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RESEARCH ARTICLE

Effect of serum ammonia, TNF-alfa and IL-6 levels on the degree and outcome of hepatic encephalopathy in Egyptian cirrhotic patients

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Abstract

Background and Objective : Hepatic encephalopathy (HE) is a major complication characterized with neuropsychiatric symptoms It's etiology is multifactorial . So we aimed to study serum ammonia, TNF-alfa and IL-6 levels on the degree and outcome of hepatic encephalopathy in Egyptian cirrhotic patients.

Patient and Method : The study included 89 patients with liver cirrhosis complicated with HE (Patients group) and 60 cirrhotic patients without HE as control group (group 3), the patients group divided into subgroups according to the grade of encephalopathy from the beginning the study, group A encephalopathy: grade 1 and 2 and group B encephalopathy: grade 3 and 4.

All patients were followed up for 7 days, then divided into 2 groups according to the response to treatment ; group 1: complete recovery and group 2: with improper response.

Results:There were statistical difference between patients groups (group 1 and 2) and control group (group 3) in terms of blood ammonia levels (66.5 ± 22.5, 13 ± 4.8), serum TNF-α levels (21.6 ± 3.2, 2.3 ±.2) and mean serum IL-6 (63.9 ± 15.9, 4 ± 1.29) respectively. There were statistical difference between group 1 and group 2 in terms blood ammonia levels (46 ± 12.8, 86.5 ± 30.5), mean serum TNF-α levels (7.1 ± 2.9, 29.4 ± 13.3) and mean serum IL-6 (19 ± 9.2, 93.9 ± 20.9). Results also, showed statistical significant difference between group 2A and group 2B in terms of blood ammonia level (79.2 ± 20.6, 173.7 ± 38.7), mean serum TNF-α levels (18.1 ± 5.3, 39.9 ± 14.5) and mean serum IL-6 levels (69.2 ± 11.1, 118.2 ± 13.8). In group 2 there were significant positive correlation between serum TNF with blood ammonia level (r = 0.843, P = 0.001), serum IL-6 (r = 0.732, P = 0.001) and between IL-6 and blood ammonia level (r = 0.699, P = 0.001).

Conclusion: there is a strong relation between high blood level of ammonia, TNF alfa, IL-6 and grade and outcome of HE in cirrhotic patients.

Introduction

Hepatic encephalopathy (HE) is a neurocognitive disorder in which brain function is impaired and is associated with both acute and chronic liver dysfunction [1]. According to the newest data, HE occurs as one of four types. Therefore, this encephalopathy syndrome might be classified into four groups: A, B, C and D [2-4]. Type A HE is associated with acute liver failure. Type B HE is associated with portosystemic shunt or by pass. Type C is associated with (cirrhosis) and portal hypertension with portosystemic shunts.. Type D is associatedwith disorders of the urea cycle [5,6]. One of toxins possibly implicated in the aetiology of HE is ammonia [7], however, HE,

hepatocellular failure and portosystemic shunting disable the ability of the liver to neutralize ammonia, leading to increasing its arterial level. Ammonia can induce astrocyte swelling as the result of osmotic imbalance leading to a major role in the pathogenesis of HE [8-10]. There was suggestion that inflammatory response (such as elevation of pro-inflammatory cytokines)is increased in response to infection and/or systemic inflammation, and oxidative stress, participate in a synergistic relationship with ammonia in the pathogenesis of HE [11-13]. Cytokines are molecules secreted from immune cells as a part of immune regulatory system, and had many effects on the inflammatory process. IL-6 is an interleukin that act as both pro-inflammatory and anti-inflammatory cytokine. It is secreted from macrophages and T-cells to stimulate immune response. [14]. There were studies indicate that mediators of inflammation (tumor necrosis factor-alpha (TNF- α), interleukin-I beta (IL- I β), interleukin-6 (IL-6) may exacerbate the effects of ammonia on the brain leading to more exacerbation of encephalopathy [13,15].
Also TNF-α is released early during infection and can influence the permeability of the blood brain barrier [16]. Moreover, an association between circulating TNF-α levels in patients with acute [17] and chronic liver failure and the severity of HE, regardless of aetiology, has been recognized [18]. Bémeur et al. [19] investigated the effect of IL-1β, TNF-α and interferon-α (IFN-α) gene deletions on the onset of HE.

In this study we aim to demonstrate the relationship between the proinflammatory marker TNF, interlukin-6 and serum ammonia level already with their relationship with the outcome and the degree of hepatic encephalopathy

Patients and methods

This study was performed in the Department of Internal Medicine, Zagazig University Hospitals, Egypt, between June 2013 to July 2014.
The study included one hundrad patients with liver cirrhosis complicated with HE (Patients group) and about 60 hepatic cirrhotic patients without HE (control group).
We obtained informed consent from each patient or from an immediate family member if the patient was unable to give consent.

Inclusion criteria:
The diagnosis of hepatic encephalopathy was made when mental status was altered and appropriate laboratory and diagnostic testing excluded other causes of mental status changes. Symptoms of hepatic encephalopathy is performed according to the so-called West Haven Classification system [20].

Exclusion criteria:
Other causes of mental status changes (cerebro-vascular stroke, organs failure, endocrinal and metabolic causes) are excluded.
Research steps: Cirrhosis was diagnosed with history, clinical, laboratory and ultrasonographic findings. Patients group (cirrhotics with hepatic encephalopathy) were divided into subgroups according to the grade of encephalopathy from the beginning the study; group A: encephalopathy grade 1 and 2 and group B: encephalopathy grade 3 and 4.
All patients were followed up for 7 days with proper management for HE, then divided into 2 groups according to the response to treatment; group 1: Complete recovery and group 2: with improper response. Beside that we selected 60 cirrhotic patients without HE as control group (group 3).
The following were done for all patients: full history (precipitated factors for HE, viral hepatitis, drug intake, previous variceal bleeding) clinical examination including signs of portal hypertension such as ascites.
All participants were subjected to laboratory tests in form of liver and kidney function tests, Fasting glucose, lipid profile and blood ammonia level on a Roche Diagnostics Cobas 6000 (c 501 module) autoanalyzer. Coagulation screen (PT, INR) on Roche Diagnostics CA1500 autoanalyzer. Viral markers on Roche Diagnostics Cobas e411 autoanalyzer.
The serum IL-6 levels were studied with ELISA (eBioscience, USA) with overall intra-assay coefficient of variation was 3.4% and inter-assay coefficient of variation was 5.2%.
The serum TNF-α levels were studied with ELISA (eBioscience, USA) with overall intra-assay coefficient of variation was 6% and inter-assay coefficient of variation was 7.4%.

Blood Ammonia Determination:
Fasting venous blood samples were obtained immediately after mental status assessment. Samples were drawn into K2-EDTA plasma (free from hemolysis and lipemia), placed immediately on ice, and taken to the clinical laboratory where they were processed and analyzed within 30 minutes of having been obtained. Total ammonia levels were determined in venous plasma by the enzymatic method, using the glutamate dehydrogenase reaction with reagents

obtained from Roche Diagnostics (GmbH, Germany) according to the manufacturer's protocol on a Roche Diagnostics Cobas 6000 (c 501 module) autoanalyzer.
Blood ammonia level, TNF-α and IL-6 was done in the blood before and after follow up period.

Other investigations

Liver cirrhosis was diagnosed by ultrasound scan of liver and possibly CT, MRI scan and or liver biopsy. Liver biopsy also used for diagnosis of steatohepatitis (NASH) if suspected, rectal snip for diagnosis of bilharisis. Also upper endoscopy was done for diagnosis or treatment of gastroesophageal varices.

Statistical analysis

SPSS 13.0 was used for statistical evaluations.One-way ANOVA and Post Hoc (Bonferroni test) was used for comparison of independent groups.Values were given mean ± SD. Mann-Whitney U test and independent t-test was used for comparisons of patients and healthy subjects.$P<0.05$ was accepted as statistically meaningful difference.

Results

The number of patients became eighty nine due to death of eleven patients during the study, cause of cirrhosis was mostly due to HCV as HCV antibodies were positive in about 57 from all the patients, there was combined lesion of bilhriasis with HCV infection in about 10 patients, HBV infection with HCV infection in 9 patients, followed by HBV infection in about 7 patients and 6 patients was diagnosed as cirrhosis secondary to NASH.
Patients who showed good response with nearly complete recovery from the encephalopathy was 57 (group 1), in this group 30 patients were classified at time of admission as encephalopathy grade 1 to 2 (group 1A) and 27 patients were classified as encephalopathy grade 3 to 4 (group 1 B), patients in this group were 30 Child B and 27 Child C, the precipitating factor of HE in this group was due to unknown cause in 4 patients, spontaneous bacterial peritonitis in 6 patients, constipation in 8 patients, hepatocellular carcinoma in 2 patients, variceal bleeding in 15 patients, diet in 10 patients and electrolyte imbalance in 12 patients.
On the other hand patients who showed improper response to treatment with prolonged state of encephalopathy was 32 patients and, in this group 8 patients were classified at time of admission as
encephalopathy grade 1 to 2 (group 2A) and 24 patients were classified as encephalopathy grade 3 to 4 (group 2 B) patients in this group were 17 Child B and 15 Child C, the precipitating factor of HE in this group was due to unknown cause in 3 patients, spontaneous bacterial peritonitis in 13 patients, constipation in 2 patients, hepatocellular carcinoma in 4 patients, variceal bleeding in 7 patients, diet in 0 patients and electrolyte imbalance in 3 patients.
There were statistical significant differences between patient group (group 1 and 2) and control group(group 3) in terms of blood ammonia levels, serum TNF-α levels and mean serum IL-6 respectively (Table 1).
There was no statistical significance between male/female ratio and mean age of the groups. There were statistical significant difference between group 1 and group 2 in terms blood ammonia levels,mean serum TNF- levels and mean serum IL-6 (Table 2).
Our results, showed statistical significance difference between group 2A and group 2B in terms of blood ammonia level, mean serum TNF-α levels, and mean serum IL-6 (Table 3).
There were no statistical significant difference between patients Child classification B and C in terms of mean serum TNF-α, serum ammonia levels and mean serum IL-6 levels at time of admission.
In group 2 there were significant positive correlation between serum TNF with blood ammonia level, serum IL-6 and between IL-6 and blood ammonia level. But In group 1 there were no significant correlation between this measures (Table 4).

Table 1 : Difference between control group and patients group as regard blood ammonia, TNF, and IL-6.

	Control group N = 60	**Patient group N = 89**	**P**
Ammonia (μg/dL)	13 ± 4.8	66.5 ± 22.5	0,0001
TNF (pg/ml)	2.3 ± 0.2	21.6 ± 3.2	0,0001
IL-6 (pg/ml)	4 ± 1.2	63.9 ± 15.9	0,0001

Table 2: difference of laboratory data between group 1 and group 2 patients

	Group1 N= 57	**Group2 N=32**	**P**
Ammonia (µg/dL)	46±12.8	86.5±30.5	0.0001
TNF (pg/ml)	7.1 ± 2.9	29.4 ± 133	0.0001
IL-6 (pg/ml)	19±9.2	93.9 ±20.9	0.0001
INR	1.8±0.5	2.8±1.0	0.03
Albumin (g/dl)	2.9±0.6	2.1±0.3	0.02
Bilirubin (mg/dl)	3.2±1.9	3.6±2.0	NS
Creatinine (mg/dl)	1.1±0.3	1.3±0.5	NS
BUN (mg/dl)	28.7±5.0	23±4.7	NS

Table 3: Difference between TNF, Ammonia and IL-6 in subgroup A and B (2A and 2B).

	Group 2A	**Group 2B**	**P**
	N = 8	**N = 24**	
Ammonia (µg/dL)	79.2± 20.6	173.7 ± 38.7	0.0001
TNF (pg/ml)	18.1±5.3	39.9±14.5	0.0001
IL-6 (pg/ml)	69.2±11.1	118.2±13.8	0.0001

Table 4: Correlation between TNF, Ammonia and IL-6 in group 2 (A&B)

	N	R	P
TNF and Ammonia	32	0.843	0.001
TNF and IL-6	32	0.732	0.001
Ammonia and IL-6	32	0.699	0.001

DISCUSSION

Hepatic encephalopathy (HE) is a major complication characterized with neuropsychiatric symptoms.It's etiology and pathogenetical mechanisms are not clearly understood and probably it is multifactorial.

The relation between several cytokines and HE pathogenesis were evaluated in many studies.Most of these studies are focused on TNF-α [21].

In our study, results showed statistical significant difference between patients who showed good response with nearly complete recovery (group 1) and patients who showed improper response to treatment with prolonged state of encephalopathy (group 2) and between hepatic encephalopathy grade 1 to 2 patients (group 2A) and hepatic encephalopathy grade 3 to 4 patients (group 2B) as regard serum ammonia level and this correlated with Ong et al who demonstrated that all four measures of ammonia; arterial total ammonia, venous total ammonia, arterial partial pressure of ammonia, and venous partial pressure of ammonia increased with the severity of hepatic encephalopathy [22]. Other studies [23,24] found the relation between plasma ammonia and the severity of the encephalopathy was variable. This discrepancy could be resolved by accounting for the frequent use of venous ammonia levels, which are appreciably lower than arterial ammonia, to which the brain is exposed [25]. Shawcross et al explained the hypothesis of ammonia in HE by that Hyperammonemia leads to the accumulation of glutamine within astrocytes,

which exerts an osmotic stress that causes astrocytes to take in water and swell [13]. But this relation is not apparent in group 1, in which there is no significant correlation between serum ammonia and degree of encephalopathy
Also our results showed statistical significant difference between group 1 and group 2 in terms of mean serum TNF-α level and mean serum IL-6 level in which this results agreed with Odeh, who investigated the relation between clinical severity of HE and serum cytokines and TNF-α level, and they found that there was a positive correlation between TNF-α levels and the severity of the HE [21] and, in addition, mean TNF-α level of a patient group, consisted of 40 patients with grade 3 HE at the beginning of the study were significantly decreased when they improved to stage1.
In our study, we similarly found a relation between clinical stages of HE and serum TNF-α and IL-6 levels in group 2 with statistical difference between group 2A and group 2B in terms of mean serum TNF-α levels and mean serum IL-6, in which this explained by de Vries et al, who demonestrated that elevated level of inflammatory cytokines such as TNF, IL-1β and IL-6 which have been shown in vitro to compromise the integrity of the blood brain barrier. This is mediated through the cyclo-oxygenase (COX) pathway within the endothelial cell [26].
Interestingly we found that no statistical significant difference between group 1A and group 1B in terms of mean serum TNF-α level although that there were different grades of encephalopathy in this group. On the other hand in group 2 there were significant positive correlation between serum TNF and blood ammonia level and serum IL-6 and between IL-6 and blood ammonia level, on the other hand this group of patients responded to treatment after follow up period, so we could notice the correlation between TNF, ammonia and IL-6 with each other in group 2, in which this phenomenon may be due to the synergistic effect of them with each other.
As elevated level of inflammatory cytokines and ammonia have been shown to be important in the pathogenesis of HE in cirrhosis, the question were the infection and/or the inflammation had a synergistic relationship with ammonia ? [27]. Marini and Broussard used mice with a deficiency in a critical urea cycle enzyme conferring chronic hyperammonemia, to demonstrate an increased sensitivity to inflammation. Furthermore, the hyperammonemic mice developed longer lasting and stronger cognitive defects when exposed to an inflammatory stimulus [28].
The peripheral immune system communicates with the brain in response to infection and inflammation as astrocytes and microgial cells release cytokines in response to injury or inflammation [29]. Findings from studies in rats found that the rise in blood levels of TNF that occurs during inflammation stimulated glial cells to secrete the cytokines IL1and IL-6 [30]. TNF also compromised the endothelial blood–brain barrier and IL-1B affects the integrity of the glial side of the blood–brain barrier [27,31]. Both TNF and IL-6 enhances fluid–phase permeability of isolated brain endothelial cells in vitro, and TNF also increases the diffusion of ammonia into astrocytes [32].
Microglial activation is followed by highly increased levels of proinflammatory cytokines (TNF, IL-1 and IL-6) in the brain [33,34]. This discovery revealed a new aspect of HE, provided a new look at the pathophysiology of HE and opened a new gate into the pharmacotherapy of HE.
This neuroinflammatory concept is also supported by the therapeutic effect of mild hypothermia and indomethacin, which reduces the activation of microglial cells and simultaneously prevents the central proinflammatory process in mild HE [33,35].
Other study obtained, once SIRS (Systemic Inflammatory Response Syndrome) and the infection had been successfully treated, and patient's levels of the inflammatory markers as tumor necrosis factor (TNF), interleukin (IL-1) and IL-6 had returned to normal, their psychometric test results did not deteriorate after hyperammonemia was induced [13].
On the other hand, Shawcross, et al told that the presence and severity of mild HE were independent of both serum levels of ammonia and the severity of liver disease; but serum levels of inflammatory markers (such as C reactive protein, white blood cell count, IL-6) were much higher in patients with mild HE than in patients without mild HE [36].
Our results revealed a new aspect of HE, provided a new look at the pathophysiology of HE and opened a new gate into the pharmacotherapy of HE in the future and dealing with HE perfectly and directly or indirectly target the proinflammatory milieu. So we recommended other researchers to study other inflammatory markers in prognosis of HE to know the more important one and deal with it.

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