

*Research Article***Study the level Of micro RNA146 a in human serum in the diabetic patients****Sahar H. El-Hini, Atef F. El-Akad and Mariam Y. Nagib**

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**Abstract**

**Introduction:** Diabetes mellitus is an endocrine disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced which results in increased concentration of glucose in the blood. Diabetic nephropathy (DN) is considered as one of the diabetic complications affecting up to 40% of patients with type 1 or type 2 diabetes. **Aim of the study:** The aim of our study is to evaluate the level of microRNA-1460a in human serum in diabetic nephropathy in prediabetic and diabetic patients in comparison to the apparent healthy individuals group. **Patients and Methods: Subjects:** The current study, Acase control study was conducted on 100 persons, 24males (24%) and 76 females (76%) of the same age group (40-65 yrs) were selected from outpatient clinic of diabetes of Internal Medicine Department at Minia University Hospital from June 2017 to June 2018. **Results:** The serum level of miR-146a of was significantly increased in diabetic group more than that of the Pre-diabetic and control groups, With p value ( $< 0.001$ ). In addition, the levels of circulating serum miR-146a gradually and markedly increased and was significantly higher in T2DM with macroal-buminuria group than that in T2DM with microal-buminuria ,T2DM with normal-buminuria and control groups. The results revealed that circulating serum miR-146a was positively correlated to UACR ( $r=0.724, p<0.001$ ), Scr ( $r=0.436, p<0.001$ ) and was negatively correlated to eGFR ( $r=-0.450, p<0.001$ ). **Conclusion:** We recommend using serum level of miR-146a as new biomarkers for diagnosis of diabetes ,early detection of DN and monitor the progression of DN .

**Keywords:** Diabetic nephropathy, Micro RNA, Prediabetic, Diabetic**Introduction**

Diabetes mellitus is an endocrine disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced which results in increased concentration of glucose in the blood, which in turn damage many of the body's systems in particular the blood vessels and nerves <sup>(1)</sup>.

In 2019, the International Diabetes Federation estimated that 463 million people aged 20-79yrs with diabetes in the world. That number is projected to rise to 700 million by 2045.The majority of the DM burden in Africa appears to be type 2 DM, with less than 10% of DM cases being type 1 DM <sup>(2)</sup>.

In Egypt is around 15.6% of all adults aged 20 to 79yrs <sup>(3)</sup>. Diabetes is classified to type 1 and type2, Type 1 diabetes mellitus (T1DM) is an

autoimmune disorder resulting from lymphocyte-mediated destruction of insulin producing  $\beta$  cells, whereas, Type2 diabetes mellitus (Formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by high blood sugar in the context of insulin resistance and relative lack of insulin secretion <sup>(4)</sup>.

Individuals with Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are described as "Pre-diabetes" indicating the relatively high risk for development of diabetes in these patients <sup>(5)</sup>.

Diabetes cause many complications, macrovascular complications linked to arteries that nourish the myocardium, brain and limbs; as well as micro vascular complications, such as retinopathies, nephropathies, and neuropathies <sup>(6)</sup>.

Diabetic nephropathy (DN) is the most serious microvascular complications of both type 1 and type 2 diabetes, affect approximately 20% to 40% of Diabetic patients and is the leading cause of CKD and ESRD Development . DN accounts for approximately 40% of diagnosed end-stage kidney failure <sup>(7,8)</sup>.

MicroRNAs, short noncoding RNAs with 22–25 nucleotides long, is able bind to the 3' untranslated region (3' UTR) of its target messenger RNA (mRNA) by imperfect complementary manner, leading to post transcriptional gene silencing <sup>(9)</sup>.

More than 2,000 human mature miRNAs have now been identified, and at least 60% of all human protein-coding genes are known to be regulated by miRNAs <sup>(10)</sup>.

### Aim of the study

The aim of our study is to evaluate the the level of microRNA-146a in human serum in diabetic nephropathy in prediabetic and diabetic patients in comparison to the apparent healthy individuals group.

### Patients and Methods

#### Subjects:

The current study, Acase control study was conducted on 100 persons, 24 males (24%) and 76 females (76%) of the same age group (40-65 yrs) were selected from outpatient clinic of diabetes of Internal Medicine Department at Minia University Hospital from June 2017 to June 2018.

#### This study subject comprises the following groups:

**Group I:** prediabetic group, It included 20 patients 4(20%) males and 16(80%) females, their age ranges from (44-62) years. this group was selected according: to criteria of ADA 2017:

Screen all adults for prediabetes starting at age 45 and all adults of any age who are overweight (BMI  $\geq 25$ ) + one of the risk factor,(habitual physical inactivity, first degree relative with diabetes history of gestational diabetes mellitus., polycystic ovary syndrome, H.T.N: 140/90 or on medications, HDL  $\geq 35$  mg/dl or triglyceride  $\geq 250$  mg/dl, Hb A1C  $\geq 5.7 - 6.4\%$ , impaired Fasting blood Glucose (IFG) = FPG

100-125mg/dl, impaired Glucose Tole-rance (IGT) = 2hPG140 -199mg/dl, other clinical conditions associated with insulin resistance e.g. obesity, High risk ethnic popu-lation, for all patients, start testing at age 45yrs, If results normal, repeat test at 3 year intervals or more frequently if high risk (i.e. prediabetes).

**Group II:** Diabetic group, It included 60 diabetic patients 14(23.3%) males and 46 (67.7%) females, their age ranges from (40-62 years), this group divided into three groups according to the level of albuminuria:

Normoalbuminuria group, (UACR  $< 30$  mg/g) It included 20 diabetic patiants,4(20%) males and 16(80%) females.

Microalbuminuria group, (UACR 30– 300 mg/g) It included 20 diabetic patiants,4(20%) males and 16(80%) females.

Macroalbuminuria group, (UACR $> 300$  mg/g) It included 20 diabetic patients with macroalbuminuria, 6(30%) males and 14(70%) females.

#### This group was selected according: to criteria of ADA 2017:

Hb A1C  $\geq 6.5\%$ .

FPG  $\geq 126$  mg/dl.

Random plasma glucose at any time of day without regard to time since last meal  $\geq 200$ mg/dl plus symptoms suggestive of DM as (polyuria, polydepsia,and unexplained wt loss.). Two-hour plasma glucose (2hPG)  $\geq 200$  mg/dl

**Group III:** controlled group, It included 20 healthy volunteers persons ( free of any acute or chronic medical disease) 6 (30%) males and (70%) females, their age ranges from (44-60 years).

### Results

The current study, Across sectional study was conducted on 100 persons, 24 males (24%) and 76 females (76%) of the same age group (25-60 yrs) were selected from outpatient clinic of diabetes of Internal Medicine Department at Minia University Hospital from June 2017 to June 2018 .

**Table (1)** The table showed the comparison between the studied groups regarding MicroRNA146a level:

As regarding MicroRNA 146a level was significantly increase in diabetic group compared to pre diabetic and control group with (p <0.001 ).

**Table (1):** Comparison between the studied groups regarding MicroRNA 146a level.

		Group I (Control)	Group II (Pre-DM)	Group III (DM)	P value		
		N=20	N=20	N=60			
<b>Micro RNA 146a</b>	<b>Range Mean ± SD</b>	(22-25.5)	(44-55)	(51-117)	<0.001*		
		24.5±1	49.9±3.3	82.9±19.7	I vs II	I vs III	II vs III
					<0.001*	<0.001*	<0.001*

- *Student t test , One-way ANOVA test for parametric quantitative data between the three groups followed by post hoc analysis between each two groups*
- *\*: Significant level at P value < 0.05.*

**Table (2)** The table showed the comparison of MicroRNA146a level of the diabetic patients according to the level albuminuria (normo-albuminuria, microalbuminuria, and macro albuminuria):

As regarding micro RNA 146a there was significant increase in micro group compared to normal group, and in macro group compared with other two groups (p<0.001)

**Table (2):** comparison of MicroRNA146 a level of the diabetic patients according to the level albuminuria:

		Normal	Micro- albuminuria	Macro- albuminuria	P value		
		N=20	N=20	N=60			
<b>Micro RNA 146a</b>	<b>Range Mean±SD</b>	(51-71)	(69-99)	(66-117)	<0.001*		
		61.6±6.3	84.5±9.2	102.5±14.1	I vs II	I vs III	II vs III
					<0.001*	<0.001*	<0.001*

**Discussion**

Diabetes mellitus (DM) is a chronic serious metabolic disease which is a growing problem worldwide with many complications, The global diabetes prevalence according to International Diabetes Federation (IDF) in 2019 is estimated to be 9.3% (463 million people aged 20–79 years), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The majority of the DM burden in Africa appears to be type (2) DM, with less than 10% of DM cases being type (1) DM (2).

DN is found in 20-40% of the individuals with DM and ranks as the main cause of end-stage renal disease and one of the main reasons for prescribing dialysis to individuals with DM . It has been described as one of the main causes of death of diabetic patient.<sup>(11,12)</sup>

Diabetes cause many complications, macrovascular complications linked to arteries that nourish the myocardium, brain and limbs; as well as microvascular complications, such as retinopathies, nephropathies and neuropathies<sup>(8)</sup>.

Early diagnosis of DN can prevent the progression of renal disease and the onset of cardiovascular events. New markers are required to assess renal function, since glomerular filtration rate (GFR) and urinary albumin excretion (UAE) have limited use in detecting early-stage DKD. because urinary microalbuminuria, it is not sensitive, as tissue damage and inflammation have already occurred by the time that microalbuminuria is detectable<sup>(8,13)</sup>

Furthermore, microalbuminuria is not specific to DN but is merely a hallmark of glomerular lesions. Renal biopsy is the gold standard for diagnosing DN, However, it is a highly invasive and expensive procedure, with a potential risk of biopsy-associated bleeding complications. Thus, sensitive and reliable biomarkers are needed for DN. <sup>(14)</sup>

MicroRNAs are small non-coding RNA molecules containing about 22 nucleotides. They are responsible for the post-transcriptional regulation of gene expression. MiRNAs recognize complementary sequences in the 3'-untranslated region (3'-UTR) of target mRNAs leading to decreased protein expression either by mRNA degradation and/or by translational repression of protein synthesis. <sup>(11,13,15)</sup>

MiRNA-146a, one of the inflammation-related miRNAs, located on chromosome 5q33. It has been extensively studied in the fields of inflammation, immunity, and cancer development. The role of miRNA-146a in the pathogenesis of inflammation and other degenerative aspects may make it participate in the course of T2DM.<sup>(16)</sup>

### Conclusion and Recommendations

In this study, we had revealed that the increase level of miR-146a have an association with diabetic renal damage and development of DN in T2DM patients. and they may serve also as a potential biomarkers for the identification of T2DM.

In conclusion, our results suggest that increase serum miR-146a, may become a new biomarkers of early diagnosis of DN and maybe better than urinary albumin and could predict the development of microalbuminuria at least 2 years before it occurred. Additionally, its levels change with the progression of DN, thus possibly making it useful markers to monitor the progression of DN.

We recommend using serum level miR-146a as new biomarkers for diagnosis of diabetes, early detection of DN and monitor the progression of DN .

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