


Research Article

A Comparative Study of Pro & Anti-Inflammatory Cytokine Levels in Children with Febrile Seizure

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Abstract

Objective: To compare the levels of pro and anti-inflammatory cytokines in children presenting with febrile seizures v/s only febrile illness in age group of 6 to 60 months.

Study design: In this hospital based observational case-control study; conducted in Department of Pediatric Medicine, Sanjay Gandhi Memorial Hospital, New Delhi 50 children (of age 6 to 60 months) were included. Patients undergo relevant investigation to find out abnormality in complete blood count, liver function tests and renal function tests. Pro and anti-inflammatory cytokine levels including IL-1 β , IL-6, IL-10 & TNF- α were assessed by ELISA by using TECAN INFINITE F50 absorbance microplate reader.

Results: In febrile seizure cases; 72% children had simple febrile seizure, followed by complex febrile seizure in 28%. The median (25th-75th percentile) values of pro-inflammatory cytokines (IL-1 β , TNF- α and IL-6) were significantly higher in febrile seizure cases as compared to control group. The median value of anti-inflammatory cytokine IL-10 showed no significant difference in cases v/s control group.

Conclusions: A comparative analysis of different cytokines in febrile seizure can be useful as a diagnostic marker. Role of cytokines in pathogenesis of different other diseases has been studied previously and then pharmaceutical drugs were formulated against particular cytokines. On the contrary, in febrile seizure, there is still a lack of sufficient studies, so the role of drugs against cytokines in febrile seizures still needs to be studied.

Keywords: Febrile seizure, Children, Cytokines, Inflammation

Objective: Febrile seizure (FS) is the most common form of seizures in children. A febrile episode (core body temperature $>100.4^{\circ}\text{F}$ or 38°C) causing seizure in children aged between 6 months to 60 months, who don't have any CNS infection, metabolic imbalance or history of afebrile seizure, comes under febrile seizure. In Europe and USA, the incidence of febrile seizure is around 2-5% and in India it is around 5-10% [1-3]. Pathophysiology of febrile seizures remains unclear; it is suggested that febrile seizure is an age-dependent response of the immature brain to fever, as studies in animal models have suggested that during the brain maturation process, there is enhanced neuronal excitability [4]. Over the past two decades, there has been emerging evidence from both clinical and experimental studies that components of the immune response involved in FS may play a role in their pathogenesis [5]. In particular, studies have focused on the pro-inflammatory cytokines, which are released during fever (both peripherally and centrally), and their possible role in FS. Specifically, several studies have focused on the pro-

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inflammatory cytokine interleukin-1beta (IL-1 β), although other studies have also examined additional cytokines such as TNF- α , interleukin-1alpha (IL-1 α) and interleukin-6 [5]. Pro-inflammatory and anti-inflammatory cytokines regulate immune response. During infection, both pro-inflammatory and anti-inflammatory cytokines are produced [6]. IL-1 β , TNF- α and IL-6 are pro-inflammatory cytokines that participate in the induction of acute-phase inflammation reactions, including fever. Interleukin-1 receptor antagonist (IL-1RA) and IL-10 are anti-inflammatory cytokines and have a negative feedback effect during fever [6,7]. The balance between these two cytokine groups influences the severity of the fever. Complex interactions among immune-inflammatory process, cytokine activation, and genetic factors are involved in the pathogenesis of FS [8]. Experimental studies demonstrate that inflammation and inflammatory mediators are the main causes and propagators of both febrile and epileptic seizures [9]. So, there is lack of sufficient data regarding studies of cytokine in febrile seizure patients, some of the studies showed positive association of cytokine in febrile seizure but some of the studies did not favor any association between cytokine and febrile seizure. Hence this study is being conducted to compare the levels of pro and anti-inflammatory cytokines in febrile seizures patients v/s control groups.

Methods

This hospital based observational case-control study was conducted in Department of Pediatric Medicine, Sanjay Gandhi Memorial Hospital, New Delhi from January 2020 to December 2020. A total of 50 children (25 case and 25 control) of age 6 to 60 months were enrolled. Febrile seizure was defined as a febrile episode (core body temperature >100.4° F or 38°C) causing seizure in children aged between 6 months to 60 months, who don't have any CNS infection, metabolic imbalance or history of afebrile seizures. Case group included 25 children of either sex presented with only febrile illness, without any seizure.

Children having any sign of central nervous system infection, RAT / RTPCR positive for COVID-19, any sign of systemic disease like heart failure, liver failure, renal failure, tumor, metabolic encephalopathy were excluded. Children with any chronic neurodevelopment problem, persistent neurological deficit, history of birth asphyxia were also excluded.

After taking informed consent from the parents, detailed history was elicited and physical examination was carried out. Personal information about the children, including age, gender and family history of seizure were collected from parents through an interviewed questionnaire. In study population, core body temperature was measured and recorded. All children before admission in hospital were tested for covid-19 and both nasopharyngeal and oropharyngeal

swab were sent for RAT and RTPCR test. 5 ml of blood sample was collected in plain vial for baseline biochemical assessment of each patient. The sample was centrifuged and collected serum stored at 2° to 8°C till sample reached via ice-packs container to department of biochemistry, GIPMER institute, New Delhi. Their serum samples were stored at -80°C Centigrade in different aliquots till further analysis of pro & anti-inflammatory cytokine levels was done. At a final step sample were tested by commercially available ELISA test for cytokines. Rest of the serum was used in estimation of serum electrolytes, liver and kidney function test. Another sample in EDTA vial was sent for complete blood count and in gray vial for random blood-plasma sugar estimation.

Assessment of cytokines IL-1 β , IL-6 IL-10 & TNF- α –

A capture Antibody highly specific according to the particular interleukin to be tested; was coated to the wells of the microtiter strip plate. Binding of specific testing interleukin samples and known standards to the capture antibodies and subsequent binding of the respective biotinylated anti-interleukin secondary antibody to the analyte were completed during the same incubation period. Any excess unbound analyte and secondary antibody was removed. The HRP (horseradish peroxidase) conjugate solution was then added to every well including the zero wells, following incubation excess conjugate was removed by careful washing by hydroflex microplate washer. A chromogen substrate was added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development was then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex was directly proportional to the concentration of specific testing IL present in the samples and standards. The absorbance of the color complex was then measured by the microplate reader for ELISA and the generated OD (optical density) values for each standard were plotted against Expected concentration forming a standard curve. This standard curve was used to accurately. Determine the concentration of respective interleukin in any sample tested. Clearance from Departmental Ethics Committee and Scientific Committee was taken prior to the start of the study. All participants had the option to withdraw from the study anytime during their hospital stay.

The presentation of the Categorical variables was done in the form of number and Percentage (%). On the other hand, the quantitative data was presented as the mean \pm SD and as Median with 25th and 75th percentiles (interquartile range). The data normality was checked by, Using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non Parametric tests. The comparison of those variables which were quantitative and not normally distributed in nature were analyzed using Mann-Whitney Test and

normally distributed data was analyzed using Independent t test. Variables which were qualitative in nature, were analyzed using Chi-Square test. If any cell had an expected value of less than 5, then Fisher’s exact test was used. The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, version 21.0. For statistical significance, p value of less than 0.05 was considered statistically significant (Figure 1).

Results

In this hospital-based case control study 25 children of either sex falling in age group of 6 to 60 months presented with febrile seizure were included as cases and 25 children of either sex presented with acute febrile illness but without any seizure were included as control.



Figure 1: TECAN INFINITE F50 Absorbance Microplate reader for ELISA.

Distribution of age (months) was comparable between case and control group. (6-12 months:- 12% v/s 16% respectively, 13-24 months:- 36% v/s 20% respectively, 25-36 months:- 28% v/s 20% respectively, 37-48 months:- 8% v/s 16% respectively, 49-60 months:- 16% v/s 28% respectively) (p value=0.564). So, most cases were of 13-24 months age group. Mean \pm SD of age (months) in cases was 29.8 ± 16.52 and control group was 34.72 ± 17.85 with no significant difference between them (p value=0.317). Distribution of gender was comparable between cases and control (Female:- 44% v/s 56% respectively, Male:- 56% v/s 44% respectively) (p value=0.396). No significant difference was seen in anthropometry parameters between cases and control group in weight (kg) (p value=0.443), height(cm) (p value=0.425). Mean \pm SD of height (cm) in cases was 91.4 ± 16.34 and in control group was 95.02 ± 15.52 with no significant difference between them. Median (25th-75th percentile) of weight (kg) in cases was 12(9.4-15) and in control group was 13.4(10.2-18.6) with no significant difference between them. In cases, type of seizure was simple FS in majority of patients 18 (72%) followed by complex FS 7 (28%). Among case group; 60% children had previous history of seizure and no one had family history of seizure while on the other side in control group; no one patient had previous history of seizure or family history of seizure. Median (25th-75th percentile) of duration of fever (days) in cases was 1(1-1) and control was 2(1-3). Median (25th-75th percentile) measurement of fever ($^{\circ}$ F) in cases was 101.5 (100.8-102.2) and in C1 was 102.4 (101-102.8). Mean \pm SD of hemoglobin (gm %) in cases was 9.64 ± 1.55 which was significantly lower as compared to control group 11.19 ± 1.51 (p value=0.0008). No significant difference was seen between cases & control group in liver function test parameters. For SGOT(U/L) (p value=0.126) and for SGPT(U/L) (p value=0.453). Mean \pm SD of SGOT (U/L) and SGPT(U/L) in cases was 31.36 ± 10.05 and 38.36

Table 1: Comparison of demographic characteristics between Cases and control group

Demographic characteristics	Cases(n=25)	C1(n=25)	Total	P value
Age(months)				
6-12 months	3 (12%)	4 (16%)	7 (14%)	0.564 [‡]
13-24 months	9 (36%)	5 (20%)	14 (28%)	
25-36 months	7 (28%)	5 (20%)	12 (24%)	
37-48 months	2 (8%)	4 (16%)	6 (12%)	
49-60 months	4 (16%)	7 (28%)	11 (22%)	
Mean \pm SD	29.8 ± 16.52	34.72 ± 17.85	32.26 ± 17.2	0.317 [*]
Median(25th-75th percentile)	25(15-36)	34(20-54)	29(17.25-48)	
Range	11-60	8-58	8-60	
Gender				
Female	11 (44%)	14 (56%)	25 (50%)	0.396 [§]
Male	14 (56%)	11 (44%)	25 (50%)	

* Independent t test, [‡] Fisher's exact test, [§] Chi square test

Table 2: History in Cases and Control

History	Cases(n=25)	C1(n=25)	Total
Type of seizure			
No	0 (0%)	25 (100%)	25 (50%)
Atypical FS	7 (28%)	0 (0%)	7 (14%)
Typical FS	18 (72%)	0 (0%)	18 (36%)
Previous history of seizure			
No	10 (40%)	25 (100%)	35 (70%)
Yes	15 (60%)	0 (0%)	15 (30%)
Family history of seizure			
No	25 (100%)	25 (100%)	50 (100%)
Duration of fever(days)			
Mean ± SD	1.24 ± 0.44	2.04 ± 0.93	1.64 ± 0.83
Median(25th-75th percentile)	1(1-1)	2(1-3)	1(1-2)
Range	1-2	1-4	1-4
History related to fever(°F)			
Mean ± SD	101.58 ± 0.84	101.98 ± 1.02	101.78 ± 0.95
Median(25th-75th percentile)	101.5(100.8-102.2)	102.4(101-102.8)	101.75(100.9-102.6)
Range	100.5-103.2	100.4-103.5	100.4-103.5

Table 3: Comparison of Pro inflammatory cytokines between Cases and Control

Pro inflammatory Cytokine	Cases(n=25)	C1(n=25)	Total	P value
IL-1β (pg/mL)				
Mean ± SD	232.28 ± 296.11	85.24 ± 42.96	158.76 ± 222.19	0.001†
Median(25th-75th percentile)	125(110-232)	87(56-125)	120(63.25-127.5)	
Range	60-1200	14-175	14-1200	
IL-6 (pg/mL)				
Mean ± SD	710.04 ± 1182.37	80.4 ± 90.17	395.22 ± 888.74	0.002†
Median(25th-75th percentile)	232(48-690)	47(27-80)	65.5(36-310.25)	
Range	30-5000	16-366	16-5000	
TNF-α (pg/mL)				
Mean ± SD	204.36 ± 285.22	36.12 ± 53.34	120.24 ± 220.13	<.0001†
Median(25th-75th percentile)	100(30-210)	10(5-40)	40(10-137.5)	
Range	10-1200	4-175	4-1200	

† Mann Whitney test

± 15.9 respectively and in control group was 36.4 ± 12.68 and 41.88 ± 17 respectively with no significant difference between them. No significant difference was seen between cases & control group in kidney function test Parameters; for blood urea (mg%) (p value=0.648) and for serum creatinine (mg/dl) (p value = 0.93). Mean ± SD of blood urea (mg%) in cases was (21 ± 8.35) and in control group was (19.84 ± 9.47) without significant difference between them. Median (25th-75th percentile) of serum creatinine (mg/dl) in cases was 0.6(0.4-0.7) and in control group was 0.5(0.5-0.7) without significant difference between them.

Median (25th-75th percentile) of pro inflammatory cytokines IL-1β (pg/mL), TNF-α (pg/mL) and IL-6 (pg/mL) in cases was 125(110-232), 100(30-210), and 232(48-690) respectively which was significantly higher as compared

to control group 87(56-125) (p value=0.001), 10(5-40) (p-value<.0001), and 47(27-80) (p-value=0.002) respectively.

Median (25th-75th percentile) of anti inflammatory cytokine IL-10 (pg/mL) in cases was 10(8-20) and in control group was 10(9-30) with no significant difference between them. (p value=0.66).

Discussion

This hospital-based case control study was conducted in Department of Pediatrics, Sanjay Gandhi Memorial Hospital, Delhi. Twenty-five (N=25) children of either sex falling in age group of 6 to 60 months presented with febrile seizure were included as cases. Twenty-five (N=25) children who came with only febrile illness without seizure were included as control group. Level of pro & anti-inflammatory cytokines were compared between case and control group.

In our present study demographic and anthropometry parameters such as age, gender, weight and height were comparable between case and control group. In our present study mean \pm SD of hemoglobin (gm%) in cases was 9.64 ± 1.55 which was significantly lower as compared to control group [(11.19 ± 1.51) (p value=0.0008)]. Low hemoglobin in febrile seizure cases may be because of anemia as it is the potential risk factor for febrile seizure found in recent studies [10-13]. Iron deficiency reduces the metabolism of some neurotransmitters [11,12]. Several lines of evidence led to the hypothesis that iron deficiency can have a role in the onset of a convulsion. Some studies have reported that in the patients with iron deficiency, febrile convulsion was significantly higher than that in the control group. In our present study there was no significant difference seen between cases v/s control group in liver function test and no significant difference was seen between cases control group in kidney function test parameters. The main focus in this study was to compare serum pro-inflammatory and anti-inflammatory cytokine levels between febrile seizure cases and control group. Median (25th-75th percentile) values of pro inflammatory cytokines IL-1, TNF- α , IL-6 was significantly higher as compared to control group while median value of anti-inflammatory cytokine IL-10 didn't show statistically significant difference between them.

Study found increased pro inflammatory cytokine IL-1 production of peripheral blood mononuclear cells of Febrile seizure patients after stimulation with lipopolysaccharide [5]. Also, in their study found that during the acute phase of febrile seizure, children had significantly elevated plasma levels of IL-1 β , CSF levels of TNF- α . Similar findings were also seen in our study; that levels of pro inflammatory cytokine IL-1 β were increased in febrile seizure cases as compared to control group [14]. Studied and examined levels of the pro inflammatory cytokine IL-1 β associated with a febrile seizure [15]. They found significantly increased levels of IL-1 β in the hippocampus of animals with FS vs. without FS ($p < 0.01$). Conclusively they found increased hippocampal and hypothalamic pro inflammatory cytokine IL-1 β compared to equally treated animals without febrile seizure, which was first evident at onset of febrile seizure in the hippocampus. There were no significant differences seen in anti-inflammatory cytokine IL-1RA levels. Similar finding was also found in our present study while comparing pro inflammatory cytokine IL-1 β , and increased levels of IL-1 β seen in febrile seizure cases comparative to control group. Instead of IL-1RA as an anti-inflammatory cytokine, in our present study we analyzed IL-10 levels. Similar results were found in our present study, as no significant difference was seen in anti-inflammatory cytokine levels between cases and control group. In their study reported that serum IL-6 was higher in children with FS than in healthy controls who had only fever [16]. In addition, the serum IL-6 levels in FS patients were much higher than

those in patients who had undergone an afebrile seizure attack. These findings suggested about pro-convulsant action of IL-6 in febrile seizures. Their study pattern was similar to our study design.

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