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Serum uric acid as a biological marker for assessment of progression of glycemic status and polyneuropathy in experimentally induced type 2 diabetic rats

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Abstract

Background: Screening for laboratory indicators and modifiable risk factors for diabetic polyneuropathy (DPN) is crucial for early detection and development of novel treatments. This study investigated the possible correlation of serum uric acid to the progression of glycemic status and polyneuropathy in experimentally induced type 2 diabetic rats. Methods: Sixty rats were divided into non-diabetic, diabetic 4-week non-treated, diabetic 8-week non-treated, diabetic 4-week metformin-treated, diabetic 8-week metformin-treated. Rats were evaluated for glycemic state, serum uric acid, lipid profile, inflammatory and oxidative stress parameters. Hot plate test, sciatic nerve conduction speed, and sciatic nerve histopathological changes were assessed. Results: Four weeks after high-fat diet (HFD) and low dose of streptozotocin (STZ) injection, rats showed significant elevation in blood glucose, HbA1c and HOMA-IR, uric acid, malondialdehyde, TNF-α, IL-6, thermal hyperalgesia and significant reduction of nerve conduction speed and total antioxidant capacity associated with significant changes in lipid profile and histopathological structure of sciatic nerve. These changes were more prominent after 8 weeks of STZ injection than in 4-week diabetic-non-treated group. Metformin treatment significantly improved all parameters, meanwhile the improvement was more prominent in 8-week than 4-week group. Conclusion: Serum uric acid can be taken as a useful biological marker for assessment of progression of diabetic status and polyneuropathy in type 2 diabetes.

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INTRODUCTION

Diabetes mellitus is a category of metabolic diseases characterized by persistent high blood sugar level. It may be due to an absolute or near absolute deficiency of insulin, presence of insulin resistance, or both. Chronic hyperglycemia can cause tissue damage that eventually results in disabling and life-threatening diabetic complications most prominent of which are vascular complications that affect small blood vessels (retinopathy, nephropathy, and neuropathy) or large blood vessels or both, leading to increased risk of cardiovascular diseases [1].

Diabetic polyneuropathy (DPN) is the most common and early complication associated with diabetes. DPN is a type of nerve damage that can alter autonomic, motor, or sensory functions. It is characterized by progressive, distal to proximal degeneration of peripheral nerves. Positive neurosensory symptoms, primarily in the toes, feet, or legs, such as sleep numbness, tingling, burning, or pain, are directly caused by DPN. The prevalence of DPN among type 2 diabetic patients showed a rather wide range (2–50%) as shown in the previous studies. Age, country, glycemic control, diabetes duration, and examination procedures all play a role. The high prevalence of DPN is a significant factor in diabetes-related death and disability [2, 3]. The risk factors involved in the pathogenesis of diabetic neuropathy have not been understood completely. Studies have shown that neuronal dysfunction, inflammation, and oxidative stress play significant role in the progression of DPN [4].

In recent years, studies have found that there is a significant relationship between serum uric acid levels and DPN. However, there is still no agreement among them, and further studies are recommended in this area [5]. Serum uric acid is the product of purine catalysis by xanthine oxidase. It is predominantly recognized as a predictor of gout. Previous studies have proposed that uric acid is a marker of oxidative stress, and hyperuricemia-induced oxidative stress may be a cause of insulin resistance, diabetes, and cardiovascular disease. High serum uric acid induces platelet adhesiveness and aggregation [6]. Hyperuricemia could induce vascular smooth muscle cells migration and inhibition of nitric oxide release from endothelial cells, leading to vascular dysfunction with irreversible damage, resulting in tissue ischemia and impaired peripheral nerve function [7]. The current methods of investigation and diagnosis, such as flare reaction and biopsy from nerve or skin, are difficult to use and unpopular among patients. So, screening for laboratory indicators for DPN is important for detecting the earliest stages of neuropathy and preventing diabetic neuropathy [3]. Hence, the aim of the present investigation is to study the correlation between serum uric acid and the severity of diabetes mellitus and its complications as peripheral polyneuropathy.

Methods

The present investigation was carried out in Medical Physiology Department, Faculty of Medicine, Menoufia and Mansoura Universities, Egypt. Animal care and use were approved by the Ethics Committee of Menoufia University, (IRB number is 4/2023PHYS13).

Rats:

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Sixty adult male albino Wistar rats of

the local strain, weighing 150-200 grams each, were used in this investigation. Rats were housed (10 per cage) in fully ventilated cages (80x40x30 cm), at normal room temperature, with natural day/night cycle and free access to water. They were fed with rat normal pellet diet (NPD) and tap water for 1 week for acclimatization before experiments. Then rats were randomly divided into three experimental groups:

I. Non- Diabetic group (ND) (20 rats):

Rats of this group were fed with normal pellet diet (NPD): 12% of calories as fat with free access to water. Rats were injected once with 0.1 ml citrate buffer (vehicle of STZ). Then they were given oral daily dose of 1 ml of saline (vehicle of metformin). This group was further subdivided into 2 subgroups:

- I-a. Non-diabetic 4-week group (ND-4W) (10 rats): rats left in their cages for 4 weeks.
- I-b. Non-diabetic 8-week group (ND-8W) (10 rats): rats left in their cages for 8 weeks.

II. Diabetic Non-treated (DNT) group (20 rats):

Diabetes was induced in these rats by offering them free access to high fat diet (HFD): 58% of calories as fat for 2 weeks [8]. Then they were injected (i.p) with a single low dose of Streptozotocin (STZ) (Sigma-Aldrich, USA) (35 mg/Kg BW) dissolved in citrate buffer (pH 4.4) after 12 hours of fasting. This was followed by administration of 5% glucose solution to prevent hypoglycemia [8]. 72 hours after STZ injection, fasting blood glucose was measured by glucometer (ACCU-CHEK) through tail pricking. Rats with more than 150 mg/dl were considered diabetic and were selected. Rats of this group had free access to HFD during the whole experimental period with daily oral administration of 1 ml of saline (vehicle of metformin). This group was further subdivided into 2 subgroups:

- II-a. Diabetic 4-week non-treated (DNT-4W) (10 rats): after being diabetic rats were left in their cages for 4 weeks with free access to water and HFD.
- II-b. Diabetic 8-week non-treated (DNT-8W) (10 rats): Diabetic rats were left in their cages for 8 weeks with free access to water and HFD.

III. Diabetic metformin-treated group (D+Met) (20 rats):

Type II DM was induced as with group II followed by daily oral administration by gavage of 200 mg/Kg metformin (Sigma–Aldrich), dissolved in saline. This group was further subdivided into 2 subgroups:

- III-a. Diabetic 4-week metformin-treated (D+Met-4W): Diabetic rats were left in their cages for 4 weeks with free access to water and HFD and daily oral administration of metformin (200 mg/Kg).
- III-b. Diabetic 8-week metformin-treated (D+Met-8W): Diabetic rats were left in their cages for 8 weeks with free access to water and HFD and daily oral administration of metformin (200 mg/Kg).

Experimental design:

At the end of experimental period, all rats were exposed to hot plate latency test to assess the painful diabetic neuropathy through assessment of paw licking and jumping. Then, after overnight fasting, retro-orbital blood samples were collected to measure:

- Glycemic state indices: glycated hemoglobin (HbA1C), fasting serum glucose and serum

insulin. Then HOMA-IR was calculated for assessment of insulin resistance.

- Serum uric acid.
- Lipid profile (total cholesterol, triglycerides, and high-density lipoprotein (HDL-c) cholesterol levels. Then low-density lipoprotein (F-LDL) was calculated.
- Serum malondialdehyde (MDA) and total antioxidant capacity (TAC).
- Tumor necrosis factor alpha (TNF-α)
- Pro-inflammatory marker: interleukin-6 (IL-6).

Rats were anesthetized and killed by cervical dislocation then, the sciatic nerves were dissected, removed, and placed in moist nerve chamber then stimulated by Power Lab 4/30 for recording compound action potential, and nerve conduction speed (NCS) (m/sec) was calculated. After that, nerves were removed for histopathological examination. Finally, the dead rats were collected in biological containers and burnt in private plants that is operated in compliance with local laws and ordinances.

Hot Plate Test

This test used latency measurements to assess acute cutaneous pain sensitivity. The rat was placed in the middle of the heated surface of plate inside a transparent glass cylinder (Hotplate 602001; TSE Systems) at a temperature of 52°C. Time to elicit jumping or paw licking was measured. To avoid tissue damage, the cut-off time was 30 seconds. The time of latency was measured from the time when the rat is placed on the hot plate surface, and the time when it licks its paw or jumps off to avoid thermal pain [9].

Nerve Conduction Speed (NCS)

Rats were anesthetized by pentobarbital sodium (100 mg/kg, i.p) and killed by cervical dislocation. Then, Sciatic nerve was exposed from the spinal emergence to the knee and removed by cutting at the proximal and distal ends. Nerve stimulation and recording were carried out using the Power Lab 4/30. The isolated Sciatic nerve was placed in a nerve chamber filled with Ringer's solution and connected to Power Lab recording unit and further analysis was done by lab chart software. NCS was calculated by dividing the distance (in meters) between the stimulating and recording electrodes by the time between a stimulus artifact and the start of the response (seconds).

Collection of blood samples:

After overnight fasting, retro-orbital blood sample (4 ml from each rat) was collected, in 2 clean graduated tubes; the first contains EDTA to measure HbA1c, the second was plain and left for clotting, then centrifuged and the supernatant serum was collected and preserved at -80 °C to measure the previously mentioned serum parameters.

Serum glucose, Insulin, and HbA1c

Serum glucose concentrations were assayed following glucose oxidase method [10]. Rat-ELISA kits were used to measure serum levels of insulin (DRG Instruments GmbH, Germany). HbA1c were estimated according to the Nayak and Pattabiraman method [11].

Homeostatic Model Assessment

HOMA-IR index was calculated by the formula: [fasting blood insulin (μU/ml) x fasting blood glucose (mg/dl) / 405] [12].

Lipid Profile

Serum cholesterol, triglycerides, and HDL were measured by colorimetric kits from Sigma-Aldrich, USA. LDL-cholesterol was calculated by Friedewald formula [F-LDL = Total Cholesterol - (HDL + TG/5)] [13].

Serum uric acid and inflammatory and oxidative stress parameters

Serum uric acid was measured using standard diagnostic kit utilizing enzymatic colorimetric approaches (BioLab., France). Serum level of IL-6 and TNF-α (Quantikine® ELISA, R&D Systems Inc., MN, USA) assayed ELISA kits according to manufacturer's instructions. MDA and TAC levels were estimated following Draper and Hadley [14] and Erel's methods respectively [15].

Histopathological Examination

About 10 mm piece of sciatic nerve were taken. Serial 5-µm thick coronal sections from sciatic nerve embedded in paraffin were collected followed by H&E staining to assess histological changes. The tissues were observed under an Olympus optical microscope (BX51, Olympus, Japan) and histopathological analysis was performed by a histopathologist blinded to the groups.

Statistical analysis:

The data were expressed as mean ± standard deviation (X±SD). Values were subjected to Pearson correlation analysis and analyzed by SPSS Statistical Package Software v.20 using one-way analysis of variance (ANOVA) followed by applying Mann-Whitney U test for testing significance. All P-values <0.05 were considered as significant.

Results

Glycemic state in all experimental groups:

Table 1 revealed a significant increase in serum glucose, HbA1c, and HOMA-IR levels accompanied by insignificant change in serum level of insulin in DNT-4W and DNT-8W groups as compared with nondiabetic group. These parameters except serum insulin were significantly changed in DNT-8W as compared with DNT-4W group. Treatment of diabetic rats with metformin alleviated all the measured glycemic state indices relative to diabetic non-treated groups. But these values were still significantly different compared to the non-diabetic animals.

Lipid profile changes in all experimental groups.

Table 2 revealed a significant increase in total serum cholesterol, serum triglycerides, F-LDL, and a significant decrease in serum HDL-c in DNT-4W and DNT-8W as compared to diabetic non-treated groups. The same parameters were significantly changed in DNT-8W as compared with DNT-4W group. Treatment of the diabetic rats with metformin alleviated all the measured lipid profile parameters relative to corresponding diabetic non-treated groups, but these values were still significantly different compared to the non-diabetic animals.

Time of latency as detected by the hot plate test in all experimental groups.

Table 3 revealed a significant decrease in the time of latency as detected by the hot plate test in DNT-4W and DNT-8W groups compared to non-diabetic groups. This decrease was more significantly prominent in DNT-8W group when compared to DNT-4W group. Time of latency was significantly restored in D+Met-4W and D+Met-8W compared to the corresponding diabetic non-treated groups however, these values were still significantly lower than the corresponding non-diabetic groups.

Sciatic nerve conduction speed.

As shown in Table 4 and Figure 1, NCS was significantly lower in DNT-4W and DNT-8W groups compared to corresponding non-diabetic

groups. DNT-8W group showed significantly lower level when compared to DNT-4W group. NCS was significantly reversed in D+Met-4W and D+Met-8W groups when compared to the corresponding diabetic non-treated groups however, these values were still significantly lower than the corresponding non-diabetic groups.

Table (1): Fasting blood glucose (mg/dl), serum insulin (µU/ml), HbA1c (% of normal Hb) and HOMA-IR index among all studied groups.

	ND-4W	ND-8W	DNT-4W	DNT-8W	D+Met-4W	D+Met-8W
Blood glucose	92.1±5.5	98.8±10.1	287.6±14.8	341.2±17	160.2±5.5	149.8±7.8
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
P2					*P<0.001*	*P<0.001*
P3				*P<0.001*		
Serum insulin	14.6±2.1	16.2±2.3	16.7±2.3	17.7±3.5	17.5±3.7	16.4±3.5
P1			*P=0.061*	*P=0.223*	*P=0.052*	*P=1.000*
P2					*P=0.468*	*P=0.340*
P3				*P=0.359*		
HbA1c	6.2±0.3	6.3±0.6	10.1±0.3	12±0.9	7.5±0.7	7.43±1
P1			*P<0.001*	*P<0.001*	*P=0.001*	*P=0.018*
P2					*P<0.001*	*P<0.001*
P3				*P=0.001*		
HOMA-IR	3.3±0.6	4±0.9	11.9±1.8	14.9±3	6.9±1.5	6.1±1.3
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P=0.001*
P2					*P<0.001*	*P<0.001*
P3				*P=0.030*		

Data are expressed as mean±SD (n=10). P value < 0.05 is considered significant. P_1: compared to corresponding non-diabetic group. P_2: compared to corresponding diabetic non-treated group. P_3: compared to corresponding DNT-4W group. ND-4W: non-diabetic-4W; ND-8W: non-diabetic-8W; DNT-4W: Diabetic 4-week non-treated; D+Met-4W: Diabetic 4-week metformin-treated group; DNT-8W: Diabetic 8-week non-treated; D+Met-8W: Diabetic 8-week metformin-treated. HbA1c: Hemoglobin A1c (Glycated hemoglobin); HOMA-IR: homeostasis model assessment-estimated insulin resistance.

Table (2): Serum lipid profile components (mg/dl) among all experimental groups.

	ND-4W	ND-8W	DNT-4W	DNT-8W	D+Met-4W	D+Met-8W
Cholesterol	89.4±5.4	92.2±8.5	124.3±9.2	142.9±12.3	111.4±11.2	108±11.5
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P=0.004*
P2					*P=0.021*	*P<0.001*
P3				*P=0.004*		
Triglycerides	61.2±2.6	63.4±5.2	80.7±6.6	91.5±4.7	74.4±10.8	73.1±6.2
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P=0.004*
P2					*P=0.028*	*P<0.001*
P3				*P=0.002*		
F-LDL	33.4±7.9	32.6±4.5	77.3±12.2	99.5±15.5	59.2±13.7	56.6±10.8
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P=0.001*
P2					*P=0.01*	*P<0.001*
P3				*P=0.005*		
HDL-c	43.8±5.6	46.9±5.7	30.9±4.4	25.1±5.6	37.3±5.5	36.8±6.4
P1			*P<0.001*	*P<0.001*	*P=0.028*	*P=0.006*
P2					P_2=0.011	P_2=0.002
P3				*P=0.033*		

Data are expressed as mean±SD (n=10). P value < 0.05 is considered significant. P_1: compared to corresponding non-diabetic group. P_2: compared to corresponding diabetic non-treated group. P_3: compared to corresponding DNT-4W group. ND-4W: non-diabetic-4W; ND-8W: non-diabetic-8W; DNT-4W: Diabetic 4-week non-treated; D+Met-4W: Diabetic 4-week metformin-treated group; DNT-8W: Diabetic 8-week non-treated; D+Met-8W: Diabetic 8-week metformin-treated. F-LDL: Friedewald-calculated low-density lipoprotein; HDL-c: high-density lipoprotein-cholesterol.

Table (3): Serum uric acid (mg/dl) and Time of latency using hot plate test (seconds) among all studied groups.

	ND-4W	ND-8W	DNT-4W	DNT-8W	D+Met-4W	D+Met-8W
Uric acid	1.9±0.3	1.98±0.3	4.01±0.4	4.93±0.4	2.92±0.2	2.6±0.4
P1			*P<0.001*	*P<0.001*	*P<0.001*	*P=0.003*
P2					*P<0.001*	*P<0.001*
P3				*P<0.001*		
Time of latency	17.4±2.3	17.3±2.4	12.2±2.2	9.2±1.9	14.3±1.6	15.3±1.3
P1			*P=0.001*	*P<0.001*	*P=0.005*	*P=0.03*
P2					*P=0.031*	*P<0.001*
P3				*P=0.008*		

Data are expressed as mean±SD (n=10). P value < 0.05 is considered significant. P_1: compared to corresponding non-diabetic group. P_2: compared to corresponding diabetic non-treated group. P_3: compared to corresponding DNT-4W group. ND-4W: non-diabetic-4W; ND-8W: non-diabetic-8W; DNT-4W: Diabetic 4-week non-treated; D+Met-4W: Diabetic 4-week metformin-treated group; DNT-8W: Diabetic 8-week non-treated; D+Met-8W: Diabetic 8-week metformin-treated.

Table (4): Sciatic NCS (m/sec) among all studied groups.

	ND-4W	ND-8W	DNT-4W	DNT-8W	D+Met-4W	D+Met-8W
Distance (m)	0.04	0.04	0.04	0.04	0.04	0.04
Latent period (sec)	0.033±0.004	0.034±0.004	0.048±0.008	0.07±0.014	0.039±0.003	0.038±0.004
NCS (m/sec)	1.22±0.16	1.19±0.14	0.86±0.14	0.59±0.1	1.02±0.08	1.06±0.11
P1			*P<0.001*	*P<0.001*	*P=0.004*	*P=0.033*
P2					*P=0.011*	*P<0.001*
P3				*P=0.001*		

Data are expressed as mean±SD (n=10). P value < 0.05 is considered significant. P_1: compared to corresponding non-diabetic group. P_2: compared to corresponding diabetic non-treated group. P_3: compared to corresponding DNT-4W group. ND-4W: non-diabetic-4W; ND-8W: non-diabetic-8W; DNT-4W: Diabetic 4-week non-treated; D+Met-4W: Diabetic 4-week metformin-treated group; DNT-8W: Diabetic 8-week non-treated; D+Met-8W: Diabetic 8-week metformin-treated. NCS: Nerve conduction speed.

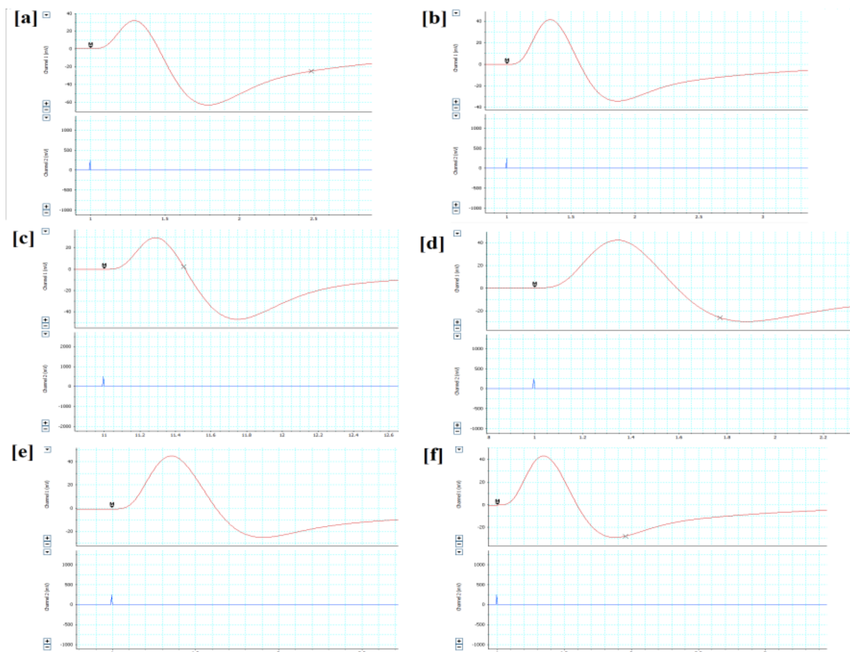

Figure 1: Recorded sciatic nerve biphasic action potential among the different studied groups via Power Lab 4/30 [a] 4-week non-diabetic rat, [**b**] 8-week non-diabetic rat [**c**] 4-week diabetic non-treated rat, [**d**] 8-week diabetic non-treated rat [**e**] 4-week diabetic metformin-treated rat, and [**f**] 8-week diabetic metformin-treated rat.

Histopathological changes in sciatic nerve in all experimental groups.

Figure 2 shows the architecture of sciatic nerve tissue. HFD-STZ produced histopathological changes including edema and degeneration in sciatic nerve tissue after 4 weeks of STZ injection with severe sciatic nerve degeneration after 8 weeks of STZ injection. Metformin-treated rats showed reduction of HFD-STZ- induced changes but still there are degenerated fibers.

Serum uric acid.

Level of serum uric acid was significantly higher in DNT-4W and DNT-8W groups than corresponding non-diabetic groups. Uric acid was significantly higher in DNT-8W group when compared to DNT-4W group. The same parameter was significantly lower in D+Met-4W and D+Met-8W groups when compared to the corresponding diabetic non-treated groups but its value was still significantly higher when both groups were compared to the corresponding non-diabetic groups (Table 3). Figure 3 and Table 5 show a strong positive correlation between uric acid and glycemic state represented by HbA1c. Also, there was a strong negative correlation between serum uric acid and time of latency detected by hot plate test, and serum uric acid and sciatic NCS in all experimental groups.

Serum oxidative and inflammatory markers.

Table 6 illustrated that serum TNF-α and IL6 were significantly higher in DNT-4W and DNT-8W groups than the corresponding non-diabetic groups. DNT-8W group showed significantly higher levels of both parameters when compared to DNT-4W group. Significantly lower levels of TNF-α and IL6 were observed when D+Met-4W and D+Met-8W groups were compared to diabetic non-treated groups however their levels in these groups were significantly higher than values in non-diabetic groups.

The DNT-4W and DNT-8W groups showed significantly higher level of MDA and significantly lower level of TAC when compared to non-diabetic groups. Both parameters were also significantly different in DNT-8W when compared to DNT-4W group. Metformin treatment significantly restored serum MDA and serum TAC in D+Met-4W and D+Met-8W groups when compared to the non-treated groups however, their values were still significantly higher than values in non-diabetic groups (Table 6).

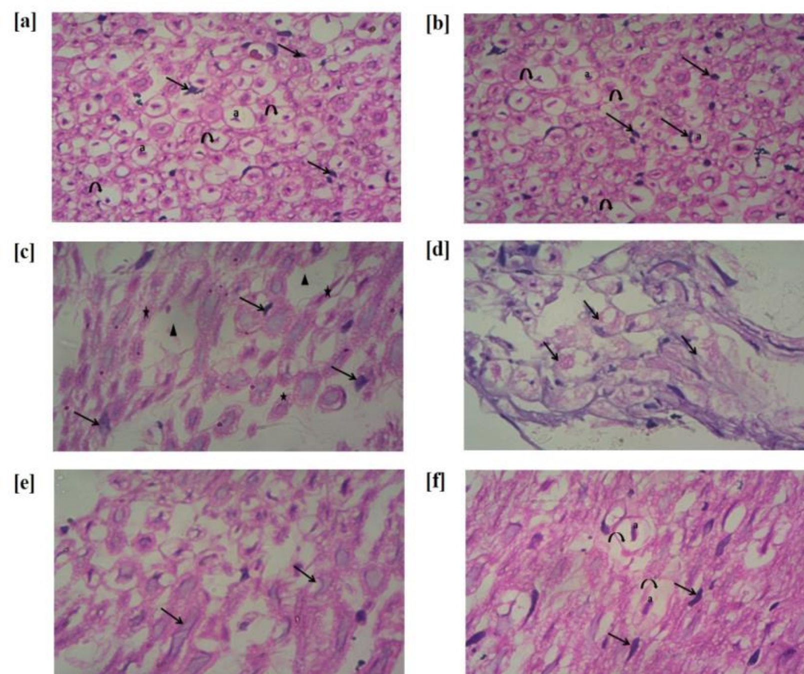

Figure 2: Photomicrograph of sections in the nerve bundle of the **[a]** 4-week non-diabetic rat (H&E, x400), showing nerve bundle with closely packed fibers formed of axons (a) unstained myelin (crossed arrow) and nuclei of Schwann cells (arrow); **[b]** 8-week non-diabetic rat (H&E, x400), showing nerve bundle with closely packed fibers formed of axons (a) unstained myelin (crossed arrow) and nuclei of Schwann cells (arrow); **[c]** diabetic 4-week non-treated rat (H&E, x400), showing multiple degenerated nerve fibers (star) with empty spaces (arrow head) faintly stained nuclei of Schwann cells (arrow); **[d]** diabetic 8-week non-treated rat (H&E, x400), showing severe degenerated nerve fibers (arrow); **[e]** diabetic 4-week metformin-treated rat (H&E, x400), showing improvement but still there are degenerated fibers(arrow); and **[f]** diabetic 8-week metformin-treated rat (H&E, x400), showing improvement of the picture as nerve fiber formed of axon (a) surrounded by myelin sheath (crossed arrow) and Schwann cells (arrow) but still there are degenerated fibers.

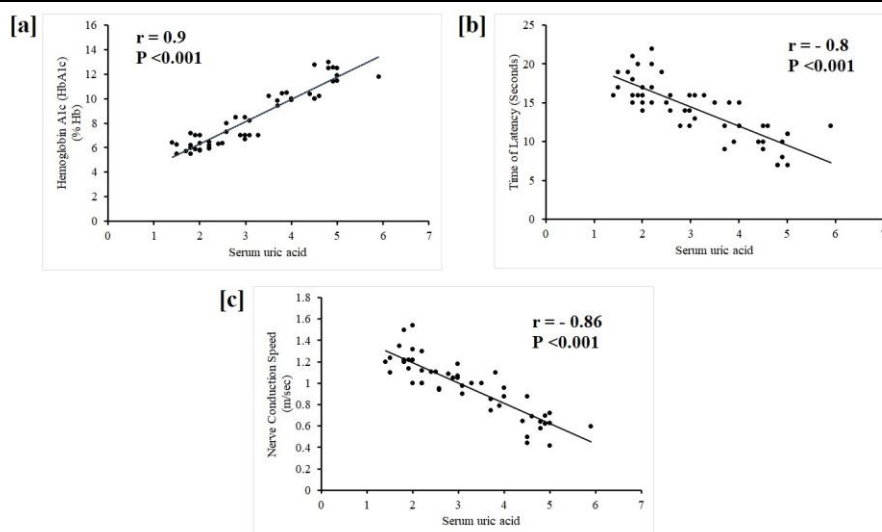

Figure 3: Correlations (r) between **[a]** serum uric acid and glycemic state presented by HbA1c, **[b]** serum uric acid and time of latency using the hot plate test, and **[c]** serum uric acid and NCS in all experimental groups.

Table (5): Coefficient (r) and significance of the correlation (P) between serum uric acid and glycemic state presented by HbA1c, serum uric acid and time of latency using hot plate test, and serum uric acid and NCS in all experimental groups.

	HbA1c	Time of latency	NCS
Serum uric acid			
r	0.9	- 0.8	- 0.859
P value	P <0.001	P <0.001	P <0.001
n	60	60	60

Table (6): Serum inflammatory and oxidative stress markers in all experimental groups.

		ND-4W	ND-8W	DNT-4W	DNT-8W	D+Met-4W	D+Met-8W
TNF-α		16.8±1	17.14±1.1	31.29±1.8	46.97±1.4	22.41±1.8	19.21±1.6
	P1			*P=0.006*	*P<0.001*	*P<0.001*	*P=0.006*
	P2					*P<0.001*	*P<0.001*
	P3				*P<0.001*		
IL-6		208.4±25.6	210±25.8	401.24±47.9	592±46.6	290±66.6	277.7±38.2
	P1			*P<0.001*	*P<0.001*	*P=0.01*	*P<0.001*
	P2					*P=0.001*	*P<0.001*
	P3				*P<0.001*		
MDA		2.04±0.4	2.17±0.4	4.62±0.5	5.43±0.6	3.55±0.4	3.17±0.7
	P1			*P=0.002*	*P<0.001*	*P<0.001*	*P=0.002*
	P2					*P<0.001*	*P<0.001*
	P3				*P=0.014*		
TAC		1.8±0.13	1.71±0.2	0.7±0.09	0.61±0.1	1.31±0.12	1.44±0.09
	P1			*P=0.006*	*P<0.001*	*P<0.001*	*P=0.006*
	P2					P_2<0.001	P_2<0.001
	P3				*P=0.03*		

Data are expressed as mean±SD (n=10). P value < 0.05 is considered significant. P_1: compared to corresponding non-diabetic group. P_2: compared to corresponding diabetic non-treated group. P_3: compared to corresponding DNT-4W group. ND-4W: non-diabetic-4W; ND-8W: non-diabetic-8W; DNT-4W: Diabetic 4-week non-treated; D+Met-4W: Diabetic 4-week metformin-treated group; DNT-8W: Diabetic 8-week non-treated; D+Met-8W: Diabetic 8-week metformin-treated. TNF-α: tumor necrosis factor-alpha; IL-6: interleukin-6; MDA: malondialdehyde; TAC: total antioxidant capacity.

Discussion

The widespread prevalence of DPN is one of the main factors contributing to death and disability. Screening for laboratory indicators for DPN and identification of connections between DPN and modifiable risk factors is crucial for detection for diabetic neuropathy in its earliest stages, prevention and development of novel treatments for DPN. In this context, we studied the

correlation of serum uric acid with the progression of DPN in type 2 diabetic rats.

In the current study, HFD and low dosage STZ successfully induced type 2 diabetes and diabetic polyneuropathy [8, 16]. Due to lipotoxicity, HFD induces insulin resistance in the peripheral tissues, whereas low dose of STZ causes mild decrease in the secretion of insulin. Consequently, HFD and low dose STZ model successfully matches metabolic features of human type 2 diabetes [8].

Induction of Type 2 diabetes was confirmed 4 weeks following the injection of STZ by obtained significant elevation in levels of glucose and HbA1c which was accompanied by increase in HOMA-IR indicating insulin resistance in the experimentally induced diabetic rats. And these parameters were significantly changed to a higher level at 8 weeks after STZ injection however; there was insignificant change in serum insulin level. Metformin treatment reduced these parameters, but it was still higher than control rats. These findings are consistent with other studies [8, 17]. Insulin resistance induced by HFD is characterized by mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and compensatory hyperinsulinemia, which is identical to prediabetic state in Humans. A low dose of STZ A declined the secretory function of pancreatic beta cells that results in frank hyperglycemia with normal blood insulin levels (relative insulin deficiency) [18]. HbA1c is created when there is excess glucose in blood. Glucose reacts with hemoglobin to form HbA1c. The amount of HbA1c reflects the blood glucose level for a 4–6 weeks period. Glycation itself may trigger the production of oxygen-derived free radicals, which may be the main factor contributing to neurological complications [19-21]. Metformin treatment reduced these parameters, but it was still higher than the control rats.

One of the major risk factors for DPN is dyslipidemia [22, 23]. Diabetic non-treated rats showed abnormalities in lipid metabolism indicated by elevated serum total cholesterol, LDL and triglycerides levels and declined HDL-c after 4 weeks and after 8 weeks of STZ injection. These findings support other studies conclusions [8, 17, 24]. Insulin resistance can alter systemic lipid metabolism which leads to the establishment of dyslipidemia. In addition, nutritional overload can quickly induce insulin resistance in skeletal muscle as well as in the liver [25]. Dyslipidemia may play a role in DPN development and excess lipids promote damage of peripheral nerves [26]. Early dyslipidemia and increased triglycerides are two important independent risk factors for DPN development, which may help to explain why type 2 diabetes patients experience DPN earlier than those with type 1 diabetes. This may be explained by dysregulated lipid metabolism within the neurons [27]. Metformin treatment showed a hypolipidemic effect in the present study. Mechanism of action of metformin on amelioration of dyslipidemia is well established [28].

Persistent hyperglycemia and dyslipidemia result in oxidative stress, lipotoxicity, and endothelial abnormalities, which finally causes nerve degeneration through reduction of nerve blood flow and oxygen supply [29, 30]. We observed a significant increase in MDA and significant decrease in TAC in diabetic non-treated rats that were accompanied by significant increase in serum TNF-α and IL-6 at 4 weeks of STZ injection. These parameters changed more

significantly at 8 weeks of STZ injection. As oxidative stress and inflammation are linked, levels of TNF- α and IL6 were found to be higher in diabetic rats [31]. Acute and chronic hyperglycemia can increase synthesis TNF-α which adversely impacts glucose uptake and metabolism via impairment of insulin signal transduction, induces generation of ROS and stimulates of hepatic lipogenesis that leads to development of insulin resistance [32,33, 34]. Metformin treatment significantly improved serum oxidative stress and inflammatory markers due to its antioxidant and anti-inflammatory effects [35, 36].

In the present investigation development of DPN in diabetic rats was evident from significant reduction in NCS, thermal hyperalgesia, manifested as reduced latencies in hot plate test, and histopathological findings after 4 weeks of developing diabetes with more prominent significant changes after 8 weeks of STZ injection. These changes were significantly ameliorated in diabetic metformin-treated groups.

The present results are consistent with the earlier findings of other authors regarding NCS and thermal hyperalgesia in HFD-STZ-induced diabetic rats [37-39]. Pain is one of the most elusive symptoms of polyneuropathy that is characterized by mechanical, thermal and chemical hyperalgesia [40, 41]. Large, myelinated nerve fibers function is assessed by NCS, but it is not sensitive enough to detect small nerve fibers or injury to unmyelinated nerve fibers. Early DPN changes mainly occur in small, unmyelinated, and thinly myelinated fibers, which are more sensitive to thermal sensation and present as hypoalgesia or hyperalgesia [42]. Hot plate test is a simple, quick and inexpensive behavioral screen used to assess acute, thermal pain [43]. Morphological study of sciatic nerve indicated that diabetes induced histological damage of the sciatic nerve fibers, endoneurial edema and axonal degeneration with occasional secondary segmental demyelination. These results were in accordance with the earlier studies [37, 38, 44].

Serum uric acid is the final oxidation product of purine catabolism. We observed significant elevation of serum uric acid in diabetic non-treated group after 4 weeks of STZ injection that was significantly higher after 8 weeks. This elevation was corrected in diabetic metformin-treated groups. Hyperuricemia has long been associated with high-fat diets [45]. There was a positive association of serum uric acid levels with hyperglycemia and dyslipidemia. However, the underlying pathophysiological mechanisms linking hyperglycemia, hypertriglyceridemia and hyperuricemia are currently unknown. The high serum urate level was related to the incidence of macro and microvascular complications in patients with diabetes mellitus and a higher risk of developing DPN [5, 47]. It is still unclear if uric acid serves as a risk indicator or a risk factor for diabetic complications. Uric acid acts as an antioxidant and pro-oxidant at its normal and high concentration respectively [48]. Uric acid is a powerful scavenger of oxygen, peroxyl, and hydroxyl radicals and high urate concentration was proposed to be one of the major antioxidants of the plasma that protects cells from oxidative damage [47]. It is possible that the increase in urate level is an adaptive response to defend against the negative consequences of excessive free radicals and oxidative stress. However, according to earlier research, uric acid enters cells where it boosts the activity of xanthine oxidase

and NADPH oxidase and encourages the production of superoxide [49]. Uric acid also activates the complement system and exerts proinflammatory effects stimulating human mononuclear cells to produce inflammatory cytokines. In the present investigation it was observed that there was a strong positive correlation between uric acid and hyperglycemic state indicated by HbA1c. Also, there was a strong negative correlation between serum uric acid and time of latency using hot plate test and sciatic NCS. So, it was concluded that serum uric acid can be taken as a useful biological marker for assessing the degree of progression of glycemic state and DPN in type 2 diabetes mellitus.

Author Contribution

DEA and RE designed the experiments; MET, SA, and RA performed experiments and collected data; DEA and RE analyzed, interpreted, and discussed the results and strategy; DEA, RE, and RA drafted the manuscript; DEA and RE supervised, directed and managed the study; DEA, RE, MET, SA, and RA Final approved of the version to be published.

Competing interest

No competing interests.

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