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Prevalence and etiology of anemias in the adult Turkish population

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Aim: Anemia is common in the general population and one of the most frequently observed nutritional deficiency diseases in the world today. The aim of this study was to investigate the prevalence and etiology of anemias in a large cohort representing the Turkish nation.

Materials and methods: The study population comprised 2187 subjects. Serum iron, iron binding capacity, ferritin, vitamin B_{12}, and folic acid analyses were conducted with autoanalyzers using commercial kits.

Results: A total of 565 (25.8%) subjects (423 females and 142 males) were diagnosed as anemic. The prevalence of anemia was 30.0% in female and 18.2% in male participants. Iron deficiency was present in 26.7%. Two hundred sixty-five (46.9%) had microcytic, 297 (52.6%) had normocytic, and 3 (0.5%) had macrocytic anemia. Although 29.3% had B_{12} and 2.2% had folic acid deficiency, in only 3 cases was macrocytosis determined.

Conclusion: The results of the present study showed that the prevalence of anemia in Turkey is higher than that reported in previous studies. Moreover, mean corpuscular volume is not always an available guide, and should not be used alone in the diagnosis of megaloblastic anemia, and it should be kept in mind that iron deficiency can cover macrocytosis.

Key words: Anemia, iron deficiency, vitamin B_{12} deficiency, folic acid deficiency, mean corpuscular volume

Introduction

Anemia is a public health problem that affects populations in both undeveloped and developing countries (1-4). The impact of anemia on the physical and psychological health status is well known. Anemia is a disorder characterized by a blood hemoglobin concentration lower than the defined normal level, and is usually associated with a decrease in the circulating mass of red blood cells (1,2). The World Health Organization (WHO) suggests that the serum hemoglobin concentration should be <13 mg/dL in males and <12 mg/dL in females and serum hematocrit should be <39% in males and <36% in females for the diagnosis of anemia (4). Anemia may result from decreased generation of red blood cells or from their premature destruction, or from loss through chronic blood loss or hemorrhage (4). Treatment of anemias in the early period prevents permanent damage; therefore early diagnosis of anemia is important. In order to combat anemia,

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epidemiologic data on anemia is vital. On the other hand, deficiency of vitamin B_{12} or folic acid generally causes macrocytic anemia. However, many subjects with vitamin B_{12} or folic acid deficiency do not have macrocytosis (5-7). The prevalence, incidence, and etiology of anemias have not been extensively studied in the general population in Turkey. The aim of this study was to investigate the prevalence and etiology of anemias and to investigate the relation of vitamin B_{12}, folic acid, or iron deficiencies with macrocytosis or microcytosis in a large cohort representing the Turkish nation.

Materials and methods

Study population

The MELEN Study is a prospectively designed survey on the prevalence of cardiometabolic risk factors in Turkish adults. The baseline visits were carried out in May and June 2010 and biennial follow-up visits were planned. The name of the study comes from the valley in northeast Düzce, Turkey, which is inhabited by 21,000 people. There is a town (Yığılca) and 39 villages. Health services in the region are provided by 6 family physicians, each responsible for almost 2500 adults. The study was conducted in May and June 2010 in the Social Health Center located in the town center. Four hundred subjects from each family physician representatively stratified for sex, age, and rural-urban distribution were randomly chosen and invited to participate in the study. A total of 2298 (1471 females and 827 males) subjects with a mean age of 50 (age range 18 to 92) were surveyed. Data were obtained by a questionnaire, physical examination, and sampling of blood. The study protocol was approved by the Ethics Committee of Düzce University and every subject signed a consent form.

Subjects who refused to give blood samples and who did not have all the results of anemia parameters were excluded. Thus 2187 subjects (1407 females and 780 males) were included in the final analysis.

Definitions

Hemoglobin concentration below 13 g/dL in males and 12 g/dL in females was accepted as anemia according to WHO recommendations (4). The diagnosis of iron deficiency was determined by one of the following conditions:

a. Serum ferritin level should be <20 ng/mL.

b. Serum ferritin level between 20 and 40 ng/mL and serum iron level <60 μg/dL.

According to mean corpuscular volume (MCV) subjects with anemia were divided into 3 groups, being microcytic if MCV was <80 fL, normocytic if MCV was 80-95 fL, and macrocytic anemic if MCV was >95 fL, respectively.

Vitamin B_{12} deficiency was defined as a level of <200 pg/mL. Folic acid deficiency was defined as a level of <2 ng/mL (8,9).

A TSH level below 0.35 IU/mL or above 4.5 IU/mL indicated thyroid disease. Decreased renal function was defined as a serum creatinine level of >1.4 mg/dL in males and >1.1 mg/dL in females. All individuals were scored according to meat consumption as follows: once daily = 1 point; once a week = 2 points; once a month = 3 points; once a year = 4 points. Total points and meat consumption are inversely proportional.

Transferrin saturation index % (TSI%) was calculated as follows: TSI% = [Serum iron level/total iron binding capacity (TIBC)] × 100.

Sample collection

Ten milliliters of blood were drawn from the antecubital vein of each subject by applying minimal tourniquet force. The first 2 mL of blood, which was used for the complete blood count, was drawn into a vacutainer tube containing 0.04 mL of the 7.5% K3 salt of ethylene-diamine-tetraacetic-acid (EDTA). The remaining 8 mL of blood was drawn into a vacutainer tube without anticoagulant. These blood samples were allowed to clot for 20 min prior to centrifugation. The blood tubes were centrifuged for 10 min at 1500 × *g* and were processed within 30 min in place. Sera were aliquoted and divided into 4-6 Eppendorf tubes and shipped within a few hours on cooled gel packs at 2-5 °C to the Düzce University Hospital Central Laboratory. Aliquots of the serum were frozen at −80 °C until analyses.

Biochemical analysis

A complete blood count was performed on the same day by a CELL-DYN 3700 SL analyzer (Abbott Diagnostics, Chicago, USA).

Serum iron, total iron binding capacity, and ferritin analyses were conducted in 5 batches and were measured with commercial kits using a Cobas 6000 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Intra-assay variability (CV%) was 0.6% for iron, 2.1% for iron binding capacity, and 1.4% for ferritin. Inter-assay variability was 1.5% for iron, 3.5% for iron binding capacity, and 2.2% for ferritin.

Serum vitamin B_{12} and folic acid levels were measured by Siemens IMMULITE 2000 (Siemens Healthcare Diagnostics Inc., Flanders, NJ, USA) competitive chemiluminescent-enzyme immunoassay method and were conducted in 6 batches. Intra-assay variability was (CV%) 7.0% for vitamin B_{12} and 4.0% for folic acid. Inter-assay variability was 6.0% for vitamin B_{12} and 5.2% for folic acid.

Quality control of biochemical analyses

In the laboratory, periodical quality control was performed to detect errors that affect test results, as described previously (10).

Statistical analysis

Statistical Package for the Social Sciences version 12 (Chicago, IL, USA) was used for analysis. Descriptive parameters were shown as mean ± standard deviation (SD) or in percentages. Two-sided t-tests and Pearson's chi-square tests were used to analyze the differences in means and proportions between groups. Abnormally distributed variables were compared using the Mann-Whitney U test. A P value < 0.05 was considered significant.

Results

In the final analysis 2187 subjects (1407 females and 780 males) were included. A total of 565 (25.8%) subjects (423 females and 142 males) were diagnosed as anemic. The prevalence of anemia was 30.0% in female and 18.2% in male participants. Biochemical characteristics of the anemic group are shown in Table 1.

Iron deficiency was present in 151 (26.7%) of the total anemic subjects (Table 2). Two hundred sixty-five (221 females, 44 males; 46.9%) of the 565 anemic subjects had microcytic, 297 (200 females, 97 males; 52.6%) had normocytic, and 3 (2 females, 1 male; 0.5%) subjects had macrocytic anemia, respectively.

Table 1. Biochemical characteristics of anemic group (n = 565).

Analytes	Mean ± SD
Serum iron level (μg/dL)	61 ± 33
Serum ferritin level (ng/mL)	203 ± 184
TIBC (μg/dL)	400 ± 78
TSI (%)	16 ± 9
Serum vitamin B_{12} level (pg/mL)	288 ± 142
Serum folic acid level (ng/mL)	8.5 ± 3.9
Sex (females/males)	421/141
Hemoglobin (g/dL)	11.2 ± 1.2
Hematocrit (%)	34.4 ± 4.1
MCV (fL)	78.9 ± 8.3
RDW (%)	15.5 ± 1.9
Platelets (10^3/μL)	267.6 ± 80.6
MPV (fL)	8.9 ± 1.6

TIBC: total iron binding capacity; TSI: transferrin saturation index; MCV: mean corpuscular volume; RDW: red cell distribution width; MPV: mean platelet volume

Taking 90 fL as the cut-off value for MCV, the number of subjects with macrocytic anemia increased to 121. Demographic and biochemical differences between microcytic anemia and normocytic anemia groups are presented in Table 3. Characteristics of the normocytic group are shown in Table 4.

Although 29.3% of the anemics had B_{12} and 2.2% had folic acid deficiency, only 3 had macrocytosis. Subjects with anemia were further analyzed according to vitamin B_{12} and folic acid levels. Mean MCV, ferritin, hemoglobin level, TSI%, and age were not significantly different between the vitamin B_{12} deficient and nondeficient groups (78.5 ± 9.3 fL, 198.0 ± 192.6 ng/mL, 11.2 ± 1.1 g/dL, 15.6 (9.8)%, and 48.7 ± 16.7 years versus 79.1 ± 7.4 fL, 211.2 ± 180.5 ng/mL, 11.2 ± 1.3 g/dL, 16.3 (9.5)%, and 50.7 ± 16.8 years, respectively). The differences were also nonsignificant in groups with folate deficiency and with normal folate level (80.8 ± 8.4 fL, 257.9 ± 191.0 ng/mL, 11.4 ± 1.2 g/dL, 15.0 (7.7)%, and 49.6 ± 19.7 years versus 78.7 ± 8.2 fL, 203.5 ± 183.9 ng/mL, 11.3 ± 1.2 g/dL, 16.0 (9.3)%, and 50.4 ± 16.6 years, respectively).

Table 2. Demographic and biochemical features in iron deficiency and nondeficiency groups.

	Anemia with iron deficiency (n = 151)	Anemia without iron deficiency (n = 414)	P
Sex (females/males)	129/22	279/135	**<0.001**
Urban living (n)	23 (15.2%)	63 (15.2%)	0.997
Women in menopause (n)	42 (32.8%)	131 (43.7%)	**0.036**
The number of abortions	0.8 ± 1.2	0.8 ± 1.0	0.953
The number of births	4.1 ± 2.1	4.7 ± 2.9	**0.048**
Alcohol addict (n)	2 (1.3%)	7 (1.6%)	0.758
Smoker (n)	20 (13.2%)	40 (9.7%)	0.058
Subjects with chronic disease (n)	66 (43.7%)	191 (46.1%)	0.537
Age (years)	47 ± 16	52 ± 17	**0.010**
TV watching (h/day)	1.9 ± 1.8	1.2 ± 1.8	0.881
Meat consumption	3.3 ± 0.9	3.1 ± 0.9	**0.015**
Systolic blood pressure (mmHg)	126 ± 27	127 ± 25	0.711
Diastolic blood pressure (mmHg)	79 ± 14	78 ± 14	0.294
Body mass index (kg/m^2)	30 ± 8	29 ± 8	0.067
Heart rate (pulse/h)	73 ±11	72 ± 12	0.748
Depression (n)	50 (33.1%)	69 (16.7%)	**0.010**
Serum vitamin B_{12} level (pg/mL)	268 ± 134	294 ± 143	0.091
Serum folic acid level (ng/mL)	8.5 ± 3.8	8.4 ± 3.9	0.838
The number of subjects with low vitamin B_{12}	43 (28.5%)	80 (19.3%)	**0.029**
The number of subjects with low folic acid	2 (1.3%)	10 (2.4%)	0.356

Discussion

The results of the present study showed that most of the subjects with anemia that had B_{12} or folic acid deficiency were not macrocytic. Although 29.3% of anemics had B_{12} deficiency (6.0% of the whole study population) and 2.2% had folic acid deficiency (0.6% of the whole study population), macrocytic anemia was found in only 3 subjects. When anemics were divided into groups according to MCV, most of the anemics were found to have normocytic anemia. Many studies emphasized that patients with B_{12} or folic acid deficiency frequently had anemia but did not have macrocytosis, since megaloblasts were seen in the late phase of these vitamin deficiencies (5-7). These data were concordant with our findings. Mean corpuscular volume is not always a guide and should not be used alone in the diagnosis of megaloblastic anemia. It should be kept in mind that iron deficiency can cover macrocytosis. As seen in Table 2, in the current survey 28.5% of iron deficiency anemia had coexistent vitamin B_{12} deficiency.

Different ranges of normal values were reported for vitamin B_{12} and folic acid in the literature. In the present study, the authors accepted the lower limit as 200 pg/mL for vitamin B_{12} and 2 ng/mL for folic acid (8,9). The prevalence of vitamin B_{12} deficiency

Table 3. Demographic and biochemical differences between microcytic anemia and normocytic anemia group.

	Microcytic anemia (n = 265)	**Normocytic anemia (n = 297)**	**P**
Menopause (n)	67 (25.3%)	105 (35.4%)	**0.010**
Chronic renal failure (n)	0	3 (1.0%)	0.252
Hypertension (n)	60 (22.6%)	105 (35.4%)	**0.010**
Chronic obstructive lung disease (n)	16 (6.0%)	24 (8.1%)	0.365
Diabetes mellitus (n)	26 (9.8%)	40 (13.5%)	0.199
Smokers (n)	30 (11.3%)	30 (10.1%)	0.371
Drug consumption (n)	98 (37.0%)	157 (52.9%)	**<0.001**
Depression (n)	67 (25.3%)	52 (17.5%)	**0.018**
Thyroid disease (n)	42 (15.8%)	64 (21.5%)	0.187
Income status (TL*)	800 ± 686	767 ± 565	0.544
TV watching (h/day)	1.93 ± 1.9	1.93 ± 1.9	0.998
The number of abortions	0.8 ± 1.2	0.8 ± 1.0	0.865
The number of births	4.1 ± 2.5	5.0 ± 3.1	**0.020**
Fasting glucose level (mg/dL)	108 ± 34	114 ± 47	0.750
Serum creatinine level (mg/dL)	0.73 ± 0.2	0.80 ± 0.25	**0.010**
Decreased renal function	9 (3.4%)	16 (5.4%)	0.227
Number of subjects with low vitamin B_{12}	55 (20.8%)	68 (22.9%)	0.672
Number of subjects with low folic acid	4 (1.5%)	8 (2.7%)	0.342
Serum vitamin B_{12} level (pg/mL)	281 ± 134	292 ± 146	0.406
Serum folic acid level (ng/mL)	8.4 ± 3.8	8.5 ± 4.0	0.661
Serum iron level (μg/dL)	48 ± 31	71 ± 29	**<0.001**
Serum ferritin level (ng/mL)	224 ± 199	185 ± 167	**0.015**
TSI (%)	12.3 ± 8.9	19.3 ± 8.8	**<0.001**
TIBC (μg/dL)	422 ± 77	381 ± 72	**<0.001**

TIBC: total iron binding capacity;
TSI: transferrin saturation index
*1 TL = $0.55

Table 4. Biochemical analysis of the normocytic group.

	Total (n = 297)	Females (n = 97)	Males (n = 200)	P
Hemoglobin (g/dL)	11.6 ± 1	12.2 ± 1.1	11.3 ± 0.9	**0.010**
Hematocrit (%)	35 ± 3	37.4 ± 2.8	34 ± 3.3	**0.010**
White blood cells	6150 ± 1740	6290 ± 1870	6088 ± 1674	0.342
Platelets (10^3/μL)	256 ± 73	237 ± 76	266 ± 69	**<0.001**
MPV (fL)	8.7 ± 1.3	8.6 ± 1.4	8.8 ± 1.3	0.373
Serum ferritin level (ng/mL)	18.5 ± 1.67	20.8 ± 1.49	17.5 ± 1.74	0.126
TIBC (μg/dL)	381 ± 72	352 ± 64	394 ± 72	**<0.001**
Serum folic acid level (ng/mL)	8.5 ± 4	8.2 ± 4.5	8.7 ± 3.7	0.295
Serum iron level (μg/dL)	71 ± 30	77 ± 32	68 ± 28	**0.023**
Serum vitamin B_{12} level (pg/mL)	292 ± 146	313 ± 167	283 ± 135	0.178
Age (years)	54 ± 17	62 ± 13	51 ± 16	**<0.001**

TIBC: total iron binding capacity; MPV: mean platelet volume

was reported as 6.5% in Framingham and 25% in Dutch studies (8,11) in elderly persons (taking the lowest normal cut-off as 200 pg/mL). In the Turkish population, vitamin B_{12} deficiency prevalence in elderly subjects was reported as 22.6% and folic acid deficiency as 10% (12). These studies have reported only rates in the elderly population. The present study reflects a reliable stratified adult population in Turkey with an age range of 18 to 92 years.

Megaloblastic anemia may cause psychiatric, hematologic, and neurologic symptoms. Neurological findings are the most unfortunate because they may not regress despite appropriate therapy. Neurological sequelae of B_{12} deficiency without anemia may occur. Therefore, vitamin B_{12} deficiency should be treated before macrocytosis is seen (12,13).

A common etiological classification of anemia is that there are 3 main causative groups of anemia: nutritional, marrow disease, and hemolytic. Nutritional anemias, including iron, vitamin B_{12}, and folic acid deficiencies, are by far the most common type of anemia worldwide. The other causes of anemia are heavy blood loss as a result of menstruation, or parasite infections such as hookworms, ascaris, and schistosomiasis (4). Hemoglobin concentration is the most reliable indicator of anemia at the population level, as opposed to clinical measures, which are subjective. In a recently published study from Turkey the prevalence of anemia has been reported as 11.1% and it has also been reported that 51.2% of the anemics are microcytic, 45.5% are normocytic, and 3.3% are macrocytic (14). In the present study, the crude anemia rate was 25.8% in the general population and 46.9% of the anemics were microcytic (265 subjects), 52.6% were normocytic (297 subjects), and 0.5% were macrocytic (only 3 subjects). The higher prevalence of anemia in our study may be related to the fact that the proportion of female subjects in our study (64%) was higher than those in previous studies. However, we found also that prevalence of anemia in males is higher than those reported in previous studies.

The prevalence of iron deficiency anemia has been reported before as 7.8% in Turkey (15). The crude iron deficiency anemia rate was 6.9% in the general population in the present study, which was concordant with previously published data. Iron deficiency anemia was more frequent in females and increased as the number of births increased.

Anemia is one of the most important health problems in Turkey. Although many studies related to anemia have been conducted, unfortunately the number of comprehensive studies is almost negligible. There is no homogeneity among the number and age range of subjects in these studies. Some studies included only patients who were hospitalized (16), whereas others only included adolescents or school children (17). Several others included patients admitted to hospital for any reason (6), with pica (18), pre-school children (16), or healthy volunteers (5). Analytic methods also differ since in some studies a complete blood count was used for diagnosis (14), whereas more advanced diagnostic methods (serum ferritin, iron, TSI%, etc.) were applied in others (15).

Anemia was shown to occur at all stages of life, being more common in women and young children. Anemia with vitamin B_{12} deficiency is more prevalent in children and elderly subjects. Children, very elderly subjects, and pregnant women were not included in our study. In the present study a "real-world" adult cohort stratified for age and sex was studied and the blood samples were taken in the morning as recommended (19). Iron deficiency anemics were also shown to consume less meat. Depression was statistically more frequent in the microcytic anemia group. Albacar et al. (20) reported that iron plays a role in the etiology of postpartum depression. The authors think that this topic needs further investigation.

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