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Experimental Research Article

Association of serum adenosine deaminase level with nerve conduction velocity in type II diabetes patients

Manikandan Sathiyaseelan[1*], Suchitra Palve[2], Kuzhandaivelu Vengadapathy[3], Devi R Nithiya[4], Jeneth Berlin Raj[5]

1. Tutor, Department of Physiology, Mahatma Gandhi Medical college and Research Institute, Sri Balaji Vidyapeeth (Deemed to beUniversity), Pillayarkuppam, Puducherry India, India
2. Professor, Department of Physiology,Graphic Era Institute of Medical Sciences, Graphic Era (Deemed to be university), Dehradun, Uttarakhand, India
3. Associate Professor,Department of Biochemistry, Arunai Medical college and Hospital, Tiruvannamalai, India
4. Assistant professor, Department of Physiology, Indira Gandhi Medical College and Research Institute, Puducherry India, India
5. Professor and Head, Department of Physiology Arunai Medical college and Hospital, Tiruvannamalai, India

ABSTRACT

Introduction: Adenosine plays an important role in increasing glucose uptake into muscles. Adenosine deaminase (ADA) enzymes convert adenosine into inosine and 2′-deoxyinosine. Increased ADA activity leads to the reduction of adenosine, subsequently lowering glucose absorption in skeletal muscles. Uncontrolled diabetes tends to result in complications such as diabetic neuropathy.To investigate the association between serum ADA levels and lower limb nerve conduction velocity in individuals with type II diabetes mellitus.

Methods: This study included 60 participants, with 30 patients in the diabetes group and the remaining 30 in the control group. Serum ADA levels were measured, and nerve conduction recordings were performed on the lower limb's motor peroneal, tibial, and sensory sural nerves.

Results: In diabetes patients, lower limb sensory sural nerves, motor tibial, and peroneal nerves showed increased latency, reduced amplitude, and decreased nerve conduction velocity compared to the control group. ADA levels were found to be higher in diabetic patients than in the control group. A negative correlation was observed between sensory sural nerve conduction velocity and ADA levels, with females exhibiting more negative correlations than males. No association was found between motor peroneal and motor tibial nerve conduction parameters and ADA levels.

Conclusion: Sensory nerves are affected much earlier than motor nerves under hyperglycemic conditions. Elevated ADA levels indicate reduced insulin sensitivity, and the depletion of adenosine contributes to nerve damage. ADA levels could be useful in diagnosing early peripheral nerve damage.

Keywords:
Adenosine deaminase
Nerve conduction
Motor nerve
Sensory nerve
Lower limb

* Corresponding author: Manikandan Sathiyaseelan, manikandans@mgmcri.ac.in
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Introduction

Globally diabetes prevalence is estimated at 537 million among individuals aged 20-79 years. By 2030, it is projected to rise to 643 million (IDF Atlas, 2021). In India, there are currently 77 million reported cases of diabetes, and it is anticipated to reach approximately 134 million by 2045 (Pradeepa & Mohan, 2021). Diabetes mellitus affects various organs, including the heart, kidneys, liver, eyes, and nerves. Chronic diabetic conditions often modify the physiological properties of nerves and muscles. Diabetic neuropathy is reported in about 50% of diabetes patients, primarily causing peripheral nerve damage in the legs and feet, followed by the arms and hands, leading to pain, tingling sensations, numbness, and foot ulcers (Diabetic neuropathy, 2018). Nerve conduction studies aid in diagnosing peripheral neuropathy by assessing neuronal impairment through the evaluation of the size, shape, and morphology of the compound muscle action potential, determining the state of myelination (AANEM, 2015). Decreased nerve conduction velocity indicates damage to the myelin sheath, resulting in peripheral neuropathy (Krarup, 2002). In purine metabolism, the enzyme adenosine deaminase (ADA) catalyzes the conversion of adenosine to inosine and 2′-deoxyinosine. These compounds further convert into xanthine, hypoxanthine, and uric acid. Adenosine is necessary for normal insulin secretion and action, facilitating glucose uptake into skeletal muscles. Decreased adenosine levels reduce glucose uptake into cells (Niraula et al., 2018). Adenosine, normally found in all tissues at low concentrations, increases significantly during tissue damage (Haskó G,2004). Serum ADA activity increases in diabetes and inflammatory diseases, contributing to inflammation by reducing extracellular adenosine concentration. Adenosine plays a crucial role in anti-inflammatory responses and cell protection by inhibiting macrophages, cytokine production, and chemokines (Vinapamula KS,2015). Increased ADA indicates insulin resistance, which may lead to mitochondrial dysfunction and impaired neurotrophic signaling (Callaghan etal.,2012). Adenosine also influences sodium channel activity in nerve terminals, crucial for the conduction of electrical impulses through sodium and potassium channels (Ribeiro,2003). Through a literature search, an existing correlation has been found between adenosine deaminase levels and nerve conduction velocity. This study is designed to investigate the association between ADA levels and nerve conduction velocity in type II diabetes patients, addressing this identified research gap.

Objective

To compare nerve conduction velocity between individuals with type II diabetic patients and non-diabetic controls.

To find the association between serum ADA levels and lower limb nerve conduction velocity in type II diabetes mellitus.

Materials and Methods

This cross-sectional study design was conducted by the physiology department in collaboration with the neurology department at Mahatma Gandhi Medical College & Research Institute Puducherry, India. Institutional ethics committee clearance was obtained according to the Declaration of Helsinki (Faculty project /2018/06/17). In this study total of 60 participants, 30 diabetic patients were selected from the diabetic clinic OPD of general medicine. Additionally, 30 age- and gender-matched non-diabetic controls who agreed to participate were recruited. Diabetic patients were included based on complaints of pain, tingling, numbness, and weakness in limbs, aiming to include undiagnosed diabetic neuropathy patients, and the age group ranged from 35 to 55 years. Exclusion criteria encompassed individuals with foot fractures and injuries, diabetic foot ulcers, and a history of chronic diseases such as thyroid disease, liver disease, alcoholism, smoking, athletes, and pregnant women. After providing clear explanations of the procedures, written informed consent was obtained in the participants' language. Subjects were instructed to schedule appointments at their convenience. On the recording day, blood samples were collected in the morning (9:00 to 12:00) in the central laboratory, followed by nerve conduction tests recorded in the Neurology Department using the Neurostim Medicaid Systems Chandigarh instrument. Lower limb nerves, including peroneal, tibial, and sural nerves, were recorded. For motor nerve conduction, standard filter settings of 2 Hz-5 kHz, sweep speed of 2-5 ms/division, and sensitivity of -5 mV were applied. For sensory nerve conduction, the filter settings were 20 Hz-3 kHz, sweep speed of 1-2 ms/division, and sensitivity of 20 μV/division. The recording process included cleaning the sites where the active, reference, and ground electrodes were

FIGURE 1. Motor peroneal nerve recording

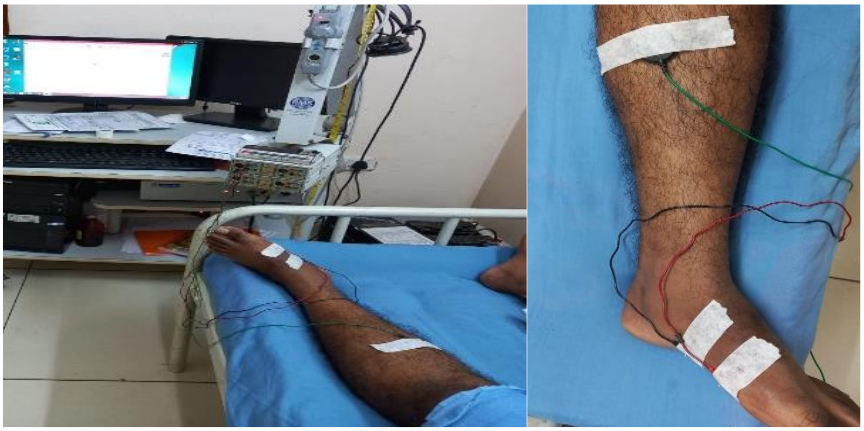


FIGURE 2. Motor tibial nerve recording

placed.

The motor peroneal nerve

recording from the extensor digitorum brevis muscle belly received an active electrode, while the reference electrode was placed on the muscle-tendon three centimeters away from the active electrode. At the ankle, between the extensor digitorum longus and extensor hallucis longus tendons, distal stimulation (S1) was applied, and proximal stimulation (S2) was applied back and proximal to the fibular head.

The motor tibial nerve

recording from the medial malleolus is stimulated distally (S1) behind it. Proximal stimulation (S2) was applied to the knee's flexor crease, the popliteal fossa, and a small distance laterally to the midline of the popliteal fossa. Between the recording electrode and the stimulating electrode for each nerve, a ground electrode was placed. A measuring tape was used to calculate the distance in millimeters between S1 and S2 for both nerves.

The sensory sural nerve

was recorded with the surface electrode placed between the tendoachillies and lateral malleolus. Specifically, the center and lower thirds of the leg, 10–16 cm proximal to the recording electrode and distal to the lower board of the gastrocnemius, were stimulated antidromically. A relaxed leg should be in a convenient lateral posture (Mishra, 1999).

ADA Measurement

2ml of venous blood was collected. The serum was separated and stored at – 20^{o}C. ADA levels were mea-

FIGURE 3. Sensory sural nerve recording

TABLE 1: Comparison of physical parameters in the diabetic group and control group

Physical parameters	Diabetic group (30)	Control group (30)	P.value
Age (yrs)	55.1 ± 12.3	53.4 ± 6.2	0.5 (NS)
Weight (kg)	70.1 ± 6.3	67 ± 6.8	0.18 (NS)
Height (cm)	159.2 ± 9.2	162.6 ± 6.8	0.14(NS)

P< 0.05 Statistically significant, P.<0.0001** - Highly Significant, (NS) - Not significant*

TABLE 2: Gender distribution between the diabetic group and the control group

	Diabetic group (30)		Control group (30)	
Gender distribution	Male	Female	Male	Female
	21	9	20	10

sured using the chemiluminescence method, and a fully automated COBAS E4 11 analyzer was employed.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess normality. A comparison between the study and control groups was conducted using an unpaired t-test. The correlation between nerve conduction parameters and ADA levels was analyzed using Pearson's correlation. Data were presented as mean±standard deviation (SD), with P values less than 0.05 considered statistically significant.

Results

In this study, age, weight, and height were expressed as mean ±SD. Unpaired t-tests were used to compare physical parameters, and no significant differences were found between the groups (Table 1).

The gender distribution in the diabetic group included 21 males & 9 females, while the control group comprised 20 males and 10 females (Table 2).

The peroneal nerve distal latency was prolonged (P<0.0075**), and reductions in amplitude and conduction velocity were observed in the diabetic group compared to the control group (P<0.0001**). The unpaired t-test revealed highly statistically significant differences in all parameters (Table 3).

Our findings on the motor tibial nerve show no significance in distal latency (P>0.382). However, the amplitude ($P<0.046$*) and conduction velocity ($P<0.0001$**) were reduced in the diabetic group. The unpaired t-test indicates a highly significant difference between the groups (Table 4).

There was a significantly increased latency and de-

TABLE 3: Comparison of the motor peroneal nerve between the diabetic group and the control group

Peroneal nerve - motor nerve			
Total (60)	Diabetic group (30)	Control group (30)	P.value
Distal latency (ms)	4.6±1.5	3.8±0.5	0.0075**
Amplitude (mv)	2.7±0.8	4.2±1.6	0.0001**
Conduction velocity (m/s)	38.7±7.1	46.2±4.2	0.0001**

P< 0.05 Statistically significant, P.<0.0001** - Highly Significant,, (NS) - Not significant.*

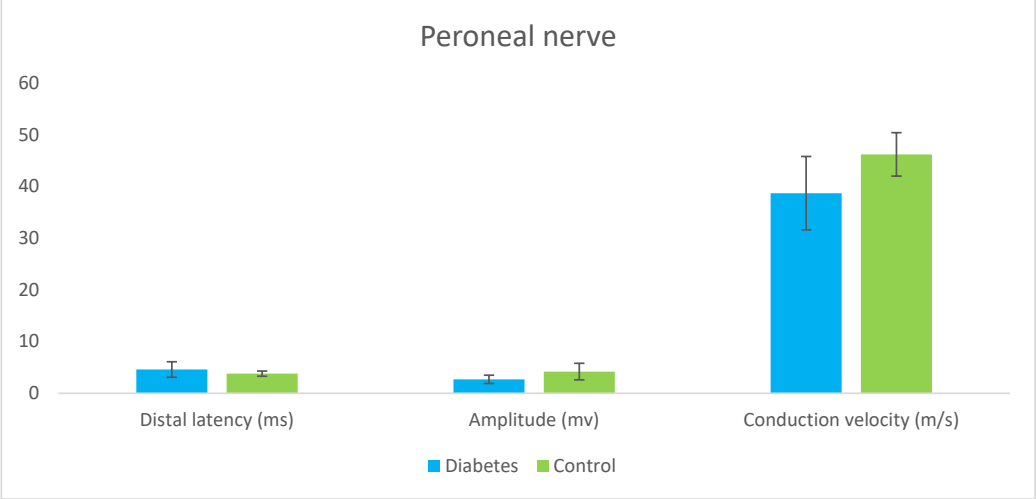


TABLE 4: Comparison of motor tibial nerves between the diabetic group and the control group

Tibial nerve - motor nerves			
Total (60)	Diabetic group (30)	Control group (30)	P.value
Distal latency (ms)	5.4±1.8	5.7± 0.5	0.382
Amplitude (mv)	2.1±0.4	3.64±0.5	0.046*
Conduction velocity (m/s)	37.3±2.7	47.5±6.9	0.0001**

P< 0.05 Statistically significant, P.<0.0001** - Highly Significant,(NS) - Not significant*

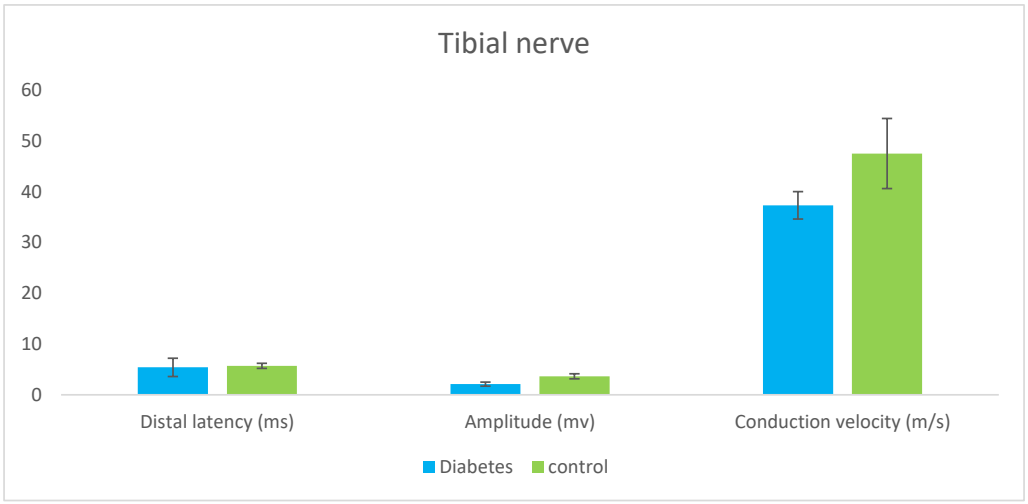


TABLE 5: Comparison of sensory sural nerves between the diabetic group and the control group

Sural - sensory nerves			
Total (60)	Diabetic group (30)	Control group (30)	P.value
Distal latency (ms)	5.6±1.8	3.1±0.4	0.0001**
Amplitude (μv)	10.1±3.5	16.5±1.6	0.0001**
Conduction velocity (m/s)	26.9±8.1	52.1±9.5	0.0001**

P< 0.05 Statistically significant, P.<0.0001** - Highly Significant,, (NS) - Not significant*

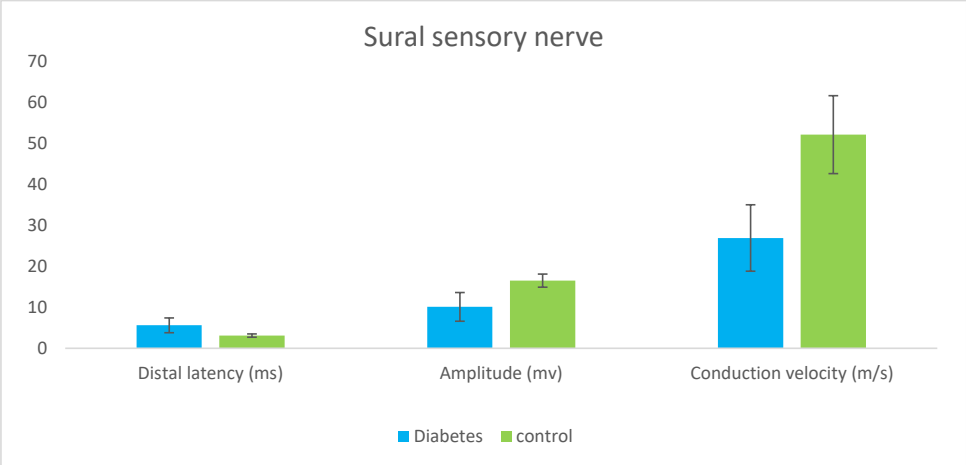
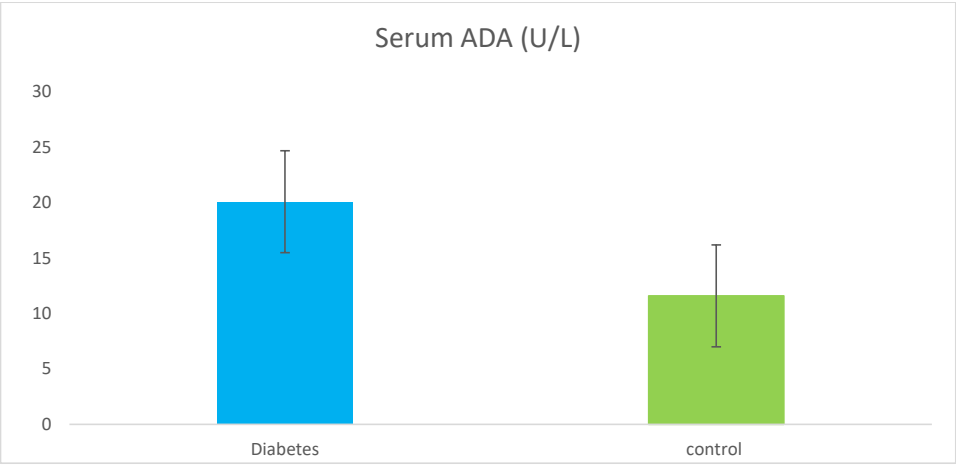


TABLE 6: Comparison of serum ADA between the diabetic group and the control group.

Total (60)	Diabetic group (30)	Control group (30)	p.value
Serum ADA (U/L)	20.1+8.7	11.6+4.6	0.0001**

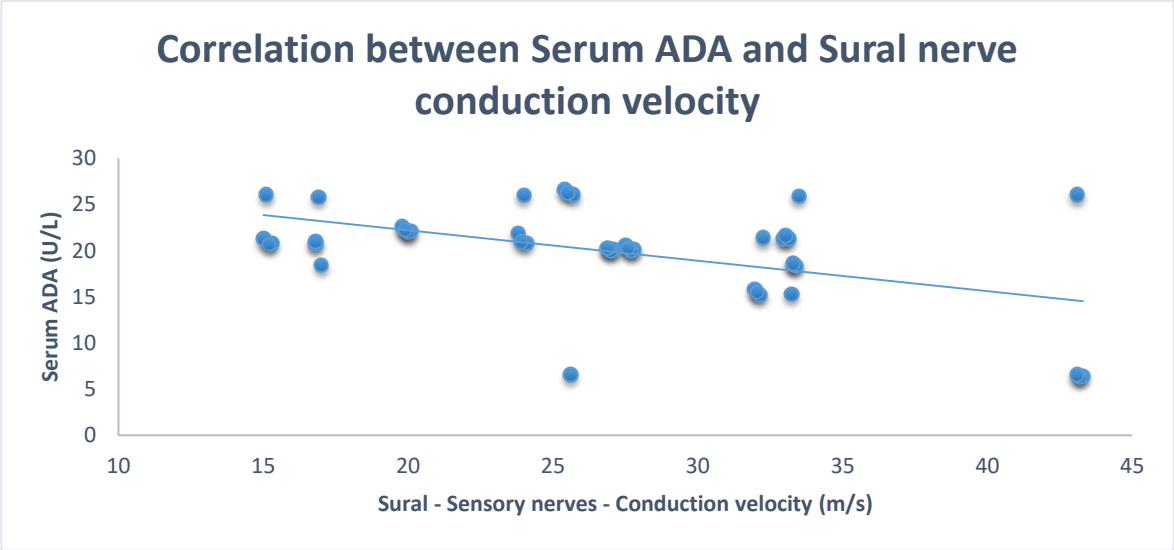


creased amplitude and velocity in sensory sural nerves in the diabetic group compared to the controls (P<0.0001), as determined by an unpaired t-test (Table 5).

The serum ADA levels were significantly increased in the diabetic group compared to the control groub (P< 0.0001**), as determined by the unpaired t-test (Table 6).

Correlation between serum ADA levels and nerve conduction velocity

Pearson's correlation coefficient indicates a negative correlation (r value -0.5) between serum ADA levels and conduction velocity of the sural nerve. The association was observed exclusively in the sural nerve conduction velocity. Increased ADA levels of activity and decreased



sural nerve conduction velocity were found. No significant association was found between ADA levels and motor peroneal or tibial nerve parameters.

In the analysis of gender variation, the correlation between serum ADA levels and sural nerve conduction velocity was found to be negatively correlated in both genders. Specifically, males exhibited an r-value of -0.4, while females showed a more pronounced negative correlation with an r-value of -0.6. The correlation in females was more negative than in males.

Discussion

The current study focused on diabetic patients with complications in the lower limb. In prolonged hyperglycemic conditions, comparatively, the lower limbs are more affected than the upper limbs. Our study found abnormal changes in all three nerves. The reduced conduction velocity in the peroneal nerve was also reported by (Meenakshi et al.,2018; Meerwaldt et al.,2005). According to (Prasad N. et al., 2013 and Kimura J. et al., 1979), motor peroneal and tibial nerves in individuals with diabetes had prolonged latency, reduced amplitude, and conduction velocity. The reduced amplitude was associated with axonal loss or malfunction, and demyelinating damage typically indicates prolonged latency and reduced nerve conduction velocity (Bansal et al.,2006). Poor glycemic control on the sensory sural nerve was found delay in latency, decreased amplitude, and conduction velocity (Nidhi Yadav et al., 2015). Demyelination changes were observed in uncontrolled diabetic patients, contributing to pain, tingling sensation, loss of sensation, and weakness in the lower limbs.

In our study, ADA levels were found to be higher in the diabetic group. This is because increased ADA activity diminishes adenosine, which, in turn, reduces the absorption of glucose into cells. In the previous studies documented by (Khemka et al., 2013 and Niraula et al., 2018), serum ADA levels were shown to be greater in diabetes patients, and serum ADA levels and fasting glucose levels were found to be positively linked.

Adenosine is involved in various physiological activities and molecular signaling. Glucose absorption in skeletal muscle mediated adenosine by stimulating GLUT–4 transports. Increased ADA activity leads to the depletion of adenosine and increased insulin resistance in the body (Kundu et al., 2019).The serum ADA level is used as a biomarker to assess glycemic status in diabetes patients (Sapkota LB et al., 2017). The correlation with ADA levels in sensory conduction velocity was found to be negatively correlated. Sensory nerve damage occurs earlier than motor nerves. Our study findings showed that the sensory sural nerve was more affected in females. Correlation with ADA levels was comparatively higher in females; this could be because females have soft and thin skin, making them more prone to foot ulcers, skin infections, and peripheral nerve damage.

There are various hypothesized processes by which hyperglycemia damages nerves. Hyperglycemia causes damage to endothelial cells in the blood vessels, reducing capillary blood flow, and endoneurial hypoxia leads to nerve damage (Prasad N et al., 2013). The cause of neuropathy is that hyperglycemia reduces the produc-

tion of neurotrophin-like nerve growth factors (NGF), necessary for nerve growth, repair, and regeneration. Hyperglycemia decreases myoinositol synthesis in nerve cells, limiting tissue sodium-potassium ATPase activity required for normal neuron transmission (Clayton et al., 2009). The diminished activity of the sodium-potassium ATPase pump increases Na+ retention, edema, axoglial disjunction, myelin swelling, and nerve injury, all contributing to peripheral neuropathy.

Limitation of the study

In our study, nerve conductions were performed on the lower limbs. Due to time constraints, we missed out on conducting tests in the upper limbs.

Future scope of the study

The correlation of ADA levels with electrolyte concentrations (Na^+ and K^+ ions) and nerve conduction investigations would be highly beneficial for future research in assessing peripheral nerve diseases.

Conclusion

In summary, the nerve conduction parameters in diabetic patients revealed increased latency, decreased amplitude, and reduced velocity in the motor peroneal, tibial, and sural nerves. Elevated ADA levels were observed in diabetic patients, with a negative association found between sensory sural conduction velocity and ADA levels. Females exhibited a more pronounced negative correlation between ADA levels and sensory sural nerves. Sensory nerves were affected much earlier than motor nerves under hyperglycemic conditions. ADA levels could prove useful in diagnosing early peripheral nerve damage.

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Conflict of Interest

Authors declare no conflict of interest.

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