Changes in iron profile after bariatric surgery in patients Changes in iron profile after bariatric surgery in patients referred to the obesity clinic at Hazrat Fatima Al-Zahra Hospital

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Abstract:

Background and Objective: **Obesity, as a global health crisis, not only imposes an economic burden but also increases the prevalence of obesity-related diseases. Bariatric surgery is considered the most effective treatment for weight loss in obese individuals; however, it is associated with potential complications, including nutritional deficiencies. This study aimed to assess the six-month changes in the iron profile following bariatric surgery in obese patients attending the Obesity Clinic at Hazrat Fatemeh Al-Zahra (S) Hospital.**

Materials & Methods: **This cohort study was conducted on 146 obese patients who underwent bariatric surgery between October 2023 and October 2024. Patients with anemia-related conditions or those taking iron supplements or medications affecting iron metabolism were excluded. Demographic, anthropometric, and laboratory data, including iron profile indicators (ferritin, serum iron, hemoglobin, etc.), were extracted from the National Obesity Surgery Database and recorded before surgery and at three and six months postoperatively. Data were analyzed using descriptive and analytical statistical tests, including paired t-tests, in SPSS version 25. A p-value of less than 0.05 was considered statistically significant.**

Results: **Significant changes were observed in ferritin levels before and three months after surgery in OAGB, SAGI, and SG procedures. In OAGB, ferritin increased from 68.55 ± 72.84 before surgery to 87.75 ± 73.41 at three months postoperatively (P = 0.007). In SAGI, it increased from 87.14 ± 19.8 to 152.87 ± 68.62 (P = 0.03), and in SG, from 60.05 ± 34.81 to 82.63 ± 54.41 (P = 0.001). Additionally, in OAGB, MCV increased significantly from 84.41 ± 5.46 before surgery to 85.87 ± 5.1 at three months postoperatively (P = 0.001). After six months, a significant decrease in mean hemoglobin levels was observed in OAGB patients, dropping from 13.62 ± 1.34 before surgery to 13.14 ± 1.17 postoperatively (P = 0.01).**

Conclusion: **Bariatric surgery has a significant impact on patients' iron profiles, with variations depending on factors such as age and gender. The findings emphasize the importance of monitoring and implementing nutritional interventions post-surgery to prevent deficiencies. Further studies with larger sample sizes and longer follow-up periods are recommended.**

Keywords: Obesity, Bariatric Surgery, Nutritional Deficiencies, Iron Profile

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Background and Objective

Bariatric surgery, also referred to as obesity surgery, represents the most effective intervention for achieving substantial weight loss and facilitating long-term weight maintenance. This surgical approach significantly enhances quality of life by alleviating obesity-related comorbidities, which include cardiovascular disease, respiratory disorders, type 2 diabetes, degenerative joint disease, and various forms of cancer.[1] The primary bariatric surgical techniques include gastric banding, sleeve gastrectomy, Roux-en-Y gastric bypass, and biliopancreatic diversion, which may be performed with or without the inclusion of a duodenal switch. Among these procedures, sleeve gastrectomy is the most commonly executed for the purpose of weight reduction. Iron deficiency and anemia are prevalent among individuals with obesity, making the monitoring of iron status before bariatric surgery imperative.[2] Anemic patients typically experience prolonged hospital stays following surgery, averaging 7.2 days compared to 1.9 days[3] for non-anemic patients. Post-bariatric surgery, there is a heightened risk of iron depletion and anemia resulting from changes in the gastrointestinal tract's absorption capacity. Certain bariatric techniques, including biliopancreatic diversion, duodenal switch, and Roux-en-Y gastric bypass, are particularly associated with malabsorption processes that impede iron uptake. Following surgery, patients frequently develop anemia related to the impaired conversion of Fe3+ to Fe2+, a consequence of diminished hydrochloric acid levels.[4] Additional contributors to anemia and iron deficiency include reduced dietary intake and a prevalent aversion to meat, which is a primary source of heme iron.[5] The American Society for Metabolic and Bariatric Surgery has established guidelines aimed at restoring iron levels following these surgical procedures.[6,7]

A relevant study involving 32 women who underwent both bariatric and subsequent abdominal surgery reported a mean hemoglobin level decrease from 12.98 to 10.88 grams per deciliter two days post-surgery, which subsequently increased to 11.53 grams per deciliter after a week, with no further increases thereafter. Concurrently, declines in serum iron and transferrin levels were observed, while the average ferritin level decreased from 29.8 to 16.4 micrograms per liter over a period of 56 days following surgery, resulting in iron deficiency and anemia in 45% of participants.[8] Furthermore, post-bariatric surgery, the incidence of iron deficiency anemia is likely to escalate over time, even with adherence to iron supplementation. A decade-long follow-up study of 151 patients who underwent gastric bypass in Brazil indicated that 37.5% experienced persistent anemia with ferritin levels below 15 micrograms per liter, a figure which increased to 45% among those with ferritin levels below 30 micrograms per liter.[9] Additionally, a retrospective cohort study conducted in Portugal, examining 1,999 patients over a four-year period, found that 24.4% of individuals experienced anemia following bariatric surgery. This study identified gender and the type of bariatric surgery as significant determinants; women and individuals who underwent Roux-en-Y gastric bypass were found to have double the risk of anemia compared to their male counterparts and those who had received sleeve gastrectomy or gastric banding procedures.[10]

Conversely, given that obese patients often present with elevated serum ferritin levels associated with inflammatory processes, it is anticipated that the reduction of adipose tissue following bariatric surgery may enhance iron absorption.[11,12] Nonetheless, procedures such as gastric bypass, particularly Roux-en-Y gastric bypass and sleeve gastrectomy, are known to cause iron malabsorption, potentially exacerbating iron deficiency.[13]

Bariatric surgical techniques induce significant alterations in both the anatomy and physiology of the gastrointestinal tract, promoting weight loss through mechanisms that include restricted food intake, selective malabsorption of nutrients, and hormonal changes. However, these modifications result in critical nutritional consequences, notably micronutrient deficiencies—especially iron—and associated anemia. Such deficiencies can severely impair patients’ quality of life and may lead to serious complications, including compromised immune function, diminished cognitive abilities, and an increased risk of cardiovascular disease.[14]

Current literature addressing alterations in iron profiles among patients following bariatric surgery, particularly over extended periods, remains limited. Comprehensive long-term studies in this domain are essential to enhance nutritional care and therapeutic strategies post-surgery, ultimately aiming to improve patients' quality of life and mitigate complications associated with iron deficiency. Acknowledging this necessity, the present study endeavors to assess the prevalence and characteristics of iron deficiency, along with the resulting anemia, in obese patients subsequent to bariatric surgery.

Materials and Methods

Study Design

This investigation was conducted as a retrospective cohort study.

Study Population

The study population comprised patients aged 15 years and older, diagnosed with obesity (defined as a body mass index (BMI) greater than 35) and associated comorbid conditions, who presented at the surgical clinic of Fatemeh Zahra Hospital in Tehran between October 2023 and October 2024 and were candidates for bariatric surgery.

Study Methodology

This retrospective cohort study focused on obese patients with comorbidities seeking bariatric surgery during the designated timeframe.

Eligible participants were required to have no history of bleeding or anemia-related conditions (e.g., reticulocytosis, autoimmune diseases) and were not on medications affecting iron metabolism, including iron supplements, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or immunosuppressants. Only patients who provided informed consent during their initial consultation were included in the study. In collaboration with the surgical clinic, the investigator accessed medical records to identify 146 patients who met the inclusion criteria. These individuals underwent evaluations at multiple stages before and after the surgical procedure.

Data pertaining to weight, body composition indices, anthropometric measurements, and biochemical parameters were extracted from routine postoperative care records maintained in the national obesity surgery database of Iran. Laboratory indices measured included serum iron levels, ferritin, total iron binding capacity (TIBC), total body water (TBW), mean hemoglobin concentration, mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), hemoglobin, hematocrit, and body mass index (BMI), all recorded preoperatively and at three and six months post-surgery. A researcher-developed checklist was employed as the data collection tool, encompassing demographic information (age, gender, marital status, education level), medical history, medication usage, and pertinent laboratory indices at various time points surrounding the surgery.

Data Analysis

Quantitative descriptive data were reported as means and standard deviations, while qualitative variables were expressed as frequencies and percentages. One-way analysis of variance (ANOVA) and repeated measures ANOVA were utilized to compare qualitative variables. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted utilizing SPSS software version 25.

Findings

Patient Characteristics

The study comprised a total of 146 patients aged 15 years and older, all possessing a minimum BMI of 40 kg/m², who underwent bariatric surgical procedures. Within the participant cohort, 127 individuals were female (87%) and 19 were male (13%). The mean age of the participants was 41.1 ± 17 years, while the average BMI was 44.06 ± 5.69. Preoperative comorbidities included diabetes in 17 individuals (11.6%) and hypertension in 31 individuals (21.2%). Additional preoperative characteristics of the patients are summarized in Table 1.

Table 1- Pre -operative characteristics of patients undergoing obesity surgery

Variables	*Number=146*
Gender (female), number (percent)	*127(87)*
Age (year)	*11.1±41.17*
Body mass index (kg/square meter)	*5.69±44.06*
Weight (kg)	*17.88±117.91*
Diabetes Number (percent)	*17(11.6)*
Blood pressure number (percent)	*31(21.2)*
Dys Lipidemmia (percent)	*22(15.1)*
Hypothyroidism Number (percent)	*25(17.1)*

Changes in Iron Profile Three and Six Months After Bariatric Surgery by Surgical Type

The results derived from the repeated measures analysis indicated that alterations in serum iron levels over time did not achieve statistical significance (p = 0.08). However, the differences among the various surgical procedures were found to be significant (p = 0.02), while the interaction between time and type of surgery did not reach statistical significance (p = 0.06). Conversely, the differences in ferritin levels among the surgical groups were significant (p = 0.03), with a notable interaction identified between time and type of surgery (p = 0.02). Consequently, post-hoc comparative analyses were conducted for each surgical type individually. In the cohort of patients who underwent mini gastric bypass with single anastomosis, the changes in ferritin levels over time were statistically significant. Specifically, the mean ferritin level increased from 72.84 ± 68.55 nanograms per milliliter (ng/mL) prior to surgery to 73.41 ± 87.75 ng/mL at three months, followed by a slight decrease to 69.45 ± 25.69 ng/mL at six months post-surgery. Pairwise comparisons indicated that the increase in ferritin levels from pre-surgery to three months, as well as from three months to six months post-surgery, were both statistically significant (p = 0.02). In contrast, no statistically significant changes in ferritin levels were observed in other surgical groups, including Roux-en-Y gastric bypass, sleeve gastrectomy, and ileogastric bypass. The analysis also revealed a significant effect of time on vitamin B12 levels (p = 0.03), indicating an increase in mean vitamin B12 levels across all groups at various intervals. Additionally, the interaction between time and type of surgery was significant (p = 0.004). Pairwise comparisons illustrated that, within the mini gastric bypass group, the increase in vitamin B12 levels from pre-surgery to both three and six months post-surgery was significant (p = 0.02). Similarly, the sleeve gastrectomy group demonstrated a significant increase in vitamin B12 levels from pre-surgery to both three and six months post-surgery (p = 0.02). However, no significant changes in vitamin B12 levels were observed in the Roux-en-Y gastric bypass and ileogastric bypass groups (refer to Table 2).

Changes in Iron Profile by Gender

Upon assessing the changes in the iron profile three and six months post-surgery, gender-specific analyses revealed that variations in ferritin and vitamin B12 levels among female patients were statistically significant. Notably, the results of the Bonferroni post-hoc test indicated that the observed differences in ferritin were primarily attributable to increases noted at three months post-surgery. For vitamin B12, significant variations were observed across all time points.

In contrast, no statistically significant differences were identified among male participants for any of the studied variables. Further details regarding these findings can be found in Table 3.

Table 2 - Changes in iron, ferritin, and hemoglobin profiles after bariatric surgery based on type of surgery

Type of operation	*Before intervention*	*3 months later*	*6 months later*	*Between-group probability value*	*Time probability value*	*Interaction probability value*
Iron						
Mini bypass single anastomosis	*78.21±30*	*71.8±22.83*	*78.27±20.03*	*0.02*	*0.08*	*0.06*
Ro-n-Y gastric bypass	*20.93±85.28*	*27.81±65.32*	*70.22±47.61*			
Sleeve gastrectomy	*73.33±26.3*	*76.45±26.56*	*82.57±22.23*			
Ileogastric bypass	*81.26±40.96*	*88.5±16.05*	*62.67±15.53*			
Ferritin						
Mini bypass single anastomosis	*68.55±72.84*	*87.75±73.41*	*90.37±79*	*0.03*	*0.11*	*0.02*
Ro-n-Y gastric bypass	*120.1±91.48*	*49.32±64.31*	*69±25.45*			
Sleeve gastrectomy	*60.05±34.81*	*82.63±54.41*	*108.53±89.03*			
Ileogastric bypass	*87.14±19.8**	*152.87±68.62**	*99.33±70.21**			
Mean hemoglobin concentration						
Mini bypass single anastomosis	*84.41±5.46*	*85.87±5.1*	*84.18±10.75*	*0.02*	*0.11*	*0.06*
Ro-n-Y gastric bypass	*84.81±7.18*	*86.21±6.57*	*88.35±9.54*			
Sleeve gastrectomy	*83.67±6.02*	*84.98±6.31*	*86.93±5.2*			
Ileogastric bypass	*83.96±7.79*	*83.96±7.79*	*82.12±11.44*			
Hemoglobin						
Mini bypass single anastomosis	*13.62±1.34*	*13.57±1.19*	*13.14±1.17*	*0.04*	*0.06*	*0.08*
Ro-n-Y gastric bypass	*13.28±1.57*	*13.06±1.28*	*13.55±1.48*			
Sleeve gastrectomy	*13.17±1.22*	*13.48±1.18*	*13.52±1.28*			
Ileogastric bypass	*14.37±1.56*	*41.14±2.05*	*12.75±2.1*			
Vitamin B12						
Mini bypass single anastomosis	*358.67±218.55**	*588.75±66.61**	*635.84±428.03**	*0.14*	*0.03*	*0.004*
Ro-n-Y gastric bypass	*366.94±164.27*	*708.09±493.41*	*1020.33±850.64*			
Sleeve gastrectomy	*402.28±322.94**	*643.17±38.95**	*794.83±624.87**			
Ileogastric bypass	*321.35±122.45*	*799.8±716.31*	*695.83±262.47*			

*Significant difference based on Bonferroni post hoc test results

Table 3 - Changes in iron, ferritin, and hemoglobin profiles after bariatric surgery based on type of surgery

Type of operation	*Before intervention*	*3 months later*	*6 months later*	*Between-group probability value*
Iron Profile				
Women				
Iron	*76.44±28.81*	*71.2±23.89*	*78.65±20.3*	*0.73*
Ferritin	*63.75±60.88**	*80.02±65.13**	*78.24±79.66*	*0.04*
Total Iron Binding Capacity	*322.87±62.04*	*309.67±56.79*	*322.96±49.71*	*0.45*
Mean Hemoglobin Concentration	*84.12±6.13*	*85.52±6.06*	*84.68±9.84*	*0.65*
Hemoglobin	*13.25±1.18*	*13.31±1.13*	*13.04±1.06*	*0.64*
Folic Acid	*11.00±5.69*	*12.57±9.25*	*10.93±5.32*	*0.43*
Vitamin B12	*373.29±244.50**	*640.56±431.11*	*713.66±495.64*	*0.04*
Men				
Iron Profile	***6 months after surgery***	***3 months after surgery***	***Before surgery***	
Iron	*84.39±27.25*	*82.28±26.28*	*76.22±25.5*	*0.92*
Ferritin	*108.22±110.85*	*134.68±66.25*	*190.12±95.46*	*0.75*
Total Iron Binding Capacity	*344.62±43.78*	*299.06±35.23*	*301.10±33.17*	*0.23*
Mean Hemoglobin Concentration	*85.02±3.41*	*85.94±2.95*	*86.85±1.77*	*0.32*
Hemoglobin	*15.14±1.38*	*14.89±1.23*	*15.52±1.84*	*0.16*
Folic Acid	*9.34±4.76*	*10.94±5.65*	*10.88±5.59*	*0.17*
Vitamin B12	*329.34±222.56*	*519.16±273.09*	*495.5±270.82*	*0.13*

**Significant difference based on Bonferroni post hoc test results*

Table 3 (a)- Comparison of iron profile changes between different age groups 3 and 6 months after bariatric surgery

Age Group	Iron 3 months	Iron 6 months	Ferritin 3 months	Ferritin 6 months	Total Iron Binding Capacity 3 months	Total Iron Binding Capacity 6 months
15-29	70.36±35.71	74±16.55	68.75±67.93	47.7±39.41	321.44±50.53	352±79.98
30-49	72.91±22.93	80.52±22.15	82.26±61.72	79.1±88.56	307.39±58.48	321.06±37.23
50-69	74.59±19.93	76.72±19.66	109.61±76.62	117.42±88.07	300±42.44	311.12±60.85
Probability value	0.85	0.77	0.07	0.25	0.46	0.48

Table 3 (b)- Table 3 continued

Age Group	Average hemoglobin concentration 3 months	Average hemoglobin concentration 6 months	Hemoglobin 3 months	Hemoglobin 6 months	Folic acid 3 months	Folic acid 6 months
15-29	84.74±5.46	72.02±23.41*$	13.41±1.19	12.68±0.19	11.95±3.94	11.72±5.85
30-49	85.18±6.03	85.7±6.14*	13.51±1.19	13.2±1.12	12.34±10.2	9.11±3.22*
50-69	87.15±4.85	87±6.09$	13.64±1.51	13.24±1.7	12.49±6.22	14.47±6.84*
Probability value	0.199	0.01	0.799	0.616	0.985	0.03

*,$ *are significant differences based on the results of the Bonferroni post hoc test.*

Changes in Iron Profile by Age

The mean iron profiles of patients three and six months after surgery were compared across three distinct age groups, as summarized in Table 3. Significant differences were observed in mean hemoglobin and folic acid concentrations six months post-surgery among these age groups. Specifically, the changes in mean hemoglobin concentration were significant for the first age group compared to both the second and third groups ($p < 0.05$). However, no statistically significant differences were found between the second and third age groups. Additionally, comparisons of folic acid levels revealed significant differences between the second and third groups, suggesting that younger patients tended to exhibit lower iron profiles following surgery. Overall, these findings indicate that the iron profile in younger age cohorts tends to be less favorable (refer to Table 3 for further details).

Table 3 (b)- Table 3 continued

Age Group	Vitamin B12 3 months	Vitamin B12 6 months
15-29	416.35±229.37	455.46±174.63
30-49	626.75±392.52	841.82±572.56
50-69	718.74±507.24	509.57±192.99
Probability value	0.083	0.10

Discussion

The present study was conducted to elucidate the patterns of iron deficiency, identify influential factors, and ascertain critical time frames for intervention following bariatric surgery in obese patients. Given the significance of this topic, we examined the six-month changes in iron profiles among patients attending the Obesity Clinic of Fatemeh Zahra Hospital at Iran University of Medical Sciences. This investigation aims to clarify the role of iron supplementation, dietary modifications, and other therapeutic interventions in enhancing the nutritional status of patients following surgery. In recent decades, obesity has escalated to epidemic proportions in both developing and developed nations, posing a substantial public health threat and representing a significant economic burden. This increase contributes greatly to the growing global prevalence of obesity-related diseases. Regrettably, surgical interventions often fail to yield long-term weight loss success, underscoring the necessity for effective treatment options. Given that non-surgical methods for weight reduction have proven less effective, bariatric surgery has gained recognition as a viable solution, known for its ability to facilitate substantial weight loss and address severe obesity.[15] However, the success of bariatric surgery is heavily contingent upon diligent long-term follow-up, as the procedures can precipitate various complications.

These complications may include intraoperative events (e.g., trocar injuries, splenic injury, portal vein injury, intestinal ischemia), short-term postoperative complications (e.g., bleeding, wound infections, anastomotic leaks, pulmonary embolism, cardiovascular, and respiratory issues), and long-term complications (e.g., kidney and biliary stones, nutritional deficiencies, neurological, and psychological effects).[16] Iron is one of approximately twenty essential trace elements and plays critical roles in human physiology as well as in nearly all biological systems.[17] Post-bariatric surgery iron deficiency arises from multiple factors: reduced iron intake due to impaired absorption, limited tolerance to iron-rich foods, inadequate adherence to iron supplementation, diminished gastric acid secretion resulting from gastric bypass, and duodenal bypass leading to impaired absorption. Depending on the type of surgery and postoperative care, studies have reported that iron deficiency affects 18% to 53.3% of patients, with iron deficiency anemia observed in 52% to 54% of these individuals.[18]

Our findings indicate that bariatric procedures, particularly mini gastric bypass with single anastomosis and sleeve gastrectomy, significantly influence patients' iron profiles. The observed alterations in ferritin levels, hemoglobin, and mean hemoglobin concentration underscore the impact of these surgeries on iron metabolism. However, these results must be contextualized within the framework of previous research. Interestingly, while serum iron levels remained relatively stable three months post-surgery, ferritin levels exhibited significant increases. In contrast, a study entitled "Anemia Post-Bariatric Surgery" found it challenging to attribute anemia solely to iron deficiency, highlighting a concurrent reduction in hemoglobin and ferritin levels alongside an increase in serum iron. These findings suggest that post-bariatric surgery anemia may arise from other mechanisms such as inflammation or altered nutrient absorption.[19]

A separate study investigating the prevalence and causes of anemia in seventy patients following bariatric surgery reported that sixteen individuals—exclusively women—developed anemia postoperatively. This underscores the particular vulnerability of women to anemia and related nutritional deficiencies.[20] However, in the current study, gender-based analyses yielded differing results. While ferritin and mean hemoglobin concentrations rose significantly in women at

three months, they nearly returned to preoperative levels by six months, with hemoglobin levels showing a significant decrease at this later time point. To enhance the reliability of future findings, we recommend conducting studies with larger sample sizes and extended follow-up periods. Our results demonstrate notable differences in mean ferritin and hemoglobin levels post-surgery between women and men, with females exhibiting lower averages. This discrepancy may be attributed to physiological variances, particularly those associated with reproductive age and the consequent demands on iron stores.

In this study, we investigated alterations in iron profiles following bariatric surgery in patients with pre-existing conditions such as diabetes, hypertension, dyslipidemia, or hypothyroidism, contrasting these changes with those observed in individuals without underlying diseases. Notably, we observed a significant reduction in mean ferritin levels among diabetic patients three months post-surgery, in stark contrast to non-diabetic individuals. This finding corroborates results from a previous study that assessed serum ferritin levels in women with diabetes and hypertension both prior to and six months following bariatric surgery.[21]

Furthermore, an additional study examining one-year outcomes of bariatric surgery across two distinct age groups—adolescents and young adults—demonstrated that the increase in hemoglobin levels was more pronounced in the young adult cohort, whereas adolescents manifested a greater decrease in ferritin levels. Our study similarly compared changes in iron profiles across three age groups and revealed a more significant reduction in mean hemoglobin concentration among younger patients. This suggests that younger populations may be at an elevated risk for iron deficiency, potentially due to increased nutritional demands associated with growth or lower adherence to postoperative care protocols.[22] Additionally, research investigating weight loss and health status in adolescents three years following bariatric surgery indicated noteworthy improvements in comorbidities and overall quality of life. However, it was found that 57% of these adolescents continued to experience ferritin deficiency. This finding underscores that, despite achieving substantial weight loss and advancements in cardiometabolic health, bariatric surgery among adolescents also presents a risk for micronutrient deficiencies and highlights the necessity for ongoing nutritional interventions.[23] Numerous studies have documented nutritional deficiencies among obese individuals both prior to and subsequent to bariatric surgery, revealing common deficiencies in iron, vitamin D, and other essential nutrients.[14,24,25] The consistency of these findings with our results emphasizes the critical need for regular screening for nutritional deficiencies both before and after surgery.

Conclusion

The findings of our study concerning changes in iron profiles following bariatric surgery largely align with those from previous research, although some discrepancies were noted. These variations may be attributed to differences in study populations, duration of follow-up, surgical techniques, or methodologies for measurement. Overall, our results accentuate the necessity for vigilant screening and monitoring of patients after bariatric surgery to prevent nutritional deficiencies and anemia. This study underscores the importance of continuously evaluating the iron status of obese patients post-surgery, in conjunction with the consistent use of appropriate and recommended iron supplements. Such interventions are vital for optimizing patient outcomes and sustaining nutritional health.

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Conflict of Interest

All authors declare that there are no conflicts of interest regarding the publication of this article.

Ethical Considerations

This study was approved by the Ethics Committee of the University under the code IR.IUMS.FMD.REC.1402.371.

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