International Research Journal of Medical Sciences _____ ISSN 2320 – 7353
Vol. **7(2),** 1-5, May **(2019)** *Int. Res. J. Medical Sci.*

Review Paper

Dengue infection and molecular mechanism of spreading

Gulshan Dhra*, Jitendra Kumar and P.K. Patanjali
Institute of Pesticide Formulation Technology (IPFT), Sec-20, UdyogVihar, Gurugram-122016, Haryana, India
gdhraster@gmail.com

Available online at: www.isca.in
Received 14th February 2019, revised 5th May 2019, accepted 26th May 2019

Abstract

This article highlights a brief overview about the Dengue Fever (DF), its distribution around the world, symptoms and cuse of spreading mechanism. Also, using of bio-pesticides have been discussed with the beneficial effects to control the disease. Dengue fever (DF) is a well known and serious disease, spreaded by Aedesmosquitoes. The virus which is responsible for the transmission of the disease made its host to the Aedesmosquito. The four serotypes of the dengue viruses are responsible for the infection, which results in the acute fever, skin rashes, heamorage and sometimes even shock to the patient. We are also highlighting the molecular ineteractions mechanism of virus and the host cell with emphasizing the receptor molecuels of the host cell surface, which is considered to be a very first step for the pathogenesis of dengue. But till date, our knowledge is not that much about these receptor molecules in human monocytes, which are the prime targets of the dengue virus (DV). The envelop proteins from the virus and heparin sulfate of the host cell surface facilitates the binding between the virus and host cell through the receptors and play a key role in virus-cell attachment and binding. Controlling stretegies are also discussed with the vector control, chemical therapeutic antiviral agents and antiviral vaccines etc., along with brief introduction of neem based pesticide formulations developed at IPFT recently which are eco friendly and safe for humans as well.

Keywords: DF, DHF, serotypes, receptors, heparinsulfate, envelop proteins, virus-cell fusion, botanical pesticides.

Introduction

Diseases like, Dengue, malaria, yellow fever and filariasis etc., are spreaded by vector and known as vector borne diseases. Dengue is known from long back in history and is recorded in the encyclopaedia of disease, China. First time in the history dengue virus was isolated in Japan. This was done by the inoculation of the serum[1]. Dengue heamorage fever is the critical form of infection and observed in Manila, Philippines[2]. The disease vector *Aedesaegypti* and the virus covers the new areas across the globe causing the epidemic. According to a data 40-45% humans are at the risk of infection and 50-100 million infections reported every year (WHO). A lot of money is being spent to control the disease worldwide.

Dengue Fever (DF): A Vector borne virus disease

Dengue Fever (DF) is an infectious disease and the virus is spreaded by the vector- mosquito. It is the most devastating disease of virus (Dengue Virus: DV) known to date. There are a number of individuals infected worldwide and tens of thousands are prone to the fatal conditions of the disease. The virus (genus *Flavivirus* and family *Flaviviridae*)[3] of the infection is a positive stranded RNA. The disease is particularly spreaded by the four serotypes (DV-1, DV-2, DV-3 and DV-4) of the virus and have a antigenic correlations with each others. On the other hand, followed by the infection, the human body becomes resistant to the disease but the with the only one particular serotype and not to the other serotypes. In real situation, cross-reactive antibodies can increase the serious conditions[4] of the diseae. At present, there is no any antivirus drug available and licensed to treat the disease and pose a serious problem in designing the controlling strategies against the virus. So, this is also a factor that more and more infections occurs to the new geographic areas in the world.

Transmission and Symptoms

The transmission occurs through biting of the infected mosquito and starting with normal disease to infection with the dengue virus. Later symptoms includes: acute fever, frontal headache followed by the retroorbital pain and many others like, vomiting, myalgia, arthralgia and severe weakness, joint/muscle pain etc. Hence DF is not fatal and generally patient can recover in a week or more. In addition to above form, the disease becomes more severe in many cases and is comparatively fatal and known as DHF (Dengue Hemorage Fever). Though its beginning symptoms are not distinguished from common dengue fever (DF). A confirmatory test tells about the increased amount of fragility of the blood capillaries, petechie, bleeding at venipuncture and at gums etc. Also, high fever hemorrhagic are observed along with the platelets count around 100,000/mm^3 or lower. These are characterized dengue hemorrhage fever (DHF). On the other hand, leakage of the plasma also determines the severity of the disease including other symptoms such as circulatory failure, restlessness etc.

Mechanism of Infection

Dengue virus (DV) enters the human body and targets the host cells for its multiplication. The entry of the virus to the host cell is eased by many factors at the host cell surface like, surface receptors of the cell for the virus-cell interactions. Also, endocytic, cellular transport and signals are initiated for the entry of the DV to the host cell. However, receptors reconition is not easy for the virus due to many reasons like, viral protein and receptor affinity for each other is low as well as number of receptor molecules are not sufficient on the host cell surface. On the other hand, it is now possible to recognize receptor molecules with the help of radioactive labelling as well as by modern immunochemical methods. Although, after conducting of various types of studies on receptors recognition, it is now established that there are a number of inhibitors which inhibits the entry of the dengue virus(DV) to the host cell.

This virus belongs to genus *flavivirus* and there are four types of dengue viruses which are related to each other serologically. These four types are transmitted by mosquitoes and ticks (Arthropods) to the vertebrate hosts. Based on the structures of the flaviviruses, it is now concluded that it uses a fusion mode with the host cell. In that barrels (beta) of domain-II of protein (glycoprotein-E) is fused with the host cell membrane.

Transmission and Infection

Human infections are occurred through the infected *Aedes* mosquitoes. These feed on human blood during daylight. Female mosquitoes feed on for a very short time on a single person and return to another host, hence it spreads the viruses to number of hosts within a very short time[5]. So, this type of spreading mechanism makes the *Aeaegypti* an efficient vector.

The spreading of the infection depends on how efficiently the virus genome and related proteins are transferred to the healthy host cell and required multi steps process. These comprises, virus assemblies into the infected host cell and infective virions are released into the extracellular space. Receptor-virus attachment/binding on the cell surface and internalization through endocytosis, decoating of the viral genome. Intra-cytoplasmic transportation are also responsible for the spreading of the infection and changing of the disease pattern is established by virus strain (Genetic variation).Infection occurs by the interactions and binding of the surface virions on virus and various specified receptor molecules on the host cell and viruses have identicle proteins which interacts with the host cell and are versatile in nature.

Entry of Flavivirus/Dengue virus

The entry of the viruses to the host cell was studied with the West Nile Virus (WNV). This internalization through coated vesicles followed by endosomes transportation[6]. On the other hand, virus infection is inhibited by some weak bases, but facilitated by acids, hence this is known as acid-induced fusion[7]. But, the penetration of the virus is pH dependent and the range reported is about pH 6.5.

Macrophase entry is facilitated by antibodies and occurs through Fc receptors[8]. Also penetration is hindered/blocked by one of the neutralizing monoclonal antibody and this is happened without interfering the attachment. Therefore, this antibody interacts with the virus, which is active for fusion process and block the penetration to the endosomes.

On the other hand, the DV entered to the host cell through the monocytes macrophase, while E-protein from the virus facilitates the virus-cell attachment. Also cell receptors play a key role in the attachment process. The molecules of receptors are mainly glycosaminogly can and lipopolysaccharides molecules[9]. However, entry of dengue virus (DV) is followed by adsorption and penetration along with the fusion to the host cell and infection accomplished within two hours of the adsorption of virus to the cell surface of the host. It concluded after many studies that one monoclonal antibody inhibits the entry of the virus to the cell and it is thought that functional domain of protein-E is involved during the attachment process but, penetration may have other correlation.

The α-mannose (carbohydrate) which is present on glycoprotein of the virus also play a role in the binding/penetration. Studies also indicate that heparin hinders the penetration process as well as the attachment. So this is concluded that heparin sulfate of the cell surface is involved in many steps mentioned above[10]. There are some specific regions of the virus envelop protein which are important for the binding of host cell through the heparin sulfate glycosaminogly can (GAG). These GAG-bindings complexes are composed of amino acids residues, which turn themselves in a manner that basic portions face each other. Although, flavivirus envelop proteins are more or less same[11].

Virus-Host Cell Fusion

As far as DV is concerned, it is composed of single RNA strand with 11 kb defragmented genome, which encircled by a lipid double layer (bilayer) known as an envelope of the virus. RNA codes for ten proteins, three of which are structural proteins which include C-Nucleocapsid, M-Membrane and E-Envelop proteins and seven are non-structural proteins including NS-1 to NS-7. The infection includes entry to the host cell with the release of C-nucleocapsid protein into the infected cell.

So, all of the above said processes occur by the fusion of the viral as well as the host cell membranes[12,13]. Hence, this was concluded that the fusion process occurs at the surface of plasma membrane or at the endosomal membrane. After this, there are some changes in the conformation takes place due to the binding of the virus and cell receptor or sometimes pH also plays a role in conformation change along with the cholesterol facilitation in the fusion[14].

Conclusion: Although, we have emphasized much about the binding of virus-cell, their penetration and fusion etc., but little is known about the molecular levels of these interactions. As far as heparin sulfate (HS) is concerned, so it functions as the receptor molecules or facilitates to concentrate the viruses to the surface of the host cell[15].Also, proteins, Fc receptors, GAGs and lipopolysaccharides etc., are considered to be the receptor molecules.

Effective Controlling Stretegies

Vector Control: As the human population and transportations of goods increases, the mosquitoes-borne infections also increases, particularly DF, DHF and yellow fever (YF). Also, in recent times the epidemicity is independent of the suitable reservoirs for their effective breeding. However, we are required some of the effective stretegies to reduce/eradicate this menace in present time.

Among the various effective stretegies, one is the controlling of the vectors (Arthropods). But this is not as easy as is considered, because many developing countries have insufficient resources for the vectors control[16,17]. Also, using of synthetic chemical (toxic) pesticides is not as effective as theses cause the concern of the safety of the environment as well as increasing pesticides resistance among the vectors[18] along with the increasing rate of resistance as compared to the development of the new chemical pesticides. Cross-resistance is also a growing problem among the vectors through the detoxifying mechanism with the reduce of the use of the alternative pesticides implementation[19].

Another method, which includes the use of botanical/natural pesticides/larvicides[20]and development of new vaccines which retard their vital body functions[21].The first step to eradicate the infection is to fast destroying their habitates and larvae. Use of indoor repellants mosquitoes traps/nets etc., are the effective ways to reduce the number of the mosquitoes bites to the human.

Mosquitoes predators strategy is also being under trials in many countries with the participation of locals to place them to habitates of the mosquitoes eg., crustacean *mesocyclops*[22] as it is a green technique and harmless to the environment and cost effective too.

Vaccines Control: Vaccines against dengue viruses can be a very effective strategy to control the dengue fever. Hence, we need to develop four types of monovalent vaccines and which are then mixed finally to produce an effective vaccine against the all four serotypes of dengue viruses, which are antigenecally different from each others. The same approach was developed for the polio virus initially. It is important to mention here that dengue viruses also mediate the increased infection through the antibodies[23].

There are many different methods are being employed to develop good vaccines against dengue viruses, which include the live attenuated vaccines and the new live attenuated vaccines were developed with the use of the infectious clone concept, vaccines using virus and plasmid vectors along with the recombinant subunit technology based[24].

Antiviral Chemical Agents: Till date, there is no any approved therapeutic anti-viral drug available for dengue treatment and also many potential antiviral agents are under trials for the better development. There are some important viral targets for the attack of the drugs. But drug resistance may be a potential hurdle. Hence, more than one drugs at the same time should be developed for the trials as an antiviral chemical agents.

Further, viral targets recognition and locations are necessary for the chemical agents to attack. NS5 viral RNA dependent RNA polymerase, methyl-transferase, NS3 protease and helicase are among the easy viral targets as they are the components of the viral complex which replicates. Viral envelop protein-E is also a good target and some specific antibody (monoclonal) may be a good antiviral agent[25]. *In vivo* and *in vitro* studies have shown that specific antisense morpholino oligomers can inhibits the viral replication[26, 27].

Other methods are also being examined like, chemotherapeutic agents and screening known inhibitors belongs to other viruses, protein crystal structure and using humanized antibodies with nucleic acid based therapy[28].

Some other potential antiviral agents includes, polyoxotungstate, sulphated polysaccharides. Their main actions are to inhibit the adsorption of the virus to the cell surface and cell entry is also retarded and this was shown in a study conducted *in vitro* condition[29]. Ribavirin is used to treat RNA infections by functioning as RNA cap analogue and mutagen. This induces an error in the synthetic route[30].

Neem Based Pesticide Formulations Developed at IPFT: Here at IPFT recently, we are successful in developing many neem based formulations to control the vector (mosquitoes). These includes mosquito repellent coils, creams, suspension concentrate, spreading oil formulation, floating tablets and Bt-WP etc., which are all environmental safe and effective against adult as well as for a varities of mosquitoes larvae.

Conclusion

At this juncture, we can say that there are many effective stretegies to control the epidemy but no one is full proof. Although by using these methods to some extent the reduction in number of dendue infections is reported, but still a lot of developments are required to fully eradicate the infection. Thus, the need of the hour is to develop effective vaccines and chemical therapeutic antiviral agents. Also, if we could well understand the spreading mechanism as well as the

multiplication at molecular level, then also it will be very helpful to design new drugs and vaccines for the treatment of the disease. As far as vaccines are concerned, so till date no one is licensed for the human trials. So, there is a need to combat this disease collectively worldwide by developing the antiviral drugs which only can eradicate this problem or to achieve controlling situations.

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