Evaluation of some microRNAs dysregulation in obesity and obesity-related hypertension

Mai Nabawy[a], Weaam Gouda[b], Mie Afify[b], Waleed I. Hamimy[c], Walaa G. Hozayen[d], Mohamed D.E. Abdelmaksoud[b], Tamer Mosa[b], Amr E. Ahmed[a]

[a]Department of Biotechnology and Life Science, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt, [b]Department of Biochemistry, Biotechnology Research Institute, National Research Centre, Giza, Egypt, [c]Obesity Surgery Unit, Faculty of Medicine, Cairo University, Giza, Egypt, [d]Department of Biochemistry, Faculty of Science, Beni-suef University, Beni-Suef, Egypt

Correspondence to Weaam Gouda, PhD, National Research Centre, Giza 12622, Egypt. Tel: +20 122 294 0588; Fax: +20 33760900; e-mail: weaamgoudaali@gmail.com, weaam_gouda@yahoo.com, wg.gouda@nrc.sci.eg

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Background
MicroRNAs (miRNAs) are implicated in regulating obesity, but clinical trials have yielded conflicting results.
Objective
This study aimed to investigate the relationships between circulating levels of miRNA-221 and miRNA-222 in obesity and hypertension-related obesity in humans. This may shed light on the pathogenic pathways controlling obesity and provide noninvasive molecular indicators to identify and predict the disease. The relationships between circulating levels of the above miRNAs and variables related to adiposity and lipid profiles were further investigated.
Patients and methods
Using a quantitative real-time P technique, the expression levels of circulating miRNAs were determined in serum samples from 65 obese patients (35 without and 30 with hypertension) and 45 age-matched and sex-matched normal-weight individuals.
Results and conclusion
MiRNAs (221 and 222) have been shown to be differentially expressed in the sera of obese patients compared to controls. In addition, obese hypertensive patients were shown to have higher serum levels of miRNA-222 and miRNA-221 than healthy controls. Serum miRNA-221 was correlated with BMI, cholesterol, triglycerides, and low-density lipoprotein cholesterol. Thus, circulating levels of miRNA-221 and miRNA-222 inease in obesity and obesity-associated hypertension. These miRNAs may serve as markers for obesity and obesity-induced hypertension.

Keywords:
microRNAs, miRNA-221, miRNA-222, obesity

Abbreviations:
EC, endothelial cells, HDL, high density lipoprotein, LDL, low density lipoprotein, TG, triglycerides

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Introduction

The abnormal or excessive deposit of fat that is harmful to health is known as obesity. According to the WHO, it was identified as a disorder in 2012, and there has been an alarming rise in its cost and impact [1]. The BMI can be utilized to seen for obesity; a BMI of 30 kg/m^2 or higher is deemed obese [2]. However, this tool has some limitations: It does not show the extent of its impact on health, nor does it reflect the pathophysiology of the disease or assist in identifying the relative contributions of muscle and fat mass [3,4]. Current estimates suggest that more than two billion people worldwide are obese, and as a result, they are at ineased risk of developing a number of metabolic disorders linked to obesity, including hypertension, cardiovascular diseases, fatty liver disease, and type 2 diabetes [5–7]. As the abdominal fat disrupts the immunological and endoine systems, there is a strong link between obesity and hypertension [1]. Furthermore, regardless of race, ethnicity, or sex, obesity has been established as a significant indicator of susceptibility to elevated blood pressure in both children and adults [8,9]. The Framingham Heart Study and the Nurse's Health Study are two important investigations that examined this relationship 10,11]. Regarding the Nurses' Health Prospective Cohort Study, which comprised 83 882 females who underwent follow-up over 16 years, it demonstrated a higher BMI was linked to the start of hypertension; the probability of developing hypertension for females who gained 5–10 kg and

more than 25 kg were 1.7 and 5.2, respectively; and 40% of cases of newly diagnosed hypertension were related to overweight and obesity [10]. Similarly, ineased adiposity was responsible for ~27% of cases of hypertension in adults, according to the investigation of Framingham Heart, whose participants were followed for up to 44 years [11]. Furthermore, hypertension is more prevalent aoss all BMI levels, with obesity having the highest frequency (87%) [12]. Thus, the risk of hypertension and its effects on health are only likely to rise only when obesity reaches endemic proportions. Nevertheless, the connection between blood pressure and obesity is complex, as a number of factors interact to cause hypertension [1].

The noncoding RNA molecules known as microRNAs (miRNA) are essential for several regulatory processes [13,14]. Adipose tissue produces these miRNAs, which regulate posttransiptional gene expression, development, proliferation, and apoptosis of cells [15]. Therefore, it is now clear that miRNAs are concerned with many biological processes and that their deregulation or disruption may contribute to numerous diseases [16]. A growing number of studies in recent years have demonstrated that miRNAs are important as efficient markers to identify and evaluate the risk of obesity and related comorbidities [17–20]. Although studies regarding the role of miRNAs in hypertension have progressed recently, there is still much to be studied [21–23]. The miRNA-221/222 gene cluster lies on human DNA chromosome Xp11.3 [24]. Both genes' nucleotide sequences are extremely similar to one another. RNA polymerase II transibes then as a single long noncoding RNA precursor [25]. More research continues to demonstrate the miRNAs involvement, notably miRNA-222 and miRNA-221, in metabolic disorders and adipogenesis [15–17,26–28]. The connection between obesity and hypertension has not been sufficiently investigated, though. While the detection and measurement of miRNAs may help physicians identify obese patients who are more likely to develop hypertension complications, these miRNAs may also serve as targets in subsequent miRNA-based approaches aimed at improving patient satisfaction [15]. Hence, the present study aimed to measure circulating levels of miRNA-221 and miRNA-222 in obese patients with or without hypertension in order to assess their association with obesity and its related hypertension, which may gain insight into their possibility as molecular biomarkers for obesity and its hypertension complication. Associations between the above-mentioned miRNAs and parameters correlated to adiposity and lipid profiles were additionally evaluated.

Patients and methods

The study design

Participants

Sixty-five obese patients with a BMI more than or equal to 30 kg/m^2 (35 without hypertension and 30 with hypertension) who had undergone bariatric surgery for obesity from the surgery unit of Kasr Al-Aini Hospital, Cairo, Egypt, were reuited in this study; their ages ranged from 25 to 60 years and sexes (39% males and 61% females). Hypertension was diagnosed when blood pressure was measured on two separate days with a diastolic reading of at least 90 mmHg and a systolic reading of at least 140 mmHg. Besides, 45 sex-matched and age-matched healthy adult volunteers with normal weight and normotensive were included as controls. Prior to taking part in the research study, every participant obtained their written informed consent. If any of the following exclusion iteria were achieved, the patient was not enrolled, as they may take apart in miRNA levels: liver disease, kidney disease, steroid treatment, and history of cancer. This study was approved by the Ethics Committee of the National Research Centre (No. 19-162) in accordance with relevant guidelines and regulations or the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations, and all experimental protocols were approved by the National Research Centre.

Sampling

After a 12-h fast, about 5 ml of peripheral venous blood was taken from every individual. The blood was left to coagulate at 25°C, centrifuged at 3000 g for 10 min, and the sera were divided into two portions, the first for biochemical analysis and the second was added to QIAZol in individually labeled, sterile tubes for each patient, and then stored at 80°C until miRNA expression levels were determined.

Anthropometric measures

BMI was calculated as weight in kilograms divided by height in meters squared.

Biochemical analyses

Using a kit from Stanbio Laboratory (Boerne, Texas, USA), triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) serum levels were calorimetrically determined. Via

Friedewald's formula, low-density lipoprotein cholesterol (LDL-C) was calculated [29].

RNA isolation

Using the miRNeasy mini isolation kit from QIAgen Inc., Germantown, Maryland, USA, following the manufacturer's directions, total RNA was extracted from serum samples. Thermo Fisher Scientific Inc. (Walthman, MA, USA) nanodrop 2000c spectrophotometer was used to measure RNA quantity and quality. All RNA samples were determined to be of sufficient quality for P analysis (1.93–2.10) based on measurement of the A260/A280 ratios [30].

MicroRNAs expression levels assay

The miRNA reverse transiption kit (Applied Biosystems) and particular miRNA reverse transiption (RT) primers were used to reverse-transibe (1 μg) miRNA-221 and miRNA-222 in accordance with the manufacturer's instructions. MiRNA-221 primers were (F): CGAGATCTGA GAATTACTTGCAAGCTG; (R): CCGCTCGA GCATTGGTGAGACAGCCAATG [31] and miRNA-222 primers were (F): CGCAGATCTT TTCTTCCACAGAGCCCCTCC;(R):GGGGAT CCTCTCAGGACACTGAAGCAGA [32]. A final volume of 20 μl was obtained by mixing 2 μl of RT products, 10 μl of P master mix (SYBR green), 1 μl of miRNA assays, and additional nuclease-free water. Utilizing an Applied Biosystems, Foster, California, USA, real-time P system 2700, the following conditions were used for all reactions: 10 min at 95°C were followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. The miRNA relative expression was normalized to U6. Via the equation $2^{-\Delta\Delta Ct}$, fold changes in candidate miRNA expression were estimated [33].

Statistical analysis

Our results were interpreted via SPSS, statistical package for the social sciences, version 20 (SPSS Inc., Chicago, Illinois, USA). Mann–Whitney test was employed for nonparametric variables, and data was represented as median and interquartile range. The SE and means were used to desibe parametric quantitative variables using Student *t* test and analysis of variance to compare miRNA expression levels of the studied groups and were graphically represented by bar graphs. Multivariable linear regression analysis was employed to determine the correlations between miRNA expression levels and parameters. In addition, Spearman rank correlation, which is represented as scatter graphs, was done. When the difference was *P* value less than 0.05, it was reflected statistically significant and greatly statistically significant when there was a *P* value less than 0.01.

Results

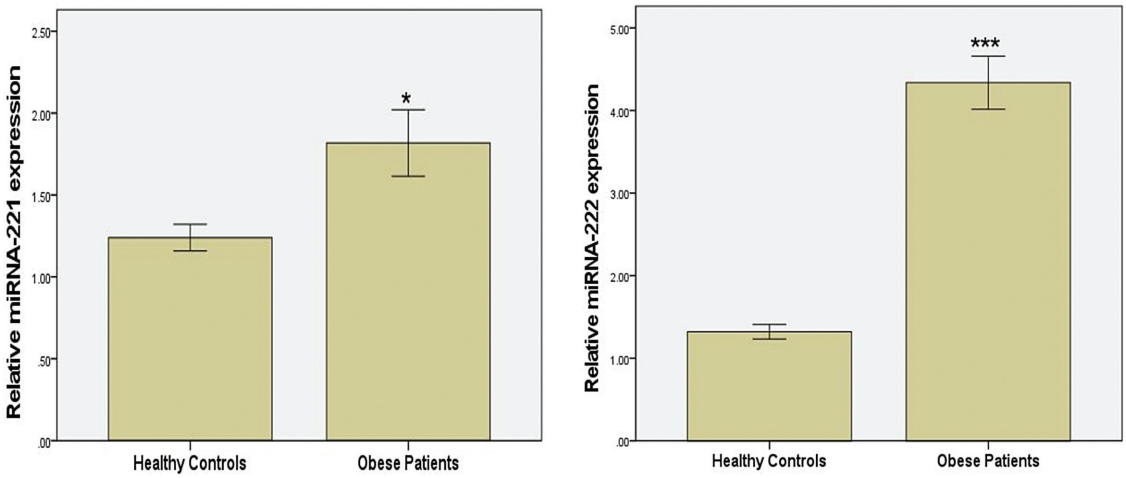
Characteristics of studied participants

Table 1 represents an overview of the anthropometric and clinical characteristics of the study participants. We found significant elevations in BMI ($P<0.001$), fasting blood glucose ($P=0.016$), TC ($P<0.001$), TGs ($P<0.001$), LDL-C ($P<0.001$), and HDL-C ($P<0.001$) between obese and control groups.

Expressions of miRNAs 221 and 222 and their relation to obesity and hypertension

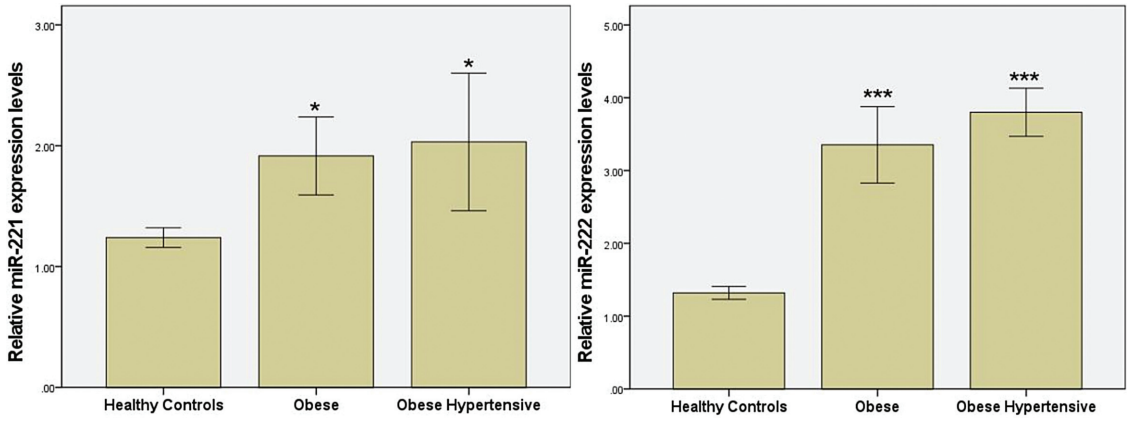
Figure 1 shows that the relative miRNA-221 expression levels deeased in the serum samples from

Figure 1



The expressions of miRNA-221 and miRNA-222 in the serum of obese patients in comparison to healthy individuals with normal body weight. The mean values are presented with the SE of the means using Student's *t* test. **P* value less than 0.05 or ****P* value less than 0.001 compared to controls.

Figure 2

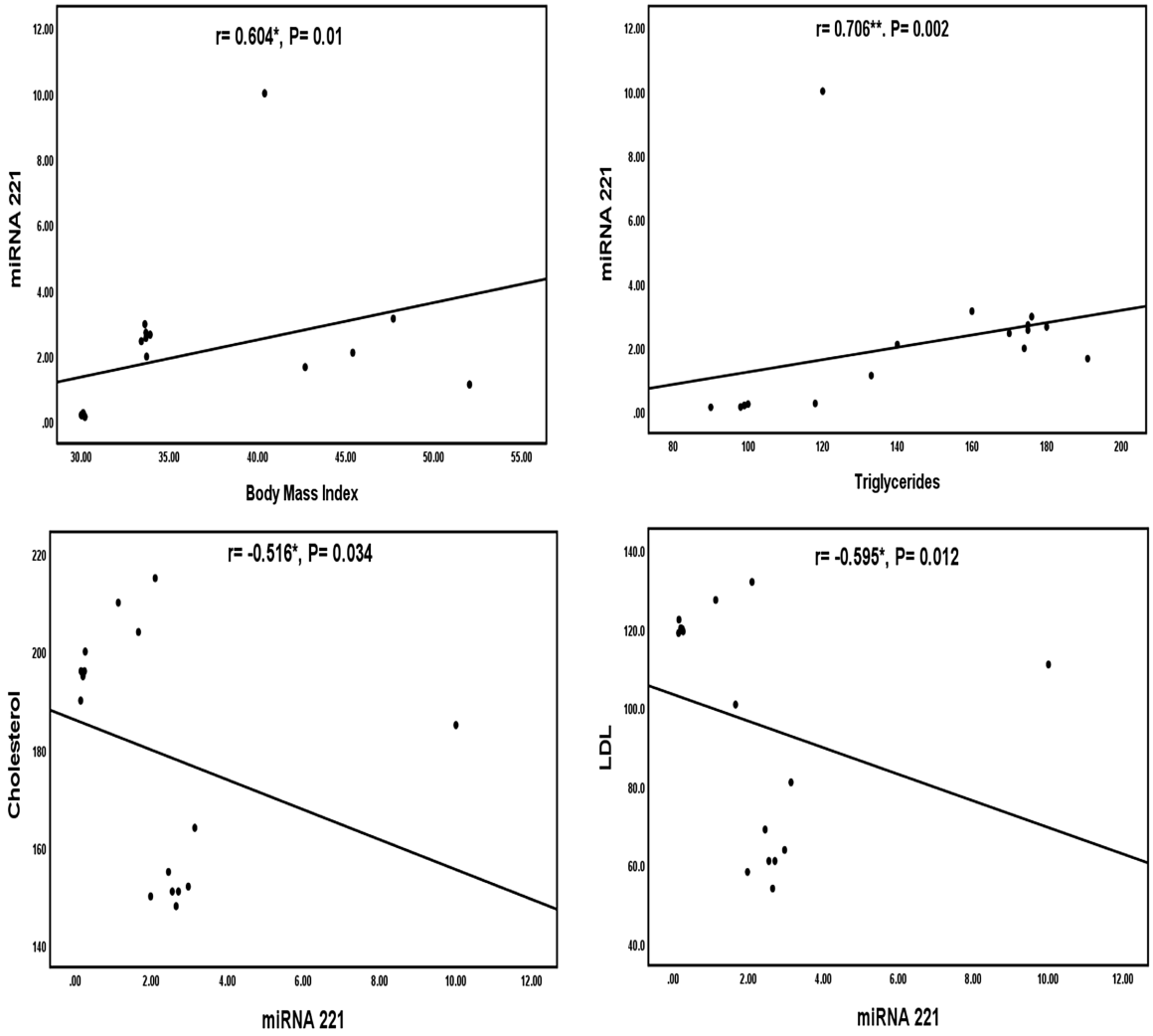


The circulating miRNA-222 and miRNA-221 expression levels for obese and obese hypertensive patients in comparison to the controls. Using one-way analysis of variance, values are demonstrated as mean±SE. ****P* value less than 0.001 and **P* value less than 0.05 versus healthy controls.

normal-weight healthy controls (1.2±0.08) than obese patients (1.8±0.2) at *P* value of 0.024. Relative miRNA-222 expression levels lowered in the sera from normal weight healthy controls (1.3±0.09) than obese patients (4.3±0.3) at *P* value less than 0.001.

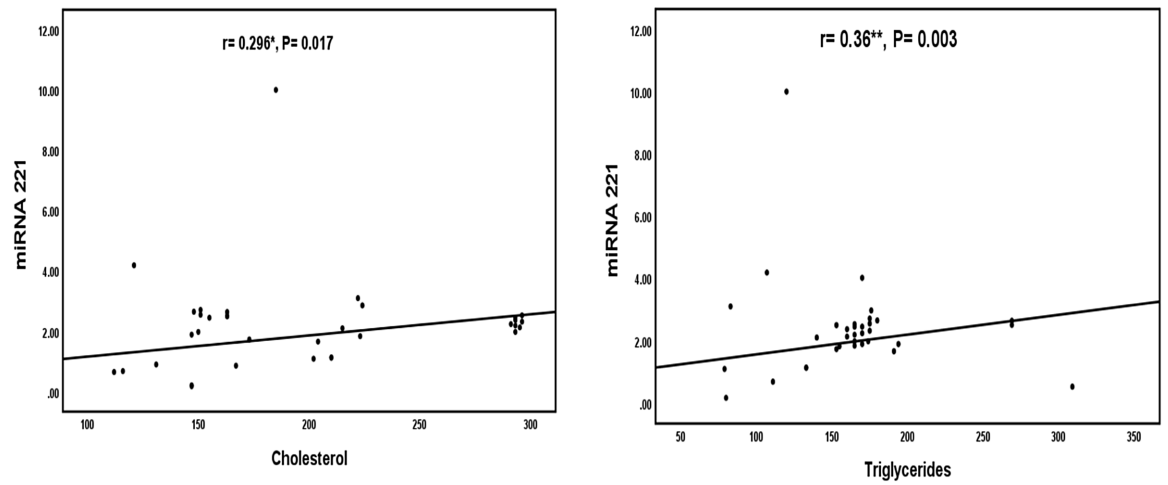
Figure 2 shows the corresponding miRNA-221 expression values reduced in serum samples from healthy controls with a normal weight (1.2±0.08) than obese patients without hypertension (1.9±0.3) and obese patients with hypertension (2.03±0.5)

Figure 3



The correlations of miRNA-221 with BMI and lipid profile in obese hypertensive patients.

Figure 4



The correlations of miRNA-221 with lipid profiles in obese patients.

with a *P* value of 0.047. Furthermore, miRNA-222 expressions were 1.3±0.09 in controls, 3.35±0.5 for obese patients, and 3.8±0.3 among obese patients with hypertension, with a lower significant difference of less than 0.001. As a result, the two obese groups had higher significant miRNA-222 expression levels than controls.

Spearman correlation analyses of miRNA-221 with different parameters in obese hypertensive patients show significant correlations in the different factors: positive correlations for TG (r=0.706; P=0.002) and BMI (r=0.604; P=0.01), however negative correlations in cholesterol (r=–0.516; P=0.034) and LDL-C (r=–0.595; P=0.012) (Fig. 3). Moreover, there were correlations with cholesterol and TG among obese patients, as shown in Fig. 4. While the correlation analyses of miRNA-222 did not show any significant difference with different parameters in obese hypertensive patients.

Multivariable linear regression analyses were performed for the relationship between miRNA expression levels and parameters; miRNA-221

Table 1 The comparison of clinical and demographic features for obese and control groups

	Obese patients (*N*=65)		Normal weight controls (*N*=45)		
Variables	Median	IQR	Median	IQR	*P* value
Age (years)	45	23.3–63.7	39	30–48.7	0.168
BMI (kg/cm^2)	39	30.1–54.5	19.8	18.8–24.8	<0.001
FBG (mg/dl)	95	76–144	90	75–105	0.016
Cholesterol (mg/dl)	190	118–294	145	130–165	<0.001
Triglycerides (mg/dl)	140	79–250	85	70–115	<0.001
LDL-C	111	66.8–205	78	71–85	<0.001
HDL-C	57	42.4–65	50	45–57	<0.001

Data displayed as median and interquartile range (IQR). FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *P* value is significant if less than 0.05.

Table 2 Linear regression analysis of miRNA-221 with demographic and lipid profile in the obese and the obese hypertensive patients

	Obese hypertensive (*N*=30)		Obese (*N*=35)	
Variables	Standardized coefficient beta	*P* values	Standardized coefficients beta	*P* values
Sex	–1.30	0.000	0.127–	0.341
BMI (kg/m^2)	1.044	0.001	–0.066	0.613
Triglycerides	0.668	0.216	0.165	0.232
LDL-cholesterol	–1.836	0.001	0.26	0.05
HDL-cholesterol	0.199	0.373	0.078	0.555

HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P* value is considered significant if less than or equal to 0.05.

Table 3 Linear regression analysis of miRNA-222 with demographic and lipid profile in studied groups

	Obese hypertensive (*N*=30)		Obese (*N*=35)	
Variables	Standardized coefficient beta	*P* values	Standardized coefficients beta	*P* values
Sex	−0.877	0.058	0.163	0.238
BMI (kg/m^2)	−0.03	0.936	0.037	0.782
Triglycerides	1.402	0.124	−0.169	0.237
LDL-cholesterol	0.174	0.803	0.083	0.543
HDL-cholesterol	−1.003	0.017	0.058	0.671

HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P* value is significant when less than 0.05.

showed a significant relation to sex, BMI, and LDL-C in the obese hypertension group but displayed an association with LDL in the obese group (Table 2). The regression analysis of miRNA-222 demonstrated significant differences with HDL-C in obese patients with hypertension (Table 3).

Discussion

The pathophysiology of obesity and related diseases focuses on impaired adipose tissue metabolism and function. The circulating miRNAs released by adipocytes and other cells have been shown to play a role in the pathogenesis of metabolic disorders. While circulating, HDL is primarily responsible for transporting certain of the different miRNAs, such as miRNA-222, miRNA-223, and miRNA-126 [34]. In the present findings, miRNA-221 and miRNA-222 expression levels were considerably greater in sera of the obese group compared to sera of normal weight controls. Additionally, compared to the control group, obese hypertension patients had considerably elevated expressions of miRNA-221 and miRNA-222. In contrast, in obese hypertension individuals, expression levels of miRNA-221 and miRNA-222 were not significantly elevated than in obese patients without hypertension complications. Hence, the above-mentioned miRNAs were considerably improved in the obese and the obese-related hypertension groups compared with the control group. These findings are in agreement with Ortega *et al*. [17], who have stated that morbidly obese individuals display a noticeable inease in circulating miRNA-222; however, contrary to our results, Ortega *et al*. [17] found a decline of miRNA-221 amounts in obesity. Consistent with our findings concerning the circulating levels of miRNA-221 and miRNA-222 as noninvasive biomarkers for obesity and hypertensive obesity, the results of Gentile and colleagues revealed that the extent of miRNA-222 and miRNA-221 expressions in human adipose tissues related to obesity and metabolic disorders [15,35]. Furthermore, it has been demonstrated that miRNA-221 contributes to adipose tissue inflammation [36,37].

Our research suggests that miRNA-221/222 cluster expression in human serum samples may be connected to obesity, even though our data are consistent with these previous findings. This miRNA controls genes involved in ucial metabolic, adiposity, inflammatory, and obesity-related pathways, indicating that miRNAs influence the cellular processes that regulate weight loss, making them reliable biomarkers for the development of obesity [38]. Moreover, Yamaguchi *et al*. [39] revealed that miRNA-222 overexpressions are linked to accelerated adipogenesis, which might become an achievable goal for miRNA-based therapeutics.

The relationship between miRNA-221 and miRNA-222, obesity, and metabolic diseases may be explained by the fact that these miRNAs stimulate adipogenesis by blocking the production of nitric oxide and matrix metalloproteinase, which in turn control the signaling pathways of activated B cells, nuclear factor kappa-light-chain enhancer, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha [40]. Even though miRNA-221 activates AdipoR, which modifies downstream adiponectin-related processes such as lipolysis, fatty acid oxidation, and ketogenesis, and suppresses the DNA damage-inducible transipt 4-mediated mammalian target of the rapamycin complex 1 pathway [15]. Hence, further research should be conducted to investigate whether miRNA-221-targeted therapy could reverse the related adipogenic process, as preliminary research on an animal model demonstrated that pharmacological suppression of miRNA-221 had a notable effect against obesity [39].

Human miRNA-221 and miRNA-222 are important players in the vascular environment, as they influence angiogenic properties of endothelial cells (ECs) and phenotypic changes in vascular smooth cells [41]. Human ECs display elevated amounts of miRNA-

221 and miRNA-222, which are believed to control the proliferation and processes of the vascular endothelium [42]. More insights revealed that miRNA-221/222 is able to sustain endothelial integrity and antiangiogenic features by means of various target-mediated processes, hence keeping ECs in their quiescent condition [43]. These findings are supported by the finding that ECs induce a proangiogenic transiptional pathway in response to vascular injury, which alters the expression of miRNA-221/222 and the EC profile [44]. Subsequently, aberrant miRNA-221/222 expression plays a ucial role in the progression of arterial wall thickness and atherogenic diseases, such as hypertension [45].

The present study found differences in BMI, fasting blood glucose, TC, TGs, HDL-C, and LDL-C between obese patients and normal weight controls. However, we did not find any variances in age between obese patients and normal weight controls. Consistent with our results, Hussain and colleagues showed that there was an insignificant correlation between BMI and LDL-C and a significant negative correlation between BMI and HDL-C. Patients with normal BMIs had considerably greater levels of HDL-C. These findings are notable because they show that BMI has a modest effect on lipid profile [46]. The study of Li *et al*. [47] demonstrated the associations of BMI, TC, TG, and HDL-C with fatty liver risk.

Our results showed that miRNA-221 correlated with different parameters (cholesterol, TG, and LDL-C) in obese hypertension patients, while it did not display any substantial association with HDL-C. On the other side, miRNA-222 did not show any correlation with different parameters in obese hypertensive patients. In line with our findings, Brandão-Lima and colleagues showed correlations of miRNA-221 with BMI, TG, and LDL-C. Also, compared to people with normolipidemia, HDL-C levels showed a slight inease in miRNA-222 levels [48]. Furthermore, Zhang *et al*. [49] revealed that cholesterol, TG, and LDL-C were positively correlated with miRNA-221. While on the contrary to our results, Zhou *et al*. [50] found that in low HDL-C phenotypes, the reversed configuration of circulating miRNAs (221 and 222) may be a clinically useful signal for molecular pathology. Karere *et al*. [51] stated that although there was a down-regulation of the miRNA-221/222 family in high LDL-C baboons, there was no appreciable difference in expression in low LDL-C samples.

Conclusion

In conclusion, the expressions of miRNA-221 and miRNA-222 were considerably up-regulated in serum samples of obese and obese-related hypertension groups compared to normal weight healthy controls. The levels of miRNA-221 expression were related to BMI, TC, TGs, and LDL-C in obese patients.

Limitations

One of the study's limitations is its limited sample size. Hence, additional larger-scale research on different individuals is essential to elucidate the links between miRNA-221 and miRNA-222 in obesity predisposition and its related hypertension.

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Authors' contributions

Concepts and Design: M.A., M.D.E.A., A.A., W.G., and W.I.H.; Definition of intellectual content: M.A, W.G.H., and W.I.H.; literature search, clinical studies, and data acquisition: M.N., W.G., A.A., and T.M.; data analysis and statistical analysis: W.G.; manuscript preparation: M.N., W.G.H., and T.M.; manuscript editing and review: M.A., M.D.E. A., A.A., and W. G.; Guarantor: M.A.

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Conflicts of interest

There are no conflicts of interest.

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