Impact of Pyridoxine Supplement on Oxidative Stress in Type 2 Diabetic Patients

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Article Info:

Received May 2023
Accepted Aug 2023
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DOI:

Abstract:

Background: The physiologically active form of vitamin B6 is pyridoxal 5-phosphate (PLP), which functions as a coenzyme in 150 enzymatic processes such amino acid, carbohydrate, and lipid metabolism and is essential for the production and/or breakdown of neurotransmitters. . It also acts as an antioxidant by quenching Reactive Oxygen Species (ROS) and counteracting the formation of Advanced Glycation End-Product (AGEs). PLP is recycled by mammals from B6 vitamins found in diet and has been linked to a number of clinically significant disorders. This study aim was to evaluate the impact of pyridoxine supplementation on oxidative stress status in type 2 diabetic patients.

Method: This prospective controlled randomized open-labeled study of newly diagnosed T2DM patients. The study was conducted from November 2022 to February 2023 at the Mesan Center for Diabetes and Endocrinology under the supervision of a specialist endocrinologist. The total number of participants whose data were collected in this study was one hundred and eight participants, eighty-eight patients newly diagnosed with type 2 diabetes were included in the study, and twenty of the participants were healthy subjects. The patients were allocated into three groups: Group 1: Control group, 20 T2DM patients were treated with non-pharmacological therapy (lifestyle modification) for one month, Group 2: 34 T2DM patients treated with metformin 500 mg/day in addition to non-pharmacological therapy (lifestyle modification) for one month, Group 3: 34 T2DM patients treated with metformin 500 mg/day plus vitamin B6 300 mg/day in addition to non-pharmacological therapy (lifestyle modification) for one month, in addition to Healthy subjects: 20 subjects were taken to compare the study parameters between type 2 diabetic patients and healthy persons at baseline. Measurement of Vitamin B6 (pyridoxine) blood level (PLP), body mass index (BMI) and Serum malondialdehyde level (MDA) was done in this study.

Results: The results of the study showed that the use of pyridoxine supplementation plus metformin decreased the level of MDA. There was significant decrease in (MDA) level ($P<0.01$) pretreatment when compared to after treatment (3.85 µmole/L vs 1.66 µmole/L) respectively.

Conclusion: Restoration of vitamin B6 level can leads to a decreased blood level of MDA in diabetic patients.

key words: Pyridoxine supplement, Oxidative stress, Serum malondialdehyde level (MDA).

تأثير مكملات البيريدوكسين على الإجهاد التأكسدي في مرضى السكري من النوع الثاني

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الخلاصة:

الخلفية: الشكل النشط من الناحية الفسيولوجية لفيتامين B6 هو بيريدوكسال ٥-فوسفات (PLP)، والذي يعمل بمثابة أنزيم في ١٥٠ عملية إنزيمية مثل الأحماض الأمينية، والكربوهيدرات، والتمثيل الغذائي للدهون، وهو ضروري لإنتاج و / أو تفكك الناقلات العصبية. كما أنه يعمل كمضاد للأكسدة عن طريق إخماد أنواع الأكسجين التفاعلية (ROS) ومقاومة تكوين المنتج النهائي المتقدم للجليكشن (AGEs). يتم إعادة تدوير PLP بواسطة الثدييات من فيتامينات B6 الموجودة في النظام الغذائي وقد تم ربطها بعدد من الاضطرابات المهمة سريريًا. هدفت هذه الدراسة إلى تقييم تأثير مكملات البيريدوكسين على حالة الإجهاد التأكسدي في مرضى السكري من النوع ٢.

الطريقة: هذه الدراسة العشوائية المفتوحة ذات العلامات المفتوحة التي يتم التحكم فيها لمرضى السكري من النوع الثاني الذين تم تشخيصهم حديثاً. أجريت الدراسة في الفترة من نوفمبر ٢٠٢٢ إلى فبراير ٢٠٢٣ في مركز ميسان للسكري والغدد الصماء تحت إشراف أخصائي الغدد الصماء. كان العدد الإجمالي للمشاركين الذين تم جمع بياناتهم في هذه الدراسة مائة وثمانية مشاركين، وتم تضمين ثمانية وثمانين مريضًا تم تشخيصهم حديثًا بمرض السكري من النوع الثاني في الدراسة، وكان عشرون من المشاركين أشخاصًا أصحاء. تم تقسيم المرضى إلى ثلاث مجموعات: المجموعة ١: المجموعة الضابطة ، ٢٠ مريضًا من T2DM تم علاجهم بالعلاج غير الدوائي (تعديل نمط الحياة) لمدة شهر واحد ، المجموعة ٢: ٣٤ مريضًا من T2DM تم علاجهم بالميتفورمين ٥٠٠ مجم / يوم بالإضافة إلى غير العلاج الدوائي (تعديل نمط الحياة) لمدة شهر ، المجموعة ٣: ٣٤ مريضًا من T2DM تم علاجهم بالميتفورمين ٥٠٠ مجم / يوم بالإضافة إلى فيتامين B6 300 مجم / يوم بالإضافة إلى العلاج غير الدوائي (تعديل نمط الحياة) لمدة شهر واحد ، بالإضافة إلى الأشخاص الأصحاء : ٢٠ شخصًا تم أخذهم لمقارنة مؤشرات الدراسة بين مرضى السكري من النوع ٢ والأشخاص الأصحاء في الأساس. تم في هذه الدراسة قياس مستوى فيتامين ب ٦ (البيريدوكسين) في الدم(PLP) ، ومؤشر كتلة الجسم (BMI) ومستوى مصل malondialdehyde (MDA).

النتائج: أظهرت نتائج الدراسة أن استخدام مكملات البيريدوكسين مع الميتفورمين قلل من مستوى .MDA كان هناك انخفاض كبير في مستوى (P <0.01) (MDA) مقارنة بالمعالجة بعد العلاج (٣,٨٥ ميكرولتر / لتر مقابل ١,٦٦ ميكرولتر / لتر) على التوالي.

الاستنتاج: استعادة مستوى فيتامين ب ٦ يمكن أن يؤدي إلى انخفاض مستوى MDA في الدم لدى مرضى السكري.

الكلمات المفتاحية: مكمل البيريدوكسين ، الإجهاد التأكسدي، مستوى مصل malondialdehyde (MDA).

Introduction

A worldwide condition, type 2 diabetes has significant negative social and economic effects. However, when it comes to managing type 2 diabetes, the emphasis is on the patient learning how to take control of their disease and prevent complications (1).

The macrovascular and microvascular effects of diabetes lead to several problems. According to (2), these deficiencies are fundamental to the tissue-damaging consequences of persistent hyperglycemia. They are particularly vulnerable to the harmful consequences of hyperglycemia because endothelial cells (as well as mesangial and Schwann cells) cannot control glucose transport like other cells. In fact, diabetes can be categorized as a cardiovascular illness from a cardiovascular standpoint (3). According to several studies, both type 1 and type 2 diabetes are linked to higher levels of free radical synthesis and lower antioxidant capacity, which causes oxidative damage to cellular constituents (4).

The primary aim of lifestyle interventions is to prevent or delay the development of type 2 diabetes and its complications (5). T2DM patients should consider non-pharmacological interventions to alter the disease's pathophysiological mechanisms. Optimal dietary and physical activity are crucial for managing T2DM, improving

glycemic outcomes and reducing associated diseases like dyslipidemia, hypertension, obstructive sleep apnea OSA, and cardiovascular diseases (6). Bariatric surgery is the best treatment option for managing diabesity, with significant improvements in all-cause and disease-specific mortality rates (٧). However, it may not be as effective in patients with T1DM, but may be an option for those with morbid obesity. Sleep hygiene is essential for T2DM prevention, and appropriate management of OSA through weight loss and continuous positive airway pressure is crucial for T2DM management (6).

Based on trials demonstrating no major adverse effects (only moderate gastrointestinal side effects were observed), metformin has been used for decades to treat diabetes. It also has positive effects on body mass index and cholesterol levels. Metformin primarily affects hepatic glucose production, which lowers fasting blood glucose levels (8). A study of people with impaired glucose tolerance (IGT) found that metformin lowers the incidence of type 2 diabetes by 45% (9).

The outcome was inferior to that of the United States Diabetes Prevention Program (10), but comparable to the lifestyle intervention in the Indian Diabetes Prevention Program-1 trial (11). In comparison to slimmer participants with lower blood glucose levels, the positive effects of metformin were larger in prediabetic subjects with higher baseline body mass index and sugar (10). Because the majority of the drug's gastrointestinal side effects were mild to moderate, the course of action seemed to be safe (11).

Salvo et al. (2011) state that pyridoxal-5-phosphate (PLP), the biologically active variant of vitamin B6, serves as a co enzyme with roughly 150 various enzyme operations, basically contributing to protein, sugar, and fatty acid metabolism as well as in neuronal processes regulated by the production and/or breakdown of vitamin B6 (12). PLP also functions as a mediator by squelching (ROS) (13), and preventing the production of (AGEs), they have been connected to aging and diabetes and are genotoxic chemicals (14). Mammals, unlike microbes, are unable to produce pyridoxal-5-phosphate; instead, they rely on vitamin B6 recycling routes present in food, such as pyridoxal, pyridoxamine, and pyridoxine (15). It is recycled pyridoxal kinase transforms pyridoxal, pyridoxamine, and pyridoxine into vitamin-5-phosphorylate in the cytoplasm. Clinically important conditions such as vitamin B6 deficiency have been linked to autism, mental illness, dementia, Parkinson's disease, seizures, developmental delays, diabetes, and malignancy (16). The emphasis of this review is on pyridoxal-5-phosphate's function in diabetes.

Diabetes and vitamin B6 have been connected. It is not clear, nevertheless, whether diabetes is a result of low PLP levels, a cause, or both. According to certain research, diabetes may develop as a result of low PLP levels (17), whereas other studies (18) demonstrate that diabetes lowers PLP levels. Although the cellular and molecular processes behind these favorable benefits on diabetes pathology and associated consequences are not fully understood, B6 treatment has been found to have positive effects by several studies (19). Different ways that pyridoxal 5-phosphate deficiency impacts diabetes exist. As an essential element for numerous enzymes that contribute to this process, pyridoxal-5-phosphate can, for example, influence the route that converts tryptophan into niacin (20). It has been proven that the metabolites produced when this pathway is damaged lessen the bioactivity of insulin and cause insulin resistance, a T2D symptom (21). By controlling the expression of genes related to adipogenesis, pyridoxal-5-phosphate may also affect insulin resistance (22). The deterioration of co-enzyme-dependent enzymes like (CBS) and (CGL), which

rely on pyridoxal-5-phosphate, may also cause insulin resistance by raising homocysteine levels (23).

The aim of the study:

This study aim was to evaluate the impact of pyridoxine supplement on oxidative stress status in type 2 diabetic patients.

Patients and methods

This prospective controlled randomized open-labeled study of newly diagnosed T2DM patients. The study was conducted from November 2022 to February 2023 at the Mesan Center for Diabetes and Endocrinology under the supervision of a specialist endocrinologist.

The proposal of the research was discussed and approved by the scientific and ethical committee at college of pharmacy/Mustansiriyah University (Research No. 17, Approval No. 5), and the agreement of health directorate in Mesan Governorate was achieved. Patient written consent was taken after full explanation of the aim of the study and ensure the reliability of the collected information. The study was implemented in Mesan Governorate; at Mesan Center for Diabetes and Endocrinology and at a specialist Private Clinic.

Participants meet the following Inclusion criteria:

- Type 2 Diabetes mellitus newly diagnosed patient with age above 30 years.
- Patients with HbA1c less than or equal to 7.5%.

Certain exclusion criteria were followed to avoid interference with the study design and include:

- Type 1 Diabetes mellitus.
- Type 2 diabetes mellitus on other treatment than the study intervention.
- Concomitant chronic diseases (Rheumatoid arthritis, anemia, bronchial asthma, alcoholics, and patient on anti-TB or anti-epileptics drugs).
- Pregnant or lactating women.
- Patients receiving vitamin or mineral supplementation.
- Should had no history of recent acute infection (within the previous two weeks), no endocrine disorders, and no renal failure

The total number of participants whose data were collected in this study was one hundred and eight participants, eighty-eight patients newly diagnosed with type 2 diabetes were included in the study, and twenty of the participants were healthy subjects, the eligible patients were allocated into three groups:

- Group 1: Control group, 20 T2DM patients were treated with non-pharmacological therapy (lifestyle modification) for one month.
- Group 2: 34 T2DM patients were treated with metformin 500 mg/day in addition to non-pharmacological therapy (lifestyle modification) for one month.
- Group 3: 34 T2DM patients were treated with metformin 500 mg/day plus vitamin B6 300 mg/day in addition to non-pharmacological therapy (lifestyle modification) for one month.

In addition to healthy subjects: 20 subjects were taken to compare the study parameters between type 2 diabetic patients and healthy persons at baseline.

Measurement of Vitamin B6 (pyridoxine) blood level (PLP), Body mass index (BMI) and Serum malondialdehyde level (MDA) was done in this study. All measurements were carried out at baseline and after 4 weeks. Measurement of (PLP) was done using (Human Vitamin B6 (PLP) ELISA Kit, Shanghai YL Biotech, Chain). To calculate the body mass index (BMI) height and weight were obtained using a standard stadiometer and electronic scale as weight/height squared (kg/m^2), the weight was measured with a light cloth and without shoes. Finally, the measurement of (MDA) was measured via (Malondialdehyde (MDA) ELISA Kit, Elabscience, USA).

Statistical analysis of data was performed using the Statistical Package for Social Sciences software (SPSS v.26). Unpaired Student's t-test (paired t-tests and unpaired t-tests) was used in this study. Paired t-tests (dependent or correlated t-test) were used to compare values obtained before and after treatment administration within each group. In contrast, unpaired t-tests (independent sample t-tests) were used to compare between study groups. Multiple comparisons were also carried out using analysis of variance (ANOVA) with least significant difference (LSD) post hoc testing to compare variable changes between groups before and after the four-week treatment period. Data presented as mean ± Standard deviation (SD). Pearson's correlation coefficient had verified the correlation. $P<0.05$ was considered statistically significant and used a two-tailed test for correlation relationships

Results

Demographic characteristics

One hundred and eight persons were included in the study, there are 55 male (51%) and 53 females (49%), aged between 30 and 61 years. The mean age ± SD is (43.4 ± 6.96 years) for females and (41.5 ±10.75 years) for males, their BMI mean was (30.52, 30.42, 30.74, 25.76) for (G1, G2, G3 and Healthy) respectively. And percentage of family history of D.M was (31, 58, 63) (G1, G2 and G3) respectively. According to exclusion criteria, no patients were included for the following conditions (smokers, have chronic diseases or use other treatments). Duration of Disease (T2DM) mean was (1.15, 1.28 and 1.43) month for (G1, G2 and G3) respectively. Twenty out of 108 were healthy subjects, while the rest (88) were diagnosed with type 2 diabetes. As shown in Table (1).

Table (1) Demographic characteristics

		G 1	G 2	G 3	Healthy	P value For G1, G2 and G3
Number of subjects		**20**	**34**	**34**	**20**	
Gender n (%)	Males	11 (55%)	17 (50%)	19 (56%)	8 (40%)	0.01
	Females	9 (45%)	17 (50%)	15 (44%)	12 (60%)	
Age for Males	Mean (Years)	40.4 ± 12.98	41.9 ± 15.66	43.8 ± 17.14	39.8 ± 14.32	0.41^{NS}
	Range	30-59	32-61	32-61	30-60	
Age for Females	Mean (Years)	42.8 ± 11.84	43.9 ± 10.36	44.7 ± 12.28	42.1 ± 11.27	
	Range	32-58	33-60	30-61	30-61	
BMI (kg/m^2)	Mean	30.52	30.42	30.74	25,76	0.01
Family history of D.M (%)		31%	58%	63%	-	
Duration of Disease (T2DM)	Mean (Month)	1.15	1.28	1.43	-	0.12^{NS}

Data presented as mean ± SD, G 1: Group 1, G 2: Group 2, G 3: Group 3, Number of patients and healthy subjects (n), Percentage (%), P value**:** represent statistically significant ($P<0.01$) difference between study groups (1,2 and 3), NS: No significant differences ($P>0.05$).

Unpaired t-test was used for statistical analysis to compare differences between study groups.

Baseline Characteristics of Study Population

The baseline of healthy subjects' measures and patients' group, as the mean level of PLP, BMI, and (MDA) are shown in Table (2).

Unpaired t-test was used to compare the baseline characters differences between the

healthy subjects and type 2 diabetes patients' group, revealed significant in all laboratory measures (P<0.01). The level of PLP was significantly lower i
n type 2 diabetes patients (22.7 nmol/L vs 39.5 nmol/L) compared to healthy subjects respectively. BMI was also significantly increased in type 2 diabetes patients (22.62 ng/ml vs 18.40 ng/ml) respectively compared to healthy subjects. Baseline higher level of MDA in type 2 diabetes patients compared to healthy subjects (2.65 µmole/L vs 0.93 µmole/L) respectively.

Table (2) Baseline parameters of type 2 diabetic patients and healthy subjects

Parameters	Diabetic patients (n=88)		Healthy subjects (n=20)		P value
	Mean	±SD	Mean	±SD	
Pyridoxine blood level (PLP) (nmol/L)	22.7	1.02	39.5	2.16	0.01**
BMI (kg/m^2)	30.25	0.90	25.76	1.79	0.01**
MDA (µmole/L)	2.65	1.33	0.93	0.36	0.01**

Data presented as mean ± SD, G 1: Group 1, G 2: Group 2, G 3: Group 3, Number of patients and healthy subjects (n), ** Statistically highly significant (P<0.01) difference.
Unpaired t-test was used for statistical analysis to compare the baseline characters differences between the healthy and type 2 diabetes patients' group.

Effect of Study Treatment on Pyridoxine Blood Level (PLP).

All of the study groups demonstrated a significant increase in the PLP at the end of 4 weeks (P<0.05) compared with baseline measurements as shown in Table (3).

Comparing with control group, the PLP level was significantly increased in groups 2 and 3 at week 4 of the study (P<0.05) (36.95% vs 76.19%) respectively. When compare the change in study groups without control group the increase in PLP was significantly more in group 3 than group 2 patients (17.63 nmol/L vs 8.45 nmol/L) respectively.

Table (3) Effect of study treatment on Pyridoxin blood level (PLP)

Groups / Parameter		G 1 (n=20)		G 2 (n=34)		G 3 (n=34)		P value a
		mean	±SD	mean	±SD	mean	±SD	
PLP (nmol/L)	Baseline	23.21	4.02	22.87	6.13	23.14	5.6	P<0.05
	4week	29.14	5.66	31.32	4.24	40.77	4.2	
	P value b	NS P>0.05		* P<0.05		** P<0.01		
	Δ PLP	5.93	0.21	8.45[a]	0.12	17.63[ab]	0.17	
	Δ PLP %	25.55%		36.95%		76.19%		

Data presented as mean ± SD, G 1: Group 1, G 2: Group 2, G 3: Group 3, Number of patients (n), NS: No significant differences (P>0.05), *: Statistically significant (P<0.05) difference after 4 weeks

compared with the baseline by using paired t-test. **: Statistically highly significant (P<0.01) difference after 4 weeks compared with the baseline by using paired t-test. P value a: Represent statistically significant (P<0.05) difference after 4 weeks between study groups (1,2 and 3) using ANOVA post hoc test, unpaired t-test. P value b: Represent statistically significant (P<0.05) difference in the change from the baseline after 4 weeks between the study treatments without control group by using ANOVA post hoc test, unpaired t-test.

Effect of study treatment on body mass index (BMI).

All of the study groups demonstrated a significant decrease in the BMI at the end of 4 weeks (P<0.05) compared with baseline measurements. as shown in Table (4).

Comparing with control group, the reductions in BMI was significantly more in G2 and G3 at the end of 4 weeks of treatment (P<0.05) (-3.48% vs -8.23%).

Table (4) Effect of study treatment on Body Mass Index (BMI)

parameter / Groups		G 1 (n=20)		G 2 (n=34)		G 3 (n=34)		P value a
		mean	±SD	mean	±SD	mean	±SD	
BMI	Baseline	30.52	0.61	30.42	0.63	30.74	0.77	P<0.05
	4week	29.47	0.59	29.36	0.61	28.21	0.72	
	P value b	* P<0.05		* P<0.05		** P<0.01		
	Δ BMI	-1.05	0.07	-1.06	0.14	-2.53[a]	0.08	
	Δ BMI %	-3.44%		-3.48%		-8.23%		

Data presented as mean ± SD, G 1: Group 1, G 2: Group 2, G 3: Group 3, Number of patients (n), NS: No significant differences (P>0.05), *: Statistically significant (P<0.05) difference after 4 weeks compared with the baseline by using paired t-test. **: Statistically highly significant (P<0.01) difference after 4 weeks compared with the baseline by using paired t-test. P value a: Represent statistically significant (P<0.05) difference after 4 weeks between study groups (1,2 and 3) using ANOVA post hoc test, unpaired t-test. P value b: Represent statistically significant (P<0.05) difference in the change from the baseline after 4 weeks between the study treatments without control group by using ANOVA post hoc test, unpaired t-test.

Effect of study treatment on Serum malondialdehyde level (MDA).

All of the study groups demonstrated a significant decrease in Serum malondialdehyde level (MDA) at the end of 4 weeks (P<0.05) compared with baseline measurements as shown in Table (5).

Comparing with control group, the reductions in Serum malondialdehyde level (MDA) were significantly more in G2 and G3 at week 4 of treatment (P<0.05) (-33.74% vs -56.88%). and when compare the change in G2 and G3 without control group the decrease in MDA was significantly more in G3 than G2 at week 4 of treatment (P<0.05) (-2.19 µmole/L vs -1.11 µmole/L).

Table (5) Effect of study treatment on Serum malondialdehyde level (MDA)

Parameters \ Groups		G 1 (n=20)		G 2 (n=34)		G 3 (n=34)		P value a
		mean	±SD	Mean	±SD	mean	±SD	
MDA (µmole/L)	Baseline	2.39	0.78	3.29	0.73	3.85	1.36	P<0.05
	4week	1.87	0.48	2.18	0.37	1.66	0.28	
	P value b	P>0.05		* P<0.05		** P<0.001		
	Δ MDA	-0.52	0.011	-1.11[a]	0.012	-2.19[ab]	0.014	
	Δ MDA %	-21.76%		-33.74%		-56.88%		

Data presented as mean ± SD, G 1: Group 1, G 2: Group 2, G 3: Group 3, Number of patients (n), NS: No significant differences (P>0.05), *: Statistically significant (P<0.05) difference after 4 weeks compared with the baseline by using paired t-test. **: Statistically highly significant (P<0.01) difference after 4 weeks compared with the baseline by using paired t-test. P value a: Represent statistically significant (P<0.05) difference after 4 weeks between study groups (1,2 and 3) using ANOVA post hoc test, unpaired t-test. P value b: Represent statistically significant (P<0.05) difference in the change from the baseline after 4 weeks between the study treatments without control group by using ANOVA post hoc test, unpaired t-test.

Analysis of the correlations between Vitamin B6 (pyridoxal 5-phosphate) blood level (PLP) and other indicators of the study.

For the purpose of correlation testing, the study would depend on the Pearson correlation coefficient, used the advanced statistical program (Spss v.26).

Correlation between PLP and BMI The Pearson correlation coefficient was used to examine the relationship between PLP and BMI. According to Table (6), it was negatively associated (-0.778), with a significance level of 0.01.

Correlation between PLP and MDA

The Pearson correlation coefficient has been used to examine the relationship between PLP and MDA. According to Table (6), it was negatively associated (-0.763), with a significance level of 0.01.

Table (6) The relationship between PLP and both of BMI and MDA

		BMI	MDA
PLP	Pearson Correlation	**-0.778****	**-0.763****
	Sig. (2-tailed)	.000	.000

**. Correlation is significant at the 0.01 level (2-tailed).

The Pearson correlation coefficient was used for statistical analysis to examine the relationship.

Discussion

The potential benefits of pyridoxine adjuvant therapy have been the subject of interest in the management of type 2 diabetes mellitus (T2DM). In this discussion, the study explores the findings of previous studies that have investigated the effects of pyridoxine on body mass index (BMI) and oxidative stress among newly diagnosed T2DM patients. Understanding these potential benefits can shed light on the therapeutic implications and contribute to optimizing the management of T2DM (24,25).

A study confirmed the association of a low level of pyridoxine in the blood in type 2 diabetes mellitus patients. The level of pyridoxine in the blood of patients (26.8 nmol/L vs 40.9 nmol/L) compared to healthy subjects respectively (24).

In the same context, another study proved that the low level of pyridoxine in the blood is related to type 2 diabetes, as the PLP level was measured before and after giving 100 mg/day of vitamin B6 for 14 days, and the result was (13.5 nmol/L to 25 nmol/L) (26).

This is consistent with the findings of the current study, as the level of pyridoxine in the blood of patients (22.7 nmol/L vs 39.5 nmol/L) compared to healthy subjects respectively. Diabetes can reduce PLP levels, as demonstrated by glucose ingestion in healthy subjects (27). Okada and coworkers suggested that diabetes may lead to vitamin B6 deficiency due to increased protein metabolism in a low-carbohydrate diet. PLP is a cofactor for many enzymes involved in protein metabolism, and increased demand causes a decrease in other tissues. In diabetic rats, aspartate amino transferase activity is four times greater in the liver, while glycogen phosphorylase activity is decreased in the muscles (28).

Demographics and Characteristics of Participants

The studies investigating the relationship between pyridoxine and T2DM have typically included a range of sample sizes. Some studies had relatively small sample sizes, involving around 50 to 100 participants, while others had larger cohorts with several hundred participants. The selection of participants was based on specific criteria, such as age, diagnosis of T2DM, and exclusion of individuals with other significant comorbidities (26).

The studies investigating the relationship between pyridoxine and T2DM have included participants across a wide age range. Typically, the age range of the participants varied from middle-aged to older individuals, reflecting the demographic profile of individuals commonly diagnosed with T2DM. Age distribution in these studies allowed for an assessment of the effects of pyridoxine on glycemic status, BMI, metabolic activity, lipid profile, and oxidative stress across different age groups (29).

Previous studies have included both male and female participants to ensure a balanced representation of gender. This approach recognizes the importance of examining potential gender-specific effects of pyridoxine on T2DM parameters. By including participants of both genders, researchers aimed to obtain a comprehensive understanding of the relationship between pyridoxine and the studied parameters in T2DM management (32,30)

The presence or absence of a family history of diabetes has been a relevant characteristic considered in previous

studies. Some studies included participants with a positive family history of diabetes, indicating a genetic predisposition to the disease. This allowed researchers to explore whether the effects of pyridoxine on glycemic status, BMI, metabolic activity, lipid profile, and oxidative stress in T2DM may vary in individuals with a familial risk compared to those without such a history (33,34).

The duration of morbidity with T2DM, referring to the length of time since the initial diagnosis, has been a crucial characteristic in previous studies. Participants in these studies were newly diagnosed with T2DM, indicating that the duration of their disease was relatively short. By focusing on newly diagnosed individuals, researchers aimed to assess the effects of pyridoxine on glycemic status, BMI, metabolic activity, lipid profile, and oxidative stress in the early stages of T2DM management, which may have distinct implications compared to longer-standing T2DM cases (25,35).

Baseline Characteristics of Study Population

The association between pyridoxine adjuvant therapy and BMI in T2DM patients has also been explored. A study by Khobrani et al. (2023) found that pyridoxine supplementation was associated with a significant reduction in BMI among T2DM patients (30). This suggests that pyridoxine may have a beneficial effect on weight management in T2DM, potentially through mechanisms involving appetite regulation, lipid metabolism, and energy expenditure.

Oxidative stress plays a crucial role in the pathogenesis of T2DM and its associated complications. Pyridoxine, with its antioxidant properties, has been investigated for its potential to mitigate oxidative stress in T2DM patients. A study by Hsu et al. (2015) reported that pyridoxine supplementation reduced markers of oxidative stress, such as malondialdehyde (MDA), among T2DM patients (36). These findings suggest that pyridoxine may have a protective effect against oxidative stress-related damage in T2DM.

Effect of Restoration of Endogenous Pyridoxine (PLP)

This study confirms previous research showing lower PLP levels in individuals with type 2 diabetes compared to healthy controls. Previous studies have found that pyridoxine supplementation can effectively raise PLP levels in this population (24,37). A study found that supplementation significantly increased PLP levels compared to placebo, suggesting that pyridoxine supplementation 150 mg/day for 4 months can effectively raise PLP levels (24.25 nmol/L to 156.25 nmol/L) (38) (33). The result of current study (23.14 nmol/L to 40.77 nmol/L) in agreement with previous study.

It's important to note that the mechanisms underlying the decrease in pyridoxine levels in patients with type 2 diabetes are complex and can involve multiple factors. The interplay between these mechanisms may vary among individuals and can be influenced by various factors such as diabetes control, dietary habits, and comorbidities (37)

Effect of Study Treatment on Body Mass Index (BMI)

The available researches suggests that pyridoxine blood levels, specifically PLP, may be inversely associated with BMI in patients with type 2 diabetes (39). The

study of Shen et al. (2010) found that the body mass index changed inversely with the increase in the level of pyridoxine in the blood (32.9 kg/m^2 to 30.7 kg/m^2). These findings align with this study (30.74 kg/m^2 to 28.21 kg/m^2).

The mechanism behind a decrease in BMI in type 2 diabetes patients taking pyridoxine (vitamin B6) is involved in various metabolic pathways, including macronutrient metabolism, which may contribute to improved energy metabolism and utilization. This may lead to weight management and a decrease in BMI. However, the direct impact of pyridoxine on BMI reduction requires further investigation (30). It is also important to consider that managing weight in individuals with type 2 diabetes involves a comprehensive approach that includes dietary modifications, physical activity, and appropriate medical management (40).

Effect of Study Treatment on Serum Malondialdehyde Level (MDA).

The study found a significant decrease in serum MDA levels in the experimental group compared to the control group after the intervention, suggesting that Pyridoxine in the form of PLP has a beneficial effect on reducing oxidative stress. This finding is consistent with previous studies that have explored the relationship between Pyridoxine and oxidative stress markers, including serum MDA levels (41,42). Previous studies have shown a significant decrease in MDA levels following Pyridoxine supplementation, Hsu et al. (2015) also experiencing a reduction in MDA levels with Pyridoxine intervention, The results showed an inverse association between PLP levels and MDA levels the decrease in MDA level (3.40 µmole/L to 1.18 µmole/L), indicating that higher PLP levels were associated with lower MDA. The results of the current study agree with these findings, as the level of decrease in MDA level (3.85 µmole/L to 1.66 µmole/L). (36). Chen et al. (2016) also found a negative correlation between PLP levels and MDA levels, suggesting that higher PLP levels were associated with lower oxidative stress levels in patients with type 2 diabetes. Aghamohammadi et al. (2011) also found a potential protective effect against oxidative stress in 68 patients with type 2 diabetes (34).

Pyridoxine, as an antioxidant, may have the potential to modulate oxidative stress and reduce MDA levels. Vitamin B6 participates in various enzymatic reactions involved in antioxidant defense mechanisms, including the metabolism of glutathione, an important cellular antioxidant. By enhancing antioxidant capacity, pyridoxine supplementation may help mitigate oxidative stress and subsequently lower serum MDA levels (44,45).

Limitations of the study:

- The first difficulty was finding people to participate in the study within the inclusion criteria, the most important of which was newly diagnosed with type 2 diabetes.
- Difficulty following up the participants in the study to take the feedback of the treatment used and the side effects and the improvement of the clinical condition.
- Difficulty assuring patients' adherence to the treatment used in the study with the prescribed doses, times, and period. This forced the researcher to communicate

directly with the patients individually to overcome this difficulty.

Conclusion

Through the results deduced in this study on the targeted samples, the following is shown:

1- The statistical analysis of PLP levels in healthy individuals and patients with type 2 diabetes revealed significantly lower levels in the latter group. These findings align with previous studies, suggesting an association between lower PLP levels and type 2 diabetes.
2- The statistical analysis of the study investigating the effect of PLP supplementation on BMI demonstrated a significant reduction in BMI among participants who received the PLP supplement compared to the control group. These findings are consistent with some previous studies, supporting the potential role of PLP in weight management.
3- The statistical analysis of this study indicates that Pyridoxine in the form of Pyridoxal 5-Phosphate (PLP) has a significant effect in reducing serum malondialdehyde (MDA) levels. These findings are consistent with previous studies that have demonstrated the antioxidant properties of Pyridoxine. The results suggest that Pyridoxine supplementation may be beneficial in reducing oxidative stress, which can have potential implications for various diseaseconditions associated with increased oxidative stress. This finding is referring that restoration of vitamin B6 lead to decrease blood level of MDA indicates that it acts as antioxidant agent.

Recommendations:

1- It is important to note that further research, including long-term studies and diverse populations, is needed to establish the efficacy and safety of PLP supplementation for clinical applications and explore the underlying mechanisms and potential therapeutic implications of optimizing PLP levels in type 2 diabetes management.
2- It is important to note that while these findings indicate a correlation, further research is needed to establish a definitive causal relationship and determine the optimal levels of pyridoxine supplementation for reducing oxidative stress in patients with type 2 diabetes.

Acknowledgment

The author would like to thank college of pharmacy/Mustansiriyah University Baghdad - Iraq for its support in the present work and special thanks to all participants for providing the practice platform of this study.

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