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ORIGINAL ARTICLE

Clinical Significance of Serum Iron in the Diagnosis of Iron Deficiency Anemia in Patients from Local Population of Punjab-Lahore

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ABSTRACT

Background: The main cause of anemia in one-third of the world's population is a lack of iron (ID). Anemia affects between 700 and 800 million individuals globally.

Methodology: Only the patients diagnosed with iron deficiency anemia was selected from Ali Fatima Hospital Lahore and Jinnah Hospital Lahore for determination of serum iron concentration in comparison with healthy controls**.**

Results: In our study male patients were 67% and females were 33%. Frequency of children with beta thalassemia major under 5 year of age is 19%, 5-9 year is 24%, 9-15 years is 31% and above 15 years is 26%

Practical implications: The current study aims to spread the awareness among community about clinical significance of serum iron in the diagnosis of iron deficiency anemia in patients and ways to control the iron deficiency. This investigation will assist in determining the severity of the condition in our community as well as the status of serum iron levels in individuals with iron deficiency anemia. The evaluation of serum iron levels in anemic individuals will be helpful to medical experts in determining the best course of treatment.

Conclusion: We concluded that renal tubular damage and hepatocellular injury may be secondary to oxidative lipid peroxidation mediated by the iron overload. However further investigations are extremely necessary for this interpretation. A better evaluation of iron toxicity and regular monitoring of renal functions, liver functions, accompanied by an improvement in chelation therapy along with the administration of selective antioxidants (vitamins E and C), may protect different organs against oxidative stress and its consequences, specifically endocrine problems.

Keywords: Anemia, Iron deficiency, Ferritin, Hemoglobin, Thalassemia, hepatocellular injury.

INTRODUCTION

One of the most prevalent health issues in the globe is anaemia. It is the sole dietary deficit that exists in affluent nations, but it is a major issue and a health burden in developing nations. Anemia affects between 700 and 800 million individuals globally. According to estimates from the World Health Organization (WHO), 40% of pregnant women and 42% of children under 5 are anaemic globally (WHO, 2022).[1,2] The most vulnerable population to anaemia is children. This has a significant impact on neonatal morbidity and death[1]. Serum ferritin is the most effective noninvasive test for the identification of iron deficiency anaemia in individuals with a variety of illnesses. In cases when a disease is suspected, this ought to be the first test ordered. Anemia comes in a variety of forms, each with a unique aetiology[2]. This ailment can be minor to severe, and it can be short-term or long-term. a lack of iron anemia Anemia due to a vitamin shortage Anemia with inflammation Aplastic anaemia linked to a bone marrow condition Anemia hemolytic If there is no evident reason of the bleeding, sickle cell anaemia should be investigated as well. Anemia can be caused by chronic inflammatory conditions, parasite infections, the inherited hemoglobin defect malaria, micronutrient deficiencies, and concurrent low levels of iron, folic acid, vitamin B12, and vitamin A[3]. The primary factor causing anaemia is an iron deficit. The following risk factors for anaemic symptoms in children have been identified by several research conducted worldwide. Iron deficiency is the most common cause of anaemia[3,4]. Various studies from across the world have shown the following risk factors for anemic symptoms in children. Male, younger, less educated mother, poorer household income Tiredness, weakness, and dizziness Shortness of breath, pale complexion, irregular heartbeat, chilly hands and feet, headache, and other symptoms of ageing According to the research, iron appears to be the most frequent dietary deficit in Pakistan[4,5]. Various national nutrition surveys in Pakistan indicated a prevalence of anaemia over 50% in children of 50.9% in 2001, 61.9% in 2011, and 53.7% in 2018, as well as a rising trend of anaemia in women of 28.3% in 2001, 48.9% in 2011, and 41.7% in 2018. (UNICEF Pakistan)[4].

The main cause of anaemia in one-third of the world's population is a lack of iron (ID). Anemia affects between 700 and 800 million individuals globally. This is a crucial component that can raise a person's morbidity and death. As a result, it is crucial to determine the serum iron levels while making an IDA diagnosis[5]. The patient must be anemic and have test evidence of iron deficiency in order for IDA to be diagnosed. When describing red blood cells with IDA, tiny erythrocytes are frequently used (i.e., corpuscular volume smaller than 80 m3).[6,7] Hypopigmented, although the signs of iron deficiency show up gradually. Patients who have serum ferritin levels below 25 mcg per mL (25 mcg per L) and serum iron levels below 60 mcg/dL are very likely to be anemic. The most reliable first diagnostic tests for IDA are serum ferritin and serum iron levels.[8] Greater than 100 mcg per mL (100 mcg per L) serum ferritin readings signify sufficient iron reserves and minimal IDA potential. Because ferritin is an acute phase reactant, these tests should be interpreted slightly differently in specific groups, such as individuals with inflammatory illness or cirrhosis. In these people, the threshold for abnormality is often greater[9].Erythropoiesis-stimulating substances can result in functional iron deficit even for erythropoiesis when iron reserves are increased. The diagnosis of iron deficiency is straightforward, with the exception of inflammatory conditions.[9,10] Because low hepcidin levels promote iron absorption, oral iron salts usually treat absolute iron deficiency in individuals. Unfortunately, oral iron's usefulness is constrained by frequent adverse effects. Since currently available S-containing preparations enable quick restoration of the body's overall iron balance, intravenous iron is being utilized more frequently[11]. It is also beneficial in situations of functional iron deficit and iron deficiency related to inflammatory illnesses, even with just one infusion. There is mounting evidence

to support the safety and efficacy of these preparations. The long-term safety of high-dose iron, however, requires more research. Furthermore, despite the fact that they may be altered, several studies have demonstrated that the effects of anemia are permanent. As a result, treating anemia is crucial and shouldn't be overlooked in any manner because it will impede growth and development[12].

Significance of Study: The current research work aims to assess the impact of the test findings because, to our knowledge, research on the assessment of blood iron levels in anemic individuals is scarce in our community. Patients from several hospitals in Lahore, Pakistan, underwent serum iron tests to determine whether they needed more iron.

Research Gap: Determined that there was not enough data to support standard iron supplementation for expectant mothers or routine testing for iron deficiency anaemia in infants or young children. In populations that are representative of the Lahore, Pakistan, the data was identified giving a substantial evidence gap linked to whether altering hematologic indices in otherwise asymptomatic pregnant women or in newborns results in improved maternal or child health outcomes. In order to fill these critical evidence gaps, future research options are presented.[6,9]

MATERIALS AND METHODS

Place of Study: Samples were collected from only the patients diagnosed with iron deficiency anemia with hypochromic, microcytic blood picture will be selected from Ali Fatima Hospital Lahore and Jinnah Hospital Lahore for determination of serum iron concentration in comparison with healthy controls.**Study Duration:** The cross-sectional study design was conducted between June 2020 to October 2020.

Population Size: About 50 Patients including women, men and children were included in the study.

Sample size: n=50

Sample calculation: Sample Collection: Diagnosed cases of anemic patients that had undergone at least ten transfusions were included after obtaining a written consent from their care takers. Those who did not give consent or had lesser number of transfusions were excluded from the study. Samples were collected from Jinnah hospital and Ali Fatima hospital Lahore. Detailed physical examination was performed and all the available blood tests reports were taken. Questionnaires were duly filled with bio data of the patient, clinical presentation of the illness, duration of illness, complete Blood Count record.

Ethical Consideration: Blood sampling was done according to the ethical considerations of biosafety and integrity of Ethical Committee School of Biochemistry, Minhaj University Lahore.

Participant selection criteria: Only the patients diagnosed with iron deficiency anemia was selected from Ali Fatima Hospital Lahore and Jinnah Hospital Lahore for determination of serum iron concentration in comparison with healthy controls.

Blood Sample Collection: About 5ml Blood Samples of selected patients was collected in Gel Vials with clot activator by a trained phlebotomist by using standardized techniques.

Serum separation from blood: Serum was separated from blood by following steps:

1. Blood was incubatedat37℃ for30 minutes in an incubator (Bio-rad).
2. After Clotting of blood in the Vials, these vials were subjected to centrifugation at 4000 rpm for 10 minutes.
3. A transparent serum was separated in an Eppendorf tube and stored at 4℃ for subsequent analysis.

Evaluation of Serum Iron: The Serum was subjected for the determination of iron concentration through spectrophotometric assays using Roche reagents on fully automated Clinical Chemistry Analyzer, Cobas A 411. Components of test kit.

Transportation of samples: Samples collected were transported in ice box to Molecular Biology Lab IMBB.

Storage of samples: All the samples were stored and preserved at -20°C for future use.

Statistical Analysis: The significance of difference between patients' serum iron was analyzed by Independent Student's t test. P value < 0.05 was considered statistically significant. All calculations were carried out with the SPSS version 19 (SPSS, Inc, Chicago, IL, USA).

RESULTS

In current study male patients were 67% and females were 33%. Frequency of children with severe anemia under 5 year of age is 19%, 5-9 year is 24%, 9-15 years is 31% and above 15 years is 26%. Almost all the patients developed severe anemia and diagnosed as anemia patients in first few months of life. Patients are on blood transfusion as they were diagnosed with a rate of two or three transfusions per month maintained by their Hb level. Anemia is seen in patients of all blood groups. All the patients were on chelation therapy.

They are taking either parental or oral chelating agents. 79% of the patients are the only child having this disease. In our study consanguinity rate among patients of anemia is 95%. They are either first or second cousins and either paternal or maternal side. Positive correlation existed between age of patients and Serum iron level. Age wise distribution of mean serum ferritin, iron, Hb, MCT and HCT levels were observed in our city with the age group of 5 to more than 15 years of anemic patients (Figure 1-4). All the biochemical parameters displayed significant alterations with p≤0.05.

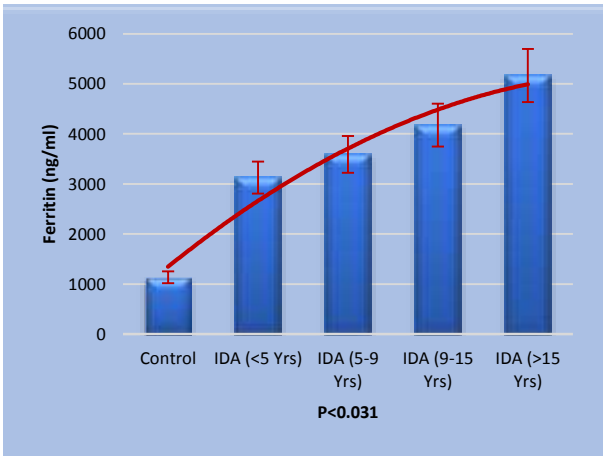

Figure 1: Age wise distribution of mean serum ferritin levels in iron deficient patients

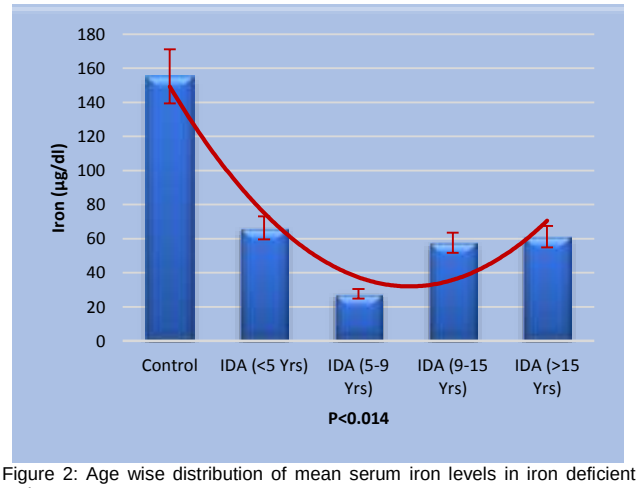

Figure 2: Age wise distribution of mean serum iron levels in iron deficient patients

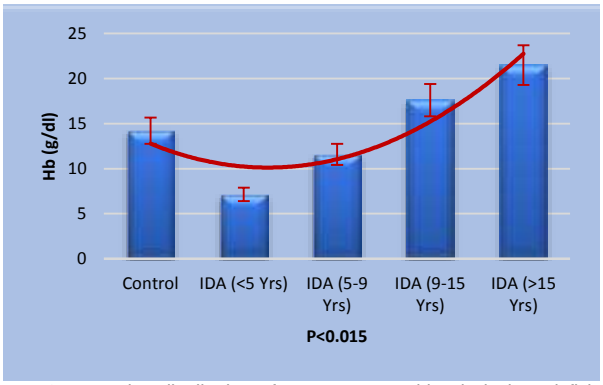

Figure 3: Age wise distribution of mean serum Hblevels in iron deficient patients

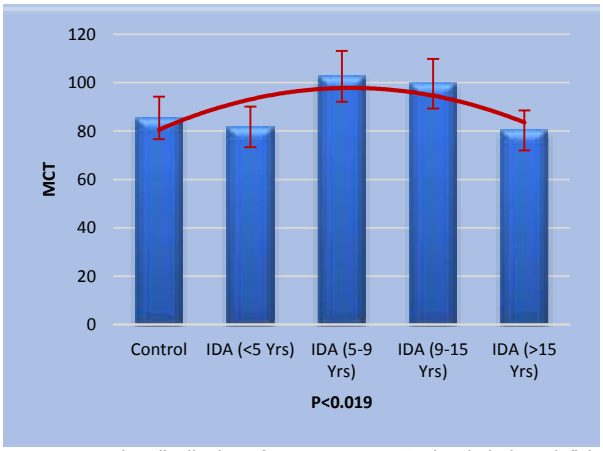

Figure 4: Age wise distribution of mean serum MCT levels in iron deficient patients

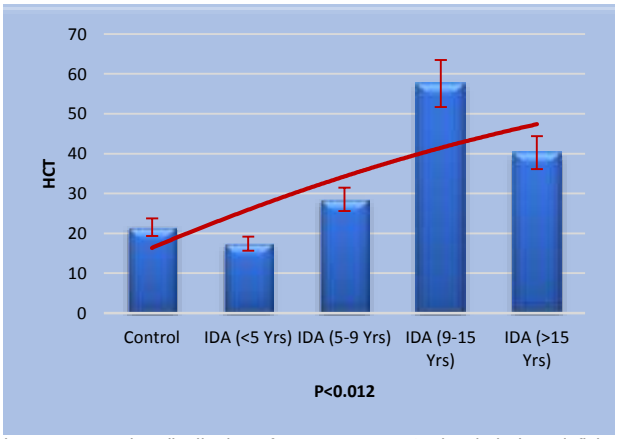

Figure 5: Age wise distribution of mean serum HCT levels in iron deficient patients

DISCUSSION

Due to frequent blood transfusions, iron overload is an inevitable consequence of thalassemia major patients. When treating the first, it is referred to as the "second illness" since it is so prevalent.[13] Due to their high cost and complexity, new noninvasive technologies of assessing iron storage in the body, such MRI or SQUID, are not widely used in impoverished nations like Pakistan. In our study, around 4/5 of the patients had serum iron levels over 2000 mg/ml, or approximately ten times the upper limit of normal. According to Borgna-Pignatti, lower ferritin levels were associated with a longer life expectancy and a decreased risk of different problems. Analysis of the rates of iron absorption and accumulation in a sample of -thalassemia intermedia patients.[14] Age causes an increase in iron absorption, According to device studies, by the time many of these patients are three or four years old, their overall iron consumption may be on level with that of homozygous people. Thalassemia is dependent on blood transfusions. Reduced gastrointestinal iron absorption is necessary for these patients in order to prevent cardiac, hepatic, and endocrine complications from iron overload in middle age. Reducing gastrointestinal iron absorption is essential for protecting these individuals against cardiac .[15]

Due to persistent anemia, chronic hypoxia, iron overload, and deferoxamine toxicity, they appear to be multifactorial.[16,17] Only 12% of patients in our study who were between the ages of 15-20 and were receiving chelation therapy for more than 15 years had high blood iron levels, according to our research. In TM patients, Grundy et al. (1994) found higher serum iron levels. Li discovered normal RCS, in contrast. Cirrhosis, hepatocellular carcinoma, and liver cancer are all made more likely by a chronic iron overload in the liver. The leading causes of death are heart disease, diabetes, hepatocellular carcinoma, and liver disease.[18,22]

Indrani comparable outcomes, too. Patients with thalassemia have been shown to have elevated levels of malondialdehyde and other plasma lipid peroxidation indicators.[19,20,21] According to a paper, unstable hemoglobin chains undergo auto-oxidation, which results in the production of H2O2 and superoxide.[23] Thus, in the presence of H2O2, this free nonheme iron can catalyse the Haber-Weiss reaction, producing a feryl moiety and a hydroxyl radical.[24] Erythrocyte lipids and membrane proteins are further harmed by it.[25] Comparable outcomes, too. Patients with thalassemia have been shown to have elevated levels of malondialdehyde and other plasma lipid peroxidation indicators .[25,26,27,28] According to a paper, unstable hemoglobin chains undergo auto-oxidation, which results in the production of H2O2 and superoxide[30,31]. Thus, in the presence of H2O2, this free nonheme iron can catalyze the Haber-Weiss reaction, producing a feral moiety and a hydroxyl radical. Erythrocyte lipids and membrane proteins are further harmed by it.[32]

CONCLUSION

We concluded that renal tubular damage and hepatocellular injury may be secondary to oxidative lipid peroxidation mediated by the iron overload. However further investigations are extremely necessary for this interpretation. A better evaluation of iron toxicity and regular monitoring of renal functions, liver functions, accompanied by an improvement in chelation therapy along with the administration of selective antioxidants (vitamins E and C), may protect different organs against oxidative stress and its consequences, specifically endocrine.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Raw data will be available on demand.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Ahmed S, Petrou M, Saleem M. Molecular genetics of beta-thalassaemia in Pakistan: a basis for prenatal diagnosis. British journal of haematology. 1996 Sep 1;94(3):476-82.
2. Aldudak B, Bayazit AK, Noyan A, Özel A, Anarat A, Sasmaz I, Kilinç Y, Gali E, Anarat R, Dikmen N. Renal function in pediatric patients with β-thalassemia major. Pediatric nephrology. 2000 Oct;15:109-12.
3. Ahmed HM, Georgy DB, Meabed MH, Botrous OE. The Association Between Plasma MicroRNA-451 Expression Levels and Chronic

Kidney Disease in Children with β-Thalassemia Major. Iranian Journal of Kidney Diseases. 2022 May 1;16(3):188.
4. Ricchi P, Ammirabile M, Spasiano A, Costantini S, Cinque P, Di Matola T, Pagano L, Prossomariti L. Combined chelation therapy in thalassemia major with deferiprone and desferrioxamine: a retrospective study. European journal of haematology. 2010 Jul;85(1):36-42.
5. Alsodani MH, Alhashemi WK. THE EFFECT OF ALLOPURINOL ON SERUM URIC ACID, BLOOD UREA AND SERUM CREATININE IN CHRONIC KIDNEY DISEASE PATIENTS WITH HIGH URIC ACID. World Bulletin of Public Health. 2022 Oct 14;15:174-81.
6. Meloni A, Pistoia L, Quota A, Messina G, Ricchi P, Bagnato S, Gerardi C, Lisi R, Cuccia L, Renne S, Vallone A. Prognostic value of multiparametric cardiac magnetic resonance in sickle cell patients. Annals of Hematology. 2022 Dec 2:1-0.
7. Kim H. Diagnosis and treatment of transfusion-related iron overload. Journal of the Korean Medical Association/Taehan Uisa Hyophoe Chi. 2022 Oct 1;65(10).
8. Kontoghiorghes GJ. Questioning Established Theories and Treatment Methods Related to Iron and Other Metal Metabolic Changes, Affecting All Major Diseases and Billions of Patients. International Journal of Molecular Sciences. 2022 Jan 25;23(3):1364.
9. Williams TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harbor perspectives in medicine. 2012 Sep 1;2(9):a011692.
10. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, Marchetti M, Piga A, Tura S. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica. 2008 May;93(5):741-52.
11. Pinto VM, Forni GL. Management of iron overload in beta-thalassemia patients: clinical practice update based on case series. International Journal of Molecular Sciences. 2020 Nov 20;21(22):8771.
12. França M, Carvalho JG. MR imaging assessment and quantification of liver iron. Abdominal radiology. 2020 Nov;45(11):3400-12.
13. Liu T, Li L, Cheng C, He B, Jiang T. Emerging prospects of protein/peptide-based nanoassemblies for drug delivery and vaccine development. Nano Research. 2022 Aug;15(8):7267-85.
14. Shahandeh A, Bui BV, Finkelstein DI, Nguyen CT. Effects of excess iron on the retina: Insights from clinical cases and animal models of iron disorders. Frontiers in Neuroscience. 2022 Feb 3;15:1852.
15. Banerjee P, Saha I, Sarkar D, Maiti AK. Contributions and limitations of mitochondria-targeted and non-targeted antioxidants in the treatment of parkinsonism: an updated review. Neurotoxicity Research. 2022 Jun;40(3):847-73.
16. Mancinelli R, Rosa L, Cutone A, Lepanto MS, Franchitto A, Onori P, Gaudio E, Valenti P. Viral hepatitis and iron dysregulation: Molecular pathways and the role of lactoferrin. Molecules. 2020 Apr 24;25(8):1997.
17. Borgna-Pignatti C. The life of patients with thalassemia major. Haematologica. 2010 Mar;95(3):345.
18. Vasilopoulou M, Stafylidis C, Politou M. The thrombotic spectrum of B-thalassemia. Thrombosis Update. 2022 Mar 21:100102.
19. Moukhadder HM, Halawi R, Cappellini MD, Taher AT. Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: a comprehensive review. Cancer. 2017 Mar 1;123(5):751-8.
20. Angoro B, Motshakeri M, Hemmaway C, Svirskis D, Sharma M. Non-transferrin bound iron. Clinica Chimica Acta. 2022 Apr 6.
21. Old J, Harteveld C. Carrier Screening for the Haemoglobinopathies: Past, Present and Future. OBM Genetics. 2017 Aug;1(3):1-40.
22. Cappellini MD, Taher A. Long-term experience with deferasirox (ICL670), a once-daily oral iron chelator, in the treatment of transfusional iron overload. Expert Opinion on Pharmacotherapy. 2008 Sep 1;9(13):2391-402.
23. Cappellini MD, Bejaoui M, Agaoglu L. Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: Efficacy and safety during 5 years' follow-up (Blood (2011) 118, 4 (884-893)). Blood. 2011 Nov 3;118(18):5060.
24. Bruzzese A, Martino EA, Mendicino F, Lucia E, Olivito V, Bova C, Filippelli G, Capodanno I, Neri A, Morabito F, Gentile M. Iron chelation therapy. European Journal of Haematology. 2023 Jan 28.
25. Shazia Q, Mohammad ZH, Rahman T, Shekhar HU. Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. Anemia. 2012 May 9;2012.
26. Bragadesh T, Bhandari S. Deferiprone, iron overload in a renal transplant patient. QJM: An International Journal of Medicine. 2014 Jun 1;107(6):465-6.
27. Basak T, Kanwar RK. Iron imbalance in cancer: Intersection of deficiency and overload. Cancer Medicine. 2022 Oct;11(20):3837-53.
28. Qiu F, Wu L, Yang G, Zhang C, Liu X, Sun X, Chen X, Wang N. The role of iron metabolism in chronic diseases related to obesity. Molecular Medicine. 2022 Dec;28(1):1-4.
29. Naseef PP, Elayadeth-Meethal M, Salim KM, Anjana A, Muhas C, Vajid KA, Kuruniyan MS. Therapeutic potential of induced iron depletion using iron chelators in Covid-19. Saudi Journal of Biological Sciences. 2022 Apr 1;29(4):1947-56.
30. Zeinivand M, Jamali-Raeufy N, Zavvari F. The beneficial role of Hepcidin peptide inhibitor in improved the symptoms of COVID-19 in diabetics: anti-inflammatory and potential therapeutic effects. Journal of Diabetes & Metabolic Disorders. 2022 Dec;21(2):1797-807.
31. Sawicki KT, De Jesus A, Ardehali H. Iron Metabolism in Cardiovascular Disease: Physiology, Mechanisms, and Therapeutic Targets. Circulation research. 2023 Feb 3;132(3):379-96.
32. Zhang J, Song Y, Li Y, Lin HB, Fang X. Iron homeostasis in the heart: Molecular mechanisms and pharmacological implications. Journal of Molecular and Cellular Cardiology. 2023 Jan 1;174:15-24.