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Histopathological Changes in a Rat Model of Diabetic Neuropathy

Hanan Kumar Gopalan

University Kuala Lumpur, Institute of Medical Science Technology, Clinical & Biomedical Laboratory Science, 43000 Kajang, Selangor, Malaysia

*Corresponding Author**: hanankumar@unikl.edu.my**

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Abstract

This study has been done to investigate the histopathological changes in a rat model of diabetic neuropathy. The 24 male rats used in this research, were splinted randomly into two groups of 12 animals each. The first group (G1; consisted of healthy control rats), was administered with normal saline, while the second group (G2; STZ treated animals) received a single intraperitoneal injection of freshly prepared streptozotocin (STZ), at a dosage of 60 mg/kg, to induce a diabetes-like state in the rats. At the third day after STZ injection, a digital glucometer was used to measure blood glucose levels of 12-hour fasting rats; the hyperglycemia state was considered if the fasting blood glucose level (FBG) was greater than 250 mg/dl. To assess neuropathic pain, cold allodynia was calculated by counting some times the foot withdrawal occurred in response to cold stimuli applied to the back of the hand. The sciatic nerve was carefully removed when rats were sacrificed (after 2 months) and preserved in 10% formalin for histopathological investigations. Normal blood glucose levels were maintained in the control rats throughout the study. However, G2 exhibited much greater FBG levels than healthy animals. In the 2-month study period, the G2 maintained FBG levels above 250 mg/dl. The score for the severity of STZ on sciatic nerves showed a significant difference between the STZ-treated group (G2) as compared to non-treated (G1). No paw reactions were seen when intact animals were subjected to plantar surface acetone. The experimental groups did not show any notable reactions to acetone on the tenth day after the diagnosis of diabetes. On days 20, 30, 40, 50 and 60 after diabetes confirmation, there were significant differences in paw withdrawal frequency between the G1 and G2 ($p<0.001$). Within the control group, nerve fibres were found to be regularly arranged and there was no evidence of axonal swelling. However, in the G2 animals, nerve fibres were found to be significantly disorganized, and there was a notable increase in the number of Schwann cells in the sciatic nerves, suggesting damage, in comparison to the control group. In conclusion, the present study confirmed different neuropathic effects on sciatic nerves in rat models of diabetic neuropathy. In addition, the histopathological changes of the nerves, including severe lipoid degeneration of axons, could be related to the chronic hyperglycemic state or to the toxicity of STZ on the peripheral nervous system, which requires further investigation.

Keywards: Histopathology, Rat, Diabetes, Neuropathy.

التغيرات النسيجية المرضية في نموذج الفئران للاعتلال العصبي السكري

الخلاصة

أجريت هذه الدراسة لمقارنة التغيرات النسيجية المرضية في الجرذان المصابة بالاعتلال العصبي السكري (المتستحدث نتيجة للاصابة بمرض السكر) مقارنة بالجرذان غير المصابة. استخدم في الدراسة 24 من الفئران الذكور تم تقسيمها إلى مجموعتين تضم كل منهما 12 حيوانًا. المجموعة الأولى مجموعة السيطرة (G1) والتي تتضمن ألجرذان الأصحاء، تم حقنها بجرعة من المحلول الملحي القياسي، عند حقن اقراد المجموعة الثانية G2 (مجموعة STZ) بجرعة 60 مجم / كجم (داخل الصفاق) من STZ ، من بعد تجويع الجرذان لمدة 12 ساعة قبل الحقن. في اليوم الثالث من اعطاء حقن STZ، تم استخدام مقياس السكر الرقمي لقياس مستويات الجلوكوز في الدم لدى الفئران الصائمة (FBG)؛ وتم تأكيد الاصابة بارتفاع السكر في الدم حين كانت القراءات أكبر من 250 ملغم / ديسيلتر. ومن أجل تقييم آلام الأعصاب المصاحبة للمرض طوال فترة الدراسة، تم استخدام طريقة حساب الألم المصاحب للبرد من خلال احصاء عدد المرات التي انسحبت فيها قدم الجرذ كاستجابةً رد فعل عند تعريضها للمنبهات الباردة على الجزء الخلفي من اليد. و بعد فترة شهرين، تم قتل الحيوانات وإزالة العصب الوركي بعناية وحفظه في 10٪ من الفورمالين لدراسة التشريح المرضي. أظهرت النتائج ان مستويات FBG في مجموعة G2 كانت أكبر بكثير من الحيوانات السليمة في مجموعة G1.

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كذلك أظهرت ان تأثير الاصابة بارتفاع السكر في الدم على الأعصاب الوركية كانت تمثل فروقًا معنوية بين اقراد المجموعة المصابة مقارنةً بمجموعة السيطرة، لم تُشاهد أي استثارة مخلبية عندما تعرضت الحيوانات السليمة للأسيتون السطحي الأخمصي، بينما لم تظهر على المجموعة المصابة أي تفاعلات ملحوظة تجاه الأسيتون في اليوم العاشر بعد تأكيد الاصابة بمرض السكري. في الأيام 20 و30 و40 و50 و60 من بعد تأكيد الإصابة بالسكري، كانت هناك اختلافات وبفروقٍ معنوية ($p<0.001$) في تكرار سحب المخلب بين افراد المجموعة المصابة ومجموعة السيطرة. أظهرت دراسة التغيرات النسيجية المرضية بالنسبة لمجموعة السيطرة أن الألياف العصبية مرتبة بانتظام ولم يظهر أي علامات تورم حول محاور الألياف العصبية الوركية. قي حين أن افراد مجموعة G2 ظهرت أن الألياف العصبية الوركية غير منتظمة وبشكل كبير، وتبين زيادة ملحوظة في اعداد خلايا شوان في الأعصاب الوركية، مما يشير إلى حدوث تورم وضرر لمجموعة الجرذان المصابة مقارنة بافراد مجموعة السيطرة. من خلال النتائج نوصي الي الحاجة للمزيد من الدراسات العلاجية مستقبلا، من خلال استخدام مضادات مستقبلات الألم، ومضادات ارتفاع السكر في الدم، ومضادات الأكسدة، وتجديد الأعصاب، التي تعزز مسارات مرض السكري المستحث عن STZ.في الختام، أثبتت الدراسة الحالية وجود تأثيرات عصبية مختلفة على الأعصاب الوركية في نموذج الجرذان المصابة بالاعتلال العصبي المصاحب لمرض السكري. بالإضافة إلى ذلك، اظهرت التغيرات النسيجية المرضية لأعصاب الجرذان، التهابات شديدة في الاغشية الدهنية للمحاور العصبية، يمكن أن تكون مرتبطة بسبب حالة ارتفاع السكر المزمن في الدم أو بسبب سمية مادة STZ على الجهاز العصبي المحيطي، الأمر الذي يتطلب مزيدا من التحقيق والدرسات المستقبلية.

Introduction :

Diabetes mellitus is a worldwide epidemic that affects people of all income levels and all nations. The International Diabetes Federation projects that the number of individuals living with diabetes would reach 700 million by 2045, with 463 million afflicted with the disease and 374 million suffering from impaired glucose tolerance (1). More over half of all type 2 diabetics with long-term disease may develop diabetic peripheral neuropathy (DPN) (2), making it the most common diabetic consequence. The fundamental illness processes of DPN remain unknown, despite the disease's high incidence (3). A rise in DPN is a direct result of the growing number of cases of diabetes and prediabetes across the world. Given the nature of DPN as a sensory dysfunction illness, patients often experience pain and discomfort, allodynia, numbness, and insensate feet. This distal symmetric polyneuropathy involves the distal part of the lower limbs in a length-dependent manner (4). It can lead to physical deficits (eg, imbalance, fall risk), foot ulcers, amputations and, eventually, death following the early somatosensory signs and symptoms (4,5). The only currently available disease-modifying therapy for DPN is strict glycaemic control, as well as the management of pain. While these are certainly useful, the devastating effects of DPN on healthcare expenditures and quality of life are enormous. Despite their well-recognized different pathophysiological processes, DPN has been shown to occur with similar frequency in both type 1 and type 2 diabetes mellitus (t1DM and t2DM, respectively) (7-9). It was not surprising that using glycaemic control, the only established disease-modifying therapy, to prevent the development of DPN symptoms in t2DM was less effective than in t1DM (10) and hence the consequence of the different pathophysiological processes. Synthetic changes have had to be made to the STZ model of t1DM to allow the development of the metabolic comorbidities associated with this disease, such as obesity, hypertension hyperlipidaemia, inflammation and insulin resistance (11-13), and this remains an important consideration when using these models. Several articles have reviewed the t1DM and t2DM models in rats and mice (14-16). The current purpose of our interest is to assess the fidelity of the rat DPN phenotype in the type 2 DM models. Therefore, this study has proposed to investigate the histopathological changes of the peripheral nerves in rat model of diabetic neuropathy.

Materials and Methods:

Twenty-four male adult rats, ranging in weight from 210g to 240g, were used in this research. The animals were provided with unlimited access to food and water.

Chemical:

Streptozotocin (STZ): freshly produced STZ was prepared by dissolving 0.1 ml in sodium citrate buffer (pH 4.5).

Experimental groups:

The 24 rats were divided into two groups of 12 animals each. The first group (G1) is control group, was administrated with 0.1 ml of normal saline. The second group (G2) is STZ-treated group, received a dosage (0.1 ml) of 60 mg/kg STZ.

A digital glucometer used to measure the fasting blood glucose (FBG) levels, of 12-hour fasting rats of both G1 and G2; the hyperglycemia state has confirmed as FBG value is greater than 250 mg/dl.

Assessment of the Neuropathic Pain:

In order to assess neuropathic pain, cold allodynia was calculated by counting the number of times the foot withdrew in response to cold stimuli applied to the back of the hand (17).

An elongated polyethylene tube used to delicately apply one drop of 100% acetone to the rat's mid-plantar surface using a syringe. As soon as acetone placed over the plantar area of the paw, a rapid retraction of the foot detected, as an indication of cold allodynia. The average break of three to five minutes between each of the ten tests.

Diabetic confirmation followed by a series of acetone drop tests on days 10, 20, 30, 40, and 50. The quantity of paw withdrawals divided by the number of trails and multiplied by 100, to indicate the acetone response frequency.

The Histopathological evaluation:

At the end of the experiment (after 2 months), all rats were sacrificed. The sciatic nerve has carefully removed and preserved in 10% formalin, until the processing, sectioning, and staining the samples.

Hematoxylin and eosin (H&E) used to stain the nerve sections. The process of detecting axonal degeneration scanning included for light microscopic observations (10X, 40X).

Evaluation of score for the severity of lesions of the sciatic nerve sections was based on the severity of the pathological changes including edema and degeneration. The following scores were given to lesions observed: 0 (none), 1 (mild), 2 (moderate) and 3 (severe).

Results and Discussion:

Blood glucose levels remained normal in the control group of rats over the whole trial. On the other hand, when compared with healthy animals, G2 had significantly higher FBG levels. Throughout the 2 months investigation, the G2 maintained FBG levels more than 250 mg/dl.

The score for severity of STZ-treated rats on sciatic nerves showed significant differences between G2 as compared with G1 rats (Table 1).

No paw reactions were seen when intact animals were subjected to plantar surface acetone. The experimental groups did not show any notable reactions to acetone on the tenth day after the diagnosis of diabetes. On days 20, 30, 40, 50 and 60 after diabetes confirmation, there were significant differences in paw withdrawal frequency between the G1 and G2 ($p<0.001$). The G1 showed 6.41±0.08%, versus 36.1±1.1% of G2, on day 20, while at day 30 were 6.53±0.34%, versus 62.4±3.7% of G2. On the day 40, G1 showed 6.44±0.16% versus 72.3±4.4% of G2, and on day 50, G1 showed 6.59±0.12% versus 76.1±2.1% of G2, furthermore, on day 60, G1 were 6.49±0.15 versus 80.2±3.7 of G2 (Table 2).

Table 1. Score for the severity of lesions of the sciatic nerve in studied groups

Groups	The severity score for sciatic nerve lesions
G1	0.0± 0.0B
G2	2.91 ± 0.13A

The different litters refer to significant differences at ($p \leq 0.05$)

Table 2. Neuropathic pain test

Groups	G1	G2
10 d	6.5±0.13A	11.46±2.3A
20 d	6.41±0.08B	36.1±1.1A
30 d	6.53±0.34B	62.4±3.7A
40 d	6.44±0.16B	72.3±4.4A
50 d	6.59±0.12B	76.1±2.1A
60 d	6.49±0.15B	80.2±3.7A

The different litters refer to significant differences at ($p \leq 0.05$)

Histopathological finding:

Within the control group, nerve fibres were found to be regularly arranged along with the absence of any signs of axonal swelling. In diabetic G2, axonal, lipid, and moderate localised peripheral axonal loss occur. G2 axons show lipoid degeneration and modest localised peripheral axonal loss with diffuse pleomorphic lymphocyte infiltration (Fig. 1).

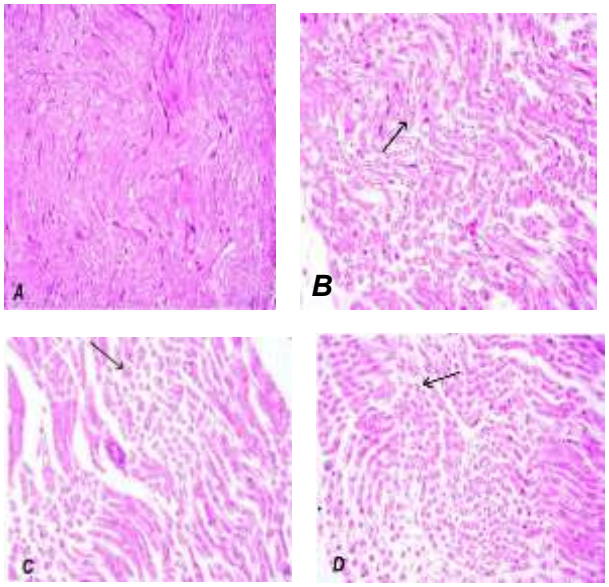

Figure 1. Histopathological characteristics of diabetic rats' sciatic nerves as a result of STZ administration. (A) control group G1, shows the nerve tissue is relatively normal and no signs of degeneration or inflammation. (B) diabetic group G2, shows mild axonal degeneration with focal peripheral axonal loss. (C) diabetic group G2, shows severe lipoid degeneration of axons along with focal peripheral axonal loss. (D) diabetic group G2, shows sciatic nerve with diffused infiltration of pleomorphic lymphocytes.

Cold allodynia, which started 20 days after STZ and persisted throughout the trial, was produced by STZ in this research. The presence of cold allodynia, as measured by the plantar surface application of acetone, is evident beginning in the second week after an injection of STZ (60 mg/kg), as previously described by (17). Among the several forms of neuropathic pain that have been documented in rats with STZ-induced diabetic neuropathy are cold allodynia, mechanical allodynia, and heat hyperalgesia (17,18). The primary pathogenic mechanisms of diabetes and its complications include chronic hyperglycemia and the glucotoxicity that is generated by STZ (19).

The histopathological changes indicated in the current study were agreed with some previous works, that the diabetic peripheral neuropathy of rats induced with STZ has reported along

with histological changes such as, fiber degeneration and edema of the sciatic nerve tissue (20), and the STZ have raised levels of malondialdehyde and lowered levels of superoxide dismutase (SOD) in rat's sciatic nerve tissues (21).

Diabetic chronic hyperglycemia is thought to be linked to mitochondrial damage, increased ROS generation, decreased nerve blood flow, reduced availability of trophic substances, and slower nerve conduction, whereas, these consequences are not adequately treated, they may cause degenerative anomalies in the peripheral nervous system (22). Neuropathy is often associated with significant alterations in axonal architecture, the number of Schwann cells, as well as nerve fiber organization. Due to the histological examinations of diabetic rats' sciatic nerves, a previous report suggested that paclitaxel treatment had the reverse effect on nerve fiber derangement, axonal enlargement, and an increased number of Schwann cells, while glibenclamide therapy reduced these symptoms (12).

Development of diabetes complications is suggested by large body of clinical and experimental data, which pointed to the importance of free radical-mediated oxidative processes. Protein glycation, hyperglycemia-induced acceleration of glucose autoxidation, and the oxidative breakdown of glycated proteins all contribute to an increase in free radical generation (23). While cellular antioxidants like SOD, GSH, and CAT may protect against nerve injury, free radicals produced by excessive oxidation may be to blame (24).

Conclusion:

Further studies for treatment, potentially via antinociceptive, antihyperglycemic, antioxidant, and neuro-regeneration boosting pathways in STZ-induced diabetic peripheral disease, are highly required.

Conflict of Interest

The authors confirmed that they had no conflicts of interest.

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