IJPSR (2022), Volume 13, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 June 2021; received in revised form, 22 July 2021; accepted, 23 July 2021; published 01 March 2022

ASSOCIATION OF LEVELS OF VITAMIN-D AND MANNOSE-BINDING LECTIN IN CASES OF DENGUE FEVER IN RURAL INDIA

V. Yadav [1], R. K. Verma [1], D. P. Singh [1], R. Rawat [2] and S. Saraswat [*1]

Department of Microbiology [1], Department of Medicine [2], Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah - 206130, Uttar Pradesh, India.

Keywords:

DENV, IgM ELISA, MBL, NS1 ELISA, Vitamin-D

Correspondence to Author:
Dr. Satender Saraswat

Junior Resident,
Department of Microbiology,
Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah - 206130, Uttar Pradesh, India.

E-mail: satt90@gmail.com

ABSTRACT: Background: Dengue is an arthropod-borne viral disease caused by the dengue virus (DENV). DENV is an RNA virus that belongs to the Flavivirus genus. The incidence of dengue has grown in the recent decade globally. The World Health Organization (WHO) considers dengue as a major global public health challenge in the tropic and sub-tropic nations. **Materials and Methods:** NS1 antigen and IgM antibody ELISA were done, and the serum levels of Vitamin-D and Mannose Binding Lectins (MBL) were determined to find out their association with the severity of Dengue, *i.e.*, Dengue fever (DF) / Dengue Haemorrhagic Fever status (DHF). **Results:** The male-to-female ratio was 1.35:1 with the most commonly affected age group of 11-20 years (31.3%). NS1 antigen ELISA alone was positive in 12 (15%) samples, IgM ELISA alone was positive in 20 (25%) samples, while NS1 and IgM ELISA both were positive in 48 (60%) samples. 64 (80%) and 16 (20%) cases were DF and DHF, respectively. **Conclusion:** Thrombocytopenia is significantly notable in DHF patients as compared to DF patients. Both NS1 and IgM ELISA are needed to effectively diagnose dengue cases. The association of low levels of MBL with DF and DHF might be related to the reduced activation of the MBL pathway of complement, leading to a higher viral load in dengue cases.

INTRODUCTION: Dengue is an arthropod-borne viral disease caused by the Dengue virus (DENV). DENV is an RNA virus belonging to the genus Flavivirus. DENV changes genetically during natural transmission and significant biological differences have been seen between strains of the same serotype. It is a debilitating condition with high fever and break-bone pain. Infection can result in Dengue fever (DF), Dengue Hemorrhagic Fever (DHF) status, or dengue Shock Syndrome (DSS). Infection with any of the serotypes produces only partial immunity to other serotypes [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(3).1259-66

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(3).1259-66

The World Health Organization (WHO) renders dengue as a major public health challenge globally, particularly in tropical and sub-tropical nations. Dengue has grown several folds worldwide, aided by the increased population growth rate, unplanned urbanization, global warming, frequent air travel, inefficient mosquito control, and lack of health care

facilities. The incidence of dengue has grown in the recent decade globally. An eightfold rise has been observed in the number of cases reported to WHO over the last two decades, from 505,430 cases in the year 2000, to over 2.4 million in the year 2010 and 5.2 million in the year 2019 [2]. According to National Vector Borne Disease Control Programme, in 2020 (till September 30), the total cases of dengue detected were 16439 out of which 12 died, and in Uttar Pradesh, a total of 212 cases of were detected, of which 1 died [3]. High incidence, ranging between 21 and 50 per million, was reported for the states of Punjab, Gujarat, Karnataka, Kerala, Tamil Nadu, and Orissa [4]. Several hypotheses have been proposed explaining the reasons for DHF.

These include changes of viral virulence, genetic susceptibility, cytokine storm, variation of lipid profile, and immunological enhancement [5]. Although the relationship between neutralizing antibodies and antigen-specific T cells and disease severity and clinical outcomes remains to be understood, high levels of neutralizing antibodies have been observed in convalescent individuals, [6] which correlate with T cell responses, particularly those of CD4+ T cells [7]. During disease progression, infected cells produce inflammatory cytokines such as TNF-α, IL-6, and IL-10 [8]. AS a result of this stimulation, endothelial cells increase the expression of adhesion molecules such as CD62E (E-Selectin), CD106 (VCAM-1), and CD62P (P-Selectin), which leads to local inflammation, endothelial damage, and plasma leakage [9].

An increase or decrease in the levels of these immune-modulators influence the outcome of viral infections [10]. Vitamin-D, an immunomodulator with the potential to affect both innate as well as adaptive immune responses, binds to its receptor (VDR), translocates to the nucleus, and influences gene expression. Vitamin-D concentration is directly proportional to increased expression of Fcγ receptors leading to higher viral load, uncontrolled inflammatory responses, and subsequent development of DHF in DENV infected cases with secondary infection. The presence of Vitamin-D during macrophage proliferation restricts DENV infection and alters the pro-inflammatory cytokine response by reducing the expression of the C-type lectin mannose receptor, a DENV receptor protein [11]. Vitamin D modulates the immune responses to several pathogens, including DENV [12, 15]. Epidemiological studies have shown that Vitamin D decreases the risk of DHF and may have a role in the management of dengue fever [16]. Elucidating the role of Vitamin-D on severe dengue disease would constitute a critical step in determining the therapeutic potential of this nutrient in patients diagnosed with DF, DHF, and DSS [17, 18]. One of the major mechanisms of activation of the lectin pathway of complement is initiated by binding of the virus to Mannose Binding Lectin (MBL) [19]. MBL is a pattern recognition molecule that concedes particular sugar molecules present on the surface of microorganisms, including DENV.

The binding of E and NS-1 proteins with MBL can activate the complement system resulting in lysis of DENV [20, 21]. An association of low MBL with an increased risk of dengue severity has also been established [22]. Altered plasma concentrations of Vitamin-D and MBL are associated with the pathogenesis of DENV [18]. Since MBL and Vitamin-D affect the innate as well as adaptive immunity and the pathogenesis of DENV infection is immune-mediated, we tested the altered levels of serum Vitamin-D and MBL suspecting their association with dengue disease severity. Therefore, the levels of serum Vitamin-D and MBL in dengue-infected patients were investigated in the context of disease severity. This study was aimed at determining the serum levels of Vitamin-D and MBL in dengue patients and to find out their association with severity (DF/DHF status).

MATERIALS AND METHODS: This was a hospital-based cross-sectional study conducted at the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah, Uttar Pradesh between November 2018 and June 2020. The study was approved by the Institutional Ethics Committee (Clearance Code: 146/2018). The patients with clinical suspicion of DF or DHF consulting in any of the Outpatient or Inpatient Department (s) at the study site during the study period were screened for Dengue by the NS1 antigen ELISA and IgM ELISA. The cases positive for NS1 antigen, or IgM antibodies with IgM antibody capture (MAC)

ELISA, or both, were contacted and explained about the study. Prior informed consent was taken. Suspected cases that turned out negative for dengue on serology and those who withdrew consent during any stage of the study were excluded. Along with patient-specific data using preformed and pretested questionnaire, blood samples (3-4 ml) were collected under aseptic precautions from the participants. The samples (serum or plasma) were separated by centrifugation for immediate serological analysis, and the remaining samples were stored at -20 °C till further processing. NS1 antigen ELISA was performed by Merilisa™ (manufactured by Meril Diagnostics Pvt Ltd, Gujarat) Dengue NS1 kit as per the manufacturer's protocol. Dengue virus IgM MICROLISA (manufactured by J. Mitra and Co. Pvt. Ltd. Okhla, New Delhi) kit was used for IgM antibody detection as per the manufacturer's instructions. EDITM Total 25-OH Vitamin-D quantitative ELISA Kit (manufactured by Epitope Diagnostics Inc. San Diego, CA 92130 USA) was used for the detection of total 25-OH Vitamin-D levels. IBM SPSS Statistics Version 20 was used for data analysis.

RESULTS: In this study, we enrolled 80 cases out of which 46 (57.5%) were males, and 34 (42.5%) were females. The male-to-female ratio was 1.35:1. Among the enrolled cases, the most commonly affected age group was 11-20 years (31.3%), followed by 21-30 years (26.2%) and 31-40 years (15%) age groups as seen in **Table 1.** Males were predominant in all age groups except for 41-50 and >60 years age groups, in which females were predominant. The mean age of study subjects was 29.73 ± 15.11 years. Among 80 samples, NS1 antigen ELISA alone was positive in 12 (15%) samples, IgM ELISA alone was positive in 20 (25%) samples while NS1 and IgM ELISA both were positive in 48 (60%) samples as seen in **Table 2.**

TABLE 1: AGE AND GENDER-WISE DISTRIBUTION OF STUDY CASES

Age group	Males (n=46)		Females (n=34)		Total (n=80)	
	Number	Percentage	Number	Percentage	Number	Percentage
0-10 years	5	10.9	3	8.8	8	10.0
11-20 years	15	32.6	10	29.4	25	31.3
21-30 years	13	28.3	8	23.5	21	26.2
31-40 years	7	15.2	5	14.7	12	15.0
41-50 years	2	4.3	3	8.8	5	6.2
51-60 years	3	6.5	1	2.9	4	5.0
>60 years	1	2.2	4	11.8	5	6.3

TABLE 2: DIAGNOSIS OF DENGUE INFECTION IN STUDY CASES (N=80)

Diagnosis of Dengue infection	Number	Percentage
Dengue NS1 Only	12	15.0
Dengue IgM ELISA positive only	20	25.0
Both NS1+ IgM ELISA positive	48	60.0

According to the WHO [2], out of 80 dengue confirmed cases, 64 (80%) and 16 (20%) cases were classified as DF and DHF, respectively. In the DF group, 36 (56.2%) cases were males, while 28 (43.8%) were females. In the DHF group, 10 (62.5%) cases were males, and 6 (37.5%) cases were females. In our study, fever was present in all cases of DF as well as DHF groups; headache in 39 (60.9%) cases of DF group and 10 (62.5%) of DHF group; chills in 32 (50%) and 9 (56.2%) cases; abdominal pain in 29 (45.3%) and 7 (43.8%) cases; retro-orbital pain in 27 (42.2%) and 4 (25%) cases; and body-ache was more frequent in DHF group (50%) as compared to DF group (35.9%), as seen in **Table 3.** Other major features were vomiting, rashes, and pruritus in declining order. However, hepatomegaly, diarrhea, and dyspnoea were rare findings. Purpura and mucosal bleeding with the distribution of 81.2% and 87.5%, respectively ($p<0.001$), were associated with DHF cases, exclusively. Value < 20 ng / ml was taken as the cut-off level for Vitamin-D deficiency and > 80 ng/ml for hypervitaminosis D [23].

The cut-off level of MBL deficiency was taken at < 500 ng/ml [18]. The association of levels of Vitamin-D, MBL, and platelet count in the DF and DHF groups has been demonstrated in **Table 4,** revealing a significant association of Vitamin-D ($p=0.01$) and platelet count ($p<0.001$) with the severity of the disease. However, No association was found in the case of MBL levels with the disease severity.

TABLE 3: CLINICAL SIGNS AND SYMPTOMS IN STUDY SUBJECTS

Clinical symptoms	DF Group (n=64)		DHF Group (n=16)		p value
	No.	%	No.	%	
Fever	64	100.0	16	100.0	-
Chills	32	50.0	9	56.2	0.65
Headache	39	60.9	10	62.5	0.91
Retro-orbital pain	27	42.2	4	25.0	0.26
Abdominal pain	29	45.3	7	43.8	0.91
Body-ache	23	35.9	8	50.0	0.30
Joint pain	7	10.9	0	0.0	0.33
Vomiting	22	34.4	4	25.0	0.56
Ascites	2	3.1	0	0.0	1.0
Pruritus	11	17.2	1	6.2	0.44
Rashes	16	25.0	3	18.8	0.75
Dyspnoea	3	4.7	0	0.0	1.0
Hepatomegaly	6	9.4	0	0.0	0.34
diarrhea	4	6.2	0	0.0	0.58
Purpura	0	0.0	13	81.2	<0.001
Mucosal bleeding	0	0.0	14	87.5	<0.001

TABLE 4: ASSOCIATION OF LEVELS OF VITAMIN-D AND MBL WITH SEVERITY OF DENGUE CASES

Median (IQR) level	DF Group (n=64)	DHF Group (n=16)	p value
Vitamin-D (ng/ml)	30.5 (22.25-43.5)	49.5 (33.25-60)	0.01
MBL (ng/ml)	107 (93.25-126.5)	122.5 (100.75-130.75)	0.10
Platelet count (/µL)	38000 (28,500-71,750)	14,000 (11,250-18000)	<0.001

Platelet Count In DF and DHF Groups: Platelet counts of all study cases were according to their severity status *i.e.*, DF and DHF and DHF (mean = 14,000) patients were found to be more thrombocytopenic than DF (mean = 38,000) patients as illustrated in **Table 5.**

Association of Vitamin-D Levels With Dengue Severity: In the DF group, 10 (15.6%) cases had a deficit of Vitamin-D, while 54 (84.4%) cases had a normal level of Vitamin-D and no case was found to have high levels of Vitamin-D. In the DHF group, 1 case (6.2%) was a deficit of Vitamin-D while 9 (56.2%) cases had normal levels and 6 (37.5%) cases had high levels of Vitamin-D. This indicates that higher levels of Vitamin-D are significantly associated DHF ($p<0.05$) **Table 5.**

TABLE 5: ASSOCIATION OF VITAMIN-D LEVELS WITH SEVERITY OF DENGUE CASES

Vitamin-D level (ng/ml)	DF Group (n=64)		DHF Group (n=16)		p value
	Number	Percentage	Number	Percentage	
Low	10	15.6	1	6.3	
Normal	54	84.4	9	56.2	
High	0	0.0	6	37.5	<0.05

Association of MBL Levels With Dengue Severity: In the DF group, 61 (95.3%) cases were deficit of MBL levels, and only 3 (4.7%) cases had normal MBL levels. On the other hand, all 16 (100%) cases in the DHF group were found to be a deficit of MBL. Thus, MBL is not associated with DHF cases **Table 6.**

TABLE 6: ASSOCIATION OF MBL LEVEL WITH SEVERITY OF DISEASE

MBL (ng/ml)	DF Group (n=64)		DHF Group (n=16)		p value
	Number	Percentage	Number	Percentage	
Low	61	95.3	16	100.0	>0.05
Normal	3	4.7	0	0.0	

DISCUSSION: 80 seropositive cases of dengue were included in the study. Among these cases, 46 (57.5%) were male, and 34 (42.5%) were female. The male dominancy could be related to the rural location of the study site. Determination of differences in infection rates across all genders is important for the correct implementation of public health programs. Studies by Murhekar M *et al.*, [24]

and Damodar T *et al*, [25] also reported higher male incidence. Kumar M *et al.*, [26] in their study on the prevalence of dengue fever in western Uttar Pradesh, found that among infected dengue cases, the proportion of males was higher than females with the ratio of (M:F) being 1.54:1. Similar results were found in our study subjects. This could be attributed to gender-related differences in health-seeking behavior. Awareness generation in general and specific interventions targeting those with lower education status can further improve the health-seeking behavior and reduce complications of dengue [27].

As the role of gender and exposure changes overtime during the human lifespan, it becomes important to examine dengue cases according to gender as well as age. The study by Murhekar M *et al*, [24] reported the highest distribution in the age group of 10-19 years (31.8%), followed by 20-29 years (31.2%). However, in our study, we found the age group of 11-20 years (31.3%) to be most affected, followed by the age group of 21-30 years (26.2%). Our results were similar to the former study.

Jayarajah *et al*., [28] reported fever in all their cases, with symptoms, such as headache 55%, body aches 52.5%, vomiting 43.1%, abdominal pain 21.6%, nausea 21.3%, and diarrhea 15.4%. In our study, fever was the most common presenting clinical feature (100%), followed by headache (60.9%), chills (50%) and abdominal pain (45.3%). Retro orbital pain is considered an important symptom in the diagnosis of dengue. The study by Anish Laul *et al*., [29] on clinical profiles of dengue infection during an Outbreak in Northern India reported that 41% of dengue patients have retro-orbital pain. It was present in 42.2% of cases in our study and was reported in 56.27% of patients in the study by Lim JK *et al* [30]. Myalgia/arthralgia was found in 36% cases, while nausea-vomiting in 34.4% of our study but was about 74.92% and 71.5% in the other two studies [30, 31], respectively. Patients with dengue syndrome showed varied presentations. It seems that the trend of clinical presentation is changing each year in dengue patients. Teoh BT *et al*., [32] in their study, found that only ELISA NS1 antigen was positive in 61.7% patients, while ELISA IgM was positive in 73% cases. Another study [33] showed that NS1 antigen positive rates were 88%-96% on days 1-5, 75%-100% on days 6-10, and 36-60% on ≥ 11days. A study [34] stated positive detection rates of NS1 antigen ELISA, IgM ELISA, and both tests as 80.9%, 68.1% and 47.9%, respectively. Higher positivity by IgM ELISA in our study may be explained by variable timing of sample collection along the disease course.

However, a fair number of patients were found positive by NS1 ELISA or IgM ELISA alone in our study, effective diagnosis of dengue requires testing by both. Panwala TH et al., reported higher positivity by IgM ELISA as compared to NS1 ELISA and also concluded that NS1 ELISA, if used in combination with IgM ELISA on a single serum sample of suspected patients, can improve the diagnosis of acute dengue cases and even treatment and control of dengue viral infection [35]. Our study compared the positivity of NS1 antigen and IgM Antibody by ELISA and found that positivity was slightly lower by NS1 ELISA (75%) than by IgM ELISA (85%). On the other hand, 60% of samples were positive for both NS1 and IgM ELISA. It is a contrasting finding as most studies have reported higher positivity by NS1 ELISA.

According to WHO criteria [36], out of a total of 80 cases, 60 (80%) and 16 (20%) cases were classified as DF and DHF, respectively. The determination of the outcome of DENV infection is based on several factors, including virulence, virus, host genetics, and host immune responses. Among the various components of host immune responses, T cells, antibodies, cytokine storm, and complement factors contribute to the pathogenesis of dengue, while various immunomodulators influence their activation [37]. An increase or decrease in the levels of these immunomodulators can influence the outcome of viral infections [5]. In this study, 87.4% of dengue cases had normal to high levels of Vitamin-D (normal range 20-80 ng/mL). Also, Vitamin-D level was significantly higher in the DHF group than that of the DF group ($p<0.05$). The increased concentration of Vitamin-D might enhance viral entry in dengue virus-infected cases [10]. Our findings were supported by various studies in different parts of the world. Alagarasu K *et al*., [18] suggested that higher concentrations of Vitamin-D might be associated with symptomatic disease and assessed that this association was more evident in

secondary DHF cases. Villamor E *et al.,* showed that low serum concentrations of 25-hydroxyVitamin-D in DF patients predicted decreased oddsof progression to DHF/DSS [38]. Several protein levels get altered in patients with severe DF.

One of the proteins that show a significant increase in DF is Vitamin-D binding protein (DBP). Because DBP is the major plasma carrier of Vitamin-D, its increase is invariably associated with higher levels of vitamin-D [38]. Infection with DENV results in activation of various components of the innate immune system. One of them is the lectin pathway of the complement system. MBL belongs to a class of molecules called Pattern Recognition Receptor (PRR) proteins that can recognize E protein and NS-1 protein of DENV [39]. MBL levels were estimated, and deficiency was found in 96.3% dengue cases (cut-off level for MBL deficiency was assumed to be < 500 ng/mL according to international standards [18]. Comparison of MBL levels in DF and DHF cases revealed that MBL level was lower in the DHF group (100%) than that of the DF group (95.3%).

Similar findings were reported by Alagarasu *et al.,* [18], who noticed the association of MBL deficiency with DF and DHF, and this association might be related to the reduced activation of the MBL pathway of the complement system, leading to higher viral load in dengue cases with primary dengue infection. Figueiredo GG *et al.,* [40] supported the hypothesis that patients carrying the genotypes or haplotypes of low production of MBL would be more susceptible to DF/ DHF. Shresta S *et al*., [41] noticed that a depressed level of MBL protein may be an independent risk factor for morbidity and mortality associated with DENV infection. A higher MBL concentration might also lead to increased inflammation by enhanced production of pro-inflammatory cytokines. Increased concentration of factor-D and decreased concentration of factor-H have been reported in DHF cases, suggesting that imbalance in the regulation of factors H and D of the alternative pathway of complement activation is associated with DHF [42]. Elucidating the role of serum Vitamin-D and MBL levels on the severity of dengue disease can be a critical first step to interrogate the potential of these tests to identify dengue patients who can progress to severe disease (DF/DSS) and require hospitalization and critical care.

CONCLUSION: In this study, we found that the ratio of male: female was 1.35: 1 in dengue cases. Younger age groups (11-20 yrs) were relatively more affected. Clinically, fever, headache, chills, retro-orbital pain, abdominal pain, and arthralgia/ myalgia were common symptoms. Purpura (81.2%) and mucosal bleeding (87.5%) were associated with DHF cases exclusively (100% association with DHF, $p<0.001$). Thrombocytopenia was significantly notable in DHF patients as compared to DF patients. Both NS1 and IgM ELISA are needed to diagnose dengue cases effectively.

The ratio of DF: DHF was 4:1. High Vitamin-D levels are significantly associated with DHF, which may be related to the inducing effect of Vitamin-D on Fcγ receptor expression, leading to higher viral load in dengue cases and hence the development of DHF. This study also suggests that low levels of MBL are associated with dengue infection (both DF and DHF). Low levels of MBL had a higher association with DHF cases than DF, though statistically insignificant. The association of low levels of MBL with DF and DHF might be related to the reduced activation of the MBL pathway of complement, leading to a higher viral load in dengue cases.

Limitations: Smaller sample size and exclusion of IgG ELISA have restricted the potential of this study, more so, given the ongoing SARS CoV-2 pandemic. By performing the IgG ELISA test, we could have differentiated between trends in primary and secondary dengue cases, additionally.

ACKNOWLEDGMENT: The authors would like to acknowledge the contribution of all the faculty and staff in aiding at every step of this study. We also appreciate the study participants for their contribution to the study.

Contribution of Authors: Concept and design of the study - Dr. R. K. Verma Data acquisition and processing - Dr. Vikas Yadav Data analysis and interpretation - Dr. Ramakant Rawat Drafting the article - Dr. Satender Saraswat; Critical review - Dr. D. P. Singh.

Funding: No financial support was sought from any individual or organization for this study.

CONFLICTS OF INTEREST: None

REFERENCES:

1. Monto AS, Pefyffer GE and Veronica V: The epidemiology of viral infections topley wilson's microbiology and microbial infections. 10th Edition Hodder Arnold 2007; p5624-5.
2. World Health Organization [Internet]. Dengue and severe dengue: fact sheet. WHO; 2018. Available from: May 19, 2021. Accessed - July 23, 2021.https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
3. National Vector Borne Disease Control Programme [Internet]; Dengue Cases and Deaths in the Country since 2010 to May. Accessed -July23, 2021. Available from: https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715
4. Mutheneni SR, Morse AP, Caminade C and Upadhyayula SM; Dengue burden in India: recent trends and importance of climatic parameters. Emerging Microbes & Infections 2017; 6: 70-80.
5. Wang WH, Urbina AN, Chang MR, Assavalapsakul W, Lu PL, Chen YH and Wang SF: Dengue hemorrhagic fever – A systemic literature review of current perspectives on pathogenesis, prevention and control. Journal of Microbiology Immunology and Infection 2020; 53(6): 963-78.
6. Ni L: Detection of sars-cov-2-specific humoral and cellular immunity in covid-19 convalescent individuals. Immunity 2020; 52: 971-77.
7. Grifoni A: Targets of T cell responses to SARS-CoV-2 corona virus in humans with COVID-19 disease and unexposed individuals. Cell 2020; 181: 1489-01.
8. Elmy Saniathi NK, Djoko Rianto BU Juffrie M and Soetjiningsih S: Dengue hemorrhagic fever: the role of soluble e-selektin, soluble intra cellular adhesion molecule-1 (sicam-1) and soluble vascular cellular adhesion molecule -1 (svcam-1) in overweight children. Bali Med J 2018; 8: 183.
9. Vitoria WO, Thomé LS, Kanashiro-Galo L, De Carvalho LV, Penny R, Santos WLC, Vasconcelos PFDC, Sotto MN, Duarte MIS and Quaresma JAS: Up regulation of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in renal tissue in severe dengue in humans: Effects on endothelial activation/dysfunction. Rev Soc Bras Med Trop 2019; 52: e20180353.
10. Beard AJ, Bearden A and Striker R: Vitamin D and the antiviral state. J Clin Virology 2011; 50: 194-200.
11. ArboledaAlzate JF, Rodenhuis-Zybert IA, Hernandez JC, Smit JM and Urcuqui-Inchima S: Human macrophages differentiated in the presence of vitamin D3 restrict dengue virus infection and innate responses by down regulating mannose receptor expression. PLoS Negl Trop Dis 2017; 11: e0005904.
12. Kumar R, Rathi H, Haq A, Wimalawansa SJ and Sharma A: Putative roles of vitamin D in modulating immune response and immunopathology associated with COVID-19. Virus Res 2021; 292: 198235.
13. Jantan I, Ahmad W and Bukhari SNA: Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front Plant Sci 2015; 6: 655.
14. Giraldo DM, Cardona A and Urcuqui-Inchima S: High-dose of vitamin D supplement is associated with reduced susceptibility of monocyte-derived macrophages to dengue virus infection and pro-inflammatory cytokine production: An exploratory study. Clin Chim Acta 2018; 478: 140-51.
15. Jadhav NJ, Gokhale S, Seervi M, Patil PS and Alagarasu K: Immunomodulatory effect of 1, 25 dihydroxy vitamin D3 on the expression of RNA sensing pattern recognition receptor genes and cytokine response in dengue virus infected U937-DC-SIGN cells and THP-1 macrophages. Int Immunopharmacol 2018; 62: 237-43.
16. Zaman S, Mahmud MR, Khalid MA, Zahid A, Khalid S, Kabir I, Manzoor S and Baqai HZ: Effectiveness of vitamin D in prevention of dengue haemorrhagic fever and dengue shock syndrome. J Rawalpindi Med Coll 2017; 21: 205-07.
17. Langerman SD and Ververs M: Micronutrient Supplementation and Clinical Outcomes in Patients with Dengue Fever. Am J Trop Med Hyg 2021; 104(1): 45-51.
18. Alagarasu K, Bachal RV, Bhagat AB, Shah PS and Dayaraj C: Elevated levels of vitamin D and deficiency of mannose binding lectin in dengue hemorrhagic fever. Virol J 2012; 9: 86.
19. Giang NT, Van Tong H and Quyet D: Complement protein levels and MBL2 polymorphisms are associated with dengue and disease severity. Sci Rep 2020; 10: 14923.
20. Takahashi K and Ezekowitz RAB: The role of mannose Binding Lectin in innate immunity. Clinical Infectious Diseases 2005; 41: 440-4.
21. Fuchs A, Lin TY, Beasley DW, Stover CM, Schwaeble WJ, Pierson TC and Diamond MS: Direct complement restriction of flavivirus infection requires glycan recognition by mannose- binding lectin. Cell Host Microbe 2010; 8: 186-95.
22. Ornelas AMM: Association between MBL2 haplotypes and dengue severity in children from Rio de janeiro brazil mem. Inst Oswaldo Cruz 2019; 114: e190004.
23. Hernando VU: Vitamin D nutritional status in the adult population in Colombia-An analytical cross-sectional study. Heliyon 2020; 6(2): e03479.
24. Murhekar M, Joshua V, Kanagasabai K, Shete V, Ravi M and Ramachandran R: Epidemiology of dengue fever in india, based on laboratory surveillance data. International Journal of Infectious Diseases 2019; 84: S10-4.
25. Damodar T, Dias M, Mani R, Shilpa KA, Anand AM and Ravi V: Clinical and laboratory profile of dengue viral infections in and around Mangalore. Indian J Med Microbiol 2017; 35: 256-61.
26. Kumar M, Verma RK, Nirjhar S and Singh M: Dengue in children and young adults, a cross-sectional study from the western part of Uttar Pradesh. J Family Med Prim Care 2020; 9: 293-7.
27. Sujatha C, Sudha RR and Krishnankutty SL: Health seeking in people later diagnosed as having dengue fever during the epidemic of 2018 in kerala, India. J Evid Based Med Health 2021; 8(1):1-5.
28. Jayarajah U, Madarasinghe M, Hapugoda D, Dissanayake U, Perera L and Kannangara V: Clinical and biochemical characteristics of dengue infections in children from sri lanka. Glob Pediatr Health 2020; 7: 2333794X20974207.
29. Laul A, Laul P, Merugumala V, Pathak R, Miglani U and Saxena P: Clinical profiles of dengue infection during an outbreak in northern india. J Trop Med 2016; 5917934.
30. Lim JK, Matendechero SH, Alexander N, Lee JS, Lee KS and Namkung S: Clinical and epidemiologic characteristics associated with dengue fever in mombasa, kenya. Int J Infect Dis 2020; 100: 207-15.
31. Rafi A, Mousumi AN, Ahmed R, Chowdhury RH, Wadood A and Hossain G: Dengue epidemic in a non-

endemic zone of Bangladesh: Clinical and laboratory profiles of patients. PLoS Negl Trop Dis 2020; 14(10): e0008567.
32. Teoh BT, Sam SS and Tan KK: The use of ns1 rapid diagnostic test and qrt-pcr to complement igm elisa for improved dengue diagnosis from single specimen. Sci Rep 2016; 6: 27663.
33. Maheshwari V, Kaore NM, Ramnani VK and Sarda S: A Comparative evaluation of different diagnostic modalities in the diagnosis of typhoid fever using a composite reference standard: a tertiary hospital based study in central India. JCDR 2016; 10: 1-4.
34. Anand AM, Sistla S, Dhodapkar R, Hamide A, Biswal N, Srinivasan B: Evaluation of NS1 antigen detection for early diagnosis of dengue in a tertiary hospital in southern India. J Clin Diagn Res 2016; 10(4): DC01-4.
35. Panwala TH and Mulla SA: Evaluation of two diagnostic methods for dengue virus infection and its correlation with thrombocytopenia. Int J Health Allied Sci 2016; 5: 88-92.
36. World Health Organization. Geneva, Switzerland: WHO; Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control 2009.
37. Martina BE, Koraka P and Osterhaus AD: Dengue virus pathogenesis: an integrated view. Clinical Microbiology Reviews 2009; 22(4): 564-81.
38. Villamor E, Villar L, Loxano A, Herrera V and Herran O: Vitamin D serostatus and dengue fever progression to dengue hemorrhagic fever/dengue shock syndrome. Epidemiology and Infection 2017; 145(14): 2961-70.
39. Kraivong R, Punyadee N, Liszewski MK, Atkinson JP and Avirutnan P: Dengue and the Lectin pathway of the Complement System. Viruses 2021; 13: 1219.
40. Figueiredo GG, Cezar RD, Freire NM, Teixeira VG, Baptista P and Cordeiro M: Mannose-binding lectin gene (MBL2) polymorphisms related to the mannose-binding lectin low levels are associated to dengue disease severity. Hum Immunol 2016; 77(7): 571-5
41. Shresta S: Role of complement in dengue virus infection: protection or pathogenesis. M Bio 2012; 3(1): e00003-12.
42. Nascimento EJM, Silva AM, Cordeiro MT, Brito CA, Gil LHVG, Brago-Neto U and Marques ETA: Alternative complement pathway deregulation is correlated with dengue severity. PLoS ONE 2009; 4(8): e6782.

How to cite this article:
Yadav V, Verma RK, Singh DP, Rawat R and Saraswat S: Association of levels of vitamin-d and mannose binding lectin in cases of dengue fever in rural India. Int J Pharm Sci & Res 2022; 13(3): 1259-66. doi: 10.13040/IJPSR.0975-8232.13(3).1259-66.