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Let-7b miRNA expression in Patients with Iron Deficiency Anemia after Bariatric surgery.

Said Abdel Baky Gad[1], Ayman Fathy Arafa[1], Samia Hussein[2], Abdallah S. Abdelazem[3], Aliaa A. Y. Ateya[1], Mayada M. Mousa [1]

[1]Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

[2]Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University Zagazig, Egypt

[3]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Suez University, P.O. Box 43221, Suez, Egypt.

[*]The corresponding author.

Email of the corresponding author: samiahussein82@hotmail.com

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The corresponding author's address is the Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

The corresponding author's email is samiahussein82@hotmail.com.

The telephone number of the corresponding author is +2 01062725981.

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Abstract

Aims: This study aimed to evaluate the expression of *miR-let-7b* in patients with anemia after bariatric surgery. Also, to investigate the correlation between *miR-Let-7b* expression and iron profile and copper serum levels.

Patients and Methods: This study included 50 participants from both sexes divided into 25 anemic subjects and 25 healthy individuals. All participants underwent entire history taking, thorough clinical examination, and laboratory investigations, including serum copper and iron

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measurements. Serum *miR-let-7b* expression was estimated by real-time polymerase chain reaction.

Results: There was a statistically significant increase in *miR-let-7b* expression in anemic patients after bariatric surgery compared to healthy individuals. Also, there were highly statistically significant negative correlations between *miR-let-7b* expression and each of serum iron (r=-0.883, $P<0.001$) and serum copper (r=-0.797, $P<0.001$). Receiver operating characteristic (ROC) curve results for *miR-let-7b* expression demonstrated that at a cut-off> 1.22, the area under the curve (AUC) equals 1.0 (P <0.001), the sensitivity was 100%, the specificity was 98%, positive predictive value (PPV) was 96.6%, negative predictive value (NPV) was 100%, and accuracy was 98%.

Conclusions: *Mir-let-7b* upregulation is associated with iron and copper deficiency in anemic patients after bariatric surgery.

Keywords: Bariatric surgery; copper, iron deficiency anemia; *Mir-let-7b* expression; real-time polymerase chain reaction; iron profile; Receiver operating curve.

Introduction

Bariatric surgery is recommended for morbidly obese subjects after failure of medical management. Bariatric surgery aims to improve health, life quality, and survival by maintaining weight reduction to decrease obesity-related medical problems. Bariatric surgery is an adjunct to the other treatment modalities rather than a separate independent therapy **[1].**

Copper is the body's third most common trace element after iron and zinc **[2].** It is widely distributed in vegetables, legumes, grains, and animal products. Thus, acquired hypocupremia due to malnutrition among healthy people is relatively infrequent **[3].** Dietary copper absorption occurs in the stomach and proximal duodenum **[4].**

Hepatocytes incorporate copper into superoxide dismutase, cytochrome-c oxidase, and ceruloplasmin. Ceruloplasmin transports copper from the liver to the tissues, where it binds to peripheral cell surface receptors and releases copper into the cell **[5].** Ceruloplasmin also facilitates iron metabolism via copper-dependent ferroxidase activity **[6].** Hephaestin is a copper-dependent ferroxidase expressed in the duodenal mucosa and facilitates ferric iron

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transport across it for transferrin loading **[7].** Patients with microcytic hypochromic anemia resistant to iron therapy may have copper deficiency **[8].**

Additionally, copper participates in the redox reactions. Oxidoreductases and monooxygenases require copper for their catalytic activity. In copper deficiency, these cuproenzymes have decreased activity **[9].**

Anemia is a typical result of micronutrient deficiency after bariatric surgery. It develops due to changing food habits or impaired absorption of micronutrients such as iron, zinc, selenium, folate, B12, and copper. To avoid the complications of vitamin and trace element deficiency, periodic follow-up is recommended with the administration of nutritional supplements and a balanced diet **[10].**

MiRNAs are single-stranded, noncoding RNA molecules. They have about 22 nucleotides. They regulate gene expression, which is responsible for the responses to environmental conditions **[11].** They regulate mRNA translation and, subsequently, protein synthesis. Due to their complementarity with messenger RNA (mRNA) sequences, they induce degradation of the transcripts or inhibition of their translation **[12].**

microRNAs (miRNAs) have been involved in the regulation of iron hemostasis. miRNAs regulate the post-transcriptional process of the genes associated with iron metabolism and storage (ferritin). Thus, factors that change miRNA expression will cause subsequent changes in iron homeostasis **[13]**.

MiRNAs regulate multiple cell functions and are considered biomarkers in many diseases **[14].** Humans modulate about 60% of the protein-coding transcriptome and are involved in various biological processes, such as inflammation **[15].**

miRNAs synthesis is under control by post-translational or post-transcriptional factors. Epigenetic and transcription factors regulate miRNA transcription, leading to changed miRNA levels. Changed miRNA levels may be caused by regulatory proteins affecting miRNA processing, acquired variations in the miRNA transcript, and changes in the nuclear export efficiency. Also, single nucleotide polymorphisms (SNPs) can affect the efficiency of the miRNA processing **[16].**

miRNAs also regulate adipocyte differentiation, metabolic integration, insulin resistance, and appetite regulation. Dysregulation of miRNAs is related to developing multiple pathological events such as neurological and cardiovascular diseases **[17].** Until now, no miRNA-based therapeutic agent has been approved by the FDA. Many are still in different stages of clinical trials **[18].**

This study evaluated the expression of miR-let-7b in patients with anemia after bariatric surgery. Also, to investigate the correlation between miR-Let-7b expression and iron profile and copper serum levels.

Subjects and methods

This is a case-control study. The total sample size was 50 participants calculated by Epi info 7 with a power of 80% and CI of 95% and divided into 25 anemic subjects after bariatric surgery and 25 healthy individuals of both sexes after bariatric surgery. All participants gave consent. Participants less than eighteen years old were excluded from the study. Patients with known hematological diseases were excluded from the study.

All the participants were subjected to taking their entire histories and completing a complete clinical examination. Blood samples were taken from all participants, and sera were prepared. The hemoglobin (Hb), serum copper, serum iron, ferritin, total iron binding capacity (TIBC), and transferrin saturation were measured. The kits were provided from Spectrum, Egypt, and measured by Sunostik, China.

Total RNA was isolated from serum using GENEzol™ (Geneaid, Taiwan). The integrity of the harvested RNA was assessed and measured on Nanodrop spectrophotometry (ND 1000-NanoDrop®). Reverse mRNA transcription to complementary DNA (cDNA) was performed using TaqMan® Small RNA Assays kit (Thermofisher, USA). The real-time RT-PCR was performed in a Mx3005P Real-Time PCR System (Agilent Stratagene, USA) using 10 µL TOPreal syberGreen (Enzynomics, Korea), 1 µL of each forward and reverse primer, 1 µL of DNA template and RNase free water up to 20 µL final reaction volume. The primers for hsa-*miR-let-7b* quantification were forward: GGGTGAGGTAGTAGGTTGT and reverse: CAGTGCGTGTCGTGGAGT. U6 served as the internal control for the relative quantification

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of *miR-let-7b* expression. The primers for U6 were F: CTCGCTTCGGCAGCACA and R: AACGCTTCACGAATTTGCGT **[19].**

The PCR cycling conditions included an initial denaturation at 95°C for 12 minutes followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The oligonucleotide-specific primers were synthesized by Sangon Biotech (Beijing, China). A melting curve analysis was performed following PCR amplification.

Results:

Both cases and controls were matched in age and sex; there was no statistically significant difference between them (*P*=0.11 and 0.544, respectively). There was a statistically significant decline in hemoglobin, serum iron, serum copper, and ferritin levels in the cases compared with the control group (P <0.001 for each). In contrast, transferrin level and TIBC were significantly higher in cases than their controls (P <0.001 for each) (Tab 1).

Tab1. ***Demographic, biochemical, and molecular results in the studied groups.***

	Cases N = 25	Controls N = 25	t-test x^{2*}	*P*
Age	32.4 ± 5.2	34.5 ± 3.8	1.63	0.11
Sex (M/F)	9/16	7/18	0.37	0.544
Serum copper (ug/dL)	73.7 ± 10.1	122.5 ± 15.7	13.1	<0.001**
Hb (gm/dL)	9.8 ± 0.2	12.5±0.7	12.5	<0.001**
Serum ferritin (ug/L)	39.4 ± 6.1	191 ± 10.3	49.3	<0.001**
Transferrin (mg/mL)	395.2 ± 5.1	222 ± 15.6	50.1	<0.001**
TIBC (ug/dL)	381.2 ± 19.1	253.6 ± 8.2	25.6	<0.001**
Serum iron	60.3 ± 5.4	132.1 ± 16.5	20.7	<0.001**
Let-7b expression	5.24 ± 0.9	1.04 ± 0.2	22.7	<0.001**

***: a highly statistically significant difference; TIBC: total iron binding capacity.*

There was a highly statistically significant increase in *miR-let-7b* expression in cases compared to controls (*P*<0.001) (Table 1, Figure 1A). Receiver operating characteristic (ROC) curve results for *miR-let-7b* expression demonstrated that at a cut-off> 1.22, the area under the curve (AUC) equals 1.0 (*P* <0.001), the sensitivity was 100%, the specificity was 98%, positive predictive value (PPV) was 96.6%, negative predictive value (NPV) was 100%. Accuracy was 98% (Fig 1, tab 2).

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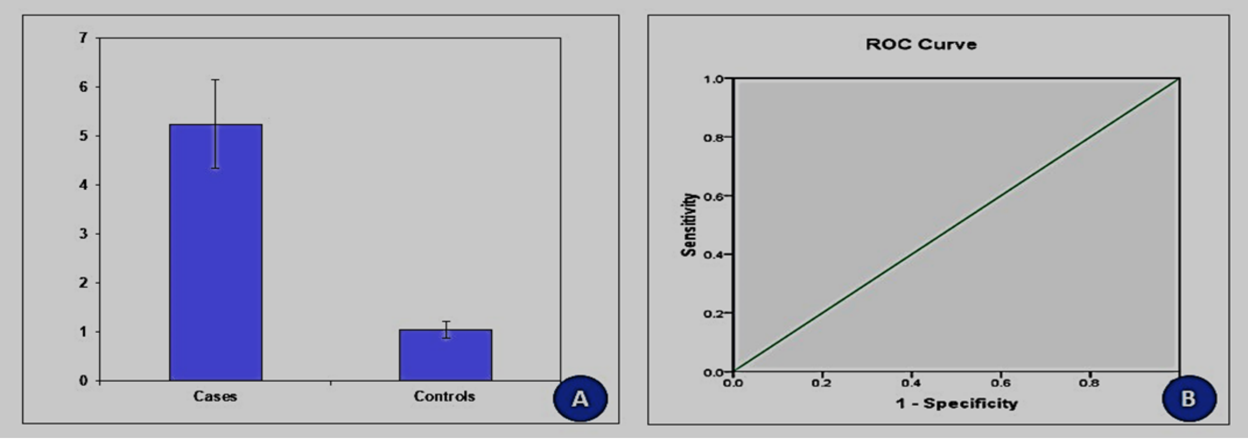

*Fig1. **Let-7b fold expression in the studied groups. B: ROC curve** of Let-7b as a diagnostic marker in anemic cases after bariatric surgery.*

*Tab2. **Roc curve Let-7b expression as a diagnostic marker after bariatric surgery.***

Cut off	AUC	*P*	Sensitivity	Specificity	PPV	NPV	Accuracy
1.22	1.0	<0.001	100%	98%	96.6%	100%	98%

There were highly statistically significant negative correlations between *miR-Let-7b* expression levels and serum iron (r=-0.883, *P*<0.001) and copper levels (r=-0.797, *P*<0.001) (Figure 2, tab 3).

*Tab3. **Correlation of Let-7b expression with iron profile and serum copper.***

	miRNA Let-7b	
	r	**P**
Serum copper	-0.797	<0.001**
Serum iron	-0.883	<0.001**
Hb	-0.168	0.401
Serum ferritin	-0.023	0.921
Transferrin	0.315	0.241
TIBC	0.213	0.221

***: a highly statistically significant difference; TIBC: total iron binding capacity.*

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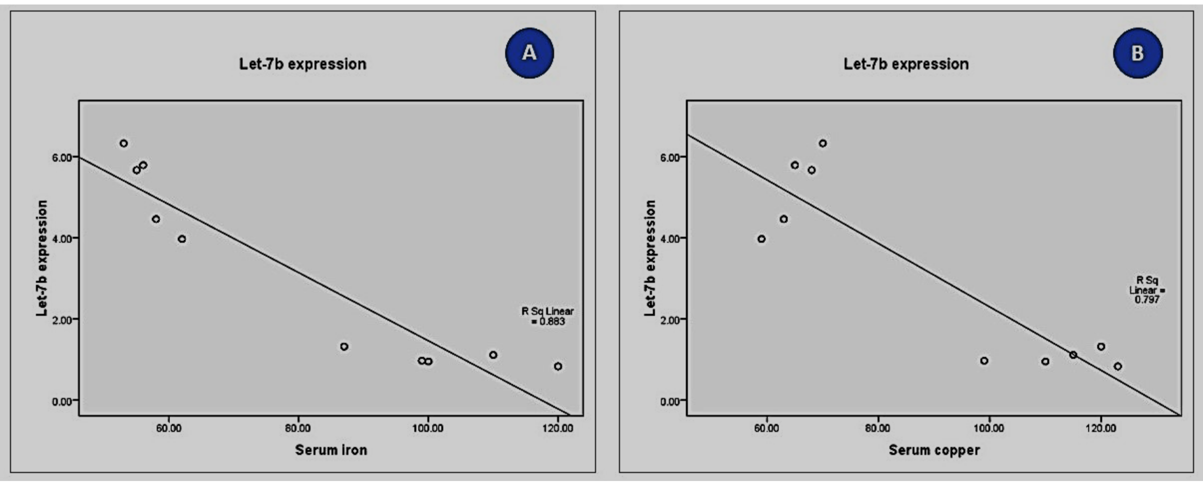

*Fig2. **Correlations of Let-7b expression with copper and iron levels.***

Discussion

Epigenetic changes (DNA methylation, histone modifications, and non-coding RNAs) after bariatric surgery may explain its metabolic beneficial effects. Epigenetic changes can respond to external environmental factors by changing gene expression levels without changing the DNA sequence. miRNAs are epigenetic factors **[20].**

Our study showed a significant decline in serum copper levels in cases compared to controls. Like our finding, copper deficiency was reported due to decreased absorption after surgery **[21, 22].** On the other hand, ***Facharztmagazine (2020)*** stated that copper deficiency after gastrointestinal tract surgery is uncommon, with a 9.6% prevalence rate 0-60 months following Roux-en-Y gastric bypass **[23].** Also, ***Myint et al. (2018)*** stated that clinical manifestations of copper deficiency appear decades after surgery **[24].**

Copper deficiency due to inadequate copper intake among healthy people is relatively infrequent **[3].** Risk factors of copper deficiency include gastrointestinal tract surgery, zinc excess, prolonged total parenteral nutrition, and malabsorption enteropathies **[22].** Upper gastrointestinal tract surgery, gastrectomy, bariatric surgery, and small bowel resection or bypass all increase the risk of hypocupremia. Surgeries that bypass the duodenum, the primary site of copper absorption, cause copper malabsorption **[25].**

Our study showed a statistically significant decline in hemoglobin, serum iron, and ferritin levels among the studied cases. In contrast, transferrin level and TIBC were significantly higher

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among cases than their controls. This finding is similar to that of ***Gowanlock et al. (2020).*** Their study evaluated the hematological parameters, including iron, in 388 patients with a mean follow-up of 31 months. Iron deficiency was reported in 43% of participants **[26].**

After bariatric surgery, iron deficiency anemia results from alterations in the gastrointestinal anatomical and physiological characteristics. Factors involved in the development of iron deficiency after bariatric surgery include decreased iron intake, decreased secretion of hydrochloric acid, and decreased surface area for absorption **[27].**

Copper deficiency anemia (often microcytic) results from the interaction between copper-dependent ferroxidases and iron **[28].** Decreased ceruloplasmin activity leads to impaired iron absorption from the small intestine and diminished conversion of ferrous to ferric iron. These changes disrupt systemic transport by transferrin and inadequate incorporation of iron into protoporphyrin **[6].** Poor heme synthesis, in conjunction with erythrocyte membrane fragility due to decreased copper-zinc superoxide dismutase activity, leads to mitochondrial iron accumulation and ringed sideroblast formation **[29].**

Several studies showed that many miRNAs were dysregulated in the white adipose tissue of obese patients compared to healthy individuals. Micro RNAs are involved in the regulation of adipogenesis and the regulation of endocrine and metabolic functions. Some of them are stimulators of human adipocyte differentiation including miR-21, miR-26b, miR-30, miR-103, miR-143, miR-148, miR-181a, miR-199a, miR-378 while the others are inhibitors including miR-let-7, miR-22, miR-125a, miR-224 **[30].** Also, circulating exosomal microRNAs derived from adipocytes may regulate metabolism and mRNA translation in other cells or tissues. Thus, they could be important biomarkers for obesity with potential therapeutic targets **[31].**

Our study showed a significant upregulation in *miR-let-7b* levels in cases compared to healthy controls. This finding was like that of ***Bae et al. (2019)***. They found an upregulation of the miRNAs, including miR-let-7b, in obese patients before surgery compared with healthy individuals. Interestingly, previous studies stated that the expression of *miR-let-7b* was downregulated after bariatric surgery compared with before the surgery **[32,33].**

Our study demonstrated highly statistically significant negative correlations between *miR-let-7b* and serum copper and iron levels.

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The expression of *miR-let-7b* changes in inflammatory diseases. Downregulated *miR-let-7b* was detected in septic shock with decreased IL-6 and TNF-α. This finding suggests that *miR-let-7b* may act as a regulator for the duration and the magnitude of inflammation ***(Ellett et al., 2022).*** It also plays a role in metabolism, regulation of glucose metabolism, and metabolic functions of obesity-derived adipocytes **[33].**

Conclusion: *Mir-let-7b* expression upregulation is associated with iron and copper deficiency in anemic patients after bariatric surgery.

Footnotes.

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Authors' contributions

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AF and SH were responsible for conception and revision, and AAYA and SH were responsible for the interpretation and analysis of data. SH wrote the manuscript that was revised and approved by all co-authors.

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