Prevalence and type of anaemia in young Egyptian patients with type 1 diabetes mellitus

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انتشار ونمط الإصابة بفقر الدم لدى صغار المصريِّين المصابين بالسكّري من النمط الأول

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الخلاصة: تم على مدى شهرَيْن، تحرِّي فقر الدم والمضاعفات الأخرى للسكَّري لدى مئتَيْن من المرضى بالسكَّري من النمط الأوَّل، والذين يراجعون إحدى عيادات رعاية الأطفال المصابين بالسكَّري في القاهرة، بمصر. وكان متوسط أعمار المشاركين 11.2 سنة ومتوسط مدة الإصابة بالسكَّري 4.0 سنوات. وقد شُخِّص فقر الدم لدى 75 مريضاً (بنسبة 37.5٪)، بصفة عامة: 45 كان لديهم فقر دم صغير الكريات، ناقص الصِّباغ، و18 كان لديهم فقر دم سويُّ الكريات، سويُّ الصِّباغ، و12 كان لديهم فقر دم كبير الكريات، مفرط الصِّباغ. وكان من بين الـ 75 مريضاً، واحد وأربعون (18.7٪) لديهم تَلاثيمية صغرى. وكان ثلاثة من المرضى (4٪) مصابين بالزُّلاق *celiac* (زَلَق الأمعاء)، كما كان 18 من المرضى (24٪) مصابين بعدوى طفيلية. ولم يكن أيٌّ من هؤلاء المرضى مصاباً بقصور الدرقية أو بالفشل الكلوي أو بعَوَز الفيتامين ب 12. ويُوصي الباحثون بتحري وجود فقر الدم واتخاذ التدابير العلاجية الصحيحة للحالات، من أجل تحسين نوعية حياة مرضى السكَّري.

ABSTRACT Over a 2-month period, 200 type 1 diabetic patients attending a paediatric diabetic clinic in Cairo, Egypt were screened for anaemia and other complications of diabetes. The mean age was 11.2 years and the mean duration of diabetes was 4.0 years. Anaemia was diagnosed in 75 patients (37.5%) overall: 45 had microcytic hypochromic anaemia, 18 normocytic normochromic and 12 macrocytic hyperchromic. Of the 75, 41 patients (54.7%) had iron deficiency, 14 (18.7%) had folate deficiency and 14 (18.7%) had thalassaemia minor. Three patients (4%) had coeliac disease, and 18 patients (24%) had parasitic infections. None of the patients had hypothyroidism, renal failure or vitamin B_{12} deficiency.

Prévalence et type d'anémie chez de jeunes patients égyptiens atteints de diabète sucré de type 1

RÉSUMÉ Sur une période de 2 mois, 200 patients diabétiques de type 1 consultant dans une clinique du diabète pédiatrique au Caire (Égypte) ont été examinés à la recherche d'une anémie et d'autres complications du diabète. L'âge moyen était de 11,2 ans et la durée moyenne du diabète était de 4,0 ans. Une anémie a été diagnostiquée chez 75 patients (37,5 %) en tout : 45 avaient une anémie hypochrome microcytaire, 18 une anémie normochrome normocytaire et 12 une anémie hyperchrome macrocytaire. Sur les 75 patients, 41 (54,7 %) avaient une carence en fer, 14 (18,7 %) avaient une carence en folates et 14 (18,7 %) avaient une thalassémie mineure. Trois patients (4 %) avaient une maladie cœliaque, et 18 patients (24 %) avaient une parasitose. Aucun des patients n'avait d'hypothyroïdie, d'insuffisance rénale ou de carence en vitamine B_{12}.

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Introduction

The etiology of anaemia in type 1 diabetes is diverse. Diabetic patients who, through neglect or ignorance, do not follow the appropriate dietary regimes, are at-risk of developing nutritional deficiency anaemia, especially iron and folate deficiency. Moreover, diabetics with poor glycaemic control are susceptible to recurrent attacks of ketoacidosis which may be accompanied by anorexia, severe vomiting with frequent hospitalization and excessive calorie loss [*1*]. The occurrence of diabetic nephropathy with ultimate renal failure is an important cause of anaemia in these patients [*2*]. The association of type 1 diabetes and coeliac disease has been widely reported, the latter being associated with iron, folic acid and vitamin B_{12} deficiencies. Hashimoto thyroiditis, resulting in acquired hypothyroidism, is strongly associated with type 1 diabetes and is commonly accompanied by anaemia [*3*]. Thalassaemia minor is relatively common in the Mediterranean area and should be considered in the differential diagnosis of anaemia [*4*].

The aim of this study was to record the prevalence of anaemia, its type as well as its possible causes among a group of young Egyptians with type 1 diabetes attending a paediatric diabetic clinic in Cairo.

Methods

Patients

The study was carried out at the Diabetic Endocrine and Metabolic Paediatric Unit of the University of Cairo, Children's Hospital. This clinic receives patients referred from all over Egypt, all of whom are characterized by being of low or intermediate social class. There is no maximum age for follow-up in the unit for type 1 diabetics, as patients who start follow-up care in the children's age group are allowed to continue follow-up indefinitely if they wish, in order not to change the protocol of therapy. Between January and December 2002, 200 patients attending the clinic were screened for the presence of anaemia. The patients were of different age groups and had been diagnosed with type 1 diabetes for different durations.

To obtain reference values for blood levels, a control group of 20 normal subjects (10 male, 10 female) were recruited from healthy relatives accompanying patients to the hospital. They were living in the same area and of the same socioeconomic class and the mean (SD) age was not significantly different from the study patients [11.2 (5.1) versus 11.7 (4.7) years].

Informed consent was taken from all patients before they entered the study.

Laboratory tests: all patients

Diabetic patients were screened for the following:

- *Presence and type of anaemia*. Complete blood count was performed on all 200 diabetic patients and 20 controls. The cut-off values for the diagnosis of anaemia were based on mean ± 2 SD values of controls. For microcytic hypochromic anaemia, the cut-offs were: haemoglobin (Hb) < 10.94 g/dL, mean cell volume (MCV) < 68.5 fL and mean cell haemoglobin concentration (MCHC) < 25.5 g/dL. For macrocytic hyperchromic anaemia, the cut-offs were: MCV < 84.5 fL and MCHC < 41.5 g/dL. Red cell distribution width (RDW) was calculated using an electronic cell counter utilizing the formula: RDW = SD / MCV × 100. Normal ranges of RDW were 11.5%–14.5% for children aged 0–2 years and 11.5%–15.0% for children aged 2–12 years according to

values obtained from control patients and other reference values [*5*].

- *Metabolic control*. Glycosylated haemoglobin (HbA_{1c}) levels of diabetic patients were measured routinely every 3 months using an automated ion capture assay (Abbott IMx, Abbott Laboratories, Illinois, USA). Mean values over the preceding 6 months were calculated. The normal value in our laboratory is < 6.5%.
- *Microvascular complications*. Diabetic patients were screened for chronic microvascular complications of type 1 diabetes: microalbuminuria (urine albumin/creatinine ratio > 30 µg/mg); retinopathy (by fundus examination); peripheral neuropathy (using electrophysiological studies); and cardiovascular autonomic neuropathy (using the methods of Ewing and Clarke [*6*].

Laboratory tests: anaemic patients

Diabetic patients with anaemia were screened for the following:

- *Iron deficiency*. Serum iron and total iron binding capacity (TIBC) were assessed using standard colorimetric kits (bioMérieux, Marcy l'Etoile, France). Cut-off points (based on ± 2 SD from the mean of control patients) were: decreased iron < 50.43 µg/dL and increased TIBC > 413.89 µg/dL.
- *Folate deficiency*. Serum folate and vitamin B_{12} levels were measured in patients with macrocytic or normocytic anaemia and the controls. A radioassay method designed for simultaneous measurement of both parameters in serum [*7*]. Cut-off points (± 2 SD of control means) for decreased levels were: serum folate < 3.04 ng/mL and serum vitamin B_{12} < 68.2 pg/mL.
- *Haemoglobinopathies*. All anaemic patients were given haemoglobin (Hb) electrophoresis to exclude the presence of haemoglobinopathies. Normal values were defined as: HbA_1 > 95%, HbA_2 1.5%–3.5 % and HbF < 2%. Thalassaemia minor was diagnosed by elevated HbA_2 > 3.5%.
- *Coeliac disease*. All anaemic patients were assessed for the presence of antigliadin, anti-reticulin and anti-endomysium autoantibodies as a screening for coeliac disease. Anti-gliadin IgG antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) kit (ImmuLisa, IMMCO Diagnostics, Buffalo, New York, USA). Anti-reticulin antibodies were analysed with an indirect immunofluorescence method using rat kidney sections (ImmuGlo, IMMCO Diagnostics,). Anti-endomysium antibodies (EMA) were analysed with an indirect immunofluorescence method using monkey oesophagus tissue sections (ImmuGlo, IMMCO Diagnostics).
- *Chronic renal failure*. Serum creatinine levels were measured to exclude chronic renal failure as a cause of anaemia.
- *Thyroid status*. Serum thyroid stimulating hormone (TSH) levels were quantitatively measured using an immunoradiometric assay kit (DSL-5300, Diagnostic Systems Laboratories, Texas, USA). Free T3 and free T4 were quantitatively assessed using solid phase I_{125} radioimmunoassay kit (Coat-A Count, Diagnostic Products Corporation, Los Angeles, USA).
- *Stool analysis*. Stool samples were obtained to test for occult blood and parasites that may be a cause of anaemia.

Therapy

Iron supplementation was given to patients with iron deficiency in a dose of 6 mg/kg/day and follow-up of Hb levels as well as mean HbA_{1c} levels (%) were compared before and 6 months after supplementation.

Statistical analysis

Quantitative data were expressed as mean and standard deviation (SD), while qualitative data were expressed as percentages. Comparison between mean values of patients and controls was done using Student *t*-test. *P* values less than 0.05 were considered significant.

Results

Of the 200 diabetic patients screened, 75 patients were anaemic (37 males, 38 females), giving a prevalence of anaemia among the diabetics of 37.5%. The mean (SD) age of anaemic patients was 11.2 (5.1) years (range 1.6 to 26.0 years) with a male to female ratio 1:1, while the mean (SD) age of controls was 11.7 (4.7) years (range 1.3 to 26.0 years) and their male to female ratio 1:1. There were no statistically significant differences in either mean age or sex distribution between anaemic diabetic patients and the control group ($P > 0.05$ for each). The mean (SD) duration of diabetes was 4.0 (3.3) years (range 0.1 to 15.3 years). The mean (SD) of the standard deviation score for height was –1.0 (0.75) (range –5.0 to 1.0) and for weight was –1.2 (1.5) (range –4.2 to 2.0).

Table 1 shows the demographic and clinical data of anaemic patients, as well as the morphologic and etiologic classification of anaemia in these patients. Of the 75 patients, 41 (54.7%) had iron deficiency, 14 (18.7%) had folate deficiency and 14 (18.7%) had thalassaemia minor. Three patients (4.0%) had coeliac disease. Among the 14 patients with thalassaemia minor, 8 (57.1%) had associated iron-deficiency anaemia.

Table 1 **Demographic and clinical data and classification of 75 diabetic patients with anaemia**

Variable	No.	%
Sex		
Female	38	50.7
Male	37	49.3
Pubertal stage		
Pre-puberty	22	29.3
Post-puberty	53	70.7
Morphologic type of anaemia		
Microcytic hypochromic	45	60.0
Normocytic normochromic	18	24.0
Macrocytic hyperchromic	12	16.0
Etiologic type of anaemia		
Iron deficiency	41	54.7
Folate deficiency	14	18.7
Coeliac disease	3	4.0
Thalassaemia minor	14	18.7
Chronic complications		
Microalbuminuria	6	8.0
Peripheral neuropathy	30	40.0
Autonomic neuropathy	30	40.0
Retinopathy	3	4.0
Parasitic infection	18	24.0

In addition, 18 patients (24%) had evidence of parasitic infection in the form of *Entamoeba histolytica* (8 patients), *Giardia* spp. (4 patients) or *Oxyuris* spp. infection (6 patients). All patients with parasite infection had normocytic normochromic anaemia.

The mean (SD) serum iron level in iron-deficient anaemia patients was 57.0 (3.5) µg/dL, while mean (SD) serum folate in folate-deficient patients was 0.9 (1.5) ng/mL. None of the patients had vitamin B_{12} deficiency. The mean (SD) vitamin B_{12}

level in serum was 371.2 (7.4) pg/mL, range 200–500 pg/mL.

By examining the red cell distribution width (RDW) in the blood picture of anaemic patients, it was apparent that RDW was increased in 95.1% (39/41) of patients with iron deficiency, 100% of patients with folate deficiency and 57.1% (8/14) of patients with thalassaemia minor. The latter patients had associated iron deficiency.

By observing the degree of anaemia in relation to the presence of chronic microvascular complications, it was found that mean levels of Hb did not differ statistically significantly between the groups with or without chronic complications ($P > 0.05$). All patients were similarly anaemic (Table 2). Table 3 shows that the mean age of patients with diabetic autonomic neuropathy was around 14.1 years, duration of diabetes was more than 5 years and mean HbA_{1c} level was above 9%.

Of the 3 patients with coeliac disease, one case with normocytic normochromic anaemia had both iron and folate deficiency, increased RDW and consequent stunted growth and underweight. The second case had microcytic hypochromic anaemia, severe iron deficiency, increased RDW and consequent stunted growth and underweight. The third case had microcytic hypochromic anaemia, thalassaemia minor, normal RDW, with not much effect on the height and weight. Coeliac disease was diagnosed from the laboratory and histopathology data with no evidence of malabsorption symptoms. The patients had positive anti-gliadin IgG, anti-reticulin IgA and anti-endomysial IgA antibodies and intestinal biopsy revealed absent villi and hyperplastic crypts with increased numbers of intraepithelial lymphocytes and plasma cells and lymphocytes in the lamina propria. The patients were put on a gluten-free diet and received nutritional supplementation for the deficient elements.

HbA_{1c} levels were statistically significantly higher in iron-deficient than in non-iron-deficient patients ($P < 0.01$) (Table 4). However, mean random blood glucose va-lues in the previous 6 months did not show a statistically significant difference between the groups ($P > 0.05$). Table 4 shows that Hb concentration was statistically significantly increased after 6

Table 2 **Degree of anaemia of diabetic patients according to type of complication**

Variable	Mean (SD) haemoglobin level	*P*-value
Microalbuminuria		
Yes	10.4 (1.5)	> 0.05
No	10.9 (0.7)	
Peripheral neuropathy		
Yes	10.6 (1.1)	> 0.05
No	11.0 (0.7)	
Autonomic neuropathy		
Yes	10.6 (1.1)	> 0.05
No	11.0 (0.7)	
Retinopathy		
Yes	10.8 (0.7)	> 0.05
No	10.8 (0.9)	

SD = standard deviation.

Table 3 **Descriptive data of diabetic patients with autonomic neuropathy**

Variable	Mean (SD)	Range
Age (years)	14.1 (4.6)	6.75–26.0
Duration of diabetes (years)	7.0 (3.1)	5.9–15.3
Haemoglobin (g/dL)	10.6 (1.1)	7.2–11.7
Mean cell volume (fL)	114.7(13.2)	50.0–125.0
Mean cell Hb (g/dL)	29.9 (9.1)	20.0–56.0
Glycosylated haemoglobin (%)	9.3 (1.1)	7.5–11.3

SD = standard deviation.

Table 4 **Comparison of haemoglobin levels in diabetic patients with iron-deficiency and non-iron-deficiency anaemia, and in iron-deficiency patients before and after iron supplementation**

Parameters	Non-iron-deficiency anaemia (*n* = 34)	Iron-deficiency anaemia (*n* = 41) Before supplements	After supplements
Haemoglobin (g/dL)	9.2 (2.1)	9.8 (2.5)	11.2 (1.6)[b]
Glycosylated haemoglobin (%)	8.6 (0.7)	10.1 (0.7)[a]	8.4 (0.7)[b]
Random blood glucose (mg/dL)	147.0 (23.6)	145.5 (20.0)	146.8 (20.1)

Values shown are mean (standard deviation).
[a]P < 0.01 iron-deficiency versus non-iron-deficiency patients; [b]P < 0.01 before versus after supplements.
n = number of patients.

months of iron supplementation in patients with iron deficiency ($P < 0.01$). On the other hand, HbA_{1c} was significantly decreased after iron supplementation ($P < 0.01$). However, there was no significant difference in mean random blood glucose values before and after iron supplementation ($P > 0.05$).

Discussion

To our knowledge, the prevalence of anaemia in type 1 diabetes has rarely been precisely determined. This could be attributed to the heterogeneous nature of its etiology. Moreover, anaemia is not present in diabetes mellitus per se unless related to another disorder or complication. In fact, anaemia in diabetes is more often than not an example of the anaemia of chronic disorders [*8*].

In this study, the prevalence of anaemia in type 1 diabetics was 37.5%. More than two-thirds (70.7%) of the anaemic patients were post-pubertal, a figure which reflects the increased nutritional demands in this age group and points to the need for correct management of nutrition. The overall prevalence of anaemia among preschool children in a national survey of 488 children across Egypt in 1986 was 51.6%, declining from 74.4% in the first year of life to 35.1% at school entry at 6 years. In Egyptian adolescents, the figure was estimated at 47%, with little variation in levels of anaemia across socioeconomic status or between the sexes [*9*].

The morphological classification of anaemia in the screened patients showed that microcytic, hypochromic anaemia was the most predominant type (60.0% of patients), which corresponds with the fact that iron deficiency was the most prevalent nutritional deficiency (54.7%) among the patients, followed by thalassaemia minor (18.7%). In Egypt, the prevalence of iron-deficiency anaemia was reported to be 60% in toddlers aged 18 to 24 months and 49% during the following 6 months in rural areas, and was related to low socioeconomic class and deficient intake of iron from animal sources and vitamin C [*10*]. Similarly, another study reported a prevalence of iron-deficiency anaemia of 48.4% in preschool age children in rural areas of Mansoura [*11*]. Others have reported that iron-deficiency anaemia affects approximately 30% of the world's population [*12*] and 27% of Egyptian children [*13*]. Iron deficiency in type 1 diabetics may be due

to inadequate dietary iron intake, malabsorption of iron due to associated coeliac disease or gastric autoimmunity, chronic blood loss, intravascular haemolysis due to associated ketoacidosis or associated parasitic or other infections [*14*].

The proportion of patients with normocytic and macrocytic anaemia was 24.0% and 16.0% respectively; a total of 30 patients. All were screened for folate and vitamin B_{12} deficiency and only about half of them (14 patients) showed folate deficiency while none of the patients showed vitamin B_{12} deficiency. The etiology of folate deficiency can be deficient intake mostly due to dietary restrictions caused by low socioeconomic standards, and less commonly due to malabsorption associated with coeliac disease (1 patient in our series). Dietary deficiency in children occurs due to rapid growth or infection, which increase folic acid requirements. On the other hand, vitamin B_{12} deficiency in children is rare because vitamin B_{12} is present in many foods of animal origin. It may be seen in cases of extreme dietary restriction, such as strict vegetarians. Cases occur in breast-fed infants whose mothers have deficient diets or pernicious anaemia. In older children, pernicious anaemia has occasionally been reported in adolescent ages where there is vitamin B_{12} malabsorption, atrophy of the gastric mucosa and achlorhydria [*15*].

Thalassaemia minor was diagnosed by Hb electrophoresis in 18.7% of patients. These patients presented with microcytic hypochromic anaemia and 8 of them had associated iron-deficiency anaemia. In Egypt, the prevalence of thalassaemia minor had been reported to be 10%–13% in different studies. Temtami et al. studied 40 children with microcytic anaemia (MCV < 80 fL) randomly selected from a paediatric haematology outpatient clinic: 5 cases had beta-thalassaemia trait (12.5%), and the other 35 had iron-deficiency anaemia [*16*]. Similarly, El Beshlawy et al. reported a rate of beta-thalassaemia trait in Egypt of 10% [*17*].

The RDW, a measure of red cell heterogeneity, can be used to differentiate thalassaemia traits from other non-thalassaemic conditions with microcytosis due to iron deficiency [*18*]. In this study, RDW was high in 95% of iron-deficient patients, in 100% of folate deficient patients and in only 57.1% of patients with thalassaemia minor; the latter were mostly the patients who had associated iron-deficiency as well. This was previously confirmed by El Bardisi who found that RDW levels measured in conjunction with MCV add specific information that allows the diagnosis of thalassaemia minor with almost complete certainty [*19*].

The prevalence of coeliac disease associated with type 1 diabetes in this study was 4%. This is in accordance with other studies reporting the prevalence of coeliac disease with type 1 diabetes at approximately 3%–8% [*20,21*]. It has been widely reported that nutritional deficiencies are common presentations of coeliac disease in older children and adolescents, anaemia being the most common deficiency [*3*]. The anaemia is most commonly due to iron deficiency, which frequently occurs in the absence of intestinal symptoms. Macrocytic anaemia due to folate deficiency is also common. Vitamin B_{12} concentrations are only low in patients with extensive involvement of the small intestine and so are usually normal. Although the haemoglobin level is low, the mean corpuscular volume can be low (iron deficiency), high (vitamin B_{12} or folate deficiency) or within a normal range due to mixed deficiency of iron and folate [*22*].

HbA_{1c} levels were statistically significantly higher in iron-deficient than in non-iron-deficient patients and they decreased

significantly after iron supplementation. These findings are in agreement with Tarim et al. who concluded that among type 1 diabetic patients with similar levels of glycaemia, iron-deficiency anaemia is associated with higher concentrations of HbA_{1c} [*23*]. In addition, iron replacement therapy leads to a decline in HbA_{1c} level in both diabetic and non-diabetic patients. Therefore, the iron status of the patient must be considered during the interpretation of HbA_{1c} concentrations in type 1 diabetes.

We can conclude that anaemia in patients with type 1 diabetes is a relatively common problem and has diverse aetiologies. Therefore, diabetic patients should be screened for the presence of anaemia yearly or at any time if they have suggestive symptoms or predisposing causes. Nutritional programmes should assure the supply of nutritional supplements to anaemic patients and improved awareness about the need for a healthy balanced diet for prevention of anaemia in type 1 diabetes. Moreover, study of the effect of anaemia on the interpretation of HbA_{1c} levels can help with better monitoring of metabolic control and hence prevention of chronic complications of diabetes with the ultimate aim of a better lifestyle for diabetic patients.

References

1. Dikow R et al. How should we manage anaemia in patients with diabetes? *Nephrology, dialysis, transplantation*, 2002, 17(suppl. 1):67–72.
2. Bosman DR et al. Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. *Diabetic medicine*, 2002, 19(1):65–9.
3. Lorini R et al. Clinical aspects of coeliac disease in children with insulin-dependent diabetes mellitus. *Journal of pediatric endocrinology & metabolism*, 1996, 9(suppl. 1):101–11.
4. Schwartz E. The anemias. In: Behrman RE, Kliegman RM, Jenson HB, eds. *Nelson textbook of pediatrics*, 16th ed. Philadelphia, WB Saunders, 2000:1461–3.
5. Bessman JD, Gilmer PR Jr, Gardner FH. Improved classification of anemias by MCV and RDW. *American journal of clinical pathology*, 1983, 80:322.
6. Ewing DJ, Clarke BF. Autonomic neuropathy: its diagnosis and prognosis. *Clinics in endocrinology and metabolism*, 1986, 15:855–8.
7. Dualcount SPB. *A radioassay for simultaneous measurement of B_{12} and folic acid in serum*. Los Angeles, Diagnostic Products Corporation, 1995.
8. An approach to anemic patients. In: Nathan DG, Orkin SH, eds. *Nathan and Oski's hematology of infancy and childhood*, 5th ed. Philadelphia, WB Saunders, 1998: 378–83.
9. El Deeb B et al. *The state of Egyptian children*. Cairo, Central Agency for Public Mobilization and Statistics and United Nations Children's Fund, 1988.
10. Ahmad SM. *Causes and functional consequences of iron deficiency anemia and parasitic infestations of toddlers in an Egyptian village* [PhD thesis]. Cairo, Ain Shams University, Department of Home Economics, 1997.
11. Yamama L, El-Damhougi T, Qadri Z. Prevalence of anemia among children of Egyptian villages: socioeconomic study and trial of therapy. *Mansoura medical journal*, 1996, 26(1):321–35.
12. Fairbankis VF, Beutler E. Iron deficiency. In: Beutler E, Lichtman MA, Coller BS,

eds. *William's hematology*. New York, McGraw–Hill, 2001:447–53.

13. El Beshlawy A et al. Microcytic hypochromic anemia in Egyptian children. *Egyptian journal of haematology*, 2000, 25(4): 585–96.
14. Worwood M. The laboratory assessment of iron status: an update. *Clinica chimica acta*, 1997, 259(1–2):3–23.
15. Behrman RE, Kliegman RM, Jenson HB. The anemias. In: Behrman RE, Kliegman RM, Jenson HB, eds. *Nelson textbook of pediatrics*, 16th ed. Philadelphia, WB Saunders, 2000:1461–63.
16. Temtami SA, Aumran N, Farag MK. *Detection of thalassemia trait in Egyptian children with hypochromic microcytic anemia. Data from the National Research Centre, Giza (Egypt)*. Paper presented at the 1st International Conference of Human Genetics and Physical Anthropology, University of Cairo, 1989.
17. El Beshlawy A et al. Thalassemia prevalence and status in Egypt. *Pediatric research*, 1999, 46(5):102.
18. Aslan D et al. Importance of RDW value in differential diagnosis of hypochromic anemias. *American journal of hematology*, 2002, 69(1):31–3.
19. El Bardisi HA. *Discrimination between thalassemia minor and iron deficiency anemia using erythrocyte measurements* [MSc thesis]. Cairo, Egypt, Faculty of Medicine, Ain-Shams University, 1991.
20. Cronin CC et al. High prevalence of celiac disease among patients with insulin-dependent (type I) diabetes mellitus. *American journal of gastroenterology*, 1997, 92: 2210–2.
21. Sjoberg K et al. Screening for coeliac disease in adult insulin-dependent diabetes mellitus. *Journal of internal medicine*, 1998, 243:133–40.
22. Cronin CC, Shanahan F. Insulin-dependent diabetes mellitus and celiac disease. *Lancet*, 1997, 349:1096–7.
23. Tarim O et al. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatrics international*, 1999, 41(4):257–62.