Original Research Article

Study of clinical profile and inflammatory markers in dengue fever with thrombocytopenia

Arun Kumar[1], Ramesh S Hiremath[2*], Deepika T[3]

[1,2]Assistant Professor, [3]Junior Resident
Department of General Medicine, Rajarajeshwari Medical College and Hospital, Bangalore, Karnataka, India
[*]Corresponding author email: **drrshiremathhiremath@gmail.com**

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Abstract

Background: Dengue is emerging as a serious public health problem globally, with 2.5 billion people at risk and 50 million dengue infections occurring annually.

Aim: The study aimed to correlate the disease progression with respect to clinical profile and inflammatory markers and to assess the correlation between dengue fever with platelet count, LFT, and inflammatory markers.

Materials and methods: An observational study was carried out on individuals admitted to a tertiary care centre over a period of 2 years, with the sample of 70 dengue patients. All suspected patients with dengue fever above 16 years of age were assessed and a detailed history and a thorough clinical examination were done on all the cases. With all the aseptic precautions, venous blood samples collected and immediately transferred to freezer and stored at -70c. The optical densities thus obtained were plotted on a graph obtained from the pilot standardization and serum concentration of IL-6, IL-8, TNF-α and IFN-ϒ were calculated using software Curve Expert Pro 2.01.

Results: The principal finding in the present study was that various inflammatory markers like IL-6, IL-8 and TNF-α were significantly elevated in dengue hemorrhagic fever than in dengue fever. DHF cases had the mean IL-6 value of 210.79 pg/ml while DF had 90.34 pg/ml and mean IL-8 value of 604.95 pg/ml in DHF while DF had 196.50 pg/ml and TNF-α mean value of 242.50 pg/ml in DHF while DF had 33.05 pg/ml indicating a major role of these inflammatory markers in the pathogenesis of the severe form of the disease. Also there was significant association between raised liver enzymes and inflammatory markers. ALT levels were significantly correlating with TNF-α and IL-8 inflammatory markers. AST levels were significantly correlating with IL-6, IL-8 and TNF-α level indicating the role of cytokines in liver pathology.

Conclusion: Increased production of inflammatory markers IL-6, IL-8 and TNF-α may have a significant role in the pathogenesis of DHF which is a severe form of the dengue infection. So these inflammatory cytokines can be taken as the markers for the prediction of the severity of the dengue infection and liver pathology.

Key words

Dengue Fever, Inflammatory markers, Liver enzymes.

Introduction

Dengue is emerging as a serious public health problem globally, with 2.5 billion people at risk and 50 million dengue infections occurring annually [1]. Dengue is becoming a serious public health problem in India [2]. Although dengue infection has been endemic in India since the nineteenth century, DHF has become endemic in various parts of India since 1987 [2]. Dengue, primarily caused by four different Dengue viruses (DENV) serotypes, DENV-1, DENV- 2, DENV-3, and DENV-4 [3] presents clinically as self-resolving Dengue Fever (DF) to the more severe Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Immunity to one serotype does not provide protection against other serotypes [4]. DHF occurs mostly in individuals who acquire a heterotypic secondary infection [5, 6]. The host innate immune responses plays a primary role in limiting the disease, however the immunological mechanisms exploited by the dengue virus to trick the host immune response still needs to be explored. Dengue virus has been known to escape from host immune responses through multiple routes and triggering of inflammation associated with host tissue damage is one such approach. The significance of circulating levels of cytokines as inflammatory mediators in dengue patients is controversial and difficult to interpret. Only few studies have correlated cytokine levels with severity of dengue. The present study aimed to correlate the disease progression with respect to clinical profile and inflammatory markers and to assess the correlation between dengue fever with platelet count, LFT, and inflammatory markers.

Materials and methods

An observational study was designed at a tertiary care centre. 70 patients with signs of fever and symptoms suggestive of Dengue fever with thrombocytopenia confirmed by dengue serology NS1 Ag, IgM, ELISA positive were included in the study. All the study patients were included after obtaining informed consent. Ethical clearance was obtained by the institutional ethics committee. Fever with thrombocytopenia of other causes such as Leptospirosis, Scrub typhus, Typhoid fever, Malaria, CMV, Influenza, Chronic inflammatory disease, Connective tissue disorder, Collagen vascular disorder, vasculitis and Malignancy were excluded from the study.

All suspected patients with dengue fever above 16 years of age were assessed and a detailed history and thorough clinical examinations were done. Data was collected using a structured proforma, which included age, sex, IP number, detailed present, past and personal history. Patients were screened for following investigations - Dengue NS1Ag, IgG ELISA, IgM ELISA, complete hemogram. Only serologically confirmed cases by dengue NS1 Ag, IgM ELISA were included, when their platelet count dropped below 150000/dl during the acute stage of the illness. With all the aseptic precautions, venous blood samples collected after fulfilling the inclusion and exclusion criteria and immediately transferred to freezer and stored at -70^0c.

Estimation of IL-6, IL-8, TNF-α and IFN-ϒ (procedure details) –

- Pilot standardization and titration was done.

- 0.1ml of each properly diluted sample was added to the precoated plate.
- Seal the plate with the cover and incubate at 37^0c for 30 min.
- Remove the cover, discard the plate content and blot the plate onto paper towels.
- Add 0.1ml of substrate A into each well and incubate the plate a 37^0c for 60 min.
- Wash the plate for 3 times with buffer.
- Add 0.1ml of substrate B into each well and incubate the plate at 37^0cfor 30 min.
- Wash the plate for 5 times with buffer.
- Add substrate C into each well and incubate plate at 37^0c in dark for 15-20 min.
- 0.1ml of stop solution was added and Optical Density read at 450nm immediately.

The optical densities thus obtained were plotted on a graph obtained from the pilot standardization and serum concentration of IL-6, IL-8, TNF-α and IFN-ϒ were calculated using software Curve Expert Pro 2.01.

Data collected were analyzed by using SPSS – version 18. Pearson`s correlation, one way anova and Independent t test were used. All the statistical methods were carried out through the SPSS for windows (version 18.0).

Results

A total of 70 cases formed the study group. The study group constituted cases aged between 16 to 60 years. The majority of cases were in the age group of 21-30 years which constituted 37.1% of the total study group. This study group consisted of 35(50%) Dengue fever cases and 35 (50%) dengue hemorrhagic fever cases. 49(70%) cases were NS1 antigen positive, 12 (17%) cases were IGM antibody positive and 9(12.9%) cases which were positive for both (**Table - 1**).

Clinical profile of the patients

47(67%) of the cases had normal pulse and 23(33%) of the cases had bradycardia. 27 (38.6%) cases of had diarrhoea and 13(18.6%) cases had vomiting. Mean platelet count at admission showed 51857 and mean platelet count at discharge was 108228 (**Table - 2**).

Table – 1: Distribution of respondents according age, sex, type of fever and Serological results.

A. Age of the respondents		
Age group	**Frequency**	**Percent**
<20 yr	20	28.6
21-30 yr	**26**	**37.1**
31-40 yr	12	17.1
41-50 yr	7	10.0
>51 yr	5	7.1
Total	**70**	**100.0**
B. Sex distribution of cases		
	Frequency	**Percent**
Female	36	51.4
Male	34	48.6
Total	**70**	**100.0**
C. Distribution of cases according to the type of fever		
	Frequency	**Percent**
Dengue Fever	35	50.0
Dengue Hemorrhagic Fever	35	50.0
Total	**70**	**100.0**
D. Distribution of cases according to serological results		
	Frequency	**Percent**
NS1 Antigen	49	69.7
IGM Antibody	12	16.9
NS1+IGM	9	12.9
Total	**70**	**100.0**

Table – 2: Clinical profiles of the cases.

Pulse rate in dengue cases		
	Frequency	**Percent**
Normal	47	67.1
Bradycardia	23	32.9
Total	70	100.0
Distribution of cases with diarrhoea and vomiting		
Diarrhoea	27	38.6
Vomiting	13	18.6
Total	70	100.0

Table - 3: Comparisons of levels of inflammatory markers in dengue fever and dengue hemorrhagic fever.

	Type of fever	N	Mean	SD	P
IL - 6	Dengue Fever	35	90.34	75.94	<0.0001
	Dengue Hemorrhagic Fever	35	210.79	55.84	
TNF - ALPHA	Dengue Fever	35	33.05	37.91	<0.0001
	Dengue Hemorrhagic Fever	35	242.50	50.91	
IFN - GAMMA	Dengue Fever	35	21.37	18.26	0.5
	Dengue Hemorrhagic Fever	35	24.0	11.90	
IL - 8	Dengue Fever	35	196.50	36.20	<0.0001
	Dengue Hemorrhagic Fever	35	604.95	38.29	

Table – 4: Correlation of platelet count with inflammatory markers.

		IL - 6	TNF - ALPHA	IFN - GAMMA	IL - 8
Platelet count	r	.019	-.122	.066	-.174
	p	.873	.313	.587	.150
	N	70	70	70	70

Table – 5: Correlation between Alanine transaminase (ALT) with inflammatory markers.

		IL - 6	TNF - ALPHA	IFN - GAMMA	IL - 8
ALT	Pearson Correlation r	.101	.264*	.016	.296*
	P	.404	**.027**	.894	**.013**
	N	70	70	70	70

Table - 6: Correlation between various inflammatory markers and Aspartate aminotransferase (AST).

		IL - 6	TNF - ALPHA	IFN - GAMMA	IL - 8
AST	Pearson Correlation r	.238*	.399**	.014	.491**
	P	**.047**	**.001**	.907	**<.0001**
	N	70	70	70	70

Levels of inflammatory markers in dengue fever and dengue hemorrhagic fever

Mean values of Interleukin-6 (IL-6) were increased in dengue hemorrhagic fever (210.79) as compared to dengue fever (90.34) and was statistically significant (p-0.0001). Mean values of Tumor necrosis factor (TNF-α) were increased in dengue hemorrhagic fever (242.5) as compared to dengue fever (33.5) and was statistically significant (p-0.0001). Mean values of Interleukin-8 (IL-8) were increased in dengue hemorrhagic fever (604.95) as compared to dengue fever (196.5) and was statistically significant (p-0.0001). Mean values of Interferon gamma (IFN-γ) in dengue hemorrhagic fever was 24 and dengue fever was 21.37 and did not show statistical significance (p -0.5).

Levels of IL-6 IL-8 and TNF-α were increased significantly in dengue hemorrhagic fever compared to dengue fever. IFN-γ levels were almost similar and were not statistically significant (**Table - 3**).

Correlation of Platelet count with inflammatory markers

Pearson's correlation test was applied to establish the correlation between platelet count and various inflammatory markers (IL-6, TNF-α, IFN-γ and IL-8). There was no significant correlation between any of the markers and platelet count (**Table - 4**).

Correlation between Alanine transaminase (ALT) with inflammatory markers

Pearson's correlation test was applied to establish the correlation between Alanine transaminase (ALT) and various inflammatory markers (IL-6, TNF-α, IFN-γ and IL-8). Out of all the four markers TNF-α (p-0.027 r-0.264) and IL-8 (p-0.013 r- 0.296) showed statistically significant correlation (**Table - 5**).

Correlation between Aspartate aminotransferase (AST) with inflammatory markers

Pearson's correlation test was applied to establish the correlation between Aspartate aminotransferase (AST) and various inflammatory markers (IL-6, TNF-α, IFN-γ and IL-8). Out of all four markers, IL-6 with (p-0.047 r-0.238), TNF-α with (p-0.001 r-0.399) and IL-8 with (p-<0.0001 r-0.491) showed statistically significant correlation (**Table - 6**).

Discussion

In the present study clinical profile of dengue infection was correlated with inflammatory markers. 70 patients fulfilling inclusion and exclusion criteria were observed. Majority of cases were in the age group of 21-30 years which constituted 37.1% of the total study group. 49% of the study groups were males and 51% of the study group was females. The males and females were almost equally distributed with a male to female ratio of 1:1. The study consisted of 35 Dengue fever cases i.e. 50% of the total cases and 35 Dengue hemorrhagic fever cases i.e. 50% of the total cases. It included 49 NS1 antigen positive cases i.e. about 70% total cases, 12 IGM antibody positive cases i.e. about 17% and 9 cases which are positive for both.

Assessment of Inflammatory markers in DF and DHF

Interleukin-6: In the present study, levels of IL-6 were significantly elevated in the DHF cases than DF cases. DHF cases had the mean value of 210.79 pg/ml while DF had 90.34 pg/ml. (p <0.0001). Similarly a study done by Priyadarshini, et al. [7] in western India revealed that the levels of IL-6 in DHF were higher than in DF patients (p = 0.02).

Interleukin-8: In the present study levels of IL-8 was statistically elevated in the DHF cases than DF cases (p <0.0001). DHF cases had the mean value of 604.95 pg/ml while DF had 196.50 pg/ml. this finding is also similar to the findings of study by Priyadarshini, et al. [7].

In the study done by Yng-huey huang, et al. showed that increased serum levels of IL-6 and IL-8 were observed in patients with dengue hemorrhagic fever [8]. Both IL-6 and IL-8 levels were statistically significant in their study and the study concluded that Dengue Virus can infect human endothelial cells and induce IL-6 and IL-8 production which may contribute to the pathogenesis of DHF.

Tumor necrosis factor – α: In the present study, levels of TNF-α was statistically elevated in the DHF cases than DF cases. DHF cases had the mean value of 242.50 pg/ml while DF had 33.05 pg/ml. This indicates TNF-α level play a significant role in DHF and also can be taken as predictor of severity of dengue infection. In a study conducted by Anita chakravarti, et al. [9] and U C Chaturvedi, et al. [10], TNF-α levels were significantly elevated in DHF in both the studies and the studies concluded that increased production of TNF-α have a role in the immune pathogenesis of DHF by acting at the level of vascular endothelium.

Interferon – γ: In the present study, levels of INF-γ were almost same in the both DF and DHF cases. DF cases had a mean IFN-γ of 21.34 pg/ml and DHF cases had a mean IFN-γ of 24 pg/ml and was statistically not significant. Present study did not find any correlation with Interferon –γ and severity of dengue infection. In contrast to the studies conducted by U C Chaturvedi, et al. [10], Priyadarshini, et al. [7] and Anita, et al. [9], where the levels of IFN-γ were compared between both DF and DHF groups, it was found that IFN-γ was significantly elevated in DF cases compared to DHF cases in the early phase of the

disease. These studies concluded that IFN-γ is elevated in the early phase of the disease and it can be used as the early marker of dengue infection.

Platelet count and inflammatory markers: In the present study, there was no statistically significant correlation between platelet count and inflammatory markers. However in contrast, study conducted by Priyadarshini, et al. [7] and U C Chaturvedi, et al. [10] showed significant correlation between IL-8 and platelet count. They concluded that high levels of IL-8 causes intravascular damage and causes plasma leakage and hence thrombocytopenia.

Liver enzymes and inflammatory markers: AST: In the present study, AST was correlated with IL-6, IL-8, TNF-α and Interferon-γ. Out of all four markers, IL-6 with (p-0.047 r-0.238), TNF-α with (p-0.001 r-0.399) and IL-8 with (p-<0.0001 r-0.491) had statistically significant correlation. **ALT**: Out of all the four markers TNF-α (p-0.027 r-0.264) and IL-8 (p-0.013 r-0.296) showed statistically significant correlation. In a study conducted in western India by Priyadarshini, et al. [7] and Anon srikiatkhachorn [11] revealed that there will be increased ALT and AST levels during the early phase of the disease and was significantly associated with IL-8. So they concluded that IL-8 plays a significant role in raised AST and ALT levels.

Conclusion

The present study assessed the levels of inflammatory mediators - IFN-γ, TNF-α, IL-6 and IL-8 in with dengue clinical profile. Inflammatory markers like IL-6, IL-8 and TNF-α were significantly elevated in DHF as compared to DF. This indicates IL-6, IL-8 and TNF-α plays a significant role in the pathogenesis of DHF which is the severe form of the disease. These cytokines predicts the severity of dengue infection and can be considered as markers for the severity of the dengue fever. However, IFN-γ levels were not statistically significant in dengue fever and dengue hemorrhagic fever. Platelet counts were correlated with all four inflammatory markers, but there was no statistically significant correlation between them. The study also compared the LFT with inflammatory markers and there was significant association between raised liver enzymes and inflammatory markers. ALT levels were significantly correlating with TNF-α and IL-8 inflammatory markers. AST levels were significantly correlating with IL-6, IL-8 and TNF-α level. This indicates that we can consider IL-6, IL-8 and TNF-α as the markers for liver pathology in dengue fever.

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