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Original article

Estimation of serum neopterin level as an early marker for detecting severe dengue infection

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ABSTRACT

Background: This study aimed to determine the role of neopterin in the early assessment of dengue severity in children of age one month to 18 years admitted for fever and confirmed by dengue serology.
Method: Two sets of samples were collected from 77 confirmed dengue patients aged one month to 18 years for serum neopterin determination. The first sample was collected at 2-3 days of fever onset, and the second sample was collected at 24–36 h after the first sample was collected and followed up cases and correlated serum neopterin levels.
Results: Among the 77 patients enrolled, 19 were diagnosed with severe dengue, 15 patients as having dengue with warning signs, and 43 patients as having dengue fever.
Conclusion: In this study, we found that the serum neopterin levels were significantly higher in the severe dengue category (mean: 58.75(±4.91) nmol/l) than in the non-severe dengue category (mean: 48.11(±8.80) nmol/l). Furthermore, a positive correlation was observed between neopterin concentration and duration of fever hence, higher serum neopterin levels may indicate dengue severity. The determination of serum neopterin concentration may be used for the early assessment of severe dengue.

1. Introduction

Dengue is an acute febrile illness caused by the *Aedes aegypti* mosquito-borne dengue viruses involving antigenically related flaviviruses of four serotypes [1]. The World Health Organization (WHO) recognizes the dengue virus as a major and emergent concern because of its expanding distribution and the increased frequency of epidemics [2,3]. This disease is now predominant in America, Africa, the eastern Mediterranean, South East Asia, and the western Pacific [4]. Dengue hemorrhagic fever (DHF) is a prominent reason for death and serious illness in children in some Asian countries [1]. 90% of DHF victims are children under the age of 15 years [5].

Dengue virus infections can manifest as dengue fever or DHF/dengue shock syndrome (DSS) and can also end in multi-organ failure [1]. In dengue virus infection, cytokines may be released either directly from virus-infected cells such as monocytes/macrophages or indirectly upon the interaction of virus-infected cells with other immunocompetent cells such as activated T lymphocytes [6]. During the immune response, 6-D erythro neopterin is generated and released in increased amounts by human macrophages upon activation by interferon-gamma, released by T-lymphocytes and natural killer cells. Therefore, the determination of neopterin concentration in body fluids is useful for the monitoring of cellular (=Th1-type) immune activation in infections, especially viral and intracellular bacterial infections, autoimmune diseases, malignant disorders, and early detection of allograft rejection episodes [7].

Only two studies are available in the literature stating the importance of determining neopterin concentration in dengue-infected patients. The CPY Chan et al. study showed that neopterin concentration was found to be higher in patients with dengue than in those with other viral infections [8]. The K. Babb et al. study revealed that neopterin level in patients at the early stage of dengue fever is a sensitive indicator for the estimation of

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the severity of the diseases [9]. Our hospital is a tertiary care health center, and many children with dengue infection are admitted every year at various stages of the clinical spectrum. Hence, this study aims to determine the role of neopterin in the early assessment of dengue severity in children of age one month to 18 years admitted for fever and confirmed by dengue serology. The study will help in the early diagnosis of severe dengue cases requiring more intensive care.

2. Methods

This prospective observational study was conducted in the Department of Pediatrics for 18 months (October 2016 to March 2018) after receiving due approval from the institutional human ethical committee.

2.1. Study criteria

In this study, we obtained informed consent and enrolled all inpatient cases of dengue virus infection confirmed by at least one of the three tests: NS1 antigen detection test, ELISA IgM, and ELISA IgG, and who were aged between one month and 18 years. Patients were excluded from the study if they were diagnosed to have severe dengue at the time of admission, dengue with coexisting infections proved by blood culture, urine routine, smear for MP, chest X-ray, Widal test, and Weil-Felix test.

2.2. Study procedure

Taking α as 0.05 and confidence interval as 95%, the sample size for the study was calculated using the formula, n = Z^2p.q/L^2 (Z = 1.96 (confidence interval), p = 28% [10], q = (100-28)%, L (marginal error) of 10%) and was estimated to be 77. All patients who met the study criteria were enrolled after obtaining informed consent. Five milliliters of venous blood was collected from the enrolled patients by aseptic venepuncture at 48–72 h from the onset of symptoms and the second sample was collected at 24–36 h after the first sample was collected. Serum was separated by centrifugation, and serum neopterin levels were estimated by ELISA.

2.3. Statistical analysis

The data collected were analyzed using EpiData and SPSS 21.0 for windows software. $P < .05$ was considered significant. Appropriate statistical tests were applied for analyzing the data, such as descriptive statistics, one-way ANOVA with post-hoc test, Mann–Whitney test, Kruskal–Wallis test, and Pearson correlation analysis.

3. Results

The majority (37.7%) of the study subjects were in the age group of <5 years, followed by 32.5% in the age group of 6–10 years and 29.9% in the age group of 11–18 years. Of the total 77 study participants, 42 (42.55%) were boys and 35 (32.45%) were girls (**Table 1**). In the first sample, mean neopterin concentration was 49.7 (9.16) nmol/l and median neopterin concentration was 48 nmol/l. In the second sample, mean neopterin concentration was 55 (10.55) nmol/l, and median neopterin concentration was 54.4 nmol/l. The mean and median of the raised neopterin value between the first and the second sample was 5.23 (5.23) nmol/l and 4.9 nmol/l, respectively (**Table 2**). Out of the 77 study patients, 55.8% (43) 43 (55.8%) were diagnosed as having “dengue without signs,” 15 (19.5%) as having “dengue with warning signs,” and 19 (24.7%) as having “severe dengue” according to the WHO severity criteria for dengue virus infection [11].

3.1. Comparison of mean neopterin levels of the first and second samples by severe and nonsevere dengue fever

The mean (SD) neopterin levels in non-severe dengue and severe dengue in the first sample (48–72 h of fever) were 48.11 (8.80) nmol/l and 58.75 (4.91) nmol/l, respectively. This variation in mean neopterin levels between non-severe dengue and severe dengue groups was found to be statistically significant.

On the other hand, the mean (SD) neopterin levels in nonsevere dengue and severe dengue in the second sample were 53.10 (10.25) nmol/l and 65.32.92 (4.41) nmol/l, respectively. This variation was found to be statistically significant (**Table 3**).

3.2. Comparison of raised neopterin levels in severe and nonsevere dengue

The median raised neopterin levels in non-severe Dengue and severe dengue were 4.20 nmol/l and 6.65 nmol/l, respectively. This difference in median raised neopterin levels between nonsevere dengue and severe dengue was not significant ($P = .06$).

3.3. Comparison of duration of fever by neopterin values of the first and second samples

The correlation between duration of fever and neopterin levels was found to be positive by Pearson correlation, and the coefficients and *P* values were found to be significant (**Table 4**).

Relationship of serum neopterin values of the first and second samples with the lowest platelet count of subjects.

No correlation was found between the lowest platelet count and neopterin level (*P* value = .053 and .013).

4. Discussion

Neopterin is a nonspecific marker of activated cell-mediated immunity involving the release of interferon-gamma. It may be a useful marker for more accurate estimation of the extent of disease and hence prognosis. Knowledge of all potential causes of its elevation can help overcome problems with reduced specificity in a patient known to have a specific infectious disease. Neopterin level

Table 1
Distribution of study subjects by different age groups.

Age category (Year)	Sex				Total	
	Male		Female			
	Count	Column, N (%)	Count	Column, N (%)	Count	Column, N (%)
<5	18	42.9	11	31.4%	29	37.7%
6–10	14	33.3	11	31.4%	25	32.5%
>11	10	23.8	13	37.1%	23	29.9%

Table 2
Comparison of mean and median neopterin values of the first and second samples.

Neopterin (nmol/l)	Mean	SD	Median	25th Percentile	75th Percentile
First sample	49.77	9.16	48.80	42.20	57.70
Second sample	55.00	10.55	54.40	45.00	64.00
Raised value between the first and second samples	5.23	5.12	4.90	2.20	7.60

Table 3
Comparison of mean neopterin levels of the first and second samples of severe and nonsevere dengue fever.

Neopterin Samples	Nonsevere Dengue		Severe Dengue		*P* value
	Mean	Standard Deviation	Mean	Standard Deviation	
First sample	48.11	8.80	58.75	4.91	<.00001
Second sample	53.10	10.25	65.32	4.41	<.00001

Table 4
Comparison of duration of fever by neopterin values of the first and second samples.

		Neopterin in the first sample (nmol/l)	Neopterin in the second sample (nmol/l)
Duration of fever, in days	r (correlation coefficient)	.439**	.474**
	P value	0.000	0.000
	N	77	77

in the early stage of dengue infection may be a sensitive indicator for the estimation of the severity of the disease. Neopterin measurement could serve as an early predictor of the future course of dengue virus infection. Technological advances may further enhance the usefulness of neopterin measurement in acute infection [12,13].

CPY Chan et al. showed in their study the elevation of serum neopterin concentrations from day 1 of fever onset with a mean neopterin value of 44.2 (2.3)nmol/l to a peak value of 54.3 (3.0) nmol/l on day 4 of fever, and neopterin remained at a higher level, with a mean neopterin value of 30 nmol/l [7]. Similarly, in our study, we observed that the serum neopterin concentration of the second sample with a mean value of 55.0 (10.55) nmol/l was elevated when compared with that of the first sample with a mean value of 49.77 (9.16) nmol/l. K. Babb et al. showed in their study that serum neopterin levels were significantly higher ($P = .009$) with a median value of 29 nmol/l in patients diagnosed with severe dengue when compared with that of nonsevere dengue with a median value of 13.5 nmol/l [8]. In the first sample of our study, the mean serum neopterin level of 58.75 (4.91) nmol/l of severe dengue was significantly ($P = .00001$) higher than that of 48.11 (8.80) nmol/l of nonsevere dengue. In the second sample, the mean serum neopterin level of 65.32 (4.41) nmol/l of severe dengue was significantly ($P = .00001$) higher than that of 53.10 (10.25) nmol/l of nonsevere dengue but with insignificant raised serum neopterin level in severe dengue (median: 6.65 nmol/l) as compared to that in nonsevere dengue (median: 4.20).

Furthermore, we also observed a positive correlation between higher levels of serum neopterin and duration of fever (r: 0.439), in line with the observation by K. Babb et al. in their study. However, we did not observe any correlation between the lowest platelet count and serum neopterin levels. The serum neopterin levels in severe dengue were significantly higher than those in no-severe dengue, but the raised serum neopterin levels between severe and non-severe dengue were not statistically significant.

5. Conclusion

Our study showed that serum neopterin levels in severe dengue were significantly higher than those in non-severe dengue, but raised neopterin values between severe and non-severe dengue were not significant. Further studies are required to determine the clinical significance of serum neopterin levels in the early assessment of severe dengue.

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Ethical approval

This study was approved by the Human Ethical Committee, JSS Medical College and Hospital, Mysuru, Karnataka, India.

Declaration of competing interest

The authors declare no conflict of interest.

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