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Role of MicroRNA in Obesity and Its Hypertension Complication

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Abstract

Background: Obesity is a major problem for world health and a leading factor in morbidity and mortality. Investigating microRNA (miRNA) profiling may aid in advancing research on obesity and related diseases. The aim of this study was to investigate the expression levels of miRNA-344 and miRNA-365 in obese patients with and without hypertension compared to normal weight healthy controls to assess the potential use of these miRNAs as early and effective diagnostic markers for obesity and could be used as predictive markers for hypertension-related obesity. We also determined the relationship between the above-mentioned miRNAs and different biochemical parameters.

Subjects and methods: We studied the expression of miRNA-344 and miRNA-365 in serum samples of 63 obese patients (29 obese patients with hypertension and 34 without hypertension) and 35 non-obese healthy individuals using quantitative real-time PCR.

Results: The expression of miRNA-365 was down-regulated in the sera of obese patients with or without hypertension compared to controls. Also, the present study found that miRNA-344 expression levels were related to obesity and its related hypertension. Furthermore, the correlation analysis showed that the lipid profile was related to miRNA 365 and 344 expression levels in hypertensive and obese patients.

Conclusion: The expressions of miRNA-365 and miRNA-344 were related to obesity and its hypertension complications, suggesting that those miRNAs and their target genes might be involved in the development of obesity and hypertension.

Keywords: microRNA (miRNA), miRNA-365, miRNA-344, obesity, hypertension complications.

1. Introduction

Obesity has received widespread recognition as the cause of an elevated risk for disease, which raises all-cause mortality and shortens life expectancy by between 3.3 and 18.7 years (Hu *et al*., 2004; Leung *et al*., 2015; GBD Risk Factors Collaborators, 2016; D'Agati *et al*., 2016; GBD Obesity Collaborators, 2017). In fact, obesity is becoming more and more prominent everywhere, and this trend represents a critical health concern because it is related to a number of comorbidities, such as the metabolic syndrome, which comprises dyslipidemia, hypertension, insulin resistance, and glucose intolerance (Ng *et al*., 2014). Consequently, obesity enhances the probability of developing type 2 diabetes, heart disease, chronic kidney disease, non-alcoholic fatty liver disease, and several cancers (D'Agati *et al*., 2016; Ghaben and Scherer, 2019). If the trend continues, overweight and obesity in the Irish population will affect 89% of men and 85% of women by 2030. This will raise the prevalence of diabetes by 21%, malignancies by 61%, and coronary heart disease and stroke by 97%, all of which are directly associated with obesity (Keaver *et al*., 2013). The adverse consequences of obesity are partially mediated by the elevated total cholesterol and blood pressure, which have been linked with it (Lim *et al*., 2012).

For optimal disease management and keeping obesity-related comorbidities under control, early detection of alterations associated with obesity is essential. More research is currently being done on microRNAs (miRNAs), a type of small non-coding RNA molecules with 20–25 nucleotides that regulate gene expression by binding to mRNA and inducing transcript splicing or translation suppression (Bartel, 2004). In particular, mature miRNAs, known as circulating miRNA that are produced inside cells and released from the cytoplasm into the circulation, are extremely stable and resistant to storage handling. The noninvasive availability of body fluids (serum, plasma, and urine) and the existence of disease-specific circulating miRNA patterns provide the diagnostic and prognostic value of circulating miRNAs (Cortez *et al*., 2011). It is interesting to note that patients with metabolic diseases and healthy individuals have different circulating miRNA profiles (Guay and Regazzi, 2013; Pescador *et al*., 2013; Ortega *et al*., 2014; Iacomino *et al*., 2016; Ji and Guo, 2019). Since metabolic syndrome can be acquired by a small percentage of obese people, abnormal miRNA expression may play a role in the development of metabolic disease. Furthermore, alterations in miRNA expression have been observed in obese phenotypes, and some miRNAs have been linked to metabolic diseases such as hypertension and insulin resistance (Huang *et al*., 2018; Suksangrat *et al*., 2019;

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Lischka *et al*., 2021). As a result, miRNA may have an added benefit in identifying patients at risk for developing future diseases. In this regard, literature records that some miRNAs are confirmedly dysregulated in human obesity, so further investigation is required to fully understand how these miRNAs are related to metabolic disorders and how they can be used to diagnose, prevent, and treat obesity (Ortiz-Dosal *et al*., 2019; Vonhögen *et al*., 2020; Wang *et al*., 2021).

It has been illustrated that the miRNAs 344 and 365 influence adipocyte differentiation and stimulate adipogenesis via diverse pathways (Cho *et al*., 2019). Based on previous research, the regulation of adipocyte differentiation may be impacted by miRNA-344, although its exact role is unidentified (Qin *et al*., 2010). Subsequently, a prior study has suggested the fundamental role and mechanism of miRNA-344 in the suppression of adipocyte differentiation by activating the transcription of the Wnt/β-catenin signaling pathway downstream genes that decrease the expression of adipogenic genes (Chen *et al*., 2014). MiRNA-365 is located on chromosome 16p13.12 and plays a role in a number of physiological functions (Zhu *et al*., 2018); thus, its expression and distribution vary throughout tissues and organs (Wu *et al*., 2021). MiRNA-365, a mechano-responsive miRNA, has been identified to possess an intense ability to induce inflammatory symptoms (Zhao *et al*., 2021) and to develop different obesity grades (Gouda *et al*., 2023). Furthermore, patients with left ventricular hypertrophy-associated hypertension had considerably higher serum expression of miRNA-365, and there was a strong correlation between its expression differences and blood pressure (Wu *et al*., 2021).

As a result, we aimed to evaluate circulating miRNAs 344 and 365 as biomarkers for the prediction and early detection of obesity and its related hypertension. Their prediction could explain the progress of obesity and its comorbidities. Also, our study aimed to correlate miRNA-344 and miRNA-365 expression levels in obese, hypertensive, and obese patients with lipid profiles.

2. Subjects and Methods

2.1. Subjects

A total of 63 obese patients with a BMI ≥ 30 kg/m^2 (34 without hypertension and 29 with hypertension) who underwent bariatric surgery for obesity from the Surgery Unit at Kasr Al Aini Hospital, Cairo, Egypt, were recruited in this study; their ages ranged between 25 and 60 years. Besides, 35 age- and sex-matched healthy adult volunteers with no medical history of obesity or its complications were included as controls. Prior to participating in the study, all subjects provided written informed consent. We excluded patients who met at least one of the following criteria: (1) patients with cardiovascular disease or cerebrovascular disease; (2) participants with secondary hypertension (HTN) or taking blood pressure-raising drugs; and (3) participants with diabetes, liver disease, kidney disease, and a past history of cancer.

2.2. Sampling

After a 12-hour fast, 5 mL of peripheral venous blood was obtained from all participants. After allowing blood to clot at room temperature (25 °C), sera were separated into two portions: the first for biochemical analysis and the second for adding to QIAZol in specially marked, sterile tubes for each individual subject and storing at − 80 °C until miRNA expression levels were determined.

2.3. Anthropometric measures

Body mass index (BMI) was assessed as weight in kilogrammes (kg) divided by height in metres squared (m^2). A standardised electronic sphygmomanometer (OMRON, model HEM-7130) was used to measure each participant's blood pressure on the right arm while they were seated. Before the measurements, the participants rested for at least five minutes in a seated position with their arms resting at the level of their hearts. To reduce random error and give a more reliable basis for blood pressure calculation, we measured each subject's blood pressure three times, each one separated by a 10-minute delay. We then calculated the mean value of these three measurements.

2.4. Biochemical analysis

Stanbio Laboratory, USA, provided the lipid profile kits (total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c). According to Allain *et al*. (1974) and Fredrickson *et al*. (1967), serum TC and TG were measured using an enzymatic colorimetric method, HDL-c was determined using Finley *et al.'s* (1978) methodology, and low-density lipoprotein cholesterol (LDL-c) was calculated using Friedewald's *et al*. (1972) formula as TC-HDL-c-TG/5. According to Heinz and Beushausen (1981), a fasting plasma glucose test was conducted immediately using the Stanbio Laboratory (USA) kit. With the use of a kit purchased from Human Company (Germany), the serum's AST and ALT activities were assessed in accordance with Bermeyer and Horder (1980). Serum urea level was estimated using the Modified Urease-Berthlot Method (Kaplan, 1984). Creatinine serum level was determined by using Jaffe Colorimetric-Kinetic, according to Murray (1984).

2.5. MiRNA expression

According to the manufacturer's instructions, total RNA was extracted using the miRNeasy Mini isolation kit from QIAgen, Germany. The MicroRNA reverse transcription (RT) Kit (Applied Biosystems) and particular miRNA RT primers were used to reverse-transcribe miRNA-344 and miRNA-365 in accordance with the manufacturer's instructions. MiRNA-344 primers were (F): ACACTCCAGCTGGGTGATCTAGCCAAAGCCT; (R): GTGCGTGTCGTGGAGTCG; and miR-365 primers were (F): ATAGGATCCTGAGGTCCCTTTCGTG; (R): GCGAAGCTTAAAAACAGCGGAAGAGTTTGG.

Thermo Fisher Scientific Inc.'s NanoDrop 2000c spectrophotometer was used to measure the quantity and quality of RNA. All RNA samples were determined to be of sufficient quality for qPCR analysis (1.93-2.10) based on measurement of the A260/A280 ratios. A final volume of 20 μL was created by mixing 2 μL of RT products with 10 μL of SYBR green PCR master mix, 1 μL of miRNA assays, and additional nuclease-free water. On a real-time system (Applied Biosystems), all reactions were carried out under the following conditions: 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Target miRNA relative expression was normalised to U6. Using

the equation $2^{-\Delta\Delta Ct}$, fold changes in candidate miRNA expression were calculated (Livak and Schmittgen, 2001).

2.6. Statistical analysis

The statistical package for the social sciences, SPSS, version 20 (SPSS Inc., Chicago, IL, USA), was used to analyse the data. Means and standard error were used to characterise quantitative variables. To compare groups, an analysis of variance (ANOVA) test was used. For skewed and normally distributed data, respectively, Spearman ranks and Pearson correlation coefficients were used to determine correlations between miRNA expression levels and biochemical parameters. Significant expression levels were graphically represented by boxplot graphs. When the difference between the groups was less than 0.05, it was considered statistically significant, and when it was < 0.01, it was highly significant.

3. Results

3.1. Anthropometric and clinical characteristics of the studied subjects

In the current study, 63 obese patients were enrolled: 23 males and 40 females, with a mean age of 43.15 ± 1.5 years. A control group consists of 35 healthy individuals, 20 males and 15 females, with a mean age of 40.3 ± 0.94 years (Table 1). BMI, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol displayed a significant rise in obese groups with and without hypertension compared to controls (*P*-values <0.05).

3.2. The expressions of miRNAs 344 and 365 in obese patients with and without hypertension and controls

Calculated expression levels of miRNAs 344 and 365 revealed that miRNA-344 expression was significantly elevated in the sera of obese patients without hypertension compared to obese patients with hypertension and compared to controls, as its expression increased in the obese non-hypertensive group compared to the control group and then decreased again in the obese hypertensive group, while serum expression values of miRNA-365 were significantly decreased among both obese patients with and without hypertension compared to controls (Table 2 and Figure 1).

3.3. The correlations of circulating miRNAs 344 and 365 with BMI and lipid profile in obese subjects with or without hypertension

A correlation analysis was done to address the correlated variables to miRNA expression levels. The results showed that serum values of miRNA-344 were negatively correlated with TG in obese patients with and without hypertension (*r*=-0.835, *P*=0.000, and *r*=-0.352, *P*=0.026, respectively), but positively correlated to HDL-C in obese patients without hypertension (*r*=0.315, *P*=0.048), and also positively correlated to BMI, cholesterol, and LDL-C in obese patients with hypertension (*P*<0.05). On the other hand, miRNA-365 was positively correlated with BMI and TG in obese and obese hypertensive patients with *P* values less than 0.05, more so negatively correlated with LDL-C in obese patients without hypertension (*r=-0.312*, *P*=0.013), and positively correlated with HDL-C (*P*=0.038) in obese patients with hypertension (Tables 3 and 4).

Table 1. Anthropometric measurements and clinical data of obese hypertensive, obese patients and controls

Parameter	Obese Hypertensive (n=29)	Obese (n=34)	Control (n = 35)	*P-value*
Age (Years)	45.31±2	41.0±1.6	40.31±0.94	0.057
Sex, n (%)				
Male	13 (44%)	10 (29%)	20 (57%)	0.105
Female	16 (56%)	24 (71%)	15 (43%)	
Body mass index (kg/m^2)	37.56±2.2	37.27±0.98	21.15±0.43	<0.001
Fasting Glucose (mg/dL)	90±1.7	85.79±1.8	84.56±2.5	0.115
Total cholesterol (mg/dL)	175.38±6.7	180.21±9.4	145.71±1.9	<0.001
Triglycerides (mg/dL)	135.69±8.7	125.75±10.5	87.86±2.46	<0.001
HDL-cholesterol (mg/dL)	55.38±1.1	57.75±1.6	50.43±0.72	0.030
LDL- cholesterol (mg/dL)	92.86±7.7	97.31±9	77.71±0.73	<0.001
Urea (mg/dL)	22.875±0.76	23.51±0.64	24.03±0.45	0.418
Creatinine (mg/dL)	0.93±0.016	0.92±0.026	0.87±0.022	0.164
AST (IU/L)	25±0.55	23.71±0.85	23.17±0.53	0.2
ALT (IU/L)	24.25±0.68	23.92±0.65	24±0.55	0.945

Numeric variables are presented as mean ± SE.
P value for comparison between obese and control groups.
P values <0.05 are represented in bold font and considered statistically significant.
HDL: High density lipoprotein LDL: Low density lipoprotein
AST: Aspartate aminotransferase ALT: Alanine aminotransferase

Table 2. The expression of circulating miRNA 344 & miRNA 365 in obese with and without hypertension and control groups

MicroRNA	Obese Hypertensive (n=29)	Obese (n=34)	Control (n = 35)	*P-value*
	Mean ± SE	Mean ± SE	Mean ± SE	
miRNA 344	0.254±0.05[b]	0.695±0.14[a]	0.35±0.04	0.003
miRNA 365	0.53±0.3[a]	0.57±0.18[a]	1.18±0.1	0.019

P value <0.05 are represented in bold font and considered as statistically significant.
[a] Considered statistically significant from the control group.
[b] Considered statistically significant from the obese group.

Table 3. Correlation analysis of the circulating miRNAs 344 and 365 with BMI and lipid profile in obese patients without hypertension

		BMI	TC	TG	LDL-c	HDL-c
miRNA-344	*r*	0.169	-0.020	-0.352*	-0.172	0.315*
	P	0.430	0.349	0.026	0.422	0.048
miRNA-365	*r*	0.297*	0.364	0.297*	-0.312*	0.152
	P	0.018	0.080	0.018	0.013	0.479

Spearman rank correlation coefficients and Pearson correlation coefficients for skewed and normally distributed values, respectively.
The bold format represents the significant p values (*$P \leq 0.05$).
r: correlation coefficient; BMI: body mass index; TC: total cholesterol; TG: triglycerides; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol.

Table 4.0Correlation analysis of the circulating miRNA-344 and miRNA-365 with BMI and lipid profile in obese patients with hypertension

		BMI	TC	TG	LDL-c	HDL-c
miRNA-344	*r*	0.627**	0.529*	-0.835**	0.676**	-0.129
	P	0.009	0.035	0.000	0.004	0.635
miRNA-365	*r*	0.663**	-0.263	0.585*	-0.025	0.522*
	P	0.005	0.325	0.017	0.927	0.038

The bold format represents the significant p values
Significant levels: *$P \leq 0.05$, ** $P \leq 0.01$. *r*: correlation coefficient

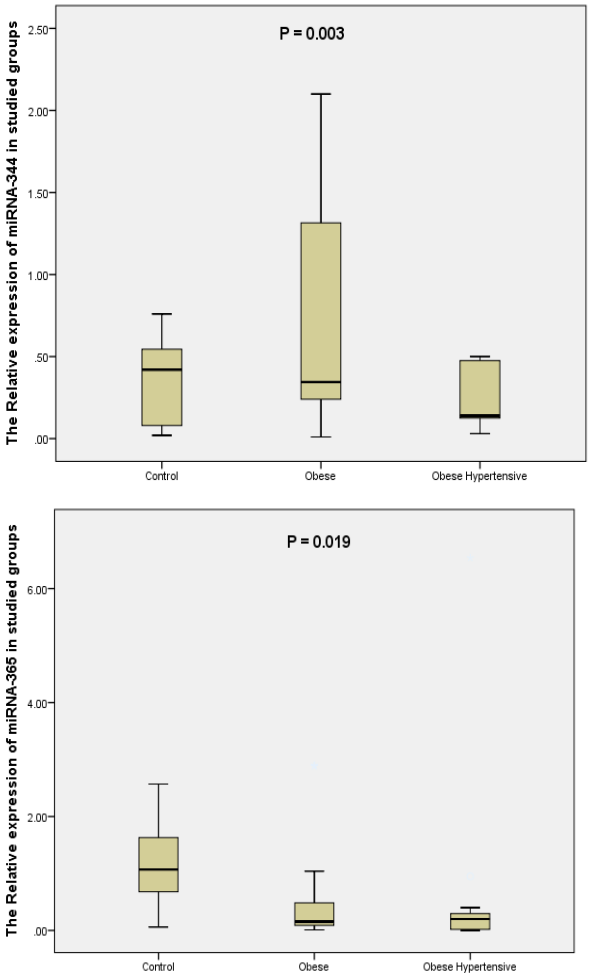

Figure 1. The relative miRNA-344 and miRNA-365 expressions in control, obese, and obese-hypertensive groups

Control group: healthy subjects with normal weight; Obese group: obese patients without hypertension; Obese Hypertensive group: obese patients with hypertension as an obesity complication.

4. Discussion

Over the last 20 years, obesity and obesity-related disorders have rapidly become a global public health concern. Recently, numerous miRNAs have been demonstrated to be important regulators of adipogenesis, more so miRNAs which are induced during adipogenesis and are down-regulated in blood samples from obese patients (Sun *et al*., 2011). MiRNA-344 and miRNA-365 have been reported to impair adipocyte differentiation (Price and Fernández-Hernando, 2016). Furthermore, Almeida and Calin (2016) have demonstrated that these miRNAs play a role in metabolic diseases and adipocyte differentiation. Based on the metabolic anomalies seen in obesity, the dysregulation of microRNA patterns, intracellular and/or extracellular, may be taken into consideration. More specifically, extracellular vesicles' altered miRNA patterns in obese patients have been thoroughly characterised, which raises the possibility that other miRNAs carried by extracellular vesicles may contribute to the development of cardiovascular problems (La Sala *et al*., 2021).

Our study investigated the expression levels of miRNA-344 and miRNA-365 in obese patients with and without hypertension and healthy controls to assess the potential of using these miRNAs as early and effective diagnostic markers for obesity and obesity-related hypertension and also to correlate these miRNAs with the lipid profiles of obese patients with and without hypertension. We found that miRNA-344 was differently expressed in the sera of obese patients compared to obese hypertensive patients and controls. Our results are in line with a recent study that revealed that miRNA-344 was up-regulated, even though not substantially, in the various obese categories based on BMI (Gouda *et al*., 2023). Moreover, the previous study by Qin *et al*. (2010) implicated that miRNA-344 had an elevated level during the process of adipogenesis as it might be involved in regulating adipocyte differentiation via the Wnt signalling pathway in a cell model. Conversely, Chen *et al.* (2014) demonstrated that miRNA 344 was significantly reduced in established culture conditions during adipogenesis and could inhibit pre-adipocyte differentiation. This could be explained by the phosphorylation of β-catenin in rats tissues, which allows it to accumulate in the cytoplasm and enter the nucleus, where it stimulates the transcription of β-catenin-dependent genes and activates the Wnt/-catenin signalling pathway by downregulating the expression of glycogen synthase kinase 3 beta (GSK3β), which results in a decrease in the expression of adipogenic genes, suggesting that miR-344 controls the process of adipocyte differentiation (Qin *et al*., 2010; Guo *et al*., 2020).

Our study showed that the expression of miRNA-365 was down-regulated in obese patients with or without hypertension compared to controls. However, till now, no research has examined the relationship between miRNA-365 and blood pressure in patients who are considered obese. Consistent with our finding regarding the expression levels of the above-mentioned miRNA in obese individuals, Gouda *et al*. (2023) showed that there was a notable decline in miRNA-365 expressions between obese patients of classes I and II compared to normal weight controls, while its expression was elevated in obese class III. Conversely to the current results concerning hypertension, according to the study of Wu *et al.* (2021), miRNA-365 serum expression was up-regulated in patients with left ventricular hypertrophy (LVH) accompanied by hypertension by targeting the S-phase kinase-associated protein 2, suggesting a clear correlation of miRNA-365 with hypertension and LVH-related blood pressure. More so, miRNA-365 has been reported to regulate the progression of the atherosclerosis process (Lin *et al*., 2016; *Surma et al*., 2020). Regarding the molecular biological assessment of the pathogenesis of hypertension, several studies have demonstrated a close relationship between the onset and progression of hypertension and miRNAs (Wu *et al*., 2017; Leimena and Qiu, 2018). According to findings from a 5-year longitudinal study, miRNAs in circulating blood vessels are linked to hypertension, suggesting that reduced serum expression levels of specific miRNAs are attributed to elevated blood pressure and new cases of hypertension (Nakamura and Sadoshima, 2018). Additionally, further investigation revealed notable differences in the expression levels of some miRNAs between healthy controls and patients with severe hypertension. Therefore, significant changes in the target genes independently regulated by these miRNAs were revealed, sparking more interest in the mechanisms expanding the variation in the expressions of circulating miRNAs (Shi *et al*., 2020), implying that miRNA expression could contribute an alternative perspective to the diagnosis and prognosis of hypertension and could be further investigated as a possible biomarker for the prediction of hypertension (Wu *et al*., 2021). Significant correlations with BMI further support the link between miR-365 and obesity and show that the biomarker is valid in obese patients without high blood pressure, which was not the case with miRNA-344. This is consistent with findings from other research looking for biomarkers associated with obesity. On the other hand, our results showed BMI correlations with miRNAs 344 and 365 in obese hypertensive patients. MiRNAs regulate lipid metabolism and also contribute to the occurrence of obesity and its complications (Kadamkode and Banerjee, 2014; Plaisance *et al*., 2014). In this regard, the correlation analysis in our study showed that miRNA-344 and miRNA-365 were correlated with lipid profiles in obese patients with and without hypertension. We demonstrate that in obese patients, miRNA-344 was positively connected with HDL, negatively associated with triglycerides, and also positively correlated with total cholesterol and LDL in obese hypertensive patients. However, miRNA-365 was associated with triglycerides and LDL, which are interconnected risk factors for metabolic illness. Excessive lipids, a low-grade systemic chronic inflammatory condition, and the accumulation of excessive visceral fat are all characteristics of obesity (Mirhafez *et al*., 2016). So, these mechanisms set off a chain of events that leads to salt retention, endothelial dysfunction, increased RAAS and sympathetic nervous system stimulation, poor control of barometric and chemoreflexes, and high blood pressure (Seravalle and Grassi, 2017; Vonhogen *et al*., 2020).

This is because the levels of many miRNAs are different in obese people compared to healthy controls, making them possible for non-invasive metabolic biomarkers (Heneghan *et al*., 2011; Ortega *et al*., 2013; Prats-Puig *et al*., 2013; Cui *et al*., 2018; Al-Rawaf, 2019; Ji and Guo, 2019). However, the first validation of circulating miRNA-344 and miRNA-365 as significantly expressed circulating serum miRNAs in obesity and its related hypertension added a novel marker to obesity miRNA signatures. The present study concludes that the expressions of miRNA-344 and miRNA-365 were altered in the sera of obese patients with or without hypertension compared to controls, suggesting that circulating miRNAs 344 and 365 have additive values as predictive markers for obesity and related hypertension. Future research can be directed towards extracting and validating their target genes that might be linked to the pathological development of obesity and hypertension complications.

5. Author Contributions

- Conceived and designed the study: Elsayed Mahdy, Mie Afify
- Diagnosis and selection of all participants in the study: Waleed Hamimy

- Contributed reagents, materials, and analysis tools: Mohamed D.E. Abdelmaksoud, Hatem A. El-Mezayen and Habeba Magdy
- Weaam Gouda contributed to the analysis of the results.
- Weaam Gouda and Soad M. Eweida contributed to the writing of the manuscript.
- All authors provided critical feedback, helped shape the research, analysis, and approved the final version of the manuscript.

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Ethical approval

The study was approved by the ethical committee of the National Research Centre, Egypt (Registration number 19-162).

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Conflicts of Interest

The authors declare no conflict of interest.

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