Neurofilament Light Chain Assessment in Type 2 Diabetic Patients with Distal Symmetrical Polyneuropathy

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Abstract:

Background: A neuron-specific cytoskeletal protein called Neurofilament light chain is present in the cytoplasm of myelinated axons especially those with a large diameter which is essential for maintaining the size as well as for structural support.

Aging, impaired renal function, liver disorders, neuropathies, and diabetes are linked to the elevation of serum neurofilament light chain. It considers a non-invasive biomarker that could aid in diagnosing distal symmetric polyneuropathy, and potentially predict its course.

Objectives: To assess serum Neurofilament light chain levels of Distal Symmetric polyneuropathic diabetic patients and compare its levels with diabetic patients without Distal Symmetric polyneuropathy using nerve study and scoring system for screening of michigan.

Materials and methods: This cross-sectional research involved 126 males and females with type 2 Diabetes Mellitus. It was run between the end of 2022 and the middle of 2023 at the Diabetic Centre of Mustansiriyah University. After obtaining verbal approval, the studied patients were evaluated for their peripheral nerve function. In addition to their blood pressure, their body mass index was calculated using their height and weight. The lipid profile and Neurofilament Light Chain were all analyzed using the serum. Formulas were used to compute Both LDL-c and VLDC.

Results: This study showed no change in the serum levels of the neurofilament light chain when comparing patients with and without distal symmetric polyneuropathy, additionally, no change was found based on the patient screening scores of Michigan. Moreover, Neurofilament Light Chain levels were substantially reduced in patients who were taking sulfonylurea alone or in combination with other anti-diabetic treatments. In patients who were taking antilipidemic statin therapy, the serum levels of the Neurofilament Light Chain were substantially reduced compared to those not receiving it.

Conclusion: The serum level of the neurofilament light chain was affected by the presence of distal symmetric polyneuropathy although as a clinical condition, it is associated with axonal damage, it was found that it could not be used for the prediction of distal symmetric polyneuropathy.

Keywords: Neurofilament Ligh Chain; Distal Symmetrical Polyneuropathy; Nerve Conduction Study; Michigan Neuropathy Screening Instrument.

تقييم سلسلة الخيوط العصبية الخفيفة في مرضى السكري من النوع الثاني الذين يعانون من اعتلال الاعصاب المتعدد المتناظر البعيد

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الخلاصة:

الخلفية: بروتين هيكلي خلوي خاص بالخلايا العصبية يطلق عليه سلسلة الخيوط العصبية الخفيفة يقع في سايتوبلازم المحاور المغلفة بالمايلين خصوصا تلك التي لديها قطر كبير ويكون ضرورياً للحفاظ على حجم هذه المحاور ويوفر دعم هيكلي لها. ترتبط الشيخوخة واضطرابات وظائف الكلى، امراض الكبد، الاعتلالات العصبية ومرض السكري بارتفاع مستويات سلسلة الخيوط العصبية الخفيفة في مصل الدم. وتعتبر علامة حيوية غير جراحية يمكن ان تساعد في تشخيص اعتلال الاعصاب المتعدد المتناظرالبعيد وربما التنبأ بمساره.

الاهداف: تقييم مستويات سلسلة الخيوط العصبية الخفيفة في امصال مرضى السكري الذين يعانون من الاعتلال الاعصاب المتعدد المتماثل البعيد ومقارنه مستوياته مع مرضى السكري الذين لا يعانون من اعتلال الاعصاب المتعدد المتناظرالبعيد باستخدام دراسة الاعصاب ونظام النقاط للتحري التحري مشيغان.

المواد والطرق: شارك 126 مريض من الذكور والاناث الذين يعانون من مرض السكري النوع الثاني في هذه الدراسة المقطعية التي امتدت من نهاية عام 2022 الى منتصف عام 2023 في مركز السكري التابع للجامعه المستنصرية.بعد الحصول على الموافقة اللفظية, تم تقييم الوظائف الاعصاب الطرفية للمرضى الخاضعين للدراسة وتم احتساب مؤشر كتلة الجسم باستخدام طولهم و وزنهم. تم تحديد مستويات جميع انواع الدهنيات وسلسلة الخيوط العصبية الخفيفة في المصل. تم استخدام المعادلات لحساب LDLوVLDL.

النتائج: اظهرت هذه الدراسة عدم وجود تغيير في مستويات مصل سلسلة الخيوط العصبية الخفيفة عند مقارنة المرضى الذين يعانون من اعتلال الاعصاب المتعدد المتناظر البعيد وبدونه. بالاضافة الى ذلك، لم يتم العثور على اي تغير بناءاً على درجات فحص المرضى مشيغان. يضاف الى ذلك، انخفضت مستويات سلسلة الخيوط العصبية الخفيفة بشكل كبير في المرضى الذين كانوا يتناولون السلفونيليوريا لوحده اومع علاجات اخرى لمرض السكري. في المرضى تاذين كانوا يتناولون علاج المضاد للدهون الستاتين, انخفضت مستويات سلسلة الخيوط العصبية بشكل كبير مقارنة مع اولئل الذين لايتناولونه.

الكلمات المفتاحية: سلسلة الخيوط العصبية الخفيفة، اعتلال الاعصاب المتعدد المتناظر البعيد، دراسة التوصيل العصبي، أداة فحص الاعتلال العصبي مشيغان.

Introduction:

Distal Symmetrical Polyneuropathy (DSPN) also known as Diabetic Peripheral Neuropathy is the most prevalent type of DPN that causes damage to the nerves found in the extremities, especially the feet (bilateral symmetric pattern), it is associated with the development of foot ulceration and leads to lower limb amputation [1,2,3]. Distal Symmetrical Polyneuropathy is mostly sensory and exists as three definite types based on the type of nerve fibers involved: mainly small fiber, mainly large fiber, and mixed (myelinated (small and large) and non-myelinated C-fibers) fibers which are the most common sensory neuropathies [4]. Diabetic Peripheral Neuropathy is commonly seen in about 40-50% of long-standing diabetic patients after the exclusion of other causes and it could be presented clinically either symptomatic or asymptomatic[5,6,7]. Clinically, it is mainly presented as symmetric sensory pain in the feet in addition to the loss of sensation, pain, numbness, and burning sensations that are worse at night and cause sleep disturbance [8,9,10]. It can also show other presentations like weakness of muscle, poor balance, and a tendency to fall

which refers to dysfunction of the nerve motor that will develop later during the course of the disease [9,10].

The Neurofilament Light Chain (NFL) is one of the five sub-units of the Neurofilament that make up the neuronal cytoskeleton [11]. As indicated in Figure 1. It is a neuron-specific cytoskeletal protein (~68 kDa) found most abundantly in axonal cytoplasm, especially large-diameter myelinated axons, where it plays a crucial role in the structural support and the preservation of the size and shape of the axons [12,13]. In addition to contributing to the development of the neuronal cytoskeleton, NFL could be crucial for the recovery from axon injury [14]. Elevated NFL is associated with aging, abnormal kidney function, liver diseases, neuropathy, and diabetes[15,16].

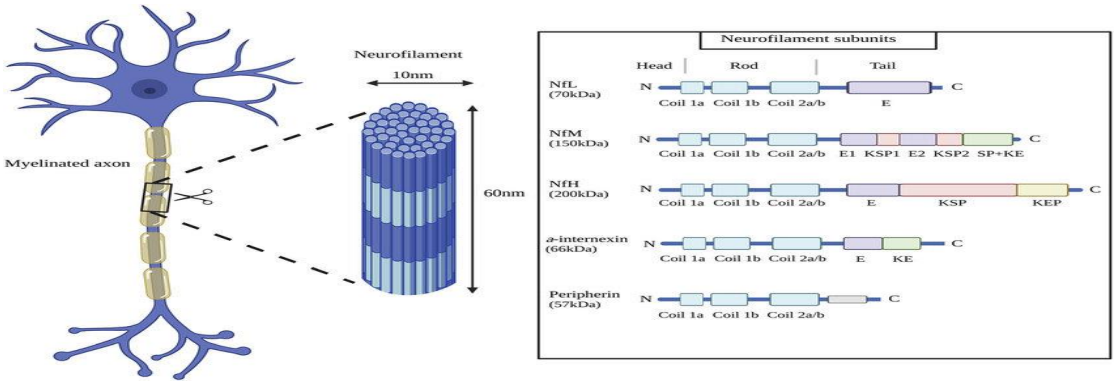

Figure 1: The structure of Neurofilament and the structure of it is sub-units (NFL, neurofilament light chain; NFM, neurofilament medium chain; NFH, neurofilament heavy chain; SP, serine-proline)[17].

Theoretically, all axonal injury-related abnormality will lead to an elevation in blood NFL levels, enabling the NFL test to be used as a tool for measurement [18]. Thus, it is considered a biomarker in many diseases associated with axonal injury in both central and peripheral nervous systems [13, 19]. As indicated in Figure 2. Thus, leading to a reduction in the nerve conducting velocity [20]. Depending on the extent of axonal injury, NFL releases into the cerebral spinal fluid (CSF) and the blood circulation during neurodegeneration, thus increasing their blood level [18,21,22] Neurofilament light chain has been utilized in the course of neurodegeneration and neuroinflammation as a general axonal damage marker [23].
Serum NFL levels, rather than CSF levels may be more reliable in PNS disorders to monitor tissue damage and disease activity [24]. Therefore, neurologists have the opportunity to use blood NFL as a supportive tool in a variety of clinical situations[25]. Several neuropathic disorders, such as amyloid neuropathy, DSPN, leprosy neuropathy, etc. they show an elevation in NFL that can serve as a predictor of the progression of peripheral neuropathy [18]. It could be used to predict the likehood of neural damage in T2DM since DM selectively attacks PNS in a widespread or diffused manner, resulting in the development of diabetic (polyneuropathy or focal) neuropathies[26]. This makes NFL, non-invasive biomarkers that could help in the DSPN diagnosis, and even forecast its development, It may have a considerable benefit in the investigation of structural

changes taking place in DSPN[27]. Neurofilament light chain may mediate inflammatory response development against the neurons since they are phagocytosed by macrophages and microglia and then presented to T-lymphocytes [28].

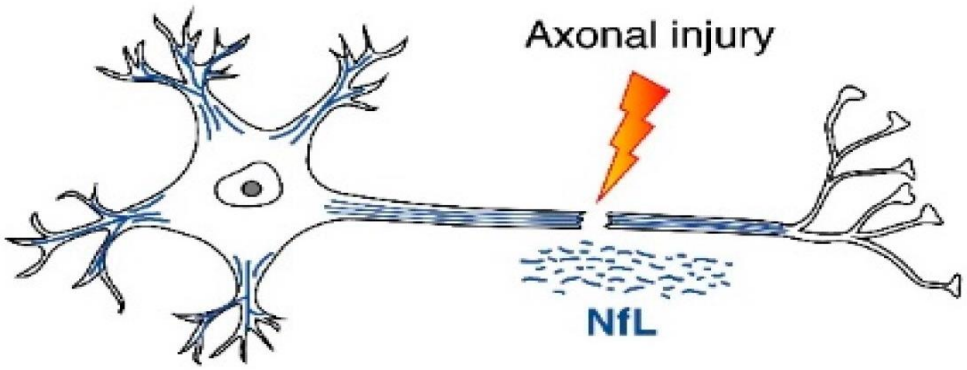

Figure 2 : Neurofilament light chains leaking out following axonal injury [29].

The aim of this study:
Measuring serum NFL level in Type 2 diabetic patients with DSPN and comparing it to those without DSPN to find out if NFL serum level is changed in patients regarding the presence of DSPN through using two DSPN identification techniques (nerve conduction study and scoring system for screening of Michigan).

Materials and Methods:

A cross-sectional study in which one hundred twenty-six of the type two diabetic patients were both male and female aged above eighteen years old. Those patients were selected randomly from patients who attended the Diabetic Research Center / Mustansiriyah University between December of 2022 till the July of 2023. After the aim of the study was explained to those patients, a verbal agreement was taken from them to take part in this cross-sectional study. Patients with neurodegenerative diseases, inflammatory diseases, smoker, renal and liver impairments were excluded. For the included patients they were not previously diagnosed with DSPN. The confirmed patients with and without DSPN were age, BMI and disease duration matched. From all the studied patients, the clinical history, and physical exam were taken. Michigan Neuropathy screening instrument and the conduction study were used for screening and confirming the existence of DSPN respectively. Serum from the fasting whole blood that was obtained from the patients was used to assess lipid profile (TG, cholesterol, and HDL) using enzymatic photometric method and Neurofilament Light Chain using ELISA technique and according to manufacturers' instructions in while VLDL and LDL were obtained mathematically [30]. As shown in Figure 3, in this study the studied patients were organized differently several times using in one of these classification the finding of the Nerve conduction study, in the second classification the screening of the sores of Michigan Neuropathy Screening Instrument (MNSI) as used in which the diabetic Patients who had score above or equal to nine and half were considered patients with possible diabetic peripheral neuropathy while the diabetic patients with a score less than nine and half were considered patients without possible diabetic peripheral neuropathy [31]. While in the last classification, both NCS and MNSI finding was used as shown in Figure 3.

Additionally, Insulin and/or oral hypoglycemic medications were used in their treatments. It is worth mentioning that of the 126 patients: 39 patients were using metformin, and 14 patients were using sulfonylurea therapy alone.

Statistical Analysis:

Independent student T-test was used to find the mean and Standard error for every single variable and for comparison of two means in addition to the ANOVA test to compare the mean among more than two groups and the Chi-squared test was used for categorical variable expression of their numbers as percentages and for analysis. Moreover, Pearson's linear correlation coefficient was utilized to ascertain the direction and degree of the correlation between two normally distributed variables. All those tests were performed using Prism as an analytical software program. The P-value of less than 0.05 was regarded as significant.

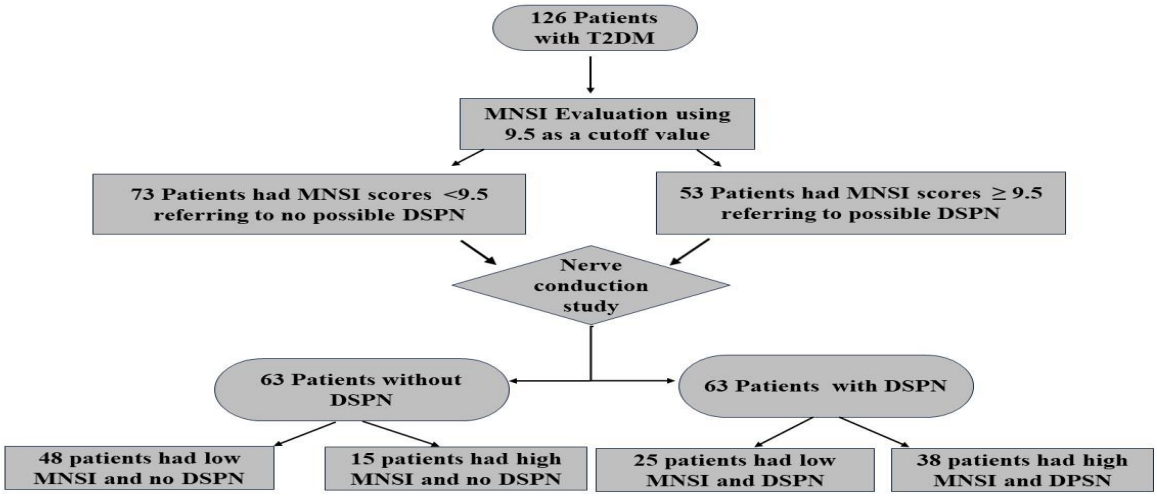

Figure 3: A flow chart explaining the study was progress with patients' classifications. (T2DM, Type 2 diabetes Mellitus; MNSI, Michigan Neuropathy Screening Instrument; DSPN, Distal Symmetric polyneuropathy)

The Results:

According to the findings of this study regarding lipid profile and as shown in Figure 4, there were no significant differences (p-value <0.00=5) between the patients with and without DSPN.

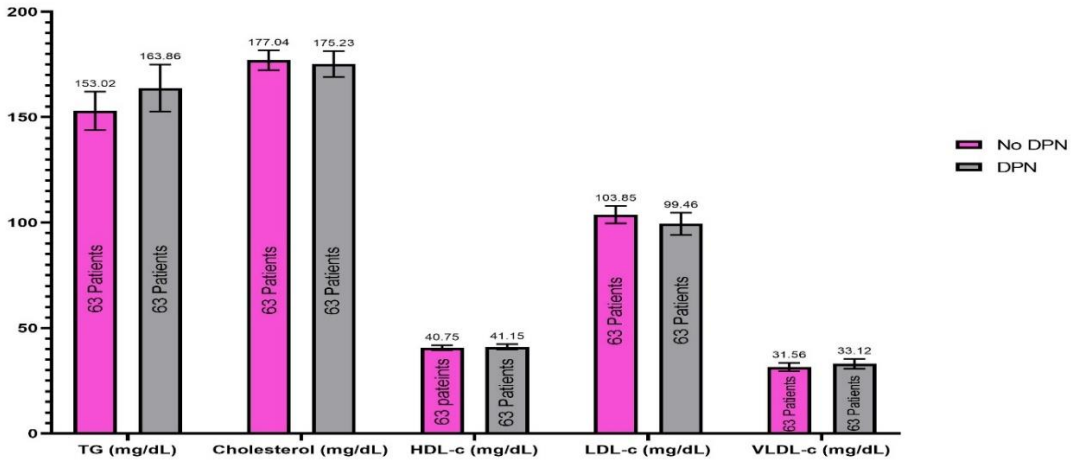

Figure 4: the comparison of the lipid profile among patients with and without DSPN (DSPN, Distal Symmetric Polyneuropathy).

Additionally, and based on the score of the diabetic patients with a score below 9.5 referred to as a lower score and equal and above 9.5 referred to as a higher score, Figure 5 demonstrated that no statistically significant differences in the mean of serum NFL levels (p-value=0.662) when compared (mean ± SE) of those with the lower scores (10.10±0.61) to those with the higher scores (10.51±0.70). Moreover, Figure 6 showed that when the same patients were compared according to the findings of the Nerve Conduction Study the mean of NFL levels had no statistically significant differences (p-value=0.793) among patients with no DSPN (10.40±0.66) and those with DSPN (10.15±0.63). Table 1 and Figure 7 both indicated that when all patients were grouped using both scores and nerve study there were no statistically significant differences in the mean of serum NFL levels (p-value=0.784) between those groups using student t-test and ANOVA test analysis

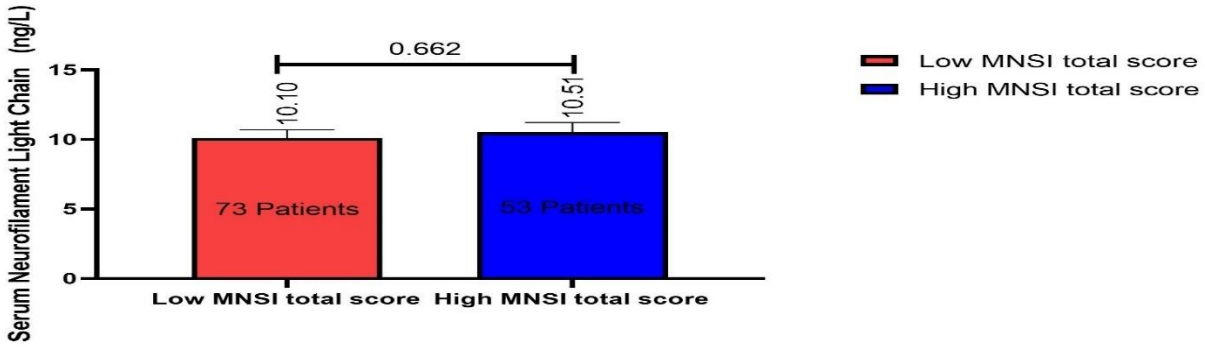

Figure 5: the comparison of patients' means of serum Neurofilament Light Chain with low and high scores of Michigan Neuropathy Screening Instrument (MNSI).

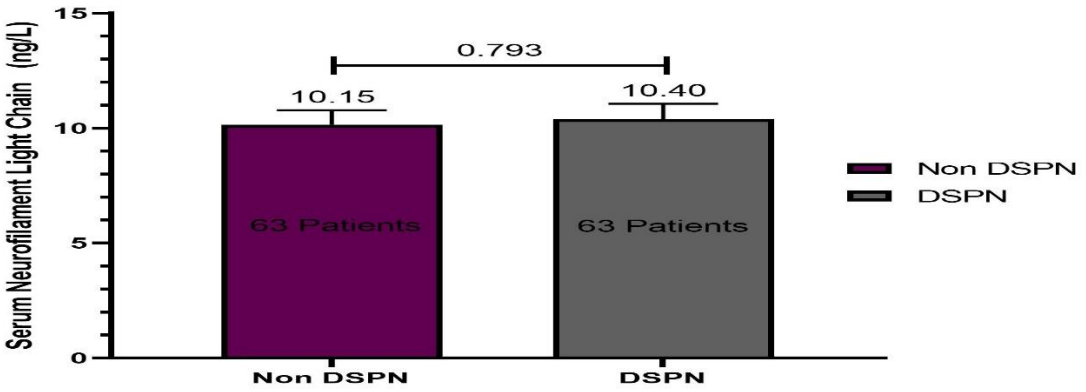

Figure 6: The differences in the mean of serum Neurofilament Light Chain (NFL) levels between diabetic patients who had no DSPN and those who had DSPN (DSPN, Distal Symmetric Polyneuropathy).

Table 1: The differences in the mean of serum Neurofilament Light Chain (NFL) levels between diabetic patients based on their scores and Nerve Conduction Study.

	Score	DSPN	Sub-group	Patients Number	Mean	±SE	P-value
Neurofilament Light Chain (NFL) ng/L	**Low**	**No**	**A**	48	10.49	0.80	0.784
		Yes	**B**	25	9.37	0.92	
	High	**No**	**C**	15	10.11	1.22	
		Yes	**D**	38	10.67	0.86	

A: Patients with low MNSI scores and had no DSPN.
B: Patients with low MNSI scores and had DSPN.
C: Patients with high MNSI scores and had no DSPN.
D: Patients with High MNSI scores and had DSPN

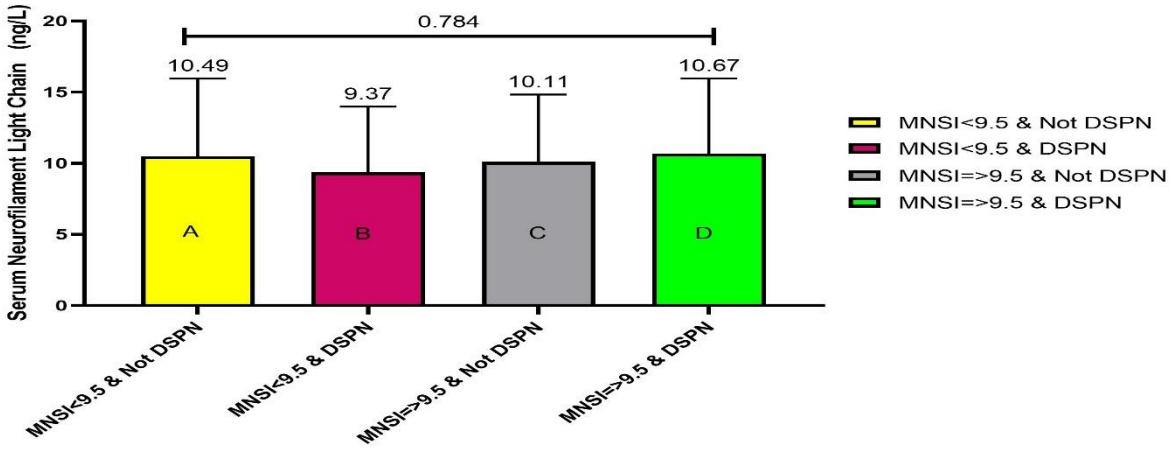

Figure 7: the comparison of serum Neurofilament Light Chain among the patients' subgroups.

Regarding whole patients' diabetic medication and as shown in Figure 8, serum NFL exhibited substantial reduction (p-value=0.046) in those who did not receive sulfonylurea compared to the others who received it. While metformin therapy (alone or with other medication), and Insulin therapy in conjugation with other therapy showed no differences between those receiving them and not.

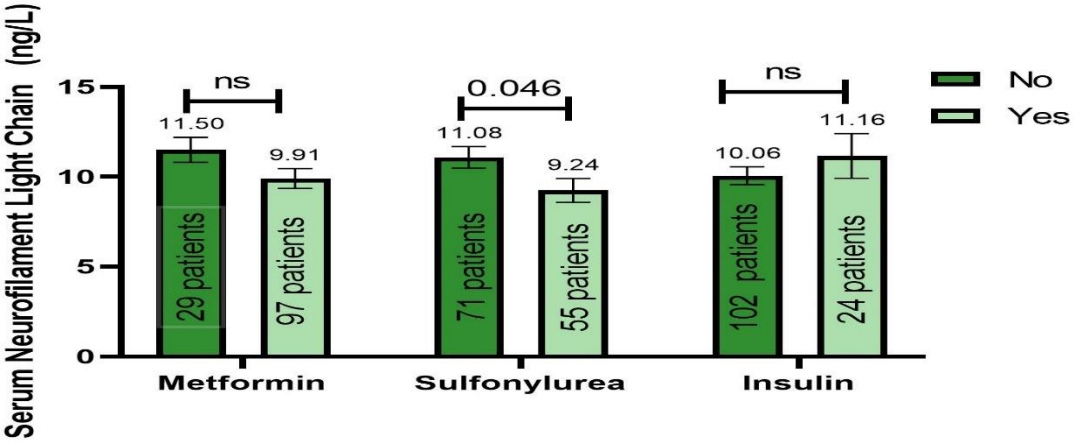

Figure 8: the comparison of serum Neurofilament Light Chain among the patients based on their diabetic medication.

Regarding statin therapy classification, this study showed that serum NFL was substantially elevated in diabetic patients who not receiving statin therapy compared to those receiving statin therapy p-value =0.015 as indicated in Figure 9.

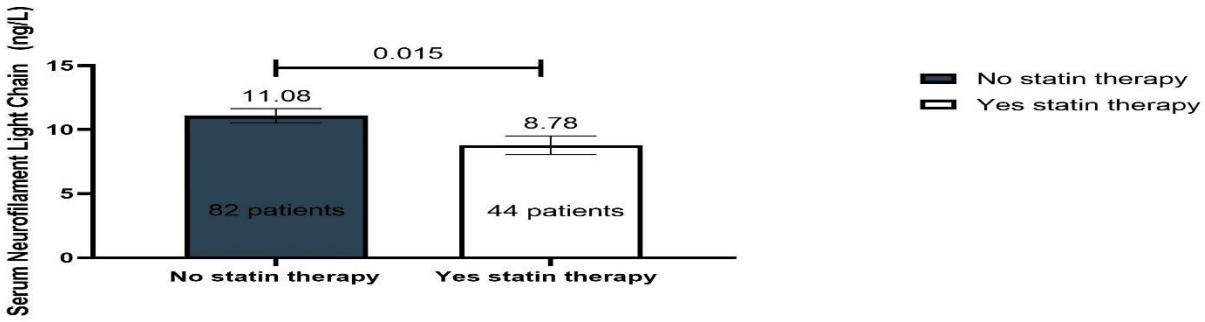

Figure 9: the comparison of serum Neurofilament Light Chain based Statin therapy.

This study showed that NFL exhibited no association with any of the studied parameters in all patients and patients with DSPN regardless of their sores and in patients who had no DSPN with low scores in which the p-values were above 0.05. However, NFL exhibited a moderate negative association with serum Triglyceride in patients who had no DSPN but had high MNSI score as indicated in Figure 10.

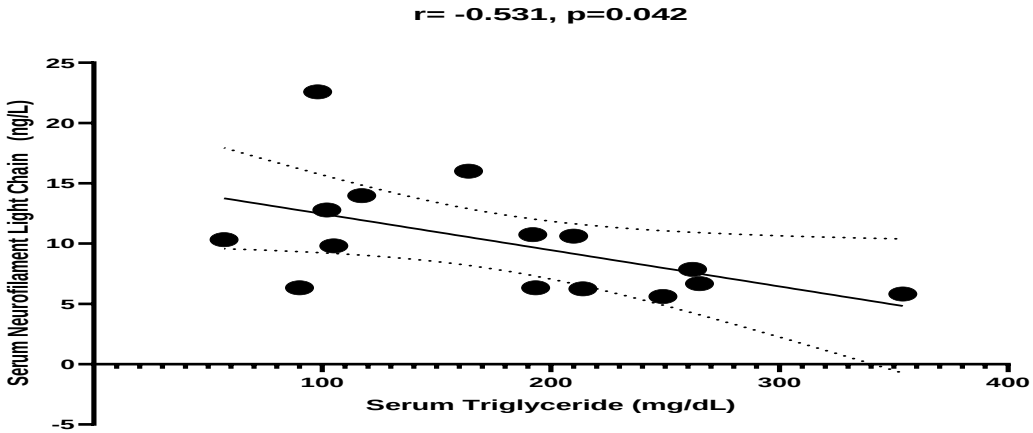

Figure 10: the correlation between serum Neurofilament light chain and serum Triglyceride in patients who had no DSPN but had high scores.

Discussion:

The neurofilament light chain is one of the neurofilament proteins that is expressed by the mature neuron in the cell body and subsequently transported to the axon to preserve structural and functional function, furthermore, when the neuron suffers from injury it will appear in the serum indication the onset of neural injury making it a significant biomarker for many illness associated with neuronal injury [32]. The determination of nerve conductivity is considered one of the most precise and accurate methods for the detection of DSPN through assessing both sensory and motor nerve integrity [33].

According to the findings of this study, the means of serum Neurofilament light chain (NFL) level exhibits no statistically substantial difference (P-value =0.793) between diabetic patients with and without DSPN as shown in Figure 6. This finding was consistent with the finding of Morgenstern et al. (2021) [27] which demonstrated the absence of substantial changes in the mean of serum NFL levels among diabetic patients with and without DSPN, however, in diabetic patients with peripheral neuropathy the levels were significantly higher than healthy control individuals, but it's notable that the duration of diabetes in studied patients was less than three years.

Conversely, the finding of this current study was inconsistency with the findings of Maalmi et al. (2023) [34] who investigated recently diagnosed diabetic patients (both type 1 and type 2) using a multiplex assay panel to assess NFL levels in serum which found that patients with DPN had the higher NFL levels, along with the inconsistently finding of Kender et al. (2023) [35] which employed electromyography alongside Magnetic Resonance for assessment of sciatic nerve microstructure, without mention of the use of Nerve Conduction Study (NCS). Additionally, the finding of the current study is inconsistent with Mä et al. (2023) flow-up study, which was conducted over ten years using ultrasensitive single molecular assay (Simoa) Technology and found that serum NFL was substantially higher in those with DPN, however, the study concluded that serum NFL could not serve as a useful marker for the presence of DPN rather than it could

be associated with the nerve injury severity of DPN [36].

Therefore, it could be said the inconstancy of the current study findings regarding NFL and DPN with the previously mentioned studies could probably be due to differences in the sample size, the use of different more sensitive methods than ELISA for NFL estimation, the differences in the duration of diabetes of patients involved, the inclusion of T1DM beside T2DM and the use of other detection methods the presence of DSPN. The current study employed a nerve conduction study to ensure the presence of nerve damage. Unfortunately, only large-diameter axons are evaluated for their integrity using NCS, while the destruction in the smaller fibers cannot be detected by NCS because they cannot produce a sufficient electrical field to be detected [37]. Moreover, Widyadharma et al. (2022) study that involved prediabetic individuals, had been related the elevation of serum NFL messenger ribonucleic acid (mRNA) to the destruction in the small nerve fibre that known also known as C- fibers due to chronic elevation in glucose blood level in individuals with peripheral neuropathy who were already prediabetic [38]. This could likely explain the lack of substantial differences among diabetic patients regarding NCS. Eventually, this could indicate and based on the previously mentioned studies that NFL is the best to use for the detection of DSPN during the early onset of DM as when the disease progresses more nerves will be involved some damages could be detected while others will not be using NCS.

Based on the participant's MNSI Total score as shown in Figure 5 diabetic patients did not exhibit any substantial change in their mean serum level of NFL (p-value=0.662). It should be noted that no previous studies were found based on our knowledge comparing serum NFL levels regarding patients' MNSI scores, but it could be suggested that NFL serum levels were not affected by MNSI scores, however, further studies are required to confirm or deny this finding.

By comparing the mean of serum NFL levels between type 2 diabetic patients' subgroups (A, B, C and D) as mentioned in Table 1 and Figure 7 no substantial differences were found between the four subgroups (P-value =0.784), however, the highest results were found in those with both a high score and DSPN (D subgroup) but statistically it was not significant. Further studies are necessary to confirm or deny these findings due to the lack of previous studies based on our knowledge in this regard.

It has been found that the use of sulfonylurea and Insulin is associated with the reduction of serum NFL among hyperglycemic rats with Alzheimer's disease [39]. This study also showed that serum NFL exhibited a substantial reduction in those who received sulfonylurea therapy (p-value=0.046), While in those using metformin and insulin, there were no substantial differences compared to those not using them (p-value >0.05) it is notable that no previous study in this regard was found thus further studies are required as shown in Figure 8.

Regarding statin therapy in this study and as shown in Figure 9, this study showed that serum NFL was substantially reduced in those patients on statin therapy (p-

value=0.015), which could indicate that statin had a beneficial effect in those patients, notably, no previous studies on our knowledge studied this relationship. The relationship between statin therapy and DPN is a subject under discussion some studies showed a positive link between them however other studies showed that the use of statin could have some advantage in DPN however more studies were suggested [40,41,42]. Additionally, previous studies also showed that statin treatment had no role in the development of peripheral neuropathy [43,44].

So far, no previous studies had been found assessing the association of serum NFL in patients with DM utilizing both the conductivity study and MNSI scoring, regarding A, B and D subgroup showed no association with any marker of lipid profile (p-value >0.05), while In the C subgroup that included patients without DPN and had an MNSI Total score of ≥9.5, NFL exhibited a moderate association with serum Triglyceride level (r= -0.531, p=0.042), to our knowledge no previous research work was found studied the relationship between those biomarkers about both NCS and MNSI scores at the same time to compare this finding to. However, a study by Ridker et al. (2008) showed two disadvantages of using fasting TG including the underestimation of the elevation of blood TG levels known as hypertriglyceridemia and the exclusion of postprandial effect [45]. **Limitations:** Small sample size, the patients being taken from one place and lack of health control due to the design of the study.

Conclusion: Although DSPN is linked to axonal injury as a clinical manifestation, the serum level of NFL could not be used to predict the development of DSPN. Moreover, in this study medication (sulfonylurea and statin) taken caused a substantial reduction in the serum level of NFL which could indicate that these medication could have benefits in patients with DSPN, however, additional studies are required to confirm those findings using a larger sample size.

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