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Evaluation of Nesfatin – 1 and Other Biochemical Markers in diabetic Neuropathy Iraqi patients before and after treatment with tegretol

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Abstract

Diabetic neuropathy is a form of nerve damage that can occur in people who have diabetes. High blood sugar (glucose) induced nerve damage in every part of the body. The nerves in the legs and feet were the most frequently affected. The extent to which a diabetic patient's body is impaired is calculated by the degree of nervosa harm.The purpose of this present study is estimation BMI,IL-10 , nesfatin-1 and HS-CRP in Iraqi DN patients before and after treatment via tegretol as well as it is the first study sheds light on the relationship between Nesfatin -1 and other parameters (BMI,IL-10 and HS-CRP) also predication of Nesfatin-1 as a newly biomarker in patients with diabetic neuropathy. The present study consist of from 30 cohort G1 as healthy group the age range from (30-50) years while diabetic neuropathy patients was divided into subunit group one of them before treatment (G2) age range (30-60) years whilst group 3(G3) age range (30-60) years after treatment ,from the results we concluded the effect of treatment by tegretol on BMI ,IL-10 and HS-CRP , but we found non-significant this treatment on Nesfatin-1 levels for patients undergo with tegretol treatment this indicated that Nesfatain -1 can't be utilize as a biomarker in those patients

Key words: ***Diabetic neuropathy ,Nesfatin-1, Interleukin -10***

Introduction

Diabetic neuropathy is a form of nerve damage that can occur in people who have diabetes. High blood sugar (glucose) induced nerve damage in every part of the body[1]. The nerves in the legs and feet were the most frequently affected. The extent to which a diabetic patient's body is impaired is calculated by the degree of nervosa harm [2] . The signs of DN range from pain and numbness in the legs and feet to intestinal, blood vessel, urinary tract, and cardiac issues. [3], The most common form of DN is distal symmetric neuropathy, which accounts for around 75% of all cases. Diabetic patients with asymmetric etiology may have set-up neuropathy. that were caused by metabolic changes and ischemia at first, but are now caused by immunological changes[3]. Two of these five tests are suggested for clinical laboratory evaluation of DN symptoms and signs: objective sensory testing, nerve conduction analysis, and autonomic testing. To regulate hyperglycemia, a cardiovascular risk factor, lipoic acid and L carnitine as non-steroidal drugs should be prescribed to unpleasant neuropathic, analgesics, or anti-inflammatory, antidepressants, and anticonvulsants. However, all diabetic patients suffer from complications such as neuropathy, although this condition may be prevented or slowed down if blood sugar levels are regulated and a proper diet is practiced [4]Interleukin-10 is a cytokine that is secreted by leukocytes (white blood cells) and other cells in the body, such as helper T cells of the second type Th2, and has properties that regulate and alter the immune response [5] , IL-10 can also decrease inflammation by blocking the production of cytokines by immune cells. Another role of Interleukin-10 is to enhance antibody production by plasma cells, allowing them to survive longer[6]. IL-10 Is Expressed Within the CNS and Limits Glial Inflammatory Responses, It is known that IL-10 plays a critical role in the resolution of peripheral inflammation and this molecule has been the most widely studied anti-inflammatory cytokineso that the biomarker IL-10 is generally considered to be the quintessential immunosuppressive cytokine produced within the CNS[7].

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Nesfatin-1 is a polypeptide consist from of 82 amino acids [8], that is excreted into peripheral tissues, the central nervous system, and the peripheral nervous system and is involved in the control of energy homeostasis in conjunction with food and water intake. In both directions, Nesfatin-1 will pass through the blood-brain barrier[9]. It prevented feeding independently of the leptin pathway and increased insulin secretion from pancreatic beta islet cells at the location[10].perhaps diabetic neuropathy (DN) is believed to be triggered by oxidative stress[11], inflammation, and neural apoptosis[12], so that Nesfatin-1 can help people with DN because of its antioxidant, anti-inflammatory, and anti-apoptotic properties [13], so that this study aimed to estimation IL-10 and nesfatin-1 as a biomarker in Iraqi diabetic DN patients before and after treatment via tegretol as well as other biomarker like HS-CRP that examines the number of high-sensitivity proteins present in the body diabetic neuropathy patients.

Patients and Methodology

The current work was carried out in The endocrinology and diabetes center of the Iraqi ministry of health in Baghdad during the period from (1/11/2020 to 1/2/2021) . The present study consist of 30 people as group(G1) age range between 35 and 65 years. While The second group consist of 30 patients with (**DN**) of type II diabetic who visited The endocrinology and diabetes center of the Iraqi ministry of health in Baghdad". before treatment with tegretol while the third group (G3) (30) patients also was selection after treatment with tegretol (200 mg per day for 3 months) also the ages range from 35 to 65 years Blood was drawn from diabetic neuropathy patients in a volume of (5 ml), then the blood was divided into two parts. A portion (2 ml) of blood was placed in the tube containing the anticoagulant (EDTA) for other measurement ,while the other part of the blood (3 ml) was placed in the tube that does not contain an anti-coagulant (EDTA) and left 30 minutes at room temperature to coagulate after that serum was obtained via separation process by a centrifuge for 10 minutesfor (3500 g) to obtain serum for determination the biomarker like (Interleukin-10 ,nesfatin-1and HS-CRP) by ELISA kit manufactured by (Human IL-10 ELISA Kit manufactured bya company (RayBiotech, Inc.) in the USA andHuman high sensitivity C-reactive protein (HS-CRP) ELISA Kit manufactured by MyBioSource company in USA andHuman Nesfatin-1 ELISA Kit manufactured by Bioassay Technology Laboratory (BT Lab) in China) the data was calculated by using T-test ,Mean and standard division . [Mean ±SD] and correlation, T-test was applied to found the difference between control group and patientsas well as between patients group before and after treatment with tegretol .The results are compared based on given $P \leq 0.05$.

RESULT

The state of association between the study groups was described as ninety samples participating in this research were studied and they were divided into three control groups as G1, and patients with diabetic neuropathy before treatment as G2 while group G3 represents patients suffering from diabetic neuropathy after treatment with (tegretol) Where they were given a treatment for three months. Results were expressed as (mean ± SD), Student's T-test was used to compare three studied groups and the extraction value P, and the posttest was also used to show the difference between the variance of groups significantly when the P- value were (P). ≤ 0.05).Result data of BMI was shown in table(1-1) and From the figure(1-1) a highly significant increase in G2(28.19±2.85) Kg/m2 comparing to G1(23.97±2.23) Kg/m2 , also a significant increase was observed in G3(25.68±3.88)Kg/m2 comparing to G1(23.97±2.23) , but a significant decrease was shown when compared between G3(25.687 ± 3.88) Kg/m2 compared to G2(28.192 ± 2.856)kg/m^2 when compared between them.Result data of Nesfatin-1 was shown in table(1-1) and From the figure(1-2) In this study a highly significant increase in G2(16.733±4.543) ng/ml comparing to G1(6.961 ± 3.996) ng/ml , also a highly significant increase was observed in G3(16.685±5.839)ng/ml comparing to G1(6.961 ± 3.996)ng/ml, but a non-significant was shown when compared between G3(16.685±5.839)ng/ml compared to G2(16.733±4.543) ng/ml when compared between them.Result data of (IL-10) was shown in table(1-1) and

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From the figure(1-3) a highly significant increase in G1(22.39 ±3.448)pg/ml comparing to G2(8.247±1.575)pg/ml, also a highly significant increase in was observed in G1(22.39 ± 3.448)pg/ml comparing to G3(13.227±2.597)pg/ml, and a highly significant increase in was shown when compared between G3(13.227±2.597)pg/ml compared toG2(8.247±1.575)pg/ml when compared between them. Result data of (HS-CRP) was shown in table(1-1) From the figure(1-4) a highly significant increase in G2(23.303±4.021) ng/ml comparing to G1(11.007±1.626) ng/ml, also a highly significant increase in was observed in G3(15.017±3.622)ng/ml comparing to G1(11.007±1.626) ng/ml, and a highly significant increase in was shown when compared between G2(23.303±4.021)ng/ml compared to G3(15.017±3.622)ng/ml when compared between them.

Table (1): Demonstrated Nesfatin-1, IL-10 , and (HS-CRP) in the studied groups

Parameters	G1 No=(30) mean ±SD	G2 No=(30) mean ±SD	G3 No=(30) mean ±SD	G2 Vs G1	G3 Vs G1	G2 Vs G3
Age years	40.4± 5.56	47.33±4.91	47.33±4.91	HS	HS	NS
BMI kg/ m^2	23.973±2.233	28.192±2.856	25.687±3.888	HS	S	HS
Nesfatin-1 (ng/ml)	6.961 ± 3.996	16.733±4.543	16.685±5.839	HS	HS	NS
IL-10 (pg/ml)	22.39 ± 3.448	8.247±1.575	13.227±2.597	HS	HS	HS
HS-CRP (ng/ml)	11.007±1.626	23.303±4.021	15.017±3.622	HS	HS	HS

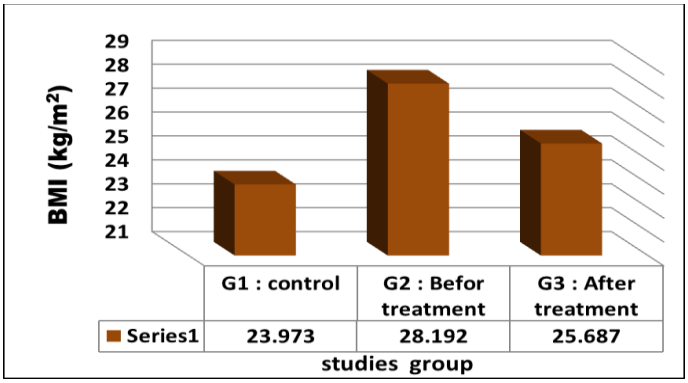

Figure (1): BMI Changes in (G1), (G2) and (G3) groups

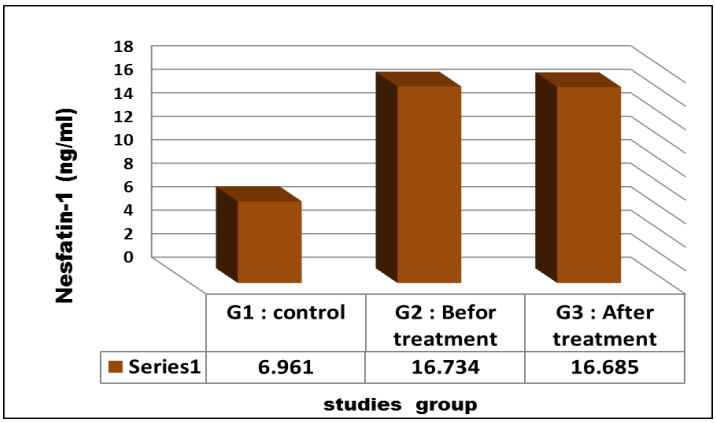

Figure (2): Nesfatin-1(ng/ml) Changes in (G1), (G2) and (G3) groups

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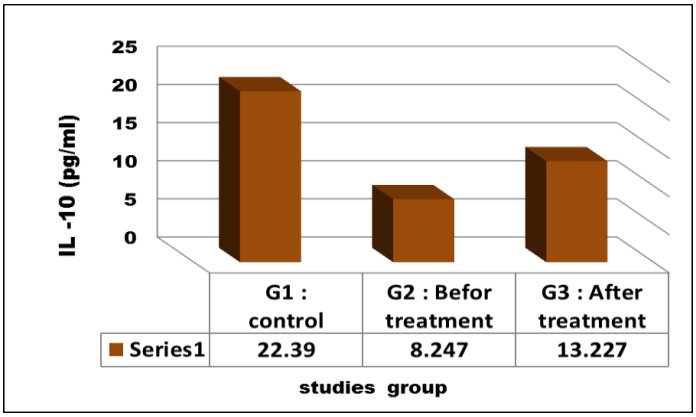

Figure (3): IL-10 (pg/ml) Changes in (G1), (G2) and (G3) groups

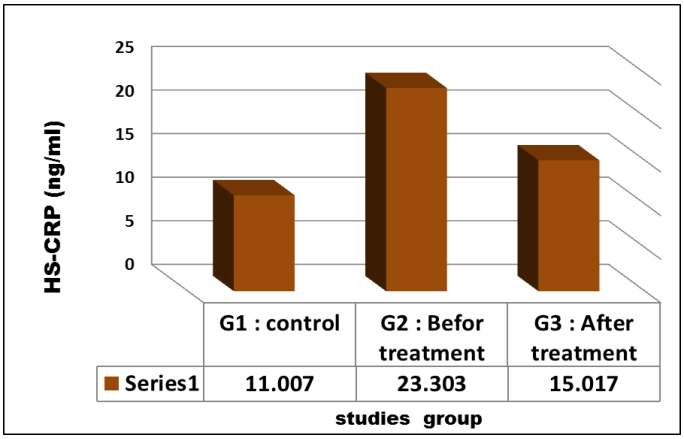

Figure (4): HS-CRP(ng/ml) Changes in (G1), (G2) and (G3) groups

Table (2) :The correlations for the present study between Nesfatin-1 with (BMI, IL-10 and HS-CRP).

parameters	Classification of Aggregates			
	G2 (Before treatment)		G3 (After treatment)	
	r	p	r	P
Nesfatin-1 VS. BMI	-0.0249	HS	-0.2412	HS
Nesfatin-1 VS. IL -10	0.03168	HS	-0.1046	HS
Nesfatin-1 VS HS-CRP	0.27436	HS	0.41636	NS

(Nesfatin-1 VS. BMI):-

The correlations for the present study between Nesfatin-1with levels BMI can present in table(1-2) and in chart -(1-1A & 1-1B) for G2&G3 respectively - which shown highly significant negative correlation When P ≤0.001 for G2(r= - 0.024) and G3(r= -0.241).

Nesfatin-1 VS. IL -10 :-

The correlations for the present study between Nesfatin-1 with levels IL -10 can present in table(1-2) and in chart (1-2A) & (1-2B) for (G2&G3) respectively which shown highly significant positive correlation When P ≤0.05 for G2(r= 0.03168) and highly significant negative correlation with G3(r= -0.1046).

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Nesfatin-1 VS. HS-CRP :-

The correlations for the present study between Nesfatin-1 with levels HS-CRP can present in table (1-2) and in chart (1-3A) & (1-3B) for (G2&G3) respectively which shown highly significant positive correlation When P ≤0.001 for G2(r= 0.27436) and no significant positive correlation G3 (r= 0.41636).

Discussion

This study is considered the first of its kind in Iraq that studied the effect of Tegretol on type 2 diabetes patients and the effect of treatment on diabetic neuropathy so that this study focused by comparing values of a set of variables before and after treatment and comprise it with the control group observing . Among the parameters that the effects were studied on are the values of (BMI,Nesfatin-1, Interleukin 10 and HS-CRP) ,Therefore, we reach a conclusion in this study that the BMI is higher in people with type 2 diabetes, and that the treatment works to increase fat burning and increase the effectiveness of insulin to reduce the body mass index, according to the results that are consistent with several studies[14].From this study, The results study for the nesfatin-1showed a very significant increase in patients with DN compared to the control group, and this indicator was consistent with [15]. The reason for this increase in type 2 diabetes patients is impaired glucose metabolism. Nesfatin-1 has been shown to have a glucose-lowering effect. It also works in the brain to increase insulin secretion from beta cells in response to high blood sugar, and to regulate insulin sensitivity [16]. Likewise, it also inhibits food intake by Nesfatin-1 through the central nervous system . The values of nesfatin-1 that we reached in this study, it confirmed its increase in patients with type 2 diabetes, which was confirmed by the study [17]. Increased secretion of nesfatin-1 correlated with BMI, weight gain, plasma insulin, and a homeostatic model assessment of insulin resistance which was also found to be elevated in our BMI study and consistent with the study [18].

There may be a paradoxical rise in nesfatin-1 in T2DM patients as a compensatory marker for the causes of obesity causing metabolic stress, as well as obesity and T2DM may be resistant to the roles of nesfatin-1, prompting to compensate them. Therefore, a decrease in nesfatin-1 was observed after treatment with a very small percentage in conjunction with a decrease in BMI after treatment. Also. Nesfatin-1 plays an important role in lowering blood sugar, even though glucose metabolism is impaired[19]. Studies have also shown that nesfatin-1 can stimulate fat metabolism. According to Li et al. In fact, eating a lot of sugar is the result of a pervasive lack of nesfatin-1 [20].The study believed that elevated Nesfatin-1 was associated with higher insulin resistance due to decreased insulin sensitivity to glucose levels in DM patients [21].However, we showed a significant increase between G3 after treatment versus the control group, because these patients were subjected to treatment to control the level of glucose and inhibit complications.While it shows a slight decrease when comparing G2 versus G3, this is due to the response of patients with DN to Tegretol treatment.From the results of the study, we showed a significant increase in the control group with DN patients compared with them. The index (IL-10) indicates an increase in the healthy control group and indicates that interleukin 10 is elevated in a healthy body as resistance, and that in the case of inflammation as in diabetic neuropathy, the role of IL-10 is antagonist. Infections in case of disease or nerve injury. The results showed that impaired production capacity of IL-10 (pro-inflammatory response) is associated with type 2 diabetes and metabolic syndrome [22]. These results are in agreement with our study. Likewise, the main biological effects of IL-10 are inhibition of the action of pro-inflammatory cytokines in neurons [23]. Therefore, its signals are low in patients with diabetic neuropathy in our study samples. The decrease in IL-10 levels gives a warning of increased effectiveness of inflammatory cytokines, which contributes to an increased risk of type 2 diabetes and tissue damage due to inflammation. Moreover, the decrease in IL-10 promotes the unbalanced regulation of pro-inflammatory cytokines and IL-10 is associated with type 2 diabetes [24] with obesity, insulin resistance and glucose intolerance [25],Therefore, the results of our study showed that IL-10 levels were low in patients with type 2 diabetes and prior to administration of Tigretol treatment, and this is consistent with studies (26) that demonstrated a link between low levels of IL-10 in patients with type 2 diabetes and supports

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its role in Peripheral nervous system that functions in cells. Neurotrophic as a treatment and anti-inflammatory, which has a role in tissue healing in neuropathy, so it gives an indication of inflammation and a clear indication [27].

Among the evidence, there is a very large increase between G1 (control group) versus G3 (after treatment) due to those patients who underwent treatment to control the level of glucose and inhibit complications, but a very large increase appears when comparing G2 Vs G3 due to the response of patients who DN suffer from Tegretol and increased levels of IL-10 after treatment (G3) indicate response and influence with treatment due to its resistance to infections caused by type 2 diabetes, control of insulin levels and lower blood sugar after treatment.From levels of HS-CRP in this study, there was a highly significant increase in the DN group of patients before treatment (G2) when compared with the control group (G1) and the group of patients after treatment (G3).T2DM has been observed to increase inflammation in patients, manifested by elevated levels of ESR and HS-CRP. Also, there was a significant positive association between HbA1c and HS-CRP blood levels. All of these results indicate a link between inflammation and glycemic control in patients with T2DM[28].(Mahajan A et al.). An association between HS-CRP and hyperglycemia was found in type 2 diabetes patients, which is in agreement with the results of our study [29]. Lima LM et al. reported that hyperglycemia is a factor related to increased serum HS-CRP levels in patients with type 2 diabetes [30]. This indicates activation of inflammatory pathways in the development of cardiovascular and kidney diseases in type 2 diabetes patients and is in agreement with our findings [31]. The inflammatory response may be due to microvascular or large vessel complications after type 2 diabetes. The level of HS-CRP in the blood is elevated in type 2 diabetes. That the concentration of HS-CRP in serum is closely related to type 2 diabetes [32]Among the evidence, a Highly significant increase between G2 (before treatment) versus G3 (after treatment) due to those patients who underwent treatment to control glucose level and inhibit complications, and this is because patients with DN had a clear response to treatment.

Conclusion

From the results we concluded the effect of treatment by tegretol on BMI ,IL-10 and HS-CRP , but we found non-significant this treatment on Nesfatin-1 levels for patients undergo with tegretol treatment this indicated that Nesfatain-1 can't be utilize as a biomarker in those patients**.**

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