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ORIGINAL ARTICLE

Unveiling Noradrenaline: Insights into Cardiac Autonomic Neuropathy in a Type 1 Diabetic Animal Model

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Background: The global surge in Type 1 Diabetes Mellitus (T1DM) cases across age groups has raised concerns about associated cardiovascular risks, warranting a deeper understanding of its complications. Diabetic Cardiovascular Autonomic Neuropathy (DCAN), characterized by cardiovascular system irregularities in diabetic patients, remains inadequately explored, particularly in T1DM. This study aimed to establish a connection between T1DM and the onset of DCAN in an animal model, focusing on the role of noradrenaline as a potential marker.

Methods: Male Wistar rats were randomly divided into Control and T1DM model groups. The diabetic group received intraperitoneal Streptozotocin injections and remained untreated until DCAN developed. Blood glucose levels and body weight were measured weekly. Vital signs monitoring, and noradrenaline measurements were conducted at specific intervals using non-invasive techniques and ELISA kits to assess the rats' cardiovascular parameters and noradrenaline levels throughout the study period.

Results: Subsequent analysis revealed distinct alterations in blood glucose, body weight, blood pressure, and heart rate in the diabetic group, mirroring clinical symptoms observed in diabetes. Notably, a significant rise in serum noradrenaline levels at week 14 suggested the emergence of DCAN.

Discussion: Findings demonstrate a parallel between T1DM and the development of DCAN, echoing observations in human diabetic patients. Importantly, this study highlights the potential of noradrenaline as an early indicator of DCAN onset. Despite limitations in sample size and duration, these groundbreaking insights pave the way for refining diagnostic strategies and developing targeted therapies for this debilitating complication in diabetic patients.

Conclusion: This investigation offers critical insights into the pathophysiology of DCAN in T1DM, emphasizing the urgent need for early detection and management strategies to mitigate associated cardiovascular risks in diabetic individuals.

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While type 1 diabetes primarily affects children, a growing number of adults either developed the condition during childhood or acquired it later in life. Consequently, a substantial portion of individuals living with type 1 diabetes face an increased risk of cardiovascular diseases as they reach adulthood. Notably, this disease's incidence is rising globally, exhibiting considerable variations across different nations and regions, possibly due to diverse environmental factors. Factors like perinatal events and hygiene in early life have been suggested as influential, while studies on body weight's link to type 1 diabetes have yielded mixed results.[1]

Surprisingly, some research indicates a rise in type 1 diabetes cases among young individuals but not in adults, yet the reasons behind this discrepancy remain inadequately explored compared to type 2 diabetes. Recent global statistics reveal that approximately 60% of the estimated 9 million people with type 1 diabetes in 2017 were over 40 years old, an age bracket predisposed to prevalent cardiovascular risk factors and emerging cardiovascular complications. Intriguingly, despite high-income countries hosting only 17% of the world's population, they accounted for 49% of new Type 1 diabetes cases.[2]

Diabetic cardiovascular autonomic neuropathy (DCAN) refers to the impairment of the autonomic regulation of the cardiovascular system in individuals with diabetes, excluding other potential causes.[3] It arises from damage to the nerve fibres that control the heart and blood vessels, leading to irregularities in cardiovascular functioning. DCAN is a prevalent chronic complication in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), linked to elevated morbidity and mortality rates in diabetic patients.[3] While the confirmed prevalence of DCAN in unselected individuals with T1DM and T2DM is approximately 20%, higher figures of up to 65% have been reported with advancing age and longer duration of diabetes. The development of diabetic CAN involves multiple factors, including heightened mitochondria production of free radicals due to hyperglycaemia-induced oxidative/nitrosative stress.[3]

Catecholamines encompass a cluster of hormones and neurotransmitters, comprising dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). These substances serve pivotal roles in the body's reaction to stress and contribute significantly to diverse physiological functions. Studies have examined the appearance rate and clearance of noradrenaline in idiopathic autonomic neuropathy and diabetic cardiovascular autonomic neuropathy (DCAN).[4] Assessing catecholamines in diabetes has revealed generally diminished responses to postural changes, like exercise, hypoglycaemia, and cardiovascular autonomic reflex tests in humans. To comprehensively gauge whole-body sympathetic activity and relevant physiological aspects (such as heart rate, blood pressure, cardiac output, and hormonal and metabolic events), it is imperative to measure plasma concentrations of noradrenaline, adrenaline, and plasma 3,4-dihydroxyphenylglycol (DHPG).[4]

Given the scarcity of studies concentrating on T1DM, our aim is to establish a connection between this disease and the emergence of diabetic cardiovascular autonomic neuropathy in an animal model, with a specific focus on noradrenaline. Consequently, these groundbreaking findings are expected to significantly aid future research aimed at diagnosing DCAN more effectively.

MATERIALS AND METHODS

Male Wistar rats weighing about 180-200g were purchased from the Comparative Medicine and Technology Unit (COMeT). The animal operation was reviewed and approved by the Institutional Animal Care and Use Committee at Universiti Putra Malaysia (IACUC). The animals were placed in the Animal Behaviour Laboratory, Human Anatomy Department, in clean cages. The room was kept at a regulated temperature and humidity, with a 12-hour light/dark cycle. All animals were given two weeks to adapt to their new surroundings before the studies began. They were housed in wooden cages in pairs and were fed freely with pellets.[5]

After adaptation, the rats were randomly divided into a Control group (n=10) and a Type 1 Diabetic model (DM) group (n=30). The DM groups were injected intraperitoneally with a high dose of Streptozotocin (STZ) 50mg/kg.[6] Subsequently, while continuing to be

fed rat pellets and housed in pairs with wood shavings, all diabetic rats remained untreated until the establishment of DCAN.

Prior to the morning blood glucose measurement using a glucometer (Accu-check Guide Meter), the rats were fasted starting at 2000H the night before.[6] Blood glucose levels were measured for Week 0, Week 3, Week 18, Week 19, and Week 20. Blood was collected from a rat's tail that had been sterilised with an alcohol swab, and the last 3 mm was snipped off before the blood glucose test paper was inserted into the glucometer.[6] Body weight from week 0 to week 20 using an (Adam Weighing Balance) was recorded weekly.[5]

Non-invasive echocardiography and blood pressure monitoring were conducted using a tail cuff and a CODA High Throughput Monitor (Kent Scientific, Torrington, CT). Measurements were taken at Week 0, 3, 10, 18, 19, and 20, with readings measured over 3 cycles for each rat. Any undesirable or erratic recordings—such as data falsely generated by the system—were excluded from the analysis. The parameters collected included systolic (SBP), diastolic (DBP), mean (M) blood pressure, and heart rate (HR) for all the rats.

Disposable test tubes were used to collect whole blood samples for noradrenaline measurement according to the kit's instructions (Elabscience Biotechnology, China) at Week 10 and Week 14. The ELISA kit uses the competitive ELISA principle. The ELISA plate provided in this kit had been pre-coated with Noradrenaline/Norepinephrine (NA/NE). During the reaction, NA in samples or standard competed with a fixed amount on the solid-phase supporter for sites on the Biotinylated Detection Ab Specific to NA. Excess conjugate and unbound samples were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) was added to the microplate well and incubated. Then, the TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by adding a stop solution, and the colour change was measured spectrophotometrically at a wavelength of 450nm. The concentration of NA in the samples was then determined by comparing the OD of the samples to the standard curve.[7]

RESULTS

The Effects of STZ on Fasting Blood Glucose Levels

Hyperglycaemia had been confirmed by Week 2 following the STZ injection. As anticipated, the negative control group (n=6) exhibited normal glucose levels compared to the experimental group (n=30) (Figure 1). Glucose levels were significantly elevated from Week 3 through to Week 18. Moreover, diabetic rats demonstrated increased urine production, leading to frequency and polyuria until Week 18.

The Effects of DM on Body Weight

In Week 0, the body weight of all the rats was recorded before the acclimatization week. The negative control group (n=6) and experimental groups (n=30) were monitored weekly for body weight changes. Despite the administration of STZ in Week 1, there was no significant increase in body weight observed for either the negative control or experimental group from Week 1 to Week 2 (Figure 2). However, starting from Week 3 until Week 18, the body weight significantly decreased in the experimental group compared to the negative control, while the body weight consistently increased in the negative control group (statistical significance not shown).

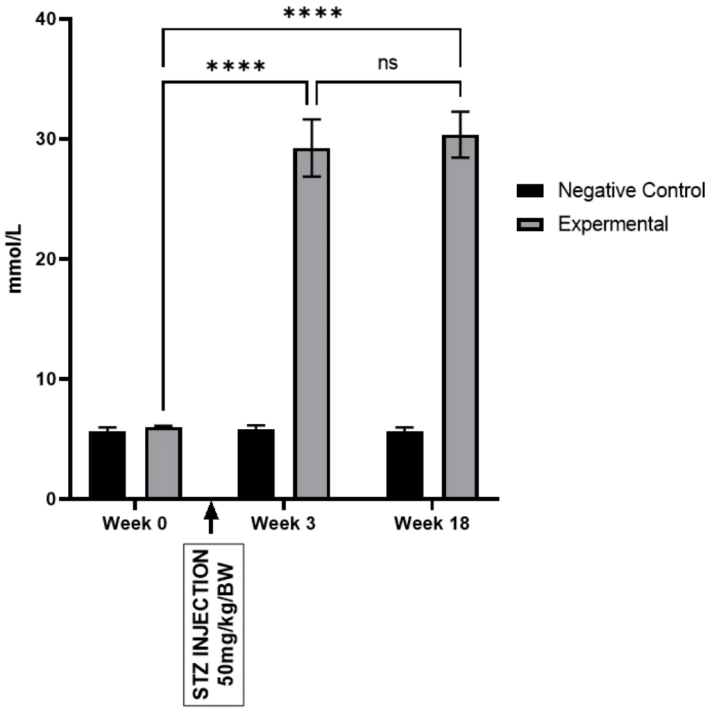

Figure 1 *STZ administration took place in Week 1. Blood glucose levels displayed a significant increase from Week 3 onwards and remained elevated until Week 18 (ns= no significant, ****p<0.0001).*

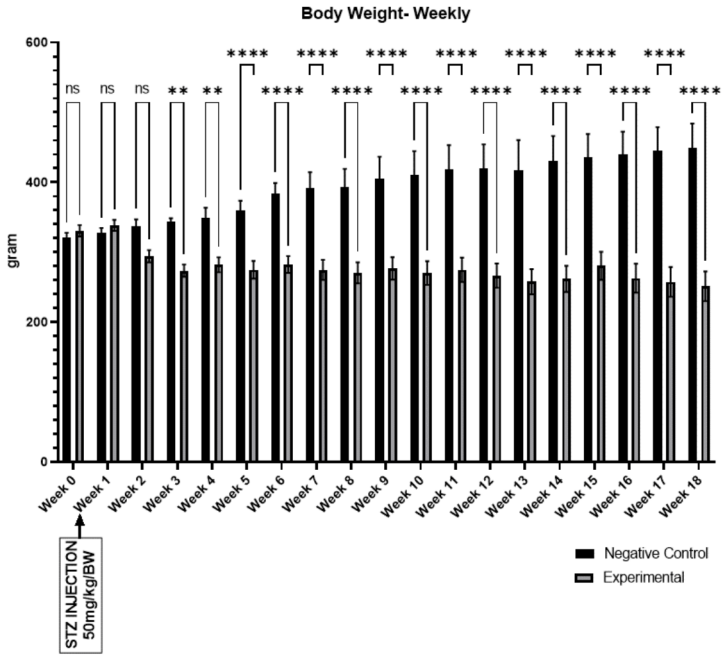

Figure 2 *The graph bars depicted a decreasing trend in body weight within the experimental group compared to the negative control group, commencing from Week 3 and persisting until Week 18 (ns= no significant, **p<0.01, ****p<0.0001).*

The Effects of DM on Blood Pressure (systolic, diastolic and mean) and Heart Rate

The effects of blood pressure and heart rate following STZ injection in Week 1 are illustrated in **Figure 3**. In the negative control group (n = 6), there were no significant changes observed in any parameters: systolic, diastolic, mean blood pressure, or heart rate (data not shown). However, both systolic and mean blood pressure exhibited a notable increase in the experimental group (n=30), commencing at Week 3 and nearly plateauing after Week 10. Additionally, the experimental group displayed bradycardia in heart rate, starting from Week 3 and maintaining a nearly constant level thereafter. No significant changes in diastolic pressure were observed within the experimental group.

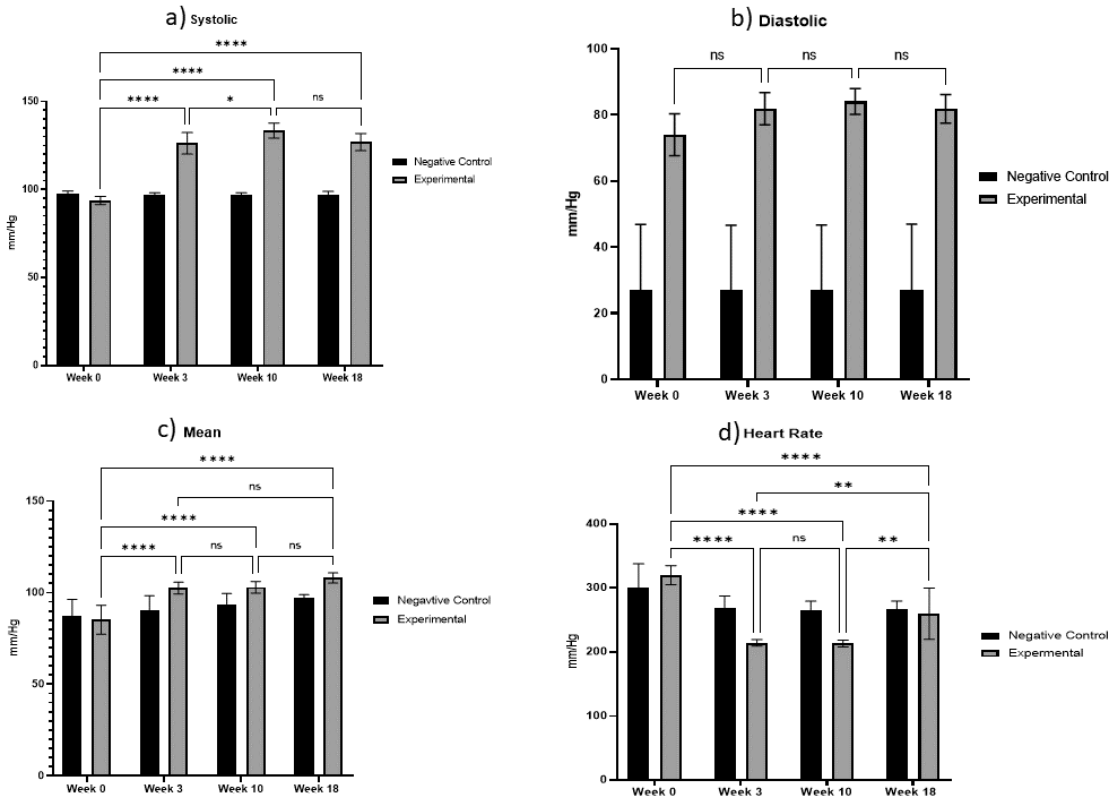

Figure 3 *The blood pressure measurements, including (a) systolic, (b) diastolic, (c) mean, and (d) heart rate, were compared to analyse the trends in both the experimental and negative control groups. Significant increases were observed in systolic and mean blood pressure within the experimental group starting from Week 3. There were no notable changes detected in diastolic blood pressure. Additionally, heart rate demonstrated a significant increase from Week 3 onwards in the experimental group (ns = no significant, **p<0.01, ****p<0.0001).including (a) systolic, (b) diastolic, (c) mean, and (d) heart rate, were compared to analyse the trends in both the experimental and negative control groups. Significant increases were observed in systolic and mean blood pressure within the experimental group starting from Week 3. There were no notable changes detected in diastolic blood pressure. Additionally, heart rate demonstrated a significant increase from Week 3 onwards in the experimental group (ns = no significant, **p<0.01, ****p<0.0001).*

The Effects of DM on Serum Noradrenaline (NA)

The ELISA results revealed a substantial rise in serum NA levels, notably higher in the experimental group (n=30) compared to the negative control group (n=6) by week 14 (Figure 4). Although there was an increased concentration of NA in Week 10, it did not reach statistical significance. The findings at week 14 suggest that the animals were already exhibiting signs of Diabetic Cardiac Autonomic Neuropathy.

DISCUSSION

The current study aimed to establish a connection between T1DM and the emergence of DCAN in an animal model, with a specific focus on noradrenaline. The investigation commenced by confirming the relevance of the study through an introductory exploration of the increasing incidence of T1DM globally. Notably, this condition, primarily considered a paediatric concern, has witnessed a surge in adulthood cases, exposing a larger population to cardiovascular risks as they age.[1]

Our findings corroborate the well-established prevalence of DCAN in both T1DM and T2DM. The confirmed prevalence of DCAN in unselected individuals with T1DM and T2DM approximates 20%, with figures soaring to 65% with prolonged diabetes duration and advancing age. This aligns with previous clinical observations of heightened morbidity and mortality rates in diabetic patients due to DCAN, underscoring the critical need for early diagnosis and effective management strategies.[3]

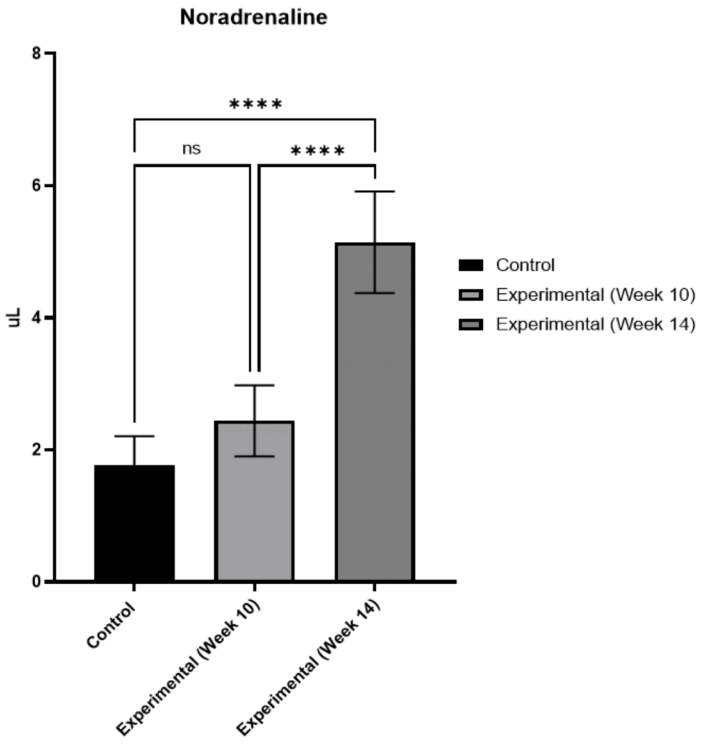

Figure 4 *The serum NA levels showed a significant increase by Week 14, indicating the establishment of DCAN. No additional measurements were conducted at Week 18 due to the limited availability of chemicals (ns= no significant, ****p<0.0001).*

SUMMARY BOX

- T1DM, once considered a childhood disease primarily, is increasingly diagnosed in adults, thereby elevating long-term cardiovascular risk. Its global incidence varies markedly across regions, possibly due to environmental influences such as perinatal factors and early-life hygiene, while associations with body weight remain inconclusive.
- The rise in cases is more pronounced among youth than adults, with high-income countries contributing disproportionately to new diagnoses.
- DCAN, prevalent in longstanding diabetes, damages autonomic nerve fibres that regulate cardiac function.
- Altered catecholamine activity, including noradrenaline, further disrupts cardiovascular responses, contributing to impaired autonomic regulation and increased morbidity over time.

The study effectively induced hyperglycaemia in the experimental group following STZ administration, simulating the diabetic condition, as evidenced by elevated blood glucose levels.[8] Concurrently, these diabetic rats displayed characteristic clinical signs such as increased urine production and reduced body weight, consistent with diabetic symptoms observed in animal models and clinical settings.[9]

An intriguing discovery from our investigation relates to the alterations observed in blood pressure and heart rate. The experimental group exhibited a notable increase in systolic and mean blood pressure levels, alongside a consistent bradycardic trend in heart rate. These alterations, akin to findings in diabetic patients, might denote an early onset of cardiovascular autonomic dysfunction in the animal model.[4] Notably, the absence of significant changes

in diastolic pressure aligns with some clinical observations in diabetic individuals, highlighting the complex nature of cardiovascular responses in DCAN.

Crucially, our study aimed to shed light on the role of NA as a potential marker for DCAN. The ELISA results revealed a substantial rise in serum NA levels, particularly pronounced at week 14, suggesting the onset or progression of DCAN. While the increase in NA concentration at week 10 did not reach statistical significance, the marked elevation at week 14 strongly indicates the establishment of DCAN in the animal model.

Our study has some limitations, including a relatively small sample size and the lack of long-term observations beyond week 18. Additionally, further exploration of other catecholamines, alongside additional biomarkers related to DCAN, could provide a more comprehensive understanding of autonomic neuropathy in diabetes.

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CONCLUSION

In conclusion, our pioneering investigation successfully established a link between T1DM and the emergence of DCAN in an animal model, emphasising the potential utility of noradrenaline as an early marker for this condition. These findings offer valuable insights into the pathophysiology of DCAN and pave the way for future research aimed at refining diagnostic approaches and developing targeted therapies for this debilitating diabetic complication.

REFERENCES

1. Waernbaum I, Lind T, Möllsten A, Dahlquist G. The incidence of childhood-onset type 1 diabetes, time trends and association with the population composition in Sweden: a 40 year follow-up. Diabetologia. 2023;66:(2)346-53.
2. Rosengren A, Dikaiou P. Cardiovascular outcomes in type 1 and type 2 diabetes. Diabetologia. 2023;66:(3)425-37.
3. Serhiyenko V, Serhiyenko A. Diabetic cardiac autonomic neuropathy. The Diabetes Textbook: Clinical Principles, Patient Management and Public Health Issues: Springer; 2023. p. 939-66.
4. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, et al. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes/metabolism research and reviews. 2011;27:(7)654-64.
5. Akinlade OM, Owoyele BV, Soladoye AO. Streptozotocin-induced type 1 and 2 diabetes in rodents: A model for studying diabetic cardiac autonomic neuropathy. African Health Sciences. 2021;21:(2)719-27.
6. Shi L, Hu Q, Li L, Yang R, Xu X, Du J, et al. Beneficial Effects of lncRNA-UC. 360+ shRNA on Diabetic Cardiac Sympathetic Damage via NLRP3 Inflammasome-Induced Pyroptosis in Stellate Ganglion. ACS omega. 2022;7:(31)27714-21.
7. Al-Assi O, Ghali R, Mroueh A, Kaplan A, Mougharbil N, Eid AH, et al. Cardiac autonomic neuropathy as a result of mild hypercaloric challenge in absence of signs of diabetes: modulation by antidiabetic drugs. Oxidative Medicine and Cellular Longevity. 2018;2018.
8. Wang-Fischer Y, Garyantes T. Improving the reliability and utility of streptozotocin-induced rat diabetic model. Journal of diabetes research. 2018;2018.
9. Sheng X, Dan Y, Dai B, Guo J, Ji H, Zhang X, et al. Knockdown the P2X3 receptor in the stellate ganglia of rats relieved the diabetic cardiac autonomic neuropathy. Neurochemistry International. 2018;120:206-12.