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Relation between IL-1 β and IL1-ra in Pathogenesis of Febrile Convulsions

Seham Elsaid,* Manal Hafez,* Eman Saif Eldeen,*Hanan A EL-Hagrasy**

Neurology department,*Clinical pathology,** Faculty of Medicine for Girls, AL-Azhar University

ABSTRACT

Background: febrile seizures are the most common form of childhood seizures. Fever is induced by proinflammatory cytokines during infection. The cytokine network may contribute to the generation of febrile seizures in children. Interleukin beta (IL-1 β) and Interleukin receptor antagonist (IL-1ra) have been implicated in the pathogenesis of febrile seizures.

Aim of the work: was to estimate the role of IL-1 β and IL-1ra in febrile convulsions and their relation to pathogenesis of febrile convulsions.

Methods: serum interleukin I beta (IL-1 β) and interleukin 1 receptor antagonist (IL-1ra) were measured by ELISA technique in twenty five children with febrile seizure and twenty five age matched controls children with febrile illness without convulsion within 24 hours from onset of fever .

Results: serum IL-1 β and IL- 1ra levels were significantly higher in febrile seizure patients than in fever in controls. Serum IL-1 β and IL- 1ra levels were highly significant higher in complex febrile seizures than in simple febrile seizures. Serum levels of IL-1 β and IL- 1ra were significantly positive correlated with duration of seizure in patients group.

Conclusions: serum levels of IL-1 β and IL 1ra were significantly higher in febrile seizure children. Our study suggest that the inflammatory cytokine may play role in the generation of febrile seizures in children. These information may allow the development of anti- inflammatory therapy targeting these cytokines to prevent febrile seizures or subsequent epileptogenesis

Key words: febrile seizures, proinflammatory cytokines, Interleukin 1- β, Interleukin receptor 1 antagonist.

INTRODUCTION

Febrile seizures (FSs), associated with the rapid rise of body temperature, are the most common convulsions in childhood, occurring in 2%-5% of children before the age of 5 years.[1] While simple FSs (duration <15 min) are generally regarded as benign and have little influence on neurological activity, both complex FSs (duration 15-30 min) and febrile status epilepticus (SE, duration >30 min) are regarded as seizures that may predispose to later epilepsies including temporal lobe epilepsy (TLE) .[2] Thus, FSs are important and should be conquered. Both clinical and experimental studies have revealed that the components of the immune response involved in FSs may play a role in their pathogenesis .[3]

Several genes have been implicated in the susceptibility to febrile seizures, including those coding sodium channels, GABAA receptors, and interleukins. [4] IL-1 β allelic polymorphism in the promoter region at the -511 position is significantly higher in febrile seizure patients than in fever only children, and this polymorphism results in an increase in IL-1 β production .[5,6]

Pro-inflammatory cytokines may trigger febrile seizures. [7] IL-1 β increases glutamatergic neurotransmission and lowers the peak magnitude of GABA-mediated currents [8], supporting the role of pro-inflammatory cytokine contribution to the generation of fever-induced seizures. [7Also, IL-1 β prolongs the duration of electroencephalographic seizure. [9]

IL-1β down-regulates AMPA receptor expression and phosphorylation in a Ca2+- and NMDA-dependent manner in hippocampal neurons .[10] It reduces the frequency of AMPA-dependent spontaneous excitatory postsynaptic currents and miniature excitatory postsynaptic currents [11], but enhances NMDA receptor-mediated currents .[10, 11] Viviani *et al.* found that IL-1β increases NMDA receptor function by activating tyrosine kinases and subsequent NR2A/B subunit phosphorylation .[12] IL-1β increases the excitability of hippocampal CA1 neurons in the p38-dependent inhibition of the outward NMDA current .[13] These results

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showed that IL-1β facilitates excitatory neurotransmission. IL-1β can also depress the inhibitory system. It mediates the depression of GABA-induced current in cultured hippocampal neurons which is dose- dependent and is blocked by IL-1ra .[8] In addition, polymorphisms of the GABAA receptor gene and the IL-1Ra gene might be associated with susceptibility to FSs .[14-15] So these findings about the effects of IL-β on ion channels and transmitter receptors support the idea that IL-1β may affect the genesis of FSs; i.e., IL-1β enhances excitation and decreases inhibition, which in turn may cause an FS. Also, the effects of IL-1β on the excitability of neurons depend on many factors, such as the concentration of IL-1β, the functional state and the type of neuron, and the duration of time that the neuron is exposed to this cytokine.[3] IL-1Ra is structurally related to IL-1α and IL-1β and competes with these molecules to occupy IL-1β cell surface receptors. Thus IL-1ra can play an anticonvulsant role in FS genesis. The production of IL-1β is accompanied by the synthesis of 100- to 1 000-fold excess of the endogenous IL-1Ra, which rapidly occludes the activation of this receptor[16], and the maximal increase after stimulation is several hours later than IL-1β .[17] This may explain why the FS is auto-restricted. Overall, these findings suggest that the balance between IL-1β and IL-1ra during seizures plays a significant role in altering neuronal network excitability, thus affecting the maintenance and spread of seizures. [3]

PATIENTS AND METHODS:

This study was carried on two groups they were selected from emergency room and pediatric department of AL Zahra university hospital.

Group 1:

Included 25 children presented by febrile seizure (13 male, 12 female) their ages ranged from 9 – 60 months. Febrile Seizure patients were classified into two types: simple FS (n= 15)for whom febrile seizures persist for < 15 minutes, are generalized , and only occur once within 24 hours; and Complex FS (n=10)for whom seizures persist for > 15 minutes, or are partial seizures, or recur within 24 hours of the initial attack.

Patient inclusion criteria include: age from 6 months to 5 years, body temperature ≥38, seizure accompanied by fever without an obvious central nervous system (CNS)-invasive infection and no other identifiable cause of the seizure.

Exclusion criteria include: patients had seizures before FS, seizures after immunization procedures, patients with acute or remote neurologic insults and marked systematic dehydration.

Clinical data for family history of febrile seizure, earlier febrile seizure attacks less than 6 months, as well as duration and semiology of febrile seizures were obtained from the patients' parents.

Group II:

Control group were collected from children with febrile illness, but without convulsion (N = 25). Control groups were matched for age, sex and grade of fever and had no convulsions during the febrile illness and no known history of previous febrile seizures.

All patients and controls were subjected to the following:

1- Full history and complete general and neurological examination

2- Routine laboratory investigations (fasting blood sugar, serum calcium, liver function and kidney function tests, C reactive protein (C R P)

3-Serum interleukin I beta (IL-1β) and interleukin 1 receptor antagonist (IL -1ra) by ELISA technique, the serum cytokines were collected from the patients and controls within 24 hours (range: 1–24 h) following an attack of seizure and onset of fever

Cytokines measurement:

Five ml blood was drown by vein puncture under complete aseptic condition from patients and control subjects. Blood was left to clot, centrifuged and serum was separated, the serum was kept at -20°C until assayed. The serum cytokines were detected as: a) Pro-inflammatory: IL-1β and anti-inflammatory: IL-1ra and compared to the controls, cytokines were measured using commercially available, enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. The kits were delivered from

ASSAYPRO, USA for Assay Max Human Interleukin I beta ELISA Kit, catalog No. E 12200-1, Lot No.11401023 and from WKEA, MED SUPPLIES, USA for Human interleukin – 1 receptor antagonist ELISA Kit.

Statistical Analysis:

The Chi-square test was used to compare the clinical characteristics between febrile seizure patients and the controls. The independent sample t test and Mann-Whitney test were used to compare serum cytokine levels and laboratory findings between controls and febrile seizure patients and between simple FS and complex FS. The Pearson Correlation Coefficient was calculated to detect significant correlations between cytokine levels and (grade of fever, duration of seizure CRP and duration of illness). SPSS for windows version 22. (SPSS Inc., Chicago, USA) was used to perform the above tests. Data analysis results are given as mean and standard deviation (SD). Statistical analysis was performed to obtain the correlation and level of significance (p value < 0.05 will be considered significant p < 0.001 will be considered high significant and p >0.05 will be considered non-significant).

RESULTS

The results and data were collected and analyzed in tables (1-4) & fig 1- 6.

The children with febrile convulsions had highly significant increase of serum levels of IL-1β and IL-1Ra than febrile children without seizures within 24 hrs from onset of fever. There were statistically highly significant increase of serum levels of IL1β and IL1ra in children with complex febrile seizure than children presented by simple febrile convulsion. Serum levels of IL1β and IL-1ra have significant positive correlation with duration of seizure and CRP in patients group. Also there was significant positive correlation between IL-1β serum levels and IL-1ra. There was no significant correlation between serum levels of IL-β, IL-1ra and (number of seizures, grade of fever, and duration of illness).

DISSCUSION

Cytokines are hormonal mediators produced in body in response to defensive and growth phenomena. The role of these mediators in infectious, immunological, and inflammatory phenomena is of special interest. Cytokines include Interleukin and chemokines, IL-1β is the important cytokine. [18,19] One of the important role of IL-1β is direct and indirect modulating effects on neurons and neurotoxic neurotransmitters released during excitation or inflammation. [20]

The role of the immune system can explain some aspects of FS pathogenesis. The immune system influences the nervous system via cytokines produced by macrophages and lymphocytes .*Cartmell et al.* [21] demonstrated that interleukin receptors, e.g. IL-1, TNF, IL-6 are found in numerous brain sites, particularly in the hippocampus, already in the early stage of development. Hippocampal sites containing cytokine receptors are involved in temperature regulation. [21]

IL-1β directly modulates ion channels, enhancing NMDA and AMPA [10,11] and reducing the effectiveness of GABAA [8], which can change the excitability of neurons and may promote FS generation. So in an integrative way, IL-1 β can both enhance and reduce neural excitability (neurotoxic and neuroprotective effects) [22]. If the enhancing and reducing effects are balanced, an FS does not occur. Moreover, a mild imbalance could cause a simple FS while a severe imbalance could cause complex FSs and febrile SE. In addition, the interaction of IL-1 β, IL-1ra and the other inflammatory cytokines is also of great significance in the generation of FS .[3] *Helminen and Vesikari* were the first to show increased IL-1β production in peripheral blood mononuclear cells from FS patients after stimulation with lipopolysaccharide (LPS). [23] Leukocytes from children with FSs have an exaggerated IL-1 β response to stimulation with double- stranded RNA . [24]

Another study showed that the IL-1ra/IL-1β ratio is significantly higher in FS patients than control children. ([18] Since the production of IL-ra is stimulated by IL-1 β, the IL-ra increase is much later than that of IL-1 β .[17]

An association between polymorphism of the IL-1β (-511) promoter and susceptibility to febrile seizures has been reported. ***Virta et al*** [5] have demonstrated a significant allelic association between the IL-1β (-511) allele 2

and febrile seizures. In addition, ***Kanemoto et al*** have reported a significant association between theIL-1β (-511) allele 2 and prolonged febrile seizures. [6]

In our study there were highly significant increase of serum levels of IL-1β and IL-1Ra within 24 hours from convulsion in the children with febrile convulsions than in febrile children without seizures (Table 2).

These results are in agreement with **Choi *et al*** who found Serum HMGB1 and IL-1b levels were significantly higher in febrile seizure patients than in fever only controls. [25] ***Also Virta et al*** found that plasma IL-1RA/IL-1β ratio and plasma IL-6 within 24 hours from convulsion, were significantly higher in FS patients compared with control children. [18] However the ***Lahat et al*** ***and Ichiyama et al*** reported no difference in serum and CSF IL-1β levels between FS and control children. [26,27] ***Wang et al***. showed that this IL-1β decreases the Gabaergic transmission in hippocampal neurons. Gabaergic inhibition also depends on the interleukin concentration and can be blocked by IL-1ra .Thus, experimental studies suggest the role of IL-1β in the genesis of febrile seizures by the increase of excitation and the decrease of inhibition.[8]

In our study there were statistically highly significant increase of serum levels of IL1β and IL1ra in children with complex febrile seizure than children presented by simple febrile convulsion (table 3).

Choi *et al* found that the IL-1β levels were at an 8.1-fold increase in patients with recurrent febrile seizure attacks than those with first febrile seizure attack .[25]

Also we found that serum levels of IL1β and IL1ra, have significant positive correlation with duration of seizure and CRP in patients group (table 4).

These finding may explained by the prolonged febrile seizure associated with more brain excitability and disturbance of BBB which leading to more brain inflammation. C-reactive protein (CRP) is a sensitive marker of inflammation, so patients with +ve CRP induce more inflammation and secrete more cytokines.

In the regulation of inflammation, balance between proinflammatory cytokines may be more critical than single cytokine concentration. In animal models 100 fold molar excess of IL-ra was needed to prevent the response to IL-1β. [17] In our study we found that serum levels of IL1β, have significant positive correlation with IL-1ra (table 5).The production of IL1-ra is stimulated among other factors by IL1-β and high level of IL1-ra could therefore indicate pervious high IL1-ra production. [17]

The studies of *Virta et al.* related to the serum level of pro- and anti-inflammatory cytokines in children with FS demonstrated high positive correlation between the diagnosis of febrile seizures and the value of IL-1ra/LI 1-beta ratio [18]

Virta *et al* demonstrated that IL-1ra rapidly reduces seizure activity in experimental models. The analysis of different brain regions affected by seizures using c-fos mRNA as a marker suggests that IL-1ra reduces seizures by inhibiting their generalization from the hippocampus to the motor cortical areas. [18].

In Conclusion: inflammatory cytokines especially IL1-β plays an important role in the genesis of FSs which may cause subsequent epilepsy. Reducing the excitability effect of IL-1β and increasing the level of IL-1ra, may have an antagonistic effect on the FS and a novel therapeutic strategy to prevent or limit febrile seizures effect on the vulnerable immature brain of children.

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Table (1): Clinical and laboratory characteristics of the studied group

	Patients group (n=25)	**control group ((n=25**		**p-value**
Age (months)	31.6 ± 14.5	36 ± 12.2	t =1.189	P>0.05 NS
Sex (M/F)	13/12	11/14	X2 =1.245	P>0.05 NS
CRP	14.28± 6.66	12.2 ± 4.07	t =1.305	P>0.05 NS
Grade of fever	39.12±.71	38.9±.67	t =1.320	P>0.05 NS
Family history of FS(n) Positive Negative	 11 (44%) 14(56%)			
FS history (n) -First attack - Recurrent attacks	 9 (36%) 16 (64%)			
Seizure type Simple febrile seizure (n) Complex febrile seizure (n)	 15 (60%) 10 (40%)	 -		
Febrile illness (n) Pharyngitis Tonsillitis Respiratory tract infection Otitis media Gastroenteritis	 6 5 6 4 4	 7 6 4 3 4		
Duration of seizure (by minutes) Mean ± SD	9.25± 3.15	-		
Duration of illness (by months Mean ± SD	12.4 ± 4.1	-		

NS; non-significant at P>0.05

Table (2): Comparison between the patients group and control group as regard the serum levels of IL1β and IL1ra within 24 hrs from onset of fever

	patients group (n=25)	**Control group (n=25)**	**t**	***P*-value**
IL-1β Mean ± SD	8.82 ± 2.03	6.35 ± 1.44	2.008	P<0.01** HS
IL-1ra Mean ± SD	24.72 ± 5.69	8.42 ± 2.02	3.824	P<0.01** HS

HS; high significant at P<0.001**

This table shows the children with febrile convulsions had highly significant higher serum levels of IL-1β and IL-1Ra than febrile children without seizures within 24 hrs from onset of fever.

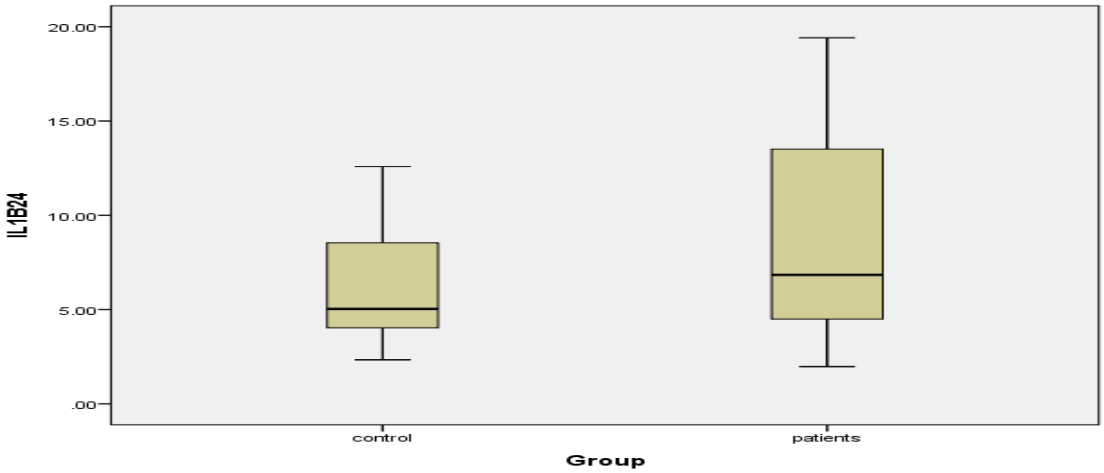

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Fig 1: serum levels of IL1β in patients and controls

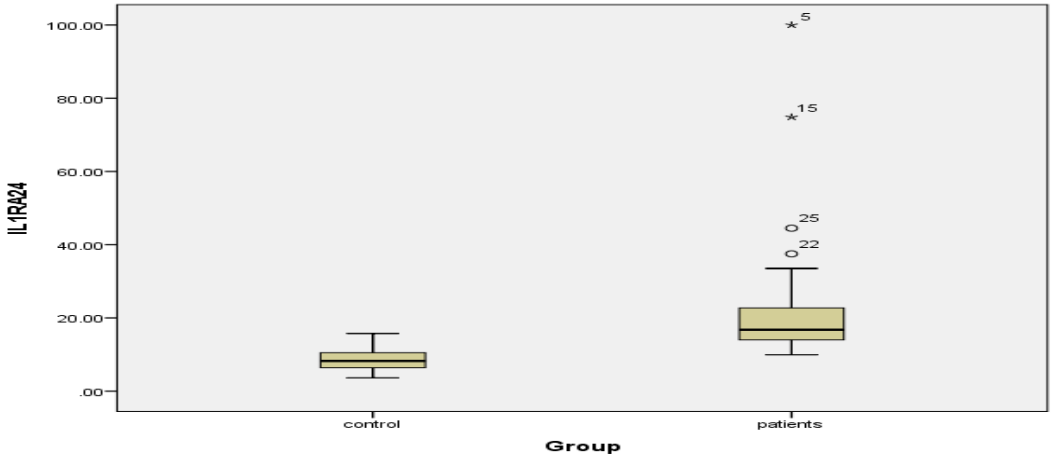

Fig 2; serum levels of IL1ra in patients and controls

Table (3): Comparison between simple FS and complex FS as regard the serum level of IL-1βandIL-1ra in patients group (n=25)

	Seizure type	**N**	**Mean ± SD**	***t***	***P* –value**
IL-1β	Simple	15	5.52 ±1.3	5.493	P<0.01** HS
	Complex	10	13.79±3.24		
IL1-ra	Simple	15	14.26±3.35	3.793	P<0.01** HS
	Complex	10	40.43±9.5		

HS; high significant at P<0.001 **

This table shows statistically highly significant increase of serum levels of IL1β and IL1ra in children with complex febrile seizure than children presented by simple febrile convulsion

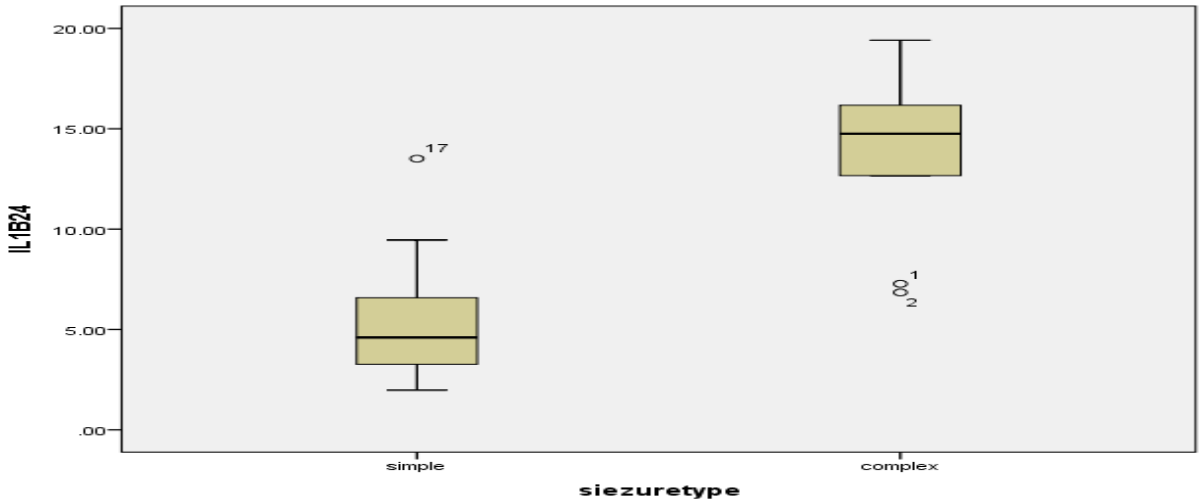

Fig 3; serum levels of IL1β in simple and complex FS patients

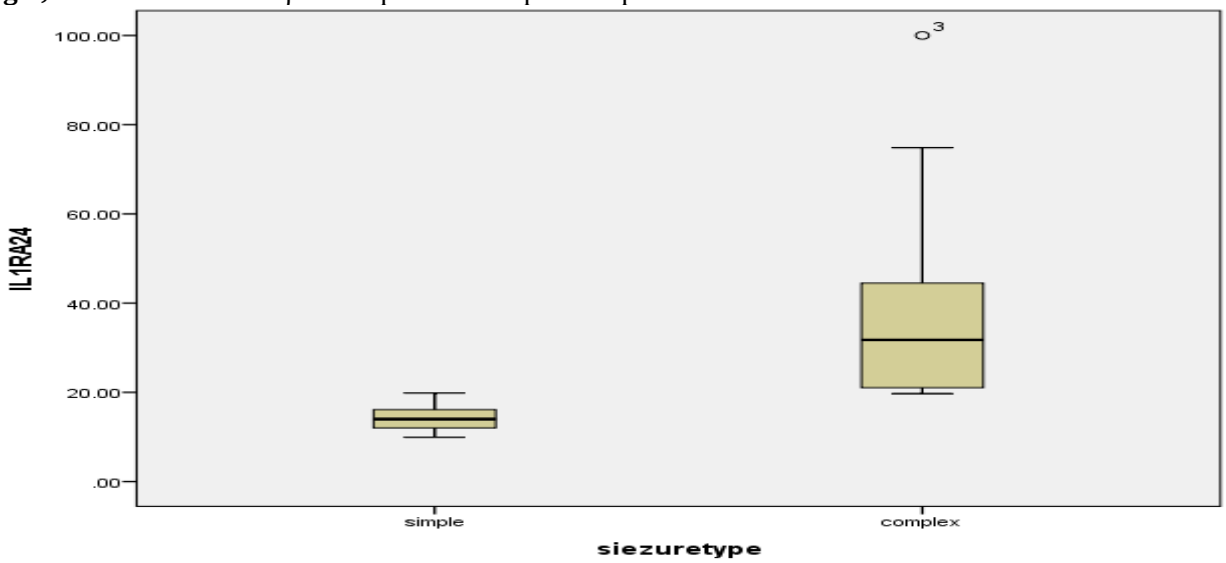

Fig 4; serum levels of IL1ra in simple and complex FS patients

Table (4): Correlation between serum level of IL-1β, IL-1ra and (number of seizures, grade of fever, CRP, duration of illness, duration of seizure) in patients group.

		Number of seizures	Grade of fever	CRP	Duration of illness	Duration of seizure
IL1β/24hr	R	.309	.108	.908**	.150	.928**
	P (n=25)	P>0.05 NS	P>0.05 NS	P<0.01** HS	P>0.05 NS	P<0.01** HS
IL1Ra/24hr	R	.141	.043	.722**	-.077	.799**
	P (n=25)	P>0.05 NS	P>0.05 NS	P<0.01** HS	P>0.05 NS	P<0.01** HS

** Correlation is high significant at P< 0.01 level * Correlation is significant at P < 0.05 level

* Correlation is non-significant at P > 0.05 level.

This table shows the serum levels of IL1β and IL1ra have significant positive correlation with duration of seizure and CRP in patients group. While there is no significant correlation between their serum levels and (number of seizures, grade of fever, duration of illness)

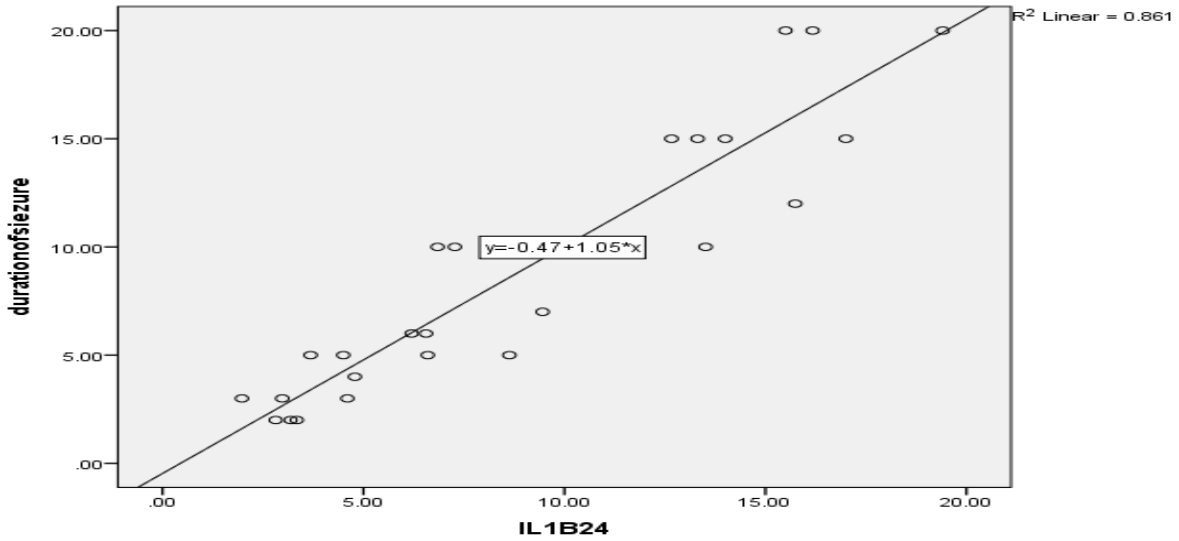

Fig 5; Correlation between serum level of IL1β and duration of seizure

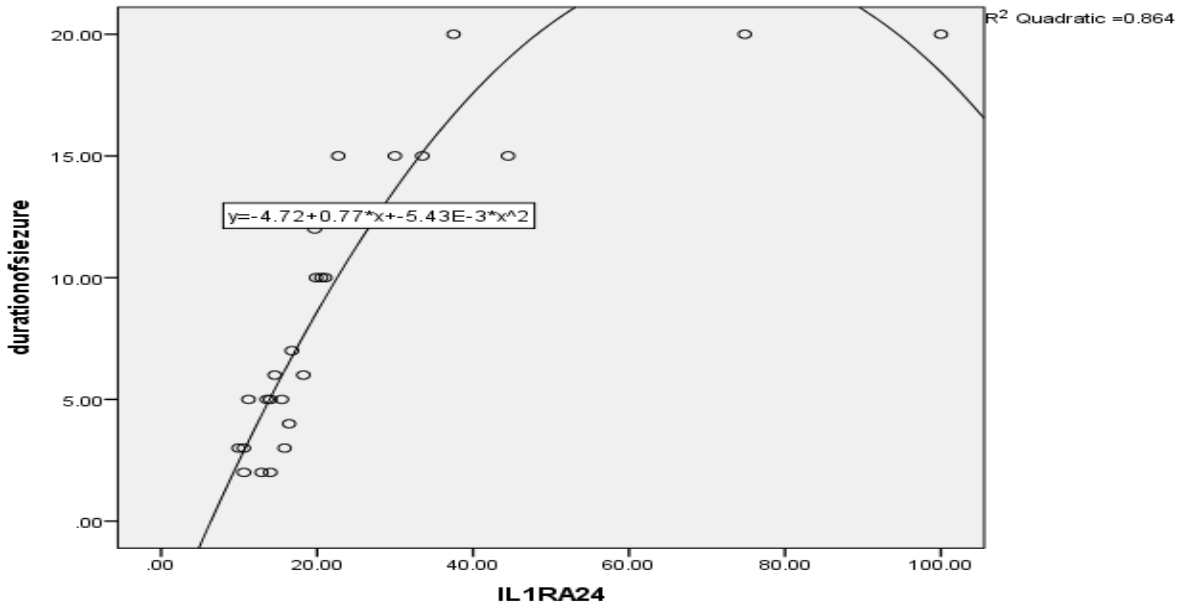

Fig 6; Correlation between serum level of IL1Ra/24hr and duration of seizure.

Table (5): Correlation between serum level of IL-1β and IL-1ra

		IL1ra
IL-β	R	0.755
	P (n=25)	P<0.01** HS

** Correlation is high significant at P< 0.01 level

This table shows significant positive correlation between serum levels of IL1β and IL1ra