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Article

Evaluation of the Impact of Advanced Glycation End-Products on Peripheral Neuropathy Outcomes in Type 2 Diabetes

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Abstract: Background: Type 2 diabetes mellitus (T2DM) affects over 500 million people worldwide, and over 50% of this group experience the most common complication, diabetic peripheral neuropathy (DPN). The presence of advanced glycation end-products (AGEs) have been linked with the development of DPN. The present study assessed for AGE levels in participants with type 2 diabetes, and explored the hypothesis that there may be increased AGE levels in more severe DPN. **Methods:** 124 participants with T2DM were consecutively recruited, and underwent skin autofluorescence, clinical assessment for peripheral neuropathy, peripheral nerve ultrasound, nerve conduction studies and axonal excitability assessment. **Results:** Elevated levels of AGE did not correlate with higher clinical neuropathy scores, sural sensory nerve amplitude, median or tibial nerve cross-sectional area or measures of axonal excitability. While higher AGE readings correlated with diabetic kidney disease, these recordings were not associated with more severe peripheral neuropathy outcomes on multiple regression analysis. **Conclusion:** The present study suggests that accumulation of AGE is not associated with clinical, electrophysiological and morphological measures of neuropathy in T2DM.

Keywords: advanced glycation end products; diabetic peripheral neuropathy

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder affecting over 500 million individuals worldwide, posing significant public health challenges. A common and debilitating complication of T2DM is diabetic peripheral neuropathy (DPN), which impacts more than 50% of diabetic patients and leads to progressive nerve injury, resulting in pain, loss of sensation, and impaired motor function [1,2]. The multifactorial pathogenesis of DPN involves complex biochemical and physiological processes, including oxidative stress, inflammation, and vascular dysfunction [3,4].

Among the numerous factors implicated in the development of DPN, advanced glycation end-products (AGEs) have attracted considerable attention as potential contributors to the development of nerve dysfunction. AGEs are formed through the non-enzymatic glycation of proteins, lipids, and nucleic acids, a process exacerbated by chronic hyperglycaemia [5]. These compounds accumulate in various tissues over time, altering their structure and function, and have been linked to diabetic complications such as retinopathy and nephropathy [6,7]. However, their role in the pathogenesis of DPN remains less well-defined in the clinical context.

AGEs contribute to nerve damage in diabetic peripheral neuropathy (DPN) through multiple interrelated mechanisms. One key mechanism is the promotion of oxidative stress, where AGEs generate reactive oxygen species that lead to cellular damage, particularly in neuronal tissues [8].

This oxidative stress not only directly injures nerve cells but also exacerbates inflammation, further contributing to neuronal injury. AGEs are known to induce chronic inflammation by activating pro-inflammatory pathways, which perpetuate and amplify nerve damage over time. Additionally, AGEs impair normal cellular repair processes by modifying proteins through glycation, which disrupts cellular signalling pathways critical for maintaining nerve integrity and function. This glycation process leads to the formation of abnormal cross-links in extracellular matrix proteins, hindering normal cellular function and repair. These effects collectively contribute to the progressive degeneration of peripheral nerves observed in DPN, resulting in symptoms such as pain, numbness, and motor dysfunction [9].

Recent studies have demonstrated the utility of skin autofluorescence (SAF) as a method of detecting elevated AGE levels [10,11]. To better understand the associations between AGE levels and the severity of DPN, this study collected comprehensive data relating to peripheral nerve structure and function from participants with T2DM, with AGE levels assessed using skin autofluorescence. This was correlated with clinical data, including age, sex, body mass index (BMI), waist circumference, duration of diabetes, HbA1c, triglycerides, LDL cholesterol, HDL cholesterol, total cholesterol, estimated glomerular filtration rate (eGFR), and the presence of chronic kidney disease.

2. Methods

A total of 124 participants with type 2 diabetes were consecutively recruited from the Diabetes Centre at Prince of Wales Hospital, Sydney. Participants underwent comprehensive assessments, including anthropometry measurements, SAF measurement, clinical evaluation for peripheral neuropathy severity, peripheral nerve ultrasound, nerve conduction studies, and axonal excitability testing. Participants were excluded if they were younger than 18 years of age, were receiving glucagon-like peptide-1 receptor agonists [12] or had peripheral neuropathy due to alternate causes such as immune-mediated disease, nitrous oxide use, vitamin B12 deficiency, excess alcohol use, or exposure to neurotoxic medications such as chemotherapy [12].

Skin autofluorescence was measured using the AGE Reader (DMU00101, DiagnOptics Technologies) to assess AGEs. Measurements were taken on the inner side of the forearm in a controlled environment with consistent ambient temperature (22-24°C) and lighting conditions. Each participant underwent three consecutive measurements, and the mean SAF value was recorded.

A single trained neurologist conducted the clinical examination for peripheral neuropathy and completed the Total Neuropathy Score (TNS) and modified Toronto Clinical Neuropathy Score (mTCNS). The evaluation included a physical examination and a review of medical history focusing on peripheral neuropathy symptoms. The modified Toronto Clinical Neuropathy Scale includes six symptom scores and five examination values, all scored from 0-3 based on severity, with a maximum score of 33 [13]. The Total Neuropathy Score yields a maximum value of 32, scoring 8 sections from 0-4 each, including sensory and motor symptoms and signs, deep tendon reflexes, and sural and tibial nerve amplitudes [14]. Nerve conduction studies were conducted following standard protocols to assess sural and tibial nerves (Natus, Middleton, WI, USA) [15].

High-resolution ultrasound was performed on median and tibial nerves by a single ultrasonographer. Images were captured with a 10-18MHz linear array transducer (MyLab™One, Esaote, Genoa, Italy). The musculoskeletal factory setting was used (acoustic power 100%, medium line density, dynamic range of 14 and persistence of 1), with constant gain and focus settings, and depth was varied as required to capture data. The probe was maintained at a 90-degree angle. The outcome measure of mean peripheral nerve cross-sectional area (CSA) was recorded for each participant after three free-hand traces of the inner margin of the endoneurium were obtained from each nerve. The median nerve CSA was tracked proximally from the wrist, and measurements were obtained at a site one third of the length up the forearm in the participant's dominant upper limb, away from any potential entrapment site [16]. The tibial nerve CSA was tracked proximally along with tibial vessels to a point 5 cm away from the medial malleolus, and measurement was recorded here away from any potential entrapment site [17].

Axonal excitability was undertaken on the median nerve and abductor pollicis brevis muscle in the participant's dominant upper limb, using nonpolarizing surface electrodes (Ambu, Sydney, AUS), DS5 Isolated Bipolar Current Stimulator and Qtrac software (Digitimer, London, UK), and TROND protocol [18]. Skin temperature was maintained above 32 degrees Celsius. Stimulus-response curves were generated by using 1ms pulses to achieve the highest CMAP amplitude, and then calculating 40% of the maximal response. The current required to achieve this target response was plotted for strength-duration, threshold electrotonus, and recovery cycle paradigms. The strength-duration relationship was tracked after using 0.2, 0.4, 0.8, and 1ms duration stimuli. The strength-duration time constant (SDTC) was obtained using Weiss' law to indirectly reflect node of Ranvier persistent Na⁺ conductance [19]. Rheobase, the minimum current of any duration needed to elicit a peripheral nerve response, was also recorded. Threshold electrotonus, which provides insight into various nodal and internodal conductances, was tracked using depolarising and hyperpolarising conditioning currents at +40 and -40% of the control threshold and percentage change in threshold was at plotted at various 10ms timepoints. For depolarising threshold electrotonus, S2 accommodation, which is the period following peak reduction and threshold reduction returns to control threshold values, was also calculated. Hyperpolarising threshold changes were recorded at 10-20ms, 20-40ms, and 90-100ms timepoints. The recovery cycle was recorded by plotting threshold changes from 2-200ms conditioning timepoints after the supramaximal stimulation of 1ms duration. The parameters that were recorded were the relative refractory period, super excitability and late sub excitability [20].

2.1. Ethical Considerations

The study protocol was reviewed and approved by the South-Eastern Sydney Local Health District Human Research Ethics Committee, ensuring all patient data was handled with strict confidentiality and in compliance with ethical research standards.

2.2. Statistical Analysis

A single investigator blinded to all participant data undertook data analysis using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY). Normally distributed data is represented as the mean and standard deviation while non-normal data is presented as median and interquartile range. By convention, nerve excitability outcomes are presented as mean and standard error (SE). Correlation analyses were applied to determine the relationship between SAF and clinical measures. Pearson correlations were used for normally distributed data while Spearman correlations were used for non-normally distributed data. Multiple linear regression analysis was applied to model the relationship between SAF and neuropathy outcomes while controlling for confounding clinical variables, namely age, sex, waist circumference, HbA1c diabetes duration and eGFR. A significance threshold of $p < 0.05$ was set for all inferential testing to determine statistically significant differences.

3. Results

Participant demographic data are summarised in Table 1. The study included 124 participants, with an average age of 66 ± 13 years. The cohort comprised 77 males and 57 females. The median skin autofluorescence, a measure reflecting the accumulation of AGEs, was 3.3 arbitrary units (IQR: 2.9–3.9). The mean body mass index (BMI) was 31.2 kg/m^2 , and the median waist circumference was 111 cm. Participants had a median diabetes duration of 144 months and a median HbA1c of 7.9%. Lipid profiles showed median triglyceride levels of 1.8 mmol/L, LDL cholesterol at 1.6 mmol/L, HDL cholesterol at 1.1 mmol/L, and total cholesterol at 3.7 mmol/L. Among the participants, 72 were receiving insulin therapy. The median estimated glomerular filtration rate (eGFR) was 77 ml/min/1.73m², and 45 participants had chronic kidney disease (CKD) compared to 89 who did not. The median potassium level was 4.4 mmol/L. Participants had a median Total Neuropathy Score (TNS) of 6 (IQR: 2–11) and a modified Toronto Clinical Neuropathy Score (mTCNS) of 4 (IQR: 2–11).

Table 1. Patient Demographics and Clinical Characteristics.

Parameter	Value
N	134
Age (years)	66 ± 13
Sex (Male)	77:57
Skin autofluorescence	3.3 (2.9–3.9)
BMI (kg/m ²)	31.2 (28.4–36.6)
Waist circumference (cm)	111 (100–117)
Diabetes duration (months)	144 (76–240)
HbA1c (%)	7.9 (7.2–9.0)
HbA1c (IFCC units)	62.8 (55.2–74.9)
Triglycerides (mmol/L)	1.8 (1.3–2.8)
LDL (mmol/L)	1.6 (1.1–2.1)
HDL (mmol/L)	1.1 (0.9–1.3)
Total cholesterol (mmol/L)	3.7 (3.3–4.6)
On insulin (Yes/No)	72:62
eGFR (ml/min/1.73m ²)	77 (50–90)
CKD (Yes/No)	45:89
Potassium (mmol/L)	4.4 (4.1–4.6)

Peripheral nerve ultrasound data are summarised in Table 2. Ultrasonography demonstrated that the median cross-sectional area (CSA) of the median nerve was 7.8 ± 1.7 mm², while the tibial nerve CSA had a median value of 14.2 mm², with an interquartile range (IQR) of 11.6–17.2 mm². The sural nerve exhibited a median sensory nerve action potential (SNAP) of 4.6 µV (IQR: 0–9.8 µV) and a conduction velocity of 40.9 ms⁻¹ (IQR: 0–50 ms⁻¹). The tibial nerve compound muscle action potential (CMAP) had a median value of 5.9 mV (IQR: 2.3–9.9 mV), with a latency of 4.0 ms (IQR: 3.7–4.5 ms).

Table 2. Peripheral Nerve Ultrasound and Nerve Conduction Data.

Parameter	Value
Median CSA (mm ²)	7.8 ± 1.7
Tibial CSA (mm ²)	14.2 (11.6–17.2)
Median CMAP (mV)	7.6 ± 2.7
Median latency (ms)	7.2 (6.6–8.0)
Sural SNAP (µV)	4.6 (0–9.8)
Sural velocity (m/s)	40.9 (0–50)
Tibial CMAP (mV)	5.9 (2.3–9.9)
Tibial latency (ms)	4.0 (3.7–4.5)
TNS	6 (2–11)
mTCNS	4 (2–11)

Nerve excitability outcome measures were obtained for the strength-duration, threshold electrotonus and recovery cycle testing paradigms. The strength-duration constant was calculated to be 0.43 ms (SE: 0.01 ms) and the rheobase was 3.5 mA (SE: 0.1 mA). In depolarising threshold electronus, threshold reduction at 10-20 ms and 90-100 ms was 64.2% (SE: 0.57%) and 42.5% (SE: 0.6%), respectively and S2 accommodation was calculated to be 21.4% (SE: 0.4%). In hyperpolarising threshold electrotonus, threshold increase at the 10-20 ms, 20-40 ms, and 90-100 ms timepoints was 72.7% (SE: 0.6%), 90.0% (SE: 1.0%) and 117.1% (SE: 2.1%), respectively. In regard to the recovery cycle parameters, the relative refractory period was 3.2 ms (SE: 0.06 ms), threshold reduction during superexcitability was 19.7% (SE: 0.6%) and threshold increase during subexcitability was 11.2% (SE: 0.4%). These values were similar to previous studies of cohorts of patients with diabetes [19].

Correlation between SAF and metabolic measures are summarised in Table 3. Significant correlations were identified between SAF and various clinical parameters. A significant negative correlation was found with body mass index (BMI) ($r = -0.180$, $p < 0.05$), triglycerides ($r = -0.265$, $p <$

0.01), total cholesterol ($r = -0.277$, $p < 0.01$), and estimated glomerular filtration rate (eGFR) ($r = -0.393$, $p < 0.01$). Conversely, a significant positive correlation was observed with diabetes duration ($r = 0.338$, $p < 0.01$) and potassium levels ($r = 0.170$, $p < 0.05$). Non-significant correlations were noted for waist circumference ($r = -0.146$), HbA1c ($r = 0.095$), LDL cholesterol ($r = -0.079$), and HDL cholesterol ($r = 0.113$).

Table 3. Correlations between AGE and Clinical Measures.

Clinical Parameter	Correlation with Skin Autofluorescence (r)
BMI	-0.180*
Waist Circumference	-0.146
Diabetes Duration	0.338**
HbA1c	0.095
Triglycerides	-0.265**
LDL Cholesterol	-0.079
HDL Cholesterol	0.113
Total Cholesterol	-0.277**
eGFR	-0.393**
Potassium (K ⁺)	0.170*

Standardized β -coefficients between SAF and neuropathy outcomes are summarised in Table 4. The data showed no significant association between SAF and tibial nerve cross-sectional area (CSA) ($\beta = 0.157$). Additionally, there was no significant associations with other neuropathy outcomes including the Total Neuropathy Score (TNS) ($\beta = 0.117$) and the modified Toronto Clinical Neuropathy Score (mTCNS) ($\beta = 0.044$). Analysis of nerve conduction data demonstrated no significant association between SAF and motor or sensory amplitudes obtained from the median, tibial or sural nerves. There was also no correlation between SAF and any of the main excitability parameters that were shown to be altered in the patient cohort.

Table 4. Standardized β -coefficients Between AGE Levels and Neuropathy Outcomes.

Parameter	β -coefficient
Median Nerve CSA (mm ²)	-0.006
Tibial Nerve CSA (mm ²)	0.157
Median Nerve CMAP (mV)	-0.147
Sural Nerve SNAP (μ V)	-0.071
Sural Nerve Velocity (ms ⁻¹)	-0.085
Tibial Nerve CMAP (mV)	-0.085
Tibial Nerve Latency (ms)	0.034
Total Neuropathy Score (TNS)	0.117
Modified Toronto Clinical Neuropathy Score (mTCNS)	0.044

Table 1: Demographic and clinical characteristics of the 134 study participants. The data include age, sex distribution, BMI, waist circumference, duration of diabetes, HbA1c levels (both in percentage and IFCC units), lipid profile, renal function (eGFR), and the prevalence of chronic kidney disease (CKD) within the cohort.

Table 2: Peripheral nerve ultrasound and nerve conduction study data for the cohort. Cross-sectional area (CSA) of the median and tibial nerves, compound muscle action potentials (CMAP), and nerve conduction latencies are displayed along with sural nerve sensory action potential (SNAP) amplitude and velocity. Additionally, Total Neuropathy Score (TNS) and modified Toronto Clinical Neuropathy Score (mTCNS) are included as measures of neuropathy severity.

Table 3 : Correlation coefficients (r) between skin autofluorescence, a measure of advanced glycation end-products (AGEs), and various clinical parameters including BMI, waist circumference, diabetes duration, HbA1c, lipid profile, eGFR, and potassium levels. Statistically significant correlations are denoted by * $P < 0.05$ and ** $P < 0.01$.

Table 4: Standardized β -coefficients from linear regression models examining the relationship between AGE levels and neuropathy outcomes, including nerve cross-sectional area (CSA), compound muscle action potential (CMAP), sensory nerve action potential (SNAP), and clinical neuropathy scores (TNS and mTCNS). The models control for confounding factors such as age, sex, diabetes duration, HbA1c, eGFR, and waist circumference. Abbreviations: CSA, cross-sectional area; CMAP, compound muscle action potential; SNAP, sensory nerve action potential; TNS, Total Neuropathy Score; mTCNS, modified Toronto Clinical Neuropathy Score.

4. Discussion

This study assessed the relationship between AGEs and the severity of DPN in a cohort of 124 individuals with type 2 diabetes. While AGEs are established contributors to various diabetic complications, our findings indicate that the accumulation of AGEs is not a robust predictor of DPN severity when compared to direct assessments of peripheral nerve structure and function.

Notably, significant correlations were observed between AGE levels and several metabolic parameters, including BMI, triglycerides, total cholesterol, and eGFR, corroborating prior research that links metabolic disturbances with increased AGE accumulation [21,30]. The positive association between AGE levels and diabetes duration further substantiates the hypothesis that prolonged hyperglycaemia fosters progressive AGE deposition in tissues [24,27]. However, our analysis did not reveal significant correlations between elevated AGE levels and key neuropathy-specific outcomes, such as clinical neuropathy scores, nerve conduction metrics, or axonal excitability measures. These findings imply that, despite their role in metabolic dysfunction, AGEs do not directly modulate the severity of neuropathy as measured by these objective parameters [23,25].

The absence of a strong relationship between AGE levels and peripheral nerve function underscores the multifactorial nature of DPN pathogenesis. It suggests that other pathogenic mechanisms, including oxidative stress, chronic inflammation, and microvascular impairment, may exert more pronounced effects on DPN development and progression than AGE accumulation alone [21,25,28]. This observation emphasises the importance of employing comprehensive peripheral nerve assessments, such as nerve conduction studies and axonal excitability tests, which are sensitive to subtle alterations in nerve health that may not be captured by AGE measurements alone.

Our findings differ from few earlier studies that reported stronger associations between AGE levels and neuropathy outcomes [28,29]. These discrepancies could be attributed to variations in study design, population characteristics, or methodological differences in AGE measurement, such as disparities in skin autofluorescence devices and calibration standards. Furthermore, our use of advanced diagnostic modalities, including nerve excitability testing and high-resolution nerve ultrasound, alongside a heterogeneous patient cohort, may have provided a more nuanced understanding of neuropathy, potentially explaining the weaker associations observed.

The specific characteristics of our study population, including prolonged diabetes duration, better glycaemic control, and varied comorbidities, may have influenced the results. It is plausible that other mechanisms of nerve damage, such as oxidative stress and vascular insufficiency, overshadowed the impact of AGE accumulation in this cohort [21]. The lack of a significant relationship between AGE levels and nerve excitability supports the hypothesis that AGEs contribute more to chronic, long-term structural alterations than to acute functional impairments [23].

Consistent with findings from similar investigations, our results underscore the complex and multifactorial nature of DPN, wherein AGEs represent just one element of the broader pathogenic landscape [23]. Our rigorous adjustment for potential confounders, including eGFR, age, sex, duration of diabetes, HbA1c, and waist circumference, further suggests that while AGEs play a role in DPN, they do not fully account for its severity, highlighting the necessity of considering multiple factors in the pathogenesis of DPN.

The observed association between higher AGE levels and diabetic kidney disease, but not neuropathy severity, suggests that AGEs may be more pertinent to other diabetes-related complications, such as nephropathy [26,27]. This finding is particularly relevant for clinical practice,

as it emphasizes the need for targeted diagnostic and therapeutic strategies tailored to the specific complications associated with diabetes.

In summary, while AGEs are critical markers of metabolic stress in diabetes, our study indicates that they are not reliable predictors of DPN severity. These findings highlight the importance of adopting a multifaceted approach that integrates direct nerve assessments to accurately evaluate and manage neuropathy. Shifting focus to a broader understanding of the pathophysiological mechanisms underlying DPN may lead to more effective strategies for improving patient outcomes [23–25,27].

Author Contributions: I.S was involved in manuscript composition, data interpretation and discussion. T.I. was involved in study design, data interpretation, discussion, and manuscript composition. R.D. was involved in study design, recruitment, data collection, data interpretation and manuscript composition. A.P. was involved in recruitment, data interpretation and discussion. K.M. was involved in recruitment, data interpretation and discussion. A.K. was involved in study design, data interpretation, manuscript composition and is the guarantor of this work. As such A.K. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the manuscript.

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