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Research Article

Modulation of MicroRNA 103 and 107 in Obese T2DM Patients Maintained on Metformin

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ABSTRACT

Metformin increases insulin sensitivity in obese patients with type 2 diabetes mellitus (T2DM) by different mechanisms. The current study was conducted to estimate and correlate the levels of microRNA (miR)-103 and 107 in obese non-diabetic subjects as well as obese T2DM patients maintained on metformin, and the development of insulin resistance. Ninety subjects were equally recruited into three groups; obese non-diabetic control (OC), obese newly diagnosed diabetic (ONDD), and obese type 2 diabetic treated with metformin (MetD). Serum levels of blood glucose, insulin, lipid profile, glycosylated hemoglobin (HbA1c), miR-103 and 107 expressions, and Dicer-1 were analyzed. Serum levels of HbA1c, fasting blood glucose (FBG), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), total cholesterol (Tch), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) were increased in ONDD ($p<0.0001$) compared to OC and MetD. A significant increase of HDL-C ($p=0.022$) was observed in MetD compared to OC and ONDD. Serum insulin levels and gene expression of the miR-103 ($p=0.004$) and 107 ($p<0.0001$) were increased significantly in ONDD but down-regulated in MetD compared to the OC group. Dicer-1 levels were decreased ($p<0.0001$) in the ONDD group and increased in the MetD group compared to the OC group. Both miR-103 and miR-107 were positively correlated with insulin and HOMA-IR but negatively correlated with Dicer-1. Depending on the estimated cutoff values of the area under the curve (AUC) of the Receiver operating characteristic (ROC) curve, miR-103 and miR-107 were excellent diagnostic biomarkers for insulin resistance. Our findings indicated the clinical utility of miR-103 and miR-107 not only in diagnosis but also in the treatment of insulin resistance. Moreover, metformin can affect miR-103 and miR-107 through modulation of the Dicer-1 level.

Keywords: Diabetes Mellitus, Dicer-1, Insulin Resistance, Metformin, MicroRNAs.

1. INTRODUCTION

Insulin resistance is a pathological condition in which target cells are unable to uptake & metabolize glucose in response to insulin leading to increase insulin and glucose levels and the development of many diseases like type 2 diabetes mellitus (T2DM).[1] Since the underlying molecular mechanisms leading to insulin resistance are partially understood, the discovery of microRNAs (miRs) and altered their expression levels may explain the development of many diseases like obesity, insulin resistance, and T2DM.[2] MiRs are small (18-22) nucleotides non-coding potent regulators of gene expression and central players in many physiological and pathological processes.[3] These miRs are highly conserved, and they have located within the pantothenate kinase (PANK) gene. PANK catalyzes the rate-limiting step of pantothenate phosphorylation during the formation of coenzymes (CoA), an important cofactor of many enzymes

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involved in various metabolic pathways. MiR-103 genes are constructed of two mature miRs: miR-103 (1) and miR-103 (2), whereas miR-107 is composed only of the miR-107 gene.[4,5]

Caveolin-1 (Cav-1) is the major component of caveolae, which are small pouches in the membrane that surrounds cells. Cav-1 is essential for multiple signals integration, stabilizing insulin receptor (INSR) structure for efficient insulin signaling and INSR compartmentalization. It has been demonstrated that miR-103 and miR-107 directly bind Cav-1 3′UTR sequence, thus controlling its expression.[6] Trajkovski et al. demonstrated that miR-103 and 107 are upregulated in obese mice and when silenced lead to improvement of glucose homeostasis and insulin sensitivity. Moreover, the increased miR-103 and 107 in either liver or fat is sufficient to induce impaired glucose homeostasis.[7] Dicer is a member of enzymes called RNase III family that specifically cleaves long double-strand RNA (dsRNA) substrates into short dsRNA fragments of defined length, typically around 21–25 nucleotides.[8] Dicer has emerged as a key regulator of cellular adaptive response to metabolic homeostasis and fluctuation of nutrient availability. Moreover, it was reported that Dicer is significantly increased in human umbilical vein endothelial cells in vitro by hyperglycemia, indicating that Dicer or miRs are sensitive to nutrient alterations. Additionally, it has been found that miR-103 and 107 can attenuate the expression levels of many miRs by targeting the Dicer enzyme.[8,9] Metformin is a biguanide derivative and is widely used as a first-line treatment for T2DM. It can decrease insulin resistance and blood glucose levels by stimulating glucose uptake in peripheral muscles, in addition to inhibiting gluconeogenesis in the liver and fatty acid oxidation in the adipose tissues. However, some studies have mentioned its function in reducing insulin resistance through affecting levels of miRs through affecting expression levels of Dicer enzyme.[10,11] Therefore, the present study aimed to estimate levels of miR-103 and miR-107 in obese non-diabetic subjects as well as obese type 2 diabetic patients maintained on metformin and to correlate between levels of miR-103 and 107 and the development of insulin resistance. Also, the present study aimed to clarify the effect of metformin on the level of miR-103 and miR-107 in obese type 2 diabetic patients.

2. MATERIALS AND METHODS

2.1. Study Design

This cross-sectional study was conducted in accordance with the local ethical standards of the Faculty of Pharmacy-Tanta University Ethical Committee of Scientific Research (No. TP/RE/11/2261), in accordance with Helsinki Declaration of 1975. An informed written consent was taken from the patients after the approval of the study. The included patients were recruited from those admitted to the outpatient clinic of the Diabetes and Endocrinology Unit, Internal Medicine Department, Tanta University Hospital, Egypt. The study started in January 2019 and ended in December 2020.

2.2. Patient inclusion criteria

The study was conducted on ninety subjects of both sexes and their age was 30-65 years. The study groups were divided into an obese non-diabetic control group (OC, n=30), obese newly diagnosed diabetics (ONDD, n=30), and obese diabetics treated with metformin (MetD, n=30). According to the World Health Organization (WHO) criteria, obese patients were included if they had body mass index (BMI)≥30 and newly diagnosed diabetic patients were defined when fasting blood glucose (FBG) levels were above 126 mg/dL.[12] The included T2DM patients in the MetD group were recruited if they received metformin with a dose of 1000 mg once daily for at least three months. Full history was taken for all patients with particular emphasis on the duration of diabetes and any other associated diseases and medications.

2.3. Patient exclusion criteria

Patients with hypertension, cancers, pancreatic diseases, kidney diseases, thyroid diseases, liver diseases, and smokers were excluded from the study.

2.4. Biochemical investigations

Blood samples (10 mL) were collected after overnight fasting. Whole blood (5 mL) was used for the determination of glycosylated hemoglobin (HbA1c) according to the method described by Hanas R. and John G.,[13] using commercial kits obtained from Biosystems (Barcelona, Spain). Serum was separated and insulin levels were assayed using enzyme-linked immunosorbent assay (ELISA) kits purchased from Chemux BioScience, Inc. (Catalogue no. 10801, Germany) according to the manufacturer's instruction, with a sensitivity of 2 µIU/mL. Homeostasis Model Assessment of Insulin Sensitivity (HOMA-IR) was estimated using the equation HOMA-IR= fasting glucose level (mg/dl) × fasting insulin level (µU/mL)/405.[14] Serum Dicer-1 levels were assayed using a human Dicer ELISA kit, purchased from SunRed Hotechnology Company (Catalogue no. 201-12-9072, Shanghai). According to the manufacturer of the Dicer assay kit, the referred sensitivity was 10-12 ng/mL and the assay range was 10-2800 ng/mL.

2.5. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) of microRNA-103 and 107

Total RNA was isolated with miRNeasy Serum/Plasma isolation kit (Qiagen, catalogue no. 217184, USA) using a QIAZOL reagent. According to the manufacturer's instructions, the total RNA was converted into cDNA using miScripT II RT kit (Qiagen, catalogue no. 21860, USA). All specific primers for miR expression were designed and synthesized by Qiagen, CA, USA, using the miScript primer assay & QuantiTect Primer Assay for miR-103 (Hs_mi

R-103a 1), miR-107 (Hs_miR-107_2) as follows, miR103 forward primer: 5'-ACACTCCAGCTGGGGGCTTCTTTACAGTGC-3', reverse primer: 5'-TGTCGTGGAGTCGGCAATTC-3'; miR107 forward primer: 5'-ACACTCCAGCTGGGAGCAGCATTGTACAGG-3', reverse primer: 5'-TGTCGTGGAGTCGGCAATTC-3'; U6 forward primer: 5'-CTCGCTTCGGCAGCACATATACTA-3', reverse primer: 5'-ACGAATTTGCGTGTCATCCTTGC-3'. The differential expression levels for miR were validated using miScript SYBR Green Quantitative PCR kit (Qiagen, catalogue no. 218073 Foster City, CA, USA). The levels of an endogenous control (U6) (Qiagen, CA, USA) were used to normalize the expression levels of each miR. The fold change in miR expression was calculated using the comparative CT method as fold change = 2 - ΔΔCT, where ΔΔCT= ΔCT sample - ΔCT control.[15]

2.6. Statistical analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21.[16] Qualitative data were described as numbers and percentages. Chi-square ($\chi 2$) test and Monte Carlo test were used for comparison between groups. Quantitative data were described as means ± standard deviation (SD) or medians, as appropriate after testing for normality by the Kolmogorov-Smirnov test. In the normally distributed variables, one-way analysis of variance (ANOVA) with LSD post-hoc multiple comparisons was used for comparison between groups, while in the non-normally distributed variables, the Kruskal-Wallis test, and Mann-Whitney U test were used for comparison between groups, as appropriate. Correlation between two continuous variables (either one or both is parametric) was done using Pearson's correlation, while non-parametric correlations were done using Spearman's rank correlation. Significant independent variables in the correlation analysis were entered into a linear regression model using enter method. Receiver Operating Characteristic(ROC) curve was plotted. The area under the ROC curve (AUC) was calculated to describe the predictive accuracy of different markers. The cutoff points were determined using Youden-Index and $p<0.05$ was statistically significant.

3. RESULTS

Demographic characteristics of the studied groups including age, sex, BMI, and treatment are shown in Table (1). Non-significant differences in age, gender, and BMI were found between the studied groups ($p>0.05$).

3.1. Effect of treatment on lipid profile

Serum levels of total cholesterol (Tch), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and very low-density lipoprotein cholesterol (VLDL-C) in studied groups are shown in Table 2. The MetD group showed a significant decrease ($p<0.001$) in serum levels of Tch, TG, LDL-C, and VLDL compared to OC and ONDD groups. However, a significant increase in serum levels of HDL-C ($p=0.022$) was observed in the MetD group compared to OC and ONDD groups.

Table 1: Demographic characteristics of studied groups**.**

Characteristic	MetD n=30	ONDD n=30	OC n=30	P-value
Age (yrs)	45.7±7.9	43.4±8.3	43.4±9	*p*=0.5
Gender	No. (%)	No. (%)	No. (%)	
Male	23 (76.7)	20 (66.7)	15 (50.0)	*p*=0.093
Female	7 (23.3)	10 (33.3)	15 (50.0)	
BMI (kg/m^2)	31.5±1.5	32.4±2.1	31.6±1.5	*p*=0.1
Drug intake	No. (%)	No. (%)	No. (%)	
Vitamins	4 (15.4)	5 (29.4)	6 (35.3)	
Statins	6 (23.1)	6 (35.3)	0 (0.0)	
Analgesic	0 (0.0)	0 (0.0)	1 (5.9)	
NSAIDs	5 (19.2)	5 (29.4)	4 (23.5)	p=0.004
Allopurinol	2 (7.7)	0 (0.0)	2 (11.8)	
Glucosamine	8 (30.8)	0 (0.0)	0 (0.0)	
H2 blockers	1 (3.8)	1 (5.9)	4 (23.5)	

Data were presented as mean ± SD (for normally distributed variables). Qualitative data were expressed as percentages and numbers. OC; obese control, ONDD; obese newly diagnosed diabetic, MetD; obese diabetic treated with metformin, BMI; body mass index= Weight (kg) x /height^2 (m). p-value: significance level between groups.

3.2. Effect of treatment on glucose homeostasis

Levels of HbA1c, FBG, insulin, and HOMA-IR of studied groups are shown in Table (2). A significant decrease ($p<0.001$) was observed in HbA1c, FBG, serum insulin, and HOMA-IR in the MetD group compared to OC and ONDD groups.

Table 2: Lipid profiles & blood glucose homeostasis in studied groups**.**

Measure	MetD n=30	ONDD n=30	OC n=30	p-value
TC (mg/dL)	182±22.8^{ab}	239.8±46.6^{a}	226.4±44.5^{b}	<0.0001
TG (mg/dL)	139.9±41.3^{a}	172.9±19.8^{ac}	150.2±38.5^{c}	0.001
LDL-C (mg/dL)	154.7±15.7^{ac}	181.2±20.1^{a}	171.9±27.4^{c}	<0.0001
HDL-C (mg/dL)	51.5±12.5^{a}	43.6±6.2^{a}	46.7±12.5	0.022
VLDL (mg/dL)	25.4±7.5^{ab}	38.9±10.5^{ac}	30.8±8.7^{bc}	<0.0001
HbA1c %	6.1±0.8^{a}	9.2±1.7^{ac}	5.9±0.5^{c}	<0.0001
FBG (mg/dL)	99.1±11.6^{a}	230.2±55.1^{ac}	102.4±12.5^{c}	<0.0001
Insulin (µu/mL) Median (range)	47.8^{a} (16.8-131.9)	81.4^{ac} (26.9-174.3)	50.2^{c} (11.2-122.7)	0.004
HOMA-IR Median (range)	11.3^{a} (3.3-37.5)	43.7^{ac} (12.5-126.5)	12.4^{c} (3.2-32.2)	<0.0001

All measures are expressed as mean±SD except for Insulin and HOMA-IR are expressed as median (range).
OC; obese control, ONDD; obese newly diagnosed diabetic, MetD; obese diabetic treated with metformin. TC; total cholesterol. TG; triglycerides, LDL-C; low-density lipoprotein, HDL; high-density lipoprotein, VLDL; very low-density lipoproteindiabetic, HOMA-IR; Homeostasis Model Assessment of Insulin Sensitivity.
p-value: significance level between groups.
[a] significance of diabetic treated group *vs* newly diagnosed obese diabetic group, [b] significance of diabetic treated group *vs* obese control group, [c] significance of the newly diagnosed obese diabetic group *vs* obese control group.

3.3. Effect of treatment on serum levels of miR-103, miR-107, and Dicer activity

Gene expression of miR-103, miR-107, and Dicer levels are shown in Figure (1). Metformin treatment significantly decreased (p<0.0001) gene expression of miR-103 and 107 in the MetD group compared to OC and ONDD groups. On the contrary, metformin significantly increased (p<0.0001) serum levels of Dicer in the MetD group compared to OC and ONDD groups.

Moreover and as shown in Table (3), there were significant positive correlations between miR-107 and insulin levels in the OC group (p=0.008, r=0.5), ONDD group (p=0.022, r=0.4), and in the MetD group (p<0.0001, r=0.6). Also, there were significant positive correlations between miR-107 and HOMA-IR in the OC group (p=0.03, r=0.4), ONDD group (p=0.004, r=0.5), and in the MetD group (p<0.0001, r= 0.4).

Regarding miR-103, there were significant positive correlations between miR-103 and insulin levels in OC group (p=0.03, r=0.4), ONDD (p=0.001, r=0.6), and MetD group (p=0.04, r=0.4). Additionally, significant positive correlations between miR-103 and HOMA-IR in OC group (p=0.03, r=0.4), ONDD group (p=0.03, r=0.4), and in MetD group (p=0.02, r=0.4) were demonstrated (Table 3).

3.5. ROC curve analysis

The cutoff values of ROC curves were used to calculate the diagnostic sensitivity (DSe), diagnostic specificity (DSp), and the area under the ROC curve (AUC) of miR-103 and miR-107. In the ONDD group versus the obese control group, the AUC value of miR-103 was 0.904 (p<0.0001) and miR-107 was 0.996 (p<0.0001). In the MetD group versus the obese control group, the AUC value of miR-103 was 0.094 (p<0.0001) and miR-107 was 0.118 (p<0.0001). The ROC-AUC, DSe, DSp, and cutoff values are presented in Tables (4 & 5) and Figure (2).

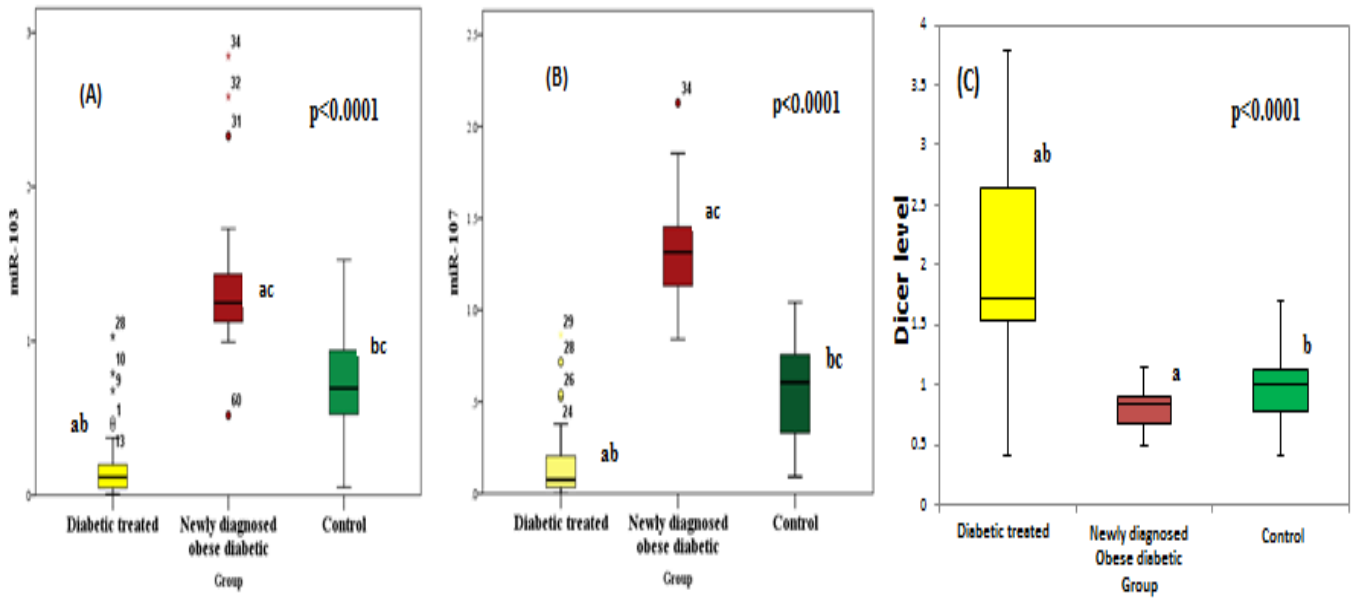


Figure 1: Serum levels of miRNA-103(A), miRNA-107 (B), and Dicer (C) in the studied groups.
All data are expressed as median using Kruskal-wallis test, significance at p<0.0001. A; significance of obese diabetic metformin-treated group *vs* obese newly diagnosed diabetic group, b; significance of obese diabetic metformin-treated group *vs* obese control group, c; significance of obese newly diagnosed diabetic group *vs* obese control group.

3.4. Correlation study

As shown in Table (3), there were significant negative correlations between miR-107 and Dicer in OC (p=0.032, r=-0.4), ONDD group (p=0.023, r=-0.4), and MetD (p=0.039, r=-0.4). In addition, there were significant negative correlations between miR-103 and Dicer in OC (p=0.013, r=-0.5), ONDD group (p=0.021, r=-0.4), and MetD (p=<0.0001, r=-0.6).

Table 3: Correlation of miR-107 & miR-103 with measured parameters in studied groups**.**

Measure	MetD n=30	ONDD n=30	OC n=30
miR-107			
TG r (p)	0.4 (0.049*)	0.1 (0.5)	-0.1 (0.5)
Insulin r (p)	0.6 (<0.0001*)	0.4 (0.022*)	0.5 (0.008*)
HOMA-IR r (p)	0.4 (0.018*)	0.5 (0.004*)	0.4 (0.03*)
Dicer r (p)	-0.4 (0.039*)	-0.4 (0.023*)	-0.4 (0.032*)
miR-103			
LDL r (p)	-0.5 (0.008*)	**0.3 (0.1)**	-0.04 (0.8)
Insulin r (p)	0.4 (0.04*)	0.6 (0.001*)	0.4 (0.03*)
HOMA-IR r (p)	0.4 (0.02*)	0.4 (0.03*)	0.4 (0.03*)
Dicer r (p)	-0.6 (<0.0001*)	-0.4 (0.021*)	-0.5 (0.013*)

MetD; obese diabetic treated with metformin, ONDD; obese newly diagnosed diabetic, OC; obese control, TG; triglycerides, LDL-C; low-density lipoprotein, HOMA-IR; Homeostasis Model Assessment of Insulin Sensitivity. r; correlation coefficient, *p; significance level.

Table 4: Area under the receiver operating characteristics curves (AUC) of miR-103 and miR-107 in MetD vs OC.

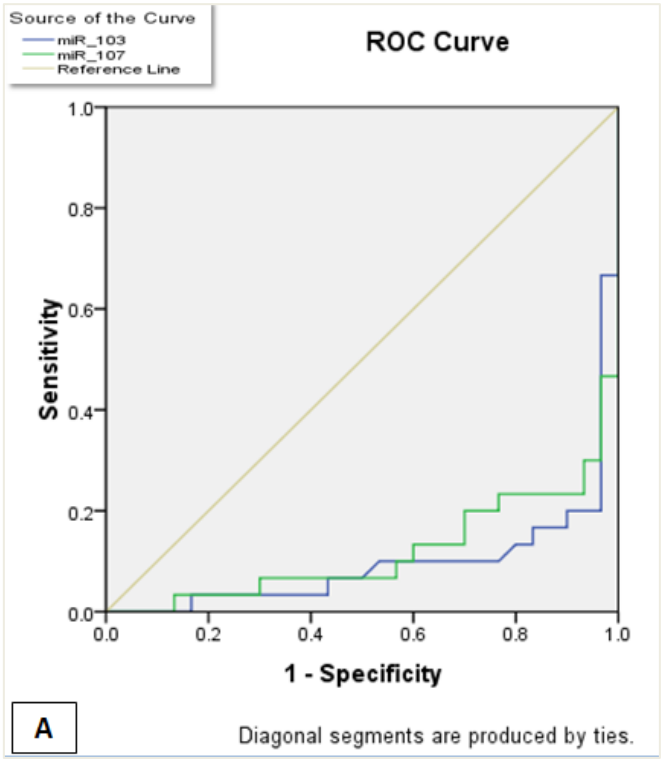
Marker	AUC	p-value	Cut off	Sensi--tivity	Specificity	PPV	NPV
miR-103	0.094	<0.0001	0.4	20%	10%	16.7%	16.7%
miR-107	0.118	<0.0001	0.3	20%	23.3%	20.7%	22.6%

PPV; positive predictive value, NPV; negative predictive value, MetD; obese diabetic treated with metformin, OC; obese control.

Table 5: Area under the receiver operating characteristics curves (AUC) of miR-103 and miR-107 in ONDD vs OC.

Marker	AUC	p-value	Cut off	Sensi--tivity	Specificity	PPV	NPV
miR-103	0.904	<0.0001	0.973	96.7%	80%	82.9%	96%
miR-107	0.996	<0.0001	1.05	96.7%	100%	100%	90.9%

PPV; positive predictive value, NPV; negative predictive value, ONDD; obese newly diagnosed diabetic group, OC; obese control.



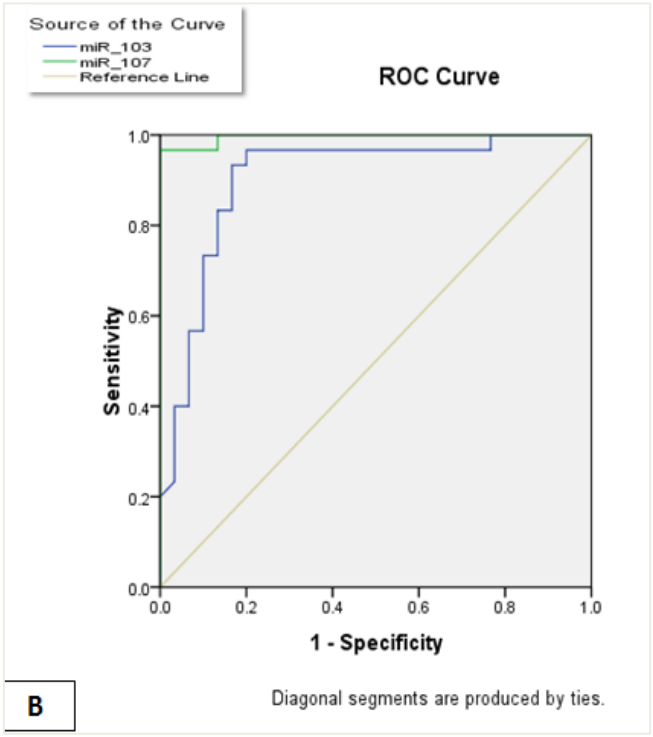


Figure 2: (A) ROC curve of miR-103 and miR-107 in MetD group vs OC group, (B) ROC curve of miR-103 and miR-107 in ONDD group vs OC group.

4. DISCUSSION

Insulin resistance is considered one of the greatest challenges faced by modern medicine. Recent studies reported that miRs have a profound role in several aspects like insulin resistance, obesity, and T2DM.[2] In the present study, metformin was used as an insulin sensitizer and hypoglycemic agent. Metformin acts through its effect on GLUT4-mediated glucose transport, AMP-activated Protein Kinase (AMPK) activation, epigenetic modifications, and enhancements in GLUT4 trafficking and translocation to the plasma membrane. However, some studies using cancer cells in culture and murine tumor xenografts have found that metformin inhibits tumorigenesis by upregulating Dicer-1, a key enzyme that processes miRs.[10,11] Therefore, the current study was conducted to estimate and correlate the levels of miR-103 and 107 in obese non-diabetic subjects as well as obese T2DM patients maintained on metformin, and the development of insulin resistance through modulation of Dicer activity. In the present study, our results showed a significant increase in the levels of miR-103 and 107 in the ONDD group compared to OC and a significant decrease in the levels of miR-103 and 107 in the MetD groups compared to the OC group. These findings agreed with those achieved by Mao et al.,[17] Maryam H et al.,[18] and Qian et al.,[6] who reported that the expression level of miR-103/107 is up-regulated in obese mice while silencing of miR-103/107 leads to improved glucose homeostasis and insulin sensitivity. These results were also concomitant with Tuba et al., who concluded that obesity and insulin resistance increase plasma miR-103 and 107 levels suggesting their involvement in glucose homeostasis.[7]

Herein, our results showed a significant increase in plasma levels of Dicer in the MetD group compared to the OC group and a significant decrease in levels of Dicer in ONDD compared to the OC group. Our results were in accordance withsyndrome Nicole et al.[19] and Giovani et al.,[20] who showed that treatment with metformin affects levels of Dicer both in mice and humans. Moreover, chronic metformin treatment of mice increased Dicer levels through altering the post-transcriptional processes that would affect the stability and/or turnover of Dicer mRNA.[19, 20] Abdelmohsen et al.[21] also showed that RNA-binding protein AUF1 (RBP AUF1) binds Dicer mRNA and negatively regulates Dicer protein levels by lowering the stability of Dicer mRNA. Treatment with metformin causes subcellular localization of AUF, disrupting its interaction with Dicer mRNA and causing stabilization of Dicer mRNA, allowing Dicer to accumulate to increase its levels.[17] The present study also showed that treatment with metformin led to a significant decrease in the expression levels of miR-103 and 107 in the MetD group.

These results were contrary to Ibrahim et al.,[10] who investigated that the expressions of miR-103 and 107 are non-significantly down-regulated by metformin. Herein, our results showed significant negative correlations between levels of miR-103, miR-107, and Dicer in all studied groups. These findings agreed with Taisuke et al.,[22] Li et al.,[23] and Graziano et al.[9] The role of miR-103 and miR-107 in insulin resistance and glucose metabolism may be mediated by their ability to inhibit the endonuclease enzyme Dicer, which is essential for processing miR precursors to mature miRs. This raised the possibility that some mature miRs could feed back to control Dicer expression. This explains the increased levels of miR-103 and 107 resulted in decreasing the levels of Dicer.[11,21] The present results also showed significant positive correlations between miR-103, miR-107, and HOMA-IR. These findings were consistent with the work done by Qian et al.[6] and Tuba et al.,[7] who demonstrated that miR-103 and miR-107 may be potential molecular markers for insulin resistance and the onset of diabetes.[4]

The role of miR-103 and miR-107 on insulin resistance and glucose metabolism may be mediated through the effect on a direct gene Cav-1. Cav-1 is essential for insulin receptor (INSR) structure stabilization for proper insulin signaling. This cholesterol/sphingolipid plays an important role as a facilitator of extracellular signal transduction events, including invaginations in rich "small caves" or plasma membranes, endocytosis, and insulin signaling.[17] Many studies of the cell and animal models showed an antagonistic effect on the modulation of caveolin-1 expression via overexpression of miR-103, miR-107, and the phosphorylation of the insulin receptor, which supports that miRs target Cav-1 and have a critical role in insulin sensitivity.[17,22] The present study also showed improvement in HbA1C, FBG, insulin levels, and HOMA IR after treatment with metformin in the MetD group compared to ONDD and OC groups. As metformin acts through improving the sensitivity of peripheral tissues to insulin, it leads to a reduction in circulating insulin levels. In addition, metformin could inhibit hepatic gluconeogenesis, increases glucose uptake by peripheral tissues, and reduces fatty acid oxidation.[23,24]

Our results showed that levels of Tch, TG, LDL-C, and VLDL-C were reduced along with increased HDL-C after treatment with metformin in the MetD group compared to ONDD and OC groups. These findings agreed with Syed et al. and Lin et al., who concluded the ability of metformin to correct dyslipidemia and lipid profiles in T2DM patients.[25,26] The sensitivity of miR-103 in ONDD was determined by the AUC values of the ROC curve in the current study to be 96.7%, and the specificity was determined to be 80%. These findings agreed with those of Mao et al.,[17] who found that miR-103 levels are increased in the pre-diabetes population with high sensitivity and specificity for identifying and predicting T2DM with high diagnostic value.

5. CONCLUSION

Our findings indicated a link between the expression levels of miR-103, miR-107, and Dicer in T2DM patients maintained on metformin treatment. Additionally, the present study illustrates a proposed molecular mechanism of metformin in the improvement of insulin resistance and T2DM through modulating Dicer-1 and down-regulation of miR-103 and 107

in obese diabetic patients. Moreover, miR-103 and miR-107 have clinical utility in diagnosis and novel therapeutic targets for insulin resistance and T2DM. However, the present study has some limitations. Firstly, the small sample size for each group. Secondly, additional experiments are necessary for further defining the precise regulatory network of metformin on Dicer and miRs.

AUTHORS` CONTRIBUTIONS

El-Ashmawy NE: conceived the presented idea and approved the manuscript in the final version for publication. Khedr NF: planned the experiments, analyzed the data, wrote and reviewed the manuscript, and approved it for publication. Amr G: designed patient groups, recruited the patients, and approved the study for publication. Hala A: conducted the research, drafted the manuscript, and approved it for publication.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest.

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LIST OF ABBREVIATIONS

- **OC:** obese control
- **ONDD**: obese newly diagnosed diabetic
- **MetD**: obese diabetic treated with metformin
- **T2DM:** type 2 diabetes mellitus
- **ELISA**: Enzyme-linked immunosorbent assay
- **q-RT PCR:** quantitative real-time polymerase chain reactions
- **ROC**: Receiver operating characteristic
- **AUC**: Area under the curve
- **DSe**: Diagnostic sensitivity
- **DSp**: Diagnostic specificity

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