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Metformin Reduces the Risk of Diabetic Neuropathy Among Egyptian Type 2 Diabetic Patients: A Case-Control Study

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ABSTRACT

This study aimed to investigate the association of different patient factors with the occurrence of diabetic peripheral neuropathy (DPN) among type 2 diabetes mellitus patients. A case-control study was conducted on a total sample of 180 Egyptian type 2 diabetic patients. A full medical, medication, social, and family history was collected for the recruited sample. Glycated hemoglobin, lipid profile, and microalbuminuria were assessed. Among 180 patients recruited, 128 patients suffered from DPN. Low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) were higher among patients suffering from DPN (p -value = 0.007 and 0.005, respectively). Metformin usage was the only factor that showed a significant decrease in the odds of developing DPN (odds ratio 0.511, 95% confidence interval 0.264- 0.911). Subgroup analysis showed that metformin decreased DPN among males rather than females (p -value = 0.006 vs 0.616, respectively). In conclusion, Serum LDL-C and TC are both determinants for increased risk of DPN among type 2 diabetic patients. Metformin usage decreases DPN in a sex-specific dimorphic fashion where females are less liable to the protective effect of metformin against DPN.

Keywords: Diabetes Mellitus; Diabetic Neuropathy; Metformin; Egyptian Population; Sex-Specific Response.

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1. Introduction

Diabetes mellitus (DM) is a significant public health concern worldwide. According to the estimation provided by the International Diabetes Federation, the global population of individuals aged 18 to 99 years affected by diabetes was approximately 451 million in 2017 [1].

In Egypt, DM comprises a significant public health problem affecting 10.9 million adults with a prevalence of 18.4% [2, 3]. Diabetic complications encompass a wide spectrum of

microvascular or macrovascular complications. Microvascular complications include nephropathy, neuropathy, and retinopathy, while macrovascular complications include stroke, cardiovascular disease, and peripheral vascular disease [4].

Diabetic peripheral neuropathy is widely recognized as the prevailing chronic consequence of diabetes, and its global prevalence has elevated its significance as a critical public health concern. Neuropathy results from both widespread and localized injury to the nervous

system and is observed in around 50% of persons with diabetes [5].

The peripheral nervous system in individuals with diabetes commonly exhibits distinct features, including degeneration of nerve fibers mostly in distal regions and those associated with sensory functions, as well as loss of axons and endoneurial microangiopathy. The mechanisms by which diabetes mellitus affects sensory neurons are still a subject of ongoing debate and discussion in academic circles [6].

Numerous theories have been suggested to clarify the etiology of pain associated with DPN. These theories encompass a range of factors, including alterations in the blood vessels responsible for supplying the peripheral nerves, metabolic and autoimmune disorders that coincide with glial cell activation, and modifications in the expression of sodium and calcium channels [7].

Progressive DPN is characterized by the retraction and degeneration of terminal sensory axons in the peripheral nervous system. The pattern of involvement in DPN, known as the "stocking and glove" pattern, is characterized by the initial destruction of the longest sensory axons. This results in the loss of distal leg epidermal axons before affecting the more proximal limbs [8]. Symptoms like tingling, numbness, orthostatic hypotension, weakness, impotence, and pain described as burning stabbing, or electrical pain may suggest the presence of peripheral neuropathy [9].

Considering the big challenge in treating DPN, it is important to know its risk factors and implement early-stage interventions for prevention. According to contemporary research, the risk factors associated with DPN include the duration of diabetes, advancing age, levels of glycosylated hemoglobin A_{1c} (HbA_{1c}), the presence of diabetic retinopathy (DR), smoking

habits, and body mass index (BMI) [10]. The duration of diabetes and hemoglobin A_{1c} are frequently associated with additional metabolic variables that exhibit a correlation with DPN, particularly in the context of type 2 diabetes mellitus (T2DM), like insulin resistance and hypertension [8].

According to the national data from Egypt, it has been estimated that over 60% of diabetic individuals experience neuropathy. The prevailing consequences associated with DPN encompass cardiac autonomic neuropathy (CAN), diabetic foot ulcers, neuromuscular impairment, and anxiety. Moreover, DPN has a significant impact on the overall quality of life (QoL) [11].

The current study aimed to investigate the association between the occurrence of DPN and various demographic, social, and clinical characteristics in Egyptian patients with type 2 diabetes with and without retinopathy. This study is a subanalysis from a large case-control study that aimed to study the association between the occurrence of diabetic retinopathy and different variables.

2. Patients and methods

2.1. Study Design

An observational, case-control, two-centered study.

2.2. Study Setting

The study was conducted at the outpatient clinics of the National Institute of Diabetes and Endocrinology and the outpatient clinics of the Department of Internal Medicine, Endocrinology Unit, and Ain Shams University hospitals.

2.3. Patients

A total of 180 diabetic patients were included in the study after being assessed for eligibility. Included patients were Egyptian patients with

type 2 DM aged 30-65 years old and suffering from DM for at least 5 years and not more than 20 years. Patients suffering from any other ophthalmic disease not related to diabetes, patients with macroalbuminuria, and patients taking medications known to cause retinopathy were excluded. The original case-control study included 92 diabetic patients suffering from clinically confirmed diabetic retinopathy and 88 diabetic patients free from diabetic retinopathy. Among those 180 patients, 128 patients suffered from DPN.

2.4. Ethical Consideration

The study was conducted following the Declaration of Helsinki as revised in 2013 [12]. The study protocol was revised and approved by the Research Ethics Committees (REC) of the Faculty of Pharmacy, Ain Shams University (REC committee number 35 held on March 22nd, 2022) and the National Institute of Diabetes and Endocrinology. The study protocol was registered at clinicaltrials.gov registry ID number NCT05344690. All patients were educated about the aim and the procedures of the study and were asked to sign a written informed consent.

2.5. Methods

2.5.1. Data Collection.

Subjects in both groups were individually interviewed for data collection related to family history of different diseases, current and previous medications used, and demographic and social history data. Social history and lifestyle data included smoking history, type of diet, level of activity, and occupation. Information related to glycemic control as body mass index (BMI) and HbA1C levels were also gathered.

Clinical history data included; diabetes duration, history of dyslipidemia or hypertension, or any other related disease together with previous exposure to gestational diabetes for females.

A full medication history was gathered including current and previous medications, especially medications associated with the occurrence of retinopathy. Medication history data also included the usage of oral hypoglycemics or insulin. Compliance with antidiabetic medications was also explored, with those committed to 80% of their medication regimen being considered compliant.

The interview included questions as well on family history of retinopathy or other metabolic diseases. Laboratory data including microalbuminuria, HbA1c, lipid profile (LDL-C, high-density lipoprotein cholesterol, TC, and triglycerides) were all assessed.

2.5.2. Blood Collection.

Blood samples were withdrawn by a professionally trained nurse and the blood samples were divided for laboratory assessment.

Serum and urine samples were collected for measuring HbA1c, lipid profile, and microalbuminuria.

2.6. Statistical Analysis

Based on the observations of this case-control study regarding the effect of metformin exposure on the risk rate of neuropathy, the study sample size provided a 92% power to detect the observed medium effect size (w) of 0.329.

Statistical analysis was done using IBM SPSS® Statistics version 28 (IBM® Corp., Armonk, NY, USA). Numerical data was expressed as mean and standard deviation or median and range as appropriate. Qualitative data was expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test were used to examine the relation between qualitative variables.

Data were tested for normality using the Kolmogorov-Smirnov test and Shapiro-Wilk test. Comparison of quantitative variables between

two groups was done using either a two-sample t-test for normally distributed data or the Mann-Whitney test (non-parametric t-test) for not distributed numerical data.

Simple binary logistic regression models were fitted to calculate the odds ratio of neuropathy as related to different variables. Hosmer and Lemeshow test were used to assess the goodness of fit of the regression models and Wald's test was used to assess the significance of the calculated odds ratio. A *p-value* < 0.05 was considered significant.

3. Results

Among the total sample size of 180 patients, the proportion of female participants was found to be 66.9%. The average age of the participants enrolled in the study was 53.8 years with a

standard deviation of 7.7 years. Additionally, the average BMI of the participants was 31.1 kg/m² with a standard deviation of 5.4 kg/m². Gender, age, microalbuminuria, and BMI were comparable between case and control groups.

The current study found a significant association between higher levels of LDL-C and TC and higher incidence of neuropathy with *p*-values 0.007 and 0.005, respectively (**Table 1**).

Similarly, a simple binary logistic regression showed a significant correlation between neuropathy and LDL-C and TC with *p*-values of 0.009 and 0.006, respectively. The odds ratio for each unit increase in LDL-C and TC and their corresponding 95% confidence intervals are shown in **Table 2**.

Table 1. Comparison of LDL-C and total cholesterol levels in patients with and without neuropathy

Neuropathy	N	Mean ± SD	<i>p</i> -value	95% Confidence Interval of the Mean Difference	
				Lower	Upper
LDL-C (mg/dL)	No	52	95.6731(±46.16885)	0.007* ^a	
	Yes	128	116.0122(±45.15739)		-35.08835 -5.58987
Total Cholesterol (mg/dL)	No	52	166.9442(±44.94224)	0.005* ^a	
	Yes	128	189.0102(±48.15282)		-37.40116 -6.73069

LDL-C, low-density lipoprotein cholesterol; N number; SD, standard deviation. Statistical test: ^aTwo Sample T-test, **p*-value less than 0.05 is considered significant.

Table 2. Simple Binary logistic regression model describing the association between diabetic neuropathy and LDL-C and total cholesterol

	<i>p</i> -value	odds ratio	95% Confidence interval for odds ratio	
			Lower	Upper
LDL-C	0.009* ^a	1.010	1.003	1.018
Total Cholesterol	0.006* ^a	1.011	1.003	1.018

LDL-C low-density lipoprotein cholesterol. ^aWald test; **p*-value less than 0.05 is considered significant

Evaluating the association between DPN and medication use, a significant association was observed between the use of metformin as an oral antidiabetic and the lower incidence of neuropathy with a *p-value* of 0.046 (**Table 3**). Simple binary logistic regression showed also a significant decrease in the odds of DPN in

patients treated with metformin (*p-value* = 0.047) (**Table 4**). When studying the effect of current metformin usage on DPN occurrence by gender, the reduction in neuropathy occurrence was statistically significant in males but not in females (*p-value* = 0.006 vs 0.616, respectively).

Table 3. Comparison of the proportion of diabetic neuropathy among those treated with metformin versus those treated by other antidiabetics

	neuropathy		<i>p-value</i>
	No	Yes	
Metformin	No	24	39
	Yes	28	89

^a Chi-square test; **p-value* less than 0.05 is considered significant

Table 4. Simple binary logistic regression models fitted to assess the association between the odds of neuropathy and metformin use

	<i>p-value</i>	odds ratio	95% Confidence interval for odds ratio	
			Lower	Upper
Metformin (both genders)	0.047 ^a	0.511	0.264	0.992
Metformin (males only)	0.008 ^a	0.205	0.063	0.661
Metformin (females only)	0.617 ^a	0.810	0.354	1.850

^a Wald test; **p-value* less than 0.05 is considered significant

4. Discussion

The current study showed significantly higher serum LDL-C and TC levels among those suffering from DPN versus those patients without neuropathy. Multiple processes, such as inflammatory scarring, cellular oxidative stress, disturbance of local lipid metabolism, and ischemia were found to contribute to a complex network in which hyperlipidemia can harmfully affect the peripheral nervous system [13].

Similarly, Zhang et al. reported a comparatively lower level of LDL-C in patients without DPN compared to those in the DPN group (*p-value* = 0.052) without a statistically significant difference in the levels of LDL-C between the two groups [14].

On the other hand, a cross-sectional cohort study examined 100 patients with type 2 diabetes and revealed a negative association between the extent of nerve lesions and total serum cholesterol levels and they interpreted this finding by the aggressive lowering of serum cholesterol levels by emerging therapies. Another explanation was that lowering serum cholesterol levels might have contributed to impairing the regeneration of peripheral nerves [15].

Also, a meta-analysis showed that serum levels of TC and LDL-C were lower in those experiencing acute pain when compared to those in asymptomatic states. This discrepancy in findings could be attributed to the obvious evidence of significant heterogeneity among studies included in this meta-analysis (reported I^2

= 84.7%) since the final analysis included results of manuscripts studying type 1 and type 2 diabetes mellitus combined. Currently, a satisfactory explanation for this occurrence remains unclear [16].

Metformin is an old oral hypoglycemic agent that is widely used for the management of patients diagnosed with type 2 diabetes [17]. In our study, we found an association between the use of metformin and the reduced odds of DPN. Multiple studies have demonstrated that metformin possesses the ability to alleviate neuropathic pain resulting from diabetes [17, 18]. A study demonstrated that metformin can effectively preserve peripheral nerves in diabetic rats to a similar extent as alpha-lipoic acid (ALA) [19]. Another animal study found that the administration of 200 mg metformin effectively inhibited the degeneration of myelinated axons and suppressed the production of the mediators of inflammation, including nitric oxide synthase and interleukin-1 β and proved that metformin has a protective effect on nerve tissue against the detrimental effects associated with prolonged elevated blood glucose levels [20]. A recent review described a potential neuroprotective and analgesic effect of metformin in DPN through its role as an activator of adenosine monophosphate-activated protein kinase (AMPK) [21]. It is worth noting that this study showed that the effect of metformin on DPN was in a sexually dimorphic fashion where metformin reduced DPN in males rather than females. This is following animal studies showing sex-specific effects of metformin in neuropathic pain in mice [22].

Yet, the deficiency of vitamin B12 caused by metformin usage which contributes to the worsening of neuropathy, should always be addressed and reported as a main goal of clinical trials. Multiple studies have demonstrated that individuals diagnosed with type 2 diabetes who

undergo long-term and high-dose treatment with metformin exhibit an exacerbation of DPN. This can be attributed to a reduction in cobalamin levels, as well as an elevation in homocysteine and methylmalonic acid concentrations [23-25].

This inconsistency merits careful consideration and scholarly investigation. Further research is necessary to assess the effectiveness and safety of metformin as a preventive intervention for DPN.

Conclusion

In conclusion, this case-control study in Egyptian diabetic patients with and without neuropathy demonstrated an association between the occurrence of neuropathy and high levels of LDL-C and TC. Also, metformin use was associated with a lower incidence of neuropathy.

Limitations and recommendations

This study is limited by being an observational case-control study which increases the susceptibility to confounders. Further prospective studies are needed to assess the prophylactic effect of metformin for the prevention and delayed progression of DPN. In addition, interventional studies assessing the effect of tight lipid profile control on the incidence and severity of DPN are required.

Declarations

Ethics approval and consent to participate

The study protocol was revised and approved by the Research Ethics Committees (REC) of the Faculty of Pharmacy, Ain Shams University, and the National Institute of Diabetes and Endocrinology. All patients were educated about the aim and the procedures of the study and were asked to sign a written informed consent.

Consent to publish: Not applicable

Availability of data and materials

Raw data for the study results are not publicly available to preserve patients' privacy and confidentiality. Data will be available upon request from the corresponding author.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

All authors contributed to the study's conception and design. Data collection and analysis were performed by Yomne Hicham. The first draft of the manuscript was written by Yomne Hicham, Rana Sayed, and Lamia El Wakeel and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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