ASSESSMENT OF NEUROMUSCULAR JUNCTION INTEGRITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

SHIRWAN H. OMER, MBCHB, MSC, PHD*

***Submitted 6/12/2018; accepted* 4/02/2019**

ABSTRACT

Background: Diabetes Mellitus produces long-term damage and failure of various tissues, in particular, diabetes-induced neural damage. Changes of neuromuscular transmission would contribute to the progressive weakness in diabetic patients; Electrophysiological studies are of recognized use in the confirmation of alterations of neuromuscular transmission and helping to differentiate them from other conditions.

The current study was performed to evaluate the integrity of neuromuscular junction (NMJ) in patients with type 2 diabetes using repetitive nerve stimulation (RNS) technique and assessment of acetylcholine receptors antibodies in the serum.

Patients and Methods: This cross-sectional study involved 103 patients with type 2 diabetes mellitus. The entire subjects met certain inclusion and exclusion criteria to exclude other possible contributing factors of neuropathy. All subjects completed a pre-requested questionnaire, then physical and neurological examinations were done, routine nerve conduction study, repetitive nerve stimulation and assessment of acetylcholine receptors antibodies were performed.

Results: Among 103 patients with type 2 DM patients 56 of them were diagnosed as peripheral polyneuropathy however, the rest (47) their NCS result were normal, 11 (10.7%) of them showed positive decrement test, All those with positive decrement test they have also peripheral polyneuropathy, serum acetylcholine receptor antibody test was negative in all the participants (those with positive and negative decrement test).

Conclusions: This preliminary study implies that type 2 diabetes contributes to the neuromuscular junction dysfunction. Further studies are indicated to explain the pathophysiology and mechanisms responsible for positive decrement test in type 2 diabetic patients.

Duhok Med J 2019; 13 (1):1-8.

Keywords: Diabetes Mellitus, Neuromuscular Junction Dysfunction, Repetitive Nerve Stimulation.

Hyperglycemia associated with diabetes mellitus (DM) produces long-term damage and failure of various tissues[1]. In particular, diabetes-induced neural damage is a predominant form of neuropathy[2]. Changes of neuromuscular transmission would contribute to the progressive muscle weakness in diabetics[3]. Therefore, the goal of this study was to further explore the effects of diabetes on the neuromuscular junction (NMJ).

Neuromuscular junction (NMJ) disorders are characterized by fluctuating muscle weakness, depending on the site of neuromuscular transmission failure, NMJ disorders have been classified as: (A) presynaptic (e.g., Lambert-Eaton myasthenic syndrome), (B) synaptic (e.g., cholinesterase inhibitor toxicity), and (C) post-synaptic (e.g., myasthenia gravis)[4]. Electrophysiological studies are ofrecognized use in the confirmation of

*Lecturer, Department of Physiology, College of Medicine, University of Sulaimani, Kurdistan Region, Iraq.

***Correspondence author to:** *Shirwan H. Omer, shirwan.omer@univsul.edu.iq Mobil* +9647701554828

alterations in neuromuscular transmission and helping to differentiate them from other conditions[5].

Electrodiagnostic techniques used for investigation of NMJ disorders include repetitive nerve stimulation (RNS) and single fiber electromyography (SFEMG)[6]. Recent literature widely explores the use of SFEMG in the diagnosis and monitoring of myasthenia gravis, but this technique is time consuming and has a lesser role in the daily clinical practice[7]. RNS is the most widely used electrodiagnostic method in the evaluation of suspected neuromuscular transmission disorders. RNS is technically easier and does not require special technical training and skill as SFEMG[7,8].

The technique of RNS is similar to that used in conventional nerve conduction studies, differing only in the application of stimuli trains or paired stimuli, the use of conditioning exercise, and the careful immobilization of the limb to reduce movement artifact. A decrement of more than 10% on slow RNS (2 or 3 Hz) is characteristically seen in patients with postsynaptic disorder, while increments usually exceeding 50-200% of the baseline value in amplitude seen in presynaptic disorder, marker of synaptic efficacy[9].

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Medical Physiology, College of Medicine, University of Sulaimania and Sulaimania Diabetes Center, from December 2017 – July 2018. The study included one hundred three patients diagnosed as type 2 diabetes mellitus (T2DM), (80 female, 23 male), with an age ranging from 38 to 72 years (mean ± SE =55.14 ± 0.67 years), Patients diagnosed as type 2 diabetes mellitus regardless of the duration of illness were included in the study. However, Patients with the following conditions were excluded: rheumatoid arthritis, thyroid disease, alcoholism, liver and kidney disease, drugs known to cause neuropathy or myopathy, pregnancy, positive family history of neuropathy or myopathy All patients gave their informed consent; the study has been approved by Ethical Committee of College of Medicine.

Physical examinations were performed to assess knee and ankle jerk, muscle power and perception to vibration. Nerve Conduction Study examinations were performed according to the standard method recommended by the American Diabetes Association[10], using Neurowerk EMG/EP measuring machine (4 channel, Germany) at 20 to 25°C room temperature, The following nerves were examined: a- Motor nerve conduction study of posterior tibial, peroneal, femoral, median, ulnar and musculocutaneous nerves, b- Median, ulnar, radial and sural sensory nerve conduction study, c- F waves minimum latency (Fmin) of posterior tibial, peroneal, median and ulnar nerves.

Standard methods and techniques for assessment and evaluation of neuromuscular junction integrity by RNS were performed[11,12,13,14].Serum acetylcholine receptors antibodies were assessed using EUROIMMUN ELISA (IgG) kit.

RESULTS

Among the 103 patients with type 2 DM, routine NCS showed that 56 of the participant have peripheral

 https://doi.org/10.31386/dmj.uod.2019.13.1

polyneuropathy; however, the rest (47) their NCS result were normal (Figure1),

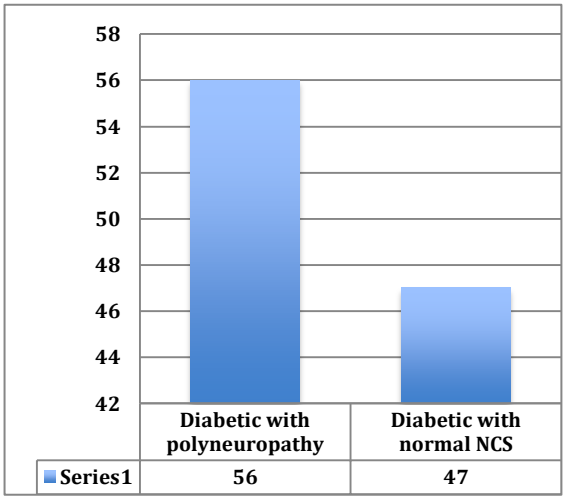

Figure 1: Number of Diabetic Patients with Polyneuropathy and Normal NCS

RNS including both increment and decrement tests were performed for the entire participant, 92 (89.3%) of patients showed negative decrement test (Table 1), and (Figures 2, 3),

Table 1: Shows Normal RNS Test in One Diabetic Patient Showing 3% Decrease of CMAP Amplitude before Exertion and 2% after Exertion.

SequenceAccessory nerve 3 Hz	Ampl. 1[mV]	Ampl. 4[mV]	Dec [%]	Stim. [mA]
Before exertion (base line)	11.6	11.4	-3	35
Immediately after 10 second of exercise (post exercise facilitation)	12	12.5	-2	35
2 minutes after 60 seconds of exercise (post exercise exhaustion)	11.7	10.9	-5	35

11 (10.7%) of them showed positive decrement test (Figure 2). As demonstrated in **Table** 2, and 3.

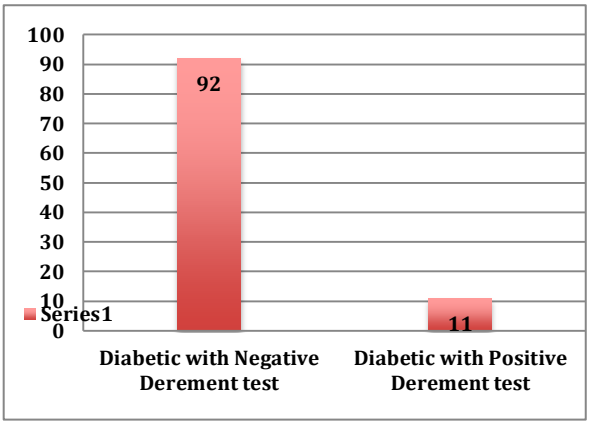

Figure2: Total Number ofDiabetic Patients with Positive and Negative Decrement Test.

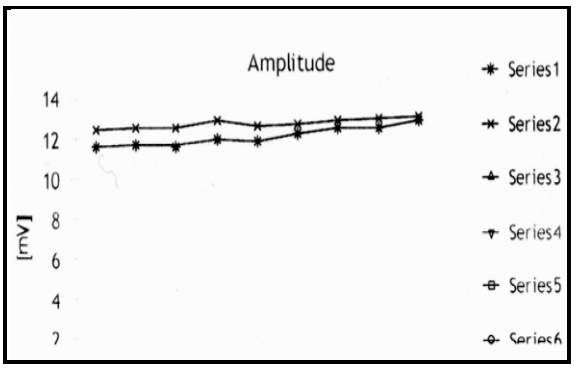

Figure 3: Normal RNS test in one diabetic patient showing 3% decrease of CMAP amplitude before exertion and 2% after exertion.

Table 2: Shows Positive Decrement Test in One Diabetic Patient Showing 29% Decrease of CMAP Amplitude before Exertion and 9% after Exertion.

Sequence Accessory nerve 3 Hz	Ampl. 1[mV]	Ampl. 4[mV]	Dec [%]	Stim. [mA]
Before exertion (base line)	10.9	7.8	-29	35
Immediately after 10 second of exercise (post exercise facilitation)	11.5	10.5	-9	35
2 minutes after 60 seconds of exercise (post exercise exhaustion)	11	6.3	-44	35

Table 3: Shows Positive Decrement Test in One Diabetic Patient Showing 30% Decrease ofCMAP Amplitude Before Exertion and 13% after Exertion.

Sequence Accessory nerve 3 Hz	Ampl. 1[mV]	Ampl. 4 [mV]	Dec [%]	Stim. [mA]
Before exertion (base line)	**11.6**	**8.1**	**-30**	**35**
Immediately after 10 second of exercise (post exercise facilitation)	**12.5**	**10.8**	**-13**	**35**
2 minutes after 60 seconds of exercise (post exercise exhaustion)	**11.3**	**7.5**	**-35**	**35**

Also,**Figure** 4 and 5 significant decrements (more than 10%) of compound muscle action potential amplitude between the first and fourth motor response were observed. Increment test was negative in all participants.

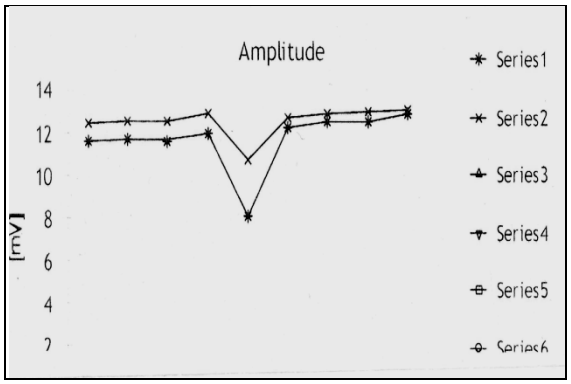

Figure 4: Positive Decrement Test in One Diabetic Patient Showing 29% Decrease ofCMAP Amplitude before Exertion and 9% after Exertion.

All those with positive decrement test they have also peripheral polyneuropathy; however, the rest of diabetic patients showed normal RNS, the gender distribution among those with positive decrement test were 7 females and 4 males.

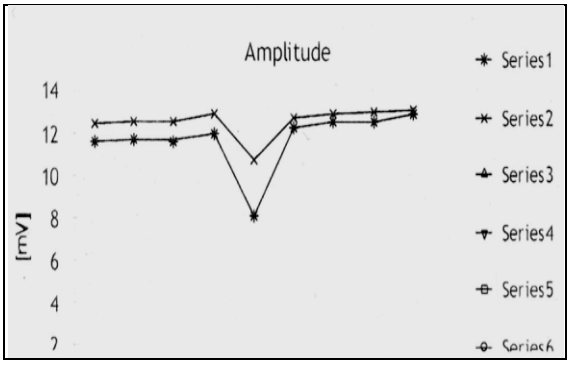

Figure 5: Positive Decrement Test in One Diabetic Patient Showing 30% Decrease ofCMAP Amplitude before Exertion and 13% after Exertion.

Estimation of serum acetylcholine receptor antibodies was done for all the patients and the results were negative in all the participants (those with positive and negative decrement test).

DISCUSSION

Target organ complications secondary to diabetes are one of the most important medical concerns[15]. A clear example is diabetic neurological complication, which is the most common and early complication of diabetes affecting up to 60% of diabetic patients[16].

Several studies have been carried out to examine the effect of type 1 diabetes on neuromuscular junction and association between myasthenia gravis and the occurrence of type 2 diabetic as a consequence of high dose of steroid therapy. To the best of the researcher's knowledge, limited investigations have examined the impact of type 2 diabetes on NMJ function, in present study we assessed neurophysiological assessment of

https://doi.org/10.31386/dmj.uod.2019.13.1

impact of neuromuscular junction function in 103 patients with type 2 diabetes, 11 of them show significant decremental response and all of these patient have coexisting diabetic polyneuropathy, a finding consistent with experimental studies conducted by Marques and Santo[17] andSouayah et al[18] on streptozotocin-induced type 1 diabetic mice, they found that diabetic neuropathy is associated with functional and morphological changes of the neuromuscular junction (NMJ), in addition to that current results may explain the clinical aspects of an experimental study on type 1 induced diabetic mice performed by Garcia et al[19], they showed that NMJ undergoes dramatic changes of function, morphology and the reduction of muscle end-plate cholinesterase (AchE) that may contribute to endplate pathology and subsequent muscle weakness during diabetes.

In present study serum acetylcholine receptor antibody was negative in all diabetic patients with polyneuropathy including those with positive decrement test, these findings are in accordance to that reported by Wakat et al[20] that type 2 diabetes associated neuromuscular junction dysfunction have no organ specific autoantibody to neuromuscular junction.

Current preliminary findings indicate that type 2 diabetes contributes to neuromuscular junction dysfunction. Further extensive investigations are recommended to elucidate the mechanism responsible for this dysfunction and positive decrement test in type 2 diabetic patient with peripheral polyneuropathy.

REFERENCES

1. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P et al. Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. Diabetes Care.2010;33:2285–2293.
2. Vinik A. Diabetic Sensory and Motor Neuropathy. N Engl J Med. 2016; 374:1 455 -64. DOI: 10.1056/NEJMcp1503948
3. Vinik A, Nevoret M-L, Casellini C, Parson H. Diabetic neuropathy. In: Poretsky L, Liao EP, eds. Acute and chronic complications of diabetes. Amsterdam: Elsevier. 2013;747-87.
4. Kimura J and Kohara N . Principles and Practice of Nerve Conduction Studies and Electromyography. 4th edition. 2013; Igaku-Shoin Lt.
5. Chumillas MJ, Cortes V. Electrodiagnosis in disorders of neuromuscular transmission. *Revista de Neurologia*. 2000; 30(1): 65-70.
6. Kimura J and Kohara N . Principles and Practice of Nerve Conduction Studies and Electromyography. 1st edition. 2003; Igaku-Shoin Ltd
7. Zivkovic S.A., Shipe C. Use of Repetitive Nerve Stimulation in the Evaluation of Neuromuscular Junction Disorders. Am J. END. *Technol*. 2005; 45:248-261.
8. Matthew P. Neurophysiological strategies for the diagnosis of disorders of the neuromuscular junction in children. Dev. Med & Child Neurol. 2008; 50: 328-333.
9. Jasper RD, Devon IR. Clinical Neurophysiology. 3rd edition. 2009; Oxford University press, Mayo Foundation for Medical Education and

Research.
10. American Diabetes Association. American Academy of Neurology: Report and recommendations of the San Antonio Conference on Diabetic Neuropathy (Consensus Statement). Diabetes Care. 1988.11:592–597.
11. Preston DC, Shapiro BE. Electromyography and Neuromuscular disorder, clinical – Electrophysiologic correlation, Second Edition; Elsevier. US.2005. P.52-61
12. AAEM Quality Assurance Committee. Practice parameter for repetitive nerve stimulation and single fiber EMG evaluation of adults with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome: summary statement. Muscle Nerve.2001; 24:1236-38.
13. Preston DC, Shapiro BE. Electromyography and Neuromuscular disorder, clinical – Electrophysiologic correlation, Third Edition; Elsevier.US.2013.p. 530-48
14. Lo YL, Leoh TH, Dan YF, Tan YE, Nurjannah S, Ratnagopal P. Repetitive stimulation of the long thoracic nerve in myasthenia gravis: clinical and electrophysio1ogical correlations. *J Neurol Neurosurg Psychiatry*.2003; 74:379-81.
15. Shaw J.E, Sicree R.A, Zimmet P.Z. Global estimate of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010.87 (1), 4-14.
16. Vincent AM, Callaghan BC, Smith AL, Feldman EL. Diabetic neuropathy: cellular mechanisms as therapeutic targets. Nat Rev Neurol. 2011;7: 573–583.
17. Marques MJ and Santo Neto H. Acetylcholine receptors and nerve terminal distribution at the neuromuscular junction of non-obese diabetic mice. Anat Rec. 2002; 267: 112–119.
18. Souayah N, Potian JG, Garcia CC, Krivitskaya N, Boone C, Routh VH et al. Motor unit number estimate as a predictor of motor dysfunction in an animal model of type 1 diabetes. Am J Physiol Endo-crinol Metab.2009; 297: E602–E608.
19. Garcia CC, Joseph GP, Kormakur H, Baskaran T, Lester GS, Nizar S, et al. Acetylcholinesterase deficiency contributes to neuromuscular junction dysfunction in type 1 diabetic neuropathy. Am J Physiol Endocrinol Metab.2012; 303: E551–E561
20. Wakata N, Nemoto H, Konno S, Nakazora H, Nomoto N, Sugimoto H, et al. Myasthenia Gravis and Diabetes Mellitus: A 35-year Retrospective Study. Intern Med. 2007. 46(9):557-9.

 https://doi.org/10.31386/dmj.uod.2019.13.1

ثوختة

هةلسةنطاندنا ثاراستنا طةهاندنا دةماروزةظلّةكى لنك نةخوَشيَن جوَرىَ دووىَ ذ دةردىَ شةكرىَ

ئامانج: دةردىَ شةكرىَ دبيتة ئةطةرىَ زيانيَن دوم دريَذ و ذ كاركةفتنا ريشاليَن هةمةجوَر. ب تايبةتى، زيانيَن دةمارى ئةويَن ذ نةخوَشيا شةكرىَ ثةيدا دبن. طوهوَرين د ظةطوهاستنا دةمارا زةظلّةكى تشكداريىَ د لاوازيا هيَدى هيَدى يا زةظلّةكان دكةت د نةخوَشيا شةكريَدا. ظةكولينيَن فيزيولوجى ييَن كارةبىَ ئةويَن دهيَنة بكارئينان دووثاتيىَ لسةر طوهوَرينيَن ظةطوهاستنا دةمارا زةظلّةكى دكةن و يا هاريكارة بو ذيَك جوداكرنا حالةتيَن دى. ظةكولينا نوكة هاتيةكرن بو هةلسةنطاندنا ئةركىَ طةهاندنا دةمارا زةظلّةكى دوان نةخوَشاندا كو ئازارىَ ذ نةخوَشيا شةكرىَ ذ جوَرىَ دووىَ دبينن، ئةوذى بكارئينانا تةكنيكا هاندانا دةمارىَ دووبارةكرى و هةلسةنطاندنا لةشيَن دذى وةرطرىَ ئةسيتيل كولين دخويناظيَدا.

بابةتو شيَواز: ئةظ ظةكولينا بةرفرةه ذ 103 نةخوَشان طرت كو توشى دةردىَ شةكرىَ ذ جوَرىَ دووىَ بووينة. ظةكولينىَ هةمى ثيظةريَن ئيَكستن و دويرئيَكستن دةست نيشانكرى بكارئيناينة بو دويرئيَكستنا هوَكاريَن دى كو ضيَدبيت تشكداريىَ نةخوَشيا زةفلّةكىَ بكةن، هةمى تشكدار راوةرطرتن تةمامكر و تشكنينيَن فيزيكى و دةمارى هاتنةكرن، ظةكولينا طةهاندنا دةمارى يا روتينى هاتةكرن و هاندانا دةمارىَ دووبارة و هةلسةنطاندنا لةشيَن دذى وةرطريَن ئةسيتيل كولين.

ئةنجام: ذناظبةرا 103 نةخوَشان ذ نةخوَشيَن دةردىَ شةكرىَ ذ جوَرىَ دووىَ، (56) ذ وان هاتنة دةست نيشانكرن كو نةخوَشيا دةماريَن دةوروبةر هةية، لىَ (47) ذ وان ئةنجام يا سروشتى بوو، 11 (10,7 %) ذ وان تاقيكرنا كيَميا ئةريَنى دياركر، هةمى ئةويَن تاقيكرنا كيَميا ئةريَنى هةى، نةخوَشيا دةمارى يا دةوروبةر هةبوو. هةر وةسا تاقيكرنا لةشيَن دذى وةرطرىَ ئةسيتيل كولين د هةمى تشكداراندا يىَ نةريَنى بوو (ئةويَن تاقيكرنا كيَميا ئةريَنى و نةريَنى لنك وان هةى).

دةرئةنجام: ئةظ ظةكولينة ئاماذة ب وىَ ضةندىَ دكةت كو نةخوَشيا شةكرىَ ذ جوَرىَ دووىَ تشكداريىَ د لاوازيا طةاندنا دةمارا زةظلّةكى دكةت و ثيَدظى ب ظةكولينيَن بةرفرةهتر هةية بو دياركرنا فيزيولوجيا نةخوَشيان و ئالاظيَن ذىَ بةرثرس ذ تاقيكرنا كيَميا ئةريَنى د نةخوَشيا شةكريَدا ذ جوَرىَ دووىَ.

الخلاصة

تقييم سلامة الاتصال العصبيالعضلي لدى مرضى النوع الثاني من داء السكري

الخلفية والأهداف:يؤدي داء السكري الى أضرار طويلةالأمد وفشل الأنسجة المختلفة ، وعلى وجه الخصوص ، الأضرار العصبية الناجمة عن مرض السكري. التغييرات في انتقال العصبي العضلي من شأنها أن تسهم في الضعف التدريجي للعضلات خلال مرض السكري. تعتبر الدراسات الفيزيولوجية الكهربية ذات استخدام معتمد في تأكيد تغيرات الانتقال العصبي العضلي وتساعد على تمييزها عن الحالات الأخرى.
تم إجراء الدراسة الحالية لتقييم وظيفةالاتصال العصبي العضلي في المرضى الذين يعانون من مرض السكري من النوع 2 باستخدام تقنية تحفيز العصب المتكرر وتقييم الأجسام المضادة لمستقبلات الأسيتيل كولين في المصل.

المواضيع و طرق البحث:شملت هذه الدراسة المستعرضة 103 مرضى يعانون من داء السكري من النوع الثاني. استوفت الدراسة جميع معايير تضمين واستبعاد معينة لاستبعاد العوامل الأخرى المحتملة التي تساهم في الاعتلال العصبي. المشاركين جميعاًأكملوا الاستبيان ، وأجريت الفحوصات الفيزيائية والعصبية ، أجريت دراسة التوصيل العصبي الروتيني، وتحفيز العصب المتكررة وتقييم الأجسام المضادة لمستقبلات الأسيتيلكولين في المصل.

النتائج:من بين 103 مريض من مرضى داء السكري من النوع الثاني تم تشخيص 56 منهم باعتلال الأعصاب المحيطية أماالبقية (47) كانت نتيجة طبيعية ،أظهر 11 (10,7 ٪) منهم اختبار تناقص إيجابي ، كل أولئك الذين لديهم اختبار تناقص إيجابي لديهم أيضا اعتلال الأعصاب المحيطية ، وكان اختبار الأجسام المضادة لمستقبلات الأسيتيلكولين سلبي في جميع المشاركين (أولئك الذين لديهم اختبار تناقص إيجابي وسلبي).

الاستنتاجات: تشير هذه الدراسة الأولية إلى أن مرض السكري من النوع الثاني يساهم في ضعف الاتصال العصبي العضلي. وتحتاج الى دراسات اوسع لبيان الفيزيولوجيا المرضية والآليات المسؤولة عن اختبار تناقص إيجابي في مرضى السكري من النوع الثاني.

 https://doi.org/10.31386/dmj.uod.2019.13.1