Brief Report

Serum Prohepcidin Levels in Children with Solid Tumors, Inflammatory Bowel Disease and Iron Deficiency Anemia

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Summary

Background: Prohepcidin is one of the regulators of iron metabolism. Few studies examined its relation with solid tumors (ST), inflammatory bowel disease (IBD) and iron deficiency anemia (IDA) in children. Methods: We measured serum prohepcidin (SP), soluble transferrin receptor (sTfR), serum ferritin (SF), serum iron (SI), transferrin saturation (TS), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels in ST ($n = 16$), IBD ($n = 15$), IDA ($n = 14$) and controls ($n = 18$). Results: The mean SP was significantly higher in ST and IBD than in IDA and controls. SP was significantly correlated with SF in ST, IBD and ESR for IBD and CRP for ST and hemoglobin for ST. Conclusion: Elevated SP may be a clinically important predictor of inflammation and leads to anemia by impairing iron utilization in IBD and ST. SP decreases in IDA and is correlated with TS but not with SF or sTfR.

Key words: prohepcidin, children, solid tumors, inflammatory bowel disease, iron deficiency anemia.

Introduction

Prohepcidin is the precursor molecule of hepcidin which is a key modulator of iron metabolism. It is mainly synthesized in liver and increases in inflammation, suggesting that it may be responsible for anemia in various inflammatory disorders [1–6]. Studies in adults with solid tumors (ST) or inflammatory bowel disease (IBD) and rheumatoid arthritis have shown elevated interleukin-6, lipopolysaccharide and hepcidin [7–14]. High prohepcidin levels have been found to be linked to anemia in children with IBD, but direct evidence for this relationship in children with cancer is lacking [15]. Furthermore, it is important to discriminate iron deficiency anemia (IDA) from anemia of inflammation in order to provide adequate treatment. Standard laboratory tests for iron status, such as ferritin, are subject to variability owing to acute phase responses of inflammation and are not reliable in detecting IDA in inflammatory diseases [7, 15].

In the present study, we determined serum prohepcidin (SP) level in ST, IBD and IDA in children to evaluate its relationships with inflammatory markers and iron parameters.

Methods

Sixteen children with ST, 15 with IBD, 14 with IDA and 18 with age-matched healthy controls were studied. Disease activity for IBD was assessed by elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Diagnosis of IDA depended on low ferritin levels. Patients in the ST group were studied at diagnosis before treatment. The study was approved by the IRB of Gazi University.

Complete blood count (CBC), ESR, serum iron (SI), total iron binding capacity (TIBC) were measured in blood samples obtained during morning hours by autoanalysers. Ferritin and CRP levels were measured by an automated chemiluminescent

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TABLE 1
Patient baseline characteristics

	ST	IBD	IDA	Control
Number of cases	16	15	14	18
Age (years)	7.8 ± 5.5	9.6 ± 2.6†	5.7 ± 3.8*	8.8 ± 3.6
Gender (F/M)	10/6	9/6	8/6	10/8
Hematologic parameters				
Hb ($g\,dl^{-1}$)	10.6 ± 2.1*,†	10.8 ± 1.1*,†	9.7 ± 0.6*	13.1 ± 0.9
MCV (fl)	75.4 ± 5.8†	70.6 ± 4.5*,‡	67.9 ± 2.8*	78.8 ± 4.8
MCH ($g\,dl^{-1}$)	25.1 ± 3.2*,†	22.9 ± 2.3*,†,‡	20.5 ± 2.4*	26.9 ± 1.8
RDW (%)	15.4 ± 3.5*	15.1 ± 2.4*,†	16.8 ± 1.2*	13.5 ± 1.9
Rtc (%)	1.4 ± 0.4*,†	1.2 ± 0.1†	0.8 ± 0.4*	1.1 ± 0.2
Iron parameters				
Serum iron ($\mu g\,dl^{-1}$)	51.81 ± 49.6*,†	50.1 ± 41.8*,†	7.4 ± 2.1*	88.1 ± 28.5
Total iron-binding capacity ($\mu g\,dl^{-1}$)	287.18 ± 71.7†	299.7 ± 72.9†	466.7 ± 52.7*	321.1 ± 48.7
TS (%)	14.4 ± 9.1*,†	16.1 ± 12.7*,†	1.5 ± 0.5*	27.5 ± 8.5
sTfR ($mg\,l^{-1}$)	4.5 ± 1.4*,†	4.1 ± 1.4*,†	6.1 ± 0.7*	2.8 ± 0.8
Inflammatory markers				
Ferritin[a] ($ng\,ml^{-1}$)	135.7 ± 119.9*,†	206.7 ± 484.1*,†	7.2 ± 2.6*	51.3 ± 14.5
Prohepcidin ($ng\,ml^{-1}$)	661.5 ± 217.2*,†	775.7 ± 395.8*,†	303.5 ± 81.5	426.1 ± 208.9
ESR ($mm\,h^{-1}$)	24.4 ± 21.4*,†	22.7 ± 29.6*,†	10.6 ± 3.6	7.8 ± 2.5

[a]Used as either inflammatory or iron parameter.
*$p < 0.05$ when comparing control subjects with IDA, ST, IBD.
†$p < 0.05$ when comparing IDA with ST, IBD.
‡$p < 0.05$ when comparing ST with IBD.

microparticle immunoassay (Abbott, Ireland) and by rate nephelometry (Beckman, USA), respectively. SP levels were measured by ELISA (DRG Instruments, Germany), and soluble transferrin receptor (sTfR) was determined with a two-step sandwich enzyme immunoassay using two independent monoclonal antibodies (DiaMed, Belgium). Statistical analysis was performed by SPSS–11.5 using the Mann–Whitney U-test and Spearman's correlation test.

Results

Clinical and laboratory data of the patients with ST, IBD, IDA and controls are shown in Table 1.

The mean values of Hb, MCH, transferrin saturation (TS) and SI were significantly lower and sTfR were significantly higher in ST, IBD and IDA than in healthy controls ($p < 0.05$).

The mean values of SP, serum ferritin (SF) and ESR were significantly higher in patients with IBD and ST compared to controls and IDA ($p < 0.05$) (Fig. 1).

Patients with ST and IBD were divided into two subgroups according to the presence of anemia (Table 2). MCV level was significantly lower in anemic patients with IBD ($p < 0.05$). Although no significant difference was seen in sTfR level, SP was found to be significantly higher in the anemic groups with ST and IBD than those without anemia. SF level was significantly higher in anemic patients with IBD ($p < 0.05$).

Prohepcidin was significantly positively correlated with SF level in ST and IBD groups, with CRP in the ST group, with ESR in the IBD group and with TS in the IDA group, while it was significantly negatively correlated with Hb in the ST group ($p < 0.05$) (Figs 2 and 3) (Table 3).

Discussion

The synthesis of hepcidin increases in iron overload or inflammatory disorders and decreases in IDA [1–6]. Although there are several studies in animals and adults investigating the role of hepcidin in inflammatory diseases and iron deficiency, little is known about its role in children [7–18].

In consistent with other studies, we found SF to be substantially lower and sTfR higher in IDA than in healthy controls. SP was reported to be unchanged in children with IDA [18–20]. However, we found a slightly decreased SP level in IDA. SP was significantly correlated with TS but not with SF, probably due to the narrow range of SF, wide variation in SP and the limited number of patients in our study group.

A major difficulty encountered in clinical practice is to differentiate IDA from ACD. Our patients with ST and IBD had mild microcytic anemia and elevated levels of SF, consistent with anemia of chronic disease (ACD) [4, 21]. sTfR is superior than ferritin to discriminate IDA from ACD [22–24]. It has been reported that sTfR level is not influenced by

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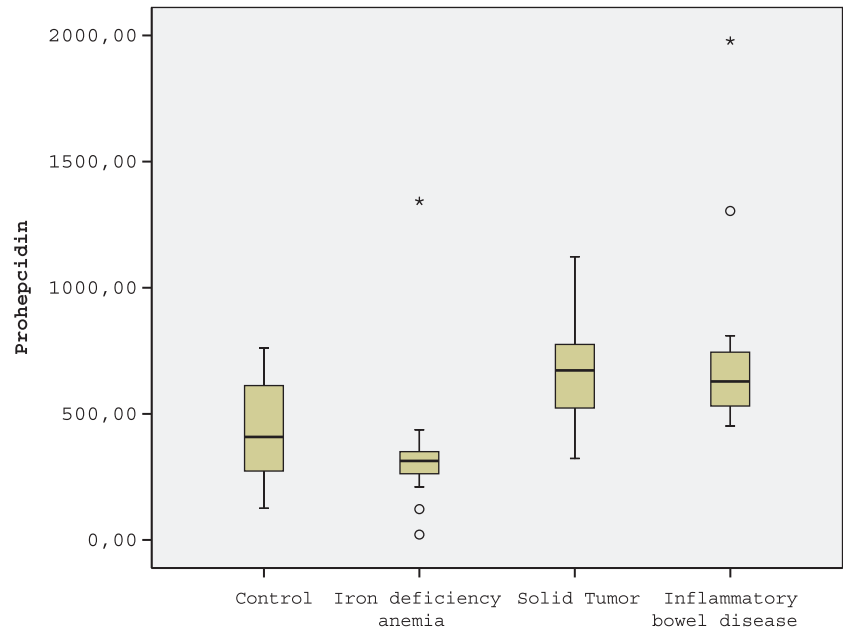


FIG. 1. Distribution of prohepcidin levels in patients with iron deficiency anemia, solid tumors, inflammatory bowel disease and healthy controls.

TABLE 2
Comparison of the laboratory tests between the anemic and nonanemic patients with ST and IBD

	ST			IBD		
Number of cases	With anemia 10	Without anemia 6	*p*	With anemia 5	Without anemia 10	*p*
Hematologic parameters						
Hb ($g\,dl^{-1}$)	9.5 ± 1.1	12.4 ± 1.9	0.001	9.6 ± 0.6	11.4 ± 0.7	0.002
MCV (fl)	74.1 ± 4.1	77.8 ± 7.9	0.19	67.5 ± 2.5	72.1 ± 4.6	0.023
MCH ($g\,dl^{-1}$)	24.7 ± 2.7	25.3 ± 4.1	0.62	22.1 ± 1.3	23.4 ± 2.6	0.19
RDW (%)	15.4 ± 4.1	15.5 ± 2.5	0.87	15.2 ± 1.6	15.1 ± 2.8	0.71
Rtc (%)	1.5 ± 0.4	1.2 ± 0.4	0.62	1.2 ± 0.1	1.1 ± 0.1	0.21
Iron parameters						
Serum iron($\mu g\,dl^{-1}$)	59.1 ± 56.6	39.6 ± 36.3	0.27	42.6 ± 49.5	53.7 ± 39.8	0.46
Total iron binding capacity ($\mu g\,dl^{-1}$)	287.5 ± 65.6	286.6 ± 87.6	0.74	253.4 ± 59.8	322.9 ± 69.9	0.086
TS (%)	14.5 ± 8.2	14.3 ± 11.1	0.82	14.3 ± 13.5	16.9 ± 13.1	0.42
sTfR ($mg\,l^{-1}$)	5.1 ± 1.3	3.7 ± 1.1	0.11	4.1 ± 1.6	4.1 ± 1.4	0.90
Inflammatory markers						
Ferritin[a] ($ng\,ml^{-1}$)	166.1 ± 137.3	85.1 ± 65.5	0.25	492.1 ± 810.7	64.3 ± 68.3	0.02
Prohepcidin ($ng\,ml^{-1}$)	756.1 ± 203.1	503.4 ± 141.6	0.013	1022.5 ± 615.8	$609.1 \pm 1–21.1$	0.04

[a]Used as either inflammatory or iron parameter.

inflammatory diseases in adults [25]. Increased sTfR level indicates the presence of iron deficiency in ACD. In our study, sTfR levels were significantly higher in IDA, ST and IBD groups than in healthy controls. However, we found no differences in sTfR levels between anemic and non-anemic patients with ST and IBD. ACD may result from inadequate erytropoietin production or diminished responsiveness of erythroid progenitor cells carrying sTfR to erytropoietin [4, 7]. Thus, sTfR expression may be

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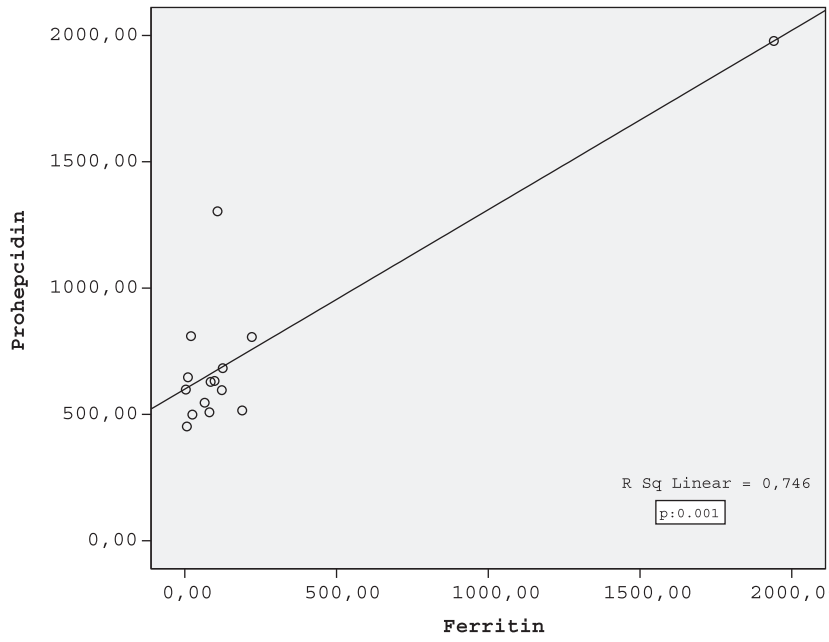


FIG. 2. Correlation between prohepcidin levels and ferritin in inflammatory bowel disease.

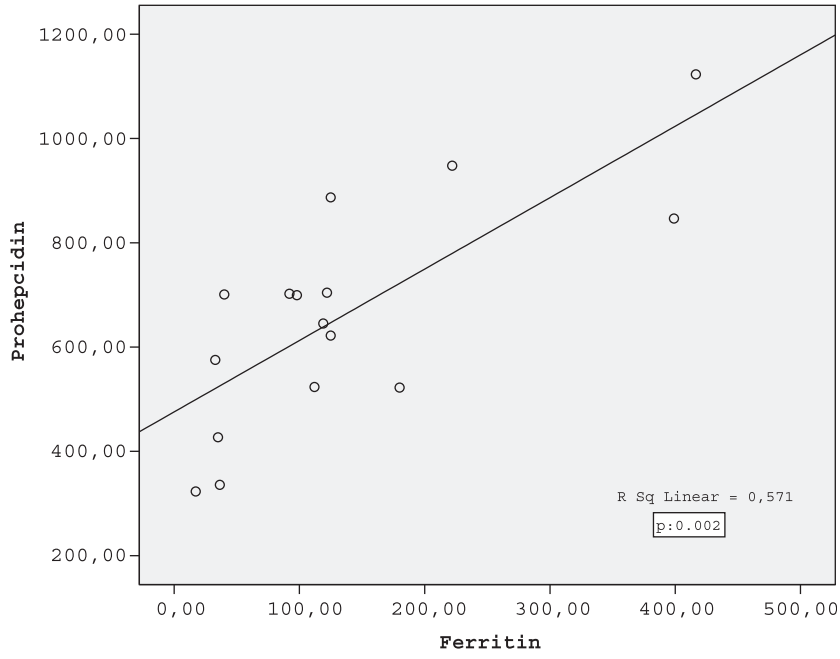


FIG. 3. Correlation between prohepcidin levels and ferritin in solid tumor patients.

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TABLE 3
Correlation of prohepcidin with hematologic and iron parameters and inflammatory markers in ST, IBD and IDA

	ST		IBD		IDA	
Number of cases	16		15		14	
Hematologic parameters	*r*	*p*	*r*	*p*	*r*	*p*
Hb ($g\,dl^{-1}$)	−0.76	0.001	−0.29	0.28	0.41	0.12
MCV (fl)	−0.27	0.30	−0.03	0.91	0.43	0.10
MCH ($g\,dl^{-1}$)	−0.19	0.26	0.04	0.88	0.66	0.007
RDW (%)	−0.38	0.14	−0.24	0.37	−0.56	0.029
Rtc (%)	−0.24	0.16	0.15	0.57	0.26	0.34
Iron parameters						
Serum iron ($\mu g\,dl^{-1}$)	0.49	0.06	−0.11	0.67	0.38	0.15
TS (%)	0.17	0.52	−0.18	0.53	0.56	0.027
sTfR ($mg\,l^{-1}$)	0.38	0.13	−0.37	0.16	−0.11	0.70
Inflammatory markers						
Ferritin[a] ($ng\,ml^{-1}$)	0.57	0.002	0.74	0.001	0.28	0.30
ESR ($mm\,h^{-1}$)	0.20	0.36	0.84	0.02	0.05	0.057
CRP ($mg\,dl^{-1}$)	0.61	0.04	0.40	0.87	–	

[a]Used as either inflammatory or iron parameter.

decreased due to reduction of erytropoesis as observed in this study during the active phase of the inflammatory disease. Therefore, normal or mildly elevated sTfR levels do not exclude the possibility of coexisting IDA in these diseases.

Serum hepcidin level is increased both in children and adults with IBD and thought to be involved in the pathogenesis of anemia of this condition [8, 15]. We found increased SP level in IBD being higher in the anemic group than those without anemia. Cytokines release from inflamed bowel might have decreased intestinal absorption and release from macrophages of iron resulting in anemia.

Anemia is also a common complication in cancer patients. There is evidence that, activation of the cellular immune system and inflammatory changes, which stimulate the production of cytokines, affect both red cell production and survival and cause functional iron deficiency [26–28]. This is supported by our results that SP level is elevated in children with ST and may be more indicative of failure of erythropoiesis rather than iron deficiency.

There was a significant correlation between SP and SF in ST and IBD groups, and strong association with ESR for IBD, and with CRP for ST. Although this incompatibility may be due to the small number of patients, studies in adults with anemia of cancer or IBD have shown a significant correlation between SP and SF as well as other acute phase proteins [8, 26]. Higher serum hepcidin have been found in patients with renal tumor, in association with the extent of disease and unfavorable clinical outcome. It is not clear whether increased hepcidin level in patients with advanced renal tumor are produced by the tumor itself or by host tissues [29]. Further studies are needed to clarify whether SP may serve as a tumor marker in some ST [29, 30].

In conclusion, hepcidin may be a clinically important predictor of inflammation in patients with ST and with IBD. The relation between SP and SF, ESR, CRP abnormalities became evident only in children with anemia of inflammation regardless of iron status. Further studies are necessary to clarify the precise role of hepcidin in various inflammatory diseases, particularly in different phases of inflammatory processes and in different tumour types.

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