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EFFECTS OF HUMAN APOLIPOPROTEIN A1, B AND E IN MODULATING THE INTERNALISATION AND REPLICATION OF DENGUE VIRUS SEROTYPES 1 AND 2 IN HUH-7 CELL LINES *IN VITRO*

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ABSTRACT

Dengue virus (DENV) causes a range of diseases, from mild fever to severe dengue, with internalisation into target cells being a critical step in infection. While previous studies have revealed a direct correlation between severe dengue disease and changes in plasma lipid profiles using clinical data, the underlying mechanisms remain unclear. Thus, this study takes a reverse approach by examining the effects of lipid profile modulation *in-vitro* to uncover these mechanisms. Specifically, this study aims to compare DENV internalisation in Huh-7 cells in the presence of apolipoproteins A1 (ApoA1), B (ApoB), and E (ApoE). Tetrazolium reduction assays were used to assess the cytotoxicity of apolipoproteins on Huh-7 cells. DENV-1 or DENV-2 at an M.O.I. of 1, along with 2 μg/mL of apoA1, apoB, or apoE, was introduced into confluent Huh-7 cells and incubated for one hour at 37 °C with 5% CO_2. Unbound virus was then removed. For direct measurement of viral attachment, the cells were lysed, and the cell lysate was collected. For indirect measurement, fresh media was added to the cells, followed by incubation for another 72 hours. The supernatant was collected to measure the virus released from Huh-7 cells. SR-B1 and LDLR knockdown was achieved by transfecting 60% confluent Huh-7 cells with specific siRNA for 48 hours. The indirect measurement experiments were then repeated after siRNA treatment. Collected samples were subjected to qPCR for viral load determination. Differences between groups were analyzed using ANOVA and an independent t-test. DENV2 RNA levels increased with each apolipoprotein treatment compared to controls, with ApoB showing the highest and significant increase ($p=0.031$), while DENV1 viral load showed no significant differences. After 72 hours, DENV2 RNA levels rose significantly in ApoA1- and ApoB-treated cells ($p=0.008$ and $p=0.015$) compared to ApoE, with ApoA1 showing the highest enhancement (101.31%). siRNA knockdown of the scavenger receptor class B type 1 (SR-B1) by 51% reduced the ApoA1-mediated increase in DENV infection by 33% ($p=0.023$). Similarly, a 39% knockdown of LDL receptor (LDLR) decreased ApoA1- and ApoB-enhanced infection by 58.2% ($p=0.014$) and 60.5% ($p=0.002$), respectively. In conclusion, ApoA1 and ApoB significantly enhance DENV internalisation, likely by facilitating initial attachment to cell surface receptors, with SR-B1 and LDLR potentially playing a role in DENV infectivity. These findings contribute to a deeper understanding of how lipid pathways facilitate viral entry and suggest that targeting apolipoprotein interactions and receptor pathways could inform therapeutic strategies against DENV infection.

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TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

1.1 Research Background

Dengue fever is one of the most prevalent mosquito-borne diseases caused by the dengue virus (DENV) and spread through the bite of female mosquitoes of the *Aedes* type, principally *Aedes aegypti* (Heilman et al., 2014). The infection produces symptoms including the sudden onset of high fever, nausea, vomiting, severe headache, muscle and joint pains, and a characteristic skin rash (Heilman et al., 2014). However, in some cases, it can lead to life-threatening complications such as dengue haemorrhagic fever and dengue shock syndrome (Alejandria, 2015; Tuiskunen Bäck & Lundkvist, 2013). Typically, it occurs in tropical and subtropical areas of the world especially in urban and semi-urban areas (Liu et al., 2021). The DENV is a single positive-stranded RNA virus of the family *Flaviviridae*; genus Flavivirus (Tuiskunen Bäck & Lundkvist, 2013). There are four serotypes of DENV (DENV1, DENV2, DENV3, and DENV4), classified based on their antigenic distinctions. Infection with one serotype usually provides lifelong, serotype-specific immunity, while offering only partial immunity to the other serotypes (Holmes & Burch, 2000). Subsequent infection with a different serotype increases the risk of severe complications (Holmes & Burch, 2000).

According to the Centre for Disease Control (CDC), almost 50% of the global population lives in areas at risk for DENV infection (CDC, 2024). According to the World Health Organization (WHO), the global incidence of dengue increases dramatically every year (WHO, 2024). About 67 to 136 million people are infected by dengue each year and approximately 10,000 to 20,000 cases are fatal (Bhatt et al., 2013). It is estimated that 3.9 billion people are at risk of DENV infection in 128 countries (Brady et al., 2012). This includes Africa, America, Eastern Mediterranean, Southeast Asia, and the Western Pacific region (Guo et al., 2017). Malaysia is not an exception with previous data showing an increased incidence of dengue cases in Malaysia since the first major outbreak in 1973 (Wallace et al., 1980). Figure 1.1