Dengue was firstly reported in Baluchistan province. Its first outbreak was reported in 1996 in Karachi city of Province Sindh. With the passage of time its cases were going to be increased. Now a days DVI outbreaks were going to be occurred constantly. The targeted cities of DVI were Lahore, twin cities Islamabad-Rawalpindi and Karachi where it causing high morbidity and mortality (Rafique, 2013). Major areas of Pakistan have dengue pandemic are Punjab and Islamabad; capital of Pakistan. After Punjab Islamabad is the most affected city. Increase in dengue cases day by day increasing the burden and pressure on health sectors either public or private. During the anti-dengue surveillance survey Islamabad sign out for the increased dengue cases. According to the National Institute of Health (NIH) in Islamabad, there were 22,938 dengue fever cases reported in Pakistan in 2017, more than 3,200 in 2018, 24,547 in 2019, and 3,442 in 2021. Since October 8, 2021, there

has been an increase in the number of cases, particularly in Lahore, Rawalpindi, and Islamabad. Between 1994 and 2017, nearly 147200 cases of dengue fever were reported, with 800 deaths. The ideal hosting weather for DVI starts from April to March and August to October (Atique et al., 2018; M. H. Butt, Ahmad, Misbah, Mallhi, & Khan, 2021; Fatima, 2019; Tahir et al., 2020). Figure 1.8 described the incidence of dengue in various provinces of Pakistan.

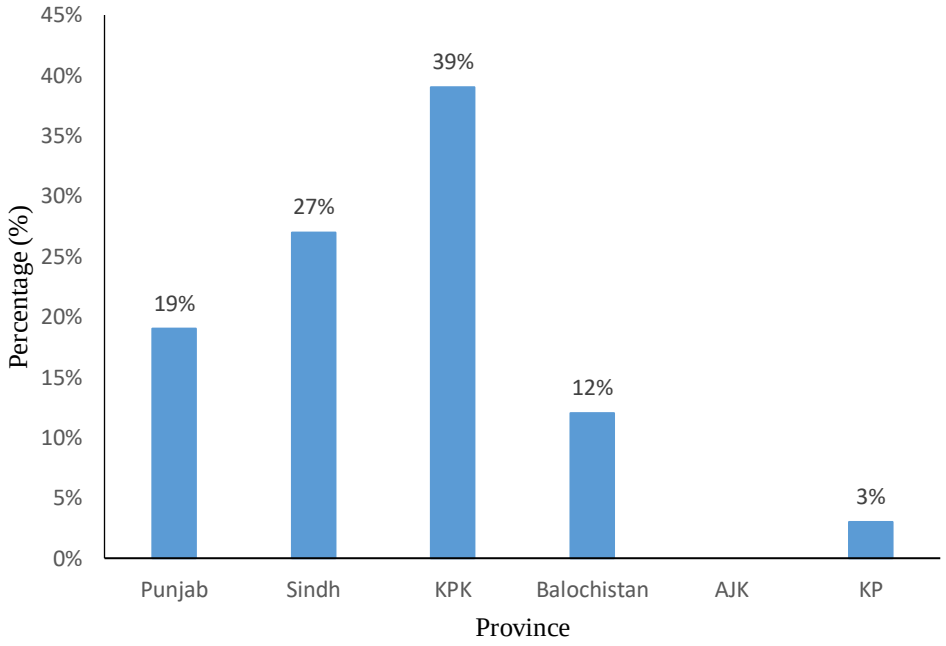

Figure 1.8 Incidence of dengue viral infection in different provinces of Pakistan during year 2020 (Figure is self-constructed)

1.8 Expanded Dengue Syndrome

DVI involving the unusual manifestation termed as expanded dengue syndrome (EDS) (figure 1.9). With the geographical distribution of DVI and involvement of more than one serotype leading to the involvement of multiple organs resulting in complication of infection. Persons with chronic diseases like diabetes, hypertension, hepatic, renal and neurological abnormality suffer from severe dengue infection and exhibiting worse symptoms. EDS is involving immune system that

target the endothelium resulting in increased vascular permeability and coagulopathies. Hepatic complications are more major one among the dengue patients. Rest of the organ involvement is seen in minority of case. However, mild to moderate renal complications also reported in dengue patients.

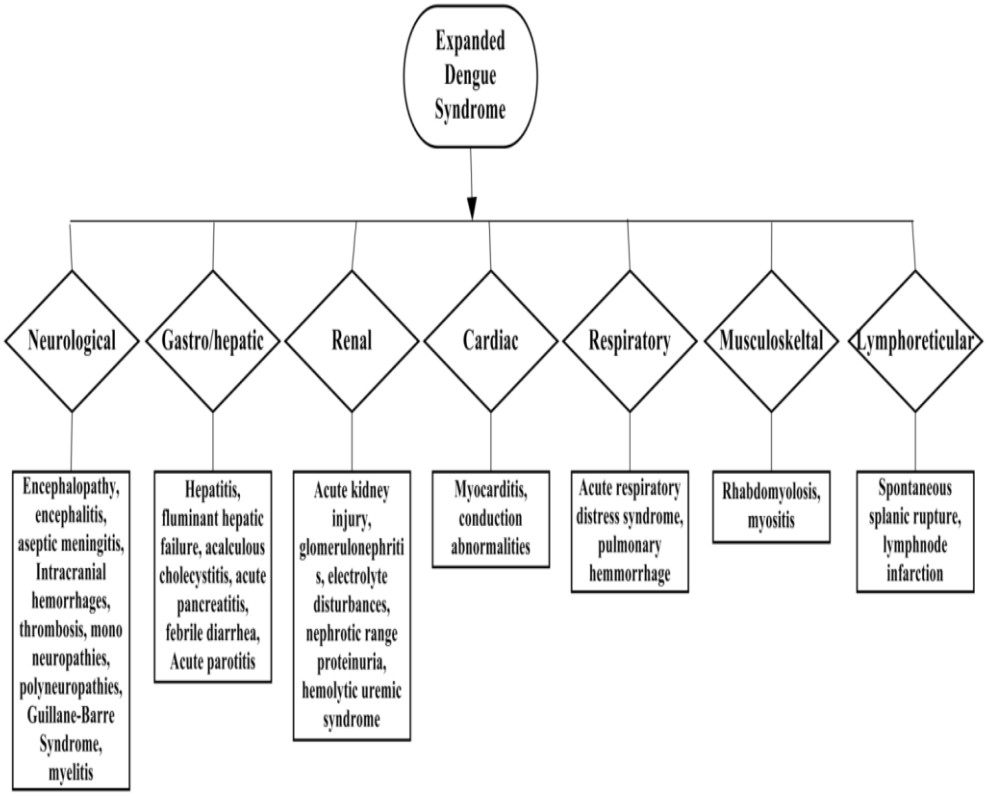

Figure 1.9 Clinical outcomes of expanded dengue syndrome (Reference: Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever (WHO, 2011)

Abnormal urinary sedimentation, glomerulonephritis, IgA nephropathy, lupus nephritis hemolytic uremic syndrome and AKI (described later on) are the arising complication of renal system during dengue fever.

1.9 Malaria

Malaria is a parasitic disease that is still a leading cause of death worldwide. This disease is endemic in 109 countries in tropical and subtropical regions. In 2017,

disease affected 219 million people globally and killed 435000 people. According to the WHO, nearly 216 million malaria cases were reported in 2010, with 665000 deaths. In 2016, 2017, and 2018, there were 445000, 435000, and 405000 deaths, respectively. Malaria is a potentially fatal disease in humans because its symptoms are similar to dengue viral infection. According to the most recent WHO report, there are nearly 241 million cases of malaria in 85 endemic countries and an increase in malarial cases from 227 to 241 million cases was observed. The decrease in malaria cases in endemic countries is due to control and prevention measures globally (Hussein, Albashir, Elawad, & Homeida, 2020; Rahim, Munajat, & Idris, 2020).

Malaria is endemic in Pakistan and has the highest disease burden in malaria endemic areas. According to WHO survey in Pakistan *Plasmodium vivax* prevalence is 84% while 14.9% and 1.1% case were due to *Plasmodium falciparum* and mixed infection. Over 3.4 million suspected cases of malaria were reported in Pakistan between January and August 2022, compared to 2.6 million reported in 2021. Over 170 000 cases were laboratory confirmed, with *Plasmodium vivax* accounting for 77% of the cases and *Plasmodium falciparum* accounting for 23% of the cases, which are the most severe and fatal (Herrel et al., 2004; Umer et al., 2018).

1.9.1 Epidemiology of Malaria

Malaria is the fifth leading cause of death globally, and the fourth leading cause of death among infectious diseases in Pakistan. Malaria cases are increasing due to Afghan refugees rather than the indigenous population. The prevalence due to *P. falciparum* species is increased from 27 to 36 percent as a result of these Afghan refugees. The national malaria control program reported a six fold increase in malaria

over the last decade due to chloroquine resistance (N. A. Qureshi, Fatima, Afzal, Khattak, & Nawaz, 2019a). A sharp turn up in malarial cases was also reported after floods. Malarial parasite exists in most regions of Pakistan but Southern and Northern Punjab and tribal areas of KPK are considered to be malarial endemic areas. In these areas infection rate is considerably higher than the other regions of Pakistan due to environment conditions, socioeconomical factors and poor malarial control measure. About 60% of population in Pakistan is living in endemic areas with 177 million peoples are at risk. Sindh and Baluchistan both provinces together presented 78% confirmed cases (Jahan et al., 2019; A. Khan & Rehan, 2018; H. Qureshi, Khan, Ambachew, Pan, & Ye, 2020; N. A. Qureshi et al., 2019a).

Malaria can infect any person throughout year but infection rate is being higher from August to November. The peak season for *P. vivax* is June to September and for *P. falciparum* is August to December. Due to extensive agriculture practice, irrigation system, urbanization and monsoon season in Pakistan infection rate is perceived to be higher as compared to the other countries. According to WHO report Pakistan is figure among the countries that has high mortality rate in the context of this pandemic (Jaleel, Saeed, NAQQASH, SAEED, & IQBAL, 2015; Umer et al., 2019).

1.9.2 Transmission of Malaria

Malarial parasite (MP) can infect human, birds and reptiles. Among the different species of MP, the five species are *P. falciparum, P. vivax, P. malariae, P. ovale curtisi, P. ovale wallikeri and P. knowlesi*. The most lethal species for human is *P. falciparum* and to some extent is *P. vivax* (Talapko, Škrlec, Alebić, Jukić, &

Včev, 2019). Mosquito anopheles bite and infect host. Within the host it completes different phases of its life cycle (Figure 1.10) and inside the host it starts pre-erythrocytic phase in liver where it infects the hepatocytes, develop in the parasitophorous vacuoles. Here it produces millions of red blood cells (RBC) infected merozoites and this process is known as schizogony. Some of MP species like *P. vivax*, *P. ovale* and *P. cynomolgi* adopt the latent phase and/or hypnozoites in which merozoites stays in host cell for prolonged time period. Latent phase may be activated at any time and initiates an infection. Parasites enters into erythrocytic phase from pre-erythrocytic phase and invade in the new red blood cells (R.B.C). These merozoites develop into PV and undergone to produce schizogony to form the daughter merozoites. These daughter merozoites burst then reinvade the new RBC and complete asexual reproductive cycle. MP produces species specific merozoites.

After completion of asexual reproduction cycle gametocyte will produce and initiate sexual reproduction cycle into intermediate vertebrate host cell. According to the type of specie, gametocytes becomes mature. Only mature gametocyte enters in blood stream while immature will be taken up by liver and spleen. Once ingested by vector, gametocytes will complete their sexual reproduction cycle into midgut where recombination will produce variation in generation. During gametogenesis divides into eight flagellated microgametes, whereas the female gametocyte develops into a single macrogamete. Fertilization of a macrogamete by a microgamete forms zygote followed meiosis and develops into an ookinete, a motile form with apical organelles. The ookinete penetrates the mosquito gut wall and comes to rest near the basal lamina of the midgut. Here the ookinete rounds up and transforms into an oocyst, within which the parasite asexually replicates and undergoes sporogony process. Upon oocyst rupture, these sporozoites migrate to and invade the salivary

glands, where they can be transmitted back to the vertebrate host during a blood meal (Ngotho et al., 2019).

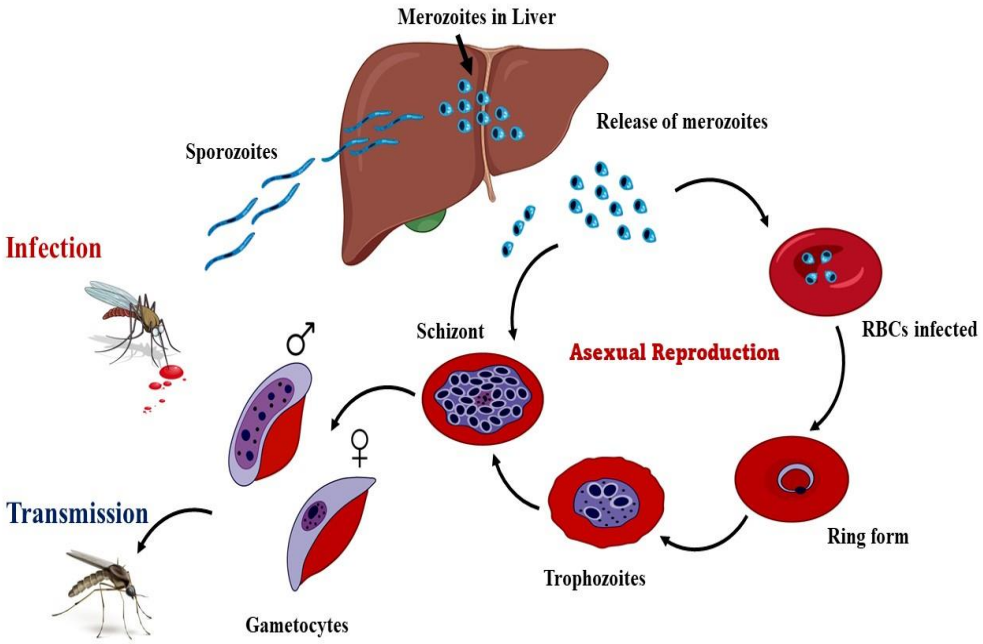

Figure 1.10 Life cycle Plasmodium parasite in human (Figure is self-constructed)

Genetic factors and genetics of persons have significant role in MP infection because in special case genetic composition of hemoglobin provide protection against malarial species by birth. Studies revealed that persons with sickle cell anemia and HbC have strongest protection against malaria. Similarly, RBC polymorphism i.e., G6PD deficiency, blood group O and duffy blood group are immune against MP infection especially *P. vivax, falciparum* and *ovale*. Genetic factor involved in controlling the immune system protect the persons from malaria (Kariuki & Williams, 2020).

1.9.3 Clinical Representations

Fever, headache, vomiting, sweating, body aches and chills are major clinical presentations of malaria. These symptoms can be mild and difficult to recognize as malaria, usually appear within 10-15 days of the infective mosquito bite. Malaria, if left untreated, can cause severe illness and death within 24 hours. In malarial non-endemic countries, these symptoms are frequently confused with the flu, common cold and other infections, whereas in malarial endemic countries, these symptoms are treated as malaria. Elevated temperature, perspiration, weakness, splenomegaly, jaundice, hepatic enlargement and increased respiratory rate are all physical findings. Infected patients also had anemia, thrombocytopenia, elevated LFTs and bilirubin levels (Boushab, Ould Ahmedou Salem, Ould Mohamed Salem Boukhary, Parola, & Basco, 2020; Lopez-Perez, van der Puije, Castberg, Ofori, & Hviid, 2020).

Malaria may be uncomplicated or complicated depending upon the type of specie infecting to human. Patients with uncomplicated and/or classical malaria presented with the disease in three stages: cold, hot and sweating. Shivering and a cold sensation are common during the cold stage. Only in young children, hot stage includes fever, headache, vomiting and seizures. Sweating, a return to normal body temperature and tiredness are the final stages. Malaria attacks typically last 6-10 hours. Attacks occur every second day with the "tertian" parasites (*P. falciparum*, *P. vivax, and P. ovale*) and every third day with the "quartan" parasites (*P. falciparum, P. vivax, and P. ovale P. malariae*) (Lopez-Perez et al., 2020).

In case of severe infection patient may suffers from single or multiple organ failure, metabolism abnormalities and blood parameters disturbances. The manifestations of severe malaria include hemolysis due to severe anemia,