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ORIGINAL ARTICLE

Haematological findings in children with inborn errors of metabolism

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Summary Early detection and therapy of haematological abnormalities and/or diseases may improve the prognosis of metabolic disorders. Accordingly, we aimed to evaluate the frequency and types of haematological abnormalities in children[-31pc] with various inherited metabolic disorders. The study group comprised 46 children with metabolic disorders who were followed at the Pediatric Metabolism Unit and were referred to the Pediatric Hematology Unit for evaluation of anaemia between June 2000 and 2005. The mean age of the children was 55.2 ± 64.8 months at haematological evaluation (range 1 month–18 years, median 22.0 months); 16 were female and 30 were male. Of these 46 patients with anaemia, 25 of (54.3%) had anaemia of chronic disease (ACD), 9 (19.6%) had iron-deficiency anaemia (IDA), 7 (15.2%) had megaloblastic anaemia due to vitamin B_{12} deficiency, 3 (6.5%) had chronic haemolytic anaemia, 2 (4.3%) had autoimmune haemolytic anaemia, 1 had β-thalassaemia major, and 1 had hereditary spherocytosis. In addition to the anaemia, bicytopenia or pancytopenia was found in 8 of 46 children (17.4%). The study indicated that in organic acidaemias including methylmalonic acidaemia, propionic acidaemia, isovaleric acidaemia, and argininosuccinic acidaemia, the majority of patients had ACD (75%), which was followed by vitamin B_{12} deficiency anaemia and IDA ($p < 0.001$). In PKU, both nutritional anaemias and ACD were present at about same frequency: 46.7% and 40%, respectively ($p > 0.05$). This study suggested that congenital anaemias such as hereditary spherocytosis or thalassaemias should be kept in mind as a coexisting haematological diseases in young patients with inborn errors of metabolism. In conclusion, ACD and nutritional anaemias are the most prevalent anaemias seen in patients with inborn errors of metabolism. Early detection of the disease, early administration of specific diet, and close monitoring of the patients are very important factors to prevent the development of haematological diseases in patients with inborn errors of metabolism.

Abbreviations

ACD	anaemia of chronic disease
AIHA	autoimmune haemolytic anaemia
IDA	iron deficiency anaemia
IEM	inborn error of metabolism
PKU	phenylketonuria

Introduction

Turkey has a high rate of consanguineous marriage. As a result, many rare autosomal recessive diseases are quite common in Turkey (Ozalp et al 1990; Tuncbilek 2001). Recent advances in the diagnosis and treatment of inborn errors of metabolism (IEMs) have substantially improved the prognosis for many of these disorders. However, haematological abnormalities that can be seen in many of the metabolic disorders due to congenital or acquired

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haematological diseases are still neglected. Early detection and therapy of the haematological abnormalities may improve the prognosis of metabolic disorders. Accordingly, we aimed to evaluate the frequency and types of haematological abnormalities in children with various inherited metabolic disorders.

Material and methods

Between June 2000 and June 2005, more than 2000 children with IEMs were diagnosed, either during nationwide neonatal screening programmes, such as for phenylketonuria, or in the neonatal period during evaluation of clinical conditions such as metabolic acidosis, failure to thrive or neurological findings, at Hacettepe University Faculty of Medicine, Pediatric Nutrition & Metabolism Unit, one of the largest metabolic centres in Turkey. Many patients with IEMs and anaemia were followed and selected arbitrarily by physicians at the Pediatric Nutrition & Metabolism Unit and 46 of them participated in our study for evaluation of anaemia and other haematological abnormalities.

A complete blood count and peripheral blood smears, serum iron concentration, serum iron binding capacity, vitamin B_{12} and folate concentrations, and bone marrow aspiration were carried out for all of the children by conventional methods. Those with Hb concentrations below 10 g/dl, transferrin saturation level below 12%, and ferritin below 12 ng/ml were considered as having iron deficiency anaemia (IDA) (WHO criteria; WHO/UNICEF/UNU 2001). A vitamin B_{12} concentration below 160 pg/ml (normal range 160–800 pg/ml) was accepted as vitamin B_{12} deficiency. Anaemia of chronic disease (ACD) is characterized by decreased plasma iron concentrations, decreased total iron binding capacity, decreased transferrin saturation, and normal or increased concentration of ferritin (Ezekowitz and Stockman 2003). Bicytopenia is cytopenia in two lineages and pancytopenia in three lineages including anaemia, neutropenia, and thrombocytopenia.

Statistical analysis was performed with a statistical software package, SPSS for Windows, version 10.0. Chi square test, Fisher's exact test, Kruskal–Wallis test, and Mann-Whitney *U* test were used for evaluation of the data. Statistical significance was established at a p value <0.05.

Results

The mean age of the children with metabolic diseases was 55.2 ± 64.8 months at haematological evaluation (range 1 month–18 years, median 22.0 months); 16 subjects (34.8%) were female and 30 (65.2%) were male. Of these 46 patients with anaemia, 25 (54.3%) had anaemia of chronic diseases (ACD), 9 (19.6%) had IDA, 7 (15.2%) had megaloblastic anaemia due to vitamin B_{12} deficiency, 3 (6.5%) had chronic haemolytic anaemia, 2 (4.3%) had autoimmune haemolytic anaemia (AIHA), 1 had β-thalassaemia major, and 1 had hereditary spherocytosis. In addition to the anaemia, bicytopenia was found in 5 of 46 children (10.9%) (anaemia and thrombocytopenia in 4 children, anaemia and neutropenia in 1 child) and pancytopenia in 3 children (6.5%) (Tables 1 and 2). Increased methylmalonic acid excretion was detected in all patients with vitamin B_{12} deficiency.

The haematological abnormalities of the patients with various metabolic disorders are shown in Table 1. In addition to the children with PKU shown in Table 1, hereditary spherocytosis was detected in one child and β-thalassaemia major was detected in another child with PKU. Statistical analysis of the haematological parameters indicated that there was a significant difference in serum iron concentration, serum iron binding capacity, and ferritin concentration between the patients with ACD and those with IDA ($p < 0.001$). The patients with ACD had high ferritin concentration and low serum iron concentration and serum iron binding capacity, which was suggestive of anaemia of chronic disease. In the comparison of the groups with respect to haematological parameters, the mean MCV (101.2 ± 14.0 fl) was higher ($p < 0.05$) and the mean vitamin B_{12} concentration (114.9 ± 45.9 pg/ml) was lower ($p < 0.05$) in the vitamin B_{12} deficiency group ($p < 0.05$), and the mean ferritin concentration (7.08 ± 4.9 ng/dl) was lower in the IDA group ($p < 0.05$).

In our study, 28.5% of the patients with various metabolic disorders did not comply properly with the recommended diet and 37% of the patients had malnutrition of various degrees. The patients with ACD had higher rate of malnutrition (13/25, 52%) than the patients with nutritional anaemias (3/16, 18.8%) ($p < 0.001$). The nutritional status of the patients is shown in Table 2. After the patients received treatment for anaemia, all of the patients with nutritional anaemias were responsive to the treatment, whereas the patients with ACD were unresponsive.

Discussion

Metabolic diseases are not rare in Turkey, due to the high incidence of consanguineous marriages (Ozalp et al 1990; Tuncbilek 2001). Nationwide use of neonatal screening studies and development of new methods allowing early definite diagnosis of several metabolic disorders have made it possible to initiate necessary treatment of affected newborns starting from the early days of life. Therefore, one could expect to observe fewer complications, including anaemia and other haematological abnormalities related to underlying disorders of IEM on comparison with previous literature reports. This study is a descriptive and cross-sectional study that evaluates

Table 1 The haematological abnormalities of the patients with various metabolic disorders[a]

Metabolic disorders	*n*	Age	ACD	IDA	anaemia	Cytopenias				Chronic haemolytic anaemia
						Vitamin B_{12} deficiency Pancytopenia	Neutropenia	Thrombocytopenia	AIHA	
Organic acidaemias										
Methylmalonic acidaemia	11	1–29 months mean10.7 ± 10.4 months	8	2	1	1[b]	–	1[b]	–	–
Propionic acidaemia	6	2–27 months mean 10.6 ± 9.6 months	6	–	–	1[b]	–	2[a]	–	–
Isovaleric acidaemia	2	5 and 18 years	–	–	1	–	–	–	–	2[c]
Argininosuccinic acidaemia	1	65 months	1	–	–	1[b]	–	–	–	–
Phenylketonuria	13[a]	8–180 months mean 97.6 ± 69.2 months	5	4	3	–	–	1[a]	2[c]	–
Maple syrup urine disease	4	2–3 months	3	1	–	–	–	–	–	–
Cystinosis	3	5–12 years	2	1	–	–	–	–	–	–
Homocystinuria	1	6 years	–	–	1	–	–	–	–	–
Citrullinaemia	1	16 years	–	–	1	–	1[b]	–	–	–
Galactosaemia	1	10 years	–	–	–	–	–	–	–	1
Fructose 1,6-diphosphatase deficiency	1	22 months	–	1	–	–	–	–	–	–
Total	44[a]		25	9	7	3	1	4	2	3

[a]This table does not include the patients with PKU-β thalassemia major and PKU-hereditary spherocytosis
[b]The patient is also included in ACD
[b]The patient is also included in vitamin B_{12} deficiency anaemia
[c]One of the patients is also included in vitamin B_{12} deficiency anaemia

Table 2 Nutritional status of the patients

Classification of the protein energy status (PEM)[a]	*n* (46)	ACD	IDA	Vitamin B_{12} deficiency anaemia	Chronic haemolytic anaemia
Mild	10[c]	7[b]	2	–	1
Moderate	6[d]	5	1	–	–
Severe	1[e]	1	–	–	–
Total	17	13	3	–	1

[a]Child's actual weight (kg) = 90–100%: normal. 50th centile weight/height (kg) = 80–90%: mild. 50th centile weight/height (kg) =70–80%: moderate. 50th centile weight/height (kg) <70%:severe
[b]One of the patients also had pancytopenia
[c]PKU ($n = 6$), organic acidaemia ($n = 3$), galactosaemia ($n = 1$)
[d]PKU ($n = 3$), organic acidaemia ($n = 3$)
[e]Cystinosis ($n = 1$)

the frequency and types of haematological abnormalities in children with IEMs in this new era.

Various cytopenias, including neutropenia, thrombocytopenia and pancytopenia, have been reported in association with organic acidaemias, particularly methylmalonic, propionic and isovaleric acidaemias (Gilbert-Barness and Barness 1999; Guerra-Moreno et al 2003; Inoue et al 1981; Kelleher et al 1980; Meerman 2000; Stork et al 1986). In organic acidaemias, accumulation of CoA esters inhibits the maturation of bone marrow precursors, which results most notably in neutropenia and also thrombocytopenia or pancytopenia. This haematological picture results from the metabolic imbalance of the patient with organic acidaemias (Hoffmann et al 2002). Indeed, in the present study, 30% of the patients with organic acidaemias had cytopenias and all of them were in metabolic imbalance. Several other studies were conducted to explain the mechanisms responsible for the haematological abnormalities in organic acidaemias. In these studies, it was suggested that increase of several substances derived from abnormalities in related enzyme pathways might be responsible for maturation arrest in haematopoietic precursors, inhibition of the bone marrow proliferation, and shortened red blood cell survival, leading to various types of cytopenias (Gilbert-Barness and Barness 1999; Guerra-Moreno et al 2003; Inoue et al 1981; Kelleher et al 1980; Meerman 2000; Stork et al 1986). In one of these studies, it was observed that when methylmalonic acid was added to the culture dishes in concentrations comparable to those in plasma of methylmalonic acidaemic patients, growth of bone marrow stem cells was inhibited (Inoue et al 1981).

In the other metabolic diseases, including galactosaemia, fructose 1,6-diphosphotase deficiency, maple syrup urine disease and homocystinuria, the accumulation of toxic metabolites and the generation of free radicals were reported to be responsible for haematological abnormalities including anaemias and cytopenias (Gilbert-Barness and Barness 1999; Guerra-Moreno et al 2003; Inoue et al 1981; Kelleher et al 1980; Meerman 2000; Stork et al 1986).

It was interesting that among our eight patients with bicytopenia or pancytopenia, only one of them with citrullinaemia had vitamin B_{12} deficiency and only two of the 11 patients with methylmalonic acidaemia had pancytopenia or bicytopenia. These observations may indicate that in the majority of the patients with bicytopenia or pancytopenia, causes other than an increase in the methylmalonic acid concentration and vitamin B_{12} deficiency could be operating. Additionally, the study indicated that in organic acidaemias, the majority of patients had ACD (75%), followed by vitamin B_{12} deficiency anaemia and IDA (25%) ($p < 0.001$).

Arnold and colleagues reported that 6 (15%) out of 41 children with PKU had anaemia. They considered that restricted diet and combined depletion of iron and protein stores were most likely to result in anaemia (Arnold et al 2001). Hanley and colleagues reported that 6 (16%) out of 37 adolescent and young adult PKU patients had subnormal serum vitamin B_{12} concentrations. They recommend that complete blood count, serum vitamin B_{12}, and RBC folate concentrations be routinely measured in adolescents and young adults with PKU (Hanley et al 1996). Early assessment and therapy of iron deficiency may improve cognitive and behavioural outcomes of children with PKU (Acosta et al 2004). It was shown in this study that in PKU patients with anaemia, the nutritional anaemias (46.7%) and ACD (40%) were present at about the same frequency ($p > 0.05$) (Table 1). It was noted that the latter type of anaemia is accompanied by thrombocytopenia in one of the patients (Table 1). Additionally, it should be kept in mind that hereditary spherocytosis and β-thalassaemia major might be associated with various metabolic diseases in countries like Turkey where these types of congenital anaemias are quite common in the general population. In several studies, routine monitoring of ferritin, complete blood count and prealbumin; a more sensitive marker of protein sufficiency; was recommended for children with PKU at all ages (Arnold et al 2001; Bodley et al 1993; Hanley et al 1996). In this study, presence of both IDA and vitamin B_{12} deficiency anaemia in patients with PKU, especially during

the adolescent period, also indicate the importance of close monitoring of nutritional status of the patients with PKU (Table 1). Megaloblastic anaemia results from metabolic diseases in which the absorption or transport of cobalamin and its conversion to methylcobalamin are interrupted by toxic metabolites. It may also result from abnormalities in folate metabolism (Hoffmann et al 2002). In addition to the usage of multivitamins, vitamin B_{12} is routinely added to the amino acid mixture of PKU patients. However, if the patient with PKU does not comply with the diet and does not use the amino acid mixture, vitamin B_{12} deficiency anaemia, and also IDA, may develop. Using the special formula decreases the risk of development of vitamin B_{12} deficiency anaemia and IDA.

In a study conducted by Evangeliou and colleagues (2002) in Greece, it was suggested that the unexplained haematological findings may have a key role in the diagnosis of IEMs in daily practice. The incidence of such an observation would be low if the diagnosis and proper prompt treatment were available at early ages. Additionally, if patients with various metabolic disorders are closely monitored and comply with the recommended diet, they might not have any nutritional anaemias. This study showed that majority of the patients with various metabolic disorders present with ACD (54.3%), followed by IDA (19.6%) and vitamin B_{12} deficiency anaemia (15.2%). Pancytopenia, neutropenia or thrombocytopenia are less common haematological abnormalities and they are usually accompanied by ACD or vitamin B_{12} deficiency (Table 1). The majority of the patients with organic acidaemia (75%) had ACD starting at an early age, although all of them were noted to be in remission at the time of diagnosis of anaemia; an increase in some undetected toxic metabolites and restriction of protein in their diet might effect bone marrow precursors (Hoffmann et al 2002).

Among 46 patients with various metabolic disorders, 37% had malnutrition. The rate of malnutrition was much higher in patients with ACD (52%) than in the patients with nutritional anaemias (18.8%) ($p < 0.001$). Therefore, malnutrition itself might be responsible for ACD, or the underlying metabolic disorder may be the primary factor for presentation of both malnutrition and ACD. Observation of bicytopenia or pancytopenia, mostly in patients with ACD, may also support the above hypothesis.

Another interesting aspect of this study is the presence of chronic haemolytic anaemia in patients with isovaleric acidaemia and galactosaemia starting in childhood or the adolescent period. We believe that this information could be helpful in clinical follow-up of patients with similar metabolic disorders.

In conclusion, ACD and nutritional anaemias are the most prevalent anaemias seen in patients with IEMs. It should be kept in mind that metabolic disorders may be associated with several congenital or acquired anaemias such as hereditary spherocytosis or thalassaemias, and AIHA. The presence of nutritional anaemias, especially in the adolescent and childhood period, indicates the importance of close monitoring of patients who are not taking proper diets. Early detection of the disease, early administration of specific diet, and close monitoring of the patients are very important factors in preventing the development of haematological diseases in patients with IEMs.

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