

## Mitochondrial Copies Number and Some Renal Function Biomarkers in Type 2 Diabetes Mellitus on Metformin

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### Abstract

One of the most common metabolic illnesses in the world is diabetes mellitus. This metabolic disease is responsible for a large percentage of the burden of kidney damage and dysfunction. This study aims to explain mitochondrial copies number changes in relation to both glycemic index and renal status in T2DM treated with metformin monotherapy visiting Al-Wafaa Diabetes Research Center in Mosul. During the period 1st January to 30 May 2021, 47 patients with T2DM (Mean age  $50.48 \pm 7.74$  years) were participate in this case-control study. These patients' results were compare to a control group of 47 seemingly healthy people (Mean age  $45.89 \pm 9.06$  years). All participants' demographic and medical histories were acquire through the delivery of a questionnaire. Blood samples collected and tested for the ubiquinone oxidoreductase chain 1 gene, HbA1c, uric acid, urea, and creatinine, among other things. In diabetics, there were extremely significant increases in Blood HbA1c, Serum urea, and creatinine ( $p < 0.001$ ,  $0.003$ , and  $0.043$ , respectively) when compared to the control group. In diabetic group, serum uric acid levels did not change significantly. HbA1c and uric acid had a strong negative correlation ( $r = -0.045$  and  $p < 0.05$ , respectively). In diabetic individuals, the number of mitochondrial copies was substantially lower than in the control group ( $p < 0.001$ ). In comparison to non-diabetic controls, diabetic patients treated with mono-metformin treatment had a lower mitochondrial copy number and moderate renal impairment.

**Keywords:** Mitochondria, H-ubiquinone oxidoreductase chain 1, HbA1c, Creatinine, Diabetes mellitus, Kidney dysfunction, Urea, Uric acid

عدد نسخ الميتوكوندريا وبعض المؤشرات الحيوية للوظيفة الكلوية عند المصابين

داء السكري من النوع الثاني على الميتفورمين

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### الخلاصة

يعد مرض السكري أحد أكثر أمراض التمثيل الغذائي شيوعاً في جميع أنحاء العالم. يؤثر هذا الاضطراب الأيضي بشكل كبير على وظائف الكلية. تهدف هذه الدراسة إلى شرح تغييرات عدد نسخ الميتوكوندريا فيما يتعلق بكل من مؤشر نسبة السكر في الدم والحالة الكلوية في T2DM تم علاجهم بالميتفورمين. الذين يزورون مركز الوفاء لبحوث مرض السكري في الموصل للفترة من 1 كانون الثاني إلى 30 آذار 2021. تتضمن دراسة الحالة والشواهد هذه 47 مريض (بمعدل اعمار  $50,48 \pm 7,74$  سنة) تم تشخيصهم بالفعل من مرضى T2DM. نتائج هؤلاء المرضى مقارنة بمجموعة السيطرة المكونة من 47 شخصاً بصحة جيدة (بمعدل اعمار  $45,89 \pm 9,06$  عاماً). البيانات الديموغرافية وكذلك التاريخ الطبي التي تم الحصول عليها من خلال إدارة استبيان لجميع المشاركين. تم أخذ القياسات الأنتروبومترية وتحليل عينات الدم لجين mt-ND1 و HbA1c وحامض اليوريك وجوهر البول والكرياتينين أظهرت النتائج وجود زيادات معنوية عالية في النسبة المئوية للسكر التراكمي وجوهر البول والكرياتينين ( $0,001 < p < 0,003$ ,  $0,043$ ،  $0,001$  على التوالي) في مرضى السكري مقارنة بمجموعة السيطرة. في حين لم يظهر حامض اليوريك أي تغير معنوي في مرضى السكري. كان هناك ارتباط سلبي معنوي بين HbA1c وحامض البوليك ( $r = -0,045$ ،  $0,05 < p < 0,001$ ). عدد نسخة الميتوكوندريا بشكل كبير في مرضى السكري مقارنة بمجموعة السيطرة ( $p < 0,001$ ).

الخلاصة كان مرضى السكري الذين تم علاجهم بالميتفورمين فقط يعانون من انخفاض في عدد نسخ بيوت الطاقة مع ضعف خفيف في وظائف الكلية، مقارنة بمجموعة السيطرة غير المصابة بالسكري.

الكلمات المفتاحية: بيوت الطاقة، NDI-gene، السكر التراكمي، الكرياتينين، السكري، ضعف الكلية، جوهر البول، حامض اليوريك

### Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia of carbohydrates, lipids, and proteins<sup>(1)</sup>. Globally, DM affects around 415 million individuals of all ages, with the number predicted to grow to 642 million by

2040. Diabetes has become a pandemic in poor countries, with low and middle-income economy nations accounting for 75 percent of DM cases. Furthermore, diabetes affects working age in low- and middle-income countries<sup>(2)</sup>.

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Diabetes mellitus classified into two types: type 1 (juvenile) and type 2 (adult) diabetes mellitus. Diabetes mellitus type 2 is the most common. T2DM is characterized by hyperglycemia, insulin resistance, and insulin insufficiency<sup>(4)</sup>. Diabetes is a long-term hyperglycemia that affects organs such as the heart, kidneys, eyes, nerves, and blood vessels<sup>(5)</sup>. T2DM is caused by a mix of genetic variables linked to insulin resistance and decreased insulin synthesis, as well as environmental influences such as obesity, overeating, inactivity, stress, and aging<sup>(6)</sup>. Metformin, a biguanide derivative, is one of the most often prescribed medications for the treatment of type 2 diabetes (T2D) and has been in use for almost sixty years<sup>(7)</sup>. While it has a minimal risk of hypoglycemia, it has a higher risk of gastrointestinal side effects and is not recommended for persons with renal failure<sup>8</sup>. Because renal function declines with age, elderly metformin users should be evaluated on a regular basis.<sup>(9)</sup> Metformin is contraindicated or should be taken at reduced dosages depending on renal function, according to clinical recommendations in the United Kingdom, Canada, and Australia.<sup>(10)</sup> Metformin usage has also been linked to an increased risk of lactic acidosis, but this has not been widely documented.<sup>(11)</sup> Diabetic nephropathy (DN), one of the most prevalent and dangerous complications of diabetes mellitus, affects approximately one-third of diabetic patients and is associated with increased morbidity and mortality in diabetic patients. DN occurs during long periods of inactivity, which can last several years. The biggest risk factors for developing DN are high blood pressure and persistent hyperglycemia. Poor treatment outcomes are the result of a difficult and insufficient knowledge of the pathogenesis of DN.<sup>(12)</sup> In addition to excreting waste products and poisons such as urea, creatinine, and uric acid, the kidneys controlled the volume of extracellular fluid, osmolality of the serum, electrolyte concentrations, and the production of hormones such as erythropoietin, 1, 25-dihydroxyvitamin D, and renin. The findings of renal function tests determine how persons with kidney disease or diseases that impair renal function are treated. Renal function tests are useful for detecting renal disease, assessing the kidneys' response to therapy, and determining the course of renal sickness. The National Institutes of Health estimate that 14% of people have chronic kidney disease (CKD).<sup>(13-16)</sup> The best indicators of progression and prognosis are serum creatinine and blood urea nitrogen levels, instituting dietary restrictions in the renal disease in type 2 DM.<sup>(17)</sup> In the degradation of purine nucleotides, uric acid is the final enzymatic product. High uric acid serum concentration is a risk factor for T2DM, according to a major epidemiological research on adult Japanese males. Despite the fact that hyperuricemia has been identified as a risk factor for T2DM in

studies, there are conflicting views on the relationship between the two.<sup>(18)</sup> Mitochondria are the power houses, due to ability to produce high-energy compound in form of ATP and any change in mitochondrial function will reflect on metabolic status. T2DM patients have high blood sugar and insulin resistance and both condition associated with elevated ROS formation that in turn significantly affect mt-DNA copies number.<sup>(19)</sup>

This study aimed at explaining the mitochondrial copies number changes in relation to both glycemic index and renal status in T2DM on metformin monotherapy.

## Subjects and Method

The Al-Wafaa Center for Diabetes Management and Research in Mosul conducted this case-control research, between first of January to 30 May 2021. Screening and management of patients were done as per American Diabetes Association guidelines (ADA, 2018).<sup>(20)</sup> Subjects with HbA1c 6.5% were considered as cases, and HbA1c 4.7-5.4% were also considered as controls. Study included 47 subjects (23 Males and 24 Females) which were diagnosed type 2 Diabetes mellitus with age range between (25-65) years, on metformin therapy with dose range between (500mg/day-1000mg/day) with durations from two to six months. A control group of 47 apparently healthy participants (20 Males and 27 Females) with ages ranging from (30-60) years old and no illness or treatment was included. For all of the patients, demographic information such as age, height, weight, and body mass index (BMI) were recorded. Body mass index (BMI) was computed according to the following equation: BMI (kg/m<sup>2</sup>) is calculated by dividing body weight (kg) by the square of body height in meters (m<sup>2</sup>).<sup>(21)</sup>

Documented renal or hepatic illnesses, a history of alcohol use, hepatitis B and C virus infection HBsAg positive and HCV antibody positive, pregnant women, Patients with autoimmune disorders were also excluded from the study.

## Blood sample collection

After an eight-hour Overnight fasting, five milliliters (5ml) of venous blood were taken from patients on metformin monotherapy and healthy controls using a disposable syringe. The blood was then split into two aliquots, one for clotting in a gel tube at room temperature and the other for mt-DNA extraction and HbA1c testing in an EDTA tube. The serum was tested for urea, creatinine, and serum uric acid using a multi-chemical fully automated chemistry analyzer and the GIESSE DIAGNOSTICS manufacturer's kit (Italy). Biohermes kit was used to test HbA1c (China), hemoglobin A1c was determined by the Boronate Affinity Chromatography method.<sup>22</sup> Urea, by the urease-hypochlorite method, Creatinine by alkaline picrate method, and uric acid, by the uricase-

peroxidase method. Mitochondrial genome extracted using AddPrep Genomic DNA Extraction Kit (China). 100ng/µl sample used for q-PCR analysis with Go-Taq qPCR master mix from Promega (A6000).<sup>23</sup> Homo sapiens mt-ND1 primers was design using NCBI program. Forward sequence, (ATTATCGCCCAACCCTCTC) and Reverse sequence (GCTCGTAGGGCTCCGAATAG). GAPDH gene used as housekeeping gene with Forward sequence (CGGGTCTTTGCAGTCGTATG) and Reverse sequence (CTGTTTCTGGGGACTA GGG G). Software for Eco studies analyzes data. The mean and standard deviations are used to depict the data in this investigation. All statistical analyses were performed using Excel 2010. The differences in parameters between the two groups were investigated using the Student's t-test. A p-value of 0.05 or less is regarded as statistically significant.

**Results**

According to this study, the number of mitochondrial copies significantly decreased in

T2DM patients by 32.8 times compared to the control group (p 0.01). The mean level of HbA1c was significantly higher in the diabetic subjects when compared with control group (7.76 ± 1.94 Vs 5.18 ± 0.49; p=0.001).

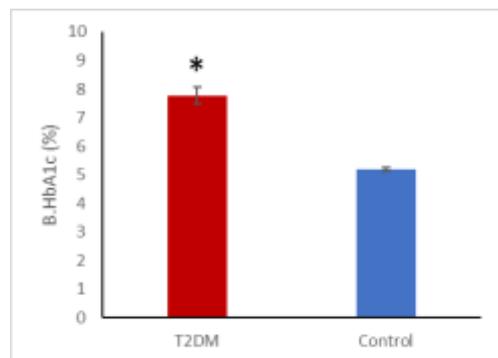
The mean level of urea and creatinine was significantly higher in the diabetic subjects when compared with the control group. (28.31 ± 8.24 Vs 24.27 ± 5.81; p=0.003 and 0.94 ± 0.20 Vs 0.88 ± 0.08; p=0.043, respectively). Whereas no significant changes in uric acid levels (4.91 ± 0.82 Vs 4.67 ± 1.34; p=0.252), There was also no significant difference in their body mass index, (p = 0.542). in our study as shown in (Table 1; Figures 1–5). In addition, Blood HbA1c and Serum uric acid had a significant negative association (r = -0.045, p<0.05). uric acid and urea have a significant positive association (r = 0.019, p< 0.05). uric acid and creatinine had a substantial negative association (r = -0.00048, p< 0.05). in our study as shown in (Table 2).

**Table 1. Comparison of mean values of variables in T2DM and control groups (N=94)**

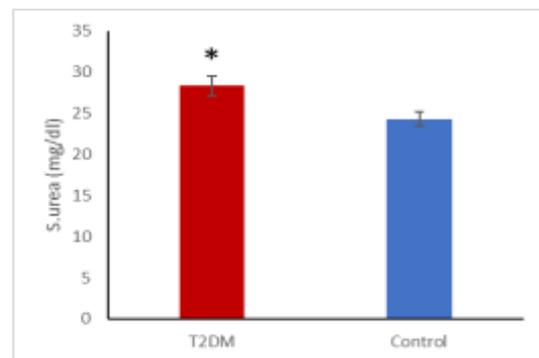
Variables	Mean ± SD		
	T2DM N=47	Control N=47	P value
Age (Years)	7.74 ± 50.48	9.06 ± 45.89	0.011 *
BMI (K/m <sup>2</sup> )	33.5 ± 6.33	21.87 ± 1.82	0.542
HbA1c (%)	7.76 ± 1.94	5.18 ± 0.49	0.001 *
Urea (mg/dl)	28.31 ± 8.24	24.27 ± 5.81	0.003 *
Creatinine (mg/dl)	0.94 ± 0.20	0.88 ± 0.08	0.043 *
Uric Acid (mg/dl)	4.91 ± 0.82	4.67 ± 1.34	0.252

**Table 2. Correlation of parameters in diabetic patients.**

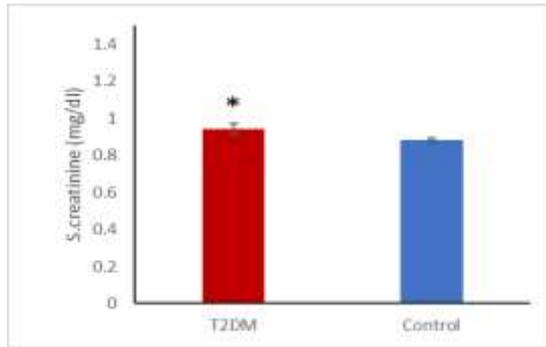
Parameters	r-value	p-value
HbA1c Vs Uric Acid	-0.054	< 0.05
Uric Acid Vs Urea	0.019	< 0.05
Uric Acid Vs Creatinine	-0.00048	< 0.05



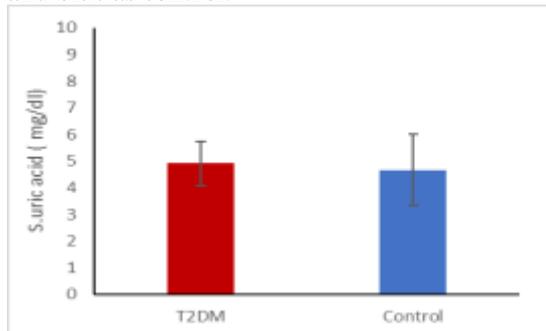
**Figure 1. Blood HbA1c percentage levels in T2DM and control individuals, the red color bar as T2DM and blue as control.**



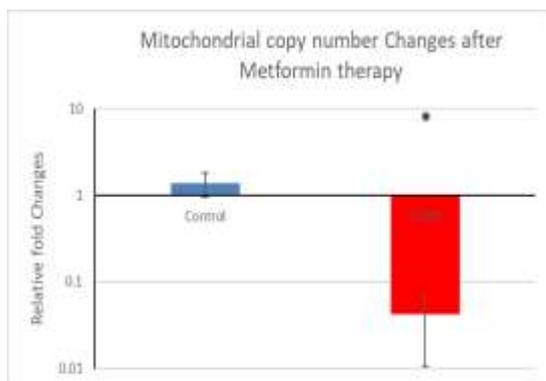
**Figure 2. Serum urea levels in T2DM and control individuals, the red color bar as T2DM and blue as control.**



**Figure 3.** Serum creatinine levels in T2DM and control individuals, the red color bar as T2DM and blue as control.



**Figure 4.** Serum uric acid levels in T2DM and control individuals, the red color bar as T2DM and blue as control.



**Figure 5.** Measurement of mitochondrial DNA copies number (mtDNA) in control and in T2DM group on metformin monotherapy.  $n = 47$ ;  $*p < 0.01$ . the red color bar as T2DM and blue as control.

## Discussion

This study found that the number of mitochondrial copies in T2DM patients was 32.8 times lower than in the control group ( $p < 0.01$ ). This is consistent with the findings of (Cho *et al.*, 2017), who discovered a substantial reduction in mitochondrial DNA (mtDNA) copy number in individuals with type 2 diabetes ( $p < 0.01$ ).<sup>(24)</sup>

In This work results showed that there were highly significant increases in the mean of HbA1c ( $p < 0.001$ ), Urea ( $p = 0.003$ ) and Creatinine ( $p = 0.043$ ) in diabetic population when compared to control as shown in (Table 1)

HbA1c ( $p = 0.0001$ ) was significantly higher in T2DM patients compared to controls in a research by Kashinakunti, S. *et al.*<sup>(25)</sup>

Additionally, Mishra *et al* discovered that diabetics had substantially higher levels of blood urea and serum creatinine compared to controls ( $p < 0.001$ ).<sup>(26)</sup>

The serum uric acid levels in Type 2 diabetes patients were not significantly higher than in healthy control subjects, according to this study. Various other studies, such as those done by Fazlani *et al*<sup>(27)</sup>, Kumari and Sankar<sup>Narayana(28)</sup> showed conflicting results which indicated that patients with Type 2 diabetes have higher serum uric acid levels than healthy people.<sup>(27,28)</sup>

The results of a previous work found that serum uric acid negatively correlation to HbA1c in both male and female groups (Male:  $r = -0.224$ ,  $p = 0.000$ ; Female:  $r = -0.245$ ,  $p = 0.000$ ). In the current study, there is a significant negative correlation between HbA1c and Uric acid in the patient group ( $r = -0.045$ ,  $p < 0.05$  Serum uric acid decreased by 3.868 units in the male group and 6.036 units in the female group when HbA1c increased by one unit after adjusting for age, BMI, SBP, DBP, TG, and creatinine.

The current study's findings contrasted with those of Babikr *et al*<sup>(30)</sup>, who reported that blood uric acid levels in diabetics were positively connected with HbA1c ( $r = 0.135$ ,  $p = 0.026$ ). In healthy controls, there was a statistically insignificant positive relationship between uric acid and HbA1c ( $r = 0.037$ ,  $p = 0.106$ ).<sup>(30)</sup>

As demonstrated in the graph, there was a substantial negative association between uric acid and creatinine ( $r = -0.00048$ ,  $p < 0.05$ ) and a significant positive correlation between uric acid and urea ( $r = 0.019$ ,  $p < 0.05$ ) in the current study (Table 2). According to Hu *et al*<sup>(31)</sup>, serum uric acid levels were favorably associated with blood urea levels. Serum creatinine ( $r = 0.23$ ,  $p < 0.0001$ ;  $r = 0.35$ ,  $p < 0.0001$ ) in Male and Female, respectively. 31 The major limited of this work was the small sample size.

## Conclusion

T2DM patients treated with mono-metformin therapy were experiencing low mitochondrial copies number with mild kidney dysfunction, compared to non-diabetic controls in relation to renal function.

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