REVIEW ARTICLE

The Role of Apolipoproteins in Dengue Infection: A Review

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ABSTRACT

The re-emergence of the dengue virus in recent decades has significantly increased with almost 40%-50% of the world's population being at risk. Meanwhile, cholesterol and its components, apolipoproteins, were found to play a vital role in dengue infectivity and the development of severe dengue. This review attempts to address the functional importance of cholesterol and related apolipoproteins in dengue virus pathogenesis and to identify the potential utilisation of this relationship in future diagnosis and management of dengue. The literature search was conducted using a computer-based electronic search on dengue infection with cholesterol and human lipoproteins from September 2017 to June 2019 through three main search engines: MEDLINE (OVID), PubMed, and Science Direct using the keywords including *Flaviviruses*, characteristics of dengue virus, the pathogenesis of dengue, enhancement of dengue, metabolism of cholesterol, cholesterol pathway and human lipoproteins in association with dengue. Dengue virus manipulates lipid raft integrity and utilizes cholesterol components and apolipoproteins for virus internalisation through LDLr and SR-BI receptors. Infectivity of the dengue virus correlated with a decrease in the cholesterol content of the virions. High cholesterol levels in the endoplasmic reticulum promote replication complexes formation of dengue virus. Cholesterol is needed for NS1 secretion which is essential in viral replication, dengue pathogenesis, and host immune evasion. Levels of cholesterol and its related components contributed to the development of severe dengue. The interplay between cholesterol and cellular proteins lead to significant effect in all aspects of the dengue virus replication cycle from viral entry to release.

KEYWORDS: dengue, cholesterol, apolipoproteins

INTRODUCTION

Dengue fever is the most prevalent mosquito-borne illness in humans, mainly spread by two important vectors, *Aedes aegypti, and Aedes albopictus*. It is caused by infection with one of the four antigenically distinct serotypes of DENV (DENV-1 to DENV-4). Dengue virus belongs to the Flaviviridae family, a single-stranded non-segmented RNA virus. In recent decades, the incidence of dengue has increased significantly with estimates of 40%-50% of the world's population being at risk of infection in tropical, subtropical, and more temperate climate areas being affected.[1] Infection with one dengue serotype confers lifelong homotypic immunity to that particular serotype with partial heterotypic immunity to other serotypes for a brief period. Based on the World Health Organization (WHO) guidelines in 2009, the clinical manifestation of dengue is categorised into i) dengue without warning signs, ii) dengue with warning signs and iii) severe dengue.[2] Cholesterol has been found to play a vital role

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in different stages of a virus's life cycle. However, not all enveloped viruses are dependent on the presence of cholesterol in both the viral and cellular membranes to establish the proper infection.

Apolipoproteins are proteins that transport lipids by binding to them and similarly act as enzyme cofactors and cell-surface receptor ligands. In general, apolipoproteins contain a spherical protein with a hydrophobic pocket in the centre containing lipid contents, while the external aspect of the sphere is largely a hydrophilic surface. Apolipoproteins are categorised into six major types namely A, B, C, D, E, and H, and each apolipoprotein has a unique structure and function.

Apolipoprotein A (Apo-A) makes up approximately 90% of the proteins in high-density lipoprotein (HDL). There are sub-classes of Apo-A namely Apo-A1, Apo-A2, Apo-A4, and apolipoprotein A-V (Apo-A5). The levels of different sub-classes of Apo-A are important in controlling heart disease. Apolipoprotein B (Apo-B) is the major protein found in low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL). Increased levels of Apo-B typically mean increased LDL and VLDL levels, which indicate an increased risk of coronary artery disease (CAD). This association has prompted the use of Apo A to Apo B ratios in predicting CAD-related diseases, particularly ischaemic stroke, the ratio being superior to measuring cholesterol levels alone or even HDL/LDL ratios.[3]

Apolipoprotein C (Apo-C) helps control lipid metabolism. Apo-C1 activates lipid lipases and releases fatty acids from chylomicrons to be broken down in the muscles. Inversely, Apo-C3 inhibits lipid lipases, so they work reciprocally to regulate lipid metabolism. Apolipoprotein D (Apo-D) on the other hand, is a part of HDL that differs from other apolipoprotein sequences. Apo-D is involved in the modulation of lecithin: cholesterol acyltransferase activity, an enzyme involved in lipoprotein metabolism.[4] Apolipoprotein E (Apo-E) is a multifunctional protein with central roles in lipid metabolism, neurobiology, and neurodegenerative diseases. It is associated with VLDL, intermediate-density lipoproteins (IDL), chylomicron remnants, and certain HDL. Apo-E plays a crucial role in mediating the clearance and uptake of circulating lipoproteins by serving as the ligand for specific cell-surface receptor attachment, including the LDL receptor family members and heparan sulfate proteoglycans (HSPGs).[5] Apolipoprotein H (Apo-H), previously known as beta-2 glycoprotein I, appears to be involved in the binding of agglutinating and inhibiting agglutination by the contact activation of the intrinsic blood coagulation pathway.[6]

In 2013, Li *et al.* successfully confirmed that Apo-A1 enhanced DENV infection via promoting initial attachment of the virus to cells and siRNA knockdown of the scavenger receptor class B type I (SR-BI), the cell receptor of Apo-A1.[7] This novel finding suggests a significant role of Apo-A1 in bridging DENV particles and host cell receptor, SR-BI, to facilitate entry of DENV into cells.

This review was based on two research questions which were i) Do levels of cholesterol and its related components contribute to severe dengue? and ii) How do cholesterol and its related components cause severe dengue? Thus, in this review, we aim to address the functional importance of cholesterol and related apolipoproteins in causing DENV infection, hence its involvement in DENV pathogenesis. This could assist in identifying the gaps in our current knowledge on DENV infectivity that represents critical challenges in the clinical management of dengue. This review also attempts to amalgamate various research findings on the influence apolipoproteins have on viral entry to further understand how we can utilise these proteins in future diagnosis, assessment, and management of dengue patients.

MATERIAL & METHODS

Data Search

The literature search for this review was conducted in a systematic process, which began in September 2017 and was completed in June 2019, allowing the identification of any research published throughout this study. A computer-based electronic search was performed to access all online dengue infections with cholesterol and human lipoproteins-related articles. Three main search engines were explored via MEDLINE (OVID), PubMed, and Science Direct. These electronic databases professional journals were searched for relevant pieces of literature based on the citations made in key

publications. The online search was carried out by inserting keywords either separately or in combination, that included *i) virology of flaviviruses, ii) characteristics of dengue virus, iii) pathogenesis and enhancement of dengue, iv) metabolism of cholesterol, v) cholesterol pathway, and vi) human lipoproteins in association with dengue.*

Data Extraction

A total of 55 articles were recognised by these three main databases, however, only 34 were included in this review as these articles specifically described the association between dengue, cholesterol, and human lipoproteins. The inclusion criteria included were i) studies describing pathogenesis of dengue, ii) all forms of research or study designs, and iii) original studies evaluating dengue virus infection with involvement of cholesterol and human lipoproteins. The exclusion criteria included were i) articles that were not available in English on the basis that translation will take up more time for the validation process, ii) abstracts or proceedings, iii) review articles, and iv) study protocol.

RESULTS

The pathogenesis of dengue is mainly related to the result of antibody-dependent enhancement (ADE), which involves secondary infections with heterologous serotypes reportedly leading to severe disease.[8] The ADE phenomenon postulates that DENV interacts with non-neutralizing antibodies which assist the viral entry into monocytes and macrophages via Fc receptors.[9,10] The increased uptake of DENV leads to an inappropriate immune reaction and this induces cytokines elevation and complement activation. The affected macrophages increase the release of vasoactive mediators and may also increase vascular permeability, causing vascular leakage, which may then lead to severe dengue (SD). Moreover, disease severity has been associated with elevated cytokines such as TNF-alpha, TNF receptors, soluble IL-2, and soluble CD8 receptors.[11]

Dengue infection has been found to promote the activation of the intrinsic pathway of apoptosis, with increased surface phosphatidylserine exposure, mitochondrial depolarization, and activation of mediators of programmed cell death such as caspases 3 and 9.[12] The virulence hypothesis stated that certain DENV strains can cause severe disease and DENV-2 has been suggested to be more virulent compared to others, in part because the majority of SD epidemics have been associated with DENV-2 compared with other serotypes. The emergence or lineages in the DENV-2 genotype was because of its antigenic diversity due to episodic positive selection on the envelope and non-structural genes during translation.[13] Epidemiological observations suggested that the chain of infection with a different serotype and the time gap between primary and secondary infection may be crucial in the development of SD. These studies reported that the longer the gap between the first infection (primary) and second infection (secondary), the higher the risk of developing SD.[14]

Cholesterol and Lipoproteins Interplay in DEN Viral Entry

The cholesterol pathway appears to be a crucial modulator of the host response to several flavivirus infections including hepatitis C virus (HCV), Japanese encephalitis virus (JEV), West Nile virus, and DENV. It was reported that cholesterol metabolism is essential for flavivirus internalisation and replication with the host's immune response to the virus.[15-17] The researchers successfully evaluated the requirement of cholesterol for DENV entry in Huh-7 cells, whereby the cells were pre-treated with any one of the three lipid rafts disruptors compound, namely Mβcd, nystatin, or filipin. Pre-treated Huh-7 cells exhibit a drop in viral yield, suggesting that the viral entry is cholesterol-dependent, specifically dependent on the integrity of lipid rafts.[18] Furthermore, both lipid rafts integrity and cholesterol were required during viral internalisation in certain cells, such as hepatic Huh-7 cells, monocytes/macrophages, K562, HMEC, N18.[18] However, some studies have reported that cholesterol does not play a role in Vero (green monkey epithelial kidney cell line), HepG2 (Hepatocarcinoma cell line) and ECV304 (human endothelial cell line) cell entry, suggesting that the cholesterol-dependent DENV entry and post-entry steps may not be involved in the general event.[19-21]

Soto-Acosta *et al.*, in 2013 described that the cholesterol levels were elevated in infected mammalian cells that correlated with an increase of surface low-density lipoprotein receptor (LDLr) on infected cells, suggesting that cholesterol is essential during the early stage of infection.[18] Interestingly, Li *et al.* in the same year reported that Apo-A1 interacts with DENV particles and assists viral internalisation through the SR-BI receptor.[7] The crucial role of Apo-A1 in DENV entry is regulated through a complex interaction between SR-BI receptor, HDL, and DENV envelope glycoproteins, highlighting the functional importance of lipoproteins and cholesterol regulation through cholesterol receptors during DENV infection. Manchala *et al.* reported that based on a proteomic analysis, Apo-A1 exists in the lipid -free form in the serum of dengue viral multiple infections, and increment of dengue virus infectivity was observed when the DENV is pre-incubated with the lipid-free form of Apo-A1.[22] These findings implicated DENV having a direct interaction with lipid-free Apo-A1. In 2014, Faustino *et al.* demonstrated that the dengue viral capsid (C protein) binds to very-low-density lipoprotein (VLDL) surfaces and this suggested the formation of lipoviroparticles (LVPs) in DENV infection.[19]

In 2016, another researcher genetically inhibited the cholesterol synthesis using HMGCR (3-Hydroxy-3-Methylglutaryl-CoA Reductase)-siRNA in DENV infected cells to clarify the DENV infection mechanism.[20] It showed that blocking the HMGCR enzyme slightly decreased the viral titre, and this indicates the complexity of DENV internalisation which involves other host proteins and mechanisms. A high concentration of intracellular cholesterol was observed in infected cells in contrast to uninfected cells after 48 hours of infection with DENV-2. However, this observation needed to be further clarified as to whether it was due to the stimulation of cholesterol transport or its biosynthesis.

Role of Cholesterol in DEN Viral Fusion

Flaviviruses are an enveloped virus that needs to be uncoated for the viral proteins to be activated into a fusion-active state and release the viral RNA genome into the cytoplasm by initiating the fusion of the viral envelope with the endosomal membrane.[23] The crucial part is the presence of cholesterol in DENV virions, where Carro and Damonte (2013) observed that depletion of the cholesterol content of the virions following exposure to MbCD led to a decrease in infectivity of all four DENV serotypes.[24] The infectivity of DENV virions was not fully recovered with the addition of foetal bovine serum except when simultaneous MbCD incubation with serum cholesterol was performed. Although it has been shown that the construction of viral membrane formed in the endoplasmic reticulum (ER), further studies need to be conducted to identify the association of cholesterol to the biophysical properties of the viral particle.

Cholesterol Modulation in DEN Viral Replication

Viral RNA is translated into a large polyprotein which is cleaved by viral and/or cellular proteases to form structural and non-structural proteins, following the uncoating and internalization of the virus. The synthesised viral proteins, such as the NS4A protein, initiate the formation of membrane curvatures by inducing ER-membrane remodelling. Due to the high consumption of cholesterol during viral replication, viruses increase the host cell uptake and biosynthesis of cholesterol by directly manipulating the host cell cholesterol transport system. The cholesterol sensor SREBP-SCAP (Sterol regulatory element-binding protein - SREP cleavage activating protein) complex is transported to the Golgi apparatus when the cholesterol level is low in the ER, whereby the cytoplasmic domain of SREBP is cleaved. In the nucleus, the protein is translocated and acts as a transcription factor to induce HMGCR and LDLr gene transcriptions.[25]

It was found that DENV modulates the lipid and cholesterol transport by the expression of viral genes to trigger ER expansion and the formation of replication micro-domains occur in the early stages of the infection.[19] During the early stage of infection, DENV protein expression will up-regulate LDL particle uptake and promote HMGCR activity to elevate the formation of lipid and total cholesterol levels. The upregulation of HMGCR and mevalonate diphosphodecarboxylase (MVD) leads to higher cholesterol levels in the ER which favours the replication of WNV and

DENV.[18,21,26] Alternatively, the regulation of extracellular cholesterol uptake by LDLr is crucial in flavivirus replication.[18] Furthermore, by using drugs or bioactive compound that reduce cholesterol biosynthesis pathway, the modification of ER membranes for virus protection or known as replication complexes (RC) formation can be altered and lead to restrain virus infection.[21,26]

Significance of Cholesterol in Viral Assembly

The assembly phase of the viral particles is accomplished through the RC induced in the ER which contains the high activity of HMGCR and MVD.[18,21,26] The flavivirus assembly involves the initial step of nucleocapsid formation, where multiple copies of capsid (C) protein is inserted with one copy of the viral RNA.[27] The viral C protein is located close to the ER surrounding the structures called lipid droplets (LDs).[28] It has been implied that, during viral infection, the LDs become the accumulation site for the C protein-RNA complex before being mobilised to viral assembly sites.[29] The nucleocapsid will then be covered by a lipid membrane to complete the assembly of the immature virion.[28,30] The viral particle undergoes its maturation phase within the Golgi complex.[31,32]

The Functional Importance of Cholesterol in DEN Viral Release

According to Barrows *et al.*, the release of viral particles by exocytosis is the final step in the flavivirus replicative cycle. The non-structural protein 1 (NS1) is secreted during flavivirus infection.[32] DENV NS1 protein is a lipoprotein particle that is secreted in a form of the hexamer, consisting of an open-barrel protein shell with a central channel rich in lipids.[34] NS1 is involved in vascular homeostasis due to the presence of triglycerides that bound at an equimolar ratio, as well as cholesterol and phospholipid esters, a composition that evokes plasma lipoproteins in humans. Thus, in DENV infection, NS1 plays essential roles in viral replication, dengue pathogenesis, and host immune evasion. This remarkable finding indicates that cholesterol is a necessary component for NS1secretion.

CONCLUSION

The risk factors of developing SD include prior infection with a heterotypic dengue virus and living in tropical and subtropical areas where dengue is predominantly endemic with DENV-2 strain. On the other hand, individuals with premorbid conditions such as CAD, hypertension, diabetes mellitus, and hypercholesterolaemia may also predispose to SD through a certain complex mechanism. In this review, we delved into the role of cholesterol and its related components that may directly or indirectly contribute to SD.

Based on more than 30 selected original articles, it was apparent that levels of cholesterol and its related components including LDL, HDL, VLDL, HMGCR, LDLr, and certain human lipoproteins were highly suggestive in their contribution towards the development of SD. These are evident through findings of the interplay between cholesterol and cellular proteins leading up to significant effects in all aspects of the DENV replication cycle from viral entry to release. Despite this current information, future studies to validate these results and to determine its utility in identifying infected patients at risk of SD as well as considering the possibility of advocating the use of lipid-lowering medication as a means of limiting the disease progression would be considered translational.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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