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Assessment of Interleukin-10 and NS 1 as Markers of Disease Progression in Dengue at a Tertiary Care Hospital in Western Uttar Pradesh

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Abstract

Introduction

Dengue is a viral illness, major cause of morbidity and mortality worldwide, transmitted by *Aedes* mosquito. Disease clinically present as Dengue fever (DF), Dengue Hemorrhagic fever (DHF) and Dengue shock Syndrome (DSS). Early detection of severity of the disease is helpful in managing the disease.

Objective

Aim of our study was to detect the plasma interleukin-10 (IL-10) levels and NS1 (Pan Bio Units) in DF, DHF, and DSS. We are also detecting the co-relation of plasma IL-10 levels and NS1 with dengue disease severity.

Methods

A total of 80 registered Dengue cases of all age groups were taken. Plasma IL-10 levels and NS1 were measured using ELISA. Patients were also categorized based on severity.

Results

Plasma IL-10 levels were raised in DHF patients and associated with fatal outcomes. The IL-10 (pg/ml), NS1 (Panbio units) with DF/DHF using Receiver Operating Characteristic curve (RoC), in this IL-10 pg/ml with DF/DHF shows the area of the curve is 0.886, p- value= 0.0005<0.01 with 95% C.I 0.792 to 0.981, which is highly statistically significant, with a cut-off of 61.05%, sensitivity of 80.0%, and specificity of 80.0%, whereas in NS1 Panbio units with DF/DHF, the area of the curve is 0.461, p- value= 0.602>0.05 with 95% C.I 0.325 to 0.597, which is not statistical significant with a cut-off of 55.77%, sensitivity of 50.0%, and specificity 48.3%.

Conclusion

Raised plasma IL-10 levels are potential predictors of disease severity and fatal outcome in DHF patients, whereas NS1, has no association with disease progression.

Keywords: IL-10, NS 1,dengue hemorrhagic fever

Introduction

Dengue is a febrile illness, transmitted by *Aedes* mosquito. Around 390 million infections occur per year increasing the global burden on healthcare facilities [1].

Clinically dengue virus infection (DENV) ranges from asymptomatic infection to self-limited dengue fever (DF) with less severe to fatal cases. The most common symptoms are fever, headache, chills, fatigue, nausea, and joint pain. In some patients, the infection may result in severe dengue, which can be life-threatening and is characterized by vascular leak causing shock, internal hemorrhage, and organ dysfunction even can cause death.

This virus has been classified into four different serotypes DENV-1, DENV-2, DENV-3, and DENV-4 [2]. When one of the four viral serotypes infects a person, that particular serotype alone provides immunity that protects against re-infection, whereas further infections with other serotypes result in severe dengue through an antibody-dependent enhancement (ADE) that triggers a cytokine storm, which suggests that infection with DENV doesn't give lifelong immunity and a person can be infected with the same virus again [3]. The predominant immune response to viruses after DENV infection is mediated by the innate immune system, which causes the production of inflammatory and antiviral molecules. An exacerbated host immune response characterized by antibody-dependent enhancement has a substantial impact on the progression of severe dengue [4].

All of these hypotheses support the theory that the cytokine storm in ADE is caused by the pathophysiology of soluble inflammatory mediators. Pro-inflammatory cytokines including TNF, IFN, and others are produced in excess in individuals with dengue infection, which leads to increased vascular leakage and poor organ perfusion [5,6].

There is no specific treatment for dengue virus infection. Sanofi Pasteur's Dengvaxia®, is a dengue vaccine that was recently developed (WHO, 2017), has created new hope for lowering the disease burden. The vaccine is likely to be available soon [7].

In this study, we wanted to identify cytokines associated with varied degrees of dengue infection severity in order to find those that might serve as indicators of severe clinical illness.

We found raised levels of IL-10 along with NS1 raised in patients with DHF when compared to dengue fever (DF). Few researches have shown that IL-10 could be used as a potential predictor of severe dengue [8]. More recent studies have investigated serial changes in cytokine patterns in patients with acute dengue infection. In our study where all patients had primary dengue, IL-10 levels were higher in patients with DHF when compared to those with DF. Some other longitudinal studies have also shown that serum IL-10 levels increased in dengue patients with warning signs, but IL-10 levels fell in those with no warning signs [9].

We also measured levels of NS1 and evaluated its association with disease severity. DENV NS1 is a 48-kDa glycoprotein that is highly conserved among all flaviviruses [10]. NS1 is essential for viral replication with an unknown mechanism that possibly involves interactions with NS4A and NS4B [11]. NS1 antigen is a non-structural protein of the dengue virus. The structural proteins, and RNA genome of the dengue virus encode 7 nonstructural proteins that are essential for viral replication (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [12]. DENV NS1 antigen is detected in the blood circulation as early as viral RNA. Thus, its detection is useful for early dengue diagnosis and could be used as an easy, fast and feasible alternative to RT-PCR [13].

Methods

Study Population

From January 2021 to October 2022, 80 subjects presented at this Hospital, were enrolled in the study. The maximum numbers of cases enrolled in our study were from rural backgrounds. In accordance with WHO criteria from 2009, patients with bleeding, vascular system injury, fever, nausea, joint pain, disorientation, shock like state, rash, drop in blood pressure, and amplification of primary symptoms were included in the study.[14,15] Blood samples were taken and immediately transported to the lab in a cool box in simple vacutainers so that serum samples could be separated. Serum samples were used for cytokine assay in ELISA. The samples were aliquoted and were stored at −80 ◦C for further use. Ethical Committee approval was obtained from Institute Ethical Review Committee. After receiving written informed consent, blood samples were taken.

Detection of Dengue Virus

Detection of the Dengue NS1 antigen and IgM on serum samples were done using Panbio®Dengue IgM Capture ELISA kit (01PE20, 96 well formats, Panbio, MA, USA) according to manufacturer's instructions. Qualitative detection of IgM antibodies to dengue antigens for recent infection in serum samples was performed using J. Mitra & Co. Pvt. Ltd, (New Delhi, India) according to the manufacturer's instructions.

Quantitative Estimation of IL-10

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We used Diaclone Human IL-10 ELISA kit™ (Manufactured by Diaclone SAS France). It is an ELISA-based kit for the in-vitro qualitative and quantitative determination of IL-10. It is done according to the manufacturer's instructions.

Statistical analysis

The collected data were analyzed with IBM SPSS Statistics for Windows, Version 23.0.(Armonk, NY: IBM Corp). Data descriptive statistics like frequency analysis, percentage analysis was used for categorical variables, and the mean & S.D were used for continuous variables. To find the significant difference between the bivariate samples in independent groups the independent sample t-test was used. To Assess the relationship between the variables, Pearson's Correlation was used. To find the efficacy of the IL-10 & NS1 to predict the DHF the Receiver Operating Characteristics curve (ROC) was used with Sensitivity, Specificity, and cut-off. In all the above statistical tools the probability value of 0.05 was considered a significant level.

Result

This study included a total of 80 dengue-positive patients who attended medical OPD or were hospitalized in various wards. The diagnosis of dengue infection was based on the presence of dengue NS1 antigen or dengue IgM antibody by ELISA.

The age distribution was,7.5% below<10 years,32.5% between 11-20 yrs, 27.5% between 21-30 yrs, 8.8% between 31-40 yrs, and also between 41-50 yrs,10.0%, between 51-60 yrs and 5.0% in more than 60 years of age.

The gender distribution shows 58.8% of males affected compared to 41.3% of females. The IgM ELISA was positive in 85.0% while negative in 15% of the cases. It was observed that all cases had fever (100.0%) in contrary to ascites which was the lowest (1.3%). DHF was reported in 25.0% and DF in 75.0% of the total number of cases.

We compared disease severity (DF/DHF) with IL-10 (pg/ml) and NS1 (Panbio units) by using Receiver Operating Characteristic curve (RoC).

Table 1: Comparison of IL-10 pg/ml between DF/DHF by Independent sample t-test

Variable	DF/DHF	N	Mean	SD	t-value	p-value
IL-10 pg/ml	DHF	20	224.14	148.31	8.226	0.0005 **
	DH	60	40.33	53.08		
** Highly Statistical Significance at p < 0.01 level						

"Table 1 : Our finding shows that the association of IL-10 (pg/ml) with DF/DHF the area of the curve is 0.886, p- value= 0.0005<0.01 with 95% C.I 0.792 to 0.981, which is highly statistical significance with a cutoff is 61.05%, sensitivity is 80.0%, and specificity 80.0%."

Table 2: Comparison of NS1 Panbio units between DF/DHF by Independent sample t-test

Variable	DF/DHF	N	Mean	SD	t-value	p-value
NS1 Panbio units	DHF	20	37.17	28.18	0.422	0.674 #
	DH	60	40.17	27.33		
# No Statistical Significance at p > 0.05 level						

"Table 2 : In case of Association of NS1 with DF/DHF shows the area of the curve is 0.461, p- value= 0.602>0.05 with 95% C.I 0.325 to 0.597, which is no statistical significance with a cutoff of 55.77%, sensitivity of 50.0%, and specificity 48.3%.

The level of IL-10 between DF/DHF by Independent sample t-test were t-value=8.226, p-value=0.0005<0.01 which shows highly statistical significant difference at p < 0.01 level. The NS1 Panbio units between groups by Independent sample t-test were t-value=0.422, p-value=0.674>0.05 which shows no statistical significance difference at p > 0.05 level. The IL-10 pg/ml with NS1 Panbio units

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shows positive correlation were r-value=0.144, p-value=0.201>0.05 which shows no statistical significance at p > 0.05 level."

Discussion

In our study, among all cases 47 (58.8%) were male and 33 (42.5%) were female. More or less similar findings were reported in study by Kumar M *et al*., (2020) [16]. The clinical profile of dengue showed that fever was the most common presenting symptom (100%), Chills (61.30%), headache (57.5%), abdominal pain (47.5%) and retro-orbital pain (43.8%) were the next common symptoms after fever.

Using ELISA, we compared the positivity of the NS1 antigen and IgM. We found that IgM ELISA positivity (85%) was marginally lower than NS1 ELISA (100%). However, In both NS1 and IgM ELISA results were positive in 85% of samples.

We also classified dengue cases according to their severity i.e. whether they belonged to DF, DHF/ DSS (Dengue Shock Syndrome) group according to WHO criteria [17]. Out of the 80 cases, 60 (75%) were classified in DF and 20 (25%) were classified in DHF. No case of DSS was reported. Anish Laul *et al*, (2016) in their study on clinical profile of dengue in Delhi-NCR region noticed similar findings [18]. However, among cases enrolled in our study, none could be classified as DSS according to the WHO criteria [19].

The result of DENV infection depends upon various factors such as viral virulence, host genetics, and host immune responses. Components such as, host immune responses, T cells, antibodies, cytokine storm, and complement factors cause the pathogenesis of dengue [20].

T cells, antibodies, and cytokines are activated due to several immunomodulators. The outcome of viral infections depend upon the fact whether the level of these immunomodulators has increased or decreased [21].

IL-10 has multiple role on inflammation and immunoregulation. It may be involved in the pathogenesis of DENV, suggesting an immunosuppressive activity that results in IFN resistance, followed by altered immune clearance and a sustained infectious effect for acute viral infection [22].

In our study, we estimated the levels of IL-10 in sera of dengue positive patients. As compared with DF patients, DHF patients are found with higher levels of IL-10. The prevalence of IL-10 values is depicted in patient population. One-fourth of the dengue positive patients had very high levels of IL-10 level. Interleukin-10 values and clinical features were examined using Pearson's Chi Square Test and its Significance was also calculated. A significant association (p=0.0005) was located between the IL categorization and the clinical features i.e. patients with Severe Dengue or Dengue with danger signs had considerably higher number of IL-10 levels.

Alike Saishruti *et al*., 2019; in this study IL-10 was obtained using ELISA and results were interpreted, Interleukin-10 values and clinical features were analyzed. There was a significant association (p=0.025) between the IL categorization and severity [23].

These findings were similar with those made by Pandey *et al.* in 2015, who reported that levels of IL10 were markedly raised when melena was present. In DHF patients compared to DF patients, high serum levels of anti-inflammatory cytokines such IL-10 were also seen, demonstrating an improved regulatory response to the more intensive immune activation in DHF. Using Pearson's Chi square test, the correlation between IL-10 and clinical characteristics was assessed and revealed a substantial association between the two [24].

In addition, studies conducted in the past by Gathsaurie Neelika Malavige *et al.*, 2012, demonstrates a correlation between the degree of infection and the amount of plasma leakage as measured by the pleural effusion index. Therefore, by inhibiting the T cell proliferative response to mitogens, which happens in dengue patients in the early stages of infection, IL-10 may result in lymphocyte dysfunction [25].

We estimated the levels (Panbio units) of NS1 in sera of all study cases from all NS1 positive patients of all age groups. The current study, evaluates the usefulness of NS1 levels as a marker of severe clinical disease. Out of 80 samples, 58.75% showed

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results above 40 panbio units; rest all (41.25%) below 40 Panbio units.

In statistical analysis, NS1 Panbio units with DF/DHF show the area of the curve is 0.461, p-value= 0.602>0.05 with 95% C.I 0.325 to 0.597, which is statistically not significant with the cutoff 55.77%, sensitivity 50.0%, and specificity 48.3% respectively. A similar multi-country study that Guzman *et al.*, 2010 conducted did not reveal any correlation between the detection of NS1 and the severity of the disease, which supports our study [26].

A study done by Sriram P *et. al.*,2016 shows although the NS1 Ag assay is an effective early diagnostic tool for dengue fever, it cannot be used to predict a severe dengue infection in its early stages. We cannot use the Dengue NS1 positive antigen test for hospital admission, it should be based on clinical warning signs [27].

Another study by Veasna Duong *et al.*, 2011, shows significantly higher viremia correlated with NS1 antigen levels, while more severe illness was correlated with lower levels of NS1 antigen [28]. Since there is no specific treatment for this infection and there are currently few therapeutic options and vaccines available, finding new potentially modifiable risk factors for progression to severe dengue disease is an extremely important priority. Finding the relationship between serum IL-10 levels and the severity of dengue disease can be a crucial first step in examining the potential of these tests to identify dengue patients who can progress to severe disease (DF/DSS).

Conclusion

In conclusion, early identification and treatment can significantly impact the prognosis for dengue and help prevent patients from developing dengue hemorrhagic fever. Additionally, regarding prognostic indicators of dengue might aid in the early identification of the disease through warning signs. The results of this study unambiguously show that IL-10 is a highly sensitive indicator of severe dengue and that it can be taken as a screening tool in secondary dengue patients or those showing warning signs.Interleukin-10 is substantially more related with DHF than DF when it is present in high levels.

For the diagnosis of dengue infection, the NS1 Ag assay is helpful, sensitive, and specific, especially during the acute phase when antibodies are undetectable and dengue serology is negative. Complications from severe dengue infections can be decreased with early detection and prompt treatment. However, based on the results of our study, we cannot use it predict severe dengue infection early on, and the criteria for hospital admission for dengue fever cases should only be based on clinical warning signs rather than NS1 Ag positive reports.

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