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Original Paper

Simvastatin Therapy Increased miR-150-5p Expression in the Patients with Type 2 Diabetes and COVID-19

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Key Words
miR-150-5p • COVID-19 • T2DM • Statin • Simvastatin

Abstract
Background/Aims: Corona virus disease 2019 (COVID-19) has become a deadly infectious disease, especially for those with co-morbidities such as diabetes. People with diabetes developing a viral infection, seem to have harder treatments due to fluctuations in blood glucose levels therefore, effective therapeutic approaches need to be considered for them. Statins are well-known lipid-lowering drugs; they also have anti-inflammatory and immunomodulatory effects and can impact on expression of microRNAs (miRNAs). ***Methods:*** In this study we investigate the effects of simvastatin on the expression of miR-150-5p as a famous regulator of inflammation and its association with multiple cancers in 30 patients with Type 2 diabetes mellitus (T2DM) and COVID-19 compared to the COVID-19 hospitalized patients before and after treatment with simvastatin with real-time–PCR after 2month, and evaluate its targets gens and functions with the help of bioinformatics and GO enrichment analysis respectively. ***Results:*** Our results showed that simvastatin can increase miR-150-5p and therefore down regulate expression of its target genes involving in immune stimulation and decrease lipid profile including LDL-C, total cholesterol, and ApoB, especially in the group with type 2 diabetes mellitus (T2DM) and COVID-19 compared to the patients with only COVID-19. ***Conclusion:*** Simvastatin as an anti-inflammatory agent can modulate miRNAs expression; it can be suggested as an adjunct therapy especially for T2DM patients with COVID-19. Further studies may help us for developing better treatments about therapeutic manipulation of miRNAs *in vivo*.

Introduction

The corona virus disease 2019 (COVID-19) pandemic was caused by severe acute respiratory syndrome coronavirus2 (SARS-CoV-2) infections which have globally spread since 2019 and have been considered the greatest major threat worldwide [1]. The mortality

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rate of COVID-19 is higher, particularly in the elderly population and patients with other malignancy such as hypertension, diabetes mellitus, and cardiovascular disease, among them, Type 2 diabetes mellitus (T2DM) have a higher risk of development in COVID-19 patient increasing the risk of severe outcome and mortality [2, 3]. T2DM is one of the common chronic conditions and a public health problem, with increasing prevalence over the past decades [4]. Although the exact mechanism of Insulin resistance (IR) in T2DM is not clearly understood, dyslipidemia is closely considered to relate to IR in diabetes. In addition, serum lipid profiles were found to deregulated in diabetics more than in non-diabetic individuals from different ethnic groups [4]. mentioning the association between hyperlipidemia and increased risk of developing abnormal glucose metabolism in patients [5, 6]. Statins are lipid-lowering drugs, that reduce circulating lipids, and most specifically cholesterol packaged in low-density lipoprotein (LDL) particles while increasing the HDL cholesterol levels [6, 7]. Statins are also a candidate for treatment of cancer with their inhibitory effects on pro-inflammatory cytokines [8, 9]. Moreover, statins disturb viral binding by decreasing cholesterol as an essential component of lipid rafts in cellular plasma membranes and disrupting virus entry. There is a previous study that statins therapy reduced mortality in influenza viral infections [10]. It has also been proved that statins can modulate expressions of microRNAs previously [11]. For instance, statins could inhibit miR-133a expression which was robustly induced by cytokines/oxidants, and prevent endothelial dysfunction [12]. MicroRNAs (miRNAs) which are classified as short non-coding RNAs and they can regulate gene expression, metabolism, inflammation, and inhibit the viral translation with the ability to suppress the expression of targeted genes through post-transcriptional mechanisms [13–15]. Therefore, determining miRNAs as crucial factor in a diverse biological processes that are modified in COVID-19 patients significantly and targeting them might be an effective and highly promising tool for patients who are susceptible for T2DM and COVID-19 [16]. miR-150 is a regulator that suppresses inflammation and is considered as a novel biomarker based on its high expression in different main tissues, such as liver, adipose tissue and skeletal muscle [14, 17, 18]. More interestingly, the higher risk of T2DM was associated with lower levels of miR-150 and its down regulated observed in cases with obesity plus T1D, or T2D [18, 19]. Therefore, in this study, we study the statins effects on circulating levels of miR-150 in patients with T2DM and COVID-19 and evaluating its status as a biomarker to estimate the risk of developing the diseases.

Materials and Methods

Patient recruitment

In the present observational, pilot study, a group of 30 plasma samples of subjects with positive COVID-19 and T2DM compared with patients only with COVID-19 before and after treatment with 10 mg/day of statins (simvastatin) for 2 months. Between (March 1st and Feb 30th, 2021) were compared with COVID-19 patients who did not exhibit T2DM or other respiratory problems was obtained. We age-matched the groups by choosing ages between 30–66 years of age. The criteria for inclusion were PCR confirmation of SARS-CoV-2 and less than 48 hours hospitalization of each case. For exclusion were: suspected to any other infections besides COVID-19; patients with neoplastic or autoimmune diseases. Written consent was received from all patients. Our study was based on Helsinki Declaration and the ethical committee of Tehran University and the registry code (IR.IUMS.REC.1399.9223497212) The patient details are provided in Table 1.

Measurements of Biochemical parameters

Venous blood was collected in EDTA tubes after a 12-h overnight fast. Plasma were separated immediately afterward in a refrigerated centrifuge to avoid any loss of analytics. Modular analyzer DDPPII Hitachi (Roche, Switzerland) was used for lipid variables and colorimetric enzymatic methods for assessing total cholesterol (TC), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C) and triglyceride (TG) and nephelometry was used for measuring Apolipoproteins AI (apoAI) and B (apo B).

Table 1. Lipid profile before and after a 2-month treatment with simvastatin. TC: total cholesterol. LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; TG: Triglycerides; ApoAI: apolipoprotein AI; ApoB: apolipoprotein B (*p < 0.05, **p < 0.01, ***p < 0.001)

Characteristics	Covid19-T2DM (group1)	Covid19 (group2)	p-value	Post-treatment (group1)	Post-treatment (group2)	p-value
N	30	30		30	30	
Gender (F/M)	16/14	11/19	0.2	16/14	11/19	0.2
Age (years)	57.4 ± 8.4	58.3 ± 8.6	0.7	57.4 ± 8.4	58.3 ± 8.6	0.7
BMI (kg/m2)	23.98 ± 2.87	24.71 ± 2.66	0.73	23.11 ± 1. 70	24.01 ± 1.33	0.61
Total cholesterol (TC) (mg/dL)	239.4 ± 28.28	158.4 ± 33.41	0.01*	145 ± 16	155 ± 43	0.023**
HDL-c (mg/dL)	43.2 ± 1.0	43.5 ± 1.1	0.11*	44.45 ±10.09	41.20 ± 9.48	0.21*
TG (mg/dL)	157.8 ± 61.0	132.0 ± 44.1	0.55	169.6 ± 56.0	141.2 ± 53.9	0. 46
LDL-c (mg/dL)	28.0 ± 14.0	26.1 ±9.8	0.03*	36.1 ±9.8	33.1 ±1.8	0. 011*
ApoAI	144.1 +- 21.2	143 ± 30	0.11	149.2 ± 28.7	148.8 ± 37.6	0.117
ApoB	147.6 ± 28.4	108.1 ± 22.3	<0.01**	120.8 ± 18.8	90.2 ± 15.3	<0.01**

Extraction of RNA and cDNA synthesis

Anticoagulant EDTA tubes were used for blood samples collection and peripheral blood mononuclear cells (PBMCs) were obtained with centrifugation.RNA was extracted using RNX™-plus reagent (SinaClon, Iran). The cDNA was synthesized by the advanced miRNA cDNA Synthesis Kit (TaqMan). RT-qPCR with the SYBR green was used to evaluate the expression levels of selected miRNA, the reaction was set as 5 min at 95 °C, 10 s at 95 °C for 45 cycles, and 5 min at 95 °C, with specific forward primer and the universal adaptor reverse primer. The relative expression was calculated with $2^{-\Delta\Delta Ct}$ method, and U6 was used as internal controls, respectively [20].

Target Prediction of miR-150-5p and Functional Enrichment Analysis

We analyzed the potential miR-150-5p target genes with MiRWalk 3.0 (http://mirwalk.umm.uni-heidelberg.de) and evaluated the other overlapping genes with three different highly recognizable miRNA-target prediction tools miRDB: (http://mirdb.org), and Targetscan7.2: http://www.targetscan.org/vert_72/) and Miranda (http://www.microrna.org) for accurate results. 11 selected overlapping genes were obtained. Then we used Metascape (metascape.org/gp/index.html) for further GO annotation analysis. P < 0.05 was considered as significant. Data was analysied with Graph Pad Prism 6 (Graph Pad Software, USA) and are shown as mean ± standard deviation (SD). To examine the normal distribution of the variables Kolmogorov–Smirnov test was used and Mann–Whitney U test. Student's t-test was used to evaluate the differences between the two groups. P < 0.05 was considered as statistically significant.

Results

Effects of Statins supplementation on lipid levels and miR-150 expression

Lipid levels before and after simvastatin treatments are presented as mean ± SD in Table 1. Treatments significantly improved the lipid profile in both groups. Simvastatin treatments significantly improved the LDL-C and Apo B. There were no significant differences in weight, BMI TG, total cholesterol, HDL-C, or ApoA1. Next to verifying the statin effects on miR-150-5p, miR-150 expression levels were measured before and after simvastatin treatments. our data revealed that plasma levels of miR-150-5p were lower in both groups and the expression level of miR-150-5p, was significantly increased after two months of treatment with simvastatin, especially in patients with COVID-19 and T2DM (Fig. 1).

Prediction of target Genes base on Bioinformatics

To better understanding of the regulatory function and pattern of miR-150-5p, bioinformatics' analyses such as GO annotation was conduct for 15 overlapping target genes using Metascape (https://metascape.org/). As shown in the results, these 15 targets were

mainly enriched in developmental and immune system processes, blood vessel developments, and so on (Fig. 2). This indicates the important role of miR-150-5p in our immune's system activity.

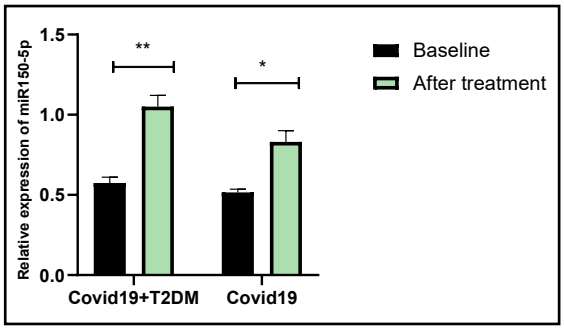

Fig. 1. The Effect of supplementation with simvastatin for two months of treatment on expression levels of miR-150-5p.

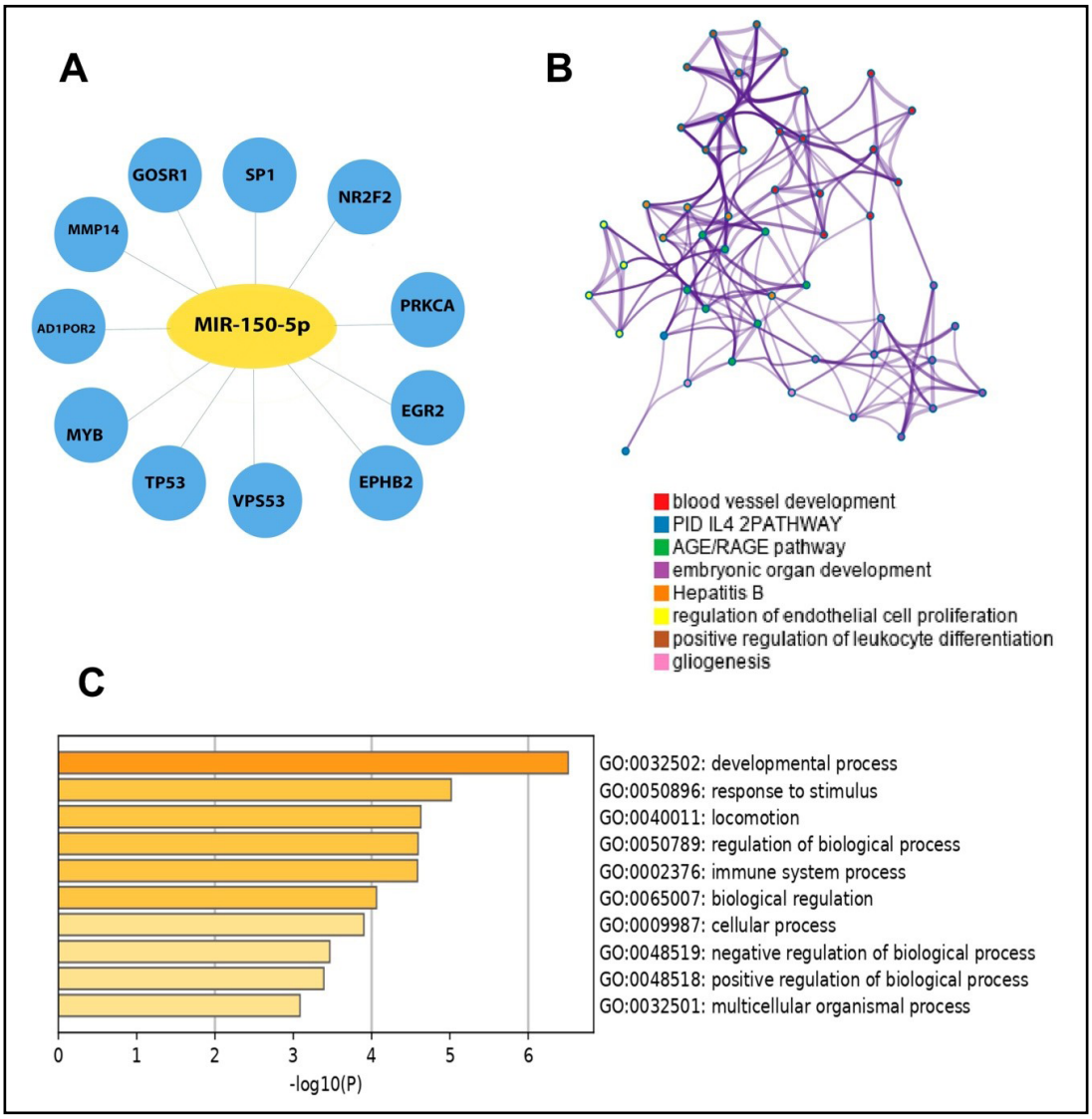

Fig. 2. Bioinformatic analysis. (A) Potential target genes for miR-150-5p predicted by multiple tools and visualized using the cytoscape program. (B) The Gene ontology (GO) annotation analysis and(C) Heat map of selected GO analysis of overlapping genes for miR-150-5p.

Discussion

Diabetic patients have a higher risk of developing a severe form of COVID-19 based on their abnormal lipid profile [21]. Different studies are showing the relation between dyslipidemia levels and the development of type 2 diabetes [22]. Cholesterol within lipid rafts is an important factor for viral entry of viruses including SARS-CoV therefore depleting cholesterol results in a significant reduction in viral mRNA [23]. Statins as lipid-lowering agents with its inhibitory role on 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG-CoA) reductase usually serve s the first line drug. Multiple studies have confirmed use of statins was associated with decreased mortality in COVID-19 patients, however, there are contradictory results about the role of statins in patients with T2DM developing COVID-19 [22, 24]. But in general, there are more studies indicating the role of statin therapy as a safe treatment in patients hospitalized with COVID-19, especially those with diabetes [25]. Emerging data also indicate that statins can differentially affect the expression of miRNAs *in vivo*, therefore understanding these modifications can lead to manipulation of statins application with miRNAs for better therapeutics approaches [26, 27]. MiRNAs are a class of ~22-nt non-coding RNAs with the ability to target (3′UTRs) of target genes leading to mRNA degradation or protein reduction [11]. A recent study revealed that human body have near 2,300 mature miRNAs and they play a role in various processes such as cell differentiation, proliferation and apoptosis [28, 29]. miRNAs can also limit the SARS-CoV-2 infection by blocking the cellular receptors and functional proteins related to viral replication [30]. Previous studies are showing that circulating levels of miRNAs can be regulated by statins [31]. Since very little is known about the potential use of miRNAs as biomarkers in response to statin therapy, in this study, we tried to reveal the association between miR-150-5p and statin treatments in a pilot study. We used COVID-19 patient's miRNA profiles based on previous reports. These data indicated that miR-150-5p is significantly lower in COVID-19 patients [32]. While over-expression of miR-150-5p showed the inhibitory effects for SARS-CoV-2 infection *in vitro* with its possible interconnection with miRNA recognition element (MRE) which is located on coding strand of SARS-CoV-2 and encoded non-structural protein 10 (nsp10) which has a crucial function on evading host immune response and viral replication [32]. On other hand, recent studies have demonstrated that miR-150 has the potential to modulate by regulating lipid metabolic activities and inflammatory responses both in cells and animals [33]. Over expression of miR-150 suppresses the expression of pro inflammatory factors including NF-κB, tumor necrosis factor-α (TNFα), IL1β, and IL6 and there is also a strong correlation between down-regulation of miR-150 and diabetes [17, 34, 35]. Our study revealed the induced changes on miR-150-5p levels after 2 months of statins treatments in both groups especially in COVID-19 and T2DM patients compared to the individuals only with COVID-19 group. Our bioinformatics study indicated the important role of miR-150-5p target genes in response to stimulus and modulating immune system process as well. Further assays are needed for better understanding the role of particular miRNAs and the miRNA-dependent pathways and their mechanisms of response to statins treatment, which may lead to better therapeutic manipulation of miRNAs for patients with diabetes and COVID-19 in future.

Acknowledgements

Author Contributions

M.A and M.A contributed equally to writing and drafting. A.P worked on editing all authors and approved the final submitted version.

Statement of Ethics

This research was confirmed by the Tehran College of Therapeutic Sciences with the code of ethics IR.IUMS.REC.1399.9223497212. Written informed consent was obtained from all patients.

Disclosure Statement

The authors declare that no conflict of interests exists.

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