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Original Article

Correlation between MicroRNA-21 Expression and Overweight/Obesity

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Abstract

Introduction: Obesity and overweight are chronic conditions characterized by excessive adiposity that negatively impact health. They are among the most significant risk factors for respiratory failure, diabetes mellitus, cardiovascular disease, and hypertension. Genetic factors may contribute to some of these conditions, but they are mainly associated with the lifestyle in the majority. However, neither genetics nor lifestyle can fully explain all cases. Recent studies have proven that microRNA-21 causes the systemic hypertension, many cardiac pathologies, and some cancers. MicroRNA-21 is viewed as an important future biomarker for many critical conditions. We explored the role of microRNA-21 in the causation of overweight and obesity. There is conflicting data about this association in the literature. Determining if there is an association may help us in better understanding and managing this condition.

Aim: To determine if there is a link between microRNA-21 expression and body mass index (BMI) in an Indian adult population. We also compared the lipid profiles (total cholesterol, HDL, LDL, and triglycerides) of participants (grouped by their BMI) to get a better understanding.

Patient and methods: The study was conducted in Pondicherry, India and had 50 participants, with 30 as controls having normal BMI, and 20 categorized as overweight or obese as per BMI.

Results: The microRNA-21 levels in circulation were analyzed using Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR). The expression of microRNA-21 was statistically higher in the overweight/obese cohort compared to the normal BMI individuals (p=0.034). A significant difference was also noted in the total cholesterol levels, with the overweight/obese group having higher values than normal BMI group (p=0.004).

Conclusion: This is the first study of its kind in the Indian population to establish that individuals classified as overweight or obese exhibit higher expression of microRNA-21 and elevated total cholesterol levels compared to those with a normal BMI.

Keywords

BMI, circulating microRNA, lipid profile, overweight, obesity, qRT-PCR

INTRODUCTION

In the last few decades, there has been a worrying increase in obesity rates worldwide. This affects people irrespective of age, socioeconomic status, and societal background, in both the developed Western world and developing nations. According to the World Health Organization (WHO), there are around 3 billion people (including 427 million children, adolescents, and teenagers) who are overweight globally, with 890 million of them falling into the category of obesity, thereby putting them at increased susceptibility for various health issues such as diabetes, hepatic patholo-

gy, hypertension, and even mental health disorders.[1] The Indian National Family Health Survey (NFHS-5; 2019-2021) places the scope of overweight or obese individuals (BMI >25 kg/m^2) in India as 22.9% for men and 24% for women, with greater disease burden in urban regions compared to rural areas (29.8% vs. 19.3% for men, and 33.2% vs. 19.7% for women).[2] Since 1990, the global prevalence of obesity in adults has increased by over 100%, and adolescent obesity has seen a fourfold increase.[1] Obesity is also a key factor in the severity of infectious diseases such as influenza and COVID-19.[3] Given the widespread nature of this problem, it is important to focus on personalized approaches to prevent and treat obesity. Obesity has major economic consequences, as it burdens healthcare systems and economies with higher healthcare costs and lower productivity levels.

Obesity has many underlying causes, including psychosocial, environmental, and heritable traits. In today's world, sedentary lifestyle and the consumption of highly processed, high-calorie foods are becoming more and more common. The situation has been worsened by increasing urbanization and 'westernization', which have increased access to inexpensive, high-calorie, low-nutrient food options.[4] These elements, along with roles in the growth of a sedentary lifestyle and decreased physical activity, lead to obesity and weight gain. Genetic obesity can be grouped into the following categories: mono-, polygenic, and syndromic.[5] Each type has specific genetic markers that can lead to obesity, impacting various bodily functions. Furthermore, the development of obesity is influenced by epigenetic factors, which include mechanisms such as microRNAs and DNA methylation that control cellular processes and gene expression.[6,7]

To diagnose overweight or obesity, we use the person's weight (in kilograms), and height (in meters) to determine the body mass index (BMI), which is computed as weight divided by height squared. BMI serves as an indicator, with the addition of measurements like waist circumference aiding in identifying obesity. For infants, children, and adolescents, the categories used to define obesity based on BMI may vary depending on age and gender. The WHO currently suggests specific ranges for BMI classification: 18.5–24.9 kg/m^2 for healthy weight, 25.0–29.9 kg/m^2 for overweight, and greater than 30 kg/m^2 for obese individuals.[1] These BMI recommendations may not be appropriate for all populations, particularly for ethnic groups like Indians, as they are mainly based on research with Western populations.[8,9] Compared to people of other ethnicities, research suggests that people of Indian descent may be more prone to developing insulin resistance and cardiovascular diseases at lower BMI levels.[10] A consensus group has proposed BMI cut-off values specific to the Indian population as follows: healthy BMI range: 18.0–22.9 kg/m^2, overweight for values between 23.0–24.9 kg/m^2, and obesity was categorized as greater than 25 kg/m^2.[11]

Elevated BMI levels have been linked to an increased risk of cardio-metabolic issues.[12] However, BMI alone does not differentiate between lean and fat tissues, leading to the observation that many overweight (~50%) and obese (~30%) individuals do not display any metabolic changes or health issues.[13] Therefore, it is important to find biomarkers for identifying obesity and its associated metabolic disorders.

Small non-coding RNA (ncRNA) molecules – known as microRNAs – composed of just 19-22 base pairs regulate gene expression, ultimately affecting cell processes like proliferation, differentiation, and apoptosis. By preventing messenger RNA (mRNA) translation and breaking down mRNA molecules, these microRNAs can silence target genes, and occasionally even have a positive impact on gene expression and translation.[14,15] MicroRNAs are crucial in biological functions, and changes in microRNA expression have been associated with diverse diseases apart from obesity, including cardiovascular diseases, hypertension, cancers, and neurological disorders.[16-19] In addition to being linked to BMI and metabolic health, circulating microRNAs also target crucial genes associated with immunometabolism by influencing the expression of cytokines and adipokines.[20] Notably, people with metabolic diseases such as obesity have different circulating microRNA profiles than people in good health. It is still unclear, nevertheless, how much microRNAs regulate human posttranscription. Human obesity is associated with an overexpression of microRNA-21, a crucial microRNA that is frequently increased in chronic disorders and that modulates transforming growth factor beta (TGF-β) signaling to promote adipogenic differentiation.[21]

Previous studies have shown a varying outcome when it comes to the expression of microRNA-21 and BMI. Ghorbani et al reported that obesity is linked to reduced microRNA-21 levels.[22] Yang et al., Mendez-Mancilla et at., and Guglielmi et al., however, concluded that microRNA-21 expression is increased in obese individuals.[23-25]

AIM

In our current research we aimed to determine if there is any link between the microRNA-21 expression and BMI in an Indian adult population. We also compared the lipid profiles (total cholesterol, high density lipoprotein -HDL, low density lipoprotein -LDL, and triglyceride levels) of the participants to get a better understanding.

MATERIALS AND METHODS

Study population

A total of 57 adult participants were enrolled from the in-patients of Vinayaka Mission's Medical College and Hospital (VMMC) in Puducherry, India. Written informed consent was obtained from all the participants and ethics

approval was secured from the institutional ethical committee of VMMC. Individuals over 18 years of age, and without any acute or chronic inflammation, cancer, or endocrine issues including diabetes were enrolled in the study. Seven individuals were excluded due to high white blood cell counts/sepsis. Fifty persons were included in the study finally and informed written consent was obtained from all of them after explaining the details of the study to them in their own language. Information on demographics, medical history, and various clinical measurements like age, gender, comorbidities, family medical history, BMI, and fasting lipid profile were collected. BMI was computed by dividing weight (in kg) by the height squared (in meters). Normal, overweight, and obesity were categorized based on the Indian Consensus Group criteria, dividing individuals into overweight/obesity (BMI 23-24.9 and >25 kg/m^2 respectively) and normal range (BMI 18.0–22.9 kg/m^2). Accurate and calibrated tools were used for anthropometric measurements. The tests for the lipid profile (triglycerides, total cholesterol, HDL, LDL – all reported in milligrams/deciliter or mg/dL) were done using fasting samples and conducted at the VMMC Clinical Laboratory using standard methods.

Micro RNA extraction, processing, and qRT-PCR

Fasting peripheral blood samples of 3 milliliters (mL) were collected in ethylenediaminetetraacetic acid (EDTA) tubes from all participants for microRNA expression analysis. The samples were centrifuged, and plasma, serum, and packed red blood cells were stored at −80°C. Following the manufacturer's instructions, 200 microliters (μL) of plasma was used to extract RNA (microRNA-21 serum/plasma kit, Qiagen, Germany). Using nanodrop spectrophotometer (Microdigital, Nabi, South Korea) the quantity and quality of RNA were evaluated. Reverse transcription and cDNA synthesis were performed using the microRNA first stand cDNA synthesis Kit (TakaraBio, USA). The quality of cDNA was confirmed using a spectrophotometer and polymerase chain reaction (PCR) with housekeeping gene primers (U6 snRNA). Using a real-time PCR (Quantstudio-5, Applied Biosystems, USA) machine, quantitative real-time PCR (qRT-PCR) was carried out in a 96-well plate for effective analysis. We used SYBR green master mix for the PCR reaction (Powerup SYBR green master mix, Applied Biosystems, USA). There was 20 μL of total volume in each reaction, and it was run in triplicates for consistency. The cycle threshold (Ct) values were recorded after amplification, representing the cycle where the fluorescence signal exceeded the background threshold. The relative levels of microRNA-21 expression were computed using the $2^{(-\Delta\Delta Ct)}$ formula.

Statistical analysis

The statistical analysis for this study was conducted using IBM® SPSS® statistics software version 25 (IBM, USA). *P*-values were calculated by chi-square test for categorical variables and by Mann-Whitney test for the numerical variables. A significant level was defined as p value less than 0.05.

RESULTS

The results obtained in our study are summarized in **Table 1**.

Demographic and biochemical profile

The demographic and biochemical profile of our participants, sorted into those with normal BMI and overweight/obese, are shown in **Table 1**. 42.4% of men and 35.3% of women in our study fell into the overweight/obesity group. 30% of those with a BMI of overweight or obesity were female.

Table 1. Clinical and biochemical profile of the study subjects, sorted into those with normal BMI and overweight/obese

Parameters	Control group (Normal BMI)	Obesity / Overweight Group	*p*-value
N	30	20	
Males (n, %)	19, 63.3%	14, 70.0%	0.625
Females (n, %)	11, 36.7%	6, 30.0%	
Age (years)	53.8±15.3	51.4±1.4	0.652
BMI (kg/m^2)	20.9±1.6	24.9±1.3	<0.001
CHOL (mg/dL)	157.7±23.186	181.6±7.839	0.004
HDL (mg/dL)	43.636±6.938	39.4±8.665	0.067
LDL (mg/dL)	96.916±29.692	111.38±36.296	0.098
TG (mg/dL)	152.643±39.216	164.81±60.279	0.238
MicroRNA-21 expression (relative units)	1.956±2.606	4.354±6.315	0.034

Values are expressed as mean ± SD; BMI: body mass index; CHOL: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides

Circulating microRNA-21 expression

Through the use of qRT-PCR, we reviewed the relative levels of circulating microRNAs among individuals classified as normal BMI and overweight or obese. The study demonstrated an increase in circulating microRNA-21 levels in the overweight/obese category (4.354±6.315 relative units) compared to the normal BMI population (1.956±2.606 relative units) and the difference was noteworthy with a p-value of 0.034 **(Fig. 1)**.

Lipid profile comparison

The overweight/obese group has higher mean values for LDL (111.38±36.296 vs. 96.916±29.692; p=0.098), total cholesterol (181.6±37.839 vs. 157.7±23.186; p=0.008), and triglycerides (164.81±60.279 vs. 152.64±39.216; p=0.238), but lower mean HDL (39.4±8.665 vs. 43.636±6.938; p=0.067) compared to the normal range BMI group. The data has been represented graphically, showing LDL, HDL, TG in **Fig. 2** and HDL in **Fig. 3**, respectively. Only the difference in the value of total cholesterol reached statistical significance.

DISCUSSION

Obesity is a well-known global health issue that stems from an inequity in the assimilation and disbursement of energy. This not only leads to the excessive accumulation of fat

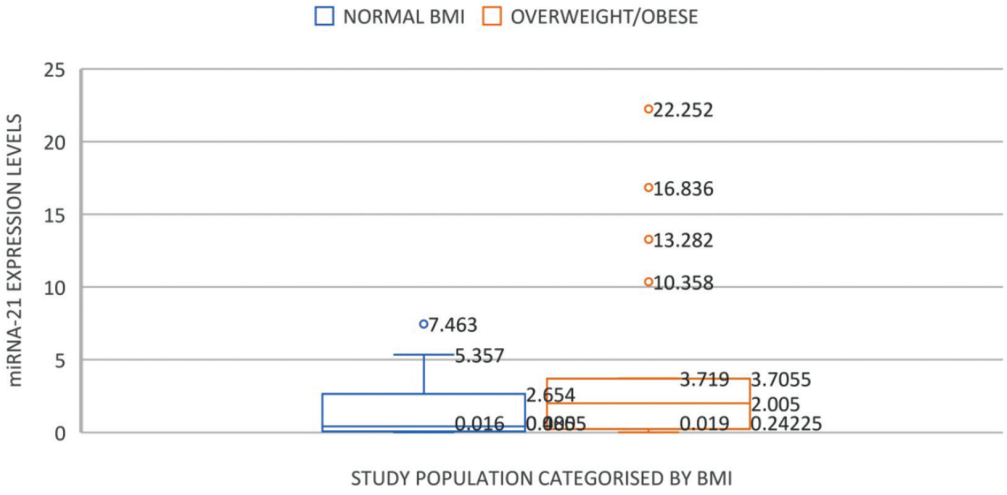

Figure 1. Comparison of circulating microRNA-21 levels between individuals with normal BMI and those classified as overweight or obese. The figure illustrates the difference in microRNA-21 levels, highlighting higher expression in the overweight/obese group relative to the normal BMI group.

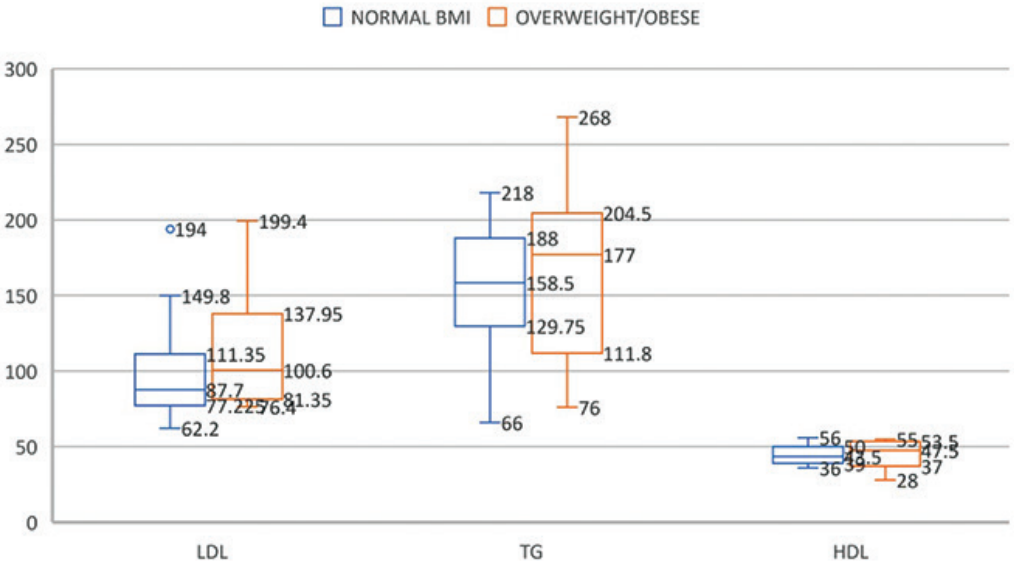

Figure 2. Comparison of LDL (Low-Density Lipoprotein), HDL (High-Density Lipoprotein), and triglyceride (TG) levels between individuals in the normal BMI group and those in the overweight/obese group. All values are shown in mg/dL, highlighting variations in lipid profiles associated with each BMI category.

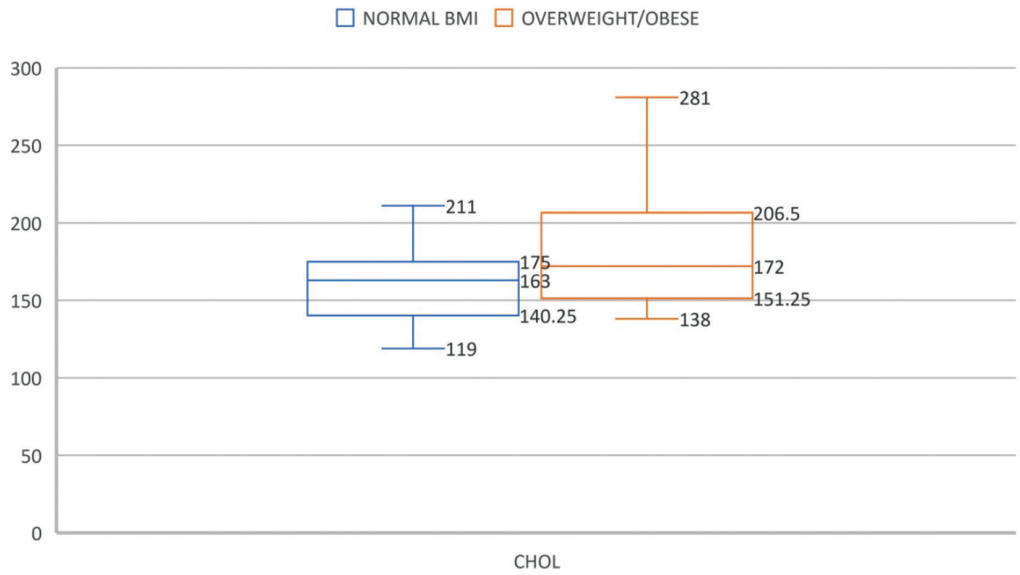

Figure 3. Comparison of total cholesterol (CHOL) values between individuals with normal BMI and those in the overweight/obese group. Total cholesterol levels are shown in mg/dL, illustrating differences between the two BMI categories.

tissue but also triggers various metabolic dysfunctions like diabetes, hypertension, and hyperlipidemia.

In our research, we utilized BMI with the Indian cut-off values as a criterion for assessing the metabolic health status of the study participants, along with including blood parameters related to renal and lipid metabolism. The primary aim of our study was to evaluate for the potential of microRNA-21 as a biomarker for obesity. The findings of our study revealed that the circulating serum microRNA-21 expression was significantly higher in overweight and obese individuals in comparison to those with normal BMI. Possible roles of microRNA-21 in the development of obesity could be by modulating the balance of lipid storage in the body – promoting adipogenesis, and reducing lipolysis.[26,27]

Chartoumpekis et al. reported an increase in microRNA-342-3p, microRNA-142-3p, microRNA-142-5p, microRNA-21, microRNA-146a, microRNA-146b, microRNA-379, and a decrease in microRNA-122, microRNA-133b, microRNA-1, microRNA-30a, microRNA-192, and microRNA-203 levels as obesity developed in mice.[28] A rise in microRNA-21 levels was discovered in the white adipose tissue of obese mice when compared to those with a leaner body mass, showing a positive correlation with BMI.[29] Lopez and colleagues reported elevated levels of microRNA-21 in participants with obesity in comparison to normal range BMI individuals.[30] Furthermore, Yang et al. showed a link between microRNA-21 and hip and waist circumference, waist-hip ratio, and BMI.[23] However, Mendez-Mancilla et al. found that microRNA-21 and microRNA-29 had a weak association with BMI.[24] Conversely, Ghorbani et al. reported that there is a decreased serum microRNA-21 level in obesity (in diabetic and non-diabetic subjects).[22] Studies by Ortega and Murri indicated a noticeable decrease in microRNA-21 levels in obese individuals, with negative correlations observed between microRNA-21 levels and parameters like waist circumference, BMI, fasting blood sugar, and glycated hemoglobin.[31,32]

Faheem et al. stated that in the general population, BMI has a positive correlation with random blood sugar levels and total cholesterol.[33] Zhu et al. found that BMI had a risk impact on altered lipid profiles (odds ratio of 1.37).[34] Lu et al. demonstrated that all lipid parameters were significantly connected to BMI even after extensive adjustment for potential factors. Notably, HDL exhibited a negative association with BMI.[35] Another large study from China showed that total cholesterol, TG, and LDL were positively linked to BMI of lactating women, but inversely related to HDL.[36] Reuter et al. reported that this association was even present in children who were overweight or obese and had a higher chance of having altered lipid profiles compared to underweight or normal-weight children.[37]

The reason behind the differences noted in various studies remains unclear. One potential explanation for this inconsistency could be the variations in where the samples were obtained from (plasma versus serum) or the differences in the groups of people studied. MicroRNAs in body fluids are transported by a variety of transporters, including high- and low-density lipoproteins, apoptotic blebs, and extracellular vesicles (exosomes, microparticles, and apoptotic blebs). They can also be found in a form that is not encased in a vesicle but is linked to RNA-binding proteins. Hence, further research utilizing more advanced technologies is necessary to distinguish between unprotected and membrane-enclosed microRNA.

CONCLUSION

Our findings show that there is a significant difference in the levels of microRNA-21 in the serum between individuals classified as overweight/obese by BMI as compared to those who had a healthy or normal BMI in the Indian population. Also, there is a significant variation in the total cholesterol levels of people with overweight/obese BMI as compared to normal BMI. However, the same association was not statistically significant for LDL, HDL, and TG.

The primary limitations of our study include the limited number of participants and the lack of follow-up. While analyzing circulating microRNA-21 against BMI has significance, it is crucial to validate its findings through larger multicenter studies to better understand its prognostic, diagnostic, and therapeutic significance in clinical environments.

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SUPPLEMENTARY MATERIALS

Table. Combined data of study subjects

Name	Age	Sex	IP No	BMI	BMI category	LDL	CHOL	HDL	TG	miRNA-21 expression
P	76	F	54xxxx	18	NORMAL	93.5	178	45	199	3.188
H	70	M	54xxxx	18.1	NORMAL	86.4	142	40	78	0.056
T	27	F	54xxxx	18.6	NORMAL	64	150	56	148	0.016
T	45	M	54xxxx	18.8	NORMAL	67	135	42	129	0.051
A	55	M	54xxxx	19	NORMAL	79.8	148	36	161	0.104
A	82	M	52xxxx	19.1	NORMAL	68	148	48	160	7.169
T	64	F	54xxxx	19.2	NORMAL	76.4	164	55	163	0.144
I	30	M	54xxxx	19.5	NORMAL	92	175	46	185	0.046
A	72	F	52xxxx	19.7	NORMAL	194	123	42	158	0.368
U	36	F	54xxxx	19.8	NORMAL	86	163	49	140	3.74
S	40	M	54xxxx	20.2	NORMAL	86.4	175	45	218	0.02
S	70	F	52xxxx	20.9	NORMAL	89	154	44	105	2.645
V	53	M	54xxxx	21	NORMAL	149.8	119	40	146	2.357
A	33	M	54xxxx	21.2	NORMAL	85	176	44	166	0.079
B	59	M	55xxxx	21.3	NORMAL	86	164	39.1	196.3	0.081
V	42	M	52xxxx	21.5	NORMAL	130	125	40	130	5.357
M	57	F	53xxxx	21.7	NORMAL	113	175	33	145	2.8
S	40	M	55xxxx	21.7	NORMAL	77.5	152	55	98	0.054
R	70	M	54xxxx	21.8	NORMAL	96.4	176	42	188	2.681
D	52	F	54xxxx	22	NORMAL	101	163	33	145	1.736
S	34	M	52xxxx	22.1	NORMAL	66.2	129	41	109	7.463
N	52	M	54xxxx	22.1	NORMAL	82.9	169	51	176	0.425
D	71	M	54xxxx	22.3	NORMAL	62.2	122	43	84	1.23
S	47	M	54xxxx	22.5	NORMAL	110.8	185	35	196	0.332
B	40	M	55xxxx	22.5	NORMAL	91	163	40	159	0.251
A	75	M	53xxxx	22.7	NORMAL	147	125	40	190	0.385
R	67	M	52xxxx	22.8	NORMAL	114.8	163	35	66	10.358
R	55	F	53xxxx	22.8	NORMAL	133.4	211	38	198	1.568
A	55	F	54xxxx	22.8	NORMAL	73	160	56	155	1.142
S	46	F	54xxxx	22.9	NORMAL	105	199	56	188	2.85
B	35	M	54xxxx	23.1	OVERWEIGHT	128.8	205	28	241	3.665
U	65	M	52xxxx	23.2	OVERWEIGHT	102	158	40	78	2.787
V	41	F	54xxxx	23.2	OVERWEIGHT	76.8	152	53	111	1.223
S	50	F	54xxxx	23.5	OVERWEIGHT	148	186	42	114.2	3.633
M	60	F	54xxxx	23.6	OVERWEIGHT	90	147	53	76	3.515
C	55	M	54xxxx	24.1	OVERWEIGHT	78.8	175	55	206	0.322
P	45	F	54xxxx	24.2	OVERWEIGHT	76.4	144	48	98	3.404
B	59	M	52xxxx	24.4	OVERWEIGHT	81.2	138	38	94	13.282
P	57	M	53xxxx	24.4	OVERWEIGHT	99.2	167	30	188	0.088
S	67	M	54xxxx	24.4	OVERWEIGHT	84.8	151	39	136	0.186
A	47	M	55xxxx	24.7	OVERWEIGHT	87	169	45	186	0.235
K	64	M	51xxxx	25.1	OBESE	110.4	184	40	168	0.604
J	40	M	52xxxx	25.4	OBESE	104.6	185	32	242	0.647
V	37	M	55xxxx	25.5	OBESE	125	209	45	196	0.019
K	58	F	52xxxx	25.9	OBESE	79.2	145	42	119	22.252
R	65	F	52xxxx	26.3	OBESE	141	207	26	200	16.836
S	30	M	54xxxx	26.3	OBESE	174.8	233	36	136	0.059
K	55	M	53xxxx	26.5	OBESE	158.4	240	32	248	3.719
R	38	M	55xxxx	27	OBESE	81.8	156	36	191	0.264
D	60	M	52xxxx	27.4	OBESE	199.4	281	28	268	10.358

BMI: body mass index; CHOL: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides

Корреляция между экспрессией MicroRNA-21 и избыточным весом/ожирением

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Резюме

Введение: Ожирение и избыточный вес – хронические состояния, характеризующиеся чрезмерным ожирением, которое негативно влияет на здоровье. Они являются одними из наиболее значимых факторов риска дыхательной недостаточности, сахарного диабета, сердечно-сосудистых заболеваний и гипертонии. Генетические факторы могут способствовать возникновению некоторых из этих состояний, но в большинстве случаев они в основном связаны с образом жизни. Однако ни генетика, ни образ жизни не могут полностью объяснить все случаи. Недавние исследования доказали, что microRNA-21 вызывает системную гипертонию, многие сердечные патологии и некоторые виды рака. MicroRNA-21 рассматривается как важный будущий биомаркер для многих критических состояний. Мы изучили роль microRNA-21 в возникновении избыточного веса и ожирения. В литературе имеются противоречивые данные об этой связи. Определение наличия связи может помочь нам лучше понять и контролировать это состояние.

Цель: Определить, существует ли связь между экспрессией microRNA-21 и индексом массы тела (ИМТ) у взрослого населения Индии. Мы также сравнили липидные профили (общий холестерин, HDL, LDL и триглицериды) участников (сгруппированных по их ИМТ), чтобы получить лучшее представление.

Пациенты и методы: Исследование проводилось в Пондичерри, Индия, и в нём приняли участие 50 человек, из которых 30 были контрольными с нормальным ИМТ, а 20 были отнесены к категории избыточного веса или ожирения в соответствии с ИМТ.

Результаты: Уровни microRNA-21 в кровотоке анализировались с помощью количественной полимеразной цепной реакции с обратной транскрипцией в реальном времени (qRT-PCR). Экспрессия microRNA-21 была статистически выше в когорте с избыточным весом/ожирением по сравнению с людьми с нормальным ИМТ (p=0.034). Значительная разница также была отмечена в уровнях общего холестерина, при этом в группе с избыточнымъ весом/ожирением значения были выше, чем в группе с нормальным ИМТ (p=0.004).

Заключение: Это первое исследование такого рода среди индийской популяции, которое установило, что у людей с избыточным весом или ожирением наблюдается более высокая экспрессия microRNA-21 и повышенный уровень общего холестерина по сравнению с людьми с нормальным ИМТ.

Ключевые слова

ИМТ, циркулирующая микроРНК, липидный профиль, избыточный вес, ожирение, qRT-PCR