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Original Article

The Significance of Mutation in *IL-1β* Gene and Circulatory Level for Prediction of Trauma Severity and Outcome in Traumatic Cerebral Hemorrhagic Contusion

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Background: Traumatic brain injury (TBI) is a leading cause of death, disability, and resources consumption. Cerebral hemorrhagic contusions are primary brain lesion and often one of the most visible lesions following TBIs. Interleukin-one beta (*IL-1β*) is pro-inflammatory cytokines it is circulatory level and gene have been implicated in secondary brain injury and worse outcome following TBIs. This study is to determine the significance role of *IL-1β* gene polymorphism (-511C/T) and circulatory level for prediction trauma severity and outcome in traumatic cerebral hemorrhagic contusion.
Methods: The study population includes 90 Sudanese patients with traumatic cerebral hemorrhagic and 90 apparently healthy individuals as control. IL-1β serum concentration was measured using enzyme-linked immunosorbent assay and *IL-1β* gene was genotyped using restriction fragment length polymorphism-polymerase chain reaction.
Results: Significant elevation of IL-1β level was seen among trauma patients compared to control (p-value < 0.001). Although there was no significant association between IL-1β level with trauma severity or death; IL-1β level was higher in severe brain injures compared with moderate and mild one, and the mean concentration of IL-1β was high (18.75 pg/mL) among patient developed poor outcome compared to survivals (15.17 pg/mL). T recessive allele of *IL-1β* gene was detected in 13.3% of participant. The highest circulatory level of IL-1β (17.8 pg/mL) was observed among patients with TT homozygous alleles. *IL-1β* gene polymorphism was not associated with trauma severity and death.
Conclusions: IL-1β circulatory level was varied according to trauma severity and highly levels were seen among patients developed unfavorable outcome. *IL-1β*-511C/T gene was not associated with trauma severity and outcome.

Key words: *cerebral hemorrhagic contusion, secondary brain injury, IL-1β*

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Introduction

Interleukin one beta gene (*IL-1β*) gene, mapped to the chromosomal locus 2q13-21, is the most important member of the IL-1 family. It is produced by numerous cell types, of the innate immune system cells as macrophages, and monocytes. This cytokine is also produced by brain parenchyma, neurons, and astrocytes after brain ischemic insult and its levels are increased after trauma.[1] IL-1β is also considered an important mediator of inflammation after cerebrovascular ischemia.[2] A C/T base substitution in the promoter region (position-511) of *IL-1β* gene has been identified; thus, individuals with TT genotype produce significantly increased IL-1β protein followed by those with TC genotype, and the least CC genotype.[3] Moreover, in clinical studies *IL-1β* gene polymorphism was used in prediction of outcome following traumatic brain injuries (TBIs).[4] Cerebral hemorrhagic contusion resulted from primary injury correlated with poor clinical prognosis, and can progress leading to delayed neurological deterioration.[5] In Sudan, traumatic cerebral hemorrhagic contusion is considered as one of the most common computed tomography finding constitute 23.5% of total types of TBIs.[6] Patients with similar amount of cerebral hemorrhage on admission imaging may end up with different neurological outcomes or variable response to therapy. The potential use of immunological, and genetics biomarkers to predict the prognosis of brain injury to choose and improve treatments options may help in reducing complications and morbidity.

The aim of the present study was to determine the significance role of *IL-1β* gene polymorphism (-511C/T) and circulatory level for prediction of trauma severity and outcome in traumatic cerebral hemorrhagic contusion.

Methods

Ethical Approval

The ethical approval was obtained from Ethical Review Board of National Center for Neurological Sciences (NCNS).

Study Populations

A total of 90 Sudanese patients presented with traumatic cerebral hemorrhagic contusion to the NCNS Khartoum, Sudan from December 2015 to January 2018 were enrolled in the present study. In addition, 90 apparently healthy individuals were recruited from blood bank donors as control. Donors were examined clinically and proved to be free from chronic diseases and blood infections (Table 1). Patients were excluded if they are non-Sudanese patients, had hemorrhagic contusion associated with other type of brain bleeding, had attending the emergency department more than three days after trauma, had infectious diseases during hospitalization, and had major systemic diseases like end stage renal diseases, conjunctive heart failure or hypertensive patient.

Table 1. Demographic variables of case and control groups

Variable	Case (n = 90)	Control (n = 90)
Mean age (years)	29.19	29.36
Gender		
Male	84	86
Female	6	4

Laboratory Assay

Two blood samples were collected from each patient upon admission to emergency department; one in ethylenediamin tetra-acetic-acid for *IL-1β* genotyping and the second in plain container for serum IL-1β measurement. Serum was separated by centrifugation at 2000 rpm for 5 minutes; then stored at -20°C for subsequent testing. The IL-1β serum concentration was measured using Sandwich Enzyme-Linked Immunosorbent Assay (TECAN Group Ltd., Männedorf, Switzerland) based on kits protocol (AssayMAX™, Assaypro LLC, St. Charles, MO, USA).

Molecular Genetic Analysis

DNA was extracted from whole blood using QIAGEN® commercial DNA extraction kites (QIAGEN, Germantown, MD, USA). DNA quantity and quality was measures using NanoDrop® spectrophotometer and gel electrophoresis (Cleaver Gel Documentation system, Cleaver Scientific Ltd., Rugby, Warwickshire, UK). *IL-1β* gene was genotyped using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). The following primers were used to amplify the target region in (5-TGGCATTGATCTGGTTCATC-3 forward, and 5-GTTTAGGAATCTTCCCACTT -3' reverse). Poly-

merase chain reaction (PCR) was conducted into 20 µL reaction volume that contains 4 µL of 5x FIREPol® master mix (Solis BioDyne, Tartu, Estonia), 1 µL forward primer, 1 µL reveres primer, 1 µL DNA and 13 µL distil water. The PCR reaction condition included (initial denaturation 95°C/3 minutes, denaturation 95°C/30 seconds, annealing 55°C/30 seconds, extension 72°C/30 seconds, and final extension 72°C/10 minutes for 35 cycles (TC-3000 Thermal Cycler-PCR, Bibby Scientific Ltd., Staffordshire, UK). PCR product was digested with AVaI restriction enzyme (New England Biolabs®, Ipswich, MA, USA). The total reaction volume was 25 µL (1 µL AVaI enzyme + PCR product + NEB buffer + H_2O). The mixture was incubated overnight at 37°C. After overnight incubation the enzyme inactivated at 80°C for 20 minutes. The digested product was separated on 2% agarose gel electrophoresis stained with ethidium bromide and visualized under ultra violet light (Fig. 1).

Statistical Analysis

Data analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19 (IBM Corp., Armonk, NY, USA). The qualitative data described as frequency and percent, while quantitative data was described as (mean ± standard deviation). The chi-square test for independence was used to test the relations between qualitative nominal variables, while the Pearson correlation test was used in cases for quantitative variables. The independent sample test used to test the difference between two groups, and analysis of variance in cases of three groups.

Results

ELISA results showed that the mean concentration of IL-1β was 15.49 pg/mL in case group compared with 3 pg/mL in the control group (p-value < 0.001) (Fig. 2). Although there was no significant association between IL-1β level with trauma severity, IL-1β level was higher in severe brain injures compared with moderate and mild one. There was no significant association between IL-1β level and patients admitted either at day one, or at day two or day three after trauma, and presence of brain edema. Although there was no significant association between high level of IL-1β and death, the mean concentration of IL-1β was high (18.75 pg/mL) among patient developed poor outcome compared to survivals (15.17 pg/mL) (Table 2). T recessive allele of *IL-1β* gene was detected in 13.33% of participant (Table 3). The highest circulatory level of IL-1β (17.8 pg/mL) was observed among patient with TT homozygous alleles, followed by (16.28 pg/mL) CC homozygous allele and (10.64 pg/mL) for CT heterozygous alleles (Fig. 3). *IL-1β* gene polymorphism was not associated with trauma severity, brain edema, and outcome (Table 4).

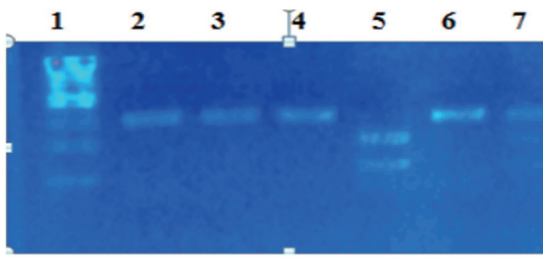

Fig. 1. Polymerase chain reaction products of *IL-1β* gene digested with AvaI restriction enzyme.
Lane l: DNA ladder. Lane 2, 3, 4, 6: CC homozygous alleles (band at 304 bp). Lane 7: CT heterozygous alleles (band at 304, 190, 114 bp). And Lane 5: TT homozygous alleles (band at 114 bp and 190 bp).

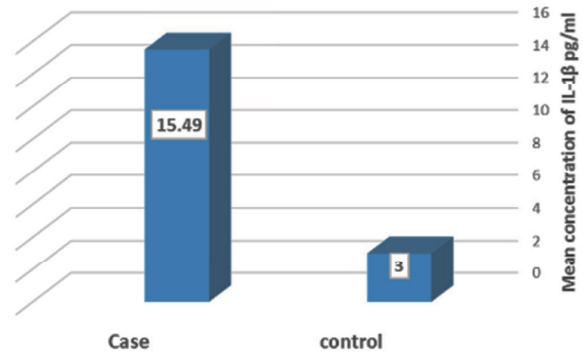

Fig. 2. Mean concentration of IL-1β, among case and control (p value < 0.001).

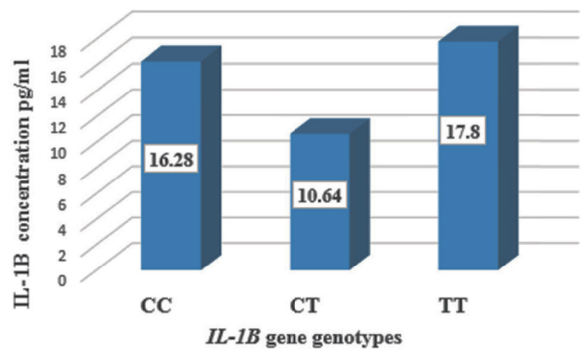

Fig. 3. Association between *IL-1β* genotypes and IL-1β circulatory levels pg/mL.

Table 2. Association between IL-1β level pg/mL with clinical features and outcome of the patients

Variable	Mean concentration pg/mL	*p*-value
Time after trauma		
Day 1	16.16	0.083
Day 2	11.82	
Day 3	16.98	
GCS		
Mild injury (13–15)	14.17	0.905
Moderate injury (8–12)	17.04	
Sever injury (< 8)	18.30	
Presence of brain edema		
Yes	11.60	0.564
No	16.60	
Outcome		
Death	18.75	0.204
Discharge	15.17	

GCS: Glasgow Coma Scale.

Table 3. Distribution of *IL-1β*-511C/T genotype and allele frequencies in patients

Characteristic	N (%)
IL-1β-511C/T genotype	
CC	71 (78.9)
CT	14 (15.6)
TT	5 (5.5)
Total	90 (100)
Allelic frequencies of I*L-1β*-511C/T gene	
C (wild allele)	156 (86.67)
T (recessive allele)	24 (13.33)
Total	180 (100)

Discussion

It is well known that in many central nervous system (CNS) disorders including stroke, Parkinson's, Alzheimer's or any disease associated with neuro-degeneration, there are increased IL-1 levels, mainly IL-1β.[7] Moreover, in clinical studies IL-1β was detected in CNS and brain post-mortem tissue of patients with head injuries.[8] Our finding demonstrated significant elevation of IL-1β in case compared to normal healthy control, and it is harmonious with previous finding.[9] Inconsistencies to our finding, Ferreira et al.[10] stated there was no significant increasing of IL-1β levels among TBIs cases compared to normal healthy controls in their study.[10] The discrepancy of results between different studies may be due to different methodology used in IL-1β detection or the time after trauma at which IL-1β level was measured. Significant association between IL-1β and injury severity was reported previously.[11] In rate model of TBIs IL-1β is heightened and prolonged when injury is followed by period of ischemia, this suggests that IL-1β may reveal various degrees of brain damage with over-lapping insult.[12] Our finding demonstrated non-significant association between IL-1β level with trauma severity; the highest concentration (18.3 pg/mL) of IL-1β among severe injuries compared to moderate injury (17.04 pg/mL) and mild one (14.17 pg/mL). However, data is still mixed with regard to the ability of cytokines to distinguish injury severity or predict outcome. There is increasing evidence for more complex roles of IL-1β in neuronal injury and survival that not only depend upon their concentration but also depend on the timing of their expression after injury so that level of IL-1β correlated with degrees of brain injury, and in case of severe injury; IL-1β is significantly enhanced and prolonged.[13] In experimental animals IL-1β was significantly induced by TBIs as early as 3 h after trauma peaking at 12 h and remaining for 48 h post-injury.[14] Gene expression study in post-mortem brain tissue from TBIs patients found that IL-1β was up regulated in individuals who died 6–12 h post injury. Thus IL-1β levels are elevat-

Table 4. Association between *IL-1β*-511C/T genotypes with clinical features and outcome

Variable	*IL-1β* gene genotype			*p*-value
	CC	CT	TT	
GCS				
Mild injury (13–15) (%)	39 (54.9)	11 (78)	3 (60)	0.087
Moderate injury (8–12) (%)	24 (33.8)	3 (21.4)	0 (0)	
Sever injury (< 8) (%)	8 (11.3)	0	2 (40)	
Total	71	14	5	
Presence of brain edema				
Yes (%)	15 (21.1)	4 (28.6)	1 (20)	0.793
No (%)	56 (78.9)	10 (71.4)	4 (80)	
Total	71	14	5	
Outcome				
Death (%)	5 (7)	1 (7.1)	2 (40)	0.102
Discharge (%)	66 (93)	13 (92.9)	3 (60)	
Total	71	14	5	

GCS: Glasgow Coma Scale.

ed during the period of secondary injury after TBIs, which has been postulated to also be an important time period for eliptogenesis process.[8] In this study no significant association between IL-1β level and patients admitted in day one, day two, or day three after trauma.

IL-1β play role in blood brain barriers breakdown and edema formation.[15] Interestingly, anti-IL-1β antibody was reported to decreased edema.[16] However, our finding demonstrated non-significant association between brain edema and high IL-1β level. Studies among severe brain injury patients reflected that high cerebral-spinal fluid concentrations of IL-1β were associated with poor outcome and increased intracranial pressure.[17] Our finding reflected non-significant association between high concentration of IL-1β and death, while the mean concentration of IL-1β among patients developed poor outcome was high (18.75 pg/mL) compared to survivals (15.17 pg/mL).[10] Previous study stated that high concentration of IL-1β was strongly associated with mortality.[18] Several lines of evidence support IL-1β single nucleotide polymorphism (SNP)-511C/T has been implicated in various cerebrovascular phenotypes, including ischemic injury.[19] Moreover, the TT genotypes of the -511C/T polymorphism was significantly associated with an increased risk aneurismal rupture among subarachnoid hemorrhage (SAH).[20] IL-1β-511 allele T was significantly associated with unfavorable outcome (dead, vegetative state or severe disability) after all types of head injuries including TBIs.[4] In contrast, our results revealed non-significant association between *IL-1β* gene -511C/T and death. Similarly, there were no significant differences in *IL-1β* allele and genotype frequencies with outcome among patients stratified traumatic brain injury.[21] Johnson et al.[22] studied the influence of the IL-1β alleles on apoptosis, evaluating hippocampus samples from patients who died following TBIs. The authors found no association between the alleles with either the measured amount of apoptosis or death.[22] However, there is evidence that polymorphisms in cytokines gene could affect the subject level of cytokine synthesis in response to injury.[23] Genetic variations within genes coding for inflammatory mediators was reported to affect plasma protein levels.[24] *IL-1β* gene was found to be significantly correlated with IL-1β levels in experimental study.[25] The minor T allele carriers considered to be high producers of IL-1β protein comparing to C allele carriers.[26] Moreover, the T allele of *IL-1β*-511 promoter SNP is correlated with enhanced IL-1β production *in vivo*.[27] Haplotypes of -511 *IL-1β* gene containing T allele has been shown to increased transcriptional activity *in vitro*.[28] Our finding revealed that although no significant association between *IL-1β*-511C/T genotypes with high mount IL-1β levels; patients carrying TT genotypes had high IL-1β circulatory levels comparing to patients carrying CC genotypes. The discrepancy of

results between different studies may be due to different methodology used in cytokine detection such as ELISA, radio immunosorbent assay, and real-time PCR. Moreover, gene functions can be modified by other genes (gene-gene interaction), proteins (protein-protein interaction), or environmental factors (gene environmental interaction).[29]

Conclusions

In this study IL-1β circulatory level was varied according to trauma severity and high levels were seen among patients developed unfavorable outcome. Mutation in *IL-1β*-511C/T gene was not associated with trauma severity and outcome.

Conflicts of Interest Statement

None.

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