Biochemical Aspects in Sera of Iraqi Patients with Trigeminal Neuralgia

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Abstract

Trigeminal Neuralgia (TN) is one of the most commonly painful cranial neuralgia characterized by paroxysmal attacks as short lasting facial pain along the trigeminal nerve branches. The aim of the present study is to innovate a biochemical relationship between (melatonin, GALNT12 and Zn) and TN and also to examine the biochemical action of tegretol (carbamazepine) as a treatment on the above biochemical parameters. Blood samples were collected from fifty four (54) trigeminal neuralgia patients diagnosed by magnetic radiation image (MRI). Patients were classified into four groups: G3 (40- 70) years composed of (12) diagnosed male (without treatment), G4 (48- 75) years composed of (12) diagnosed female (without treatment), G5 (34- 76) years composed of (15) male under treatment with tegretol (200 mg /daily) and G6 (49-65) years composed of (15) female under treatment with tegretol (200 mg/ daily). Patients were compared with healthy subjects (have approximately the same range of age) as control groups: G1 composed of (15) males (43-70) years and G2 composed of (15) females (50-55) years. The present study is the first reporting that melatonin is a novel biochemical marker in Iraqi patients with TN (with significant and highly significant decrease in males and females respectively compared with healthy subjects). Also it is the first reporting that GALNT12 and Zn are novel biochemical markers in Iraqi patients with TN (with highly significant decrease in both genders compared with healthy subjects). The present study is the first highlighting and dealing with the biochemical action of tegretol (carbamazepine) on melatonin, GALNT12 and Zn by submitting unique and novel mechanisms. Finally the present study confirms the specific role of tegretol (carbamazepine) on postmenopausal women regarding melatonin, and GALNTI2 by focusing on its interaction with female sexual hormones.

Key Words: Melatonin, GALNTI2, Zinc, Trigeminal Neuralgia.

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Introduction

Trigeminal Neuralgia (TN) is regarded as the most commonly and extremely painful cranial neuralgia which characterized by paroxysmal attacks as short lasting stabbing facial pain along the trigeminal nerve branches, [Hamdeh et al., 2020] and [Bruton et al., 2019]. This cranial nerve disease is essentially linked with inflammation which is particularly the key cause of neuropathic pain [Yao et al., 2020]. Tegretol (carbamazepine) is the highly effective and reliable medical treatment for TN with rare side effects [Louges et al., 2020]. Tegretol (Carbamazepine) is a derivative of iminodibenzyl [Ambrósio et al., 2002].

Melatonin (N-acetyl-5- methoxy tryptamin) is a pleiotropic neurohormone secreted and synthesized mainly by the pineal gland in the vertebrates [Xie et al., 2020] and [Liu et al., 2014]. Functionally, melatonin is an endogenous regulator of circadian, seasonal rhythms and sleep disorders. Moreover, melatonin has not only endocrine actions but also autocrine and paracrine effects and clinical therapeutic uses [Liu et al., 2014] and [Omar et al., 2010].

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Besides circadian rhythms, melatonin has other immune – modulatory and oncostatic characteristics, [Omar et al., 2010]. N-acetyl galactosaminyl transferase 12 (GALNT12) is the enzyme involved in mucin type glycolysation by catalyzing the synthesis of o-glycan structured which are complex and controversial [Venkitachalam et al., 2017]. Indeed, protein glycosylation involves the addition of N- linked glycans, O- linked glycans, phosphorylated glycans, glycosam inoglycans and glycosylphosphatidyl inositol anchors to the peptide back bone as well as tryptophan residue C-mannosylation [Reily et al., 2019]. Zinc is a key micro nutrient acting pivotally in the central nervous system due to crucial biochemical roles, such as in enzyme structural properties catalysis and regulation. It contributes to antioxidant factors and the dynamic functioning of the immune system, any defect in human brain, excessively accumulated zinc ions leads to neurotoxic damage to postsynaptic neurons [Choi et al., 2020]. The aim of the present study is to innovate a biochemical relationship between (melatonin, $GALNT_{12}$ and Zn) and to highlight the biochemical role of tegretol (carbamazepine) on the above parameters for both genders.

Materials and Methods

Patients Selection

Fifty four (54) trigeminal neuralgia (TN) patients were participated in the present study, each of them attended either Saad Al-witry hospital for nervous sciences or private clinics. They were diagnosed by Magnetic resonance imaging (MRI) as trigeminal neuralgia (TN) patients. Indeed, patients enrolled in the present study were classified into four (4) groups: G_3 (40 – 70) years were composed of (12) diagnosed male TN patients (without treatment), G4 (48-75) years were composed of (12) diagnosed female TN patients (without treatment), G5 (34-76) years were composed of (15) male TN patients under treatment with tegretol and G6 (49-65) years were composed of (15) female TN patients also under treatment with tegretol. According to G5 and G6, 200 mg of tegretol / dose daily are used the duration of treatment is between (3 -72) months for both G5 and G6. Remarkably, all females participated in the present study were menopausal, TN patients were compared with healthy subjects with approximately the same range of age with patients, they did not suffer from chronic diseases and regarded as control groups: G1were composed of (15) male (43-70) years and G2 were composed of (15) female (50-55) years.

Sampling

Five milliliters (5 mL) of venous blood were collected from all subjects enrolled in the present study (from September 15th 2020 to February 20th), placed into plain tubes until coagulation was performed. Serum was separated from blood cells by centrifugation at 4000 r.p.m for 3 min, subsequently blood sera were divided into small fractions and kept as frozen samples (-20 °C) until analysis.

Biochemical Measurement

Double antibody or sandwich / enzyme linked immuno sorbent assay (ELISA) technique was applied for both melatonin and GALNT12, antibody specific for melatonin and GALNT12 was pre-coated on a microplate. Color intensity was recorded at 450 nm, melatonin concentration and GALNT12 activity were calculated according to the values of optical density. Regarding zinc, a reaction occurred between zinc and the chromogen within the reagent resulting in a complex its intensity is proportional with the concentration of zinc within sample exposed to test.

Statistical Analysis

Results of the present study were expressed as mean ± SEM(standard error mean). Students t- test was used for comparison the difference between all groups. p-value ($p < 0.05$), ($p < 0.001$) and ($p \geq 0.05$) were regarded statistically as significant, highly significant and non - significant respectively.

Results and Discussion

Table 1. Melatonin levels (pg/mL) in sera of TN patients and control groups

Group	Mean± SEM	Group	Mean± SEM
G1	47.978 ± 3.077	G2	41.099 ± 3.139
G3	26.229 ± 4.689	G4	9.65125 ± 0.891
G5	27.035 ± 4.075	G6	64.404 ± 6.784
p G3/ G1: 0.03266(S)		p G4/ G2: 0.000022(H.S)	
p G5/ G3: 0.9269(N.S)		p G6/ G4: 0.000796(H.S)	
p G5/ G1: 0.0203(S)		p G6/ G2: 0.07203(N.S)	
p G4/ G3: 0.026261(S)			

Results of table (1) have reported that melatonin level was significantly decreased in sera of G3 compared G1 and highly significant decreased in

sera of G4 compared with G2. Definitely, neuropathic pain accompanied by TN is a strong indication to inflammation, but the mechanism is not clear [Yao et al., 2020]. Another recent study has linked between TN and inflammation since TN is characterized by reasonable levels of pro-inflammatory cytokines. Those cytokines are induced by demyelination following neurve injury which is the initiated factor causing neuropathic pain [Liu et al., 2019]. Regarding melatonin, a recent study has revealed an inverse relationship between melatonin level and pain caused by melatonin is both anti - oxidant and anti- inflammatory hormone that play a key role in pain modulation [Xie et al., 2020]. Interestingly, the anti – inflammatory action of melatonin is reflected by its ability to inhibit cyclooxygenase -2 (COX-2) and inducible nitric oxide synthase (INOS) which are inflammatory mediators. [Liu et al., 2014] Moreover, melatonin reduces nuclear factor kappa B (NF-kB) expression and inhibits the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α). [Xie et al., 2020]. Besides, melatonin inhibits the release of prostaglandins and suppress polymorphonuclear leukocytes integration at the inflammation site. [Danilov et al., 2016] As a result, these accumulated findings supports the data related to the lower levels of melatonin in TN patients (males and females) compared with healthy subjects.

Conversely, after treatment with tegretol, melatonin level was non - significantly increased in G5 compared with G3 and highly significant increased in G6 compared with G4. In this regard, tegretol (carbamazepine) can return the balance by its inhibitory action on pro-inflammatory mediators generated by stimulated glial cells [Matoth et al., 2000]. Moreover, carbamazepine able to inhibit many types of inflammation in rats [Bianchi et al., 1995]. Indeed, dysregulation of voltage gated sodium channels in membrane is a major factor in pain attack in TN patients [Gambeta et al., 2020]. Sodium channels have a key role in pain as an malosensory signal [Cardoso et al., 2018]. Consequently, inhibition of those channels could return melatonin to its balance. Surprisingly carbamazepine causes inactivation of sodium channels [Menezes et al., 2020], and the results is shifting of melatonin towards the balance. Remarkably, tegretol was more active on females compared with males because the difference between G6 and G4 was highly significant while it was non-significant between G5 and G3, table (1). Hence, a previous study has reported that carbamazepine increases the activity of LH hormone by enhancing its binding with its receptors [Tamura et al., 2001]. Higher activity of LH provokes a motive for melatonin to be increased because melatonin can treat sleep disturbances in menopausal women [Omar et al., 2010]. Importantly, all members of G6 are menopausal. This is why carbamazepine was more potent on females than males regarding melatonin by its effect on LH. The significant difference between G5 and G1 indicated that melatonin level was modulated by tegretol but not reached the normal balance while the non-significant difference between G6 and G2 indicated the superior role of tegretol on melatonin in females.

Melatonin level was significantly decreased in G4 compared with G3, this comparison is related to melatonin level in both sexes regardless tegretol. Since melatonin has antioxidant properties, [Xie et al., 2020] the defense antioxidant system was reduced in post-menopausal women compared with pre-menopausal [Kolesnikova et al., 2015]. This is why melatonin level was so decreased in female patients before treatment.

The present study reports that melatonin is a novel biochemical marker in Iraqi patients with TN by a unique mechanism based on pain as a response for inflammation. Also, the present. study is the first dealt with tegretol (carbamazepine) action on melatonin in TN and the potent action of carbamazepine on menopausal women by highlighting the biochemical interaction between (carbamazepine and LH) by side and (LH and melatonin) by the other side.

Table 2. N – acetyl galactosaminyl transferase12 (GALNT12) activities (pg/mL) in sera of TN patients and control groups

Group	Mean± SEM	Group	Mean± SEM
G1	239.271 ± 9.522	G2	119.697 ± 6.160
G3	41.695 ± 1.976	G4	39.965 ± 1.457
G5	84.749 ± 1.031	G6	102.464 ± 8.594
p G3/ G1: 0.00013(H.S)		p G4/ G2: 0.00052(H.S)	
p G5/ G3: 0.001036(S)		p G6/ G4: 0.029023(S)	
p G5/ G1: 0.000595(H.S)		p G6/ G2: 0.476397(N.S)	
p G4/ G3: 0.70698(N.S)			

Results of table (2) have shown that the activities of N – acetyl galactosaminyl transferase (GALNT12) were highly significantly decreased in sera of G3 and G4 compared with G1 and G2 respectively. Definitely, trigeminal neuralgia (TN) is an exclusive facial pain syndrome characterized by paroxysmal, shock-like pain attacks located exactly in the somatosensory distribution of the trigeminal nerve [Montano et al., 2015]. In this regard, pain is the definite feature of inflammation concerning TN [Lechner et al., 2015]. On the other hand glycoconjugates are biosynthesized through a biochemical process called glycosylation and can differ in their glycan sequences, connections and length [Reily et al., 2019]. Since GALNT12 catalyzes o-glycan biosynthesis and involved in mucin glycosylation [Venkitachalam et al., 2017]. Glycosylation biosynthesis is a dynamic process linked with auto-immune, infectious and chronic inflammatory diseases. For this reason any change in glycosylation can modulate immune responses and promote inflammatory status [Reily et al., 2019]. Consequently, these collected findings supports the data related to the lower activities of GALNT12 in trigeminal neuralgia patients (males and females) compared with healthy subjects, table (2).

The present study is the first highlighting GALNT12 as a novel biochemical parameter in Iraqi patients with TN on the basis of immune- inflammatory responses.

Conversely, after treatment with tegretol, N-acetylgalactosaminyltransferase12 activities were significantly increased in G5 and G6 compared with G3 and G4 respectively. Interestingly, carbamazepine can return the biochemical-immune balance by its inhibitory action on pro-inflammatory mediators produced by stimulated glial cells [Matoth et al., 2000]. Furthermore, carbamazepine dependently reduces prostaglandin E2-like activity. A previous study has suggested that carbamazepine can inhibit the development of different types of inflammation in the rats [Bianchi et al., 1995]. Another previous study has suggested that changes in voltage-gated sodium channels may play a key role in inflammatory pain, thus sodium-channel blockers may have therapeutic action [Amir et al., 2006]. Consequently, inhibition of those channels could modulate GALNT12 towards its balance. Interestingly, carbamazepine causes inactivation of sodium channels and the results is GALNT12 shifting towards the balance [Menezes et al., 2020].

The present study submits novel findings related to tegretol action on GALNT12 based on its activity to inhibit voltage gated sodium channels and other features of inflammation. The highly significant difference between G5 and G1 have shown that GALNT12 activity is slightly affected by tegretol (carbamazepine) in males which cannot return GALNT12 activity to normal balance while the non-significant difference between G6 and G2 indicates the greater role of carbamazepine on GALNT12. Carbamazepine increases LH activity by enhancing its binding with tis specific receptors [Tamura et al., 2001]. LH in implied in the regulation of adaptive immune system [Schumacher et al., 2014]. This is why tegretol (carbamazepine) was more potent on females than males.

N-acetylgalactosaminyltransferase12 activity was non-significantly decreased in G4 compared with G3, revealing that GALNT12 activity in untreated TN patients is minimally influenced by the gender.

Table 3. Zinc levels (µg/dL) in sera of TN patients and control groups

Group	Mean± SEM	Group	Mean± SEM
G1	197.071 ± 0.205	G2	169.008 ± 2.585
G3	83.342 ± 1.264	G4	76.650 ± 0.536
G5	93.667 ± 1.092	G6	90.153 ± 0.938
p G3/ G1: 9.894E-20 (H.S)		p G4/ G2: 3.49E-9 (H.S)	
p G5/ G3: 0.029915 (S)		p G6/ G4: 7.34E-5 (H.S)	
p G5/ G1: 3.92E-21 (H.S)		p G6/ G2: 4.42 E-9 (H.S)	
p G4/ G3: 0.0763 (N.S)			

Results of table (3) have elucidated that zinc level was highly significantly decreased in sera of G3 and G4 compared G1 and G2 respectively. A previous study has reported that any change in Zn level in the central nervous system may play a definite role in the development of TN pain Syndromes [Megdiatov et al., 1995]. Definitely, zinc in blood serum is bound with albumin (57)% [Uwitonze et al., 2020]. Blood albumin level is decreased during chronic inflammation (albumin is negative acute phase protein) [Komaromi et al., 2015]. Recently, it has been suggested in experimental research that, neuropathic pain shows close correlation with inflammation [Yao et al., 2019]. In this regard, zinc is an important nutrient due to its antioxidant and anti-inflammatory properties [Jung et al., 2015].

Moreover, serum zinc levels may be inversely related to inflammatory markers (IL-6, TNF-α, and C-reactive protein CRP) [Jung et al., 2015]. As a result, these accumulated findings support the data related to lower levels of zinc in trigeminal neuralgia patients (males and females) compared with healthy subjects. The present study is the first directly highlighting lower levels of zinc in sera of TN.

In contrast, after treatment with tegretol, zinc level was significantly increased in G5 compared with G3 and highly significant increased in G6 compared with G4. This point highlights the anti-inflammatory role of tegretol. Hence, the anti-inflammatory effect of tegretol (carbamazepine) may contribute to its anticonvulsive effect [Matoth et al., 2000]. Furthermore, carbamazepine reduces prostaglandin E2 activity and inhibit many aspects of inflammation in rats [Bianchi et al., 1995]. The present study is the first directly highlighting tegretol role in modulation of Zn level in TN patients.

By comparing Zn level in treated TN patients with healthy subjects, a highly significant decrease was confirmed in G5 and G6 compared with G3 and G4 respectively revealing that tegretol though able to modulate Zn level but could not approach the normal value. A previous study has a good agreement with the present result by reporting that Zn level in patients treating with anti-convulsants children is lower than healthy subjects [Abd et al., 2015].

Zinc level was non-significantly decreased in G4 compared with G3, this comparison reflected that zinc level in untreated TN patients is not affected by the gender.

Conclusions

1. The present study is the first reporting that melatonin is a novel biochemical marker in Iraqi patients with TN by a unique mechanism based on pain as a response to inflammation, also it is the first dealing with tegretol (carbamazepine) action on melatonin in TN and its potent action on menopausal women by highlighting the biochemical interaction between (carbamazepine and LH) by side and (LH and melatonin) by the other side.
2. The present study is the first highlighting GALNT12 as a novel biochemical marker in Iraqi patients with TN on the basis of glycosylation role in protection of the immune system. Also it submits novel findings related to tegretol (carbamazepine) action on GALNT12 caused by its activity to inhibit voltage gated sodium channels and other features on inflammation.
3. The present study is the first directly highlighting Zn level in Iraqi patients with TN and tegretol role in modulating of Zn level.

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