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TANAFFOS

Iron Supplementation in Anemic Patients with Cystic Fibrosis: Effects on Hematologic, Clinical, and Pulmonary Outcomes

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Background: Cystic fibrosis (CF) is an autosomal recessive multisystem disorder frequently complicated by anemia. This study aimed to evaluate the clinical, laboratory, radiological, and functional outcomes of oral iron therapy in anemic patients with CF.

Materials and Methods: In this interventional before–after study, 340 patients with confirmed CF were screened for anemia. Twenty-two patients met the diagnostic criteria for anemia, and 15 completed the study and were included in the final analysis. Participants received oral ferrous sulfate at a dose of 4–6 mg/kg/day (maximum 150 mg/day) for 6–8 weeks. Demographic characteristics, CBC, iron profile, inflammatory markers, pulmonary function tests, sputum culture, high-resolution computed tomography (HRCT) findings, and Shwachman–Kulczycki scores were evaluated before and after treatment.

Results: The prevalence of anemia among patients with CF was 6.47% (22/340). The mean age of participants was 18.8±1.24 years, and 53.3% were male. Hemoglobin and hematocrit levels increased significantly following treatment, with the greatest improvement observed among patients with combined iron deficiency anemia (IDA) and anemia of chronic inflammation (ACI) (p=0.007 and p=0.014, respectively). Serum iron levels also increased significantly after treatment (p=0.040). No significant changes were observed in pulmonary function indices, sputum culture results, or HRCT findings. Physical examination scores within the Shwachman–Kulczycki assessment improved significantly in the IDA+ACI group (p=0.039). Hemoglobin and ferritin levels showed significant direct and inverse correlations with the total Shwachman–Kulczycki score, respectively (p=0.003 and p=0.015).

Conclusion: Short-term oral ferrous sulfate therapy improved hematologic and selected clinical outcomes in anemic CF patients without evidence of worsening pulmonary infection or respiratory status. Accurate identification of anemia etiology and appropriate management may improve patient outcomes, although longer-term follow-up studies are warranted.

Keywords: Cystic Fibrosis; Anemia; Iron Deficiency Anemia; Ferrous Sulfate; Iron Supplementation

INTRODUCTION

Cystic fibrosis (CF) is a classic inherited disorder with an autosomal recessive pattern caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (1-8). The product of this gene in normal people is the chloride ion channel, whose lack of function

leads to changes in the viscosity, volume, and salt concentration of body fluids (9). Previously, life expectancy for patients with CF was only a few months, but in recent decades, life expectancy of these patients has increased dramatically to more than forty years in developed countries (8). This improvement is due to the provision of well-organized care in multidisciplinary medical centers, as well as the use of effective treatments to improve mucus secretion, and also the advent of more effective antibiotics for the management of infections (10).

CF affects various organs of the body, including lungs (bronchiectasis, hemoptysis, pneumothorax, etc.), pancreas (exocrine insufficiency and CF-dependent diabetes mellitus), and the liver (reports of cirrhosis and portal hypertension), and causes susceptibility to infections (often with *Staphylococcus aureus* and *Pseudomonas aeruginosa*) (11,12). These complications have been discussed and studied as the main problems and have always been considered, while some problems, such as anemia in these patients, are often neglected (13). Previous studies have shown a decrease in iron storage (hypoferremia) of 23-100% of subjects with CF, suggesting that iron deficiency may limit erythropoiesis (14). However, accurate assessment of iron status in CF is challenging because serum ferritin levels and total serum transferrin saturation (TSAT) levels are often affected by inflammation. In addition, although elevated levels of soluble transferrin receptor (sTfR) in CF imply hypoferremic anemia and sTfR is not affected by the acute phase of the inflammatory response to infection, sTfR cannot differentiate iron deficiency from inflammation-induced anemia (15). Therefore, no blood test can detect hypoferremia in CF, making iron supplementation essential for diagnosing anemia in CF patients.

Iron supplementation is necessary for iron-deficient anemic CF patients, but it has several challenges. Bacteria in the CF lungs need iron for their growth and have developed mechanisms for obtaining human tissues' iron (12). Iron enhances the formation of *Pseudomonas aeruginosa* (*P.aeruginosa*) biofilm, which can be detected even in patients' sputum culture (16). In an epithelial co-culture model, ΔF508-CFTR increased iron in the airway surface fluid and subsequently increased *P.aeruginosa* biofilm growth and antibiotic resistance (17). In addition, new vascular changes in the bronchial arteries may lead to hemoptysis and even import iron into the airways (18). These observations have made the use of iron supplementation in patients with CF challenging.

According to reports of the symptoms of CF pulmonary exacerbation (CFPE) in patients after intravenous iron injection, it seems that iron supplementation in CF may be harmful (8). The complex relationship among iron deficiency, inflammation, and respiratory infection in cystic fibrosis presents important challenges in the evaluation and treatment of anemia. To further clarify these issues, clinical, paraclinical, and quality-of-life parameters were assessed and compared in anemic CF patients before and after treatment of iron deficiency anemia.

MATERIALS AND METHODS

Study Population

A total of 340 subjects with proven CF (by sweat test or genetic test results) over one year of age referred to the Pediatric Clinic of the National Research Institute of Tuberculosis and Lung Disease (NRITLD) were evaluated. 22 of these patients were diagnosed with anemia according to the latest CBC test (last two months) based on the criteria of the World Health Organization (WHO) (19). These individuals were called for confirmation and final diagnosis of anemia.

Study setting

Anemia was defined according to the World Health Organization, which is age- and gender-based, and the patient was considered anemic if the Hb was lower than normal in two distinct CBC tests at least two months apart. The distinction between Iron Deficiency Anemia (IDA) and Chronic Inflammation Anemia (ACI) was made based on criteria proposed by Ganz and Nemeth (20). Patients with a history of iron supplementation 10 days before the first visit or those who were not anemic were excluded from the

study. Among the 340 patients screened, 22 subjects were initially identified as having anemia and were invited for confirmation and further evaluation. After reassessment, anemia was not confirmed in three patients. During follow-up, two patients were lost to follow-up, and two died before post-treatment evaluation. Therefore, 15 patients completed the study and were included in the final analyses. These patients received oral ferrous sulfate at a dose of 4–6 mg/kg/day (maximum 150 mg/day) for 6–8 weeks.

Finally, patients were divided into three groups based on ferritin levels and response to ferrous sulfate treatment. IDA was defined as CF subjects with iron-deficiency anemia, based on decreased Hb and ferritin within the defined normal range. ACI were anemic CF subjects with anemia due to chronic disease with decreased Hb, normal or increased ferritin, and inflammatory factors (ESR and CRP), without a good response to ferrous sulfate. IDA+ACI were anemic CF subjects with normal or increased ferritin levels who responded to the initial treatment with ferrous sulfate.

Questionnaire & Shwachman-Kulczycki Scoring

Using a standard questionnaire designed for demographic information (age, sex, height, and weight for BMI), CBC, iron status (Iron, TIBC, TSAT, and serum ferritin) and also inflammatory parameters (C reactive protein: CRP and erythrocyte sedimentation rate: ESR), sputum culture results, PFT parameters, HRCT findings and Schwachmann-Kulczycki score were collected before and after ferrous sulfate administration. The Schwachmann-Kulczycki scoring is designed to determine the clinical severity of CF patients with the evaluation of general activity, physical examinations, nutrition, and X-ray findings (21).

Statistical Analyses

Statistical Package for the Social Sciences (SPSS) v.23 software was used for statistical analysis of the present study. For qualitative variables, cross-tabulation was used. For quantitative variables with a normal distribution, the mean ± standard deviation (SD) and the One-way ANOVA were used to compare groups and evaluate the significance level. For quantitative variables without a normal distribution, the median and the non-parametric test were used. Significance level for *p*. value (*p*) was considered less than 0.05.

Ethical Considerations

The study was approved by the Research Ethics Committee of NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.NRITLD.REC.1399.145). All steps performed in this study were conducted in accordance with the terms of the Helsinki Convention. Written informed consent was obtained from all patients or their legal guardians before inclusion in the study. All data were handled confidentially, and no personal identifiers were recorded or disclosed at any stage of the study.

RESULTS

Among 22 included CF patients suspected of anemia, anemia was not confirmed according to the inclusion criteria in three cases; two cases couldn't participate in evaluations after treatment, and two cases had expired. Thus, the prevalence of anemia in the referring population was equal to 6.47% (22 out of 340 patients with CF), and the prevalence of iron deficiency anemia was 2.94% (10 out of 340 patients). Anemic CF subjects included in the study (n=15) were divided into three groups: IDA (n=2), ACI (n=5), and IDA+ACI (n=8).

Among the 15 included anemic CF patients with a mean age of 18.8 (±1.24) years, 53.3% were male (n=8). There was no statistically significant difference in terms of gender distribution between the three groups of patients (*p*=0.266).

Increased Hb, HCT, and serum iron levels in CF patients with IDA after iron supplementation

The results of the CBC test of patients did not show a significant difference after treatment in patients. However, Hb and HCT increased significantly in patients after treatment, but this increase in Hb and HCT levels in the

IDA+ACI group was observed significantly (p=0.007 and 0.0014, respectively) (Table 1).

Serum iron (SI) levels are significantly elevated after treatment (p=0.040). This SI elevation in the ACI group showed a significant increase after the use of ferrous sulfate (p=0.009). For other factors, including TIBC, TSAT, and ferritin after treatment with ferrous sulfate, no significant difference was reported (Table 2).

No changes in inflammatory screening tests and sputum culture of patients after iron supplementation

No significant differences were observed before and after treatment with ferrous sulfate in terms of serum CRP levels and ESR (Table 3).

Additionally, 10 patients had a history of positive sputum cultures for Pseudomonas aeruginosa. Among these patients, no significant differences in sputum culture results were observed following ferrous sulfate treatment (Table 4).

Administration of ferrous sulfate did not affect patients' PFT

HRCT of all patients was indicative of bronchiectasis as Cylindric in all cases (n=15), Varicoid in 80% (n=12), and Cystic in 86.7% (n=13). Also, based on HRCT's evidence, complications including Air Trapping, Mucus Impaction and Pre-bronchial Thickening were reported in 33.3% (n=5), 73.3% (n=11) and 80% (n=12) of patients, respectively. According to these observations of HRCT, PFT studies were considered for patients for whom no statistically significant differences were observed between the groups after treatment with ferrous sulfate (Table 4).

Significant correlation of the Schwachmann- Kulczycki score with Hb and ferritin levels

Assessment of pulmonary function tests revealed no statistically significant changes in spirometric parameters following ferrous sulfate treatment in any of the study groups. Changes in FEV1, FVC, and FEV1/FVC ratio before and after treatment are presented in Table 5.

Results of the Schwachmann-Kulczycki test showed a statistically significant difference in the IDA+ACI group for the physical examination after treatment with ferrous sulfate (p=0.039) (Table 6).

There was a significant correlation between increased Shwachman–Kulczycki score and increased Hb levels (p=0.003) (Table 7). Serum ferritin levels showed a significant inverse correlation with the total Shwachman–Kulczycki score (p=0.015) (Table 7).

Table 1. Factors associated with anemia in the complete blood count

(n=15)		IDA	ACI	IDA+ACI	*p*. value	
WBC	Before	9.41 (±0.90)	12.63 (±2.09)	10.16 (±1.04)	0.412	0.934
	After	8.53 (±0.38)	14.98 (±4.53)	11.04 (±0.93)	0.399	
	p. value	0.439	0.917	0.344		
RBC	Before	5.34 (±1.64)	5.25 (±0.72)	4.87 (±0.42)	0.639	0.388
	After	4.77 (±0.33)	4.82 (±0.45)	4.99 (±0.34)	0.701	
	p. value	**0.001***	0.111	0.513		
Platelets	Before	225 (210-240)	389 (172-785)	216.5 (143-493)	0.403	0.740
	After	208 (150-267)	304 (156-739)	290 (165-506)	0.472	
	p. value	1.000	0.754	0.345		
Hb	Before	12.15 (±0.64)	11.84 (±1.30)	11.46 (±0.85)	0.430	**0.011***
	After	12.75 (±1.20)	12.48 (±1.85)	13.26 (±1.41)	**0.005***	
	p. value	0.439	0.600	**0.007***		
HCT	Before	40.95 (±1.49)	38.22 (±5.26)	36.13 (±3.26)	0.764	0.093
	After	41.35 (±3.75)	38.16 (±5.03)	40.86 (±3.48)	**0.009***	
	p. value	1.000	0.834	**0.014***		

WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, HCT: Hematocrit.

Table 2. Factors related to serum iron levels

(n=15)		IDA	ACI	IDA+ACI	*p*. value	Total
	Before	36.30 (±9.70)	35.82 (±2.46)	34.18 (±5.15)	0.965	35.01(±2.94)
SI	**After**	49.00 (±17.00)	44.00 (±1.30)	41.86 (±5.45)	0.820	43.64 (±3.26)
	***p*.value**	0.439	**0.009***	0.183		**0.040***
	Before	228.50 (±16.50)	237.60 (±7.42)	240.00 (±10.02)	0.638	235.5 (±5.77)
TIBC	**After**	259.00 (±63.00)	254.40 (±13.18)	243.50 (±4.46)	0.896	255.89 (±11.33)
	***p*.value**	1.000	0.251	1.000		0.423
	Before	15.65 (±3.15)	14.33 (±0.82)	14.83 (±2.71)	0.591	14.80 (±1.38)
TSAT	**After**	-	15.00 (±0.74)	15.48 (±3.33)	0.413	15.82 (±1.51)
	***p*.value**	0.221	0.564	0.670		0.477
	Before	11.00 (±4.00)	80.60 (±25.82)	47.25 (±8.51)	**0.071**	53.53 (±10.92)
Ferritin	**After**	39.00 (±2.00)	104.80 (±25.07)	65.75 (±10.39)	0.220	73.93 (±11.95)
	***p*.value**	0.121	0.347	0.141		**0.062**

SI: Serum Iron, TIBC: Total Iron Binding Capacity, TSAT: Transferrin Saturation.

Table 3. Serum CRP levels and whole blood ESR

		IDA	ACI	IDA+ACI	*p*. value	
	Before	2.50 (±1.50)	25.67 (±3.67)	24.00 (±7.71)	0.100	0.248
CRP (n=14)	After	5.50 (±4.50)	26.75 (±6.14)	7.00 (±2.59)	0.028	
	p. value	0.683	1.000	**0.055**		
	Before	41.00 (±16.00)	66.33 (±13.32)	68.00 (±1.87)	0.315	0.280
ESR (n=13)	After	25.50 (±8.50)	62.75 (±20.48)	48.00 (±16.60)	0.501	
	p. value	0.439	0.564	0.495		

CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate.

Table 4. Sputum culture of patients before and after treatment with ferrous sulfate

Sputum Culture Positive for	Before the Treatment (n)	After the Treatment (n)	*p*. value
Pseudomonas	8	7	0.500
Staphylococcus aureus	5	6	0.500
Klebsiella spp.	1	1	0.759
Acinetobacter spp.	2	2	0.701
Others	3	2	0.500

Table 5. Spirometric test results of patients before and after treatment with ferrous sulfate

(n=15)		IDA	ACI	IDA+ACI	*p*. value	
	Before	59.50 (±11.50)	36.20 (±6.63)	47.25 (±8.76)	0.389	0.649
FEV1	After	67.00 (±13.00)	38.75 (±13.52)	49.63 (±7.79)	0.417	
	p. value	0.439	0.806	0.752		
	Before	61.00 (±8.00)	42.00 (±10.19)	46.00 (±7.74)	0.491	0.824
FVC	After	66.00 (±10.00)	35.75 (±8.98)	50.38 (±7.89)	0.216	
	p. value	0.439	0.806	0.636		
	Before	85.50 (±1.50)	76.20 (±9.94)	86.88 (±1.97)	0.966	0.237
FEV1/FVC	After	90.50 (±0.50)	75.75 (±15.67)	88.38 (±1.69)	0.711	
	p. value	0.121	1.000	0.187		

Table 6. Shwachman–Kulczycki score components before and after ferrous sulfate treatment

Score		IDA	ACI	IDA+ACI	*p*. value	
	Before	15.00 (±5.00)	15.00 (±2.74)	16.88 (±2.10)	0.802	0.520
GA	After	20.00 (±0.00)	12.00 (±3.39)	20.00 (±1.64)	0.163	
	p. value	0.317	0.262	0.251		
	Before	17.50 (±2.50)	14.00 (±1.87)	14.38 (±1.48)	0.549	0.379
PE	After	20.00 (±0.00)	10.00 (±2.74)	18.13 (±1.31)	**0.039***	
	p. value	0.317	0.192	**0.068**		
	Before	17.50 (±2.50)	13.00 (±3.00)	13.75 (±2.63)	0.474	0.948
N	After	20.00 (±5.00)	10.00 (±3.87)	16.25 (±2.95)	0.210	
	p. value	0.683	0.174	0.616		
	Before	5.00 (±0.00)	7.00 (±2.00)	5.63 (±0.63)	0.756	0.609
XF	After	10.00 (±5.00)	7.00 (±2.00)	5.63 (±0.63)	0.433	
	p. value	0.317	1.000	1.000		
	Before	55.00 (±5.00)	51.00 (±11.11)	50.63 (±5.70)	0.433	0.617
Total	After	70.00 (±10.00)	41.00 (±13.64)	60.00 (±4.63)	0.165	
	p. value	0.221	0.109	0.224		

GA: General Activity, PE: Physical Examination, N: Nutrition, XF: X-ray Findings

Table 7. Correlation of factors involved in the diagnosis of anemia with the Schwachmann-Kulczycki score

Score	Spearman rho Correlation					
	Hb		Serum Iron		Ferritin	
	Co-efficiency	*p*. value	Co-efficiency	*p*. value	Co-efficiency	*p*. value
GA	**0.501****	**0.005**	0.244	0.203	-0.260	0.165
PE	**0.540****	**0.002**	0.115	0.553	**-0.399***	**0.029**
N	**0.392***	**0.032**	0.290	0.127	**-.0465***	**0.010**
XF	**0.355**	**0.054**	0.244	0.202	-0.260	0.165
Total	**0.531****	**0.003**	0.288	0.130	**-0.438***	**0.015**

GA: General Activity, PE: Physical Examination, N: Nutrition, XF: X-ray Findings, Hb: Hemoglobin.

*Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

CF is known as a fatal disease in which multi-organ involvement, especially lung involvement, is common. In a substantial proportion of adults with CF, severe pulmonary involvement characterized by an FEV1 below 40% predicted remains associated with significantly impaired survival compared with patients with preserved lung function, although outcomes have improved considerably in the era of CFTR modulator therapies (22). Liver involvement and pancreatic dysfunction of these patients are also considered serious complications leading to malabsorption and malnutrition (23,24). Efforts to improve the CF patients' quality of life are promising to the extent that today it is no longer considered a childhood disease, and more than 40% of patients with CF are adults (25).

Recently, prevention of CF-related complications, including diabetes mellitus and malnutrition, has been considered to be essential since these complications are directly linked to poor lung function and increased mortality (24,26). The prevalence of anemia in patients with CF is increasing; however, the underlying mechanisms and severity of anemia in this population remain poorly understood (15). Recent evidence has demonstrated that iron deficiency and anemia remain common hematologic abnormalities in patients with cystic fibrosis despite advances in nutritional support and CFTR modulator therapies, highlighting their continued clinical

relevance (27). Limited studies have simultaneously investigated the role of inflammation and malabsorption/ malnutrition as mechanisms involved in anemia of CF patients (15). Thus, the relationship between the contribution of iron deficiency and inflammation in anemia, as well as its relation with other complications of the disease, especially respiratory disorders and malnutrition, is still in a state of ambiguity (28).

The predominance of male patients observed in our cohort is generally consistent with demographic patterns reported in several cystic fibrosis registries and epidemiological studies. However, sex distribution in CF varies considerably across populations and is not considered a major determinant of disease occurrence (29). In general, anemia is more prevalent among females than males, particularly during the reproductive years, largely because of menstrual blood loss, increased iron requirements, and pregnancy-related physiological demands (30,31). Although no significant difference in sex distribution was observed between the study groups (p=0.266), recent investigations have suggested that female sex may be associated with a higher risk of iron deficiency among patients with cystic fibrosis (15).

No significant changes were observed in the blood cell counts measured by the CBC test following treatment (Table 1). Of note, in IDA, there is a significant decrease in RBC count after treatment with ferrous sulfate compared to the count of RBCs before the treatment period, which does not seem to be considerable since only two patients were in this group. However, Hb and HCT increased significantly in patients after treatment, but this increase in Hb and HCT levels in IDA+ACI was observed significantly as compared to IDA and ACI (p equal to 0.007 and 0.0014, respectively) (Table 1). Additionally, the effectiveness of ferrous sulfate treatment in improving Hb and HCT in IDA+ACI patients was also significant compared to the other two groups (p equal to 0.005 and 0.009, respectively) (Table 1). These findings support the clinical classification of iron deficiency anemia. In fact, accurate assessment of the iron status of CF patients is always challenging because in CF, an increase in serum ferritin, along with a decrease in TSAT levels in the presence of inflammation, leads to misconceptions of iron stores in patients (15,27,32,33). Thus, differentiation of IDA from chronic anemia using blood tests is not easy, and therapeutic iron supplementation remains an important practical approach for distinguishing functional iron deficiency from inflammation-related anemia in selected CF patients.

Serum iron levels in patients increased significantly after treatment (p=0.040), and this increase was noticeable in ACI (p=0.009) (Table 2). No significant changes were reported for other factors, including TIBC, TSAT, and ferritin. It should be noted that serum ferritin levels showed a significant increase in patients after treatment with ferrous sulfate (p=0.062). Despite the need for iron supplementation to differentiate IDA and ACI in CF anemic patients, there are theoretical concerns about iron administration in these patients (9). In this regard, previous studies have shown that bacteria in the lungs of CF need iron to grow and have specific mechanisms for obtaining the iron (34,35). Iron increases the biofilm formation of *P.aeruginosa* and can be detected in CF patients' sputum (9,36). However, in the present study, no significant differences were observed before and after treatment with ferrous sulfate in terms of ESR and serum CRP (as screening tools for inflammation) (Table 3). Conversely, the level of serum CRP after treatment with ferrous sulfate showed a noticeable decrease in IDA+ACI, although this change was not statistically significant (p=0.055). In addition, no significant changes were reported for sputum culture of patients after ferrous sulfate administration (Table 4). These findings are also in agreement with the randomized placebo-controlled trial of Gifford et al., which demonstrated that oral ferrous sulfate improved iron indices without significant changes in sputum iron content, pulmonary exacerbation scores, or sputum microbiome composition (37).

In this study, daily administration of 325 mg of ferrous sulfate increased serum iron and TSAT, but did not affect sputum iron and microbiome, as well as the pulmonary

exacerbation score of these patients. In addition, a research study reported no significant differences between iron-deficient and iron-replete CF patients for positive sputum culture of common pathogens (8).

It is important to note that previous experimental and clinical studies have raised concerns regarding the potential of iron administration to increase susceptibility to infections, particularly in conditions characterized by chronic bacterial colonization such as cystic fibrosis (38,39). Firstly, the way of iron administration, which, if given orally, increases hepcidin levels, thus reducing the intestinal iron uptake and iron sequestration of the reticuloendothelial system. This is actually a protective mechanism against infectious agents resulting from the iron dependency of human pathogens (40,41). In addition, hepcidin is a hormone-like antimicrobial peptide synthesized by the liver that is a "master regulator" of iron metabolism (20). While intravenous iron administration can increase the iron content of the lungs, thus increasing the susceptibility to infection by suppressing iron inhibition mechanisms (39). Secondly, the applied changes in treatment protocols to improve standard treatment over time affected the results of studies. At the forefront of these changes is the recent emphasis on the need for intravenous antibiotics to improve blood Hb levels (10,11). In fact, improving serum iron levels alone is not enough for the improvement of Hb levels of anemic CF patients. In this regard, improved control of inflammation caused by infections using recent protocols also reduces interleukin-6 (IL-6) levels in these patients. This inflammatory cytokine, which inhibits the maturation of erythropoietin-dependent erythroid cells, is increased in CF patients, thus restricting Hb synthesis (20,41). Decreased IL-6 following recent improved therapies may also be a cause of Hb levels' improvement in CF patients in most recent studies, including the present study (42-44).

HRCT findings were indicative of bronchiectasis for all included patients, but there were no statistically significant differences in PFT results after treatment with ferrous sulfate (Table 4). Previous studies have demonstrated that anemia and impaired iron status in patients with CF are associated with poorer pulmonary function and lower spirometric indices compared with patients without anemia (15,27). Recent studies have also reported an association between iron deficiency and reduced FEV1 values in patients with cystic fibrosis (10,27). The difference in PFT results is probably due to the selected groups of patients. In fact, those studies were done comparing CF patients without IDA as the control, whereas the control group was anemic CF patients after treatment with ferrous sulfate, in the present study.

Performed tests (including CBC, *etc.*) are not helpful independently in the CF patient's management and should be combined with the results of clinical examinations and radiology results (45). Many advances in the clinical field are due to scoring systems such as the Shwachman & Kulczycki scoring of CF subjects, which rely on assessing disease progression through continuous examinations (46). Clinical examination is always based on the observations of the clinician, to whom the patient refers regularly (43,45). Previous studies have demonstrated that longitudinal clinical scoring systems and structured outcome measures are valuable tools for assessing disease severity, monitoring progression, and evaluating treatment response in patients with cystic fibrosis (45,47). The Shwachman-Kulczycki score is one of the earliest clinical scoring systems developed for longitudinal assessment of disease severity in patients with cystic fibrosis and has contributed substantially to the evolution of CF outcome assessment (21,48). The results of the current study only showed statistical improvement in PE of Schwachmann-Kulczycki score after treatment with ferrous sulfate (p=0.039) (Table 5). In fact, the Schwachmann-Kulczycki score showed that despite the insignificant changes in PFT, treatment with ferrous sulfate improved clinical symptoms of IDA+ACI patients. Serum iron levels did not show correlation with the Schwachmann-Kulczycki score in this study (p=0.130) (Table 7). Similarly, previous studies have reported inconsistent associations between serum ferritin concentrations and clinical severity indices in patients with

cystic fibrosis (9,15). Reliance on serum ferritin alone may be insufficient for the assessment of iron deficiency in CF because ferritin is strongly influenced by chronic inflammation and may not accurately reflect functional iron availability (15,41). In contrast, in the present study, a significant correlation was seen between the increase in the Schwachmann-Kulczycki score and the increased Hb levels (p=0.003) (Table 7). It is possible that the observed improvement in Hb levels contributed to better clinical status in these patients (15,44).

Study limitations

The relatively small sample size, particularly in some subgroups, may have reduced the statistical power of the analyses. In addition, biomarkers such as folate, vitamin B12, soluble transferrin receptor (sTfR), and hepcidin were not assessed, which might have provided a more comprehensive evaluation of anemia. Finally, loss to follow-up in a subset of patients reduced the number of participants available for post-treatment assessment.

CONCLUSION

Anemia in patients with cystic fibrosis is likely attributable to both iron deficiency and chronic inflammation. In the present study, short-term oral ferrous sulfate supplementation was associated with significant improvements in hemoglobin, hematocrit, and serum iron levels. Improvement in the physical examination component of the Shwachman–Kulczycki score was observed in patients with combined iron deficiency anemia and anemia of chronic inflammation. Importantly, no evidence of worsening pulmonary infection, pulmonary function, or radiologic findings was observed following treatment. These findings highlight the importance of accurate identification of anemia etiology and appropriate management in patients with CF. Nevertheless, larger studies with longer follow-up periods are needed to further evaluate the long-term safety and efficacy of iron supplementation and to investigate additional biomarkers of iron metabolism, including soluble transferrin receptor and hepcidin.

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Conflict of interest

There are no conflicts of interest.

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REFERENCES

1. Afifi M, Hassanzad M, Malek F, Kamfar S, Pourabdollah M, Farnia P, et al. Prevalence of Anemia and its Relation with Shwachman Score in Children with Cystic Fibrosis. ***Tanaffos*** 2023;22(1):160-6.
2. Orooji A, Valinejadi A, Hassanzad M, Boloursaz M, Velayati AA. Association Between Prevalence of Depression and Anxiety in Cystic Fibrosis Patients and Their Parents: A Cross-sectional Study in Iran. ***J Compr Ped*** 2021;12(3):e113685.
3. Hassanzad M, Farnia P, Farnia P, Arian M, Valinejadi A, Ghaffaripour H, et al. Assessment of Cystic Fibrosis Distribution Based on Air Pollution by Geographical Information System (GIS). ***Tanaffos*** 2022;21(1):31-44.
4. Hassanzad M, Ansari M, Valinejadi A, Sayedi SJ, Velayati AA. Cystic fibrosis patients evaluation in the last decades in a referral center, Tehran-Iran. ***J Pediatr Perspect*** 2019;7(8):9997-10007.
5. Hassanzad M, Orooji A, Valinejadi A, Velayati A. A fuzzy rule-based expert system for diagnosing cystic fibrosis. ***Electron Physician*** 2017;9(12):5974-5984.
6. Eslampanah S, Hassanzad M, Valinejadi A, Ghaffaripoor HA, Boloursaz M, Mahdaviani SA, et al. Evaluation of quality of life in patients with cystic fibrosis regarding smoking history in parents. ***J Compr Ped*** 2022;13(2):e122110.
7. Fadaizadeh L, Hassanzad M, Valinejadi A, Taheri SM, Farnia P, Hassanzad N, et al. Evaluation of Mobile Phone-based Tele-

monitoring of Cystic Fibrosis Patients during the COVID-19 Pandemic: A 3-year Experience in Iran. ***Biomedical and Biotechnology Research Journal (BBRJ)*** 2022;6(2):261-5.

8. Greenwald MA, Wolfgang MC. The changing landscape of the cystic fibrosis lung environment: From the perspective of Pseudomonas aeruginosa. ***Curr Opin Pharmacol*** 2022;65:102262.
9. Hazlett HF, Hampton TH, Aridgides DS, Armstrong DA, Dessaint JA, Mellinger DL, et al. Altered iron metabolism in cystic fibrosis macrophages: the impact of CFTR modulators and implications for Pseudomonas aeruginosa survival. ***Sci Rep*** 2020;10(1):10935.
10. Petrocheilou A, Moudaki A, Kaditis AG. Inflammation and Infection in Cystic Fibrosis: Update for the Clinician***. Children (Basel)*** 2022;9(12):1898.
11. Harvey C, Weldon S, Elborn S, Downey DG, Taggart C. The Effect of CFTR Modulators on Airway Infection in Cystic Fibrosis. ***Int J Mol Sci*** 2022;23(7):3513.
12. Firoz A, Haris M, Hussain K, Raza M, Verma D, Bouchama M, Namiq KS, Khan S. Can Targeting Iron Help in Combating Chronic Pseudomonas Infection? A Systematic Review. ***Cureus*** 2021;13(3):e13716.
13. Biswas L, Götz F. Molecular Mechanisms of *Staphylococcus* and *Pseudomonas* Interactions in Cystic Fibrosis. ***Front Cell Infect Microbiol*** 2022;11:824042.
14. Ryan H, Ballard E, Stockwell RE, Duplancic C, Thomson RM, Smith K, Bell SC. A systematic review of the clinical impact of small colony variants in patients with cystic fibrosis. ***BMC Pulm Med*** 2023;23(1):323.
15. Lobbes H, Durupt S, Mainbourg S, Pereira B, Nove-Josserand R, Durieu I, Reynaud Q. Iron Deficiency in Cystic Fibrosis: A Cross-Sectional Single-Centre Study in a Referral Adult Centre. ***Nutrients*** 2022;14(3):673.
16. Guillaume O, Butnarasu C, Visentin S, Reimhult E. Interplay between biofilm microenvironment and pathogenicity of *Pseudomonas aeruginosa* in cystic fibrosis lung chronic infection. ***Biofilm*** 2022;4:100089.
17. Hunter RC, Asfour F, Dingemans J, Osuna BL, Samad T, Malfroot A, et al. Ferrous iron is a significant component of bioavailable iron in cystic fibrosis airways. ***mBio*** 2013;4(4):e00557-13.
18. Blayac M, Coll P, Urbach V, Fanen P, Epaud R, Lanone S. The Impact of Air Pollution on the Course of Cystic Fibrosis: A Review. ***Front Physiol*** 2022;13:908230.
19. Guideline on haemoglobin cutoffs to define anaemia in individuals and populations [Internet]. Geneva: World Health Organization; 2024.
20. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. ***Nat Rev Immunol*** 2015;15(8):500-10.
21. Stanford GE, Jones M, Charman SC, Bilton D, Usmani OS, Davies JC, et al. Clinimetric analysis of outcome measures for airway clearance in people with cystic fibrosis: a systematic review. ***Ther Adv Respir Dis*** 2022;16:17534666221122572.
22. Ong T, Ramsey BW. Cystic Fibrosis: A Review. ***JAMA*** 2023;329(21):1859-71.
23. Eldredge JA, Oliver MR, Ooi CY. Cystic fibrosis liver disease in the new era of cystic fibrosis transmembrane conductance regulator (CFTR) modulators. ***Paediatr Respir Rev*** 2024;50:54-61.
24. Frantzen T, Barsky S, LaVecchia G, Marowitz M, Wang J. Evolving Nutritional Needs in Cystic Fibrosis. ***Life (Basel)*** 2023;13(7):1431.
25. Barry PJ, Simmonds NJ. Diagnosing Cystic Fibrosis in Adults. ***Semin Respir Crit Care Med*** 2023;44(2):242-51.
26. Prentice B, Nicholson M, Lam GY. Cystic fibrosis related diabetes (CFRD) in the era of modulators: A scoping review. ***Paediatr Respir Rev*** 2023;46:23-9.
27. Jia S, Wang Y, Ross MH, Zuckerman JB, Murray S, Han MK, et al. Association between CFTR modulators and changes in iron deficiency markers in cystic fibrosis. ***J Cyst Fibros*** 2024;23(5):878-84.
28. Patel D, Shan A, Mathews S, Sathe M. Understanding Cystic Fibrosis Comorbidities and Their Impact on Nutritional Management. ***Nutrients*** 2022;14(5):1028.
29. Gärtner C, Fallmann J, Stadler PF, Kaiser T, Berkemer SJ. Toward a Systematic Assessment of Sex Differences in Cystic Fibrosis. ***J Pers Med*** 2023;13(6):924.
30. Tang GH, Sholzberg M. Iron deficiency anemia among women: An issue of health equity. ***Blood Rev*** 2024;64:101159.

31. Alem AZ, Efendi F, McKenna L, Felipe-Dimog EB, Chilot D, Tonapa SI, et al. Prevalence and factors associated with anemia in women of reproductive age across low- and middle-income countries based on national data. ***Sci Rep*** 2023;13(1):20335.
32. Cacoub P, Choukroun G, Cohen-Solal A, Luporsi E, Peyrin-Biroulet L, Peoc'h K, et al. Iron deficiency screening is a key issue in chronic inflammatory diseases: A call to action. ***J Intern Med*** 2022;292(4):542-56.
33. Rohr M, Brandenburg V, Brunner-La Rocca HP. How to diagnose iron deficiency in chronic disease: A review of current methods and potential marker for the outcome. ***Eur J Med Res*** 2023;28(1):15.
34. Cornelis P, Tahrioui A, Lesouhaitier O, Bouffartigues E, Feuilloley M, Baysse C, et al. High affinity iron uptake by pyoverdine in Pseudomonas aeruginosa involves multiple regulators besides Fur, PvdS, and FpvI. ***Biometals*** 2023;36(2):255-61.
35. Konings AF, Martin LW, Sharples KJ, Roddam LF, Latham R, Reid DW, et al. Pseudomonas aeruginosa uses multiple pathways to acquire iron during chronic infection in cystic fibrosis lungs. ***Infect Immun*** 2013;81(8):2697-704.
36. Haas AL, Zemke AC, Melvin JA, Armbruster CR, Hendricks MR, Moore J, et al. Iron bioavailability regulates Pseudomonas aeruginosa interspecies interactions through type VI secretion expression. ***Cell Rep*** 2023;42(3):112270.
37. Gifford AH, Alexandru DM, Li Z, Dorman DB, Moulton LA, Price KE, et al. Iron supplementation does not worsen respiratory health or alter the sputum microbiome in cystic fibrosis. ***J Cyst Fibros*** 2014;13(3):311-8.
38. Pantopoulos K. Oral iron supplementation: new formulations, old questions. ***Haematologica*** 2024;109(9):2790-801.
39. Shah AA, Donovan K, Seeley C, Dickson EA, Palmer AJR, Doree C, et al. Risk of Infection Associated With Administration of Intravenous Iron: A Systematic Review and Meta-analysis. ***JAMA Netw Open*** 2021;4(11):e2133935.
40. Stoffel NU, Zeder C, Brittenham GM, Moretti D, Zimmermann MB. Iron absorption from supplements is greater with alternate day than with consecutive day dosing in iron-deficient anemic women. ***Haematologica*** 2020;105(5):1232-9.
41. Sangkhae V, Nemeth E. Regulation of the Iron Homeostatic Hormone Hepcidin. ***Adv Nutr*** 2017;8(1):126-36.
42. Halle O, Graeber SY, Kontsendorn J, Kessemeier C, Falke JN, Schwabe J, et al. Reduction of systemic inflammation by elexacaftor/tezacaftor/ivacaftor correlates with lung function improvement in cystic fibrosis. ***Eur Respir J*** 2025;66(6):2500150.
43. Graeber SY, Mall MA. The future of cystic fibrosis treatment: from disease mechanisms to novel therapeutic approaches. ***Lancet*** 2023;402(10408):1185-1198.
44. Țichil I, Mitre I, Zdrenghea MT, Bojan AS, Tomuleasa CI, Cenariu D. A Review of Key Regulators of Steady-State and Ineffective Erythropoiesis. ***J Clin Med*** 2024;13(9):2585.
45. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, et al. The future of cystic fibrosis care: a global perspective. ***Lancet Respir Med*** 2020;8(1):65-124.
46. Shteinberg M, Haq IJ, Polineni D, Davies JC. Cystic fibrosis. ***Lancet*** 2021;397(10290):2195-2211.
47. Alameeri A, Yavuz BC, Lucca F, Bambir I, Famulska P, Cohen RWF. Cystic fibrosis year in review 2024. ***J Cyst Fibros*** 2025;24(2):218-23.
48. Mall MA, Burgel PR, Castellani C, Davies JC, Salathe M, Taylor-Cousar JL. Cystic fibrosis. ***Nat Rev Dis Primers*** 2024;10(1):53.