



Dimorphism in response of sexes to dengue infection modifying course and outcome of disease

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Abstract:

Introduction: Sexes respond differently to infection, which further is modified by the 'stage of life cycle' they belong to.

Materials and methods: We studied a cohort of patients admitted in our hospital diagnosed with dengue during the year 2014-2015 and found significant dimorphism in response of males and females in different age categories to infection; this appeared to modify the disease process from infection to progression/outcome.

Results: Females in reproductive age category were found to be more inclined to developing uncomplicated dengue (DF). Severe dengue was found to occur in males and females past middle age – males appearing to be more prone to developing complications. Possible reasons based on available knowledge are discussed.

Conclusions: Dimorphism in response to infection in the sexes in different stages of their lifecycle was found to modify disease process from infection to progression and outcome of dengue.

Key words: dengue, dimorphism, infection

Introduction:

Dengue is the most common vector borne disease affecting man. About 40% of the world's population is living in areas endemic for the disease, with ~ 390 million established cases, more than 100 million infections occurring every year - 1 in 2000, resulting in death.¹

The disease may range from a self limited febrile illness to classical Dengue Fever (DF) with high pyrexia and severe joint pain or may proceed to 'Severe Dengue', with thrombocytopenia and vascular leakage- Dengue Hemorrhagic Fever (DHF) and circulatory collapse- Dengue Shock Syndrome (DSS).^{2,3}

Dengue virus (DENV) is a flavivirus belonging to family *Togaviridae*. It is a 50nm enveloped virus with an icosahedral capsid in T4 symmetry, enclosing a 11kbp single stranded positive sense RNA genome, coding for envelope proteins (E and M/ PreM) and non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5)⁴. There are four serotypes of the virus, DEN-1, DEN-2, DEN-3 and DEN-4,

with ~ 60-65% genomic sharing between them. The latter is responsible for serological cross reactions between the serotypes.^{5,6}

The antibodies may even 'cross protect' during initial period, until class switch occurs from IgM to IgG, which whereas confers lifelong immunity against homologous type, can only bind, but are unable to neutralize heterologous serotypes. This forms the basis of 'Antibody Dependent Enhancement' (ADE) leading to DHS/DSS, during secondary infection with a different strain.^{7,8}

Aedes aegypti is the primary vector of DENV. *Aedes albopictus*, albeit its wider distribution and better survival, has only a secondary role to the former- a matter of vector compliance, than compatibility per se.⁹ Humans and mosquitoes are only known hosts for the virus. Humans thus serve as host and the reservoir for the virus. The mosquito remains infected for life and may sustain the virus through trans-ovarian transmission (TOT).¹⁰

Sex and Gender influencing DENV infection:

Males and females respond differently to DENV infection which further is modified by the stage of life cycle they belong.

A. Gender roles predisposing man/ woman to DENV infection are mainly related to exposure to bite of infected vector.

B. Biological differences in sexes belonging to different age groups, influencing response to DENV infection are mainly hormonal or immunological. Differences in lipid disposition of cell membrane may also be contributory.

1. Sex hormones exert their effect via specific receptors expressed by immunocytes (in our context)^{11,12,13}. Secretion of hormones and distribution of receptors on cells differ between sexes and further fluctuates with stages of life cycle of the host.

The effect of estrogen is 'dose' dependent and differ with cell type involved.^{11,12,13}

At serum levels found in males and females in luteal phase or past menopause, it induces production of IFN- γ by CD4+ Th1 lymphocytes, promoting a 'pro inflammatory' Th1 type response. It has however the opposite effect on macrophages.^{11,13}

At high levels as in pregnancy and pre-ovulatory periods in women, estrogen promotes an anti-inflammatory, Th2 type response^{11,12,13} with prolific production of antibodies. It promotes early class switch from IgM to IgG while enhancing clonal expansion of TREG cells. Apoptosis is inhibited.^{11,12,13}

Progesterone is anti-inflammatory,^{11,12} with high levels during pregnancy and luteal phase of menstrual cycle. Estrogen and progesterone levels fall with advancing age, dropping low during perimenopause.

Androgens promote Th1 type immune response with production of pro-inflammatory cytokines viz. IL-2, IFN- γ and CTL activity^{11,12}. Androgen secretion

also wanes with age, dropping steeply after 50-60 years.

2. Immune Response: Genetic disposition and hormonal/cytokine milieu in males (and females post-menopause) favor polarization of T helper cells to Th1 type, with secretion of pro-inflammatory cytokines (viz. IFN- γ , IL-2 and lymphotoxin).

In females on the other hand, response to infection is of Th2 type, with prolific production of antibodies, while apoptosis and phagocytosis are inhibited.^{11,12,14}

Viruses have evolved ways to hijack natural pathways sub-serving physiological functions of cells to gain entry into them^{15,16,17,18}. DENV 'attaches' to C-type lectins viz. DC-SIGN (in dendritic cells) or its analogue L-SIGN (in lymph nodes/ liver)^{19,20,21,22}, CLEC5²³ and Mannose Receptors (in macrophages/ MDDCs)²⁴, that recognize mannose rich N-glycans in envelopes of 'mosquito derived' virions that initiate infection. This triggers a signal cascade that lead to production of pro-inflammatory cytokines of Th1 type with enhanced CTL activity and tissue damage.

DC derived virions (with poorly mannosylated complex glycans on their envelopes) may have other routes of entry viz. Heparan sulfate, heat shock proteins or apoptotic pathways— even direct transfection.^{15,16,17,18}

A major mode of cell infection by DENV, (both mosquito and DC derived) is via Fc gamma receptors (FcR gamma), virions being carried in as immune complexes bound to non-neutralizing antibodies, mainly involving MDDCs and macrophages of M2b type. The immune response generated is of Th2 type. This forms basis of ADE (Antibody Dependent Enhancement) seen in 'secondary' infection with virions belonging to a heterologous serotype.^{7,8} It could happen in 'primary' infection as well— antibodies formed against immature 'PreM' virions

may be 'non-neutralizing' and over 30% of virions from the mosquito are of 'PreM' phenotype.

Understandably, Fc receptor mediated infection may occur more readily in females, who are prolific producers of antibody and in whom IgM to IgG class switch happens early.

3. Lipids in DENV entry:

Cholesterol in plasma membrane is required for fusion and release by budding and/or in direct (C- dependent) genomic translocations described, of DENV. Level of cholesterol modulates the curvature and plasticity of membranes. 'Protection' of cholesterol is required for DENV in the low pH of late endosome, during 'fusion'. Differences between sexes in lipid metabolism, pertaining to expression of LDL receptors on the cells in particular, could translate to differences in 'permissiveness' between male and female cells.^{25,26,27,28,29,30,31.}

Aims and Objectives:

Aim of our study was to analyze sex based dimorphism in host response to DENV infection in different age groups, influencing incidence and progression of the disease.

Objectives: To generate data to find how the sexes responded to infection in different stages of life cycle, and how they fared during disease- DF and severe dengue, in a cohort of patients admitted in our hospital during year 2014-2015.

Materials and Methods:

This was hospital based, prospective study over a period of one year-2014-2015, with patients diagnosed with dengue, treated as in-patients in Pariyaram Medical College hospital. Informed consent from subjects and institutional clearance were obtained.

One thousand two hundred and fourteen patients, (belonging to both sexes in all age groups), had attended 'fever clinics' at Pariyaram Medical College (Academy of Medical Sciences- ACME) in 2014. Those

who gave history or presented with signs and symptoms clinically suggestive of dengue (378) were screened by rapid card test and 157(41.5%) were found positive for Dengue NS1 and/or IgM. One hundred and ten of them (110) who were found requiring hospitalization formed subjects of our study. (Out patients were not included in this study). Half of them (23 males, 32 females) had uncomplicated DF (on clinical grounds); The other half (31 males and 24 females) were admitted with or developed DHS (with hemorrhagic signs) while in hospital; 2 males and 4 females went into shock (DSS). We lost two of them (one male and one female).

Rapid chromatographic Card test, manufactured by J.Mitra and Co.Pvt. Ltd, New Delhi, to detect NS1 antigen, IgM and IgG antibodies to dengue, was used for screening, following prescribed test protocol.

IgM Capture ELISA for DENV antibody was done, using test kits manufactured by Panbio Ltd, Brisbane, Australia. The antigen used was a mix of E protein from all serotypes of DENV. Gac ELISA kits made by J.Mitra and Co.Ltd., Delhi, (with the same antigen mix) were used to find IgG titres.

Tests were performed as per manufacturer's instructions. Samples giving more than cut off of '11 units' (OD>0. 4) was taken as POSITIVE, in both tests.

Analysis was done with descriptive statistics like frequencies, proportions and percentages and inferential statistics namely Chi square test using Epi Info version 7. A 'p' value of less than 0.05 was taken to be significant.

Results:

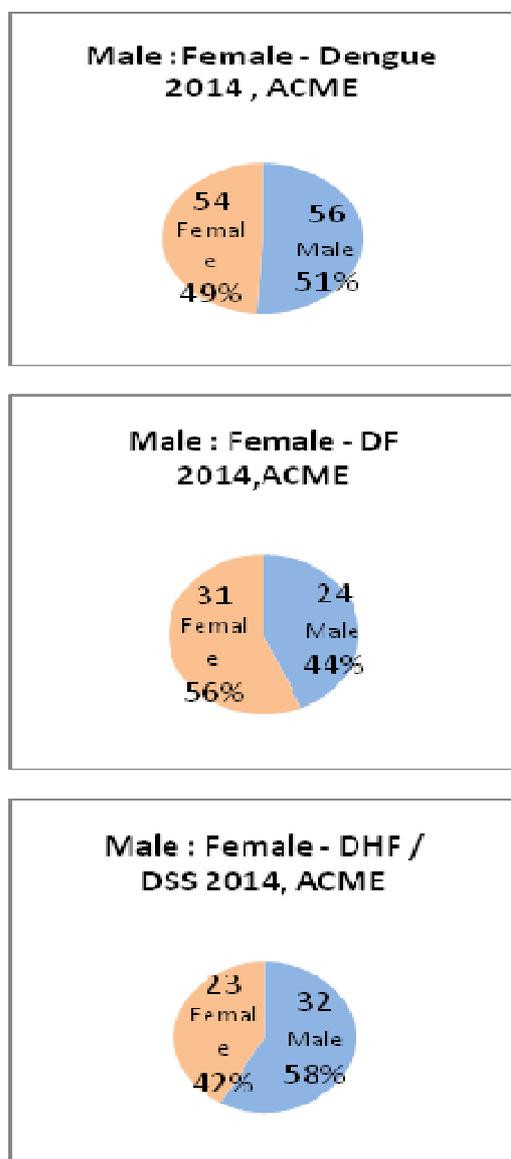
All 155 patients selected for this study, were found positive for NS1 Ag and/or IgM screening. Fifty six belonged to males

and 54 were of females. Male: female ratio was 1.03:1.

IgM capture ELISA for dengue was performed on sera of all those admitted and all 155 were found positive (OD>0.4) (except 2 giving equivocal values) – i.e. indicating 'primary infection'.

O.D. values given by IgM and IgG were compared to assess risk of ADE. 40 out of 55 cases who developed severe dengue gave a IgM/ IgG ratio below cut off 1.4 compared to 28 out of 55, in the uncomplicated DF category. The difference is significant; Chi square value being 5.54, p= 0.01.

Figure I: IgM positive males: females



Distribution of seropositive males and females categorized as DF (on clinical grounds) was in ratio 23:32 (1:1.39). It was 31:24 (1:0.77) in the group that developed signs and symptoms of DHS/DSS. **(Figure I)**

Females predominated in the uncomplicated DF category and males with majority among those who developed severe dengue. Females thus seemed to be more susceptible to infection with DENV but males seemed more prone to developing complications of dengue.

We analyzed the distribution of seropositive males and females separately, in different age groups they belonged, in both categories of the disease, which gave results as follows:

1. Dimorphism in 'stages of life cycle':

Significant variation was found in male / female distribution in the different age groups. IgM positive males and females were about equally distributed in the extreme age groups in both categories. **(Figure IIa and IIb)**

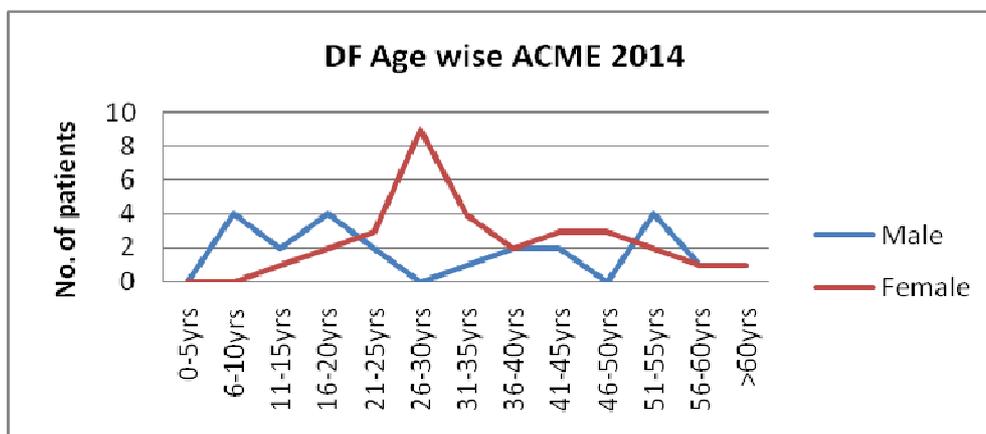
Uncomplicated Dengue: Boys appeared to be more susceptible to DENV infection in the pediatric age group, girls catching up by pubertal age. Thereafter, the graph showed a clear preponderance of females with dengue fever, the trend continuing all through young adulthood (reproductive period in females) with peak between 20 - 30 years and waning towards peri-menopause. Males and females seemed equally affected in old (post menopausal) age. The difference in proportion of males and females in the pre-menopausal, reproductive and peri-menopausal categories in DF is significant, Chi square value being 38.49, 'p' < less than 0.0001.

Severe Dengue:

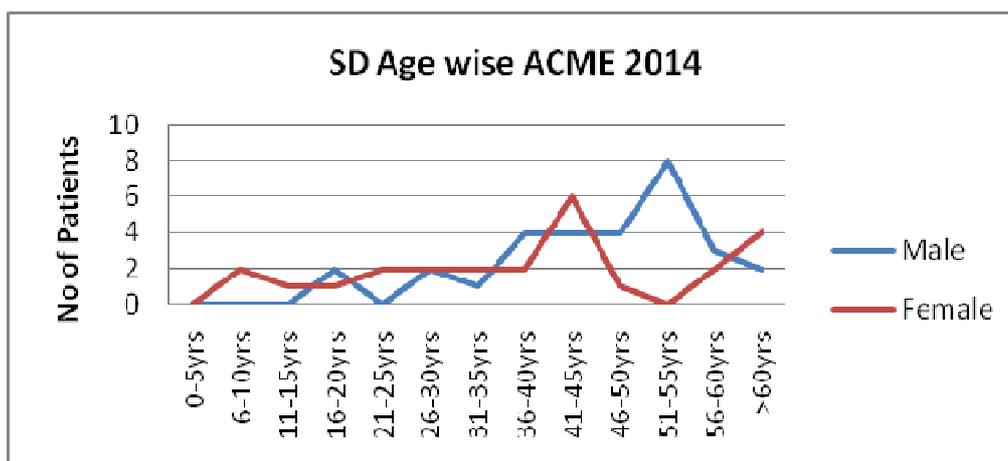
Overall, more males than females developed severe dengue. Male: Female ratio being 1: 0.77. The difference was more in age groups above 30 years when most of such cases had occurred.

Figure IIa and IIb: Distribution of seropositive males & females, and their respective proportions, in the different age groups

a) Those diagnosed as DF



b) Those who developed DHF/DSS

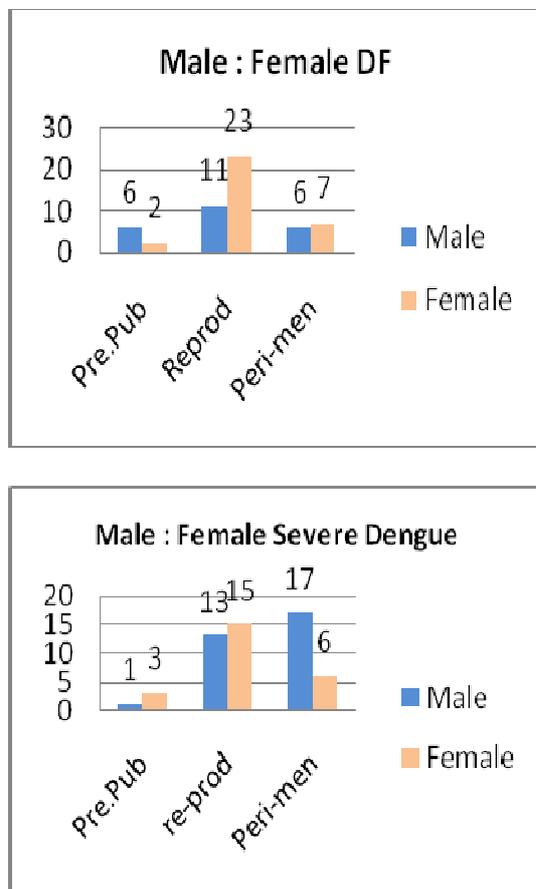


More girls developed severe dengue in pediatric age group. After that males and females were found about equally represented in the graph, till about 30 years, though in numbers, much less than in the older age group. Most of the severe disease occurred in patients above 40 years. There seemed to be a surge in number of SD, both male and female during 'early middle age' (30-35 years), with number of females dwindling through 'late middle age' (45-55yrs.) during which time, most of the male cases had occurred. Males and females seemed about equally affected after 60 years.

The difference in proportion of males and females in the pre-menopausal, reproductive and peri-menopausal categories in severe dengue is significant, Chi square value being 61.62, 'p' < 0.0001.

The same, analysed in different 'stages of life cycle'- pre-pubertal, adult/reproductive and old/post menopausal age groups, the findings were as shown in **Figure IIc and IId**.

Figure IIc & IId: Proportions of IgM+ve males and females in different stages of 'life cycle'



Discussion:

The study population consisted of 110 patients with DENV infection, near equally represented by the sexes (M:F=1.03:1).

Uncomplicated Dengue: Males seemed more susceptible to DENV infection in the pediatric age group. This could be due to inherent difference in immunity and girls having an advantage over boys in mounting a robust humoral response with neutralizing antibodies clearing the virus before they developed symptoms.

Females in reproductive age groups produce high levels of estrogen and favor polarization of CD4+T- helper cells to Th2 type, with prolific secretion of antibodies. Estrogens again promote hypermutation

and early 'class switch' of these antibodies from IgM to IgG. IgG so formed recognizes (repeating) epitopes (which are carbohydrates) by 'linear sequences', present, rather than 'stereochemical conformation', and may thus be more cross reactive. More than 30% of mosquito derived virions are of immature PreM phenotype which may give rise to 'non-neutralizing antibodies. All these factors add on to inherent disposition of females to mount Th2 type response to infections. FcR mediated infection occurs more readily in females which make them more susceptible to dengue during reproductive age period. The trend wanes with hormone levels dropping towards menopause. Males and females seemed equally affected in old (post-menopausal) age.

Severe Dengue:

Males and post-menopausal females respond to infection with a predominantly Th1 type of response, with release of pro-inflammatory cytokines like IFN- γ , IL-2, lymphotoxin and enhanced CTL mediated apoptosis and phagocytic killing are possible reasons why complications of the disease are more frequent in older age groups.

The surge in number of severe dengue in females during late middle age may be due to dwindling serum levels of estrogens. Estrogen has 'pro-inflammatory' effect in low concentration. They switch to mounting Th1 type response like men after menopause.

The sample size is too low in pediatric age group to make a meaningful comment – which incidentally is not in agreement with several reports- especially from 'endemic areas', that dengue is more common in children. There is a possibility still, that it was a novel serotype of virus that was circulating in our area of study; if so, the paucity of cases in pediatric age group may be looked upon as a cue,

pointing to importance of antibody mediated mechanism of viral cell entry in which maturity of immune system of host mattered.

Notably, the few cases of SD in female children were found clustered in ages between 10-15yrs. It may be due to early puberty or can be explained on grounds of anatomical vulnerability of female vasculature to 'leakage'.

In the reproductive age period, the slight preponderance of females over males probably was a reflection of higher number of women contracting the disease as mentioned above; this however was balanced by the fact that they responded to infection in a Th2 manner, with secretion of anti-inflammatory cytokines, recruitment of TREG cells and inhibition of CTL and phagocytic killing- reasons why most of them did not develop severe disease.

Conclusion:

Dimorphism in response to infection in the sexes in different stages of their life cycle was found to modify disease process, from infection to progression and outcome of dengue. Males and post-menopausal females who mounted a Th1 type response were found more prone to developing severe manifestations (DHF/DSS). The effect of estrogen, which is 'pro-inflammatory' in low concentrations, was observed in females with waning levels of the hormone, through middle age. Females in reproductive age period were found to be more inclined to contracting dengue fever, highlighting the importance of an antibody mediated mechanism in DENV infection.

The study emphasizes the need for segregating the sexes in separate categories based on stages of lifecycle they belong, during surveys/ studies collecting data to plan strategies for disease control – a W.H.O. guideline, which is often neglected³².

References:

- Centers for Disease Control and Prevention - Division of Vector-Borne Diseases [updated 2016 April 2]; Available from: <https://www.cdc.gov/ncezid/dvbd/>
- Bhatt,S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature* 2013; 496(7446): 504-7; <https://doi.org/10.1038/nature12060>
- World Health Organization. Dengue and severe dengue [updated 2017 April]; available from: <http://www.who.int/mediacentre/factsheets/fs117/en/>
- Vaughan K, Greenbaum J, Blythe M, Peters B, and Sette A. Meta-analysis of all immune epitope data in the *Flavivirus* genus: inventory of current immune epitope data status in the context of virus immunity and immunopathology. *Viral Immunol* 2010; 23(3):259-84; <https://doi.org/10.1089/vim.2010.0006>
- Cecilia D. Current status of dengue and chikungunya in India WHO South-East Asia Journal of Public Health 2014; 3 (1): 22-27; <https://doi.org/10.4103/224-3151.115828>
- Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JG, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *Am J Trop Med Hyg* 2015; 92(5): 999-1005; <https://doi.org/10.4269/ajtmh.14-0628>
- Flipse J, Diosa-Toro MA, Hoornweg TE, van de Pol DPI, Urcuqui-Inchima S, Smit JM. Antibody-dependent enhancement of dengue virus infection in Primary Human Macrophages; Balancing Higher Fusion against Antiviral Responses. *Sci Rep* 2016; 6: 29201; <https://doi.org/10.1038/srep29201> .
- Boonnak K, Slike BM, Burgess TH, Mason RM, Wu SJ, Sun P, et al. Role of Dendritic Cells in Antibody-Dependent Enhancement of Dengue Virus Infection. *J Virol* 2008; 82(8): 3939-51; <https://doi.org/10.1128/JVI.02484-07>
- Carrington LB, Simmons CP. Human to Mosquito Transmission of Dengue Viruses *Front Immunol* 2014; 5:290; <https://doi.org/10.3389/fimmu.2014.00290>

10. Mourya DT, Yadav P. Vector biology of dengue & chikungunya viruses. *Indian J Med Res* 2006;124: 475-80.
11. Fairweather DL, Frisancho-Kiss S, Rose NR. Sex Differences in Autoimmune Disease from a Pathological Perspective. *Am J Pathol* 2008; 173(3): 600–609; <https://doi.org/10.2353/ajpath.2008.071008>.
12. Oliver JE, Silman AJ. Why are women predisposed to autoimmune rheumatic diseases? *Arthritis Res Ther* 2009; 11(5): 252; <https://doi.org/10.1186/ar2825>
13. Lang TJ. Estrogen as an immunomodulator. *Clin Immunol* 2004; 113(3):224-30; <https://doi.org/10.1016/j.clim.2004.05.011>
14. Spellberg B, Edwards JE Jr. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis* 2001; 32(1):76-102; <https://doi.org/10.1086/317537>
15. De La Guardia C and Leonart R. Progress in the Identification of Dengue Virus Entry/Fusion Inhibitors *BioMed Research International*, Volume 2014, article ID 825039, 13 pages, (2014); <https://doi.org/10.1155/2014/825039>
16. Cruz-Oliveira C, Freire JM, Conceição TM, Higa LM, Castanho MA, Da Poian AT. Receptors and routes of dengue virus entry into the host cells. *FEMS Microbiol Rev* 2015; 39(2):155-70; <https://doi.org/10.1093/femsre/fuu004>
17. Alen MMF and Schols D. Dengue Virus Entry as Target for Antiviral Therapy. *Journal of Tropical Medicine*, Volume 2012, article ID 628475,13 pages, 2012; <https://doi.org/10.1155/2012/628475>
18. Acosta EG, Talarico LB, Damonte EB. Cell entry of dengue virus. *Future Virology* 2008; 3(5); <https://doi.org/10.2217/17460794.3.5.471>
19. Watterson D, Kobe B, Young PR. Residues in domain III of the dengue virus envelope glycoprotein involved in cell-surface glycosaminoglycan binding. *J Gen Virol* 2012; 93(Pt 1):72-82; <https://doi.org/10.1099/vir.0.037317-0>
20. Navarro-Sánchez E, Desprès P, Cedillo-Barrón L. Innate immune response to dengue virus. *Arch Med Res* 2005;36(5): 425-35; <https://doi.org/10.1016/j.arcmed.2005.04.007>.
21. Guabiraba R and Ryffel B. Dengue virus infection: current concepts in immune mechanisms and lessons from murine models *Immunology* 2014; 141(2): 143-56; <https://doi.org/10.1111/imm.12188>
22. Navarro-Sanchez E, Altmeyer R, Amara A, Schwartz O, Fieschi F, Virelizier JL, et al. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep* 2003; 4(7):723-8; <https://doi.org/10.1038/sj.embor.embor866>
23. Chen ST, Lin YL, Huang MT, Wu MF, Cheng SC, Lei HY, et al. CLEC5A is critical for dengue-virus-induced lethal disease. *Nature* 2008; 453(7195):672-6; <https://doi.org/10.1038/nature07013>
24. Miller JL, deWet BJM, Martinez-Pomares L, Radcliffe CM, Dwek RA, Rudd PM, et al. The Mannose Receptor Mediates Dengue Virus Infection of Macrophages. *PLoS Pathog* 2008; 4(2): e17; <https://doi.org/10.1371/journal.ppat.0040017>
25. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997; 387(6633): 569-72; <https://doi.org/10.1038/42408>
26. Subtil A, Gaidarov I, Kobylarz K, Michael A, Lampson MA, Keen JH and McGraw TE. Acute cholesterol depletion inhibits clathrin-coated pit budding *Proc Natl Acad Sci USA* 1999; 96: 6775-6780.
27. Lee CJ, Lin HR, Liao CL, and Lin YL. Cholesterol effectively blocks entry of Flavivirus. *J Virol* 2008; 82 (13): 6470-6480; <https://doi.org/10.1128/JVI.00117-08>
28. Rothwell C, Lebreton A, Young Ng C, Lim JY, Liu W, Vasudevan S, et al. Cholesterol biosynthesis modulation regulates dengue viral replication. *Virology* 2009; 389 (1-2): 8-19; <https://doi.org/10.1016/j.virol.2009.03.025>
29. Soto-Acosta R, Mosso C, Cervantes-Salazar M, Guardo HP, Medina F, Favari L, et al. The increase in cholesterol levels at early stages after dengue virus infection correlates with an augment in LDL particle uptake and HMG-CoA reductase activity *Virology* 2013; 442(2):132-47; <https://doi.org/10.1016/j.virol.2013.04.003>
30. Lindenbach BD, Rice CM. The ins and outs of hepatitis C virus entry and assembly *Nat Rev Microbiol* 2013;11(10): 688-700; <https://doi.org/10.1038/nrmicro3098>

31. Jin Ye. Reliance of Host Cholesterol Metabolic Pathways for the Life Cycle of Hepatitis C Virus. PLOS pathog 2007 3(8):e108;

<https://doi.org/10.1371/journal.ppat.0030108>

32. Martha A. Addressing sex and gender in epidemic-prone infectious diseases. WHO

Publication 2008 by Departments of Gender, Women and Health, and Epidemic and Pandemic Alert and Response, W.H.O.; http://www.who.int/iris/handle/10665/43644/9789241595346_eng.pdf

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