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Case Report

Single-center experience of four cases with iron-refractory iron deficiency anemia (IRIDA)

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ABSTRACT

Background. Iron refractory iron deficiency anemia (IRIDA) is a rare autosomal recessive type of anemia characterized by unresponsiveness to oral iron therapy and partial response to parenteral iron therapy. In this article, we report the clinical presentation of four patients with IRIDA admitted to our clinic, including their laboratory values at admission and after oral and parenteral iron treatment, and the analysis of their mutation(s) in *TMPRSS6* gene.

Case. Four patients from different families, aged between 3 and 14 years, two girls and two boys, two of whom were from consanguineous marriages, who were diagnosed with iron deficiency anemia in primary health care institutions and referred to our clinic because of inadequate response to oral iron treatment were included. Patients were evaluated for the differential diagnosis of microcytic, hypochromic anemia and investigated for the etiology of IDA. Homozygous or compound heterozygous mutations causing defective matriptase-2 protein expression were detected in the *TMPRSS6* gene; these mutations included four frameshift mutations-two of which were the same in two cases and causing premature terminal stop codons-and a nonsense mutation, all of which were previously demonstrated in the literature. The response to parental iron therapy ranged from complete non-response to mild to good response in hemoglobin levels, but none of the patients showed improvement in iron parameters.

Conclusions. Increased awareness of IRIDA and keeping it in mind in the differential diagnosis in the presence of hypochromic microcytic anemia that does not respond to iron treatment will be crucial in improving the diagnosis and treatment of the disease and ultimately enhancing the quality of care for affected individuals.

Key words: iron refractory iron deficiency anemia, IRIDA, *TMPRSS6*, parenteral iron.

Iron deficiency anemia (IDA) is the most common type of anemia worldwide, especially in children and adolescents, due to increased physiological iron demand, inadequate intake, chronic blood loss or underlying malabsorptive pathologies.[1,2] The clinical distribution of signs and symptoms of the disease largely depends on the degree of anemia, and oral iron medications are the first-line treatment that should be initiated after laboratory diagnosis of IDA.[1,3] However, there are genetic/hereditary forms of iron-related anemias due to inherited defects in different stages of iron utilization, which complicate the treatment and management of iron deficiency anemia.[4-6]

Iron-resistant iron deficiency anemia (IRIDA) is a rare form of autosomal recessive anemia caused by various mutations in the *TMPRSS6* (MIM #609862) gene, which encodes matriptase-2 (MT2).[7] MT2 is a type II transmembrane serine

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protease responsible for the downregulation of hepcidin expression; it induces the degradation of hemojuvelin and reduces the activity of the BMP/SMAD pathway.[8-11]

This clinical condition is manifested with microcytic, hypochromic anemia, very low mean corpuscular volume (MCV), low serum iron and transferrin saturation index (TSI), normal or high serum ferritin levels and high urinary hepcidin levels; in addition, patients with IRIDA respond poorly to oral iron and only partially to intravenous iron therapy.[6,12,13]

Patients with IRIDA have mild to moderate anemia with a degree of hypochromic microcytosis, and growth and development are usually normal.[13,14] Because of this inconsistency of clinical and laboratory findings, in the absence of routine laboratory screening for anemia, many cases may go unnoticed clinically due to the normal growth and development of affected individuals , hence the estimate of IRIDA frequency in the community.[10]

In this article, the clinical picture, laboratory values at presentation and after iron therapy, and analysis of *TMPRSS6* mutations in four patients referred to our clinic with a diagnosis of iron deficiency unresponsive to oral iron therapy are presented.

Case presentations

Four patients from different families, aged between 3 and 14 years, two girls and two boys, two of whom were from consanguineous marriages (Case-1 and Case-4) were diagnosed with IDA in primary health care institutions and referred to our clinic because of an inadequate response to oral iron treatment.

Medical history revealed that none of the patients had undergone bone marrow examination and only Case-2 had received an erythrocyte transfusion once. When the family history was questioned, it was noted that only the cousins of the father of Case-2 received intravenous iron treatment regularly; however, no further details could be obtained.

Firstly, it was confirmed that all patients had a varied iron-rich diet, and avoided foods that could lead to iron malabsorption. None of the patients had any recent infections and C-reactive protein values were in the normal range. Except for Case-4 (who presented with dizziness), the other patients had no significant complaints other than mild pallor and fatigue; no abnormal physical examination findings were found in any patient. Furthermore, blood loss was not suspected in patients whose medical histories were taken and fecal occult blood tests were performed. Liver, kidney and thyroid function tests were normal in all patients and no pathological findings were found in urinalyses. IgA and IgG antibodies against tissue transglutaminase were negative and serum IgA levels were within the normal range, making celiac disease unlikely.

Since there was microcytic, hypochromic anemia with low serum iron and low TSI, and no pathological findings were found in the peripheral smear except for hypochromia and poikilocytosis, oral iron (II)-glycine sulfate treatment of 4-6 mg/kg/day for 2 to 3 months was initiated in all patients except Case-4. Since it was confirmed that Case-4 had received oral iron treatment at the appropriate dose and duration in the previous center and the patient was still symptomatic; therefore, intravenous iron treatment was preferred instead of oral iron treatment.

In follow-up examinations of the patients in whom oral iron treatment was initiated, it was observed that there was no adequate response to the treatment, and no improvement in their fatigue. Similar results were observed in the follow-up blood tests of Case-4, in whom parenteral iron therapy was initiated. Hemoglobin electrophoresis was performed for the differential diagnosis of hypochromic microcytic anemia due to a lack of response to iron treatment, and thalassemia was ruled out with laboratory results shown in Table I.

Table I. Results of hemoglobin electrophoresis of all cases.

	Case-1	Case-2	Case-3	Case-4
Hb A (%)	98.6	97.7	98.4	97.8
Hb A2 (%)	1.4	2.3	1.6	2.1
Hb F (%)	0	0	0	0.1

Hb, hemoglobin.

Table II. Detected mutations in *TMPRSS6* gene.

Case	*TMPRSS6* Variation	Amino acid Change	Region	Reference Sequence	rsID	Genotype
1	c.[1877_1878dupGC]	p.[Lys627fs]	Exon 16	NM_001374504.1	rs869320724	Homozygous
2	c.[188del]	p.[Leu63fs]	Exon 2	NM_153609.3	rs1438085143	Heterozygous
	c.[580G>T]	p.[Glu194*]	Exon 5	NM_153609.3	rs761779631	Heterozygous
3	c.[1904_1905dupGC]	p.[Lys636Alafs*17]	Exon 16	NM_153609.3	N/A	Homozygous
4	c.[1904_1905dupGC]	p.[Lys636Alafs*17]	Exon 16	NM_153609.3	N/A	Homozygous

rsID, reference single nuclear polymorphism identification.

Therefore, IRIDA was suspected for iron deficiency anemia resistant to oral iron therapy (Case-1 and Case-3) and unresponsive to parenteral iron therapy (Case-4), and the *TMPRSS6* gene was investigated.

For mutation analyses, peripheral blood samples from each patient were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and genomic DNA was extracted. All 18 exons and exon-intron boundaries of the *TMPRSS6* gene were amplified by polymerase chain reaction (PCR). GenBank accession numbers NM_001374504.1 (for Case-1) and NM_153609.3 (for Case-2 and Case-4) were used as reference sequences. Direct sequencing was performed and the results were analyzed using NextGENe (Version 2.4.2.3/SoftGenetics LLC-USA) and Geneticist Assistant (Version 1.8.1.0/SoftGenetics LLC-USA).

TMPRSS6 gene sequence analysis revealed homozygous frameshift in exon 16 in Case-1, compound heterozygous frameshift in exon 2 and heterozygous nonsense mutation in exon 5 in Case-2, and a homozygous frameshift mutation causing an early stop codon in exon 16 in Case-3 and Case-4 (Table II).

Case-1and Case-3 were started on intravenous ferric hydroxide sucrose complex, and it was observed that they benefited from parenteral iron therapy compared to oral iron therapy. Case-4 did not respond to intravenous iron treatment and 6 mg/kg/day oral iron (II)-glycine sulfate treatment with vitamin C supplementation was initiated; the patient did not respond to this treatment either and the patient did not attend the follow-up visits in our center and continued his follow-up in his hometown.

Complete blood count, serum iron, ferritin and TSI values of the patients at admission, after oral iron treatment and parenteral iron treatment are shown in Table III.

All the studies were performed in accordance with the Declaration of Helsinki and guidelines for good clinical practice. All legal representatives of the patients were informed, and their informed consent was obtained.

Discussion

Iron deficiency anemia is a global health problem and is the leading cause of anemia manifested by microcytic and hypochromic erythrocytes.[15]

Other common causes of microcytic anemia that should be excluded when IDA is suspected include anemia of chronic disease, hemoglobinopathies, thalassemia syndromes

Table III. Demographic features, complete blood count and serum iron parameters of four patients at the time of presentation and after oral and parenteral iron therapy.

		Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-CV (%)	RBC (x10^6/µL)	WBC (x10^3/µL)	Platelets (x10^3/µL)	Serum iron (µg/dL)	TSI (%)	Ferritin (µg/L)[a]
Case-1	Admission	8.1	27.4	55.8	16.4	29.4	19.4	4.91	7.6	582	10	3	62.9
(3 years, M)	After 2 months of oral iron treatment	8.8	31.6	62.2	17.3	27.8	19	5.08	7.72	521	14	5	81.2
	After 2 months of parenteral iron treatment	10.4	35.3	65.5	19.3	29.5	21.0	5.39	7.57	388	13	5	165.7
Case-2	Admission	8.8	31.1	57.2	16.2	28.3	18.6	5.44	5.8	465	8	2	44.1
(5 years, M)	After 3 months of oral iron treatment	9.8	34.5	61.8	17.6	28.6	18.3	5.58	6.7	401	11	3	108.3
	After 2 months of parenteral iron treatment	10.3	34.2	64.1	19.3	30.2	19.3	5.34	7.3	382	15	4	286.7
Case-3	Admission	7.9	26.5	52.1	15.5	29.8	20.0	5.05	6.6	653	9	2	63.2
(6 years, F)	After 10 weeks of oral iron treatment	9.3	30.4	55.3	16.9	30.5	22.4	5.49	6.5	617	12	3	44.8
	After 3 months of parenteral iron treatment	11.6	36.2	69.9	22.4	32.0	23.7	5.18	7.4	438	21	6	205
	After 6 months of parenteral iron treatment	13	42.4	79.41	24.34	30.65	16.3	5.33	8.56	407	23	9.6	524.98
Case-4	Admission	9.8	32.4	59.4	18	30.4	20.3	5.45	6.5	467	14	4	107.3
(14 years, F)	5 weeks after parenteral iron treatment	9.2	30.4	59.9	18.2	30.3	21.9	5.08	9.9	383	14	5	152.9
	6 months after parenteral iron treatment	9.6	32.3	60.8	18	29.6	21.5	5.32	7.1	419	10	4	107.1
	4 months after oral iron and vitamin C treatment	9.4	32.1	61.7	18	29.2	19.9	5.21	9.5	400	NA	NA	NA

Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width – coefficient of variation; RBC, red blood cell; WBC, white blood cell; TSI, transferrin saturation index.
[a]Normal range: 11-307 µg/L; NA, Not available; M, male; F, female.

and sideroblastic anemias, especially in congenital cases.

Following the diagnosis of IDA, acquired causes of iron deficiency, such as inadequate dietary iron intake and persistent hemorrhage, should be addressed for optimal treatment.[16] The well-established treatment strategy for IDA is oral ferrous sulphate; in addition, vitamin C has been shown to improve iron absorption.[17] However, inadequate treatment response can occur and in most cases is associated with poor treatment adherence, improper use of oral iron medication (e.g. not taking pills on an empty stomach), dosage errors and inadequate duration of treatment.[16]

Malabsorptive defects should be primarily suspected as the underlying cause of IDA if the patient is compliant with oral iron therapy at the appropriate dose and duration. Infections and inflammatory diseases that upregulate hepcidin, achlorhydria (e.g. chronic proton pump inhibitors, histamine H2 receptor antagonists or anti-acid medication, post-gastrectomy) and celiac disease are among the common causes limiting the absorption of dietary iron.[1,18]

IRIDA should be considered in the differential diagnosis of IDA patients with onset in infancy or childhood whose hematological parameters do not improve with oral iron therapy and/or abnormalities are found in oral iron absorption tests.[6] These patients clearly have hypochromic and microcytic anemia (hemoglobin 6-9 g/dL) with very low MCV (45-65 fL) and TSI (<5%).[10]

In the cases presented above, the IRIDA assessment was based on specific clinical and laboratory findings. Strikingly, the patients showed hypochromic microcytic anemia with significantly reduced serum iron levels accompanied by a low transferrin saturation index and normal serum ferritin levels. The diet of the patients investigated for iron deficiency and its possible causes was questioned and iron deficiency in the daily diet was ruled out. No abnormal findings were found in terms of chronic blood loss and malabsorption syndromes, especially celiac sprue. In addition, all patients had normal C-reactive protein levels, which excluded infectious or inflammatory conditions as acquired factors. Patients who did not respond to iron therapy underwent hemoglobin electrophoresis for the differential diagnosis of hypochromic microcytic anemia, especially thalassemia, and decreased HbA2 levels supporting IDA were noted (Table III).

Finally, an important common characteristic among these cases was the patients' history of minimal to no improvement upon oral iron supplementation, indicating the iron-refractory nature of their anemia. Consequently, the patients were selected to perform a genetic analysis in order to pinpoint the mutation resulting in the expression of the defective matriptase-2 protein.

Since the first identification of IRIDA, more than 45 different mutations, including nonsense, missense and frameshift, have been reported.[13] Of these, the presence of two nonsense mutations was noted to cause a more severe form of the disease compared to two missense mutations or one missense and one nonsense mutation, which is presumably due to the formation of a truncated version of the protein with nonsense mutations.[12] Among the pathogenic mutations identified and extracted from the dbSNP database, missense (rs770897887, s1438085143, rs1373272804, rs199474805, rs855791 and rs1449962575) and frameshift variants (rs1438085143, rs786205060, rs869320724, rs767094129, rs1384933966, rs869320724 and rs137853123) were the most frequently reported, followed by stop-gain variants (CM1411671, rs137853123 and rs137853122) and synonymous variants (rs4820268). These variants have been shown to disrupt iron homeostasis by impairing matriptase-2 autoproteolytic activation and contributing to the development of IRIDA.[13] Furthermore, several single nucleotide polymorphisms (SNPs) associated with IRIDA have been identified in the *TMPRSS6* gene, with rs1373272804, rs1430692214 and rs855791 being the most frequently recorded SNPs associated

with functional outcomes. Exons 5, 7, 13 and 15 are important hotspots for SNPs and are some of the exons that play a critical role in shaping the genetic picture by showing a high frequency of genetic variation.[11]

In our cases, four frameshift mutations—two of which were the same in two cases and caused early terminal stop codons—and a nonsense mutation were detected. Mutations in Case-1, -3, and -4 were located in exon 16, which is responsible for coding the C-terminal trypsin-like serine protease domain of MT-2.[19] Specifically, in Case-3 and Case-4, a premature stop codon was introduced and thought to result in termination of translation before the catalytic serine, as described in a previous case.[20] In Case-2, there was a frameshift mutation in exon 2, which codes for the transmembrane region, and a nonsense mutation in exon 5, which codes for the membrane-proximal SEA domain.[19] Since all the mutations were either frameshift or nonsense and there was no missense mutation, we would expect to see more severe disease in Case-2 because the mutations were in the more proximal exons of the catalytic region of the enzyme compared to Case-1, -3, and -4. Correspondingly, only Case-2 required an erythrocyte suspension transfusion prior to his referral to our clinic.

It has been known that in patients with IRIDA, the causative mutations in *TMPRSS6* occur mostly in exon 15 and exon 16.[21] Interestingly, previous reports have shown that Turkish patients with IRIDA often exhibit a duplication mutation in exon 16, resulting in a frameshift mutation with a premature stop codon (c.1904_1905dupGC).[6,22-24] Supporting this observation, we found that two of our cases (Cases-3 and Case-4) also had the same mutation. This finding might suggest the possibility of a founder effect for this mutation among patients of Turkish origin, particularly in regions like the northern part of Türkiye, where consanguineous marriages are common, as was the case in our study.

In the literature, there is no consensus on the treatment of patients with IRIDA. The patients benefit from parenteral iron treatment often only partially and temporarily. In our management of the cases, proper dosage and duration of oral iron treatment were initially ensured, which was later replaced with parenteral iron. Response to parenteral iron treatment ranged from total unresponsiveness (Case-4) to mild (Case-1) to good response (Case-2 and Case-3) in Hb levels. However, iron parameters did not improve in any of the patients.

In conclusion, IRIDA remains a rare syndrome in established literature and clinical practice, despite being believed to be one of the most common congenital anemias.[10] This discrepancy may, in part, stem from a lack of awareness of the disease and the misattribution of symptoms to non-adherence to oral iron treatment by hematologists. The steps towards diagnosing IRIDA begin with a suspicion of the disease during the differential diagnosis process, which we aim for in the present report. In future studies, we anticipate the discovery of new mutations in *TMPRSS6* and a more precise description of IRIDA's frequency. These advances in understanding and awareness will be crucial in improving the diagnosis and management of IRIDA, ultimately enhancing the quality of care for affected individuals.

Ethical approval

All the studies were performed in accordance with the Declaration of Helsinki and guidelines for good clinical practice. All legal representatives of the patients were informed, and their informed consent was obtained.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GP, SU, FG, MDA; data collection: GP, SU; analysis and interpretation of results: GP, SU; draft manuscript preparation: GP, MDA. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declares that there is no conflict of interest.

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