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# Role of Tumor Necrosis Factor Alpha, Ghrelin, Evoked Potentials in Hepatic Encephalopathy

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## Abstract

**Background**: Hepatic encephalopathy has a negative effect on patient health-related quality of life. Apart from increased blood ammonia, alterations in various other substances (Tumor necrosis Factor- Alpha (TNF-α), Ghrelin)have been implicated in the pathogenesis of hepatic encephalopathy (HE). Ghrelin and TNF-alpha have numerous metabolic actions.

**Aim of the work:** was to estimate the role of TNF -α, Ghrelin and Evoked Potentials changes in patients with hepatic encephalopathy, and their relation to grades of hepatic encephalopathy

**Methods:** We measured serum levels of TNF -α, Ghrelin in 40 patients with liver cirrhosis (20 with hepatic encephalopathy & 20 without encephalopathy) and 10 healthy controls. All subjects underwent to neurophysiological test: p300,visual and auditory evoked potentials.

**Results**: The results of this study showed highly significant increase in TNF -α, Ghrelin & levels in both groups when compared to the control group with a significant increase in the cirrhotic encephalopathic group. Also significant correlation between TNF-α, Ghrelin & grading of encephalopathy was found. Delay in latency and decrease amplitude of VEP, p 300 and ABR were significant in cirrhotic patients with hepatic encephalopathy.

**Conclusion**: TNF -α, Ghrelin levels are sensitive indicators of the severity of liver disease. Patients with liver cirrhosis can be followed up by measurement of these serum markers which might predict the development of encephalopathy. The increasing levels of Ghrelin & TNF-α is more prominent in cirrhosis with encephalopathy. The presence of nutritional and metabolic abnormalities, including malnutrition in cirrhosis, at least partly, elucidates high Ghrelin level. The applied neurophysiological tests are a simple, suitable and objective method for differentiating the degrees of encephalopathy and for identifying the preclinical stage of encephalopathy because abnormalities in these tests may prompt the clinician to initiate treatment.

**Keywords:** TNF, Ghrelin, evoked potentials, Hepatic Encephalopathy.

## Introduction:

Hepatic encephalopathy (HE) is a major complication of acute or chronic liver disease characterized by neuropsychiatric symptoms. Its etiology and pathogenetical mechanisms are not clearly understood and probably it is multifactorial. Episodes are usually precipitated by factors that increase inflammation or ammonia production [1].

There is agreement that ammonia is a key toxin involved in the disease process. Many researches aimed to characterize the effects of inflammation, oxidative stress and other factors working in synergy with ammonia to produce astrocyte swelling as a common pathway towards cerebral dysfunction [2].

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine with numerous immunologic and metabolic actions [3].The TNF-α system activity is increased in liver cirrhosis and generally thought to be associated with several known cirrhosis related complications such as hyperdynamic circulation, susceptibility to infection, and hepatic encephalopathy[4].

Ghrelin is a novel endogenous ligand for the growth hormone (GH) secretagogue receptor that has been isolated from both human and rat stomach [5]. Ghrelin controls energy balance, enhancing fat mass deposition and food intake through the activation of the hypothalamic nuclei and the promotion of neuropeptide Y (NPY) and agouti-related protein (AGRP) expression[6].

The effect of Ghrelin-like leptin is not exclusively mediated by direct hypothalamic receptor activation, but also by modulating cytokines [7]. In catabolic situations like in cirrhosis, raised Ghrelin levels may induce a combination of enhanced food intake, increased gastric emptying and food assimilation. These actions of Ghrelin are the opposite of leptin[8].


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HE is an alteration of the central nervous system as a result of hepatic insufficiency. It manifests itself as a neuropsychiatric syndrome which may result in impairment of the sleep-wake cycle, cognition, memory, consciousness, personality changes, motor-sensory abnormalities[9].

Evoked potentials (EVPs. are used (1) to diagnose HE in patients with severe liver disease and mental alteration, (2) to grade overt HE and monitor the effect of treatment for HE, (3) to diagnose minimal HE (4) to predict the occurrence of episodes of overt HE[10].

These tests demonstrate delayed latencies (a slower response) which become more prolonged in relation to the degree of encephalopathy [11].

**Aim of the study:** was to estimate the role of TNF Alpha, Ghrelin and Evoked Potentials changes in patients with hepatic encephalopathy, and their relation to grades of hepatic encephalopathy**.**

**Patients and methods:** This study was conducted on 40 adult patients with liver cirrhosis as proved by history, clinical examination and investigations who presented to Tropical Medicine Department of Al-Zahraa University Hospital, 20 of them with hepatic encephalopathy (Group I) as proved by clinical and neurophysiological testes (that were done in Neurology Department of Al-Zahraa University Hospital) and 20 of them with no hepatic encephalopathy(Group II)

The study also included 10 healthy subjects who were clinically, laboratory and ultrasonographically free served as a control group after their consent.

***The inclusion criteria include:*** Adult patients with liver cirrhosis (with and without hepatic encephalopathy).

***The exclusion criteria include:*** Alcohol consumption and sedatives intake, history or evidence of malignancies, any other organ failure and other causes of encephalopathy rather than hepatic, history of trans-jugular intrahepatic porto-systemic shunt, recent head trauma, fever, sepsis or shock, and grade 3 and 4 encephalopathy as patient is uncooperative and comatosed. Patient with error of refraction or hearing defect.

***Subjects were divided into* 3 *groups:***

**Group I:** Included 20 patients liver cirrhosis with encephalopathy (17 males, 3 females), their ages ranged from 48-59years old with a mean±SD (54.5±5.4).

***Group* II**: Included 20 patients with liver cirrhosis without hepatic encephalopathy (9 males, 11 females), their ages ranged from40-57 years old with a mean±SD (52.1±5.4)

**Group III**: Included 10 healthy subjects with no clinical, laboratory or ultrasonographic evidence of liver disease served as a control group (4 males, 6 females), their ages ranged from 25-47years old with a mean ±SD (30.4±7.01).

**All patients and controls were subjected to the following:** 1- Full history and clinical examination with stress on manifestations of hepatic encephalopathy.

2- Abdominal ultrasonography.

3- Laboratory investigations: Blood samples were taken from patients and control subjects. Each blood sample was divided into three portions as follows:

• First portion was collected into Na citrate - containing tube, and used for estimation of prothrombin time (PT) immediately.

• The second portion was collected into EDTA containing tube for CBC estimation using folly automated cell counter, and for ESR estimation by Westegren method.

• The third portion was put in a plan tube, left to clot then centrifuged at 1600 rpm for 20minutes and serum was separated and used for estimation of:

- Liver and kidney function tests were done on Hitachi 911 auto-analyzer.

-TNF and Ghrelin Assay: TNF-α, were stored at −20°C. Ghrelin, TNF-α, levels were analyzed with ELISA kits (TNF-α kit was purchased from Bio-Source International Inc, 542, Flynn Road, Camarillo, California, USA; Ghrelin kit from Phoenix International, Inc, USA kit from DRG International, Inc, USA).

**4**-Neurological assessment:

- Visual evoked potential (P100)

- Recordings were performed in an electrically shielded, dimly lit room. By (sierra wave, cadwall, 4 channels) the stimulation source was a black/white full-field checkerboard patterns. Two trials were performed under the same stimulation condition for each subject to confirm the reproducibility. The latencies and amplitudes of the N75, P100, and N145 waves, the P100 morphology, and differences in the latency and amplitude of the P100 wave between the two eyes were evaluated.

- Brainstem auditory evoked potential. The patient was asked to relax in a darkened room. Identification of waves: Identify wave V

which is the most persistent wave. It comes as IV-V complex, and wave V comes to the base line. Go in reverse order, wave IV, III, II, &I.

- Also observe their latencies, eg. Latency of wave I will be less than 2mSec. Then Find out the interpeak latencies of I – III, III – V and I – V.
- P-300 (auditory event related potential). To elicit P-300 the subjects were sitting comfortably with their eyes closed then present the subjects with two types of stimuli both clearly audible ,the P-300 component is identified as positive peak that follow the negative N200 .Analysis of the p300 has involved the wave form identification ,amplitude ,latency and number of peaks of p300.

- **Statistical analysis:** Data was analyzed using Microsoft Excel 2007. Parametric data was expressed as mean±SD and non-parametric data was expressed as number and percentage. Student's t test was done to compare between groups. Pearson Correlation Coefficient was done to correlate between different parameters among groups. Analysis of Variance (ANOVA)test was used to estimate the difference between the means of more than two groups. P value of > 0.05 considered non significant, p value of ≤ 0.05 considered significant, p value of < 0.01 was considered highly significant**.**

**Results:**

The results and data were collected and analyzed in **tables 1-7&Fig 1-4:** Grades of encephalopathy according to West Haven classification in GI were 0, I, II, in 35%,45%,20%, of patients respectively. There was statistical highly significant increase of cases number with child C in group I in comparison to group II. There was a highly significant elevation in ALT, AST, and T.Bilirubin while there were significantly higher reduction in serum albumin, PC in patients groups in comparison to control group and there were no significant difference between ALT, AST, and T.Bilirubin in GII in comparison to GI. TNF- alpha, Ghrelin were highly significant increased in GI in comparison to GIII while Ghrelin were significantly higher in GI in comparison to GII. Also there were significant correlation between TNF-**α**, Ghrelin **&**grading of encephalopathy while there is no significant correlation between their levels **&** (ALT, AST).

Also there were highly significant increase in p 100 latency and decrease in P100 amplitude in G I in comparison to G III and significant in comparison to GII. About ABR there were highly significant prolonged RT and LT (ABR III , ABR V, ABR I-III and ABR III-V , I- V latency in G I in comparison to GIII and significant in G I in comparison to G II . While as regard p300 there were highly significant increase in P 300 latency in G I in comparison to G III and GII. While there were highly significant decrease in P300 amplitude in G I in comparison to GII and G III. Regarding grading of HE . More grade of H E associated with significant prolonged latency in (p 100, ABR III, V, I-III, III-V, I- V andP300) and decrease in (p 100 amplitude and p 300 amplitude).

**Table (1): Grades of encephalopathy**

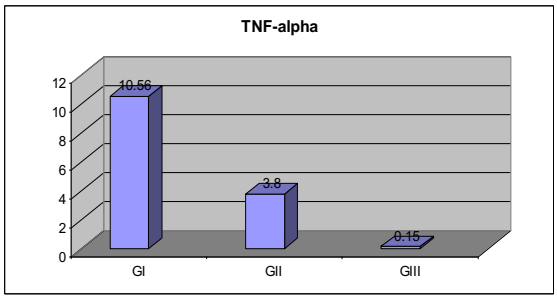
| | Grade 0 | Grade I | Grade II |
|---|---|---|---|
| ***G 1(n=20)*** | 7(35%) | 9(45 %) | 4(20%) |

***Table (2): Comparison of Biochemical characteristics among the studied groups:***

| | | ***G I (n=20)*** | ***G II (n=20)*** | ***Control (n=10)*** | ***P-value GI/Control*** | ***P-value GII/Control*** | ***P-value G I/G II*** |
|---|---|---|---|---|---|---|---|
| ***ALT*** | ***Mean*** | 44 | 45.8 | 16.4 | <0.001 ***HS**** | <0.001 ***HS**** | > 0.05 NS |
| | ***±SD*** | 19.6 | 29.2 | 5.5 | | | |
| ***AST*** | ***Mean*** | 64.8 | 65.4 | 18.7 | <0.001 ***HS**** | <0.001 ***HS**** | > 0.05 NS |
| | ***±SD*** | 35.7 | 22.9 | 4.6 | | | |
| ***PC*** | ***Mean*** | 50.7 | 56.5 | 101 | <0.001 ***HS**** | <0.001 ***HS**** | > 0.05 NS |
| | ***±SD*** | 10.6 | 9.7 | 8.4 | | | |
| ***S.Albumin*** | ***Mean*** | 2.1 | 2.5 | 4.5 | <0.001 ***HS**** | <0.001 ***HS**** | ***<0.05 S*** |
| | ***±SD*** | 0.31 | 0.4 | 0.34 | | | |
| ***T.Bilirubin*** | ***Mean*** | 2.04 | 2.2 | 0.6 | <0.001 ***HS**** | <0.001 ***HS**** | > 0.05 NS |
| | ***±SD*** | 1.2 | 1.2 | 0.2 | | | |
| ***TNF Alpha*** | ***Mean*** | 10.56 | 3.8 | 0.15 | <0.001 ***HS**** | <0.001 ***HS**** | **<0.01** ***HS**** |
| | ***±SD*** | ±0.34 | ±0.26 | ±0.32 | | | |
| ***Ghrelin*** | ***Mean*** | 9.71 | 8.1 | 7.55 | <0.001 ***HS*** | <0.05 ***S**** | ***<0.05 S*** |
| | ***±SD*** | **±1.7** | **±0.7** | **±0.97** | | | |

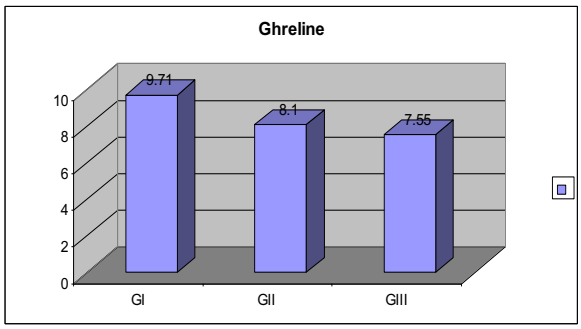
**HS: highly significant at p<0.01 S: ≤ 0.05 significant NS: non significant at p>0.05

**Fig(1):Comparison of TNF alpha among the studied groups**




It shows highly significant increase in GI in comparison to GII,GIII

**Fig(2): Comparison of Ghrelin, among the studied groups**




It shows highly significant increase in GI in comparison to GIII, significant increase in GI In comparison to GII

**Table (3): Child's – Pugh classification of the studied patients.**

| ***Child*** | ***Group I (20)*** | | ***Group II (20)*** | | |
|---|---|---|---|---|---|
| | ***N*** | ***%*** | ***N*** | ***%*** | ***P-value*** |
| ***A*** | *0* | *0* | *5* | *25* | ***(<0.001) (HS)*** |
| ***B*** | *7* | *35* | *13* | *65* | ***(<0.001) (HS)*** |
| ***C*** | *13* | *65* | *2* | *10* | ***(<0.001) (HS)*** |

HS: highly significant at p< 0.01

This table shows statistical highly significant increase of child C patients in group I in comparison to group II, while there was statistical highly significant increase of Child A/B in group II in comparison to group I.

**Table (4): Correlation between TNF-α, Ghrelin and (ALT, AST, grading of Encephalopathy):**

| | **ALT** | | **AST** | | **Grading of Encephalopathy** | |
|---|---|---|---|---|---|---|
| | R | *P-value* | R | *P-value* | R | *P-value* |
| **TNF- α** | 0.192 | **(>0.05) (NS)** | 0.661 | **(>0.05) (NS)** | 0.950 | **(<0.0001) (HS)** |
| **Ghrelin** | 0.231 | **(>0.05) (NS)** | 0.191 | **(>0.05) (NS)** | 0.841 | **(<0.05) (S)** |

**HS: highly significant at p<0.01 S: ≤ 0.05 significant NS: non significant at p>0.05

This table shows significant correlation between TNF-**α**, Ghrelin **&**grading of encephalopathy while there is no significant correlation between their levels **&** (ALT, AST).

**Table (5): Comparison of the P100 among the studied groups :**

| | Group I Mean±SD | Group II Mean±SD | Group III Mean±SD | Comp. | P-value |
|---|---|---|---|---|---|
| **RT p100 latency** | 107.08±4.41 | 103.67±4.75 | 100.75±1.60 | I & II<br>I & III<br>II & III | ***<0.05 (S)***<br>***<0.001 (HS)***<br>***>0.05 (NS)*** |
| **Lt p100 latency** | 105.77±4.12 | 102.62±4.41 | 100.23±1.59 | I & II<br>I & III<br>II & III | ***<0.05 (S)***<br>***<0.001 (HS)***<br>***>0.05 (NS)*** |
| **RT p100 amp** | 3.53±1.63 | 6.38±2.15 | 8.20±3.20 | I & II<br>I & III<br>II & III | ***<0.001 (HS)***<br>***<0.001 (HS)***<br>***>0.05 (NS)*** |
| **Lt p100 amp** | 4.20±1.54 | 5.62±2.20 | 7.20±3.30 | I & II<br>I & III<br>II & III | ***<0.05 (S)***<br>***<0.001 (HS)***<br>***>0.05 (NS)*** |

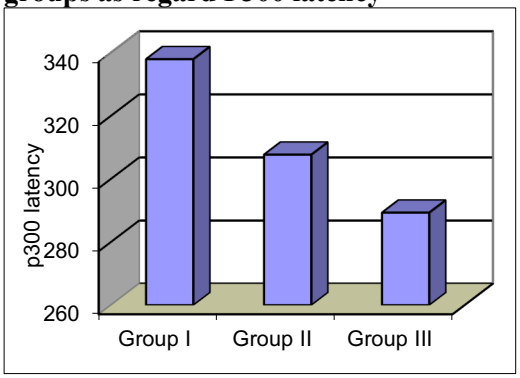
**HS: highly significant at p< 0.01 S: ≤ 0.05 significant NS: non significant at p>0.05
This table shows statistical highly significant decrease in P100 amplitude and increase latency in G I in comparison to G III while there are significant increase latency and decrease in amplitude in G I in comparison to the G II.

**Table (6): Comparison of ABR among studied groups:**

| | Group I Mean±SD | Group II Mean±SD | Group III Mean±SD | Comp. | P-value |
|---|---|---|---|---|---|
| **RT ABR I** | 1.72±0.26 | 1.67±0.17 | 1.65±0.11 | I & II<br>I & III<br>II & III | **> 0.05 (NS)**<br>**> 0.05 (NS)**<br>**> 0.05 (NS)** |
| **LT ABR I** | 1.71±0.22 | 1.76±0.15 | 1.68±0.15 | I & II<br>I & III<br>II & III | **> 0.05 (NS)**<br>**> 0.05 (NS)**<br>**> 0.05 (NS)** |
| **RT ABR III** | 3.98±0.50 | 3.68±0.34 | 3.49±0.21 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **LT ABR III** | 4.02±0.27 | 3.80±0.43 | 3.7±0.21 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **RT ABR V** | 6.3±0.57 | 5.72±0.41 | 5.56±0.26 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **LT ABR V** | 5.98±0.50 | 5.64±0.56 | 5.51±0.23 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **RT ABR I-III** | 2.26±0.24 | 2.01±0.17 | 2.02±0.11 | I & II<br>I & III<br>II & III | **< 0.001 (HS)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **LT ABR I-III** | 2.31±0.01 | 2.04±0.26 | 2.02±0.10 | I & II<br>I & III<br>II & III | **< 0.001 (HS)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **RT ABR III-V** | 2.32±0.07 | 2.04±0.07 | 2.07±0.05 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **LT ABR III-V** | 1.96±0.23 | 1.84±0.13 | 1.81±0.02 | I & II<br>I & III<br>II & III | **< 0.05 (S)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **RT I-V** | 4.58±0.31 | 4.05±0.24 | 3.91±0.15 | I & II<br>I & III<br>II & III | **< 0.001 (HS)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |
| **LT I-V** | 4.27±0.28 | 3.88±0.41 | 3.83±0.08 | I & II<br>I & III<br>II & III | **< 0.001 (HS)**<br>**< 0.001 (HS)**<br>**> 0.05 (NS)** |

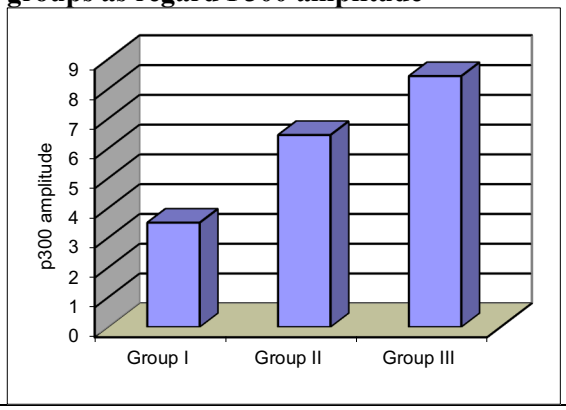
**HS: highly significant at p< 0.01 S: ≤ 0.05 significant NS: non significant at p>0.05 -This table shows a highly significant prolonged RT and LT (ABR III , ABR V, IPLs ABR I-III and III-V , I- V) latency in G I in comparison to GIII and significant prolonged in G I in comparison to G II except in IPLs III-V, I-V.

**Figure (3): Comparison between studied groups as regard P300 latency**




It shows highly significant increase in P300 latency in G1 in comparison to G II**&** G III

**Figure (4) : Comparison between studied groups as regard P300 amplitude**




It shows highly significant decrease in P300 amplitude in G1 in comparison to G II&G III

**Table (7):comparison of (VEP, ABR, P300) in different grades of H E in G I.**

| | | G0 | G1 | G2 | P-value |
|---|---|---|---|---|---|
| **RT p100 Latency** | Mean± SD | 103.25±9.14 | 106.33±6.13 | 109.72±7.22 | **< 0.05(S)** |
| **RT p100 amp** | Mean± SD | 4.23±1.32 | 3.92±1.25 | 2.31±1.3 | **<0.05(S)** |
| **Lt p 100 latency** | Mean± SD | 99.14±8.95 | 103.47±5.13 | 105.25±5.23 | **<0.05(S)** |
| **Lt p 100 amp** | Mean± SD | 4.67±1.23 | 3.22±1.12 | 1.95±0.99 | **<0.05(S)** |
| **P300 lat** | Mean ± SD | 289.5±25.32 | 315.25±27.03 | 373.22±14.32 | **<0.001(HS)** |
| **P300 amp** | Mean± SD | 4.61±0.356 | 3.37±0.92 | 2.72±0.65 | **<0.001(HS)** |
| **RT ABR I** | Mean± SD | 1.85±0.35 | 1.75±0.32 | 1.83±0.32 | **>0.05(NS)** |
| **RT ABR III** | Mean± SD | 4.72±0.72 | 4.91±0.62 | 5.12±0.52 | **<0.05(S)** |
| **RT ABR V** | Mean ± SD | 5.45±0.87 | 5.76±0.63 | 6.21±0.73 | **<0.001(HS)** |
| **RT ABR I-III** | Mean ± SD | 2.87±0.37 | 3.16±0.3 | 3.29±0.2 | **<0.05(S)** |
| **RT ABR III-V** | Mean ± SD | 0.73±0.15 | 0.85±0.01 | 1.09±0.21 | **<0.05(S)** |
| **RT ABR I-V** | Mean ± SD | 3.6±0.52 | 4.01±0.31 | 4.38±0.41 | **<0.001(HS)** |
| **LT ABR I** | Mean ± SD | 1.67±0.32 | 1.72±0.23 | 1.52±0.42 | **>0.05(NS)** |
| **LT ABR III** | Mean ± SD | 3.62±0.53 | 3.74±0.55 | 4.01±0.52 | **<0.05(S)** |
| **LT ABR V** | Mean ± SD | 5.12±0.65 | 5.42±0.62 | 6.11±0.55 | **<0.001(HS)** |
| **LT ABR I-III** | Mean ± SD | 1.95±0.21 | 2.02±0.32 | 2.49±0.1 | **<0.001(HS)** |
| **LT ABR III-V** | Mean ± SD | 1.5±0.12 | 1.68±0.07 | 2.1±0.03 | **<0.001(HS)** |
| **LT ABR I-V** | Mean ± SD | 3.45±0.33 | 3.7±0.39 | 4.59±0.13 | **<0.001(HS)** |

**HS: highly significant at p< 0.01 S: ≤ 0.05 significant NS: non significant at p>0.05

This table shows the more grade of H E associated with significant prolonged latency in (p100 )and decrease in p100 amplitude . While there is statistical highly significant increase inP300 latency and decrease in amplitude and statistical significant highly prolonged in wave V and IPLs, I-III, III-V , I-V ) .

**Discussion:**

In Hepatic encephalopathy a general consensus exists that the synergistic effects of excess ammonia and inflammation cause astrocyte swelling and cerebral edema; however, the precise molecular mechanisms that lead to these morphological changes in the brain are unclear. The different grades of HE can be diagnosed by a number of investigations, including neuropsychometric tests (such as the psychometric hepatic encephalopathy score), brain imaging and clinical scales (such as the West Haven criteria) [2].

The peripheral immune system communicates with the brain in response to infection and inflammation. Astrocytes and microglia cells release cytokines in response to injury or inflammation. Proinflammatory cytokines have the capacity to alter blood-brain barrier (BBB) integrity and preliminary studies suggest that the presence of infection in ALF (acute liver failure) results in rupture of the BBB and vasogenic brain edema. [12]

Tumor necrosis factor enhance fluid– phase permeability of isolated brain endothelial cells *in vitro*, increases the diffusion of ammonia into Astrocytes, also it influences energy homeostasis and has an anorexigenic effect on the hypothalamus [13].

Ghrelin is an important regulator of appetite and energy balance. Ghrelin increases as liver cirrhosis decompensates, which is reflected by clinical symptoms such as ascites, gastrointestinal bleeding and encephalopathy as well as by biochemical alterations, e.g. hypoglycemia, anemia, renal insufficiency and inflammation [14.]

Both TNF-alpha and Ghrelin possess anti-inflammatory properties, metabolic actions and both seem to have effects regarding the hypothalamic regulation of eating behavior, modulation of the immune response and the state of mental health[15].

In our study there were statistically highly significant differences between serum TNF-α levels of patients with liver cirrhosis with and without HE and healthy subjects, and between patients with and without HE. Also we found that TNF -alpha correlate positively with the severity of HE. This is in agreement with Goral *etal* [1] and Odeh *et al* [16]. Increased endogenous TNF-α in advanced liver disease is generally believed to be a consequence of chronic liver failure, which is associated with endotoxin-dependent macrophage stimulation and with a decrease in cytokine clearance [17].

Our study is in agreement with Srivastava *etal.*[18] who reported that there is significant increase of TNF-α and IL-6 in serum of patients with MHE (minimal hepatic encephalopathy). Also Alvarez *et al*[19]reported that proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IF-γ and ammonia induce increase of the mitochondria permeability results in reduction of ionic gradients and enhance mitochondrial dysfunction, leading to brain energetic disorders that may be an important factor in the pathogenesis of HE and could be a potential target for therapy.

Also we established increased serum Ghrelin levels in cirrhosis and much more with hepatic encephalopathy. This is in agreement with Frank *etal* [20]who reported that Ghrelin is elevated in patients with Child C liver cirrhosis. Bajaj *etal* [21] reported that Ghrelin may play an important role in the sleep disturbances seen in cirrhosis.

While Evangelos *etal* [22] reported that patients with cirrhosis had lower Ghrelin concentrations at 4 h postprandial than did the control subjects. The increase in Ghrelin from its minimal post meal value to 4 h post meal was negatively correlated with weight loss in the patients with cirrhosis.

There was significant correlation between TNF-**α**, Ghrelin and grading of encephalopathy, however no correlation was found between TNF-**α**, Ghrelin, and (AST, ALT) this is in agreement with Huseyin *etal* [23.]

Visual evoked potential (VEP) recording may be a valuable tool in assessing patients with early hepatic encephalopathy.[24]

In our study there were statistically highly significant difference delayed of latency of P100 and decrease amplitude of patients with HE more than patients without encephalopathy and control group .This in agreement with study of Ryu *et al* [25] who reported that prolonged latency of p100 and decrease amplitudes.

Also we established more prolonged latency and decrease amplitude of VEP in relation to degree of encephalopathy from G 0- G2our study did not include other grades of HE because EVPs require patient cooperation and selective attention and there for unsuitable for patients with severe HE this was in agreement with Zeneroli *et al*[26] who reported that VEP can differentiate the degrees of encephalopathy and for identifying the preclinical stage of encephalopathy and this in

agree with Ryu *et al* [25] who reported that the amplitudes of P100 VEP were significantly lowered in the cirrhotics than those of the controls before the appearance of clinical encephalopathy and disagree with Sandford and Saul[27] who reported that VEP less accurate in assessing level of consciousness.

In this study the studied groups showed statistically prolonged of latency of waves III, V this in agreement with Ryu *et al* [25]. Also we established prolonged interpeak latency of I-III , III- V and I – V and this abnormalities increase with more grading of HE. this in agreement with El-ffky and Shabana[28]who reported significant delayed IPLs I- III, III – V and absolute latency of wave V in patient with overt HE when compared to control.

In our study BAEPs demonstrated a statistically highly significant delayed interpeak latencies I-III, III–V, I- Vin patients with overt hepatic encephalopathy compared to the control and its more delayed compared to cirrhotic patients without overt encephalopathy this agree with Ryu *etal*[25].

Patients with HE may demonstrate subtle reversible cognitive difficulties, such as poor attention and concentration[29].

The studied groups showed highly statistically difference of prolonged P300 latency and lower P300 amplitude in the cirrhotic encephalopathic group (G I) of either subclinical or overt encephalopathy in comparison to control group this changes in P300 correlated with degree of encephalopathy and this in agreement with Hollerbch *et al* [30] who reported that P300-EP is a sensitive measure to detect functional cognitive impairment in cirrhotic patients with subclinical HE and clinically apparent HE. Typical changes include latency prolongation and decreased central peak amplitude.

While these results disagree with Davies *et al*[31] who reported that amplitude of the wave was decreased in both nonencephalopathic and encephalopathic.EVPs abnormalities in HE can explained by Rovira teals[32] who reported that HE originally was thought to be a metabolic disorder caused by the injured liver's inability to remove toxins effectively from the blood stream, which then were carried to the brain, altering its function. Current theories postulate that HE might also result from a variety of brain abnormalities, including vascular changes, brain cell (e.g., astrocyte) swelling, hemorrhage, and the deposition of certain metals in the brain stem.

Slower response in EVP in this study explained by the mechanism by which ammonia specially contributes to astrocytic swelling and sub- sequent brain edema and how the effects of ammonia on astrocytes and neurons are translated into the shift of balance of neurotransmission to net neural inhibition which progresses with the advancement of HE[33].

**In conclusion:** TNF-alpha, and Ghrelin levels are elevated in liver cirrhosis and markedly increased with hepatic encephalopathy than healthy individuals. There is significant correlation between TNF-α, Ghrelin &grading of encephalopathy while there is no significant correlation between their levels &(ALT,AST). Neurophysiological tests including VEP, ABRand P300 demonstrate delayed latencies (a slower response) which become more prolonged in relation to the degree of encephalopathy. They may therefore be of use in detecting minimal hepatic encephalopathy or monitoring treatment in established encephalopathy. In addition, marked prolongation or absence of various wave forms can be an indicator of poor prognosis. The most sensitive test is P300.

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