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Short Communication

Favourable improvement in haematological parameters in response to oral iron and vitamin C combination in children with Iron Refractory Iron Deficiency Anemia (IRIDA) phenotype

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ABSTRACT

Treatment in IRIDA focuses on use of intravenous iron preparations to circumvent oral absorptive defect resulting from high levels of hepcidin due to *TMPRSS6* gene variations. However, recent case reports and recommendations on atypical microcytic hypochromic anemias advocate use of oral iron and vitamin c trial before parenteral iron, as the same results in comparable improvement in haemoglobin. We prospectively evaluated our IRIDA cohort (n = 7) with oral iron and vitamin c dose over a period of 10 weeks and noted complete response in majority (6/7 = 86%) with > 2 g/dL rise in Hb along with significant improvement of other iron related indices.

1. Introduction

Iron Refractory Iron Deficiency Anemia (IRIDA) is a rare autosomal recessive orphan disease, due to pathogenic variations in *TMPRSS6* gene. The key features of IRIDA include moderate anemia (Hb 6–9 g/dL), severe microcytosis (MCV 45–65 fL), low normal to normal plasma ferritin and inappropriately high hepcidin levels [1,2]. In addition, these patients are refractory to oral iron supplementation and only partially responsive to parenteral iron therapy.

The prevalence of IRIDA is not known and only few studies worldwide have published large series on genotype phenotype correlation. We had published a study from our institute wherein IRIDA phenotype was noted in 38.3% (23/60) cases, which on further genetic evaluation of *TMPRSS6* gene revealed deleterious variations consistent with IRIDA in 12/20 (60%) cases [3]. Current expert recommendations on treatment of genetically confirmed IRIDA cases recommend an initial trial of iron (ferrous sulphate form) along with Vitamin c for 6–8 weeks before going ahead with IV iron treatment [4]. There have also been case reports wherein an infant with IRIDA has been successfully treated for anemia with oral iron and vitamin c combination [5]. Moreover, unlike adult patients, IV iron treatment is rarely indicated in children, even in those with severe iron deficiencies and thus using same as 1st line or primary treatment for IRIDA cases needs to be planned cautiously and judiciously. Hence, based on current evidence and recommendations, we prospectively evaluated our cohort of genetically proven IRIDA cases with a combination of oral iron (ferrous ascorbate salt) along with Vitamin C and noted response with repeat haemogram evaluations at 4 and 10 weeks respectively.

2. Methods

The study was duly approved by Institute's ethics committee and departmental review board. We have a cohort of 12 confirmed cases of IRIDA [3], however, of these three refused consent for inclusion in study and two cases had already been given parenteral iron in the last year. Hence 7 cases were enrolled and 2 ml EDTA sample was collected at baseline for complete blood count and reticulocyte indices. The sample was run on Sysmex XN-1000 system (Japan) and the patient was started on oral iron and vitamin c trial with detailed information on compliance and dosage. Since most children were < 5 years hence, oral iron syrup (ferrous ascorbate- 33 mg/5 mL) was prescribed at a dose of 6 mg/kg OD along with vitamin C at a dose of 250 mg per day (half tablet-500 mg). Repeat assessment of complete blood count and reticulocyte indices was performed at 4 weeks and 10 weeks to note for response.

2.1. Interpretation of direct response indicator i.e. haemoglobin levels (Hb)

Anemia at baseline was classified as mild (10–10.9 g/dL), moderate (7–10 g/dL) or severe (< 7 g/dL). Normal Hb levels as per age used in

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Table 1
Haematological parameters at baseline, 4 weeks and 10 weeks in IRIDA cohort (n = 7).

S.NO.	Haematological parameters and indices Baseline/Week 4/Week 10									Type of response	Reported genetic variations [3]
Case number	Hb (gm/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (CV)	Reticulocyte count (%)	Ret-He (pg)	% Hypo-He	%Microcytes	Category[a]	Variations
Case 1 (1.9 y)	10.9/11.3/12	67.4/6 6.6/76.8	20.6/20.7/24.1	30.6/31.1/32.3	18.7/18.1/15.2	0.73/0.97/2.2	18.5/19.8/25	16.7/16.7/5	20/16/4	CRC	Intronic: g.11184 A > G (exon splicing silencer (HM) g.25335 T > A (UTR binding element loss (HM)
Case 2 (2 y)	10/11.1/12.3	70.7/76.8/80.8	22.2/24.3/26.3	31.3/32.4/33.1	13.9/13/13.1	0.55/1.5/1.8	22.4/26.8/30.8	12.4/6.4/1.2	23/16.5/4.3	CRC	Exonic:- g.38612C > T [P555S] (HM) g.42668T > C [V736A] (HM) Intronic: g.23404 T > A (transcriptional regulatory motif (HM) g.35000 C > T (C-U RNA editing site generation (HM)
Case 3 (6 y)	8.7/9.3/11.05	53.3/55.2/72.4	15.5/16.1/24.8	29/29.2/32.1	23.5/22/15.7	0.95/1.2/1.79	18/22.8/27.3	45/35/8.5	54/43/12	CRC	Intronic:- g.23404 T > A (transcriptional regulatory motif (HM)
Case 4 (2.5 y)	9.6/11.3/11.7	69.1/70.2/73	21.8/23.8/24.2	31.6/34.1/33.6	16.2/15.9/15.4	0.79/0.88/1.17	21.5/28.2/29	20/6.4/0.8	25/11/3.3	CRC	Intronic:- g.35000 C > T (C-U RNA editing site generation (HT)
Case 5 (1.8 y)	7.8/8.5/10.1	61.2/67.6/74.4	19.5/23.2/26.3	28.2/29.1/31.2	17.8/17.3/16.4	0.38/1.1/1.86	16/21.2/28.1	48/36/10.1	59/40/15	CRC	Exonic:- g.42668T > C [V736A] (HM) Intronic:- g.11184 A > G (exon splicing silencer (HT) g.35000 C > T (C-U RNA editing site generation (HT)
Case 6 (2 y)	8.9/9.4/10.5	65.2/68.2/71.6	22.4/24.1/26.1	28.3/29.3/30.8	16.8/16.5/15.7	1.1/1.21/1.67	22.5/24.3/28.9	16/10.1/3.4	12/13/5.3	PSRC	Intronic:- g.40632T > A (loss of intron splicing enhancer)
Case 7 (1.8 y)	9.1/9.8/11.3	67.8/70.3/75.8	23.3/26/28.2	28.6/31.3/32.4	15.8/14.6/13.6	1.04/2.15/1.88	24.8/29/30.8	11.8/2.2/1.2	22.3/7.3/3.5	CRC	Intronic:- g.42484 G > T (relocation of splice site donor (HM)

[a] PSRC:- partial sustained response category; CRC:- complete response category; HM:- homozygous.

Table 2
Comparison of means ± SD of haematological parameters at baseline, 4 weeks and 10 weeks.

Parameter	Day-0	4 weeks	Mean diff	p-Value (Day 0 vs. Week 4)	10 weeks	Mean diff	p-Value (Day 0 vs. Week 10)
Hb-gm/dl	9.28 ± 0.99	10.1 ± 1.12	0.82	0.172	11.27 ± 0.79	1.99	**0.0013**
MCV-fl	64.9 ± 5.97	67.84 ± 6.49	2.94	0.3951	74.97 ± 3.16	10.07	**0.0019**
MCH-pg	20.75 ± 2.63	22.6 ± 3.27	1.85	0.2661	25.3 ± 1.45	4.55	**0.0017**
MCHC	29.65 ± 1.46	30.92 ± 1.88	1.27	0.1835	32.21 ± 0.98	2.56	**0.0023**
RDW-CV	17.52 ± 3.04	16.77 ± 2.86	−0.75	0.643	15.01 ± 1.20	−2.51	0.0649
Retic-%	0.79 ± 0.26	1.28 ± 0.42	0.49	**0.0222**	1.76 ± 0.30	0.97	< **0.0001**
Ret-He-pg	20.52 ± 3.09	24.58 ± 3.53	4.06	**0.041**	28.55 ± 2.03	8.03	**0.0001**
% Hypo-He	24.27 ± 15.45	16.11 ± 13.97	−8.16	0.3204	4.31 ± 3.74	−19.96	**0.0061**
% Microcytes	30.75 ± 18.12	20.97 ± 14.38	−9.78	0.2852	6.77 ± 4.72	−23.98	**0.0054**

our population are based on WHO criteria i.e. Hb 11.0 g/dL or higher for children between 6 months–59 months of age and Hb 11.5 g/dL or higher for children aged 60 months–11 years is considered as normal cut-off. Iron refractoriness criteria (< 1 g/dL Hb rise at 4 weeks) and initial haemoglobin levels were used to define various response dynamics at the different time periods in the current study and cases were appropriately classified into one of the following groups:

a) Complete response category (CRC):- rise of Hb at 4 weeks ≥1 g/dL than the initial pre-treatment Hb and at 10 weeks ≥1 g/dL than at 4 weeks. A rise of Hb ≥ 2 g/dL at 10 weeks irrespective of status at 14 weeks was also taken under this category as complete late response. In addition any degree of rise in Hb if initial anemia was mild but final Hb at 4 or 10 weeks came within normal range for age.
b) Partial sustained response category (PSRC):- rise of Hb < 1 g/dL at 4 weeks than the pre-treatment level and any rise in Hb (upto 1.9 g/dL) at 10 weeks than the 4 week level.
c) Partial incomplete response category (PIRC):- rise of Hb < 1 g/dL at 4 weeks than the pre-treatment level and a fall in Hb at 10 weeks but less than the degree of rise seen at 4 weeks OR no rise of Hb at 4 weeks and any amount of rise in Hb at 10 weeks (upto 1.9 g/dL) OR any amount of rise in Hb at 4 weeks (upto 1 g/dL) but no change or fall in Hb at 10 weeks so that the final Hb at 10 weeks is still more than pre-treatment level.
d) No response/absent response category (NRC):- no rise in Hb at either 4 or 10 weeks than pre-treatment level OR rise of Hb < 1 g/dL at 4 weeks than the pre-treatment level and a fall in Hb at 10 weeks greater than the degree of rise seen at 4 weeks.

However, for statistical purposes category b) and c) were clubbed together as partial response cases.

2.2. Interpretation of indirect response indicators (novel reticulocyte and RBC markers)

a) Reticulated haemoglobin (Ret-He) levels:- normal (29–35 pg); levels below 28 pg are suggestive of functional iron deficiency and below 25 pg classical iron deficiency. Data in IRIDA cases is insufficient as this is a new parameter offered by automated analyzers, but levels are expected to rise in response to treatment in parallel with Hb rise as it is an acute marker for analyzing iron delivery to bone marrow for erythropoiesis.
b) % Microcytic cells:- normal (3–5%); usually high in IRIDA due to moderately severe microcytosis but data and evidence lacking. Following response to treatment it is expected to fall and hence a fall > 10% at any time period was taken as significant indicator of response in parallel with rise in Hb.
c) % Hypochromic cells (Hypo-He):- normal (< 2%); data in IRIDA cases is insufficient as this is a new parameter offered by automated analyzers but likely to be moderately increased due to low MCH values seen in IRIDA. Hence, a fall > 5% than baseline values at 4 weeks and > 15% at 10 weeks in parallel with rise in Hb at any time period was taken as significant indirect response to treatment.

2.3. Statistical analysis

Results are reported as mean and SD. Means of all parameters were compared at different time intervals with baseline means using paired student's *t*-test. Logistic regression analysis was performed between the dependent nominal variable of response and the baseline independent variables in the study. A p-value < 0.05 were considered significant. Data analysis was done on SPSS statistical software (version 21.0, SPSS Inc. Chicago, IL, USA).

3. Results

The mean age of the cohort (n = 7) was 3.5 years (2.8–5 years) and all cases were male gender. On baseline complete blood count evaluation, 2 cases (29%) had mild anemia (Mean Hb- 10.4 g/dL) while 5 (71%) had moderate anemia (Mean Hb- 8.8 g/dL). The detailed haematological parameters for all cases at baseline, 4 weeks and 10 weeks are highlighted in Table 1.

On comparison of direct response based on haemoglobin levels at 4 weeks and 10 weeks, 6 cases (86%) had complete response (including 3 with late response) of rise of > 2 g/dL of Hb than baseline or with rise of Hb to acceptable normogram level with respect to age. Only one case (14%) had partial sustained response. On comparison of means of all parameters at different time intervals, reticulocyte count (p = 0.02) and reticulated haemoglobin (Ret-He) levels (p = 0.04) showed a significant improvement at 4 weeks in parallel with rise in Hb. However at 10 weeks all parameters had a significant improvement except for RDW (p = 0.06) (Table 2). Chisquare or Fishers exact test for comparison of rise or fall of different parameters in response to increase or no change in Hb at 4/10 weeks could not be performed due to limited number of cases in the cohort.

In addition, we performed logistic regression analysis to note whether any of the baseline parameters could predict the final complete response. On analysis none of the parameters except baseline reticulocyte count were able to predict a complete response (refer Supplementary Table S1). The baseline reticulocyte count could predict the complete response with an odds ratio of 0.00 and chi-square value of 5.74 and p-value 0.016.

None of the cases reported having any adverse side effects to higher iron dosage or vitamin C supplement.

4. Discussion

This is one of the first studies to evaluate a series of IRIDA cases with oral iron and vitamin c combination prospectively and highlights the significant improvement in most of the haematological parameters at 10 weeks. The study had been designed based on recent recommendations by experts in the field that IRIDA cases should be evaluated with an oral iron and vitamin C trial before parenteral iron

therapy. Moreover it has also been highlighted that since the long term effects of parenteral iron therapy on tissue damage and risk for infections is not available in IRIDA, the cases should first be treated with prolonged oral iron or with an oral iron and vitamin c combination [6].

The role of vitamin C in enhancing non haeme iron absorption in iron deficiency is well established since long, however the exact mechanism behind the same is not yet known [7]. It is suggested that ascorbic acid not only reduces formation of insoluble iron complexes but also acts at cellular level in duodenal cells to limit ferritin autophagy, increases conversion of ferric to ferrous form for better iron absorption via DMT-1 and also more importantly increases aconitase thereby reducing iron responsive protein activity and enhancing ferroportin expression for increased transfer of iron across duodenal cells into plasma [8–10]. The case highlight by Cau et al., responded to oral ferrous sulphate and vitamin c with rise in Hb from 8.2 to 9.5 g/dL at 4 weeks and 12.1 g/dL at 3 months [5]. However in their report, the dose for oral iron used was 3 mg/kg/d and vitamin c was 30 mg per day. In current study we evaluated a higher iron dose of 6 mg/kg/d along with a vitamin c dose of 250 mg/d. The possible reason for starting higher iron and vitamin c dose was to override the hepcidin block by enhancing the ferroportin expression via higher ascorbic acid and simultaneously making higher ferrous iron available for transport across the channel. Though the study is limited by absence of hepcidin level and ferroportin expression analysis and quantification, nevertheless the response with improvement in haemoglobin in our cohort should pave way for further trials at relatively lower standard doses to achieve possible uniform dosing guidelines for children. None of the relevant parameters in our study except baseline reticulocyte count could predict the final complete response on logistic regression analysis. A reticulocyte count of $\leq 1\%$ is more likely to predict complete response than a higher count at baseline in cases with IRIDA phenotype. However this is needs to be validated in further prospective studies. Current study is the one of the first prospective studies on IRIDA that focuses on the novel oral iron treatment approach in a good number of cases with a structured response evaluation.

5. Conclusion

The study not only strengthens the available guideline on use of oral iron and vitamin C for IRIDA cases but also highlights the utility of novel reticulocyte and RBC markers like Ret-He and % Hypo-He & % microcytes in evaluating response to acute (< 4 weeks) and chronic iron therapy (10 weeks or $>$) respectively. Ret-He in our study showed significant improvement ($p = 0.04$) at 4 weeks compared to all other novel parameters and is suggested as a very sensitive marker in iron response evaluation [11].

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcmd.2018.12.002.

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Authorship contributions

SS enrolled cases and carried out necessary investigational work up and follow up. PB designed the trial, analyzed results and critically evaluated and drafted the manuscript. RJ was the clinician who prescribed the treatment and also analyzed the results.

Disclosure of conflicts of interest

None.

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