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The Levels of Some Interleukins and Serum Copper Levels in Patients with Head Injury

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ABSTRACT

Background and Objective: Head injury is caused by server physical force to the head, and it can be open or closed. In a closed trauma, the skull is not broken, but the impact may damage the brain, skull, scalp and underlying tissue and blood vessels of the head. Therefore, the aim of this study is to find the relationship between head injury and several parameters including the serum level of interleukin-6, interleukin-8 and copper (Cu) in these patients.

Methods: This study was conducted at the Neurosurgical Teaching Hospital in Baghdad/Iraq and comprised 60 patients with head injuries in the age range of 20-80 years, who were matched to 30 healthy controls. Five mL blood samples were taken around 8 hours after the injury. Serum IL-6 and IL-8 were measured by the Enzyme Linked Immunosorbent Assay (ELISA), whereas Cu was measured by the Atomic Absorption Spectrophotometer (AAS). The results were compared withween the two groups.

Findings: The results revealed that there were no significant differences in age (p=0.169), gender (p=0.434) and BMI (p=0.102) between head injury patients and healthy subjects. The results revealed a significant increase in serum IL-6 (177.45±55.12 & 83.43±22.98) and IL-8 (181.19±61.77 & 49.99±15.32) levels in patients as compared to healthy subjects (p<0.001), and a significant decline in Cu levels in patients (0.84±0.16) compared to the healthy group (1.34±0.15) (p<0.001).

Conclusion: Based on the results of this study, increased levels of interleukins and copper element can be used as markers related to head injury.

Keywords: *Head injury, Interleukin, Copper.*

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Introduction

Trauma injuries are a serious healthcare issue and one of the major causes of mortality globally (1). Head injury is a prevalent type of trauma (2). The yearly global incidence of head injury needing medical care, hospitalization, or death is estimated to be over 10 million, with a substantial risk of morbidity, mortality, and disability (3). Physical cognitive and psychological factors can cause disability after an accident of head injury. Many studies have discussed that the psychosocial complications can be particularly severe or cause prolonged experience of depression (4-8).

Cytokine-mediated inflammation is most likely critical in the development of illness after traumatic brain injury or head injury (9). Following a significant head injury, pro-and anti-inflammatory cytokines can be abundantly produced and released into the blood and central nervous system (CNS) (10). Pro-inflammatory cytokines are critical for maintaining normal brain function as well as healing following TBI. Massive and unregulated production of these cytokines, notably Interleukin-1β (IL-1β), IL-8, IL-6, and tumor necrosis factor alpha (TNF-α) which link with the inflammation in the body, might, however, cause significant further brain damage. The levels of these cytokines in the brain may be thousands of times higher than in the serum (11, 12).

Trace metals, such as copper play an important role and are a cofactor for many important enzymes involved in production of energy, metabolism of iron, activation of neuropeptide, and synthesis of neurotransmitter and connective tissue (13). Copper metabolism dysfunction or regulatory pathways cause brain an imbalance in copper homeostasis, and can lead to huge consequences of chronic and acute pathological disorders on the function of neurological system (13). Copper plays an important role in the organization of various physiological processes. In particular, they actively contribute to the metabolism of carbohydrate, fat and protein, leading to the final production of usable form of energy (14). Cu has a universal distribution in the brain but is concentrated in the striatum, hippocampus, synaptic membranes, cerebellum, and cerebellar granules and cortical pyramidal neurons (15). The functions of several enzymes in the CNS are largely dependent on Cu (16). The development of oxidative stress has been linked to the etiology of acute CNS injury (17). An imbalance in trace element levels is of great concern since it can cause disruption in the intrinsic capability of antioxidant systems, ultimately leading to the spread of primary injuries. Thus, trace element levels in the serum of patients with head injuries can be used to predict oxidative stress and, consequently, the degree of head injury (15, 18).

Methods

The current case control study included 60 head injury patients with a Glasco Coma Scale≤8 (GCS is used to give a reliable description of the level of consciousness in all sorts of acute medical and trauma patients), who were admitted to the Neurosurgical Teaching Hospital in Baghdad, and 30 healthy subjects matched in age and gender to patients group. Patients with other disorders such as Alzheimer, and cerebrovascular diseases (neuroinfections, stroke, migraine and headache disorders, Parkinson's disease, multiple sclerosis, head trauma due to nervous system disorders), were excluded, and they had no further medical issues between the time of sampling and the assessment of laboratory tests. Five mL of blood was obtained during the first 8 hours after the injury, then centrifuged (for 15 minutes at 3000 rpm), and the supernatant was immediately stored at -80 °C until analysis. The enzyme-linked immunosorbent assay (ELISA) was used to assess IL-6 and IL-8 levels in serum. Copper was quantified using an Atomic Absorption Spectrophotometer (AAS).

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The SPSS statistical program (version 18) was used to conduct the Spearman's rho, Mann-Whitney Test, and Independent T-Test on data including GCS on admission, gender, age, results of interleukin and copper measurement, and their relationship with patient outcome. At $p \leq 0.05$, differences were considered statistically significant.

Results

The results revealed that there were no significant differences in age, gender, and BMI between head injury patients and healthy subjects, as shown in Table 1. Table 2 shows the percentages of severe and mild trauma for the injured body region, injury causes, and injury type. The head had the largest proportion of injuries as a body region; military attacks have had the highest percentage of injuries as a reason for injury; and penetrating injuries have had the largest proportion of injuries as a type of injury.

Table 1. Clinical and non-clinical characteristics of head injury patients and healthy subjects

Parameters	Healthy subjects (n=30) Number (%)	Patients with head injury (n=60) Number (%)	p-value*	Sig.
Age (year)				
<20	2(6.6)	-		
20-40	18(60)	40(66.6	0.169	N.S
41-60	8(26.6)	17(28.3)		
61-80	2(6.6)	3(5)		
Gender				
Male	25(83.3)	44(73.3)	0.434	N.S
Female	5(16.7)	16(26.7)		
BMI ** **(kg/m^2)**				
Lean	1(3.3)	1(1.7)		
Obese Normal	8(26.7)	18(30.0)	0.102	N.S
Weight	10(33.3)	20(33.3)		
Overweight	11(36.7)	21(35.0)		

*T-test, **Body Mass Index.

Table 2. Injury characteristics of the study population by intensity of trauma among trauma patients

Characteristic	Severe trauma (n=40) Number (%)	Mild trauma (n=20) Number (%)
Body region injured		
Head and/or Neck	20(50)	11(55)
Face	7(17.5)	5(25)
Thorax	6(15)	3(15)
Abdomen/visceral pelvis	7(17.5)	1(5)
Cause of injury		
Military attacks	24(60)	12(60)
Road traffic crush	8(20)	4(20)
Assault	4(10)	2(10)
Falls	3(7.5)	1(5)
Burns	1(2.5)	1(5)
Type of injury		
Penetrating	20(50)	9(45)
Blunt force	15(37.5)	7(35)
Burn	5(12.5)	4(20)

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Table 3 shows the findings of 60 patients with head injuries and 30 healthy subjects. The results revealed a significant increase in serum IL-6 and IL-8 levels in patients as compared to healthy subjects ($p<0.001$), and a significant decline in Cu levels in patients compared to the healthy group ($p<0.001$).

Table 3. Serum IL-6, IL-8, and Cu Levels in head injury patients and healthy subjects

Parameter	Healthy subjects (n=30) Mean±SD	Patients with Head injury (n=60) Mean±SD	p-value*
IL-6** (pg/mL)	83.43±22.98	177.45±55.12	0.001
IL-8*** (pg/mL)	49.99±15.32	181.19±61.77	0.001
Cu**** (µg/mL)	1.34±0.15	0.84±0.16	0.001

***T-test, **Interleukin-6, ***Interleukin-8, ****Copper.**

Discussion

Results showed a significant elevation in serum of measured interleukins level in patients as compared to healthy subjects with $p<0.001$, and a significant decline in Cu levels in patients compared to the healthy group with $p<0.001$.

The accumulation of neuronal injury causes an inflammatory response, which can be advantageous and give the central nervous system neuroprotection (19). There is a relationship between the severity of trauma and the immune response. Such a reaction is characterized in part by cytokine production in the periphery, which leads to the promotion of an acute inflammatory response that is seen after trauma (18).

In this study, the results demonstrate a significant increase in serum IL-6 and IL-8 levels when compared to healthy subjects. The results of this study were consistent with those of Xuet al. (20). Following head injury, IL-6, a major regulator of the inflammatory response, increases in serum, CSF, and brain tissue (21). This cytokine has both pro- and anti-inflammatory actions; hence, it serves a dual-opposing function (21, 22). Previous studies on head injury serum IL-6 have revealed contradictory results. According to Kalabalikis et al., there is no link between IL-6 levels and neurological outcomes (23).

Singhal et al. observed that increasing levels of IL-6 resulted in a favorable outcome (24). Others have shown that a high IL-6 level is related to poor results and a greater risk of mortality (22, 25). Raheja et al. hypothesized that increased levels of these proinflammatory cytokines are an adaptive response of the brain to injury, causing transitory destruction and apoptosis of injured neural cells and laying the way for the reparative process. As a result, an initial rise in IL-6 levels may be beneficial in the long term, but a delayed rise may be disastrous since it generally signals more serious diseases such as raised intracranial pressure, multiorgan dysfunction syndrome, sepsis, and shock (26). As a result, several authors have recommended reducing inflammatory mediators as a therapy strategy for TBI (27, 28). IL-8, a pro-inflammatory cytokine, has been demonstrated to increase neurotrophin production following head trauma. This cytokine also promotes neutrophil chemotaxis and phagocytosis, drawing them to the site of neural damage (29). Several studies show that acute and persistent IL-8 levels rise after severe traumatic head trauma (22, 30, 31). They hypothesize that this cytokine plays a crucial role in both damage and regenerative processes following head injury and that its high concentration relates to mortality (22, 31). In this study, copper levels were found to be significantly lower in patients with head injuries compared to healthy people. The findings of this study correspond with those of Pu et al. (32) and Belatar et al. (33) studies. Copper is essential for many neural activities, and copper metabolism problems commonly affect the central nervous system. Copper excess and copper deficiency are both linked to brain dysfunction (13). Given the vital function of copper in a wide

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range of cellular activities, local concentrations of copper and the cellular distribution of copper transporter proteins in the brain are critical for maintaining the internal environment's steady state (34). A disruption in copper metabolic or regulatory pathways disrupts copper balance in the brain, which can have a variety of acute and chronic degenerative consequences on neurological function (34). Maintaining Cu homeostasis in the brain might be exploited as a therapy target for TBI (35). Previous research found that patients with a persistently low GCS score also have persistently low serum copper levels, while the level of copper was substantially increased from day 0 to day 10 in moderate GCS patients, implying that the relationship between the patient's outcome and serum Cu is most likely due to the critical role of Cu in eliminating oxidative stress, which is the most detrimental factor in severe head injury (36).

In this study, the high levels of IL-6 and IL-8 in patients, as well as the low level of Cu, suggest that these markers can be utilized as markers to prognosticate head injury. However, further research involving patient outcomes is required to assess its overall clinical benefits.

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