MicroRNA29-a as Diabetic Nephropathy Biomarker in Diabetic Patients (Type 2)

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Abstract

Background: Diabetic nephropathy (DN) is one of the main causes of renal failure. Recent research demonstrated that the serial microbes (miRNA) could be used as a marker to detect pathogenic disease; the DN-related miRNAs could not be studied.

Aim of Study: In this study the aim was to investigate the predictive value of miRNA29-a in diabetes mellitus type 2 (T2DM) patients in relation to nephropathy risk onset prediction.

Subjects and Methods: In the present study a total of (120) persons were divided into three groups as Group (I): (N=20) control group, Group (II): (N=50) T2DM patients without renal symptoms Group (III): (N=50) T2DM patients with renal insufficiency, RT-PCR method was used to determine the relative quantitative of miRNA29-a.

Results: Increased levels of HbA1c, FBS, urea, creatinine and Albumin/creatinine ratio (ACR) were observed in subjects under study. An increased levels of miRNA 29-a in group III, DN (51.97 ± 61.78) and group II, DM (7.06 ± 6.83) when compared with group I, control subjects (0.8 ± 0.2). The result of the present study implied that miRNA 29-a could be served as an early indicator of DM-mediated renal pathogenesis.

Key Words: Diabetes mellitus type 2 – Diabetic nephropathy – Circulating microRNA – Biomarker.

Introduction

DIABETES mellitus (DM) is a group of metabolic problems propertied by chronic high blood sugar condition caused by insufficiency of insulin secretion, insulin activity or both [1]. Diabetes mellitus (T2DM) is a dominant type of diabetes and represents at least 90% of all cases of diabetes [2]. The incidence of T2DM has increased exponentially, especially in developed countries [3,4]. Macro and microvascular complications of diabetes are mainly due to prolongation of exposure to hyperglycaemia [5].

Diabetes enhances the individual’s risk of developing cardiovascular Disease (CVD) [6,7]. Diabetes mellitus is also a strong independent prediction of risk stroke and cerebrovascular disease, such as coronary artery disease [8]. Diabetic retinopathy can be the most common microvascular complication of diabetes [9]. Diabetes neuropathy also accompanies diabetes mellitus complications which leads to significant diseases and mortality even in patients with diabetes [10]. Diabetic nephropathy (DN) is a renal complication of diabetes mellitus. The pathogenesis of DN includes the deregulation of various biological functions [11]. A study has estimated about 40% of T2DM patients develop DN [12].

Among clinical characteristics of DN They include persistent albuminuria and a gradual decrease in glomerular filtration rate (GFR), although low level of albuminuria (30-300mg/day) could be detected in patients with early and reversible DN, evident proteinuria (>300mg/day) represents irreversible level of DN [13]. Analysis of microbiobacteria is an ideal method for detecting DN in early stages, but it has around by problems such as micro albuminuria can develop with advanced medical conditions [14].

Indeed, a new and more sensitive biomarker for early detection of renal failure with relative minimal invasive sampling and simple experimentation procedure is required [15,16]. Many studies have suggested the new family of indigenous, small (about 19-22 nucleotides), single stranded non-code RNA molecules known as microRNAs (miRNAs) as observers development. These molecules
have been shown to play important roles in modu-
lating gene expression \[17,18\]. It is estimated that
about 60% of human protein-gene coding can be
controlled by miRNAs \[19\]. Several studies have
estimated that miRNAs have vital regulators of
gene expression, including cancer, hepatitis, and
diabetes. \[20,21\]. MiRNA29 is involved in patho-
genicity DN targets Spry1 in db/db mice or collagen
in diabetes mellitus caused by STZ \[22,23\].

Subjects and Methods

Subjects:
The current study was conducted on a total of
(120) persons, the study was previously approved
by The Ethical Committee of Molecular Biology
Dept., Genetic Engineering & Biotechnology Re-
search Institute (GEBRI), university of Sadaat
City. Written informed consent was obtained from
all patients. The patient's samples (serum) were
collected under the approval of National Kidney
Institute, Matarya, Cairo, Egypt from May 2018 –
July 2018. All patient samples were recruited from
the emergency department. Individual were divided
into three groups as the following: Group (I):
Included (20) healthy volunteers, control group,
Group (II): Included (50) consecutive Egyptian
T2DM without renal symptoms were constituted
as predictive group for renal impairment developing
symptoms. Group (III): Included (50) Egyptian
T2DM patients with renal insufficiency were sug-
gested of renal impairment.

Blood sampling and serum preparation:
A 7ml Peripheral venous blood was collected
and distributed into a 2ml was collected into EDTA
tube, stored as a whole blood at 4°C until used to
determine HBA1c level. A 5ml blood was collected
into uncoated tube; serum was collected and stored
in aliquots at –80°C until used. Serum samples
were divided into two parts. (i) Serum was used
to determine (F.B.S, urea & creatinine). (ii) Serum
was used to isolate total RNA containing miRNA
29-a.

Urine samples collection & preparation:
Random urine samples were collected, centrifuga-
ed; supernatant was collected, and stored at
20°C until used to determine albumin/creatinine
ratio level.

Methods:
Biochemical parameters:
Estimation of fasting blood sugar level:
Serum glucose was assayed by using Diamond
diagnostics Kit (Diamond Diagnostics Company,
Cairo, Egypt) according to \[24\].

Estimation Glycosylated Hemoglobin (HBA1c)
level:
HBA1c was determined by using BIOMED
diagnostics Kit (EGY-CHEM Diagnostics Compa-
y, Cairo, Egypt) according to \[25\].

Estimation of serum urea level:
Serum urea was determined by using Diamond
diagnostics Kit (Diamond Diagnostics Company,
Cairo, Egypt) according to \[26\].

Estimation serum creatinine level:
Serum creatinine level was determined by using Diamond
diagnostics Kit (Diamond Diagnostics Compa-
y, Cairo, Egypt). Jaffe, colorimetric Kinetic
method according to \[26\].

Estimation of albumin/creatinine ratio level:
Albumin/Creatinine Ratio level in urine was
determined by using BioVision (ACR) kit, (Sunrise
TECAN, Austria) according to the manufacturer's
instructions.

Real-time reverse transcriptase (RT)- PCR:
In the present study the real-time reverse trans-
scription (RT)-PCR method was used to detect and
determine the relative quantitative (RQ) miR29-
a in subjects serum under study. Total RNA con-
taining small RNA was isolated from 250×1 of
serum using miRNeasy Mini Kit (QIAGEN). Total
RNA was converted to cDNA by using the miS-
script®ll RT. Kit (QIAGEN). Real-Time PCR was
performed using ViiATM 7 system (Applied Bio-
systems) by using 2x Quanti Tect SYBR Green
PCR Master Mix and RT-PCR reagents Kit to
amplify the miRNA29-a cDNA. Briefly, Atypical
PCR reaction mix was Prepared as a 25×1 per well
reaction volume as following 2x Quanti Tect SYBR
green PCR master mix 12.5×1. 10x miScript univer-
sal primer 2.5×1. 10x miScript primer assay
2.5×1. RNase-freewater 5×1.TemplatecDNA 2.5×1.
The amplification of the samples were carried out
with the following cycling 15min heat start at 95°C,
followed by 40 cycles of denaturation at 94°C for
15 second, annealing/fluorescence detection at
60°C for 1min. The internal control gene, non-
coding small RNA Hs-SNORD68-11, was used
according to the applied biosystems application
note. The Relative quantitative (RQ) of miR29-a
in the studied subjects were calculated using non-
coding small RNA Hs-SNORD68 as an internal
control. The point at which the amplification plot
crossed the threshold was defined as threshold
cycle CT, ACT was determined by subtraction CT
that of Hs-SNORD68 (calibrator) from CT of target
miR29-a, ΔΔCT was calculated by subtraction (ΔCT) of control subjects from (ΔCT) of target miR29-a, finally, Relative quantitative (RQ) of miR29-a=2(ΔΔCT).

**Statistical analysis:**

Comparisons among different groups were performed by one way analysis of variance (ANOVA). It is a parametric statistical analysis that compares between-and within-groups variance to measure differences between two or more groups. All statistical analysis were performed by using statistical software packages namely (SPSS, version 17) (SPSS Inc.Chicago, USA). p-values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as mean ± SD.

**Results**

**Diabetes and kidney functions markers:**

The miRNA29-a was assayed and correlated to HBA1c, fasting blood sugar and renal functions including serum urea & creatinine levels and albumin/creatinine ratio. The present study showed a significantly increased levels of miRNA29a (RQ) (p-value<0.05) in subjects that had a high level of HBA1c & FBS, also an elevated levels of miRNA29-a were observed in diabetic subjects that had a high levels of urea, creatinine & ACR. We reported that serum miR-29-a was significantly elevated in T2DM patients with aggressive DN, based on ACR, urea and creatinine.

Data represented in Table (1) show the levels of diabetes markers i.e. (FBS, HBA1c) and kidney function markers i.e. (urea, Creatinine & alb/creat ratio) in the serum of control and other studied groups. Marked significant elevation (p<0.05) in the activities of these markers were observed in group II, DM & group III, DN when compared with group I, control subjects.

<table>
<thead>
<tr>
<th>Analytical parameter</th>
<th>Group I N=20</th>
<th>Group II N=50</th>
<th>Group III N=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dl)</td>
<td>89.10±11.58</td>
<td>187.02±82.44</td>
<td>216.64±75.10</td>
</tr>
<tr>
<td>HBA1c (%)</td>
<td>5.41±0.41</td>
<td>8.33±1.86</td>
<td>8.5±1.8</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>29.35±8.49</td>
<td>35.82±12.46</td>
<td>183.2±62.39</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.77±0.2</td>
<td>1.03±0.32</td>
<td>5.46±2.01</td>
</tr>
<tr>
<td>ACR (mg alb/g creat)</td>
<td>9.1±6.85</td>
<td>32.4±19.84</td>
<td>366.62±228.10</td>
</tr>
</tbody>
</table>

Table (1): Clinical information of subjects recruited in the present study.

- Results are expressed as mean ± SD
- Comparisons are made between group II, DM patients & group III, DN patients with group I, control subjects.
- Activity is expressed as: mg/dl for FBS, urea & creatinine; % for HBA1c; mg alb/g creatinine for ACR

**Relative quantitative of miRNA29-a**

Data represented in Table (2) show increased in relative quantitative of miRNA29-a in group III, DN (51.97±61.78) and group II, DM (7.06±6.83) when compared with group I, control subjects (0.8±0.2). Also Data represented in Figs. (1A & 1B) show a positive significant correlation between miRNA29-a (RQ) and (ACR & HBA1C), this explains to us the covariant relationship between them.

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>Group I N=20</th>
<th>Group II N=50</th>
<th>Group III N=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-29a (RQ)</td>
<td>0.8±0.2</td>
<td>7.06±6.83</td>
<td>51.97±61.78</td>
</tr>
</tbody>
</table>

Table (2): Levels of relative quantitative (RQ) for miR29-a in different studied groups.

- Results are expressed as mean ± SD.
- Comparisons are made between group II, DM patients & group III, DN patients with group I, control subjects.

![Fig. (1A): Correlation between HBA1c and miR29-a in diabetic nephropathy patient](image1.png)

Fig. (1A): Correlation between HBA1c and miR29-a in diabetic nephropathy patient.

![Fig. (1B): Correlation between ACR and miR29-a in diabetic nephropathy patients.](image2.png)

Feg. (1B): Correlation between ACR and miR29-a in diabetic nephropathy patients.

Feg. (1): Correlation of serum miR29a levels in patients with diabetic nephropathy. Pearson's correlation coefficient analysis was performed and p<0.05 is considered as significant difference. The result showed that the subjects with greater level of serum miR-29a exhibited more rapid ACR & HBA1c changes in contrast to those with lower miR29a levels. The X-axis represented changing rates of ACR & HBA1c, the Y-axis referred to the relative quantitative of miRNA29-a level (versus Hs-SNORD68-11).
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Discussion

DN causes significant diseases and even deaths in patients with diabetes. Although the detection of protein in urine samples is considered the best standard to detect one of the first and reversible DN, the sensitivity to diagnosing the exact disease, is dissatisfied [27]. There are many other signs that demonstrate performance in DN analysis or authentication those at risk of development. However, some of these tags cannot be truly special DN and may indicate other types of kidney disease, such as transport and primary glomerulonephritis [28]. The need for highly stable and varied labeling DN and in advance DN individuals are required. The miRNA is well prepared to meet this need. The miRNA is very constant at room temperature [29].

A recent finding demonstrated that hyperglycemias-induced podocyte dysfunction was ameliorated by miRNA29-a promotion of nephrin acetylation [30]. According to this moment study, a recent study suggesting a series of urinary miRNAs that had not been previously associated kidney disease in patients with T1DM with different levels of albuminuria/DN. Among these miRNAs related to DN, miRNA29 is produced in different ways between patients who developed to DN after a long time in relation to non-patient patients [31]. An increase in the expression of many miRNAs has been observed in diabetes mellitus GK rats model, as increased expression of the myRNA-29 family in muscles, fat and liver, associated with insulin resistance [32]. According to this study is a recent study showed that an elevated level of miR-29a in urine, but not miR-29b and miR-29c, of T2DM patients with microalbuminuria and macroalbuminuria compared to those with normoalbuminuria [33].

On the contrary, Lv 2013 showed that urinary activities of miR-29 and miR-200 Family decreased in patients with chronic kidney disease, including certain anatomy DN, but the expression miR-29c closely followed the glomerular filtration rate and association not related to the extent of tubulointerstitial fibrosis [34].

Conclusion

The present data of this study implied that miRNA 29-a could serve as early indicator of DM-mediated renal pathogenesis, which can be of importance in the aspect of preventive medicine.

References


