The Egyptian Journal of Hospital Medicine (October 2021) Vol. 85 (2), Page 4046-4051

Study of MicroRNA192 as an Early Marker of Nephropathy in Type 2 Diabetic Patients

Esam Eldin Mahmoud Lotfy[1], Mohammed Fouad Ahmed Ayoub[1], Lamiaa Abd Elwahab Mohamed[2], Hesham Mohamed Mitwally Elsayed[3]
Departments of [1]Internal Medicine and Nephrology and [2]Clinical Pathology,
Faculty of Medicine Zagazig University, Egypt
Department of [3]Internal Medicine and Nephrology, Suis Canal Authority Hospital, Egypt
***Corresponding Author:** Hesham Mohamed Mitwally Elsayed, **Mobile:** 01222192645 **Email:** hishammitwally@gmail.com

ABSTRACT

Background: MicroRNAs (miRNAs) are endogenous ubiquitous non-coding single-stranded (ss) RNA transcripts, frequently of 19–25 nucleotides in length. They alter the differentiation, growth, apoptosis and proliferation of cells by interfering with protein synthesis by either inducing mRNA degradation or repressing translation. miRNAs are expressed in many diseases and different cancers such as diabetes and have the potential to become new kinds of diagnostic markers. miRNA-192 is highly expressed in kidney especially in renal cortex. Many studies have confirmed that miRNA-192 played important roles in the fibrosis of kidney and liver.

Objective: This study aimed to evaluate the use of microRNA-192 as early predictor in cases of diabetic nephropathy.

Patients and methods: This study was conducted on volunteers from Internal Medicine Department - Suez Canal Authority Hospitals. 80 subjects were divided into: 60 patients with type 2 diabetes mellitus (T2DM) who were further subdivided into (normal albuminuria group (n= 20), microalbuminuria group (n= 20), and macroalbuminuria group (n=20)), and 20 healthy control group. MicroRNA-192 was quantified in blood using Reverse Transcription TaqMan MicroRNA Assay.

Results: There was a statistical significance decrease in micro RNA-192 level in macro-albuminuria group compared to other groups and also in microalbuminuria group compared to normal albuminuria group and healthy control group.

Conclusions: We concluded that there is a possible role of miRNA-192 in the pathogenesis and progression of diabetic kidney disease in humans. Also, blood miRNA-192 may be a useful biomarker for predicting the development and the stage of diabetic kidney disease.

Keywords: T2DM, MicroRNAs, Nephropathy, Diabetes.

INTRODUCTION

The incidence of type 2 diabetes mellitus (T2DM) has increased significantly, especially in developed countries. Many studies have speculated that diabetes mellitus causing microvascular and macrovascular pathological conditions could result in various complications leading to a sever morbidity in T2DM subjects. Approximately, 40% of T2DM patients develop diabetic nephropathy. Diabetic nephropathy (DN) is a progressive kidney disease caused capillaries damage of the kidneys' glomeruli **(1).**

Microalbuminuria is widely-used as an early marker for nephropathy in diabetic patients. TGF- β families are essential for regulation of cellular growth, differentiation and apoptosis, as well as immune suppression. TGF-β1 has been known as a key mediator in extracellular matrix formation. In fibrosis and in tissue remodeling during disease progression in different organs, up-regulation of TGF-β1 is informed to be necessary, in which glomerular fibrosis in the kidney is included **(2)**.

Inflammatory cytokines implicated in the pathogenesis of diabetes play a significant role in several renal disorders development and progression, including diabetic nephropathy. Effects of them on renal disease involved the expression of different molecules, intraglomerular abnormalities, alteration of extracellular matrix, apoptosis and necrosis, endothelial permeability and oxidative stress, which cause the development of microvascular diabetic complications as neuropathy, retinopathy, and nephropathy. IL-18 is a member of the IL-1 family and was primarily described as an interferon gamma inducing factor. It has been associated with obesity, insulin resistance, and dyslipidemia. Circulating levels of IL-18 have consistently been reported to be elevated in patients with T2DM in different studies, and have also been suggested to participate in microangiopathy such as nephropathy in T2DM**(3).**

MicroRNAs (miRNAs) are endogenous ubiquitous non-coding single-stranded (ss) RNA transcripts, frequently of 19–25 nucleotides in length that alter the differentiation, growth, apoptosis and proliferation of cells by interfering with protein synthesis by either inducing mRNA degradation or repressing translation **(4).** Specifically, miRNAs are expressed in many diseases and different cancers such as diabetes, hepatic cancer, prostatic cancer, breast cancer, gastric cancer, squamous cell carcinoma, lymphoma, colon cancer, and lung cancer. Serum miRNAs phenotypes have the potential to become new kinds of diagnostic markers. MiRNA-192 is highly expressed in kidney especially in renal cortex. Many studies have confirmed that miRNA-192 played important roles in the fibrosis of

Received: 25/7/2021
Accepted: 21/9/2021

kidney and liver, but the effect of miRNA-192 in DN are still controversial [5].

In the present study, we aimed to investigate the ability of early prediction of diabetic nephropathy by use of micRNA-192.

PATIENTS AND METHODS:

This study was conducted on 80 volunteers (49 male patients and 31 female patients) from Internal Medicine Department - Suez Canal Authority Hospitals.
Inclusion criteria: Age > 18 years males and females. Patients with T2DM at least 5 years ago. Patients with stable renal function without doubling of serum creatinine in the last 5 months. Diabetic patients on oral hypoglycemic medications or insulin therapy.
Exclusion criteria: Age <18 years. Patient with Type 1 diabetes mellitus. Patients with Serum creatinine above 2.5 mg/dl and Serum potassium above 5.5 mEq/L. Allergy or intolerance to ACEI or ARB. Class III or IV heart failure or angina. Hospitalization in the last 3 months. Pregnant females. Ongoing chemotherapy. Patients with hematuria or any clinical or laboratorial findings suggestive of associated non-diabetic glomerulopathy.

Subjects and Grouping:

The study included a total number of 80 subjects that were divided into four groups:
A- Sixty T2DM patients were subdivided according to their urinary (ACR) into 3 groups:
Group I: with normal albuminuria (ACR < 30 mg/g, n= 20), group II: with microalbuminuria (ACR: 30-300 mg/g, n=20), and group III: with macro albuminuria (ACR >300mg/g, n=20).
B- Twenty control healthy subjects were matched for age and sex as control group (Group IV).

Measurements of some parameters:

All participants were subjected to the following: Full history taking including (age, sex) and full clinical examination. Blood samples: 5CC of venous blood were taken from each patient and divided into 2 parts: The 1st part for complete blood count, fasting blood glucose, glycosylated hemoglobin, serum ALT, AST, bilirubin and albumin, serum creatinine, blood urea, serum total cholesterol level, serum triglycerides, high density lipoproteins and low-density lipoproteins and Estimated glomerular filtration rate (eGFR) according to MDRD. The 2nd part was used for miRNA detection. It was centrifuged at 10000 rpm for 10 minutes then serum was separated and kept frozen at -80 °C till analysis. Early morning urine sample for testing for albumin/creatinine ratio (ACR) repeated twice 3 months apart.

MicroRNA was isolated using miRNA extraction kit supplied by mirVanaTM PARISTM Kit, ambion, USA. Nanodrop® spectrophotometer was used to measure the absorbance of isolated RNA at 260 nm, 280 nm and 230 nm. The Nanodrop® instrument can measure using only 0.5 -2 ng/µl up to 12,000 ng/µl. Absorbance at 260 nm was used to measure the amount of nucleic acid present in the sample, absorbance at 280 nm was used to estimate the amount of protein in the sample, and absorbance at 230 nm was used to determine the amount of other contaminants that may be present in the sample such as guanidine thiocyanate common in nucleic acid purification kits. Detection and quantification of the amplified miRNA were achieved using real time PCR by The TaqMan® MicroRNA Assays. Amplification of cDNA was carried out on step-one real- time PCR system (Applied Biosystems). It was hold at 50°C for 2 minutes with an initial step of enzyme activation at 94 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing and extension at 60 v for 60 seconds.

Ethical approval:

This study was ethically approved by The Ethics Committee, Faculty of Medicine, Zagazig University. Informed consent was obtained from every patient in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). One-way ANOVA for continuous normally distributed variables. Post hoc analysis after ANOVA was performed using the Tukey test. with post hoc analysis by means of the Mann–Whitney U test. P value < 0.05 was considered significant.

RESULTS

Table (1) showed that there was no statistically significant difference between the studied groups in height or sex distribution. There was a statistically significant increase in age in group III compared to other groups and also in group II compared to groups I & IV. Regarding weight and BMI, there was a statistically significant increase in both in group III compared to other groups and also in group II compared to groups I & IV and in group I compared to IV.

Table (1): Comparison of demographic data of the studied groups

Variable	Group I (normo-albuminuria) (n=20)		Group II (Micro-albuminuria) (n=20)		Group III (Macro-albuminuria) (n=20)		Group IV (Control) (n=20)		P
Age (years) Mean ± SD	51.85 ± 3.47		54.70 ± 3.76		58 ± 5.05		51.5 ± 3.47		< **0.001****
Range	45 - 57		46 - 61		49 - 67		45 - 57		
Weight (Kg): Mean ± SD	82.85 ± 7.09		88.9 ± 10.72		97.75 ± 9.39		74.05 ± 7.94		< **0.001****
Height: (m) Mean ± SD	171.7 ± 5.81		171 ± 6.32		171.35 ± 5.61		171.55 ± 6.05		0.98 NS
BMI (Kg/m^2): Mean ± SD	28.15 ± 2.64		30.40 ± 3.34		33.31 ± 3.03		25.14 ± 2.12		< **0.001 ****
Variable	**No**	**%**	**No**	**%**	**No**	**%**	**N**	**%**	**P**
Sex									
Female	9	45	7	35	7	35	8	40	0.90 NS
Male	11	55	13	65	13	65	12	60	

SD: Stander deviation, F: ANOVA test, χ^2: Chai square test NS: Non-significant (P > 0.05) **: highly significant (P < 0.001) LSD: Groups with different letters are statistically significant (P < 0.05)

Table (2) showed that there was no statistically significant difference between the studied groups in Hb, WBCs, platelets or bilirubin. But, there was a statistically significant increase in albumin in group III compare to other groups and also in group II compared to group I & IV. Regarding AST & ALT, there was a statistically significant increase in both in group III & II compared to other groups and also in group I compared to group IV.

Table (2): Comparison of CBC and LFT among the studied groups

Variable	Group I (normo-albuminuria) (n=20)	Group II (Micro-albuminuria) (n=20)	Group III (Macro-albuminuria) (n=20)	Group IV (Control) (n=20)	P
Hb: (gm/dl) Mean ± SD	12.76±1.25	13.02±1.26	13.24±1.27	13.63±0.94	0.13 NS
WBCs: (×10^3/ul) Mean ± SD	5.88±1.35	6.1±1.37	6.02±1.36	5.88±0.93	0.93 NS
Platelets:(×10^3/ul) Mean ± SD	294.05±6.69	311.5±6.83	317.65±6.97	345.95±6.35	0.06 NS
Albumin: (g/dL) Mean ± SD	6.65±0.72	108.95±0.72	359.4±12.12	5.45±0.72	<**0.001 ****
AST: (u/L) Mean ± SD	26.8±4.46	32.1±4.39	33.85±7.08	20.65±4.28	<**0.001 ****
ALT: (u/L) Mean ± SD	22.3±4.16	26.65±4.60	29.45±7.13	16.9±3.31	<**0.001 ****
T. bilir: (mg/dl) Mean ± SD	0.87±0.19	0.81±0.16	0.82±0.16	0.93±0.16	0.07 NS

SD: Stander deviation, F: ANOVA test, NS: Non-significant (P > 0.05) **: highly significant (P < 0.001)
LSD: Groups with different letters are statistically significant (P < 0.05)

Table (3) showed that there was a statistically significant increase in FBS & cholesterol level in group III compared to other groups and also in group II compared to groups I & IV and in group I compared to group IV. Regarding HbA1c & LDL, there was a statistically significant increase in both in groups III & II compared to other groups and also in group I compared to group IV. IN TG, there was a statistically significant increase among all cases groups compared to group I. Finally in HDL, there was a statistical significance decrease in its level in group III compared to other groups. Also, this table showed that there was a statistically significant increase in creatinine level and albumin create ratio in group III compared to other groups and also in group II compared to groups I & IV. This table also showed that there was a statistically significant increase in urea and a statistically significant decrease in eGFR in group III compared to other groups and also in group II compared to groups I & IV and in group I compared to group IV. Regarding uric acid, there was a statistically significant increase in its level in group III compared to other groups. And there was a

statistically significant decrease in micro RNA-192 level in group III compared to other groups and also in group II compare to groups I & IV. No difference was found between group I & group IV.

Table (3): Comparison of blood sugar parameters, lipid profile, kidney function and micro RNA-192 among the studied groups

Variable	Group I (normo-albuminuria) (n=20)	Group II (Micro-albuminuria) (n=20)	Group III (Macro-albuminuria) (n=20)	Group IV (Control) (n=20)	P
FBS: (mg/dl) Mean ± SD	182.85±6.06	207.3±6.4	218.3±6.4	93.85±6.06	<**0.001****
HbA1c: (%) Mean ± SD	7.72±0.31	8.42 ± 0.31	8.52±0.31	5.02±0.31	<**0.001****
Cholesterol:(mg/dL) Mean ± SD	155.45±6.98	175.45±6.89	185.45±6.98	150.45±7.01	<**0.001****
TG:(mg/dL) Mean ± SD	144.35±7.94	148.35±7.90	148.2±9.24	131.35±7.94	<**0.001****
HDL: (mg/dL) Mean ± SD	40.35±4.08	41.36±4.00	34.35±4.09	43.35±4.10	<**0.001****
LDL: (mg/dL) Mean ± SD	97.15±7.21	108.05±6.4	111.05±6.4	86.05±6.4	<**0.001****
Creatinine:(mg/dL) Mean ± SD	0.71±0.05	0.81±0.08	0.93±0.09	0.70±0.05	<**0.001****
Urea: (mg/dl) Mean ± SD	32.25 ± 4.29	37.4±4.07	44.1±3.96	19.55±3.95	<**0.001****
Uric acid: (mg/dl) Mean ± SD	3.96±0.82	3.91±0.78	5.31±1.17	3.95±0.81	<**0.001****
eGFR: (ml/min) Mean ± SD	103.42±6.65	96.67±8.23	79.67±8.23	115.07±8.23	<**0.001****
Albu.creat.ratio:(mg/g) Mean ± SD	1.04±0.23	15.65±1.37	33.12±2.31	0.73±0.03	<**0.001****
Micro RNA 192: Mean ± SD	0.95±0.09	0.61±0.09	0.32±0.09	1.01 ± 0.09	<**0.001****

SD: Stander deviation, F: ANOVA test, **: highly significant (P<0.001)LSD: Groups with different letters are statistically significant (P<0.05)

Table (4) showed that at cut off < 0.95 micro RNA had sensitivity of 88.3%, specificity of 75% and accuracy of 86.3% in diagnosis of DM.

Table (4): Validity of micro RNA-192 in diagnosis of DM among the studied groups:

Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	95% CI	P
<0.95	88.3	75	93	69.6	**86.3**	**0.90**	**0.83-0.96**	**<0.001 ****
<0.95	88.3	75	93	69.6	**86.3**	**0.90**	**0.83-0.96**	**<0.001 ****

AUC: Area under curve CI: Confidence interval **: highly significant (P<0.001)

Table (5) showed that at cut off < 0.255 micro RNA had sensitivity of 90%, specificity of 100% and accuracy of 93.3% in diagnosis of abnormal albuminuria.

Table (5): Validity of micro RNA 192 in diagnosis of abnormal albuminuria among the studied cases groups

Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	95% CI	P
<0.255	90	100	100	83.3	**93.3**	**0.99**	**0.99-1**	**<0.001 ****

AUC: Area under curve CI: Confidence interval **: highly significant (P<0.001)

Table (6) showed that at cut off < 0.205 micro RNA had sensitivity of 95%, specificity of 100% and accuracy of 97.5% in diagnosis of micro albuminuria.

Table (6): Validity of micro RNA 192 in diagnosis of micro albuminuria among the studied cases groups

Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	95% CI	P
<0.205	95	100	100	95.2	**97.5**	**0.99**	**0.99-1**	**<0.001 ****

AUC: Area under curve CI: Confidence interval **: highly significant (P < 0.001)

DISCUSSION

In the present study, there was no statistically significant difference between the studied groups in height or sex distribution while there was a statistically significant increase in age in group III compared to other groups and also in group II compared to groups I & IV. Regarding weight and BMI, there was a statistically significant increase in both in group III compared to other groups and also in group II compared to groups I & IV and in group I compared to group IV. This is in agreement with study of **Al-Kafaji and Al-Muhtaresh** [6] who found that the mean age was significantly higher in the diabetic patients compared to the healthy controls ($P < 0.05$). They found that the values of BMI, was significantly higher in patients with microalbuminuria and macroalbuminuria compared to patients with normo-albuminuria and controls ($P < 0.05$). **Saadi *et al.*** [7] found that there were no significant differences in gender and age distribution among all study groups.

In the present study, there was a statistically significant increase in disease duration in group III compared to other groups and also in group II compared to group I. This comes in agreement with **Ma *et al.*** [8] who found that there was significant differences in duration between NA group and MA group, between NA group and LA group and between MA group and LA group.

In the present study, there was a statistically significant increase in FBS & cholesterol level in group III compared to other groups, in group II compared to groups I & IV and in group I compared to group IV. Regarding HbA1c & LDL, there was a statistically significant increase in both in groups III & II compared to other groups and also in group I compared to group IV. Concerning TG, there was a statistically significant increase among all groups compared to group I. Finally, in HDL there was a statistically significant decrease in its level in group III compared to other groups. This comes in agreement with **Saadi *et al.*** [7] who found that the mean HbA1c was significantly higher in patients with macroalbuminuria than those with normo-albuminuria and microalbuminuria ($P < 0.001$, 0.003, respectively), but there was no significant difference in mean HbA1c between diabetic patients with normo-albuminuria and those with microalbuminuria.

In the current study, there was a statistically significant increase in creatinine level and albumin creatinine ratio in group III compared to other groups and also in group II compared to groups I & IV. Also, there was a statistically significant increase in urea and a statistically significant decrease in eGFR in group III compared to other groups, in group II compare to groups I & IV and in group I compared to group IV. Regarding uric acid, there was a statistically significant increase in its level in group III compared to other groups. This comes in agreement with **Saadi *et al.*** [7] who found that the mean eGFR was significantly lower in patients with macroalbuminuria and microalbuminuria than in controls ($P < 0.001$, < 0.016, respectively), but there was no significant difference between the control group and patients with normoalbuminuria ($P=0.67$). Serum creatinine was significantly higher in diabetic patients with macroalbuminuria than in controls ($P < 0.001$), but there was no significant difference between the control group and each of those with micro or normo-albuminuria ($P=0.154$, 0.082, respectively).

In the present study, there was a statistically significant decrease in micro RNA-192 level in group III compared to other groups and also in group II compared to groups I & IV. No difference was found between group I & IV. This is in agreement with **Krupa *et al.*** [9] who suggested that the relationship between miR-192 and renal fibrosis is complicated. They found that miR-192 in human renal biopsies was significantly lower in patients with advanced DN, correlating with tubulointerstitial fibrosis and low GFR. Also, **Ma *et al.*** [8] found that the expression of miR-192 in LA group was significantly lower than in MA and NA groups and the miR-192 was lower in MA group than in NA group. Additionally, there were no significant differences in miR-192 between NC group and NA group. Besides, **Al-Kafaji and Al-Muhtaresh** [6] revealed that the expression of miR-192 was significantly lower in overall diabetic patients compared to healthy controls, and was significantly lower in patients with microalbuminuria compared to patients with normo-albuminuria. However, the decrease in miR-192 expression was not significant between microalbuminuria and normo-albuminuria. **Ma *et al.*** [8] demonstrated that miR-192 was significantly decreased in patients with macroalbuminuria (n=148) compared to patients with normo-albuminuria (n=159). However, there are several studies with opposite conclusions **Deshpande *et al.*** [10], **Putta *et al.*** [11], **Zhong *et al.*** [12], **Kato *et al.*** [13] and **Chung *et al.*** [14]. These studies found that the renal miRNA-192 is overexpressed in MCs and TECs of db/db mice as well as T2DM patients and that miR-192 increases in parallel with increased TGF-beta. Deletion of inhibition of miR-192 can attenuate proteinuria and renal fibrosis and the renal function can be improved. The possible mechanisms include Smad and Akt signaling pathways. The discrepancy in these studies may be due to differences in animal species, cell types (including podocyte, mesangial cells, and renal tube cells), and experiment conditions. Our study disagrees with **Saadi *et al.*** [7] who found that the level of miRNA-192 is more significantly elevated in patients with macroalbuminuria and microalbuminuria than those with normo-albuminuria. They found that the miR-192 blood level is upregulated in participants with more progressive DN compared to T2DM patients with normal albuminuria. It is apparent that the blood level of miR-192 is related to the stage of DN as the level of miR-192 is significantly higher in patients with

macroalbuminuria than in patients with microalbuminuria. Our results also disagrees with **Hamdia *et al.*** [15] who found a significantly higher level of blood miR-192 in diabetics than in non-diabetics with again significantly higher levels in patients with long-standing disease and in patients with retinopathy and nephropathy than the newly diagnosed patients without microvascular complications**. Chien *et al.*** [16] reported increased expression of miR-192 in overt proteinuria patients compared to microalbuminuria patients. **Cai *et al.*** [17] found that miR-192 and miR-205 levels were significantly higher in the sera obtained from patients with primary focal segmental glomerulosclerosis and correlated with proteinuria and interstitial fibrosis. Significantly increased expression of miR-192 was observed in glomeruli isolated from streptozotocin-induced diabetes and diabetic db/db mouse in parallel with increased TGF-β1 and collagen 1a2 (Col1a2) levels [18]**.**

When the present study evaluated the possibility of using blood miR-192 as a biomarker for DN, ROC analysis revealed that the miR-192 was significantly able to discriminate overall patients from healthy subjects. This comes in agreement with **Al-Kafaji and Al-Muhtaresh**[6] who found that the miR-192 exhibited a significant ability to discriminate patients with normoalbuminuria from patients with microalbuminuria/macroalbuminuria. Conversely, previous studies of miRNA biomarkers of renal disease revealed that miR-192 in urine extracellular vesicles is a useful biomarker for the early stage of DN [19].

CONCLUSION

The results of our study suggest a possible role of miR-192 in the pathogenesis and progression of diabetic kidney disease in humans. Also, blood miR-192 may be a useful biomarker for predicting the development and the stage of diabetic kidney disease. Owing to its role in the pathogenesis and progression of diabetic kidney disease miR-192 may provide a novel therapeutic target for preventing the progression of diabetic nephropathy.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Gnudi L, Coward R, Long D (2016):** Diabetic nephropathy: perspective on novel molecular mechanisms. Trends in Endocrinology & Metabolism, 27(11): 820-830.
2. **Wahab N (2004):** The role of CTGF in mediating the pathogenesis of diabetic nephropathy. International Journal of Experimental Pathology, 85 (1): 7-8.
3. **Fujita T, Ogihara N, Kamura Y *et al.* (2012):** Interleukin-18 contributes more closely to the progression of diabetic nephropathy than other diabetic complications. Acta Diabetol., 49 (2): 111-117.
4. **Simpson K, Wonnacott A, Fraser D *et al.* (2016):** MicroRNAs in Diabetic Nephropathy: From Biomarkers to Therapy. Curr Diab Rep., 16: 35-39.
5. **Gu Y, Wei X, Sun Y *et al.* (2019):** miR-192-5p silencing by genetic aberrations is a key event in hepatocellular carcinomas with cancer stem cell features. Cancer Res., 79: 941–953.
6. **Al-Kafaji G, Al-Muhtaresh H (2018):** Expression of microRNA-377 and microRNA-192 and their potential as blood-based biomarkers for early detection of type 2 diabetic nephropathy. Molecular Medicine Reports, 18 (1): 1171-1180.
7. **Saadi G, El Meligi A, El-Ansary M *et al.* (2019):** Evaluation of microRNA-192 in patients with diabetic nephropathy. The Egyptian Journal of Internal Medicine, 31 (2): 122-128.
8. **Ma X, Lu C, Lv C *et al.* (2016):** The expression of miR-192 and its signifcance in diabetic nephropathy patients with different urine albumin creatinine ratio. J Diabetes Res., 16: 6789402.
9. **Krupa A, Jenkins R, Luo D *et al.* (2010):** Loss of microRNA-192 promotes fibrogenesis in diabetic nephropathy. J Am Soc Nephrol., 21: 438–447.
10. **Deshpande S, Putta S, Wang M *et al.* (2013):** Transforming growth factor-β–induced cross talk between p53 and a microRNA in the pathogenesis of diabetic nephropathy. Diabetes, 62 (9): 3151-3162.
11. **Putta S, Lanting L, Sun G *et al.* (2012):** Inhibiting MicroRNA-192 ameliorates renal fibrosis in diabetic nephropathy. J Am Soc Nephrol., 23: 458–469.
12. **Zhong X, Chung A, Chen H *et al.* (2011):** Smad3-mediated upregulation of miR-21 promotes renal fibrosis. Journal of the American Society of Nephrology, 22 (9): 1668-1681.
13. **Kato M, Castro N, Natarajan R (2013):** MicroRNAs: potential mediators and biomarkers of diabetic complications. Free Radical Biology and Medicine, 64: 85-94.
14. **Chung A, Huang X, Meng X *et al.* (2010):** miR-192 mediates TGF-β/Smad3-driven renal fibrosis. Journal of the American Society of Nephrology, 21 (8): 1317-1325.
15. **Hamdia E, Abdelwahab M, Fatma A *et al.* (2013):** Expression of micro RNA192 in type 2 diabetes mellitus relation to glycemic control, metabolic abnormalities, renal and ocular complications. Am J Biochem., 3: 97–106.
16. **Chien H, Chen C, Chiu Y *et al.* (2016):** Differential microRNA profles predict diabetic nephropathy progression in Taiwan. Int J Med Sci., 13: 457-465.
17. **Cai X, Xia Z, Zhang C *et al.* (2013):** Serum microRNAs levels in primary focal segmental glomerulosclerosis. Pediatr Nephrol., 28: 1797–1801**.**
18. **Kato M, Arce L, Wang M *et al.* (2011):** A microRNA circuit mediates transforming growth factor-β1 autoregulation in renal glomerular mesangial cells. Kidney International, 80 (4): 358-368.
19. **Jia Y, Guan M, Zheng Z *et al.* (2016):** miRNAs in urine extracellular vesicles as predictors of early-stage diabetic nephropathy. J Diabetes Res., 16: 7932765.