ORIGINAL ARTICLE

Ultrasonic Evaluation of Muscle Echogenicity in the Lower Limbs for the Detection of Diabetic Peripheral Neuropathy

Sally H. Abdulkareem * , **Abdulnasir H. Ameer**

Department of Physiology, College of Medicine, University of Baghdad, Iraq

*Corresponding Author: Sally H. Abdulkareem
Email: sallyrasheed9944@gmail.com

Received: 23 June 2023 / Accepted: 27 August 2023

Abstract

Purpose: This study aimed to test the ability of high-frequency muscle ultrasound in detecting changes in muscle Echo Intensity (EI) in patients with type 2 Diabetes Mellitus (T2DM) and to correlate muscle ultrasonography findings with Nerve Conduction Study (NCS) parameters in those patients. Additionally, we aimed to assess the usefulness of muscle ultrasound in diagnosing diabetic peripheral neuropathy.

Materials and Methods: In this case-control study, 26 diabetic patients with Diabetic Peripheral Neuropathy (DPN) and 25 controls were enrolled. Among the controls, 15 were healthy individuals, and the remaining 10 were diabetic patients without DPN. All participants underwent Nerve Conduction Studies (NCS) of the peroneal and tibial motor nerves, as well as quantitative muscle ultrasound. Ultrasound (US) images of the Abductor Hallucis (AH) muscle, Tibialis Anterior (TA) muscle, and Rectus Femoris (RF) muscle were taken and analyzed using grayscale analysis to measure quantitative Echo Intensity (EI). A comparison between the groups regarding EI was made, and correlations between NCS and quantitative US results were assessed.

Results: Our Study unveiled a statistically significant augmentation in muscular EI within two of the scrutinized muscle groups among individuals afflicted by DPN, relative to the control cohorts. Moreover, a noteworthy correlation was discerned between the parameters of NCS and muscular EI.

Conclusion: Quantitating muscle EI using grayscale analysis of US images is a useful supplementary test for the detection of DPN.

Keywords: Diabetic Peripheral Neuropathy; Muscle Ultrasound; Echo Intensity; Nerve Conduction Study; Greyscale Analysis.

DOI: https://doi.org/10.18502/fbt.v11i3.15889

1. Introduction

The diabetes epidemic and its complications constitute a significant threat to global health [1]. In 2021, the International Diabetes Federation (IDF) predicted that the prevalence of diabetes mellitus among individuals aged 20-79 is estimated to be one in ten (537 million adults); this estimate is expected to grow to 783 million by 2045. Diabetes can lead to a wide range of complications [2]. Diabetic Peripheral Neuropathy (DPN) is a prevalent complication of Diabetes Mellitus (DM). It is characterized as a length-dependent neuropathy and affects at least half of all people who have diabetes during the course of their lifetime. Severe complications can arise from advanced DPN, including diabetic foot ulcers, gangrene, and the necessity for amputation, leading to impaired quality of life for individuals with diabetes. Thus, early recognition of this condition is of great importance [3, 4].

Asymptomatic diabetic neuropathies represent around 50% of diabetic neuropathies [4]. Multiple types of diabetic peripheral neuropathy exist. The most prevalent is distal symmetric polyneuropathy [5]. The spread of its symptoms is described as "stocking and glove," where the hands and lower limbs are frequently involved [4]. Its primary diagnoses heavily rely on the patient's clinical history and thorough physical examination, complemented by essential electrophysiological studies, particularly Nerve Conduction Studies (NCS). The NCS proves to be exceptionally valuable, not only in confirming the presence of polyneuropathy but also in excluding any potential mimics and revealing its characteristic symmetrical length-dependent pattern. Nevertheless, it remains incapable of offering insights into the muscles' morphological assessment or the surrounding tissues' condition and structures [6].

Over the past two decades, Neuromuscular Ultrasound (NMUS) has evolved into a reliable and essential diagnostic technique. Needle electromyography and nerve conduction investigations are utilized together worldwide to enhance the diagnostic capabilities of clinicians. It provides dynamic structural information that aids in refining diagnoses and determining the structural etiology. It has a high success rate for assessing muscle size, thickness, shape, and echogenicity. NMUS has the potential to enhance patient care by providing valuable insights for individuals with muscle disorders, motor neuron disease, mononeuropathies, and polyneuropathies [7, 8].

Motor nerve dysfunction is a well-established characteristic of DPN, leading to observable distal muscle alterations, including muscle weakness and atrophy [9]. Normal muscle tissue allows ultrasound waves to travel easily through it, giving it a hypoechoic appearance, whereas fibrous tissue appears hyperechoic due to the reflection of ultrasound. Echo Intensity (EI) can be used to illustrate muscle alterations arising from elevated intramuscular fibers and adipose tissues. The presence of muscle atrophy and fat deposition within muscle fibers leads to hyperechoic and heterogeneous muscle tissue, increasing EI. Consequently, denervated muscles exhibit elevated EI levels [10], while a high muscle EI has been suggested as a muscle alteration indicator arising from myopathy and neuropathy [11].

Our study introduces a novel approach by harnessing the power of Ultrasound (US) imaging and quantitative analysis. Our primary objective is to explore the effectiveness of ultrasound in identifying echo intensity alterations in the muscles of individuals with DPN, while concurrently comparing these findings to NCS. Furthermore, we seek to unravel the intricate relationship between the quantitative muscle ultrasound results and the parameters derived from NCS, thereby enhancing our understanding of the complex interplay between diabetes-related neuropathy and musculoskeletal changes. This innovative methodology not only extends our understanding of the nuanced progression of DPN but also promises a reliable diagnostic tool for its early detection. Our investigation marks a significant advancement in the field, offering a fresh perspective on the multifaceted nature of DPN and its impact on both nerves and muscles.

2. Materials and Methods

2.1. Patients

Our case-control study took place at the neurophysiology unit of Al-Shaheed Ghazi Al-Hariri Hospital in Baghdad from October 2022 to February 2023. Prior to the procedure, we provided detailed explanations about the test to all patients, and their informed consent was obtained. Additionally, the study received approval from the ethical committee of the University of Baghdad – College of Medicine. The patient group comprised twenty-six individuals

diagnosed with type 2 DM, consisting of 6 females and 20 males. These patients reported symptoms such as paresthesia, pain, and/or weakness in their extremities, with a particular emphasis on the lower limbs. After a clinical history was taken, they underwent a neurological examination and nerve conduction study. Patients who had abnormal NCS results were included in the study as patients with DPN. Regarding the control group, we obtained two groups: control group 1 included diabetic patients without DPN, which comprised 10 participants, and control group 2 included 15 apparently healthy individuals. Group 1 participants were chosen to have type 2 DM but had no complaints of peripheral nerve dysfunction and had normal physical examinations and nerve conduction studies. Both case and control groups' ages ranged from 40 to 65 years old. Patients with neuropathy caused by endocrine/metabolic triggers (hypothyroidism, liver dysfunction, and chronic renal impairment), autoimmune medical conditions (SLE, RA, and Sjogren syndrome), drugs (amiodarone, isoniazid, and chemotherapy), tumors (lymphoma, leukemia, and direct tumor infiltration), or alcohol intake were omitted from the study. After explaining the purpose of the study to each participant, verbal consent was obtained.

The assessment was conducted for each patient, which involved recording their medical history, measuring their body mass index (BMI), and obtaining HbA1c levels, and all participants underwent a general examination, including an upper and lower limb examination from the neurological perspective. NCS of the tibial and peroneal nerves as motor nerves and sural as sensory nerves were tested in each participant. Limb temperature was measured and kept between 33 and 36 degrees Celsius, and an abrasive skin cleanser was used to prepare the skin. Electrical stimulation of the peripheral nerves was done to induce maximal responses. Various nerve parameters were measured, including waveform amplitude, distal latency, and conduction velocity for each nerve [12]. Ultrasound examination was performed in the ultrasound unit on the same day as the electrophysiological examination, using an HD11XE Philips ultrasound device, 2009 model, and a linear probe with a frequency range of 5-12 MHz [13, 14]. The US examination was conducted by the same examiner for all the participants to decrease inter-observer variation. Each participant was lying relaxed in a supine position with the knees extended. A considerable amount of ultrasound gel was used so that full contact occurred between the probe and the skin without compressing the skin and the tissues below. To examine the AH, the probe was positioned on the foot medially midpoint between the heel and the ball of the foot while positioning the probe laterally to the tibial crest on the belly muscle, it was placed two-thirds of the way between the knee and the ankle. RF was visualized by positioning the probe on the anterior thigh, at the midpoint between the hip and the knee, as shown in Figure 1. Transverse ultrasound images of the rectus femoris muscle, the tibialis anterior muscle, and the abductor hallucis muscle were obtained by applying the probe on the muscle belly showing as much of the muscle as possible to assess the echo intensity of those muscles. The gain was not changed between participants, while the depth and focal point were adjusted based on the muscle thickness and distance from the skin to the inferior border.

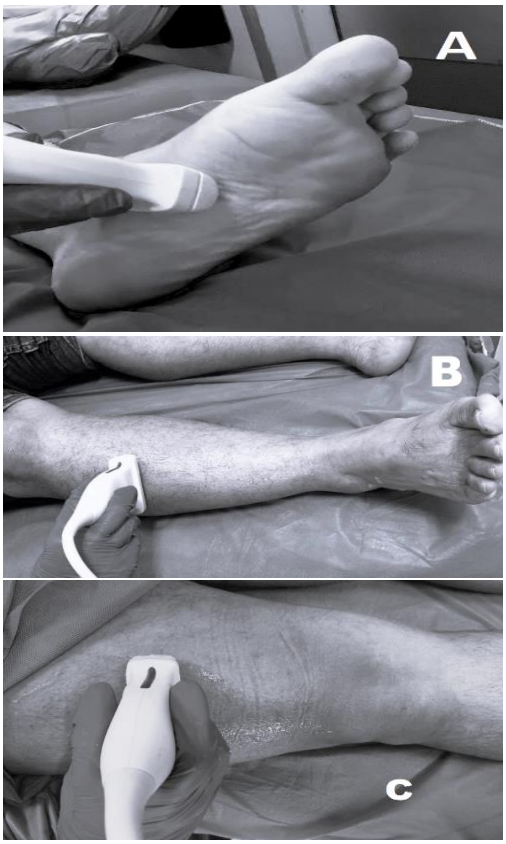

Figure 1. Showing the placement of the probe during (A) AH muscle examination, (B) TA muscle examination, and (C) RF muscle examination

To determine the value of EI, grayscale analysis was employed. EI was defined as the mean pixel

intensity within the muscle, and its calculation involved utilizing the standard histogram function in MATLAB (Student License). The region of interest (ROI) for each muscle was selected using the Image Segmenter Application, ensuring the inclusion of as much muscle as possible while excluding surrounding bone and fascia. The range of values from 0 to 255 represents pure black and pure white, respectively, and is used to express the EI in the ROI (see Figures 2, 3). EI measurements for each muscle were conducted on three images of the same muscle, and then the mean of those three measurements was calculated. EI increased in muscles of DPN patients (see Figure 2, Figure 3).

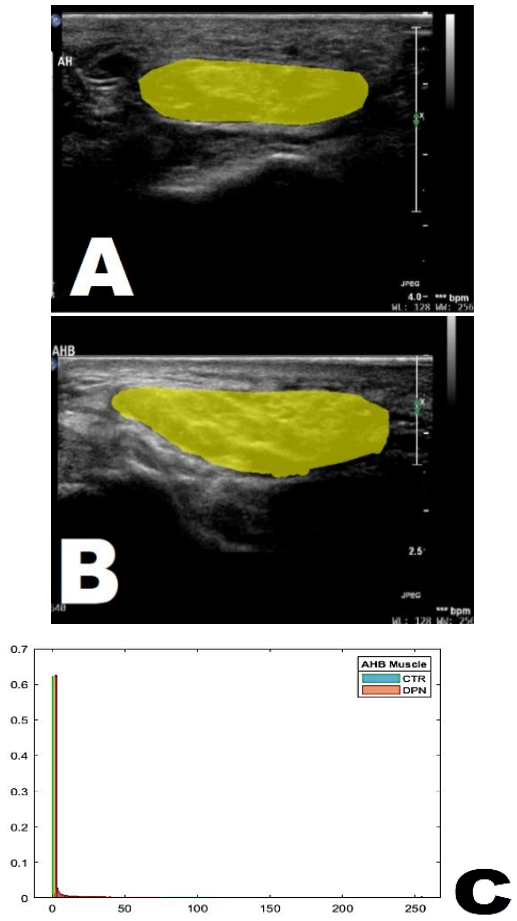

Figure 2. Showing AH muscle in (A) a DPN patient, (B) a normal individual, and (C) the shifting in EI value between DPN patients and normal individuals

2.2. Statistical Analysis

The data analysis was conducted using the 25th version of the statistical package SPSS. For categorical variables, frequencies and relative frequencies were employed to summarize the data,

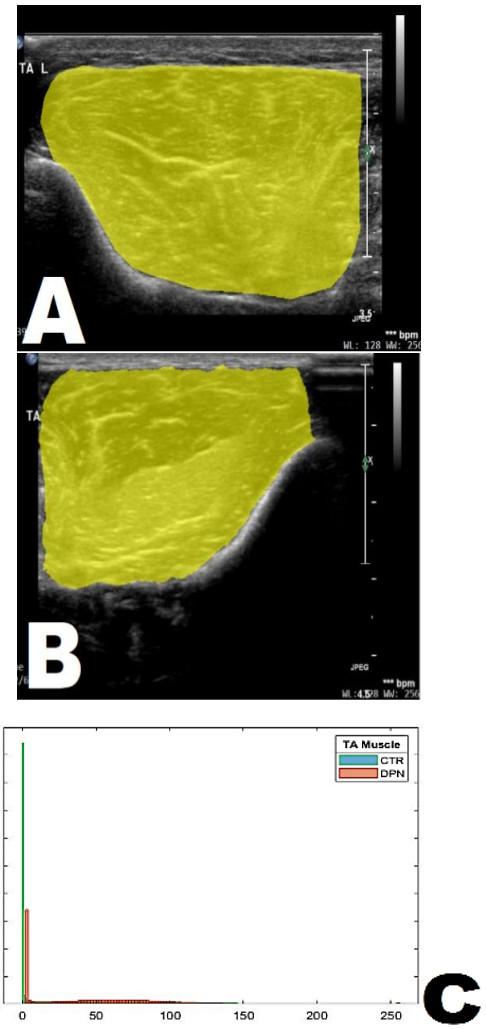

Figure 3. Showing TA muscle in (A) a DPN patient, (B) a normal individual, and (C) the shifting in EI value between DPN patients and normal individuals

while quantitative variables were summarized using mean, standard deviation, minimum, and maximum values. To compare groups for quantitative variables with normal distributions, one-way ANOVA and unpaired t-tests were utilized, whereas non-parametric Mann-Whitney tests were used for those with non-normal distributions. For comparing categorical data, the Chi-square (χ^2) test was employed. In cases where the expected frequency was less than 5, the likelihood ratio was applied instead. To determine correlations between quantitative variables, Pearson's correlation coefficient was used. A p-value of ≤ 0.05 was considered significant, $p > 0.05$ was considered non-significant, and $p \leq 0.001$ was regarded as highly significant.

3. Results

The results of this study were presented in three groups: diabetic patients with DPN, diabetic patients without DPN, and healthy participants. The characteristics of these three study groups are shown in Table 1 and Table 2.

Patients with diabetic peripheral neuropathy had statistically significant ($p < 0.001$) higher levels of glycated hemoglobin (HbA1c) as shown in Table 1, and a statistically significant ($p < 0.001$) increase in the duration of diabetes (Table 1 and Figure 1).

Echo intensity of the Abductor Hallucis (AH), Tibialis Anterior (TA), and Rectus Femoris (RF) muscles for the three study groups was measured using grayscale analysis of these muscles. When comparing EI between diabetic patients with DPN and normal individuals, there was a statistically significant difference in two of the muscles (TA and AH) with a p-value < 0.05 for TA and p-value < 0.001 for AH, while RF revealed a not statistically significant difference ($P > 0.05$). Similar results were found when comparing diabetic patients with DPN and those without DPN, as shown in Table 3 and Figure 4.

Table 1. Characteristics of the three study groups

Characteristic	Diabetic Patients with DPN				Diabetic Patients without DPN				Healthy Participants				Sig.	
	min	max	mean	SD	min	Max	mean	SD	min	max	mean	SD	P-value	
Age	40.0	65.0	51.69	7.92	40.0	61.0	45.9	6.83	41.0	58	48.27	5.35	0.066	NS
BMI	16.9	45.2	28.41	5.80	22.7	30.1	27.5	2.73	24.1	37.3	28.89	3.07	0.768	NS
Dm duration	4.0	20.0	10.19	4.69	3.0	6.0	4.3	1.05	-	-	-	-	<0.001	HS
HbA1c	7.5	13.5	10.11	1.72	4.9	7.5	6.2	0.79	-	-	-	-	<0.001	HS

HS: highly significant; NS: non-significant; SD: Standard deviation; HbA1c: glycated hemoglobin; DM: diabetes mellitus; BMI: body mass index; DPN: diabetic peripheral neuropathy;

Table 2. Distribution of the study groups by gender

Gender	Diabetic Patients with DPN		Diabetic Patients without DPN		Healthy Participants		P-value	Significance
	N.	%	N.	%	N.	%		
male	20	76.92	6	60.0	9	60.0	0.829	NS
female	6	23.08	4	40.0	6	40.0		

DPN: diabetic peripheral neuropathy; N: number; NS: non-significant; SD: Standard deviation.

Table 3. Comparing the study groups concerning echogenicity

EI of Muscles	Diabetic Patients with DPN	Diabetic Patients without DPN	Normal Individuals	Sig. between diabetics with DPN& Normal		Sig. between diabetics with & without DPN	
	Mean±SD	Mean±SD	Mean±SD	p-value		p-value	
Rectus Femoris Muscle	71.28±27.32	54.97±21.81	59.22±19.08	0.095	NS	0.381	NS
Tibialis Anterior Muscle	82.91±25.31	49.45±10.14	58.28±12.28	0.004*	S	0.006*	S
Abductor Hallusis Muscle	88.36±32.95	34.16±12.34	32.52±14.31	<0.001**	HS	<0.001**	HS

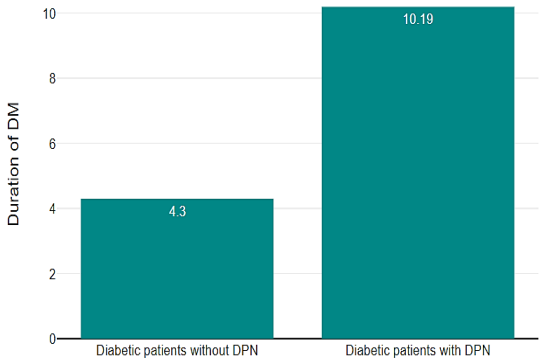

Figure 4. Distribution of patients based on the duration of diabetes mellitus

A highly significant (P < 0.001) positive correlation was found between the duration of diabetes and the EI of TA and AH, and a significant (P < 0.05) positive correlation was found between HbA1c levels and the EI of those muscles. However, the correlation with the same parameters revealed a not statistically significant correlation. These results are shown in Table 4.

Table 4. Correlation between muscle echogenicity and (duration of diabetes and glycated hemoglobin)

Muscle EI	Duration of DM (years)			HbA1c levels (%)		
	r	p	Sig.	r	p	Sig.
Rectus Femoris Muscle	0.25	0.227	NS	0.34	0.091	NS
Tibialis Anterior Muscle	0.77	<0.001	HS	0.51	0.008	S
Abductor Halluces Muscle	0.86	<0.001	HS	0.54	0.004	S

EI: echo intensity; DM: diabetes mellitus; HbA1c

Nerve conduction study parameters showed a significant (p < 0.05) negative correlation between tibial nerve amplitude and AH EI, and also a significant (p < 0.05) positive correlation was found between the latency of the tibial nerve and AH EI. No correlation was found between the tibial nerve conduction velocity and EI of this muscle, as shown in Table 5.

TA EI showed a highly significant (p < 0.001) negative correlation with the amplitude of the peroneal nerve, a significant (p < 0.05) negative correlation with the conduction velocity of the peroneal nerve, and no significant (p > 0.05) correlation with peroneal nerve latency, as shown in Table 6.

Table 5. Correlation between abductor hallucis muscle and tibial nerve conduction study parameters

	EI of Abductor Hallucis		
	r	p	Sig.
Tibial Nerve Amplitude	-0.49	0.01	S
Tibial NerveLatency	0.44	0.026	S
Tibial Nerve Conduction Velocity	-0.36	0.068	NS

Table 6. Correlation between tibialis anterior muscle echogenicity and peroneal nerve conduction study parameters

	EI of Tibialis Anterior Muscle		
	r	p	Sig.
Peroneal Nerve Amplitude	-0.87	<0.001	HS
Peroneal Nerve Latency	0.34	0.091	NS
Peroneal Nerve Conduction Velocity	-0.43	0.028	S

4. Discussion

It is well known that DPN is characterized by progressive nerve function loss in a distal-proximal fashion, with sensory deficits predominating in the early stages of the disease, and motor deficits becoming more clinically evident in later stages [15, 16].

Recent studies show that muscle impairment in DPN results from a combined impact between the condition of diabetes and motor nerve injury. These weaknesses usually appear in the earliest stages of DPN and worsen as the extent of neural damage to the peripheral nervous system increases. Functional motor unit loss, impairment of neuromuscular signal transmissions, and myofibrillar protein glycation were postulated to be the main causes of muscular system destruction in diabetic patients [17, 18].

Denervation not only causes muscle atrophy but also impairs the metabolic functions of muscles [19]. Among the numerous US parameters proposed to detect muscle changes, muscle EI was considered a reliable indicator of muscle changes caused by denervation [9, 11, 20-22]. Normal muscle tissue is echo lucent; therefore, the US is easily transmitted through it (Mayans, Cartwright, and

Walker, 2012). Nevertheless, in neurogenic disorders, they appear hyperechoic owing to muscle tissue undergoing atrophy, fibrosis, fatty infiltration, inflammation, and necrosis, which give rise to numerous new planes of sound reflection within the muscle, causing the affected muscle to become more echogenic [23]. The present study revealed that quantitative muscle echogenicity measurements of two of the three muscles we examined, AH and TA, were significantly different between DPN patients and controls, while RF muscle didn't show a significant difference between the cases and controls. This distribution of muscle involvement with a proximo-distal gradient indicates that muscles located distally in the lower leg were affected, while the proximal muscle, rectus femoris, remained unaffected. This mostly reveals the effect of a length-dependent neuropathic process within motor fibers. This is similar to a study by Andersen *et al.* [24], who concluded that muscle atrophy in diabetic neuropathy is most prominent distally.

Previous studies measured quantitative EI, such as Soliman *et al.* [25] and El Hefnawy *et al.* [26], who found a significant difference in muscle EI between diabetic patients and healthy participants. Lee *et al.* [27] found that quantitative grayscale analysis of abductor pollicis brevis muscles in CTS patients showed a significantly different EI from the control group muscle EI while Pillen *et al.* [28], who studied 150 children with different neuromuscular disorders, found that quantifying EI facilitates comparisons of changes across various muscles, describes pathology distribution throughout the body, and distinguishes myopathic from neurogenic disorders, as neurogenic disorders showed increased EI and decreased size of distal muscles of lower limbs [29]. Kim and his coworkers [29] examined the EI of thenar and hypothenar muscles in 11 healthy subjects and 35 patients diagnosed with CTS by measuring mean and Standard Deviation (SD) of EI and found that ultrasound evaluation of muscle EI can identify muscle changes caused by neuropathy.

A study by Hokkoku *et al.* [30] stated that quantitative muscle US could be regarded as a supplementary tool to NCSs in patients with CIDP because it can identify axonal degeneration by evaluating alterations in muscle structure caused by denervation. However, in another study by Klawitter *et al.* [31], quantitative grayscale analysis of muscle echo intensity was not able to identify muscle weakness in intensive care unit patients when compared to qualitative analysis. There was a highly significant ($P < 0.001$) negative correlation between TA and AHB muscles' EI and diabetes duration and a significant ($p < 0.05$) negative correlation between the EI of these muscles and HbA1c levels. This correlation was more significant with AHB than TA, which may be explained by the more distal location of AHB compared to TA, making it more liable to denervation and atrophy due to the dying-back pattern of DPN. While the EI of the RF muscle did not have a significant correlation with HbA1c levels and diabetes duration, this could be attributed to its proximal location. Furthermore, we succeeded in finding a significant correlation between TA EI and the peroneal nerve conduction study parameters, and another significant correlation between Ah EI and tibial nerve conduction study parameters was found. These results are similar to the results by Lee *et al.* [27] when studying patients with CTS symptoms; EI revealed a highly significant positive relationship with the NCS severity and distal latency of the motor and sensory median nerve. Hokkoku *et al.* [30] found a substantial inverse relationship between muscle echo intensity and CMAP amplitude in patients with CIDP with secondary axonal degeneration. Likewise, Shahrizaila *et al.* [32] made an interesting observation, noting a correlation between the EI of the FDI and CMAP amplitude in patients diagnosed with Charcot-Marie-Tooth neuropathy.

It is worth noting that by incorporating diabetic patients without neuropathy, we established a comprehensive baseline for assessing the distinct muscle echogenicity changes directly attributable to DPN that allowed us to discern and highlight the specific impact of peripheral nerve dysfunction on muscle tissue.

The study findings suggest that quantitative muscle Echointensity (EI) could complement nerve conduction studies for assessing muscle changes in DPN. This could aid early detection and monitoring of motor unit dysfunction. Future research with larger cohorts and multiple examiners is needed to enhance clinical applicability and understanding.

Our study had some limitations. First, the number of participants was insufficient, so we couldn't perform a subgroup analysis for age, BMI, and gender, as US images of muscle texture vary with these parameters. Secondly, the limitation of having only one experienced US examiner prevented us from evaluating the inter-rater reliability of quantitative US. This evaluation

necessitates two examiners conducting US examinations on the same subjects on the same day. Additionally, we did not assess muscle thickness and CSA. Further studies with a larger sample size are required to increase the understanding of this method's clinical usefulness and applicability.

5. Conclusion

In comparison to control groups, diabetic patients with DPN exhibited elevated muscle echogenicity, and this increase was found to have a significant correlation with various NCS parameters. As a result, physicians can utilize Ultrasound (US) to aid in diagnosing DPN while interpreting the observed echo patterns in ultrasonography of DPN patients.

Acknowledgements

No financial support was received by the authors for conducting this research and publishing it.

References

1- Dina AM Al-Khafajy, Maysaa J Majeed, Omar F Al-Azzawi, and Ayad Ismael Khaleel, "Role of CoQ10 and IGFBP-1 in Obese Male Patients with Diabetic Mellitus Type II." *Indian Journal of Forensic Medicine & Toxicology*, Vol. 15 (No. 1), pp. 2204-09, (2021).

2- Numan Salman Dawood, Numan S Dawood, and Maan H Al-khalisy, "Evaluation of the effect of type II diabetes mellitus on Bone Mineral Density of Upper and Lower Limbs by Dual-Energy X-Ray Absorptiometry." *Journal of the Faculty of Medicine Baghdad*, Vol. 65 (No. 1), pp. 27-33, (2023).

3- Hailun Huang and Shan Wu, "Application of high-resolution ultrasound on diagnosing diabetic peripheral neuropathy." *Diabetes, Metabolic Syndrome and Obesity*, pp. 139-52, (2021).

4- Kamakshi Patel, Holli Horak, and Ezgi Tiryaki, "Diabetic neuropathies." *Muscle & Nerve*, Vol. 63 (No. 1), pp. 22-30, (2021).

5- Caitlin W Hicks and Elizabeth Selvin, "Epidemiology of peripheral neuropathy and lower extremity disease in diabetes." *Current diabetes reports*, Vol. 19pp. 1-8, (2019).

6- Jennifer Miller, Henrik Gollee, and Mariel Purcell, "Ultrasound imaging as a diagnostic tool to assess the functional status of muscles after a spinal cord injury." *Ultrasound in Medicine & Biology*, Vol. 47 (No. 3), pp. 386-97, (2021).

7- Natalia L Gonzalez and Lisa D Hobson-Webb, "Neuromuscular ultrasound in clinical practice: A review." *Clinical neurophysiology practice*, Vol. 4pp. 148-63, (2019).

8- A.J.A. Al-Sarray, "Molecular and electronic properties of Schiff bases derived from different aniline derivatives: density functional theory study." *Eurasian Chemical Communications*, Vol. 5 (No. 4), pp. 317-26, (2023).

9- Arne Schwennicke, Meikel Bargfrede, and Carl D Reimers, "Clinical, electromyographic, and ultrasonographic assessment of focal neuropathies." *Journal of neuroimaging*, Vol. 8 (No. 3), pp. 136-43, (1998).

10- Ilse MP Arts, Sigrid Pillen, H Jurgen Schelhaas, Sebastiaan Overeem, and Machiel J Zwarts, "Normal values for quantitative muscle ultrasonography in adults." *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, Vol. 41 (No. 1), pp. 32-41, (2010).

11- Natalia Maria Maurits, Anna Elizabeth Bollen, Alphons Windhausen, Aeiko Eppo Jurjen De Jager, and Johannes Harmen Van Der Hoeven, "Muscle ultrasound analysis: normal values and differentiation between myopathies and neuropathies." *Ultrasound in Medicine & Biology*, Vol. 29 (No. 2), pp. 215-25, (2003).

12- Zenji Makita *et al.*, "Advanced glycosylation end products in patients with diabetic nephropathy." *New England Journal of Medicine*, Vol. 325 (No. 12), pp. 836-42, (1991).

13- Cheng-Yin Tan, Thaarani Arumugam, Siti Nur Omaira Razali, Mohd Azly Yahya, Khean-Jin Goh, and Nortina Shahrizaila, "Nerve ultrasound can distinguish chronic inflammatory demyelinating polyneuropathy from demyelinating diabetic sensorimotor polyneuropathy." *Journal of Clinical Neuroscience*, Vol. 57pp. 198-201, (2018).

14- Ali J. A. Al-Sarray, Tarik Al-Kayat, Borhan Mustafa Mohammed, Mohammed Jassim Bader Al-assadi, and Yousif Abu-Zaid, "Dielectric and Electrical Properties of Intercalated 1-(4-nitrophenyl)-N-(p-tolyl) methanimine into the Interlayers of Bentonite Clay." *Journal of Medicinal and Chemical Sciences*, Vol. 5 (No. 7 (Special Issue)), pp. 1321-30, (2022).

15- Ihsan M Ajeena, "Effect of hypertension on diabetic peripheral neuropathy." *Journal of the Faculty of Medicine Baghdad*, Vol. 50 (No. 2), pp. 160-65, (2008).

16- Steven J Brown, Joseph C Handsaker, Frank L Bowling, Costantinos N Maganaris, Andrew JM Boulton, and Neil D Reeves, "Do patients with diabetic neuropathy use a higher proportion of their maximum strength when walking?" *Journal of biomechanics*, Vol. 47 (No. 15), pp. 3639-44, (2014).

17- Omar Farooq Nafea Al-Azzawi, Mohammed Waheeb Alobaidy, and Momtaz M Saham, "Nephrotic range proteinuria; does it predict lung involvement in patients with type 2 diabetes." *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, Vol. 13 (No. 1), pp. 622-25, (2019).

18- Giorgio Orlando, Stefano Balducci, Andrew JM Boulton, Hans Degens, and Neil D Reeves, "Neuromuscular dysfunction and exercise training in people with diabetic peripheral neuropathy: a narrative review." *Diabetes Research and Clinical Practice*, Vol. 183p. 109183, (2022).

19- Tatiana Y Kostrominova, "Skeletal Muscle Denervation: Past, Present and Future." *International Journal of Molecular Sciences*, Vol. 23 (No. 14), p. 7489, (2022).

20- Gerd Gunreben and Ulrich Bogdahn, "Real-time sonography of acute and chronic muscle denervation." *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, Vol. 14 (No. 7), pp. 654-64, (1991).

21- Karim Reimers, Carl D Reimers, Stefen Wagner, I Paetzke, and DE Pongratz, "Skeletal muscle sonography: a correlative study of echogenicity and morphology." *Journal of Ultrasound in Medicine*, Vol. 12 (No. 2), pp. 73-77, (1993).

22- Sigrid Pillen *et al.*, "Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity." *Ultrasound in Medicine & Biology*, Vol. 35 (No. 3), pp. 443-46, (2009).

23- David Mayans, Michael S Cartwright, and Francis O Walker, "Neuromuscular ultrasonography: quantifying muscle and nerve measurements." *Physical Medicine and Rehabilitation Clinics*, Vol. 23 (No. 1), pp. 133-48, (2012).

24- H Andersen, PC Gadeberg, B Brock, and J Jakobsen, "Muscular atrophy in diabetic neuropathy: a stereological magnetic resonance imaging study." *Diabetologia*, Vol. 40pp. 1062-69, (1997).

25- Steven B Soliman *et al.*, "The hyperechoic appearance of the deltoid muscle on shoulder ultrasound imaging as a predictor of diabetes and prediabetes." *Journal of Ultrasound in Medicine*, Vol. 39 (No. 2), pp. 323-29, (2020).

26- F Zidan Howayda, E Hanan, Amr M Gamil, S Soad, and H Agyad, "Correlation between Quantitative Ultrasonography on Small Muscles of the Foot in Diabetic Peripheral Neuropathy and Electrodiagnostic Study." *The Medical Journal of Cairo University*, Vol. 88 (No. December), pp. 1975-84, (2020).

27- Hyewon Lee, Sungju Jee, Soo Ho Park, Seung-Chan Ahn, Juneho Im, and Min Kyun Sohn, "Quantitative muscle ultrasonography in carpal tunnel syndrome." *Annals of Rehabilitation Medicine*, Vol. 40 (No. 6), pp. 1048-56, (2016).

28- S Pillen, A Verrips, N Van Alfen, IMP Arts, LTL Sie, and MJ Zwarts, "Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease." *Neuromuscular disorders*, Vol. 17 (No. 7), pp. 509-16, (2007).

29- Ji-Sun Kim, Hung Youl Seok, and Byung-Jo Kim, "The significance of muscle echo intensity on ultrasound for focal neuropathy: the median-to ulnar-innervated muscle echo intensity ratio in carpal tunnel syndrome." *Clinical Neurophysiology*, Vol. 127 (No. 1), pp. 880-85, (2016).

30- Keiichi Hokkoku *et al.*, "Quantitative muscle ultrasound is useful for evaluating secondary axonal degeneration in chronic inflammatory demyelinating polyneuropathy." *Brain and Behavior*, Vol. 7 (No. 10), p. e00812, (2017).

31- Felix Klawitter, Uwe Walter, Robert Patejdl, Josefine Endler, Daniel A Reuter, and Johannes Ehler, "Sonographic evaluation of muscle echogenicity for the detection of intensive care unit-acquired weakness: a pilot single-center prospective cohort study." *Diagnostics*, Vol. 12 (No. 6), p. 1378, (2022).

32- N Shahrizaila *et al.*, "Quantitative muscle ultrasound as a biomarker in Charcot-Marie-Tooth neuropathy." *Clinical Neurophysiology*, Vol. 128 (No. 1), pp. 227-32, (2017).