JJBS

Volume 17, Number 3,September 2024
ISSN 1995-6673
Pages 487 – 494
https://doi.org/10.54319/jjbs/170311

Jordan Journal of Biological Sciences

Prevalence of Anemia Among Patients with Subclinical and Clinical Hypothyroidism in Jordan

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Received: October 13, 2023; Revised: February 16, 2024; Accepted: March 13, 2024

Abstract

Malabsorption of iron and/or vitamin B_{12} and low thyroid hormone levels may cause anemia in patients with hypothyroidism. This cross-sectional study investigates anemia prevalence, types, and potential causes in 69 patients with hypothyroidism. The control group consisted of 31 healthy volunteers. Diagnostic assessments for hypothyroidism and anemia, in addition to participant-completed questionnaires, were conducted. Females with hypothyroidism exhibited a higher anemia prevalence (12:1 ratio) than males. Among L-thyroxine-treated patients, 22.8% had anemia compared to 11.5% in the untreated subclinical group with hypothyroidism ($p>0.05$). Females under L-thyroxine therapy had significantly lower hemoglobin level than females in the control group ($p=0.0001$). Also, L-thyroxine-treated females had lower ferritin level compared to the untreated females with hypothyroidism ($p=0.026$) and females in the control group ($p=0.007$) while having higher red blood cell distribution width (RDW) compared to the untreated females ($p=0.012$) and the control group females ($p=0.0001$). No significant differences in hemoglobin, ferritin, or RDW were found between males with hypothyroidism and the control group. Also, no significant differences were found among patients with hypothyroidism and the control group in Vitamin B_{12} level. Blood film examination revealed that normochromic normocytic anemia was present in 53.8% of anemic cases with hypothyroidism while hypochromic microcytic anemia was evident in 46.2% of cases. None of the anemic patients had macrocytic anemia. Ferritin level correlated negatively with RDW in patients with hypothyroidism treated with L-thyroxine ($r =-0.517$, $p <0.01$). In conclusion, screening and treating anemia in patients with hypothyroidism is recommended. Treatment with L-thyroxine alone may not be sufficient to prevent anemia in hypothyroidism.

Keywords: Anemia, Hashimoto, ferritin, hypothyroidism, L-thyroxine, red blood cell distribution width.

1. Introduction

According to a recent report, anemia in Eastern Mediterranean Region ranged between 22.6% - 63% amongst pregnant women, 27% - 69.6% amongst women of reproductive age, and 23.8% - 83.5% among children under the age of five (Abdo et al., 2019). Several factors contribute to the high prevalence of anemia in developing countries including the economic situation, health-care costs and poor dietary habits (Abu-Baker et al., 2021). Hypothyroidism is a common endocrine condition characterized by decreased thyroid hormone production. Most cases of primary hypothyroidism are caused by auto-immune thyroiditis (Hashimoto's thyroiditis), idiopathic atrophy, or earlier treatment of hyperthyroidism with radioactive iodine or surgery (Gittoes et al., 1997). Based on large number of studies, anemia is a common, although often underestimated, clinical condition accompanying thyroid diseases (Antonijević et al., 1999; Peraka et al., 2019; Shah et al., 2020).

In addition to the direct impact of low thyroid hormone on erythropoiesis, people with hypothyroidism may be more susceptible to anemia due to other conditions like heavy menstrual bleeding or difficulty absorbing vital nutrients like iron, Vitamin B_{12} and folate (Yadav et al., 2019). The severity of anemia in hypothyroidism was related to the degree of hypothyroidism (Yadav et al., 2019).

In hypothyroidism, anemia may manifest as normocytic, microcytic, or macrocytic (Antonijević et al., 1999). Microcytic anemia is typically attributed to iron malabsorption and iron loss through conditions like hypermenorrhea or menorrhagia, both of which are clinical manifestations associated with a deficiency in thyroid hormones (Szczepanek-Parulska et al., 2017). On the other hand, malabsorption of Vitamin B_{12}, folic acid and pernicious anemia are common causes of macrocytic anemia. Pernicious anemia occurs 20 times more frequently in individuals with hypothyroidism compared to the general population (Antonijević et al., 1999; Lippi et al., 2008). Normocytic anemia emerges due to the deficiency of thyroid hormones without accompanying nutritional deficits (Antonijević et al., 1999; Yadav et al., 2019).

Elevated rates of anemia in patients with hypothyroidism were documented in poor countries such as India and Bangladesh (Peraka et al., 2019; Shah et al., 2020). In contrast, no such association between anemia and hypothyroidism was identified in certain developed

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nations like the Netherlands (Du Puy et al., 2022). Given the scarcity of prior research on the prevalence of anemia among individuals with hypothyroidism in Jordan, this study was undertaken to explore its occurrence and identify its specific types. Identifying the type and cause of anemia will facilitate the implementation of appropriate treatments, supplements, and interventions.

2. Material and Method

2.1. Participants and ethical consideration

This cross-sectional study included 69 Jordanian adults with hypothyroidism of both sexes; thirty-four of them were untreated, newly diagnosed cases, and thirty-five patients were under L-thyroxine hormone replacement therapy. A 31 healthy, age-matched control subjects were included in the study. Exclusion criteria were: medical history of anemia of any type diagnosed before the diagnosis of hypothyroidism including pernicious anemia, pregnancy, cancer, thyroidectomy, radioactive iodine treatment, celiac disease, renal disease or any platelet dysfunction. The study was approved by the research ethical committee at Al-Ahliyya Amman University (ethical approval number IRB: AAU/1/10/2021-2022). Blood was collected after signing an informed consent form by participants. Also, a questionnaire was filled by each of them. The questionnaire included information about age, sex, body mass index (BMI), smoking, presence of chronic or autoimmune diseases, thyroidectomy, iron intake, vitamin intake, red meat intake, blood transfusion, COVID-19 infection and vaccination, menstrual cycle irregularities and bleeding extent, symptoms of hypothyroidism and medications.

2.2. Blood collection and processing

Two milliliters of blood were collected in EDTA tubes for complete blood count (CBC) and blood film testing. Plain; anticoagulant-free, tubes were used to collect 5 ml blood from participants between 10 am – 12 pm. After clot formation, blood samples were centrifuged at 5000 rpm for 7 minutes. The serum was then separated and assayed for Vitamin B_{12}, ferritin, thyroid peroxidase antibody (TPO), thyroid stimulating hormone (TSH), free tri-iodothyronine (FT_3) and free thyroxine (FT_4) testing within 2 hours of blood collection.

Kits for Vitamin B_{12}, TSH, TPO, FT_3 and FT_4 were purchased from Roche Diagnostics, USA and analyzed with Cobas e_{411} – Roche while ferritin was analyzed using (Cobas C_{311}) – Roche. For CBC Sysmex (XS-500i) analyzer. Blood film was stained with Wright's stain.

The precision assessment of the TPO assay involved the utilization of commercially available quality control material (Elecsys PreciControl ThyroAB) within two concentration ranges (level 1 and 2). BioRad standards were employed for Vitamin B_{12}, TSH, FT_3, and FT_4 assays. According to product information, the specificity of Roche Diagnostics' Elecsys was determined to be 95%, 99%, 99.5%, and 99.5% for Vitamin B_{12}, TSH, FT_4, and FT_3 tests, respectively. The sensitivity of Roche Diagnostics' Elecsys for the Vitamin B_{12} assay was established at 225 pg/mL. Additionally, the functional sensitivity values for TSH, FT_4, and FT_3 were measured at 0.014 µIU/mL, 0.2 pmol/L, and 0.15 pmol/L, respectively.

2.3. Statistical analysis

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 26.0 (Chicago, IL, USA) was used. Normality of the distribution of variables was checked using the Kolmogorov-Smirnov and Shapiro-Wilk test. The difference in means for the 3 groups (newly diagnosed, untreated cases with hypothyroidism, L-thyroxine-treated cases of hypothyroidism and control) was carried out using one-way analysis of variance (ANOVA) for normally distributed variables followed by Tukey's post-hoc test. For variables which were not normally distributed, independent-sample Kruskal-Wallis test was used. Spearman's correlation coefficient was obtained after 2-tailed bivariate correlation analysis was performed. An on-line version of Fisher's Exact test (https://www.socscistatistics.com/tests/fisher/default2.aspx) was used to compare the symptoms of anemia and hypothyroidism in the treated and untreated groups. In all tests, a $p<0.05$ was considered significant.

3. Results

3.1. Demographic and clinical characteristics of the study's population

A total of 100 participants were recruited voluntarily in the study including newly diagnosed, untreated cases of hypothyroidism (n = 34), patients with hypothyroidism under L-thyroxine therapy (n = 35), and healthy controls (n = 31). About 82.4% of the untreated group had subclinical hypothyroidism while overt hypothyroidism was present in 17.6% of them.

The demographic and clinical data of the 3 studied groups is illustrated in Table 1**.** The age of the studied subjects ranged from 18-45 years with an average age of 33.4, 35.2 and 34.6 years for untreated, L-thyroxine-treated patients with hypothyroidism and healthy controls, respectively. No significant difference in age and gender was found between different groups (Table 1). Also, no significant differences in BMI, smoking, COVID-19 previous infection or vaccination were encountered. None of the participants had autoimmune disease or any other hormonal imbalance. Chronic diseases were present in 5.7% of the untreated group and 22.8% of the L-thyroxine-treated group but not in the healthy control group (Table 1).

In the untreated group, all females experienced regular menstrual cycles, with 34.7% of them reporting heavy bleeding. Conversely, among L-thyroxine-treated females with hypothyroidism, 79.3% had regular periods, and 24.1% experienced heavy bleeding. In the control group, all females had regular periods, and only 4.3% of them reported heavy bleeding. A significant difference in the regularity of menstrual period was found between the 3 studied groups (Table 1).

Table 1. Demographic and clinical data of untreated cases of hypothyroidism, treated patients with hypothyroidism, and control subjects. Data is represented as number (%).

	Variable	Untreated cases of hypothyroidism Number (%)	Cases of hypothyroidism under L-thyroxine treatment Number (%)	Control Number (%)	*p* value*
Number of participants		34	35	31	
Age (Years)	Average	33.4	35.2	34.6	0.474
	Range	19-45	21-45	18-45	
Gender	Male	11 (32.3)	6 (17.1)	9 (29.0)	0.342
	Female	23 (67.6)	29 (82.8)	22 (70.9)	
Chronic diseases	Present	2 (5.7)	8 (22.8)	-	0.005
	Absent	32 (94.1)	27 (77.1)	31 (100)	
Smoking	Cigarettes	6 (17.6)	5 (14.2)	3 (9.6)	0.462
	Hookah	11 (32.3)	13 (37.1)	6 (19.3)	
	Both	1 (2.9)	2 (5.7)	1 (3.2)	
	Stopped smoking	1 (2.9)	-	-	
	Not smoker	15 (44.1)	15 (42.8)	21 (67.7)	
Previous confirmed	Yes	29 (85.2)	29 (82.8)	23 (74.1)	0.532
COVID-19 infection	No	5 (14.7)	6 (17.1)	8 (25.8)	
Received COVID-19 Vaccine	1 dose	-	1 (2.8)	-	0.395
	2 doses	25 (73.5)	28 (80)	27 (87.0)	
	3 doses	9 (26.4)	6 (17.1)	4 (12.9)	
	None	-	-	-	
Vitamins intake	Vitamin B_{12}	9 (26.4)	12 (34.2)	12 (38.7)	0.878
	Vitamin D_3	22 (64.0)	27 (77.1)	14 (45.1)	0.017
	Iron	5 (14.7)	11 (31.4)	0	0.01
	Multivitamins	6 (17.6)	6 (17.1)	6 (19.3)	0.836
No meat intake (Vegetarians)		2 (5.9)	2 (5.7)	2 (6.4)	0.543
Autoimmune disease	Yes	-	-	-	---
	No	34 (100)	35 (100)	31 (100)	
Imbalance in other hormones	Yes	-	-	-	---
	No	34 (100)	35 (100)	31 (100)	
Regular period	Yes	23 (100)	23 (79.3)	22 (100)	0.006
(Females only)	No	-	6 (20.6)	-	
		*(out of 23)	*(out of 29)	*(out of 22)	
Period bleeding	Light	-	1 (3.4)	-	0.095
(Females only)	Medium	15 (65.2)	21 (72.1)	21 (95.5)	
	Heavy	8 (34.7)	7 (24.1)	1 (4.5)	
		*(out of 23)	*(out of 29)	*(out of 22)	
Relatives having hypothyroidism	Yes	27 (79.4)	31 (88.5)	1 (3.2)	0.0001
	No	7 (20.5)	4 (11.4)	30 (96.7)	
Type of medication	L-thyroxine	0	35 (100)	0	0.0001
Blood transfusion	Yes	0	0	0	
	No	34 (100)	35 (100)	31 (100)	
BMI	18.5-24.0	9 (26.4)	10 (28.5)	8 (25.8)	0.341
	24.5-30	14 (41.1)	18 (51.4)	15 (48.3)	
	> 30	10 (29.4)	6 (17.1)	6 (19.3)	
	NA	1 (2.9)	1 (2.8)	2 (6.4)	

Chronic diseases include: Hyper/hypotension, diabetes, hyperlipidemia, colon diseases, cancers, splenomegaly and platelets disorders.

* P value is based on chi square test results. $P<0.05$ was considered significant.

3.2. Comparison of thyroid function tests between different groups

The untreated group with hypothyroidism had higher TSH level compared to the control group (p=0.0001) and the treated group (p=0.0001) (Table 2). TSH level was within the normal range in 85.7% of the L-thyroxine-treated patients with hypothyroidism. No significant difference in TSH level between control and L-thyroxine-treated group was found (Table 2).

Both untreated and L-thyroxine-treated groups with hypothyroidism had significantly higher TPO levels compared to the control group (p=0.0001). No significant difference between untreated and L-thyroxine-treated groups in TPO level was found. High TPO levels (>16 IU/ml) were present in 70.6% of the untreated and 57.1% of L-thyroxine-treated cases with hypothyroidism with no significant difference between treated and untreated groups (p>0.05).

Patients in the treated group, receiving L-thyroxine hormone replacement therapy, had higher FT_4 levels compared to the control group (p=0.01) and untreated group (p=0.0001). No significant difference between the untreated and the control group in FT_4 level was found. On the other hand, a significantly lower FT_3 level was reported in the treated group compared to the control group (p=0.028).

Table 2. Laboratory characteristics of the study groups. Values are the mean±SD

Parameter	Normal range	Newly diagnosed, untreated group (n=34)	L-thyroxine-treated group (n=35)	Control group (n=31)	*p* value
Hb (g/dL)	Male: (13.8-18.0)	15.6±1.26	15.0± 1.16	15.6 ±1.05	0.564
	Female: (12.0-16.0)	13.0±1.41	12.2 ± 1.15	13.8±0.81	0.0001
Hct (%)	Male: (42.0-45.0)	46.7±4.23	44.4± 3.14	46.4±2.84	0.479
	Female: (37.0-47.0)	39.8±3.67	37.8 ± 3.06	43.1±3.24	0.0001
RBC (x10^12/L)	Male: (4.70-6.10)	5.23±0.37	5.06± 0.60	5.21±0.28	0.534
	Female: (4.20-5.40)	4.71±0.32	4.44± 0.35	4.80±0.28	0.001
RDW (%)	Male: (11.8-14.5)	13.9±0.74	14.0± 0.57	14.2±0.72	0.711
	Female: (12.2-16.1)	14.0±0.81	15.0± 1.35	13.7 ±0.84	0.0001
MCV (fL)	(76.0-99.0)	86.4±7.51	86.0 ± 6.44	87.5±4.74	0.601
MCH (pg)	(27.0-31.0)	28.8±2.56	28.2 ± 2.68	29.2±1.55	0.409
MCHC (g/dL)	(32.0-36.0)	32.7±1.41	32.7 ± 1.23	33.4±1.23	0.108
WBCs (x10^9/L)	(4.0-11.0)	7.45±2.00	7.03± 1.79	6.73±1.17	0.210
Neutrophils (%)	(2.0-7.0)	56.9±10.3	57.8 ± 8.14	56.8±7.38	0.921
Lymphocytes (%)	(1.0-3.0)	35.5±9.34	34.7 7.26	35.0±7.00	0.983
Monocytes (%)	(0.20-1.0)	6.5±1.88	6.45 1.88	6.90±1.59	0.702
Eosinophils (%)	(0.02-0.50)	1.02±0.1	1.02 0.16	1.16±0.72	0.712
Platelets (x10^3/µL)	(150-450)	281.6 ±64.5	281.8 ± 70.2	262.4 ±57.8	0.389
Ferritin (ng/ml)	Male: (30-400)	131.1±62.0	111.3 ± 61.3	123.2 ±53.1	0.719
	Female: (15-150)	83.6 ±104.3	41.1 ± 42.6	61.3 ±25.6	0.014
Vitamin B_{12} (pg/mL)	(200-1000)	284.3 ±162.4	291.4 ±149	352.3 ±93.9	0.104
TSH (uIU/ml)	(0.27-4.20)	18.4 ±37.6	3.39 ± 9.34	2.03 ±0.68	0.0001
FT_3 (pg/mL)	(2.0-4.4)	2.73 ±0.62	2.60 ± 0.50	2.83 ±0.38	0.037
FT_4 (ng/ml)	(0.93-1.71)	1.20 ±0.33	1.44 ± 0.23	1.26 ±0.16	0.0001
TPO (IU/ml)	(1.0-16.0)	185.3 ±188.7	250.4 ± 265	10.94 ±6.71	0.0001

SD: Standard deviation.

P values are based on ANOVA test for normally distributed variables and Kruskal-Wallis test for variables that are not distributed normally.

3.3. Comparison of hematological parameters between different groups

3.3.1. Hemoglobin (Hb) level

In females, the Hb level was significantly lower in the L-thyroxine-treated group, but not in the untreated group, compared to the control group (p=0.0001). On the other hand, no significant difference in Hb level between males with hypothyroidism, including treated and untreated groups, and the control group was observed (Table 2).

Females with hypothyroidism exhibited a higher anemia prevalence than males (12:1 ratio). No significant difference in the prevalence of anemia between treated and untreated cases of hypothyroidism was found (p>0.05). Within the L-thyroxine-treated group, anemia was present in 22.8% of cases (7 females and 1 male): 5 cases had mild anemia while 3 cases had moderate anemia. None of the patients had severe anemia. In the untreated group with overt hypothyroidism, 2 patients out of 8 (25%) had

anemia: 1 case of mild and 1 case of moderate anemia. In patients with subclinical hypothyroidism, 3 females out of 26 cases (11.5%) had anemia: 1 case was mild and 2 cases were moderate anemias. On the other hand, none of the control group had anemia.

3.3.2. Hematocrit (Hct)

L-thyroxine-treated females had lower Hct level compared to females in the control group (p=0.0001), while no significant difference was observed between untreated females and females in the control group. Also, no significant difference between males in different groups was found (Table 2).

3.3.3. Red blood cell (RBC) count

L-thyroxine-treated females had lower RBC count compared to the control females (p=0.001) and untreated females (p=0.016). No significant difference in RBC count between males in different groups was found (Table 2).

3.3.4. Red blood cell distribution width (RDW)

In females, the L-thyroxine-treated group had significantly higher RDW levels compared to the untreated female group (p=0.012) and control group (p=0.0001). No significant difference in RDW between males in different groups was found (Table 2).

3.3.5. RBC indices

No significant difference was found in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) between L-thyroxine-treated, untreated patients with hypothyroidism and control subjects according to the data set in Table 2.

3.3.6. Blood film

Blood film examination revealed that normochromic normocytic anemia was present in 53.8% of anemic cases with hypothyroidism while hypochromic microcytic anemia was evident in 46.2% of cases.

3.3.7. Ferritin levels

Low ferritin level was present in 37.1% of the L-thyroxine-treated group and 5.9 % in the untreated group with hypothyroidism with significant difference between the two groups (p=0.0027). Based on the questionnaire, 14.7% and 31.4% of the untreated and treated patients with hypothyroidism, respectively, are taking iron supplementation (Table 1). In females, the L-thyroxine-treated group had significantly lower ferritin level compared to the untreated (p=0.026) and control groups (p=0.007). No significant difference was detected between the 3 male groups in ferritin level (Table 2).

3.3.8. Vitamin B_{12}

No significant difference was found between the three studied groups in Vitamin B_{12}level (Table 2).

3.4. Correlation between ferritin and different Lab parameters in patients with hypothyroidism treated with L-thyroxine

No correlation was found between the duration of treatment with L-thyroxine and Hb or ferritin levels ($p>0.05$). On the other hand, a positive correlation was found between ferritin level and FT_4 ($r =0.362$, $p <0.05$), Hb ($r =0.637$, $p <0.01$), Hct ($r =0.601$, $p <0.01$), MCH ($r = 0.488$, $p < 0.01$) and MCV ($r = 0.555$, $p < 0.01$). Also, a negative correlation between ferritin and RDW ($r =-0.517$, $p <0.01$) was found in patients with hypothyroidism treated with L-thyroxine (Table 3).

Table 3. Correlation between ferritin and different Lab parameters in patients with hypothyroidism treated with L-thyroxine

		VitaminB12	Ferritin	TSH	TPO	T3	T4	Hb	Hct	RBC	RDW	MCH	MCHC	MCV
VitaminB12	Correlation Coefficient	1.0001	-.288	-.101	.095	-.074	-.147	-.196	-.169	-.004	.368*	-.334*	-.260	-.280
	Sig. (2-tailed)	.	.094	.564	.588	.673	.399	.259	.331	.982	.030	.050	.132	.103
Ferritin	Correlation Coefficient	-.288	1.0001	-.189	-.138	.221	.362*	.637**	.601**	.085	-.517**	.488**	.257	.555**
	Sig. (2-tailed)	.094	.	.278	.431	.202	.033	.0001	.0001	.625	.001	.003	.136	.001
TSH	Correlation Coefficient	-.101	-.189	1.0001	.097	-.152	-.529**	-.209	-.215	-.171	.160	.107	-.073	.150
	Sig. (2-tailed)	.564	.278	.	.581	.382	.001	.229	.216	.325	.358	.541	.677	.390
TPO	Correlation Coefficient	.095	-.138	.097	1.0001	.250	-.074	.114	.125	.074	.088	.121	.021	.100
	Sig. (2-tailed)	.588	.431	.581	.	.147	.674	.514	.476	.673	.617	.488	.904	.567
FT3	Correlation Coefficient	-.074	.221	-.152	.250	1.0001	.391*	.239	.321	.165	-.067	.043	-.080	.180
	Sig. (2-tailed)	.673	.202	.382	.147	.	.020	.166	.060	.342	.700	.807	.647	.299
FT4	Correlation Coefficient	-.147	.362*	-.529**	-.074	.391*	1.0001	.322	.377*	.176	-.293	.084	-.138	.158
	Sig. (2-tailed)	.399	.033	.001	.674	.020	.	.059	.025	.312	.088	.630	.429	.365
Hb	Correlation Coefficient	-.196	.637**	-.209	.114	.239	.322	1.0001	.936**	.509**	-.604**	.462**	.464**	.380*
	Sig. (2-tailed)	.259	.0001	.229	.514	.166	.059	.	.0001	.002	.0001	.005	.005	.024
Hct	Correlation Coefficient	-.169	.601**	-.215	.125	.321	.377*	.936**	1.0001	.607**	-.437**	.307	.229	.349*
	Sig. (2-tailed)	.331	.0001	.216	.476	.060	.025	.0001	.	.0001	.009	.073	.187	.040
RBC	Correlation Coefficient	-.004	.085	-.171	.074	.165	.176	.509**	.607**	1.0001	.062	-.400*	-.039	-.426*
	Sig. (2-tailed)	.982	.625	.325	.673	.342	.312	.002	.0001	.	.723	.017	.825	.011
RDW	Correlation Coefficient	.368*	-.517**	.160	.088	-.067	-.293	-.604**	-.437**	.062	1.0001	-.715**	-.661**	-.548**
	Sig. (2-tailed)	.030	.001	.358	.617	.700	.088	.0001	.009	.723	.	.0001	.0001	.001
MCH	Correlation Coefficient	-.334*	.488**	.107	.121	.043	.084	.462**	.307	-.400*	-.715**	1.0001	.623**	.870**
	Sig. (2-tailed)	.050	.003	.541	.488	.807	.630	.005	.073	.017	.0001	.	.0001	.0001
MCHC	Correlation Coefficient	-.260	.257	-.073	.021	-.080	-.138	.464**	.229	-.039	-.661**	.623**	1.0001	.311
	Sig. (2-tailed)	.132	.136	.677	.904	.647	.429	.005	.187	.825	.0001	.0001	.	.069
MCV	Correlation Coefficient	-.280	.555**	.150	.100	.180	.158	.380*	.349*	-.426*	-.548**	.870**	.311	1.0001
	Sig. (2-tailed)	.103	.001	.390	.567	.299	.365	.024	.040	.011	.001	.0001	.069	.

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

3.5. Symptoms of anemia in hypothyroidism groups.

Based on the gathered information from the questionnaire, 79.60% of the patients in the untreated group had no symptoms of anemia, while all L-thyroxine-treated patients had at least one symptom of anemia. About 8.80% of the untreated group and 33.6% of the treated group complained from weakness and tiredness which was statisticaly significant between the two groups ($p<0.05$), whereas 5.80% of the untreated group presented with palpitation, compared to 23.5% in the treated group, and it was significant between the two groups ($p<0.05$). A significant difference was present in hair loss between untreated and treated group ($p<0.05$) in which 23.0% of patients in the treated group had hair loss compared to 0% in the untreated group. Also, 2.90% of the untreated group had shortness of breath compared to 4.70% in the treated group. Also, 2.90% had pallor in the untreated group compared to 15.20% in the treated group which was significant between the two groups ($p<0.05$).

4. Discussion

Routine monitoring for anemia is imperative in individuals with hypothyroidism, as it is frequently observed in such cases (Shah et al., 2020). In light of the limited clinical manifestations of anemia in subclinical hypothyroidism, it is advised to conduct regular investigations for early detection and management as recommended earlier (Anand et al., 2018). This aligns with our findings, indicating that 79.60% of newly diagnosed, untreated cases of hypothyroidism displayed no symptoms of anemia.

In the current investigation, anemia was present in 11.5% of Jordanian patients with subclinical hypothyroidism, 25% of patients with overt untreated hypothyroidism and 22.8% among L-thyroxine-treated patients. In different countries, prevalence rates of hypothyroidism-associated anemia varied. In India, anemia was prevalent in 26.6% of patients with subclinical hypothyroidism and 73.2% of patients with overt hypothyroidism (Peraka et al., 2019). In another study in India, anemia was found in 69% of the overt group and 56% of the subclinical group with hypothyroidism (Anand et al., 2018). In Bangladesh, anemia was present in 21- 60 % of patients with hypothyroidism (Shah et al., 2020). On the other hand, no baseline associations were found between TSH and Hb levels or the presence of anemia in patients with hypothyroidism in Netherlands (Du Puy et al., 2022).A simple explanation for the contradicting findings between different studies is that it is expected that the prevalence of anemia among patients with

hypothyroidism is higher in poorer countries since it is related to the prevalence of anemia in the general population. Based on WHO reports, anemia prevalence is higher in poor countries and can reach 33% of the general population (WHO, 2017). According to a study conducted in India, anemia was present in 86.6% of the hypothyroid group and 33% in the euthyroid control group (Aparajita et al., 2022). It is important to highlight that our study excluded all cases of anemia diagnosed before the onset of hypothyroidism. Therefore, our findings reflect cases of hypothyroidism-associated anemia only.

It has been suggested that thyroid hormones promote erythropoiesis by increasing erythropoietin gene expression (Golde et al., 1977). In the current study, some patients under L-thyroxine therapy had anemia despite high FT_4 levels compared to the control group. In a recently published study, L-thyroxine treatment failed to rise Hb levels in persons aged 65 years and older with subclinical hypothyroidism (Du Puy et al., 2022). In another study, treatment with L-thyroxine plus iron was superior to treatment with L-thyroxine alone or iron alone in increasing Hb levels (Ravanbod et al., 2013). Therefore, identifying the precise nutritional deficiency in individuals with hypothyroidism is essential for the appropriate treatment of anemia.

In the current investigation, blood film examination demonstrated that 53.8% of anemic cases were normocytic normochromic. This type of anemia emerges due to the deficiency of thyroid hormones without accompanying nutritional deficits (Antonijević et al., 1999; Yadav et al., 2019). On the other hand, 46.2% of anemia cases were diagnosed as iron deficiency anemia. Anisopoikilocytosis was commonly seen. This may explain the higher RDW levels in L-thyroxine-treated patients, with lower ferritin and Hb levels, compared to the control group. These findings agree with previous studies reporting a statistically significant higher RDW value in patients with hypothyroidism (Aparajita et al., 2022). Therefore, checking RDW and RBC morphology in blood film, rather than relying solely on MCV, is essential in such cases to diagnose hypothyroidism-associated anemia.

To be diagnosed with iron deficiency anemia, the patient must have Hb levels of less than 12 g/dL in females, ferritin levels of less than 30 g/L and/or two of the appearance characteristic of iron deficiency anemia (e.g. microcytosis, hypochromia, anisocytosis, poikilocytosis) (WHO, 2007). Depending on our findings, low ferritin levels in anemic group, anisopoikilocytosis in the peripheral blood film and significantly higher RDW in L-thyroxine-treated hypothyroid group existed, confirming the diagnosis of iron deficiency anemia.

In the current investigation only mild or moderate anemia were reported. According to the questionnaire, 14.7% of untreated patients and 31.4% of treated patients with hypothyroidism reported taking iron supplementation. This could potentially reduce the severity of anemia in anemic patients with hypothyroidism.

Multiple studies have indicated that ferritin levels in individuals with hypothyroidism are lower compared to those in healthy individuals (Tripathi et al., 2019; Zwirska-Korczala et al., 1990). In our study, 37.1% of females in the L-thyroxine-treated group exhibited low ferritin levels, contrasting with the 0% observed in the females in the control group. A potential contributor to anemia in hypothyroidism is excessive bleeding in female patients. This may elucidate why L-thyroxine-treated females, in contrast to males with hypothyroidism, demonstrated lower hemoglobin and ferritin levels compared to the control group. Approximately 34.7% of untreated females and 24.1% of treated females experienced heavy bleeding, contrasting with only 4.5% in the control group. This finding aligns with prior research, suggesting an elevated risk of blood loss associated with menorrhagia in women with hypothyroidism, which was accused of iron deficiency in hypothyroidism (Christ-Crain et al., 2003; Peraka et al., 2019).

Our study did not identify any significant differences in Vitamin B_{12} levels between individuals with hypothyroidism and the control group. These results contrast with a study that reported a notable decrease in Vitamin B_{12} levels in patients with hypothyroidism compared to individuals without the condition (Tripathi et al., 2019). One potential explanation for our findings is that 26.4% of the untreated group and 34.2% of the L-thyroxine-treated group were supplementing with Vitamin B_{12}, reducing the likelihood of Vitamin B_{12} deficiency. It has been proposed that celiac disease may contribute to Vitamin B_{12} deficiency in patients with hypothyroidism (Aon et al., 2022). However, individuals with celiac disease were excluded from our study. This might clarify our observation that macrocytic anemia was not reported in patients with hypothyroidism. In a previous investigation, Vitamin B_{12} deficiency anemia was reported in 9% of patients with hypothyroidism and this percent was equal to the prevalence of macrocytic anemia in the general population (Mehmet et al., 2012).

5. Conclusion

The results of this study revealed a significant prevalence of anemia among individuals with hypothyroidism in Jordan, especially among females. The identified types of anemia included normochromic normocytic anemia and hypochromic microcytic anemia, with no instances of macrocytic anemia observed in patients with hypothyroidism. RDW emerged as a vital hematological parameter indicating the degree of erythrocyte anisocytosis, and its correlation with ferritin levels, reflecting the iron deficiency status, underscores its importance.

The present findings suggest that it is crucial to screen for anemia in hypothyroidism and to initiate timely and appropriate treatment before the condition deteriorates. Additionally, it is worth noting that relying solely on L-thyroxine treatment may not be sufficient to prevent anemia in hypothyroidism, especially in females with menorrhagia.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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