SERUM LEVEL OF HMGB1 VERSUS DEMOGRAPHIC AND FEBRILE CONVULSION CHARACTERISTICS IN IRAQI CHILDREN

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ABSTRACT

Febrile seizure (FS) is the most common type of seizure in childhood that occurs in 2-5 % of the children younger than 6 years. Fever is induced by pro-inflammatory cytokines during infection, and pro-inflammatory cytokines may trigger the development of febrileseizures.HMGB1 contributes to febrile inflammatory responses. There are conflicting results on increasing HMGB1 in serum during FS.

One group 72 febrile children (6 months to 5 years old)andother group consisting of 80 children without seizure which served ashealthy control group. Blood samples were collected from the febrileseizure child patients within 30 minutes of the time of the seizure members of both groups and serum samples were prepared; HMGB1 concentrations were measured using Enzyme-linked immunosorbent assay (ELISA) kit. Serum HMGB1levels were significantly higher in febrile seizure patients than in healthycontrols. Serum HMGB1levels were no significant for dimorphiccharacteristics(Temperature, type of FS,duration of FS, recurrent of FS,family history of FS or epilepsy).

HMGB1 was significantly higher in febrile seizure children. Although it is not possible to infer causality from descriptive human studies, our data suggest that HMGB1 may contribute to the generation of febrile seizures in children. There may be a potential role for therapy targeting HMGB1 in preventing or limiting febrile seizures or subsequent epileptogenesis in the vulnerable, developing nervous system of children. in same samples.

Keywords: febrile seizures, proinflammatory cytokines, High mobility group box-1

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INTRODUCTION

A febrile seizure is defined as a sudden change in the dynamic or behavioral activity with limited time and results from the abnormal electrical activity of the brainthat occurs in tandem with a body temperature 38°C (100.4°F) in the absence of intracranial infection, metabolic disturbance [1].febrile seizureone of the most common forms of convulsion in children are febrile seizures which occur in 2-5% of children aged between 6 months and 5 years [2]. Febrile seizures usually have a good prognosis; however, due to the increase in recurrence of such convulsions and the risk of epilepsy in the future, they are considered as serious conditions [3]. The causes of

febrile seizures are not fully understood [4]. There are suggested risk factors, however, such as developmental delay, discharge from neonatal unit after 28 days, daycare attendance, viral infections, some vaccinations, genetic predisposition, and iron and zinc deficiencies [4]. The HMGB1 protein (previously referred to as HMG-1, amphoterin or P30; is a ubiquitous and abundant non-histone chromatin binding protein first purified in the 1970s [5]. The protein belongs to the HMGBox (HMGB) family, which also includes HMGB2 and HMGB3. The proteins have a highly conserved structure and were named after their ability to migrate quickly during electrophoresis. The average cell has up to 106 HMGB1 molecules (Iori*et al.,* 2013). High mobility group box-1 (HMGB1) has been shown to be a key mediator of inflammatory diseases HMGB1 is a nuclear protein that triggers inflammation, binds to lipopolysaccharides (LPS) and IL-1, and initiates and synergizes with a Toll-like receptor (TLR) 4-mediated pro inflammatory response [7]. After pro-inflammatory stimulation, such as that by LPS, TNF-a, IL-1, IL-6 and IL-8, HMGB1 is actively released from activated monocytes and macrophages. Regulation of HMGB1 secretion is important for control of HMGB1-mediated inflammation and is dependent on various processes such as phosphorylation by calcium-dependent protein kinase C [8], as well as acetylation and methylation [9].In previous study, HMGB1 and TLR4 were involved in the generation and recurrence of seizures in experimental animals. [10]**.** Following seizure, IL-1β and HMGB1 are primarily expressed in microglia, although HMGB1 is also found in neurons [11]. Increased immunostaining for HMGB1 and its receptors, TLR4 and RAGE, has been demonstrated in the brain tissue of mice following induction of both kainate- and bicuculline-induced acute seizures microglia and astrocytes produce multiple inflammatory mediators in response to HMGB1 stimulation [12]. What is more, IL-1β can induce release of HMGB1 in human [13]. This suggests the possibility of a self-perpetuating feedback loop driven by both HMGB1 and IL-1β. In rodents, pre-treatment with intra-hippocampal HMGB1 and IL-β prior to treatment with bicuculline or kainate exacerbates seizures [12]. In contrast, intra-cerebral infusion of the endogenous IL-1R1 antagonist IL-1RA or its over-expression in astrocytes delays seizure onset and reduces duration following kainate orreduces seizure behavior following bicuculline treatment or electrically-induced SE [11]. Seizure onset is also delayed in mice lacking IL-1R1 [13].Similarly, selective inhibition of HMGB1 or TLR4 delays seizure onset and decreases seizure number and duration in both kainite and bicuculline-induced acute seizure models and reduces the number of spontaneous epileptic seizures in the kainate model of chronic epilepsy [12]. Knock-out of TLR4 or RAGE is also anticonvulsant in kainite models of acute and chronic seizures [14].Increased expression of IL-1β and HMGB1 signaling in a variety of experimental models and seizure disorders [(12- 15).]in addition to their established pro-convulsive effects [13].

MATERIALS AND METHODS

Patients

Seventy twofebrile seizure patients who visited to emergency department of Al-DiwaniyahMaternity and Children Teaching Hospital (Al-Diwaniyah, Iraq)from November2017to march2018. Blood was obtained from patients within 30 minutes of the time of seizure, and serum was immediately separated and frozen for subsequent HMGB1 assay. Patient inclusion criteria were age between 6 months and 6 years, body temperature ≥38.5°Cand presented no other identifiable cause of the seizure. Clinical data for familial febrile seizure history, earlier febrile seizure attacks, as well as duration and sings and symptoms of febrile seizures were obtained from the patients' parents. Family history was regarded as positive when febrile seizures occurred in first-degree relatives. Febrile seizure patients were classified into two types: simpletype for whom febrile seizures persist for

< 15 minutes, are generalized tonicclonic, and only occur once within 24 hours; and complex types for whom seizures persist for > 15 minutes, or are partial seizures, or recur within 24 hours of the initial attack. Control samples were collected from children(N = 80). Control groups were matched for age and sex and no known history of previous febrile seizures. Control blood serum was collected and frozen as above. For cytokine assay in order to subtract fever effects from the cytokine levels. Informed consent was obtained from each child's parents. Levels of HMGB1, were measured using commercially available, enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (for HMGB1 Wuhan Fine testChina)

RESULTS

The demographic characteristics of patients with febrile convulsion and control subjects are demonstrated in table (1).There was no significant difference in mean age between patients and control groups (P = 0.160), 25.89 ±15.70 months and 29.50 ±15.81 months, respectively, table (1). However, there was significant difference in the distribution of patients and control subjects according to one year age interval (P = 0.012), table (1). With respect to gender, there was no significant difference in the frequency distribution of patients and control subjects (P = 0.229), as shown in table 4.1. Patients' group included 29 (40.3 %) males and 43 (59.7 %) with a male: female ratio of 1.1.43.The Characteristics of febrile convulsions are shown in table (2). Mean duration of a single attack was 8.3 ±3.6 minutes with a range of 5 –15 minutes. The degree of temperature measured in Celsius ranged from 38.5 – 40 ºC with a mean of 39.0 ±0.2 ºC. According to frequency of recent attacks patients were categorized into 12 (16.7 %), 42 (58.3 %) and 18 (25.0 %) as single attack, two attacks and three attacks. According to type of febrile convulsion, patients were categorized into 23 (31.9 %) and 49 (68.1 %) as simple and complex, respectively. Positive family history of febrile convulsion was seen in 60 (83.3 %) and positive family history of epilepsy was seen in 17 (23.6 %).The serum levels of cytokines were expressed as median and inter-quartile range because they are not normally distributed quantitative variables according to Kolmogorove-Smirnov normality distribution statistical test. These levels are demonstrated in table (3) and figures (1). The serum level of HMGB1 in control group was 24.35 (4.55) ng/ml; whereas its level in patients group was 153.70 (54.83) ng/ml; the difference was highly significant (P< 0.001), being higher in patients group, table (4) and figure (1)Serum HMGB1 was not correlated to any of demographic characteristics or convulsion characteristics (P> 0.05), as shown in table (4).

Table 1: Demographic characteristics of patients and control groups

Characteristics	**Patients** ***n* = 72**	**Control** ***n* = 80**	***P***
Age (months)			
Mean ±SD	25.89 ±15.70	29.50 ±15.81	0.160 † NS
Range	5 -71	8 - 62	
1-12, *n* (%)	13 (18.1)	16 (20.0)	0.012 ¥ S
13-24, *n* (%)	31 (43.1)	20 (25.0)	
25-36, *n* (%)	13 (18.1)	20 (25.0)	

37-48, *n* (%)	6 (8.3)	16 (20.0)	
49-60, *n* (%)	5 (6.9)	0 (0.0)	
>60, *n* (%)	4 (5.6)	8 (10.0)	
Gender			
Male, *n* (%)	29 (40.3)	40 (50.0)	0.229 ¥ NS
Female, *n* (%)	43 (59.7)	40 (50.0)	
M:F ratio	1:1.48	1:1	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P \leq 0.05$; S: significant at $P \leq 0.05$

Table 2:Characteristics of febrile convulsions

Characteristic	Value
Duration (minutes)	
Range	5 - 15
Mean ±SD	8.3 ±3.6
Temperature	
Range	38.5 - 40
Mean ±SD	39.0 ±0.2
Attack	
1	12 (16.7 %)
2	42 (58.3 %)
3	18 (25.0 %)
Type	
Simple	23 (31.9 %)
Complex	49 (68.1 %)
Family history of febrile convulsion	60 (83.3 %)
Family history of epilepsy	17 (23.6 %)

Data were expressed as mean ± standard deviation, range or number (%)

Table 3: Serum levels of HMGB1 in patients and control group

Characteristic	**Control n = 80**		**Patients n = 72**		***P*** †
	Median	**IQR**	**Median**	**IQR**	
HMGB1 ng/ml	24.35	4.55	153.70	54.83	<0.001 HS

n: number of cases; IQR: inter-quartile range; †: Mann Whitney U test; HS: highly significant at $P \leq 0.01$

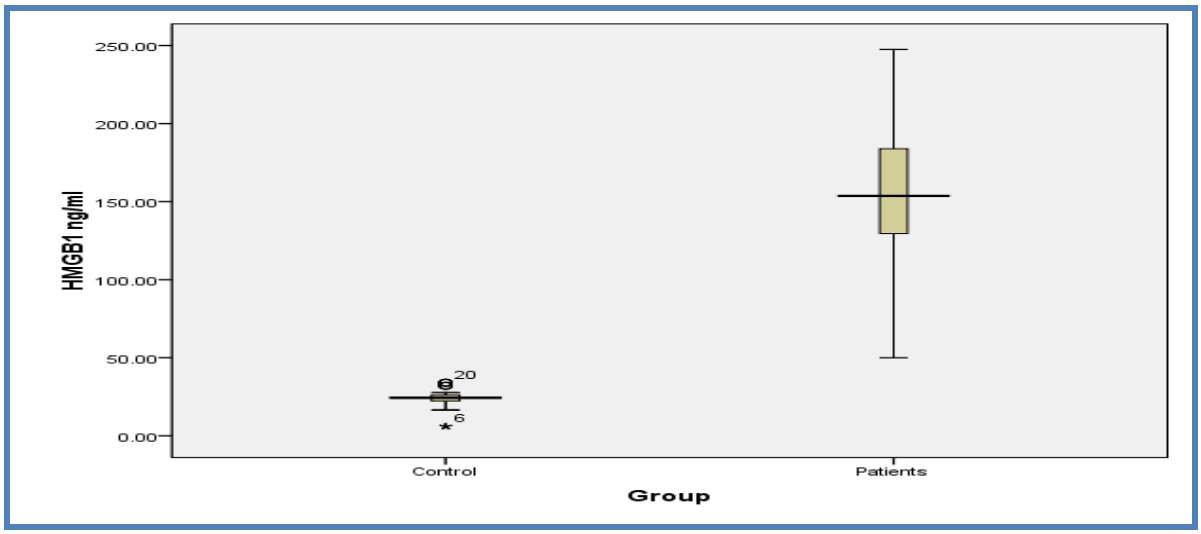

Figure 1: Serum level of HMGB1 in patients and control group

Table 4: Correlations of serum cytokine levels to demographic characteristics and febrile convulsion characteristics in patients group

Characteristic	HMGB1 ng/ml	
	r	*P*
Gender	-0.145	0.225
Age (months)	0.091	0.449
Temperature ° C	-0.145	0.225
1st FS versus recurrent	0.101	0.399
Type (simple versus complex)	-0.052	0.666
FH to FS	-0.021	0.860
FH to epilepsy	0.079	0.507
Duration of FS	0.119	0.320

*Significant at $P \leq 0.05$

DISCUSSION

The distribution of age in this study, table (1), demonstrated that the majority of cases with febrile convulsion 43.1%among children were in the age group of 13-24 month and collectively 79.3% of cases among children less than 36 month.[17]Studied paediatric patients in rural Kenya and found that the median age was 25 months.[18]Studied 520 paediatric patientswith seizure and found that the age of 74.6% of cases to be less than 6

years age. [19]Studied 200 children who were admitted with seizures as their presenting complaint, in their study 96.5% patients were in the age range of 1 month to 5 years. Most febrile seizures occur between 6 months and 3 years of age with the peak incidence at 18 months. And approximately 6–15% of it occurs after 4 years, and onset after 6 years is unusual. Regardless of the population, most data support the unique age specificity of the maturing brain's sensitivity to fever. Although the mechanism of this increased susceptibility is unclear, animal models suggest that there is enhanced neuronal excitability during the normal brain maturation[20].Regarding the genderin this study, 72 cases with febrile seizure were boys (40.3%) and the remainder were girls (59.7%). A definite female predominance was detected for febrile seizure with non-significant P value (p=0.229)with male to female ratio of 1:1.48 table (1).These results are inconsistence to the findings of a study conducted by [21] in Pakistan; he found that the male gender had a 1.3 times greater risk of febrile seizure. This is also supported by a study performed by [22] that quoted a slight predominance of febrile seizure in males.[23]Found that gender is an important factor in febrile seizure; in his study, 66% of the infants with febrile seizure were boys. [24]Also noted that febrile seizure is more frequent in boys than girls. While similar to present study, [26] revealed in their studythat 344,cases with febrile seizure were boys (42.7 %) and the remainder were girls (57.3%).Results of this study conducted that the temperature in Celsius ranged from 38.5 – 40ºC with a mean of 39.0 ±0.2ºC in febrile seizure cases table (2). By definition there has to be a febrile illness or certainly fever. Many febrile seizures occur early in the illness and may be the presenting feature;there are no data to support the rate of temperature rise as being more important than the peak temperature achieved. It is also unclear whether there may be a lower limit of fever under which it would be difficult to make a diagnosis of an FS, with some studies citing 38˚C and others, 39˚C. It is possible that the peak of the fever may be related to recurrent FS [20]. Previous study showed that high fever is a risk factor independently associated with febrile seizure recurrence. However these studies were performed in children known to be at risk for febrile seizures [24]. Also some studies suggested an association between the increased of fever and the risk of febrile seizures in general [25].and the number of fever episodes to be an independent risk factor for febrile seizures [26].Collectively 83.3% of FS cases with recurrent attack table (2), Most children with FS do not experience further FS, but one third will; age would appear to be the single, strongest, and most consistent risk factor for recurrence [28]. More than half of the risk is realized during the first year after the initial FS and over 90% recur within two years. A family history of febrile seizures (but not epilepsy) in a first degree relative, is associated with an increased risk of recurrence. Recurrences appear to be more likely in children whose initial FS occurred with a relatively low fever. Multiple initial seizures occurring during the same febrile episode alsoappear to be associated with an increased risk of recurrence [29].The present study conducted that the positive family history of seizures was seen in 60 (83.3%) cases.It is presumed that some genetic and early environmental factors increase the susceptibility for febrile seizures by lowering the seizure threshold [25].The most consistently identified risk factor for FS is the presence of a close family history (within first degree relatives) of FS. The more relatives affected, the greater the risk. In cohorts of children with FS, the risk that siblings will have an FS is 10–45%. So FS is a heterogeneous condition with an as yet unclear pathophysiological and genetic basis. Several studies have examined possible determinants of febrile seizures. Almost all have demonstrated that febrile seizures occur at a higher than expectedrate in first and second-degree relatives of children with febrile seizures, supporting a genetic factor [7].Indeed, there are a limited studies demonstrating the elevation of HMGB1 in the serum of febrile seizure patients, although the mean HMGB1 level in the serum of FS patients was found to be significantly higher than that in in healthy controls ($p < 0.001$),table (3) and figure (1).These outcomes are in accordance with the study by Choi *et al*.,(2011) which was the primary

review showing a significant height of HMGB1 in the serum of FS patients. Moreover, they announced that it was unrealistic to infer causality from distinct human reviews; however, their information recommended that HMGB1 may add to the era of FS in children. And also in accordance with the study by Mahmoud *et al.*,(2018) which they conclude that serum HMGB1 was significantly higher in patients with FSs, andsuggest that HMGB1 may contribute to the generation of FSs and may be used as good negative test for FSs. FunctionallyIt was thought that HMGB1 only act as a nuclear factor that enhances transcription; however, it was discovered to be an important cytokine that mediates the response to infection, inflammation, and injury. These observations had led to the emergence of a new field in immunology that was interested in understanding the mechanisms of HMGB1 release, its biological activities, and its pathological effects in different diseases [32].Found that the HMGB1 was act as a pro-inflammatory cytokine peripherally and evidence suggested that HMGB1 had the same action in the brain, but the role of HMGB1 in the central nervous system needs further examinations. Although many pro-inflammatory cytokines exhibit pyrogenic activity and increase interleukin-1 (IL-1) levels when injected directly into the brain, the effect of HMGB1 on core body temperature and hypothalamic IL-1 levels still need further workup [33].However some data revealed that HMGB1 may play a role as an endogenous pyrogen and supported that HMGB1 has a pro-inflammatory effect within the central nervous system [34]. And it is highly expressed in human epileptogenic brain, and antagonists of HMGB1 and TLR4 have been demonstrated to retard seizure precipitation and to decrease acute and chronic seizure recurrence in epilepsy animals so these findings suggested a role for the HMGB1- TLR4 axis in epilepsy [35]. Although it is not possible to infer causality from descriptive human studies, our data suggest that HMGB1 and the cytokine network may contribute to the generation of febrile seizures in children. Pro-inflammatory cytokine production may promote seizures, further exacerbate epilepsy, and may cause subsequent intractable epilepsy. If so, there may be a potential role for anti-inflammatory therapy targeting cytokines and HMGB1 as a novel therapeutic strategy to prevent or limit febrile seizures or subsequent epileptogenesis in the vulnerable, developing nervous system of children.

CONCLUSION

HMGB1 was significantly higher in febrile seizure children. Although it is not possible to infer causality from descriptive human studies, our data suggest that HMGB1 may contribute to the generation of febrile seizures in children. There may be a potential role for therapy targeting HMGB1 in preventing or limiting febrile seizures or subsequent epileptogenesis in the vulnerable, developing nervous system of children. in same samples.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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