DOI: 10.4149/BLL_2019_056

*Bratisl Med J 2019; 120 (5)*
344 – 348

CLINICAL STUDY

# The relationship between plasma microRNAs and serum trace elements levels in primary hyperlipidemia

Yerlikaya FH[1], Can U[2], Alpaydin MS[3], Aribas A[4]

*Necmettin Erbakan University, Meram Faculty of Medicine, Department of Biochemistry, Konya, Turkey.*
**fhumeyray@hotmail.com**

**ABSTRACT**
OBJECTIVES: The aim of the study was to investigate the relationship between plasma microRNA expression levels, which are associated with lipid metabolism and serum trace element levels in patients with primary hyperlipidemia.
METHODS: This study was performed on 46 (21M / 25F) primary hyperlipidemia patients aged 25–65 years and 37 (18 M/19 F) healthy people aged 25–65 years.
RESULTS: The following miRNAs were upregulated: miR-33a-5p, miR-370-5p, miR-378a-3p, miR-27a-3p, miR-27a-5p and miR-335-5p. Additionally, the levels of Co ($p < 0.001$), Ni ($p < 0.01$), Cd ($p < 0.001$) were significantly higher and the level of Cr ($p < 0.01$), Fe ($p < 0.05$), Mn ($p < 0.01$), Se ($p < 0.001$) and Mo ($p < 0.001$) was significantly lower in the primary hyperlipidemic patients compared to the healthy people . Also, miR-33a-5p was negatively correlated with serum Cr levels in patients with primary hyperlipidemia ($r = –0.376$, $p < 0.05$).
CONCLUSIONS: Our study demonstrates that miR-33a-5p and Cr element may regulate abnormal lipid homeostasis. Also, miR-370, miR-378, miR-27-a and miR-335 might aid in the identification of new therapies to treat patients with primary hyperlipidemia *(Tab. 3, Ref. 36)*. Text in PDF *www.elis.sk.*
KEY WORDS: primary hyperlipidemia, microRNA, trace elements.

## Introduction

Hyperlipidemia is a condition characterized by increased concentrations of lipids like low density lipoprotein (LDL- cholesterol), and triglycerides in blood (1, 2). It may be classified as either primary caused by specific genetic abnormalities, or secondary, as an acquired condition. Hyperlipidemia might be also idiopathic, that is without known cause. The fact that hyperlipidemia is a major cause of cardiovascular diseases is well established (1, 2). Despite advances in the prevention and management of cardiovascular diseases, this multifactorial disorder remains the leading cause of mortality worldwide (3).

MicroRNAs are a class of small noncoding RNAs, which post-transcriptionally regulate gene expression and control a wide range of biological functions (4). The specific role of microRNAs in regulating lipid metabolism is a new area of investigation (3). Recent reports have identified some microRNAs as major regulators of lipid metabolism, which is firmly regulated at the cellular level (3, 5).

The detection of trace elements in serum is of increasing interest in many clinical and research laboratories due to their role in physiological function and effects on health (6). It is stated that deficiencies of some trace elements cause significant changes in lipid and lipoprotein metabolism (7). The mechanisms of trace elements have not been fully elucidated and, despite extensive research, the role of these elements must be clarified further. In addition, there are some conflicting findings regarding the relationship between serum trace elements with lipid and lipoproteins (7). Also, disease-specific microRNA profiles have been identified in multiple disease states, including those with known dietary risk factors. Therefore, the role that nutritional components, in particular, trace elements, in the modulation of microRNA expression levels, and consequently health and disease, is increasingly being researched (8). Thus, the objective of this study was to evaluate the relationship between some microRNA expression levels, which are associated with lipid metabolism and serum trace elements levels in patients with primary hyperlipidemia.

## Materials and methods

### *Participants*

This study was performed on 46 (21 M/25 F) primary hyperlipidemia patients aged 25–65 years and 37 (18 M/19 F) healthy

[1]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Biochemistry, Konya, Turkey, [2]Konya Education and Research Hospital, Department of Biochemistry, Konya, Turkey, [3]Konya Education and Research Hospital, Department of Cardiology, Konya, Turkey, and [4]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Cardiology, Konya, Turkey

**Address for correspondence:** F. Hümeyra Yerlikaya, Dr, Necmettin Erbakan Universitesi, Meram Tip Fakültesi, Biyokimya Anabilim Dali, Konya, Turkey.
Phone: +0.505.4664231

**Acknowledgement:** This research was supported by Grant 131218002 from University of Necmettin Erbakan, Scientific Research Projects Department (BAP), Konya-Turkey.

people (control group) aged 25–65 years. All of the patients, who were diagnosed with primary hyperlipidemia in Cardiology Clinics of Meram Medical School and Cardiology Clinics of Konya Education and Research Hospital, were recruited into the study. There were no complaints and symptoms in the primary hyperlipidemia patients others than primary hyperlipidemia. Exclusion criteria of our study were diabetes, malignant diseases, chronic liver disease, hypertension, history of cardiovascular disease, infectious disease, pregnancy, alcohol and smoking habit, taking vitamin supplements, mineral, antioxidant and fish-oil tablets. Blood samples were obtained after an overnight fasting into plain vacuum tubes and into EDTA tubes. Plasma and serum samples were obtained after a suitable centrifugation and were stored frozen at -80 °C until the day of study. The study was approved by Ethical Committee of Meram Medical School, and informed consent was obtained from all the participants.

*Measurement of microRNA expression levels*

RNAs were isolated from plasma samples by using High Pure microRNA Isolation Kit (Roche Life Science, Mannheim, Germany). RNA samples were then converted to cDNA by using miScript II RT Kit (Qiagen, Hilden, Germany). cDNA samples were pre-amplified by using miScript Microfluidics PreAMP Kit (Qiagen, Hilden, Germany). qRT-PCR analysis was performed using miScript microRNA Assays (Qiagen, Hilden, Germany) with Dynamic Array 96.96 (Fluidigm, South San Francisco, CA, USA) on BioMark System (Fluidigm, South San Francisco, CA, USA).

*Measurement of serum trace elements levels*

An ELAN DRC-e (Perkin Elmer SCIEX Inc., Ontario, Canada) inductively coupled plasma mass spectrometry (ICP-MS) system was used for the measurements of the levels of the trace elements (Cr, Fe, Cu, Zn, Mn, Se, Co, Ni, Cd, Mo) in serum and using an multielement standard solution for IV and ICP (PerkinElmer Pure Plus, PerkinElmer Life and Analytical Sciences, USA). The MARS microwave digestion system (CEM Corporation, 3100 Smith Farm Road, Matthews, NC 28105-5044, USA) was employed for mineralization of serum samples. The instrument operating parameters are presented below: integration time: 0.6 s; RF power (W): 1100.00; plasma gas flow rate (L/min): 15.00; auxiliary gas flow rate (L/min): 1,20; nebulizer gas flow rate (L/min): 0,82; sampling depth (cm): 0,5; scanning mode: peak hopping; number of replicates: 3.

*Measurement of other analytes*

Total cholesterol, triglycerides, high density lipoprotein (HDL-cholesterol), LDL-cholesterol and blood glucose was measured by commercially available kits based on routine methods by the Abbott Architect C16000 auto-analyzer (Architect C16000 auto-analyzer; Abbott Laboratory, Abbott Park, IL, USA).

*Statistical analysis*

Statistical analysis of qRT-PCR data was made by using $2^{-DDCt}$ method (9, 10). Basic student t test was used for statistical analysis. Statistical analyses of biochemical data were done using SPSS v. 16.0 (SPSS Inc., IL, USA). All data are expressed as the mean ± standard deviations (SD). The normality of the variables was evaluated using the one-sample Kolmogorov–Smirnov test. The normal distribution of variables was examined with Independent-Samples t test, and abnormally distributed variables were examined by Mann–Whitney U test. The correlations between variables were performed by Pearson's Correlation test. We set statistical significance at $p < 0.05$.

**Results**

Baseline characteristics and biochemical parameters of the groups are presented in Table 1. As can be seen from the table, body mass index (BMI), total-cholesterol, triglycerides and LDL-cholesterol levels of the primary hyperlipidemia patients were significantly higher ($p < 0.01$ for BMI and $p < 0.001$ for the other parameters), whereas HDL-cholesterol levels were lower than those in the control group ($p < 0.01$). In addition, no significant differences were observed in systolic and diastolic blood pressure, age and glucose levels in the groups. Serum trace elements levels of the groups are presented in Table 2. As can be seen from the table, serum Co, Ni and Cd levels were significantly higher in the primary hyperlipidemia patients than those of the control group ($p < 0.01$ for Ni and $p < 0.001$ for the other parameters). Serum Cr, Fe, Mn, Se and Mo levels were significantly lower in the primary hyperlipidemia patients than those of the control group ($p < 0.05$ for Fe, $p < 0.01$ for Cr and Mn and $p < 0.001$ for Se and Mo). There were no significant differences between Zn and Cu levels of the groups.

To investigate the impact of primary hyperlipidemia on the patients and the control group on plasma microRNA expression levels, microRNAs were extracted from plasma and detected to microRNA expression levels (Sixteen microRNAs) by quantita-

**Tab. 1. Baseline characteristics and biochemical parameters of groups.**

| Parameter | Primary hyperlipidemia patient (n=46) | Control group (n=37) | p |
|---|---|---|---|
| Age (years) | 42.43±12.4 | 37.05±12.2 | 0.052 |
| BMI ($kg/m^2$) | 25.56±3.1 | 23.80±2.5 | 0.006 |
| Systolic blood pressure (mmHg) | 12.82±1.1 | 12.37±0.9 | 0.054 |
| Diastolic blood pressure (mmHg) | 8.06±0.9 | 7.86±0.8 | 0.319 |
| Glucose (mg/dL) | 91.95±8.1 | 89.91±6.4 | 0.207 |
| Total Cholesterol (mg/dL) | 304.57±81.3 | 174.78±19.5 | 0.001 |
| Triglyceride (mg/dL) | 160.26±74.6 | 100.73±50.7 | 0.001 |
| HDL-Cholesterol (mg/dL) | 51.65±9.9 | 60.64±13.6 | 0.01 |
| LDL-Cholesterol (mg/dL) | 202.72±46.9 | 98.89±14.4 | 0.001 |

**Tab. 2. Serum trace elements levels of the groups (All values are the mean ± standard error).**

| Parameter | Primary hyperlipidemia patient (n=46) | Control group (n=37) | p |
|---|---|---|---|
| Cr (µg/L) | 1.22±0.2 | 1.42±0.3 | 0.002 |
| Fe (µg/dL) | 57.8±3.3 | 126.07±26.8 | 0.016 |
| Zn (µg/dL) | 44.6±89.4 | 105.8±24.7 | 0.054 |
| Cu (µg/dL) | 167.1±4.7 | 154.2±4.7 | 0.057 |
| Mn (µg/L) | 1.51±0.08 | 3.76±0.3 | 0.001 |
| Co (µg/L) | 0.16±0.03 | 0.31±0.03 | 0.001 |
| Ni (µg/L) | 200.9±29.2 | 156.0±72.7 | 0.001 |
| Se (µg/L) | 36.9±10.1 | 308.7±81.0 | 0.002 |
| Cd (µg/L) | 7.12±1.3 | 1.2±0.7 | 0.001 |
| Mo (µg/L) | 1.42±0.1 | 1.01±1.1 | 0.001 |

**Tab. 3. Plasma microRNAs expression levels of groups.**

| Position | microRNAs | Fold Regulation | p |
|---|---|---|---|
| 1 | miR-33a-3p | –1.1204 | 0.218 |
| 2 | miR-33a-5p | 14.7945 | 0.003 |
| 3 | miR-122-3p | –1.0584 | 0.287 |
| 4 | miR-122-5p | 1.0036 | 0.258 |
| 5 | miR-758-3p | –1.2937 | 0.385 |
| 6 | miR-758-5p | –1.5929 | 0.346 |
| 7 | miR-370-3p | 1.0793 | 0.062 |
| 8 | miR-370-5p | 6.0203 | 0.034 |
| 9 | miR-378a-3p | 4.401 | 0.003 |
| 10 | miR-378a-5p | –1.346 | 0.847 |
| 11 | miR-27a-3p | 9.6274 | 0.014 |
| 12 | miR-27a-5p | 3.2672 | 0.026 |
| 13 | miR-335-3p | 1.1581 | 0.660 |
| 14 | miR-143-3p | 4.2354 | 0.442 |
| 15 | miR-143-5p | –1.0118 | 0.971 |
| 16 | miR-335-5p | 3.5296 | 0.034 |

tive real-time PCR (qRT-PCR) with the Fluidigm integrated microfluidic circuit technology. Among them, expression levels of six microRNAs were found to be significantly different ($p < 0.05$) between the groups. The following microRNAs were upregulated: miR-33a-5p, miR-370-5p, miR-378a-3p, miR-27a-3p, miR-27a-5p and miR-335-5p. Relative expression of these microRNAs in the plasma of primary hyperlipidemia patients and the control group is presented in table 3.

Simple correlation analysis was performed to investigate the association of microRNA expression levels and serum trace elements levels; miR-33a-5p was negatively correlated with serum Cr levels in patients with primary hyperlipidemia ($r = –0.376$, $p < 0.05$). miR-33a-5p was positively correlated with total cholesterol in the patients with primary hyperlipidemia ($r = 0.381$, $p < 0.05$). And miR-370 was positively correlated with total cholesterol ($r = 0.423$, $p < 0.05$), and LDL-cholesterol ($r = 0.456$, $p < 0.01$) levels in patients with primary hyperlipidemia. On the other hand, there were no correlations between our measured other microRNAs and trace elements and lipid panel parameters in patients with primary hyperlipidemia and the control group (data not shown).

## Discussion

To date several microRNAs have been described to regulate lipid metabolism including miR-33a, miR-122, miR-758, miR-370, miR-378a, miR-335, miR-27a, and miR-143. miR-33a plays a major role in various biological processes such as: cholesterol homoeostasis, HDL-cholesterol formation and fatty acid oxidation (11). Its role in the regulation of cholesterol homeostasis is regulating the ATP-binding cassette transporters (ABC-transporters) Abca1 and Abcg1 (12). Also, Rayner et al reported that anti-miR-33 was shown to directly target macrophages in mouse atherosclerotic plaques, and to cause regression of atherosclerosis characterized by a 35 % reduction in plaque size and decreases in macrophages and inflammatory gene expression (13). Parallel to the above description, Martino et al (11) and Simionescu et al (14) support our findings in their work. Namely; Martino et al found that miR-33a and miR-33b were significantly up-regulated in the plasma of 28 hypercholesterolaemic children compared to 25 healthy subjects, and for both microRNAs, a positive correlation with total cholesterol and LDL-cholesterol was found (11). Simionescu et al found that level of miR-33a was increased in hyperlipidemic patient sera and correlate positively with the levels of the main lipid and inflammation parameters (14).

Our results revealed that plasma level of miR-370, which could directly down-regulate the expression of carnitine palmitoyl transferase 1α gene, which controls fatty acid oxidation, was significantly increased in patients with primary hyperlipidemia compared to the control group. And the level of miR-370 was positively correlated with total cholesterol, and LDL-cholesterol level in patients with primary hyperlipidemia. Previous studies found that miR-370 were over-expressed in the livers of hyperlipidemia animals (15) and this microRNA was significantly increased in hyperlipidemia patients compared to the controls (16). Also it was positively correlated with total cholesterol, triglycerides, and LDL- cholesterol levels in both hyperlipidemia patients and the controls (16). These results support our findings.

In the present study, we found that miR-378a-3p was significantly upregulated in patients with primary hyperlipidemia. This finding is in accordance with that of Huang N et al (17). MiR-378-3p plays an important role in adipogenesis and lipogenesis. It induces these defined metabolic events by targeting mitogen-activated protein kinase 1 (17). Overexpression of miR-378a-3p increases the size of lipid droplets and the accumulation of triacylglycerol, whereas knockout of this microRNA reduces triacylglycerol accumulation (17). In our study, miR-27a-3p, miR-27a-5p and miR-335-5p expression levels of the patients

with primary hyperlipidemia were significantly higher than that of the control group, a finding, which constitutes a critical role of microRNAs in regulating lipid metabolism (3). Yang Z et al reported that overexpression of miR-27a accelerated adipolysis by releasing more glycerol and free fatty acids from the adipocytes and repressed lipid storage in cells (18). In addition, miR-27a inhibited the expression of many lipid metabolic genes, including fatty acid synthase (FASN), SREBP-1, SREBP-2, PPARα and PPARγ, as well as ApoA1, ApoB100 and ApoE3. Thus, miR-27a may regulate lipid metabolism by reducing lipid synthesis and increasing lipid secretion from cells (18). Also, Fernández-Hernando et al reported that miR-335 is upregulated in response to lipid loading and is highly expressed in liver and adipose tissue of obese mice (3). In another study, investigating microRNAs associated with lipid metabolism in childhood obesity, miR-27, miR-378 and miR-370 were found to be high, which supports our findings, because childhood obesity enhances the risk for dyslipidemia (19).

New findings about the effects of trace elements on human lipid metabolism are limited. Previous studies have shown that selenium in small quantities is bound to lipoproteins, particularly LDL-cholesterol and very low-density lipoprotein (20, 21). Hypercholesterolemia may affect the synthesis of selenocysteine, an important component of selenoproteins, as isopentenylation of selenocysteine tRNA and one step in the formation of cholesterol require the same substrate, isopentenyl pyrophosphate (22). In the context of these explanations, we can say that low selenium levels are predictable in patients with primary hyperlipidemia. It has been suggested that low serum levels of iron results in hypocarnitinemia impairing carnitine biosynthesis, and this effect increases serum triglyceride levels by shifting the fatty acid metabolism to glyceride synthesis (23). In the study of Choi et al, after iron supplementation, the initially reduced concentrations of total cholesterol and triglyceride returned to levels comparable to the control group (24). The findings of this researcher support our findings. There are different results at the other side; Dabbagh et aldemonstrated that iron overload caused a significant increase in plasma total and HDL-cholesterol levels in rats (25). Bristow-Craig et al (8) showed that higher dietary iron levels were associated with higher serum cholesterol levels in rats (26).

In the epidemiological study, it has been shown that high blood levels of Cd are associated with the initiation of atherosclerosis, which may be due to hypertensive effects of Cd and its ability to cause endothelial cell damage (27). Ilyas et al reported that Cd levels were noticeably higher in the blood of atherosclerosis patients in comparison with healthy subjects (28). In accordance with literature, Cd is reported higher in serum of the patients with primary hyperlipidemia in the present study. Cr is an essential element. Its deficiency may cause atherosclerosis, because Cr is an important component of the lipid and protein metabolism in human body (28). It is reported that Cr had an inhibitory effect on hydroxymethylglutaryl-CoA reductase with hypolipidemic effect on lipid metabolism in literature (29). Also, LDL- cholesterol decreased significantly, when subjects were ingesting chromium picolinate (28). This finding supports our findings. On the other hand, our results indicate that there is a significant negative correlation between serum Cr levels and miR-33a-5p expression levels in patients with primary hyperlipidemia, while there was no such correlation in the control group. According to our hypothesis, increase in cholesterol and LDL-cholesterol levels parallel to decreased Cr levels and decreased ABCA1 and ABCG1-mediated cholesterol efflux with increased miR-33a-5p expression and consequently increased intracellular lipid accumulation revealed this negative correlation between Cr and miR-33a-5p. In the literature review we made, the relationship between Cr and some microRNAs was detected. For example, chromium restriction downregulates miR-338-5p in offspring adipose. miR-338–3p is down-regulated in islets under conditions of insulin resistance, such as pregnancy and obesity (30-32). In addition, chromium restriction downregulates miR-181b-5p in offspring adipose (33).

In conclusion, for the first time, our study demonstrates that miR-33a-5p expression levels and serum Cr levels are correlated in patients with primary hyperlipidemia. This microRNA and Cr element may regulate abnormal lipid homeostasis. Also, miR-370, miR-378, miR-27-a and miR-335 may aid in the identification of new therapies to treat patients with primary hyperlipidemia. Nevertheless, the underlying mechanism of this finding needs to be investigated further.

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Received Januaey 16, 2019.
Accepted February 22, 2019.