Overview of Pathogenesis, Diagnostics, and Therapeutics of Infectious Diseases: Dengue and Zika

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ABSTRACT: The emergence of more virulent SARS virus has made scientists look back at other so-called neglected diseases such as dengue, Zika, and chikungunya, etc. Until recently these neglected diseases have not received much attention for their control or elimination from society. Over the past decade several attempts to investigate the pathogenicity, diagnostic, and therapeutic strategies for flavivirus caused diseases have been made. Herein we have reviewed the progress made toward the detection and treatment of two diseases—dengue and Zika. The above flavivirus related pathogenesis is concerned with the host immune system and known to be mediated through various receptors along with antibody-mediated disease enhancement. Moreover, researchers have been progressing toward discovering new drugs and therapeutic methods that target various stages of the flavivirus life cycle to minimize the above caused mortality and morbidity. The available diagnostics are based on serological, small molecule detection systems and point-of-care sensing devices. In this work, we have reviewed the advancements made toward understanding the pathogenesis, diagnostics, and therapeutics of the viral diseases caused by dengue and Zika.

1. INTRODUCTION

Several arthropod-borne viruses (arboviruses) have been identified to date, and many of them are known to cause infections in humans.1 An outbreak of epidemics triggered by the reemergence of infective viruses causing dengue (DENV), Zika (ZIKV), and chikungunya (CHIKV) demands developments toward the available diagnostics and therapeutic strategies.2 The therapeutic strategies including the standard and emerging drugs along with potential drug targets of other neglected tropical diseases such as Leishmaniasis and Chagas diseases were reviewed recently.3 Among the above-mentioned pathogens, ZIKV and DENV belong to the Flaviviridae family. Until the past decade, clinical issues caused by the above viral infections were mild with only a few cases reported in humans. Though horizontal transmission to humans was commonly reported, several other models such as nonvector-based transmission were also reported in the literature4 and are known to be caused through sexual contact or maternal to fetal transmission, blood transfusion, or body fluids. Both symptomatic and asymptomatic cases were reported.

Conventional diagnostic methods employing optical probes5 to diagnose viral pathogens are less sensitive and are time-consuming. Serological and other immunoreagents-based methods, such as ELISA (enzyme linked immunosorbent assay), are prone to produce false-negatives due to cross-reactions. The molecular methods of diagnosis based on PCR (polymerase chain reaction) techniques are known to have the upper hand with respect to either the serological or the virus isolation methods. The ambiguities observed with viral persistence, disease pathogenesis, and nonspecific clinical manifestations at different stages of infection by different flaviviruses6 make the diagnosis challenging and pave the way for development of novel molecular or serological testing methods. The challenges posed toward such diagnostics include the onset period of infections—being symptomatic or asymptomatic—and specificity toward virus detection,7 differentiating it from one another and other closely related viruses. Further, the majority of the epidemic prone regions lack resources for rapid diagnosis and treatment, urging the scientific community to focus on rapid and efficient methods with high sensitivity, selectivity, and specificity.

The issues on viral persistence along with the nonspecific clinical manifestations at the acute stage of viral infections make the diagnosis challenging.7 With subsidence of the above
caused pandemics, there still exists the lack of clear knowledge on pathogenesis or sero-prevalence to analyze the possible epidemics in the future. Until now, there are no specific antiviral or immunomodulatory drugs to prevent or cure infections caused by ZIKV and DENV. However, there are many well-characterized drug candidates that are reported to target the life cycle or entry of the virus into the host cells that may help in the treatment or prevention of ZIKV or DENV infection. In this review, an overview of pathogenesis, diagnostics, and therapeutic methods of infectious neglected diseases causing viruses—ZIKV and DENV—are discussed. Further the recent advancements toward the establishment of novel platforms for the diagnosis and suitable therapies available for these viral infections in humans have also been attempted.

2. PATHOGENESIS OF ZIKA AND DENGUE INFECTIONS

Infectious diseases caused by the flaviviruses such as Zika and dengue have been posing a major threat to humans globally over the past decade. All of these three viral diseases are found to show more or less the same epidemiological and clinical aspects but differ in the pathogenesis leading to several clinical manifestations. The severity of these diseases seems to be different, where DENV is associated with relatively high mortality, ZIKV leads to neurological morbidity, and CHIKV is attributed to causing severe illness in newborns. Also, DENV and ZIKV were found to result in loss of fetus, with ZIKV infections causing teratogenesis.

2.1. Zika Pathogenesis. The accurate pathogenic mechanisms of ZIKV infection are still under investigation, but there is available clinical evidence that verifies the involvement of ZIKV leading to pathogenesis of neurological syndrome in humans. In the case of ZIKV, congenital infections in fetus were reported through maternal transmission. The pathogenesis of neurological as well as congenital infections could follow two different mechanisms: direct viral attack and immune-mediated pathogenicity.

ZIKV is known to fight against the host’s first line of defense—type I interferon (IFN) signals—to replicate and cause disease in vertebrates. In vitro studies have proved that ZIKV growth in the host cells/tissues are controlled by several factors in the hosts, such as type I and type III IFNs, IFN-stimulated genes (ISGs) such as IFITM1 (interferon-induced transmembrane protein 1) and IFITM3 (interferon-induced transmembrane protein 3), that belong to the transmembrane antiviral protein family. Other ISGs involved in ZIKV caused pathogenesis are still under investigation. Moreover, the identified and reported ZIKV receptors through in vitro methods are yet to be validated in vivo. Axl is one such ZIKV receptor identified through in vitro studies and belongs to the TAM receptor family (Tyro3, Axl, and Mer). TIM1 (T-cell immunoglobulin and mucin domain 1) is another potential receptor that comprises of glycoproteins and binds to the phosphatidylinositol present on the viral membrane.

The ZIKV pathogenesis in female genital tract was reported with the presence of ZIKV RNA in cervical mucus after the appearance of symptoms and clearance of viral load from blood and urine. In vitro studies have revealed infections in uterine fibroblasts of humans which can potentially hinder fetal development. On the basis of human and animal studies, several cells and cellular signals, such as hormones and interferons, were found to be involved in the maternal—fetal ZIKV transmission. Studies have detected ZIKV cell tropism at the maternal—fetal interface that includes trophoblasts, fibroblasts, endothelial cells, Hofbauer cells, and amniotic epithelial cells. It is also reported that ZIKV could penetrate the placental syncytium and infect the Hofbauer cells leading to proliferation of these macrophages thereby accumulating the viral RNA in Hofbauer cells, which could subsequently lead to the transmission to fetus or the congenital viral symptoms. The increase in occurrence of microcephaly in those children born to infected pregnant mothers and the presence of viral RNA in the amniotic fluid, placenta, and brain tissues of those infants with microcephaly, confirmed ZIKV as an etiologic factor of CNS (central nervous system) abnormality.

ZIKV pathogenesis in male reproductive tract was speculated to occur by disrupting the blood—testis barrier (BTB), breaking immune suppression in the testes leading to dissemination of virus. Upon infecting the Sertoli cells in humans, the proliferation of cytokine signals and cell-adhesion molecules, improving leukocytes adhesion, and facilitating accessibility of the BTB were noticed to increase substantially. High viral loads of ZIKV were reported in semen and serum, which suggested viral replication in the testicles or seminal glands. An important report has revealed tyrosine kinase Axl, as an entry point receptor, in almost the entire male reproductive tract that include testes, especially the Sertoli cells, prostate, and epididymis. Recent data also demonstrated that Axl aids in the entry of ZIKV and depletes the ability of Sertoli cells to resist viral infection, along with long-term persistence of ZIKV infection in testis. Another receptor Tyro3 was found to be colocalized with ZIKV, in infected semen samples, revealing its role in viral entry, and the same receptor is also reported as the entry point for Ebola and Marburg viruses.

The overall summary of the above-discussed ZIKV pathogenesis studies with important receptors and infected cells involved are shown in Figure 1.

2.2. Dengue Pathogenesis. Dengue infections are caused by four different, antigenically related serotypes (DENV-1, -2, -3, and -4). The severity of DENV infection among the four serotypes is associated with several factors. In 2009, WHO released a clarity note on the clinical diagnosis of DENV, based
on its severity as dengue with or without warning signs and severe dengue. The dengue infection without warning signs was reported to be more like a mild flu infection, accompanied by sudden fever, vomiting, myalgia, abdominal pain, arthralgia, skin erythema, headache, flushing, and leukopenia. And the dengue with warnings involved ailments that include mucosal bleed, enlarged liver, high hematocrit level, and low platelet count. In the case of severe dengue infections, respiratory problems with severe plasma leakage, bleeding, or severe organ problems including hepatitis, myocarditis, or encephalitis are observed. DENV pathogenesis is initiated by suppression of the innate immune response in the host body, leading to enhancement of viral replication in human keratinocytes. These viruses are found to initially infect the cells, like Langerhans, dendritic cells, and other cells at the site of entry mainly by its attachment with several receptors such as dendritic cell-specific ICAM-grabbing non-integrin. DENV on infected humans is found to suppress the interferon (IFN) response. In the case of less severe infections, high levels of IFNα were found, whereas in the severe cases of infections, IFNα was observed in low levels. Asymptomatic DENV infections in humans are associated with type 1 interferon of the hosts along with suppression of other immune responses aiding in viral replication. These include high viremia, activation of complement system and T cells (nonprotective), genetic factors of the host, and also secondary infections during/after the first infection with a new serotype. High plasma leakage, a severe pathophysiological manifestation in dengue, is also found, which results in dengue hemorrhagic fever and dengue shock syndrome.

Higher susceptibility to severe dengue is reported in the case of a second infection. This severity is mainly attributed by the phenomenon of antibody-dependent enhancement (ADE), in which the antibodies produced in the first infection by DENV of a particular serotype are demonstrated to be non-neutralizing in the case of infections by other serotypes. With heterotypic infections or secondary infections with any other serotype, these non-neutralizing antibodies of first infection bind to the new heterotypic DENV, help in viral entry, and lead to infection of cells containing FcγR receptors that include monocytes and macrophages, resulting in enhancement of infected cells and also cytokines such as TNF-α, IL-6, IL-1β, and IL-10 in the host body. This phenomenon of antibody-dependent enhancement was also reported in mouse models. Development of severe dengue is observed as primary infections in newborns possessing subneutralizing antibodies obtained from dengue-immune mothers.

Vertical transmission of DENV is also reported to occur through blood, bone marrow, or any target organs upon infection. In the case of the maternal host with secondary infections involving a new serotype, the infants are found to suffer from severe ailments caused by antibody-dependent enhancement. DENV pathogenesis in pregnant women can result in placental inflammation (placentitis) leading to hypoperfusion and ultimately loss of fetus or premature or low birth weight infants. This virus apparently crosses the placenta during placentitis followed by infection of the fetus and other developmental ailments.

To date, there have been very few reports on the sexual transmission of DENV, suggesting female to male transmission and the presence of viral RNA in vaginal secretions, and contradictory reports are available for the presence of DENV in semen.

3. DIAGNOSIS FOR ZIKV AND DENV

3.1. Serological Methods. ELISA is the commonly adopted method to detect antibodies against specific infection in host systems. But challenges exist to overcome cross-reactivity between the antibodies of different viruses because of the observed similarity in the target protein sequences.

Figure 2 depicts an ELISA-based method to detect ZIKV infection with a large cohort of human sera. In this the domain III of ZIKV envelope protein (EDIII) having high similarity with DENV was targeted. The antibodies raised against ZIKV EDIII did not show any cross-reactivity with DENV EDIII. The sensitivity of 92% and specificity of 90% was reported with infected patients’ serum. In this assay the human sera samples (with or without IgG) were incubated with surface impregnated recombinant EDIII of ZIKV, followed by the addition of detection antibodies conjugated with conventional horseradish peroxidase and colorimetric substrate. The specificity of this assay was verified with RT-PCR/IgM-based assay, and other possibilities such as flavivirus-positive but ZIKV-negative, or flavivirus-negative, and so on. The assay also revealed the non-observation of cross-reactivity of ZIKV IgG with EDIII of DENV. Therefore, this study has shown the potential use of recombinant protein-based ELISA, as the basis for developing reliable, rapid, and specific assays/commercial diagnostic kits for such viral infections.

Computational strategies employing molecular dynamics, macromolecular docking, and so on are considered as the most safe and time- and cost-effective methods to screen potential diagnostic or drug candidates. In the case of DENV a simple and cost-effective computational diagnostic approach was attempted with specifically designed peptides (E1−E10) based on the envelope protein followed by screening with chemiluminescence enzyme immunoassay (CLEIA). This assay was very similar to the above-demonstrated ELISA
except that a chemiluminescent substrate was used to enhance the sensitivity. This method exhibited a sensitivity of 85% and specificity of 96.4%, within a time period of 1.5 h and further this formed the basis for development of microarrays and peptide-based ELISAs. High-density microarrays with proteomes of different infectious viruses were analyzed to obtain nonredundant peptides and tested for immunoreactivity toward ZIKV, DENV, and a few others. The NS2B peptide obtained was proven to be a diagnostic target with high immunoreactivity in ZIKV infected patients and not with other flavivirus infections. This assay was further extended to develop a concatemer of 49 amino acid peptides, with two 22 amino acids long NS2B peptide (AGDITWEKDAEVTGNSPRLDVALDEAGDITWEKDAEVTGNSPRLDVALD) joined together with a glutamic acid residue in the middle and spacers comprising two glycine and two alanine residues on terminal ends of the two NS2B peptides. This concatemer was biotinylated at the C-terminal with lysine at residues on terminal ends of the two NS2B peptides. This formed the basis for development of microarrays and peptide-based ELISAs. High-density microarrays with proteomes of different infectious viruses were analyzed to obtain nonredundant peptides and tested for immunoreactivity toward ZIKV, DENV, and a few others. The NS2B peptide obtained was proven to be a diagnostic target with high immunoreactivity in ZIKV infected patients and not with other flavivirus infections. This assay was further extended to develop a concatemer of 49 amino acid peptides, with two 22 amino acids long NS2B peptide (AGDITWEKDAEVTGNSPRLDVALDEAGDITWEKDAEVTGNSPRLDVALD) joined together with a glutamic acid residue in the middle and spacers comprising two glycine and two alanine residues on terminal ends of the two NS2B peptides. This concatemer was biotinylated at the C-terminal with lysine at the junction. Using this concatemer in ELISA, referred to as “ZIKV-NS2B-concat ELISA”, an indirect sandwich mode was established for ZIKV detection using antibiotin antibodies (Figure 3). The above studies throw insights on the use of programmable peptide array platforms. They also serve as a tool for predicting specific peptide regions in viral genomes and are considered vital for the development of diagnostics in serology.

3.2. Molecular Detection Methods. RNA viruses can be detected in the infected hosts by a sensitive, specific, and more reliable reverse transcription followed by amplification in a quantitative polymerase chain reaction (qRT-PCR). This molecular method of viral detection starts with isolation of RNA from the biological samples of patients followed by a reverse transcription and amplification using virus specific primers and probes. But the presence of inhibitory components such as urea in urine samples; lactoferrin, IgG, or hemoglobin in patient blood samples; and anticoagulants such as heparin formed the major drawbacks for this method. However, these inhibitory effects were overcome by using positive or spike-in controls. Until now, only a few commercially available qRT-PCR kits for ZIKV diagnosis with various limitations have been available. More recently, a direct RT-quantitative PCR (dirRT-qPCR) assay was reported to diagnose ZIKV without pretreatments. With this assay a ZIKV RNA detection limit of 95 copies per reaction was achieved with 5 μL sample volume in 2 h. Specific forward and reverse primers were designed on the basis of the alignment with NS5 viral protein. In this assay the limitations of inhibitors in clinical samples were overcome by optimizing DNA polymerases, enhancers, and reaction conditions. OmniTaq DNA polymerase was reported to work efficiently, through efficient amplification even in the presence of other inhibitors in clinical samples. The reverse transcriptase enzyme of ZIKV was also successfully demonstrated toward its detection. The reverse transcriptase enzyme SuperScriptIII was proven to be better with lower Ct values, than PrimeScript, in detecting ZIKV regardless of the type of samples employed.

Several multiplex assays to differentiate and detect ZIKV and DENV infections are available. A single step multiplex real-time RT-PCR assay was reported to detect ZIKV and DENV in whole blood, plasma, or serum, with existing primer and probe data, without any cross reactivity or PCR inhibition. An interesting pentaplex assay was reported to detect the viruses that cause Zika, dengue, chikungunya and West Nile virus quantitatively along with human housekeeping gene (RNaseP) via a single-step procedure.

3.3. Point-of-Care Sensing Methods. Point-of-care or lab-on-chip diagnostics are essential for rapid diagnosis of diseases with ease, specificity, and sensitivity. Recently, a novel microfluidic chip based viral RNA detection tool called “C3 system”, was developed for ZIKV (Figure 4). This is comprised of a miniaturized PCR unit and a smartphone-based fluorescence detection system. In this the chitosan coated capillary sample tubes were designated to regulate pH, adsorption/desorption of nucleic acids and allow rapid nucleic acid extraction. This miniature equipment is portable and can recover RNA at as low concentrations as 50 transducing units/mL from crude salivary samples.
A simple handy battery-operated device that can perform reverse transcriptase recombinase polymerase amplification (RT-RPA) in an isothermal field-applicable assay was reported to detect as low as five copies of viral nucleic acids with high specificity (100%) and sensitivity (83%). The device was also reported to be used in vector surveillance apart from diagnostics.

Also a miniature metal organic framework (MOF) system was reported to detect nucleic acids based on their fluorescence quenching ability. A nitrogen-doped porous carbon (NPC) formed by simple methods involving MOF-based precursors was employed to develop a nanobiosensor along with a charge-coupled imaging system (CCD) to detect nucleic acids. In this the luminescence from fluorophore tagged probe-DNA gets quenched when adsorbed over NPCs, while the fluorescence gets recovered when it hybridizes with complementary target sequence (Figure 5).

An instrument free colorimetric point-of-care detection technique to detect ZIKV on the basis of reverse transcription–loop mediated isothermal amplification (RT-LAMP) assay was reported. This method does not require sophisticated devices for detection or visualization and possessed a high sensitivity ∼ 5 pfu in a time period of 40 min.

An electrochemical capacitive-based assay for the detection of DENV was developed with NS1 protein of DENV as a biomarker, in which a ferrocene tagged peptide (Fc-Glu-Ala-Ala-Cys) modified surface containing anti-NS1 was used as the receptor, and positive/negative cases of DENV in serum samples were identified without any electrochemical reagents or labels. This paved the way for the development of a new platform to establish novel diagnostic electrochemical devices for several other infectious diseases.

4. THERAPEUTIC APPROACHES

To date there are no available methods or vaccines to treat ZIKV and DENV infections. The available treatments are only to relieve the symptoms of infection. But there are several vaccine candidates that are in clinical trials. Because ZIKV and DENV fall under the flaviviridae family, researchers working toward therapeutic developments are employing methods that are directed against flaviviruses. The life cycle of flavivirus (Figure 6) is recognized as one of the common therapeutic targets for ZIKV and DENV.

4.1. Therapeutics Targeting the Flavivirus Life Cycle and Host Entry. The open reading frame of ZIKV encodes 10 proteins of which 3 are structural proteins—envelope (E), membrane (M), and capsid (C)—and 7 are nonstructural proteins—NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Following the entry of virus and release of RNA genome into the host system, a sequence of reactions gets initiated. Briefly, the translation of ssRNA into a polyprotein complex occurs on the endoplasmic reticulum membrane, which is further cleaved by various proteases. The post-translationally modified non-structural proteins form a replication complex on the endoplasmic reticulum (ER) membrane. The above proteases are highly conserved in ZIKV and DENV strains and are considered as potential therapeutic targets. There are several inhibitors that are reported to target various stages of the virus life cycle.

Inhibitors such as duramycin and nanchangamycin (Table 1) are known to prevent viral entry into the host system by targeting the attachment, entry, and fusion of viral particles by blocking the receptors and endocytosis, respectively. Nanchangamycin is a bacterially derived natural product that inhibits the entry of ZIKV and DENV infections across several cell types. In vitro studies show that inhibition is by blocking clathrin-mediated endocytosis, the internalization pathway for these viral infections. Duramycin is a tetracyclic peptide, suggested to be a broad-spectrum antibiotic that prevents DENV, Ebola, West Nile viruses and ZIKV infection by specifically binding to phosphatidylethanolamine (PE), present on the surface of virus. Duramycin is reported to bind to the headgroup of PE, with the binding site consisting of lipophilic side chain moieties from the amino acid residues Phe-7 to Cys-14 of duramycin, which is stabilized by ionic interaction between ammonium group of PE and carboxylate group of Asp-15.

Further computational investigations have revealed that candidates possessing chains of aromatic rings, derivatives of phenyl hydrazine and thiophene-quinazoline, oxysterol 25-hydroxycholesterol and antiparasitic suramin can potentially...
inhibit the attachment, endocytosis, and fusion with host receptors.

The NS2B-NS3 proteases in ZIKV serve as one of the potential inhibitory targets. NS3 is a serine protease that cleaves the viral polyprotein into 3 structural and 7 nonstructural proteins (shown above). NS2B being a cofactor for NS3, without its association, NS3 cannot exhibit its activity. As these genes are conserved across the flavivirus genus, they can act as promising targets for therapeutic candidates. Various inhibitors like temoporfin, nitazoxanide and niclosamide, viperin, aprotinin, novobiocin, and bromocriptine (Table 2) can potentially interrupt the interaction of NS2B and NS3, but their efficacies in vivo are yet to be investigated.

Representative inhibitors that target the NS2B-NS3 protease of flavivirus along with their mechanism of action are depicted in Figure 8. Three compounds temoporfin, nitazoxanide and niclosamide were identified to exhibit inhibitory activity not only against Zika virus but also against dengue, Japanese encephalitis, yellow fever, and West Nile viruses. Temoporfin was reported to be more potent toward inhibiting the viral loads in blood and hence preventing lethal infection. In particular, structural docking suggested that two phenol groups of temoporfin are anchored into the NS3 pockets that hold NS2B residues at positions 51 and 53 (Figure 9). This eventually was found to inhibit flaviviral polyprotein processing in a noncompetitive manner. Moreover the recent reports have revealed that the active site of NS2B-NS3 protease of DENV consists of two pockets in chains A and B. The drug interactions are majorly stabilized by hydrophobic interactions with minor but significant contributions from hydrophilic and charged residues.

Viperin, an interferon-inducible iron–sulfur protein, exhibits broad antiviral activities by catalyzing the formation of 3′-

Table 1. Inhibitors of DENV and ZIKV That Block Specific Receptors and Endocytosis

| Inhibitor | Nature | Functions |
|-----------|-------|-----------|
| Duramycin | Tetracyclic peptide | - Broad-spectrum antibiotic  
|           |       | - Inhibits infection by binding to viral surface  
|           |       | - Exposed phosphatidylethanolamine |
| Nanchangamycin | Bacterially derived natural product | Blocks clathrin-mediated endocytosis |

Table 2. Protease inhibition potential (IC50 (μM)) for ZIKV and DENV inhibitors

| Inhibitor | IC50 (μM) |
|-----------|-----------|
| temoporfin | 1.1 ± 0.100 |
| niclosamide | 12.3 ± 0.600 |
| nitazoxanide | 15.9 ± 0.900 |
| aprotinin | 0.07 ± 0.012 |
| novobiocin | 26.12 ± 0.330 |
| bromocriptine | 13.04 ± 2.000 |

Figure 7. Chemical structures of potential drugs that target NS3/NS2B proteases of flavivirus.

Figure 8. Various protease inhibitors targeting NS2B-NS3 and their mechanism of inhibition.
deoxy-3′,4′-didehydro-CTP (ddhCTP) from cytidine triphosphate (CTP) (Figure 10). Here the ddhCTP formation terminates the polymerization reaction induced by the RNA-dependent RNA polymerase in several flaviviruses including West Nile, Zika and Dengue virus. Moreover, viperin was found to specifically inhibit ZIKV multiplication and was not effective against JEV or YFV. Interaction and colocalization of viperin with several ZIKV nonstructural proteins like NS2A, NS2B, and NS3, led to reduction of NS3 expression by induction of its proteasome-dependent degradation.20

Another drug novobiocin was reported to act as NS2B-NS3 inhibitor. Reports have proven increased survival rates in infected mice, decreased levels of viral loads in blood/tissue and histopathological damages. The structural interactions of novobiocin and ZIKV-NS2B-NS3 protease were reported using molecular docking which showed that three hydrogen bonds were formed between Novobiocin, the ligand and the protein via the ZIKV-NS2B-NS3 monomer A residues, (PDB ID: 5LC0) Met51, Ser81 and Lys5421 as shown in Figure 8. Also, hydrophobic interactions between the compound and residues His51 and Val155 of monomer B was found induce stabilization. Two other residues from monomer B, Ser81 and His51 were also localized at the binding site of boronate inhibitor cn-716 and the ZIKV-NS2B-NS3 protease that suggested inhibition of ZIKV-NS2B-NS3 catalytic efficiency by novobiocin. This was also proven using enzyme kinetic experiments in the presence/absence of the drug.

Molecular docking studies to investigate the interaction of bromocriptine with NS2B-NS3, have revealed the inhibition of proteolytic activity, which was further proven by cell culture assays.22 Random docking of ZIKV-NS2B-NS3 protein with bromocriptine has revealed the interaction of many active site residues like His51 and Ser135 that are localized in the proteolytic cavity. Further interactions were also observed with other residues in the monomer subunits of A (Asp129, Ala132, Gly151, Val155 and Thr208) and B (Thr28, Val37, Val52, Thr53, Lys54 and Ala132) of NS2B-NS3 complex through van der Waals interactions. In addition to the above bromocriptine was also found to form hydrogen bond (2.63 Å) with the Ser135 of subunit B (NS2B-NS3). All the above binding interactions of bromocriptine promoted its anchoring to the active site and inhibit the protease activity of ZIKV-NS2B-NS3 protein.

Sofosbuvir, a phosphoramidate nucleotide prodrug is one of the class B FDA approved drugs that can inhibit NS5 polymerase in ZIKV. It is one of the first compounds tested to possess anti-ZIKV activity. The design of natural nucleoside and nucleotide analogs has been in focus for many novel drug developments. The success of sofosbuvir as anti-ZIKV drug resides on the fast step phosphorylation process as compare to natural nucleosides. This is due to the presence of phosphate group in its structure (Figure 11). Also, several other inhibitors are reported to inhibit the unwinding of RNA by NS3 and the function of NS5 methyl transferase.

The development of direct antiviral therapeutics for viral infections has always been a daunting task. Immunotherapy or the use of immunomodulatory drugs are potential and effective alternatives that enhance the immune system responses at
different levels against viral infections, immunodeficiencies or tumors and act as immunosuppressive agents by decreasing the immune responses in the treatment of autoimmune diseases or allergies. Immunocytokines are regarded as one of the most important targets for immunomodulation in viral infections and autoimmune diseases.23

ZIKV infections are known to be associated with activated immune system responses caused by enhanced inflammatory molecules such as cytokines/chemokines.24 With DENV infections, the symptoms and immune responses were associated with enhanced pro-inflammatory cytokines. There are different kinds of immunomodulators developed specifically to enrich or inhibit the immune cells such as cytotoxic T-cells, natural killer cells, macrophages, neutrophils, and lymphocytes.

For instance, Fingolimod (FTY720), an oral drug (FDA approved) is used in the treatment of multiple sclerosis that modulates sphingosine-1-phosphate (S1P) receptor to further regulate the lymphocytes in lymph nodes and inhibit them from causing autoimmune reaction. The drug FTY720 was proven to be effective against autoimmune diseases and transplantation rejections. ZIKV infections are known to be associated with activated immune system responses caused by enhanced inflammatory molecules like cytokines/chemokines. Immunomodulatory drugs would potentially reduce the inflammatory responses in ZIKV infections by activating the regulatory T-/B-cells that secrete anti-inflammatory cytokines (IL-10, TGF-ß). A sphingosine analog of FTY720, AAL-R, was reported to limit inflammation and mortality in H1N1 influenza infected animal model, without affecting the generation of neutralizing antibodies against H1N1 influenza virus. Similarly, ZIKV infected patients with autoimmune problems could benefit out of immunomodulatory therapy with FTY720. In the case of DENV infections, several symptoms and immune responses against the virus were more associated with enhanced pro-inflammatory cytokines. Among the polyphenols such as curcumin, fisetin, resveratrol, apigenin, quercetin, and rutin that were tested for immunomodulatory properties, quercetin and fisetin were shown to inhibit DENV infections by downregulating the pro-inflammatory cytokines. This combination therapy using immunomodulators with antiviral drugs were proved to be more effective than using any other available individual drugs. The potential of repurposing the existing immunomodulators along with the available direct antiviral drugs ought to be explored for use as combinational therapeutics to treat many viral infections especially the neglected infectious endemic diseases like ZIKV and DENV.

5. FUTURE SCOPE

Extensive research is still necessary in the areas of diagnostics and treatment of ZIKV and DENV infections. The emergence of novel applications platforms in ZIKV diagnostics, such as high-density peptide arrays, metal–organic frameworks, and field-applicable nucleic acid assays, etc., can potentially establish field-ready point-of-care testing. Also, in case of molecular testing, significant developments are continuously being made in creating specific primers and probes for the viruses (ZIKV and DENV), differentiating them from other flaviviruses. However, more developments are required to reach more rapid and simpler detection approaches. In serological-/antibody-based methods, researchers ought to find ways to reduce the cross-reactivity between antibodies against different flaviviruses or secondary infections, such as the EDIII peptide-based ELISA, assuring specificity to ZIKV detection. With the available molecular/serological assays as platforms, lab-on-chip or point-of-care diagnostics should be developed. Inexpensive sensors or handy sensing tools that are easy to assemble, operate, and detect ZIKV with sensitivity and specificity will have larger impact in remote, less accessible areas and also in the areas vulnerable to epidemics or even pandemic situations. These kinds of point-of-care tools can possibly decentralize health care systems and also provide reliable diagnosis, assisting doctors toward patient care. The future of these diagnostic tools lies in bringing them into operation as simple kits for standard applications.

In the aspect of therapeutics, various new drugs and treatment methods have emerged in recent years, though they are not directly tested for specific viral infections in humans. The majority of the scoring drugs are repurposed ones. There are several other drugs, which are still in the stage of clinical trials. There are many more to be added on the drugs that are reported in the literature but have failed to inhibit or control the above-discussed viral pathogenesis in humans. With the available bioinformatics approaches and tools, it is possible to come up with reasons for failure and a chance to re-address the related issues.

6. CONCLUSIONS

Numerous diagnostic methods such as ELISA, RT-PCR, and point-of-care diagnostics are being developed by several researchers to curb the rise in infections caused by several of the neglected diseases such as dengue, Zika, and so on, so as to prevent the rise of epidemics/pandemics. At the same time, several therapeutic strategies and antimicrobial drug candidates are also developed and investigated by various experimental and computational methods such as culture-based assays that target different aspects of the viral life cycle or host entry and host–drug interactions, respectively. However, only a few of them are tested in animal models that necessitate the need to evaluate safety or efficacy before their applications in clinical trials. Therefore, there is still a long way ahead to implement these therapeutic measures against ZIKV and DENV infections.

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Musuvathi Motilal Balamurali was awarded the Master's degree in Chemistry, by Madurai Kamaraj University, Tamil Nadu, India in 1999. He pursued his doctoral research in Photophysical Chemistry at Indian Institute of Technology Kanpur, under the supervision of Prof. Sneh Kumar Dogra. In 2005, he was awarded the CSIR research associate fellowship and continued his postdoctoral research in Prof. Raghvan Varadarajan's group at Indian Institute of Science Bangalore. In 2007, he moved to University of British Columbia, Canada, and in 2009 he joined Prof. Debkumar Pain’s research group at New Jersey Medical School, USA and continued his interdisciplinary research respectively in Biophysics and Biochemistry. In 2011, he served as research scientist in the DST India funded National Hub for Healthcare Instrumentation Development, Anna University, Chennai, India. Since 2013, he has continued to serve Vellore Institute of Technology, India as Associate Professor in the Department of Chemistry. His research interests include Protein Engineering and Biophysical Chemistry.

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