

ORIGINAL

## Characteristics of anemia in subclinical and overt hypothyroid patients

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**Abstract.** Thyroid hormones stimulate directly or indirectly growth of erythroid colonies through erythropoietin. Anemia is often the first sign of hypothyroidism. Hypothyroidism can cause a wide variety of anemic disorders. Numerous mechanisms are involved in the pathogenesis of these anemias that can be microcytic, macrocytic and normocytic. We designed this study to investigate the anemia frequency and if present, etiology of anemia in hypothyroid patients. 100 patients with overt hypothyroid, 100 patients with subclinical hypothyroid, and 200 healthy controls were enrolled in this study. Overt hypothyroidism diagnosis is done when elevated TSH and low levels of free T<sub>4</sub> and/or free T<sub>3</sub> have been observed. Subclinical hypothyroidism is defined as elevated serum TSH with normal free T<sub>4</sub> and free T<sub>3</sub> levels. Peripheral smears of the anemic patients were examined. Anemia prevalence was 43% in the overt hypothyroid group, 39% in the subclinical hypothyroid group, and 26% in the control group ( $p=0.0003$  and  $p=0.021$  respectively related to controls). Thus, the frequency of anemia in subclinical hypothyroidism is as high as that in overt hypothyroidism. There was no difference between the hypothyroid groups in terms of anemia. Vitamin B12, Fe, and folic acid were similar between these groups. According to our findings, anemia of chronic disease is the most common type of anemia in hypothyroid patients. Suspicion of hypothyroidism should be considered in anemias with uncertain etiology.

*Key words:* Anemia, Subclinical hypothyroidism, Overt hypothyroidism

**ALTHOUGH** frequency of hypothyroidism differs from one society to another, 2-5% of prevalence has been reported through out the world. However, the prevalence of subclinical hypothyroidism is approximately 4-8.5%; it can reach to 20% in women aged 60 years or older [1].

There is a metabolic deceleration in hypothyroidism. All organ systems are affected; and these symptoms and findings show different characteristics depending on the occurrence age of the hypothyroidism and deficiency or inefficacy of thyroid hormones. Hematopoietic system is the primary one among these affected systems and anemia is the most important one. Mediocre anemia is commonly seen in hypothyroidism. Anemia is defined in 20-60% of the patients with

hypothyroidism [2, 3].

Anemia in hypothyroidism can be normochromic normocytic, hypochromic microcytic, and macrocytic. Anemia severity is associated with the hypothyroidism degree. Hypocellular structure of the bone marrow gives rise to thought that thyroid hormones play a role in hematopoiesis. The most frequently encountered anemia type is normochromic normocytic anemia. The most frequent reason of this is the bone marrow repression due to thyroid hormone deficiency as well as lack of erythropoietin production arising from the reduction in need of O<sub>2</sub>. Erythrocyte life cycle in hypothyroidism is normal, and there is hypoproliferative erythropoiesis. Thyroid hormones also increase 2-3 DPG (diphosphoglycerate) levels assisting in the transmission of oxygen into the tissues [4-6].

Autoimmune thyroid disorders can be seen with other autoimmune disorders. Pernicious anemia can accompany hypothyroidism as a constituent of polyglandular autoimmune syndrome. Failure of vitamin B12 absorption occurs in pernicious anemia due to

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intrinsic factor (IF) deficiency and gastric achlorhydria. This is the reason of macrocytic anemia occurrence in hypothyroidism. Macrocytosis is found in 55% of the hypothyroid patients [2].

Iron deficiency anemia is related with menorrhagia occurring as a result of various hormonal imbalances and also malabsorption which is seen in hypothyroidism [7, 8]. Folic acid is another vitamin with impaired intestinal absorption, and causing macrocytic anemia in hypothyroidism [9].

Our objective is to study the relationship between anemia frequency and anemia types in patients with subclinical and overt hypothyroidism.

## Materials and Methods

The study population was selected from 4800 patients who presented to the Endocrinology and Metabolic Diseases outpatient clinic of Numune Training and Research Hospital in Adana, Turkey, for the first time, between January and September 2008. During the creation of the protocol, it was planned to enroll 400 subjects of whom 100 patients with subclinical and 100 patients with overt hypothyroidism, and 200 healthy subjects. This study was approved by The Institutional Research Ethics Committee and informed consent was obtained from each participant.

Exclusion criteria were multifactorial anemia or anemia due to other reasons including hemolytic anemias, gastrointestinal or genitourinary losses due to malignancy and/or acute/subacute blood losses from the respiratory, gastrointestinal, or genitourinary system; prior thyroid disorder and/or treatment history; presence of any comorbid disease like renal insufficiency/failure, coronary heart disease, uncontrolled hypertension, diabetes mellitus, or any endocrine system disease other than hypothyroidism (subclinical, overt hypothyroidism); and patients who were under the treatment that might affect blood parameters such as steroids or who had received anemia treatment previously. Anemia was not the one of the inclusion criteria of the study subjects.

Of these 4800 patients, 2250 were admitted for thyroid disorders or for the suspicion of a likely thyroid disorder; 1945 were for diabetes mellitus; 350 for obesity; 175 for osteoporosis and 80 patients for other endocrinologic diseases (i.e. hypophysis disorder, surrenal disorder and etc.) (Fig. 1).

Of these 2250 patients, 276 subjects were admit-

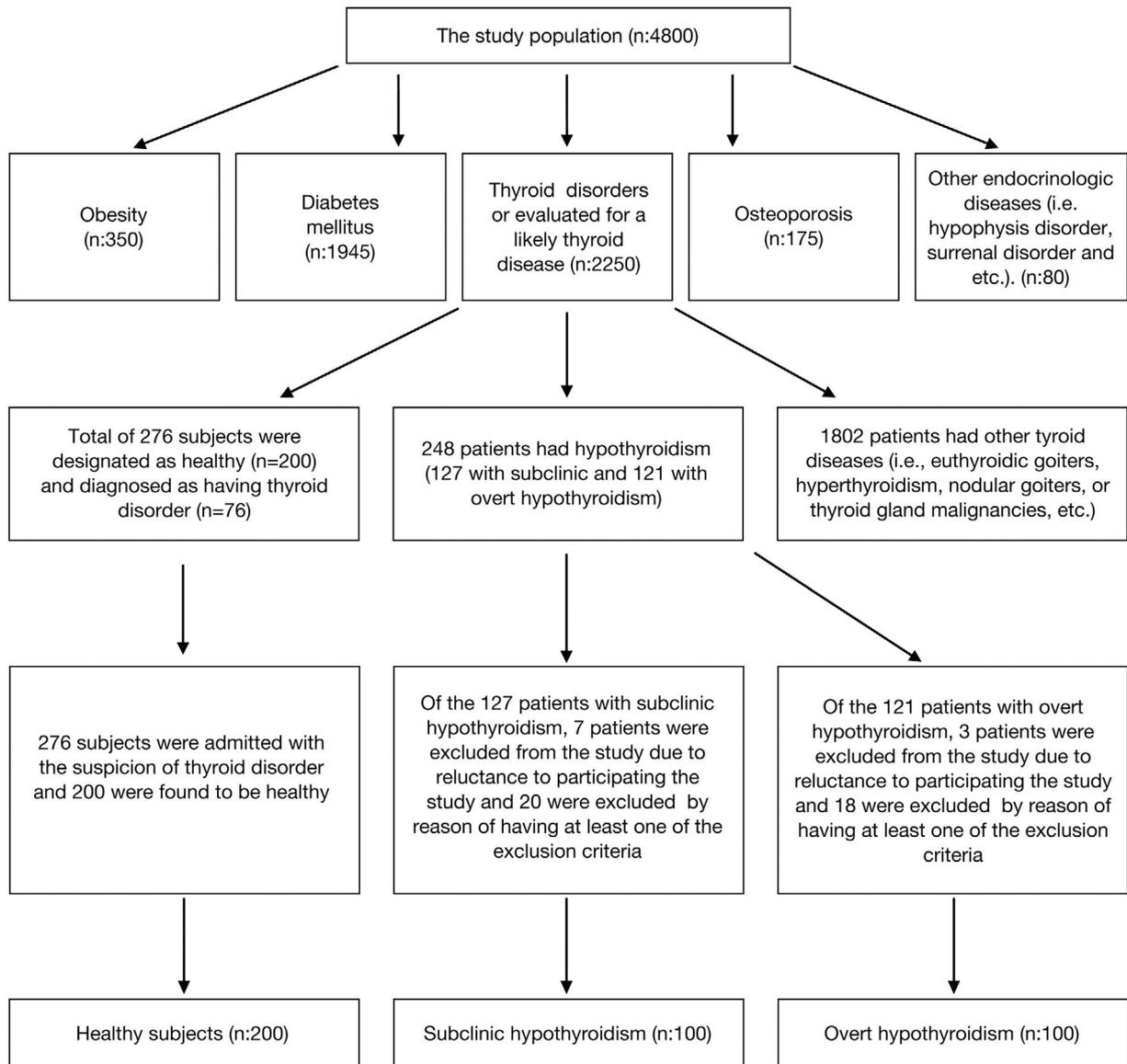
ted with possible thyroid disorder (200 were found to be healthy and 76 were diagnosed with thyroid disorder). 248 patients had hypothyroidism (127 with subclinical and 121 with overt hypothyroidism); and 1802 patients had other thyroid diseases (i.e., euthyroidic goiters, hyperthyroidism, nodular goiters, or thyroid gland malignancies, etc.) (Fig. 1).

Of the 127 patients with subclinical hypothyroidism, 7 patients were excluded from the study due to reluctance for participating to the study and 20 were by reason of having at least one of the exclusion criteria. Of the 121 patients with overt hypothyroidism 18 were excluded from the study due to unmet criteria and 3 by reason of reluctance (Fig. 1).

The data were obtained by analysing 200 patients (subclinical, overt hypothyroidism) and 200 healthy subjects. The healthy group consisted subjects referred to endocrinology outpatient clinic for the suspicion of thyroid disease and found not to have the disease (Fig. 1). Several regions of Turkey, including endemic goiter region, are well documented for increased prevalence of thyroid disorders. TSH measurement is routine in the region. The healthy control group included the subjects who admitted to the hospital but any disorders had not been detected.

Analysis of thyroid-stimulating hormone (TSH:0.27-4.2 mIU/L), free Thyroxine (FT4: 0.70-1.48 ng/dL) free T<sub>3</sub> (FT3: 3.1-6.8 pmol/L), anti-thyroid peroxidase antibody (Anti TPO: <10 IU/mL), anti-thyroglobulin (Anti Tg:<20 IU/mL) antibody, iron (40-160 mcg/dL), iron binding capacity ( 228-428 mcg/dL), ferritin (10-250 ng/mL), vitamin B12 (189-883 pg/mL), folic acid (3-34 ng/mL), and complete blood count values were carried out from blood samples which were taken from patients after a fasting of at least 10 hours. Peripheral smears of anemic patients were examined. Peripheral smears are done to confirm the type of anemia due to erythrocyte morphology and to exclude some other pathologies such as leukemia.

Measurement of the biochemical values was carried out by Beckman Coulter Synchron LX20 Clinical System Auto analyzers. Measurements of TSH, FT3, and FT4 were done with Architec i2000 SR device. The measurement of anti TPO and anti Tg values was carried out by Elecsys 2010 device. Overt hypothyroidism diagnosis was made when elevated TSH and low levels of free T<sub>4</sub> and/or free T<sub>3</sub> were observed. Subclinical hypothyroidism was defined as an elevated serum TSH with normal free T<sub>4</sub> and free T<sub>3</sub> levels.



**Fig. 1** Distribution of the patient and healthy subjects referred to Endocrinology and Metabolic Diseases outpatient clinic

Anemia is defined as hemoglobin levels lower than 12 g/dL in women and 13 g/dL in men. Iron deficiency anemia is defined as serum Fe levels lower than 40 mcg/dL, iron binding capacity greater than 428 mcg/dL, ferritin levels lower than 10 ng/dL and with microcytosis and hypochromia in peripheral blood smear. Folic acid deficiency anemia is defined as folic acid levels lower than 3 ng/mL together with macrocytosis in peripheral blood smear. Vitamin B12 deficiency anemia is defined as B12 levels lower than 189 pg/mL with increased MCV levels and with macrocytosis in

peripheral blood smear. Anemia of chronic disease is defined as low Iron, low iron binding capacity and ferritin levels normal or elevated, folic acid and vitamin B12 levels normal.

### Statistical Analysis

SPSS 11.0 packed software was used to perform statistical analyses. Descriptive analyses were used for variables in their groups. Chi square test, student-*t* tests, and Anova tests were applied for the examination

**Table 1** Clinical and laboratory datas of patients in all groups.

	Subclinical hypothyroidism	Overt hypothyroidism	Control group	<i>P</i> value
Number	100	100	200	
Gender (male/female)	15/85	12/88	32/168	0.57
Age (year)	44.9±14.2	44.5±13.9	45.3±13.7	0.90
TSH (0.27-4.2 mIU/mL)	13.9±10.9	43.1±30.2	1.7±1.9	0.0001
FT4 (0.70-1.48ng/dL)	0.8±0.1	0.5±0.1	1±0.2	0.0001
FT3 (3.1-6.8 pmol/L)	3.3±1.2	2.8±1.3	2.6±0.6	0.0001
Anti Tg positivity (<20 IU/mL) (%)	56	64	19	0.0001
Anti TPO positivity (<10 IU/mL) (%)	65	100	22.5	0.0001
Hemoglobin (gr/dL)	12.4±1.5	11.9±1.5	12.8±1.5	0.0001
Hematocrit (%)	36.5±3.9	35.9±4.1	36.9±3.9	0.105
Vitamin B12 (189-883 pg/mL)	348.5±211.8	400.2±314.5	299.1±205.5	0.002
Folic acid (3-34 ng/mL)	7.5±3.4	7.40±3.9	8.5±3.9	0.025
Iron (40-160 mcg/dL)	73.8±33.8	69.6±33.4	75.5±38.5	0.404
Ferritin (10-250 ng/mL)	42.1±47.4	43.7±52.6	48.1±58.8	0.623
Mean erythrocyte volume (80-97 fL)	83.5±7.1	84.4±9.6	84.5±6.7	0.551

of the relations between the variables. Pearson correlation test was used in correlations between parametric variables, and Spearman correlation test was used in analysis of non parametric variables. *p* values of <0.05 were accepted as statistically significant.

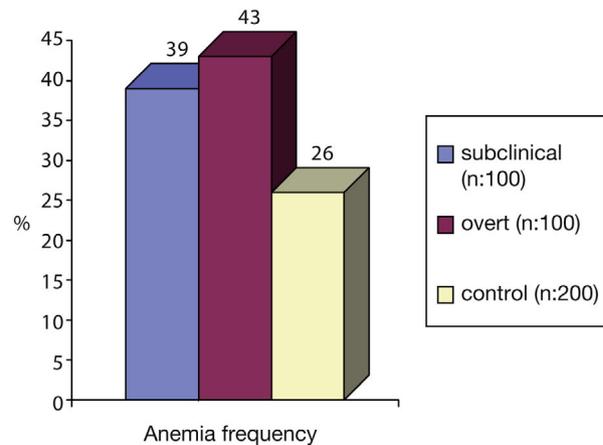
## Results

Demographic measures and biochemical values of patient and control groups participating in this study are shown in Table 1.

In our study; anemia frequency was 41% in the patient group, and 26% in the control group; and the difference between these groups was statistically meaningful ( $p=0.002$ ). Anemia was determined in 39% of those with subclinical hypothyroidism and 43% of those with overt hypothyroidism (Fig. 2). There was no statistical difference in terms of anemia frequency between subclinical and overt hypothyroid groups ( $p=0.568$ ). However, anemia frequency was different between subclinical and overt hypothyroid groups related to the controls ( $p=0.021$  and  $p=0.0003$  respectively).

Biochemical datas of the patients with anemia in hypothyroid and control group are shown at Table 2.

Anemia was present in 134 out of 400 patients included into the study (33.5%). When all groups are taken into consideration, 19 of anemic patients were male (14.2%) and 115 were female (85.8%). While 66 females and 16 males had anemia in hypothyroid group, 49 women and 3 males had anemia in control group. Sex was not related to anemia occurrence



**Fig. 2** Anemia frequency in patient (Subclinical hypothyroidism, overt hypothyroidism) and control groups  
Difference between subclinical hypothyroidism and control group  $p=0.021$   
Difference between overt hypothyroidism and control group  $p=0.0003$

( $p=0.573$ ). There was no statistical difference between mean erythrocyte volume, hematocrit, hemoglobin, ferritin, vitamin B12 level, folic acid and iron levels of the patients with anemia (Table 2). The etiologies of anemia in patients and controls are shown in Fig. 3.

In patient and control groups, anemia of chronic disease frequency was found to be statistically meaningful ( $p=0.005$ ). The frequencies of vitamin B12 deficiency, folic acid deficiency, and iron deficiency anemias were not statistically meaningful ( $p=0.686$ ,  $p=0.623$ ,  $p=0.318$ ; respectively). Also, ratio of patients

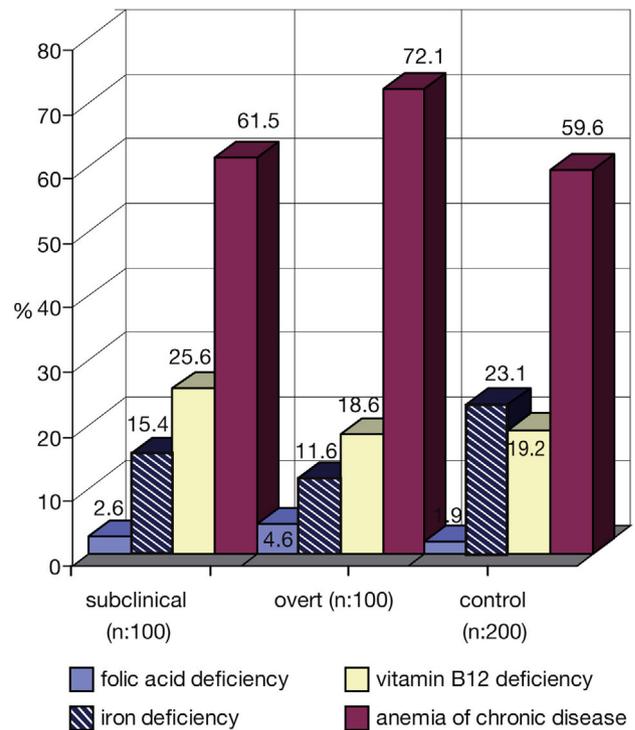
**Table 2** Laboratory values of the anemic patients in all groups

	Subclinical hypothyroidism (n:39)	Overt hypothyroidism (n:43)	Control group (n:52)	P value
Hemoglobin (gr/dL)	10.9±1.1	10.8±1.4	11±1.0	0.57
Hematocrit (%)	32.9±3.2	32.8±3.8	32.5±2.3	0.83
Vitamin B <sub>12</sub> (189-883 pg/mL)	319.7±229.6	418.9±392	345.6±293.4	0.32
Folic acid (3-34 ng/mL)	7.7±3.1	7.9±4.9	8.6±3.9	0.51
Iron (40-160 mcg/dL)	59.3±32.1	51.4±31.9	51.6±27.6	0.40
Ferritin (10-250 ng/mL)	25.5±37.3	39.9±66.1	29.8±34.6	0.36
Mean erythrocyte volume (80-97 fL)	80.3±9.0	81.2±12.2	80.2±8.0	0.87

having both B<sub>12</sub> vitamin deficiency anemia and iron deficiency anemia in the patient group was 2.5%. However, in control group, both vitamin B<sub>12</sub> deficiency anemia and iron deficiency anemia were present in 1% of the patients which were not statistically meaningful ( $p=0.449$ ).

In the subgroup analysis of the hypothyroid patients, the most frequently seen anemia type was the anemia of chronic disease in patients with overt and subclinical hypothyroidism. The ratio depending on this type of anemia was determined to be 31% in patients with clinical hypothyroidism, and 24% in patients with subclinical hypothyroidism. This ratio was found to be 15.5% in control group. The difference between overt hypothyroid and control group was found to be statistically meaningful ( $p=0.007$ ). There was no statistical difference between subclinical hypothyroid, overt hypothyroid, and the control groups in term of the anemia arising from vitamin B<sub>12</sub> deficiency, iron deficiency, and folic acid deficiency ( $p=0.252$ ,  $p=0.213$ ,  $p=0.471$ ; respectively).

There was microcytic anemia in overt hypothyroid patients, subclinical hypothyroid patients and normal subjects respectively 5%, 6%, 6% ( $p=0.933$ ). There was macrocytic anemia in overt hypothyroid patients, subclinical hypothyroid patients and normal subject respectively 10%, 11%, 5% ( $p=0.116$ ). There was normocytic anemia in overt hypothyroid patients, subclinical hypothyroid patients and normal subject respectively 34%, 26%, 16.5% ( $p=0.02$ ).



**Fig. 3** Percentages of anemia reasons in patients with anemia in all groups (Subclinical hypothyroidism, overt hypothyroidism and control)

There was no statistical difference between the distributions of subclinical hypothyroid, overt hypothyroid and control group anemia arising from iron deficiency anemia, folic acid deficiency anemia, B<sub>12</sub> deficiency anemia and anemia of chronic disease ( $p=0.323$ ,  $p=0.731$ ,  $p=0.691$  and  $p=0.420$  respectively).

## Discussion

Although hypothyroidism frequency varies between countries it is a common disease. Subclinical hypothyroidism prevalence in England was determined to be 2.8% in males and 7.5% in females [10]. On the other hand, according to the data of WHO (World Health Organization), anemia is an important public health problem. In order to carry out the treatment of the patient with anemia correctly, it is necessary to determine etiological causes. The adverse effect of hypothyroidism on the hematological system can be anemia development. To the best of our knowledge there is no published study examining subclinical hypothyroidism and anemia in such a detailed content. In our study, we examined this relationship of hypothyroidism (overt and subclinical) with anemia.

In our study, 86.5% of hypothyroid patients were female [11], and the most frequent hypothyroidism cause was determined to be primary hypothyroidism arising from chronic autoimmune thyroiditis [11] consistent with the literature. Anti TPO positivity was 82.5% and Anti Tg positivity was 60% in patients with hypothyroidism.

According to the data of WHO, anemia prevalence is 24.8% throughout the world and it is seen more frequently in underdeveloped countries [12]. Anemia prevalence is 14% in Europe and it reaches to 25% in our country [13]. In our study, anemia prevalence in healthy control group was 26%. Anemia frequency in overt hypothyroid and subclinical hypothyroid groups was determined to be 43 % and 39 %, respectively. Thus, the frequency of anemia in subclinical hypothyroidism is as high as that in overt hypothyroidism. Anemia frequency in patients with overt and subclinical hypothyroidism was found to be statistically significant when compared to control group ( $p=0.0003$  and  $p=0.021$  respectively). This result gave rise to thought that hypothyroidism presence may be a risk factor in anemia development.

Directly or indirectly, stimulation of erythroid colony development by thyroid hormones, inhibition of the latter in its absence, reduction in oxygen distribution to tissues and diminution of erythropoietin level in the absence of thyroid hormones causes normocytic anemia that this anemia forms the most frequent type of anemia in hypothyroid patients [14]. The determination made by Christ-Crain and colleagues indicated that erythropoietin values were increased as result of

levothyroxine treatment in women with subclinical hypothyroidism [3]. Also in our study, similar with the literature the most frequent anemia type was anemia of chronic disease and ratio was higher in overt hypothyroid group according to the control group ( $p=0.007$ ). However, prevalence of vitamin B12 deficiency increases along with the age and Framingham study reveals that the prevalence in old population is 12 % [15]. Wang YH. and colleagues found that vitamin B12 deficiency was 19.71% and megaloblastic anemia prevalence was 9.82% in old hospitalized patients in the neurology clinic [16]. However, the prevalence is observed as 1.6% to 10% in Europe [17]. We found vitamin B12 deficiency as 5% in healthy control group which is similar with these values. Another reason of anemia is macrocytic anemia occurring as a result of vitamin B12 deficiency. It mostly occurs as a result of malabsorption due to pernicious anemia accompanying hypothyroidism. Antibodies against gastric parietal cells were determined in 1/3 of the patients with primary hypothyroidism. It was also determined that the presence of clinical pernicious anemia was seen in 10 % of the patients [18, 19]. In the study carried out by R. Carnel and colleagues, thyroid disorder and hypothyroidism were determined respectively in 24.1 % and 11.7% of the patients with pernicious anemia [20]. Insufficient intake, absorption change arising from deceleration in intestinal motility, intestinal wall edema, and bacterial infiltration are blamed among other reasons causing vitamin B12 deficiency in hypothyroidism [18]. Jabbar A. and colleagues evaluated the prevalence of vitamin B12 deficiency and found low vitamin B12 levels in 46 of 116 (39.6%) patients consulted to endocrinology department [18]. On the other hand, Lippi and colleagues carried out a retrospective study and found that prevalence of vitamin B12 and folic acid deficiency were not different in patients with hypo/hyperthyroidism [21]. Similarly, in our study, we found vitamin B12 deficiency anemia in 9% of the patients with hypothyroidism which was not different from the control group.

Folic acid deficiency, one of the reasons of anemia, occurs as a result of intestinal malabsorption. Again hypothyroidism ruins folate mechanism by decreasing hepatic level of dihydrofolate reductase such as methylene-tetrahydrofolate reductase [22]. Folic acid deficiency almost always occurs as secondary to an underlying disease. We determined folic acid deficiency in hypothyroid patients as 1.5 % in our study. However,

in control group this ratio was determined to be 0.5 % which was not statistically significant.

One of the most frequently seen diseases in all over the world is iron deficiency anemia. In a study carried out in England, it was reported that its frequency was between 3.5-5.3% [23]. Malabsorption and iron deficiency anemia depending on menorrhagia occurring as a result of various hormonal instability are observed in hypothyroidism. In a study carried out by Cinemre H. and colleagues, they showed that the efficacy and absorption of oral iron treatment in women with subclinical hypothyroidism improved after levothyroxine replacement. This demonstrates that hypothyroidism should be assessed in patients with anemia [8].

Hypothyroidism is an endocrine disorder that is

frequently seen in the society. Anemia is frequently seen in these patients and it occurs as a result of various causes. Determination of etiological reasons of anemia and arrangement of the treatment is important. As a result, we found an elevated anemia frequency in hypothyroid patients consistent with the literature. We did not find an increase in vitamin B12 or iron deficiency anemia in hypothyroid patients. We determined that the most frequent cause was linked to anemia of chronic disease.

As a result increase of anemia frequency in hypothyroidism is detected in our study. Further studies with larger number of patients are needed to clarify this increase.

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