Homozygote A2 allele of Interleukin 4 is Associated with Poor Outcome of Traumatic Brain Injury in Sudanese Patients

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Abstract
Background: Traumatic brain injury (TBI) is a major global health concern, as the most common cause of death in the developed countries. Several studies investigated IL-4 alleles in different cases. The rare alleles of these polymorphisms were found to be associated with high IL-4 expression.
Aims of the study: In the present study we aimed to investigate IL-4 gene and some biochemical profile in 51 Sudanese of TBI patients and compare between the outcome and il-4 alleles.
Material and methods: This is a cross sectional study that had been conducted in the National Center of Neurological Sciences, during January 2016 to March 2016.
Results: molecular screening of 51 TBI patients specimens showed that, the most common allele of IL-4 VNTR was A1/A1 allele in37.3%. Calcium results showed that 72.5% of the patients were present with hypocalcaemia (< 8.5 mg/dl).
Conclusion: the most common allele of IL-4 among TBI patients was A1/A2 allele, and hypocalcaemia was detected in 72% of the patients.
Key words: TBI, IL-4 VNTR, NCNS, Sudan

I. Introduction

Traumatic brain injury (TBI) is a non degenerative, non congenital insult to the brain from an external mechanical force, possibly leading to permanent or temporary impairment of cognitive, neurological, and psychosocial functions, with an associated diminished or altered state of consciousness[1]. Traumatic brain injury (TBI) is a major global health concern, as the most common cause of death in the developed countries [2]. TBI is responsible for high morbidity and mortality around the world [3]. The principal mechanisms of TBI are classified as focal brain damage due to contact injury types resulting in contusion, laceration, and intracranial hemorrhage or diffuse brain damage due to acceleration/deceleration injury types resulting in diffuse axonal injury or brain swelling.[4] Outcome from head injury is determined by two substantially different mechanisms, the primary insult occurring at the moment of trauma, this type of injury is exclusively sensitive to preventive but not therapeutic measures, the secondary insult (secondary damage, delayed non-mechanical damage) represents consecutive pathological processes initiated at the moment of injury with delayed clinical presentation. Cerebral ischaemia and intracranial hypertension refer to secondary insults and, in treatment terms, these types of injury are sensitive to therapeutic interventions. [5]

Under these conditions, neurons may act as "first responders," secreting signaling molecules that support tissue healing and repair rather than potentially deleterious proinflammatory ones [6].
One molecule that may be a part of this endogenous neuronal defense mechanism is interleukin-4 (IL-4). IL-4, a product of select immune cells, is a pleiotropic cytokine involved in the regulation of diverse immune and inflammatory responses [7]. It is one of the major cytokines that plays an important role in truma [8]
One of the most intriguing properties of IL-4 is that it polarizes macrophages toward the phenotype that is often termed M2 (or alternatively activated). M2 macrophages aid in the resolution of inflammation via increased trophic input and the augmentation of phagocytosis and proteolysis of dead, diseased cells/proteins, ultimately paving the way for tissue repair[9] [10]Numerous animal model studies representing a vast array of neurological diseases suggest that IL-4 may act as a therapeutic factor [11]

Population-based studies in countries such as South Africa (SA), Taiwan and India suggest even higher rates in developing countries accounted for primarily by road traffic accidents or motor vehicle accidents (MVAs). [12] Indeed, males in South-East Asia and Africa have the highest and second highest incidences of road traffic injury-related fatalities in the world [13]In the United States (US), where the overall incidence of TBI is 506.4 per 100 000 population [12]

TBI has been classified into primary and secondary injury. The primary injury is the result of the external mechanical force at the moment of trauma leading to skull fractures, brain contusions, lacerations, diffuse axonal injuries, vascular tearing and intracranial hemorrhages [14]The first stages of cerebral injury after TBI are characterized by direct tissue damage and impaired regulation of cerebral blood flow CBF and metabolism. This 'ischaemia-like' pattern leads to accumulation of lactic acid due to anaerobic glycolysis, increased membrane permeability, and consecutive oedema formation. Since the anaerobic metabolism is inadequate to maintain cellular energy states, the ATP-stores deplete and failure of energy-dependent membrane ion pumps occurs. The second stage of the pathophysiological cascade is characterized by terminal membrane depolarization along with excessive release of excitatory neurotransmitters (i.e. glutamate, aspartate), activation of N-methyl-D-aspartate, a-amino-3-hydroxy-5- methyl-4-isoxazolpropionate, and voltage-dependent $Ca^{++}$and Na^+-channels. The consecutive Ca^{++}- and Na^+-influx leads to self-digesting (catabolic) intracellular processes [15].

TBI is primarily and secondarily associated with a massive release of excitatory amino acid neurotransmitters, particularly glutamate[16 17].This excess in extracellular glutamate availability affects neurons and astrocytes and results in over-stimulation of ionotropic and metabotropic glutamate receptors with consecutive Ca^{++}, Na^+ and K^+-fluxes.[18 19] Although these events trigger catabolic processes including blood–brain barrier breakdown, the cellular attempt to compensate for ionic gradients increases Na^+/K^+-ATPase activity and in turn metabolic demand, creating a vicious circle of flow metabolism uncoupling to the cell.[20 21]

II.   Material And Method

The occurrence study is a cross-sectional study that had been performed at the National Center for Neurological Sciences (NCNS) during the period from January 2016 to March 2016.fifty one TBI patients were enrolled in the this study during the aforementioned period. The all patients were clinically and radiologically diagnosed as having TBI at the National Center of Neurological Sciences. The ethical approval was obtained from the local ethical committee at the NCNS. Blood specimens were obtained from 51 TBI patients treated at the National Center for Neurological Sciences, blood samples were drawn from each patient in two containers that contains (EDTA) and LI-heparin respectively, the EDTA samples were processed for DNA extraction, and the heperinized samples were processed for calcium and glucose estimations. Clinical and demographic data were collected using predesigned structural interview questionnaire.
The demographic and clinical data concerning each case were recorded (age, gender, residence, blood genotyping of IL-4 gene VNTR.

III.   DNA Extraction From Blood Samples

The DNA extraction was done by using chemical method,[22] into Falcon tube (15 ml),the followings were added, 2ml blood from each patient, and 10 ml red cell lyses buffer, then the tubes gently were mixed , after this step the tubes were centrifuged at 6000 RPM for 10 minutes , the above mentioned step was repeated until clear white pallet appear , after this steps 2 ml from White cell lyses buffer, 1 ml from guanidine chloride , 350 µl of ammonium acetate and 20 µl of proteinase K were added ,after that the all tubes were vortexed and then incubated at 37 °C for overnight, following the incubation ,2 ml from pre chilled chloroform was added, the tubes were mixed by using vortex mixer , after that the tubes were centrifuged at 6000 RPM for 10 minutes. After that the supernatant was transferred into a new Falcon tube (15 ml) ,and then 5 ml of pre chilled ethanol was added to each tube for completion of DNA precipitation, and then the tubes were incubated at -20 °C for 2 hours , after this step, the tubes were centrifuged at 6000 RPM for 10 minutes , after pouring the ethanol, 4 ml of 70% alcohol was added to each tube and then the tubes were centrifuged at 6000 RPM for 10 minutes, the 70% alcohol was discarded into disposal bottle, and then the tubes were left to air dry, after that, 100 µl of de-ionized water was added, then the tubes were incubated at 4°C for completion of DNA elution. For polymerase chain reaction, 18µl of readymade MM and 2 µl of DNA were added into each PCR tube.

Primers for IL-4 gene was 5 gta aat agg ctg aaa ggg gga aa 3 forward primer and 5 cat ctt ttc ctc ccc tgt atc tt 3 , reverse primer were used for amplification of allele 1 and 2 at intron 3 of IL-4 gene (allele 1=340bp and allele 2=272 bp). Then the amplified PCR products were separated using 2% gel electrophoresis and then the separated DNA was visualized using UV light.

IV.   Result

A total of 51 Traumatic brain injury patients were enrolled in the present study Genotyping of IL-4 gene showed that , the most common allele of IL-4 variants was A1/A1 allele in 37.3% of TBI patients. This was followed by A1/A2 allele in 35.3% of TBI patients. (Table 1) The frequencies of TBI types showed that, acute hemorrhagic contusion was detected in 23.5% of TBI patients, followed by acute subdural hematoma in 17.6% of the patients (Table 2). Out of the 51 TBI patients, 43 of whom were experienced good outcome. And the remaining 7 patients had poor outcome according to (GCS) (table 3) .According to the biochemical

screening of TBI patients, Calcium results showed that 72.5% of the patients were present with hypocalcaemia (< 8.5 mg/dl). Hypercalcaemia (> 10.5 mg/dl) was detected in 7.8% of the TBI patients (table 4). The findings of the present study showed that, IL-4 alleles was significantly correlated with the outcomes of TBI patients P = 0.015 (Table 5). Cross-tabulation of IL-4 and types of TBI is displayed in (Table 6). Cross-tabulation results of glucose were displayed in Table (7)

Table 1. Shows the frequency of IL-4 alleles among TBI patients

IL-4 alleles	Frequency	percent
A1/A1	19	37.3%
A2/A2	13	25.5%
A1/A2	18	35.3%
Total	50	89.0%
Missing system	1	2.0%
Total	51	100.0%

Table 2: shows the frequencies of TBI types

Type of traumatic brain injury	Frequency	percent
Brain edema	3	5.9%
Heamorrhage contusion	12	23.5%
Intercerebral heamorrhage	1	2.0%
Axonal injury	2	3.9%
Extra dural heamatoma	6	11.8%
Subdural heamatoma	9	17.6%
Edema +contusion	4	7.8%
Bullet injury	1	2.0%
SDH + Contusion	3	5.9%
SAH	3	5.9%
EDH,SDH.HC	1	2.0%
SAH+edema	2	3.9%
HC+SAH	4	7.8%
Total	51	100.0

Table 3: shows the frequencies of TBI outcome

Outcome	Frequency	percent
Discharge	43	84.3
Death	7	13.7
Total	50	98.0
Missing system	1	2.0
Total	51	100.0

Table 4 shows the frequency of calcium level in TBI patients

Calcium	Frequency	percent
Valid <8.5	37	72.5%
>10.5	4	7.8%
8.5-10.5	9	17.6%
8.50	1	2.0%
Total	51	100.0%

Table 5 shows the cross-tabulation of Il-4 alleles and outcome

		Outcome		Total
		Discharge	Death	
IL-4	A1/A1	18	1	19
	A2/A2	8	5	13
	A1/A2	16	1	17
Total		42	7	49

Table 6 shows the cross-tabulation of IL-4 alleles and types of TBI

Type of TBI	IL-4			TOTAL
	A1/A1	A2/A2	A1/A2	
Brain edema	1	1	1	3
Heamorrhage contusion	3	4	5	12
Itercerebral heamorrhage	0	0	1	1
Axonal injury	1	0	1	2
Extra dural heamatoma	4	1	1	6
Subdural heamatoma	4	1	4	9
Edema +contusion	1	1	2	4
Bullet injury	0	0	1	1
SDH + Contusion	2	1	0	3
SAH	1	2	0	3
EDH,SDH.HC	0	1	0	1
SAH+edema	1	0	1	2
HC+SAH	1	1	1	3
Total	19	13	18	50

Table 7: shows the Cross-tabulation of glucose and IL-4 alleles

Glucose mg/dl	IL-4 alleles			Total
	A1/A1	A2/A2	A1/A2	
Less than 70 mg/dl	2	4	3	9
71-110 mg/dl	9	4	6	19
111-200 mg/dl	8	5	9	22
Total	19	13	18	50
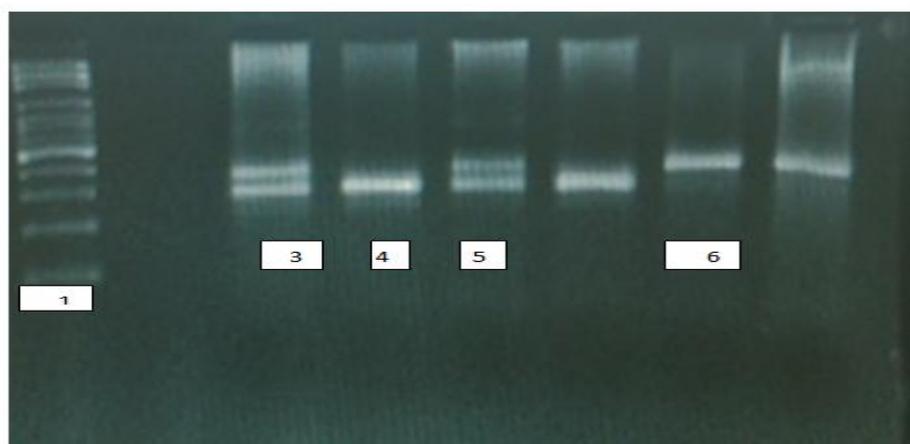
This figure shows gel electrophoresis for IL-4 alleles, lane 1 (100bp ladder),

lane 3and 5 shows heterozygote 1/2 alleles, lane 4 shows homozygote 2/2

allele and lane 6 shows homozygote 1/1 allele

V.   Discussion

Traumatic brain injury are classified into two main types, primary brain damage resulting in contusion, and intracranial hemorrhage and secondary brain damage resulting in diffuse axonal injury and brain swelling[23, 24]. Outcome from head injury can be classified into two categories, primary insult occurring at the time of impact [23.] And secondary insult (non-mechanical damage) initiated at the time of injury with delayed clinical presentation. Hypertension, cerebral ischemia and intracranial hypertension are refered to secondary insults. [23, 24]Traumatic brain injury (TBI) is a major cause of death and disability in global wide, as well as in under developing countries, 2.5 million of people in U.S were suffering from bad outcome. Many head injured patients die or survive with bad outcome even after mild or moderate head injury. [25, 26]

The results of this study showed that among the 51 TBI patients, male were 46 and females were 5.The male predominance in this study did not differ from the international one [27]. The distribution of the ages of the studied cases revealed that 82% of the patients were under the age of 40 years, this finding did not differ from the international incidence of TBI age group in male and females [28].in this study the most common type of TBI was the acute hemorrghic contusion in 23.5%. Several studies were indicated that, the major cytokines that play

an important role in trauma include tumor necrosis factor–alpha (TNF-α), and interleukins 1β, 2, 6, 8 [29, 30, 31] and IL-4 [8] .down regulation of inflammation and minimizing of cytotoxicity events can be mediated by anti-inflammatory cytokines such as IL-4, IL-10 and TGF-β1 [32, 33]. The role of microglia in the healthy CNS has shown to be, as an environmental surveillance, to maintain homeostasis [34].Once microglia recognizes a foreign substance they become activated. As macrophage-like cells of the brain, one of the main roles of activated microglia is that of regulating CNS innate immunity and initiating appropriate responses, such as inflammation. In the brain, this inflammatory response, termed neuroinflammation, is a crucial response needed to protect the CNS; uncontrolled or persistent neuroinflammation can lead to cellular damage. This is particularly relevant to neurodegenerative diseases, and evidence of microglial activation and neuroinflammation [35]. When microglia was been activated this activation can be shown into two different states [36]. The first is classical activation, which is accompanied by the production of inflammatory cytokines and reactive oxygen species, while anti inflammatory response is associated with the alternative activation, in which microglia involved in wound repair and debris clearance [37]. During neurodegenerative disease, in which the dominance feature is neuroinflammation, the alternatively activated microglia has a beneficial role in resolving pathology. Stein and colleagues observed that interleukin4 (IL-4) induced macrophages to express the mannose receptor [38]. Interferon-γ produced from Th1 cells was found to be instrumental in polarizing macrophages to M1 [39]. Microglia and astrocytes have also been observed to do same action [40,41], In many cases, this response is down regulated once the damage or pathogen has been cleared; however, unregulated, or chronic inflammation can lead to tissue destruction [42] the alternatively activated macrophages express cytokines and receptors that can inhibiting inflammation and maintaining homeostasis. M1 macrophages has been associated with proinflammatory, while in contrast the Th2 cytokine IL-4 has been associated with M2, or alternative, activation. The first induce alternative activation was IL-4 [38]. This type of activation has been classified as'M2a'. The main function of alternative activation in which the IL 4 is involved, is to suppress the inflammatory reaction and minimize cellular damage. As we mentioned before we aimed in this study, to find any correlation between IL 4 VNTR with traumatic brain injury outcome, and for our knowledge, IL-4 alleles were investigated for the first time in TBI patients in Sudan. The findings of this result showed that, the most common allele of IL 4 genotyping was A1/A1 allele followed by A2/A2 allele in TBI patients. In the present study homozygote allele (A2/A2) was significantly associated with the poor outcome according to the Glasgow coma score (GCS), p=0.015. However, our result differ from Tsai findings, the findings of Tsai showed that there is no correlation between IL-4 gene intron 3 VNTR with oral cancer patients). [42] Several studies investigated IL-4 alleles in different types of nonmalignant cases. Study done by Buchs et al found that the allele 2 is associated with protection against destructive polyartheritis.[43] Tsai et al were suggested that IL-4 intron 3 polymorphism has no role as a predictive marker for febrile seizure and epilepsy in children [44]. However, IL-4 VNTR variants were associated with the susceptibility for several diseases, including rheumatoid arthritis [45] atopic dermatitis, Graves' disease and multiple sclerosis [46]. Several investigators found that there were no association between IL-4 polymorphism and specific diseases. IL-4 alleles were seemed to be associated with susceptibility to some immunological illnesses, including multiple sclerosis [47] myasthenia gravis [48].This differences may be due to our small sample size and different biological behavior for different diseases.

In traumatic brain injury reduced blood flow and oxygen metabolism in the brain promotes a metabolic switch from aerobic process to an anaerobic program. Lactate is a marker of anaerobic respiration. The findings of this study showed that glucose level of >80 mg/dl was in 31.4 %of the TBI patients, 19% of whom were present with poor outcome according to Glasgow coma score (GCS). Several studies were investigated glucose level in TBI [49, 50, 51]. The findings of other study indicated that, in later phases of TBI pathophysiology, large variations in glucose levels have been associated with worse long-term outcomes, suggesting a more complicated metabolic relationship [52].

In the present study hypocalcaemia was indicated in 72% of TBI patients, 19.4% of those patients were associated with poor outcome according to (GCS). Different studies showed that, the accumulation of calcium intracellular is an indicator for cell death, by means of its apoptotic properties [53, 54]. Authors of different publications were mentioned that, the cellular changes can leads to excessive production of toxic reaction products, such as free radicals [53, 54, 55, 56, 57, 58, 59].Another study showed, that the accumulation of intracellular calcium does not always result in cell death, but affects the metabolic machinery of the mitochondria and subsequently rendering the cell vulnerable to energy failure [60]. In our study hypocalcaemia was present in 72% of studied material from whom 19.4% were suffering from bad outcome. Studying calcium as a prognostic factor in TBI patient's in their secondary phase outcome, becomes a significant issue, to overcome secondary insults such as increased body temperature, seizures, hypotension and hypoxia.

VI. Conclusion

In this study we concluded that, the most common allele of IL-4 among TBI patients was A1/A2 allele. Furthermore homozygote A2 allele was found to be associated with bad out come. Hypocalcaemia was detected in 72% of the patients.

Acknowledgment

Refrances

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