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Studying the Role of L-Thyroxine in Long-Term Management of Hypothyroidism: A Comparative Analysis with Healthy Control

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Abstract

Background: Hypothyroidism, characterized by insufficient thyroid hormone production by the thyroid gland, which may lead to significant challenges to metabolic health and overall well-being. Hence, studying the intricate correlation between thyroid biological functions, metabolic pathways and treatment outcomes are crucial for patients care management. Although, recent researches have clarified the complexity between thyroid hormone levels and metabolic pathways, there are still unanswered questions about the specific mechanisms and practical implications. **Patients and methods:** Across sectional study was conducted in Iraq from September 2023 to July 2022 involved 100 females with hypothyroidism undergoing L-thyroxine therapy for at least four months and 50 healthy subjects as control. Blood samples was collected after an overnight fast for plasma extraction. Various biochemical and hormonal assays were performed including TSH, TT4, FT4, TT3, FT3, TSH, fasting plasma glucose, insulin levels, HOMA-IR, cholesterol, triglycerides, HDL, LDL, VLDL, and BMI calculations for categorizations into weight groups.

Results: there were notable differences in weight, blood pressure, and BMI but not in age between patients with hypothyroidism and healthy controls. In addition, patients with hypothyroidism showed decreased FT4 levels, which suggested problems with the regulation of free thyroxine, along with increased TSH and FT3 levels, which indicated thyroid dysfunction and

hyperactivity. Furthermore, patients had lower HOMA-IR indices and impaired glucose metabolism, highlighting the complex relationship between thyroid function and metabolic parameters. Patients had dyslipidemia indicated by elevated total cholesterol and triglyceride levels, and altered lipid metabolism was suggested by lower VLDL levels. Long-term L-thyroxine therapy resulted in a tendency toward lower TSH levels, with TT3 levels fluctuating over the course of treatment.

Conclusion: The aforementioned results offer fresh perspectives on the intricate relationships among thyroid function, metabolic markers, and treatment results in hypothyroidism. They underscore the necessity for additional investigation to clarify underlying mechanisms and enhance clinical management approaches.

دراسة دور الليفوثيروكسين في الإدارة طويلة الأمد لقُصور الغدة الدرقية: تحليل مقارن مع المجموعة الضابطة السليمة

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الخلاصة:

المقدمة: قصور الغدة الدرقية، الذي يتميز بإنتاج هرمون الغدة الدرقية بشكل غير كافٍ، والذي قد يؤدي إلى تحديات كبيرة في الصحة الأيضية والعامة للفرد. لذا، فإن دراسة الارتباط المعقد بين وظائف الغدة الدرقية البيولوجية ومسارات الأيض ونتائج العلاج أمر أساسي لإدارة رعاية المرضى. على الرغم من أن الأبحاث الحديثة قد أوضحت تعقيد العلاقة بين مستويات هرمون الدرقية ومسارات الأيض، إلا أن هناك أسئلة لم يتم الإجابة عنها بعد حول الآليات الخاصة والتبعات العملية.

المرضى وطرق العمل: تم إجراء دراسة مقطعية في العراق من سبتمبر ٢٠٢٣ إلى يوليو ٢٠٢٢ شملت ١٠٠ أنثى مصابة بالنقص الدرقي تخضع لعلاج الإلثيروكسين لمدة لا تقل عن أربعة أشهر و ٥٠ شخصاً سليماً كمجموعة ضابطة. تم جمع عينات الدم بعد الصيام الليلي لاستخراج البلازما. تم إجراء مجموعة من التحاليل الكيميائية والهرمونية بما في ذلك TSH و TT4 و FT4 و TT3 و FT3 و TSH وسكر البلازما الصائم ومستويات الأنسولين ومؤشر HOMA-IR والكوليسترول والتريجليسريدات و HDLو LDLو VLDLوحسابات BMI للتصنيف في مجموعات الوزن.

النتائج: كانت هناك فروق بارزة في الوزن وضغط الدم ومؤشر كتلة الجسم ولكن ليس في العمر بين المرضى الذين يعانون من النقص الدرقي والأشخاص الأصحاء. بالإضافة إلى ذلك، أظهر المرضى الذين يعانون من النقص الدرقي انخفاضًا في مستوياتFT4 ، مما يشير إلى مشاكل في تنظيم الثايروكسين الحر، إلى جانب زيادة في مستويات TSH وFT3 ، مما يشير إلى اضطراب الغدة الدرقية وزيادة النشاط. علاوة على ذلك، كانت لدى المرضى مؤشرات HOMA-IR أقل واضطراب في استقلاب الجلوكوز، مما يسلط الضوء على العلاقة المعقدة بين وظيفة الغدة الدرقية والمعايير الأيضية. كان لدى المرضى دايسليبيديميا مشيرة إلى زيادة في مستويات الكوليسترول الكلي والتريجليسريدات، واقترحت اضطرابات في استقلاب الدهون من خلال خفض مستويات .VLDL أدى العلاج بالإلثيروكسين على المدى الطويل إلى انخفاض في مستويات TSH، مع تذبذب مستويات TT3 على مدى العلاج.

الاستنتاج: تقدم النتائج المذكورة أفاقًا جديدة حول العلاقات المعقدة بين وظيفة الغدة الدرقية والمؤشرات الأيضية ونتائج العلاج في النقص الدرقي. إنها تؤكد على ضرورة إجراء المزيد من البحوث لتوضيح الآليات الأساسية وتعزيز النهج السريري للإدارة.

1. Introduction

Hypothyroidism is a common endocrine dysfunction that characterized by inadequate production of thyroid hormones by the thyroid gland (Chiovato et al., 2019; Gottwald-Hostalek and Schulte, 2022; Leng and Razvi, 2019). This condition impacts people of all ages and both genders, with significant implications for metabolic health, cardiovascular function, and overall well-being. Understanding the intricate relationship between thyroid function, metabolic markers, and long-term treatment outcomes is essential for enhancing patient care and refining clinical management approaches (Staff, 2021).

Recently, there has been increased attention toward investigating the correlation between thyroid function and metabolic biomarkers in patients with hypothyroidism. Numerous studies have uncovered the complex interplay between thyroid hormone levels and various metabolic pathways, such as glucose metabolism, lipid regulation, and body weight control (Bensenor, 2019; Deniz et al., 2022; Fotakis et al., 2022). However, the precise mechanisms underlaying these associations and their clinical implications for patients with hypothyroidism still need to be unmasked (Al-Fatlawi, 2022; Chiu et al., 2023; Kemkem et al., 2020; Liu et al., 2019; Luo et al., 2022; Xu and Zhong, 2022).

Aim of the study: this current study aims to address gaps between thyroid function, metabolic functions and treatment outcomes in patients with hypothyroidism by exploring a comprehensive analysis of thyroid hormones and metabolic parameters compared to the healthy control population. Additionally, the study looks to address the impact of hypothyroidism on metabolic health and assess the efficacy of long-term L-thyroxine treatment in managing thyroid dysfunction and optimizing metabolic biomarkers. Furthermore, the study intends to explore the role of L-thyroxine, the primary medication for hypothyroidism patients in thyroid hormone levels and normalize metabolic parameters, which may provide valuable insights into the efficacy and safety of this treatment approach in clinical practice.

2. Materials, Patients and Methods

2.1.Patients' Constant and Enrollment

This cross-sectional study was conducted from July 2022 to September 2023. The study involved 150 female individuals included 100 patients had been diagnosed with hypothyroidism and 50 healthy control population were recruited from a private clinic in Iraq, where they sought medication and advice for their condition. The patients have been selected based on clinical assessment by specialist doctor which may include physical exam, laboratory tests and thyroid hormone levels. Patients received treatment according to the specialist endocrinologist and thyroid specialist. The study included patients had been receiving levothyroxine therapy for at least four months.

2.2.Inclusion Criteria:

1. Female patients aged 40 years or older.
2. Patients receiving levothyroxine therapy for at least four months for thyroid hormone replacement.
3. Exclusion Criteria:
4. Patients with a treatment duration of less than four months.
5. Patients under 40 years old.
6. Patients taking medications affecting THRA1 expression or levothyroxine activity.
7. Patients who had undergone thyroidectomy.

2.3. Blood Sample Collection

After an overnight fast, 5 ml of blood was drawn from each patient via vein puncture. The samples were collected in EDTA tube for plasma extraction.

2.4. Biochemical and Hormonal Assay Methods

The following techniques were used to measure each different parameter following the commercial instructions:

1. Estimation of Serum Thyroid Stimulating Hormone (TSH): Electrochemiluminescence immunoassay (ECLIA) using the Cobas e immunoassay analyzer.
2. Estimation of Serum Total Thyroxine (T4), Estimation of Serum Free Thyroxine (FT4) and Estimation of Serum Total Triiodothyronine (T3) and Free Triiodothyronine (FT3): Competitive chemiluminescence immunoassay.
3. Estimation of Fasting Serum Glucose Level: Glucose oxidase method.
4. Estimation of Fasting Serum Insulin Level: ELISA kit.
5. Estimation of Insulin Resistance (HOMA-IR): Calculated using the formula: HOMA = (Fasting serum insulin × Fasting serum glucose) / 405.
6. Estimation of Cholesterol, Triglycerides, HDL, LDL, and VLDL: Enzymatic assays.
7. Measurement of Body Mass Index (BMI): BMI was calculated using the formula: BMI = Weight / (Height)^2, and individuals were categorized as normal weight, overweight, or obese based on BMI values (Asil et al., 2014; Dewi et al., 2021).

2.5.Statistical Analysis

The present data was analyzed using Social Sciences (SPSS version 26). The results were presented as mean±SD, frequencies and percentages in appropriate tables and graphs. Independent t-test, and post hoc analysis were used where is appropriate to find out the possible association between the related variables of the current study as LSD. Tukey's method was used to calculate 95% confident interval, which used for either

pairwise comparison or comparing multiple groups. Besides, a statistical Pearson correlation test was also used to determine the relationship between the parameters under study. Statistical association was considered significant when p value equal or less than 0.05 or 0.01 (P value ≤ 0.05 , 0.01).

3. Results

3.1. Demographic Characteristics of Control and Patient Groups

The demographic data of the study's participant was presented in Table 1 and Fig.1A-E. For age, the difference between means is 1.360 ± 1.588 years with a non-significant p-value of 0.3932. interestingly, we observed a significant difference in weight (13.22 ± 2.470 kg, p < 0.0001), BMI (4.872 ± 0.9133 kg/m^2, p < 0.0001), systolic blood pressure (9.210 ± 2.361, p < 0.0001), and diastolic blood pressure (3.610 ± 1.152, p < 0.0021) between patient's population comparing to the healthy control population.

Table 1: Demographic Analyses of The Study Participants

Parameters	Difference between means ± SEM	P value
Age/Year	1.360 ± 1.588	0.3932
Control N (50)		
Patients N (100)		
Weight/Kg	13.22 ± 2.470	<0.0001
Control N (50)		
Patients N (100)		
BMI/(kg/m2)	4.872 ± 0.9133	<0.0001
Control N (50)		
Patients N (100)		
Systolic blood pressure SBP	9.210 ± 2.361	<0.0001
Control N (50)		
Patients N (100)		
Diastolic blood pressure DBP	3.610 ± 1.152	<0.0021
Control N (50)		
Patients N (100)		
Data was presented as mean±SEM, SEM refers to standard error of the mean, P<0.05 considered significant, BMI refers to body mass index.		

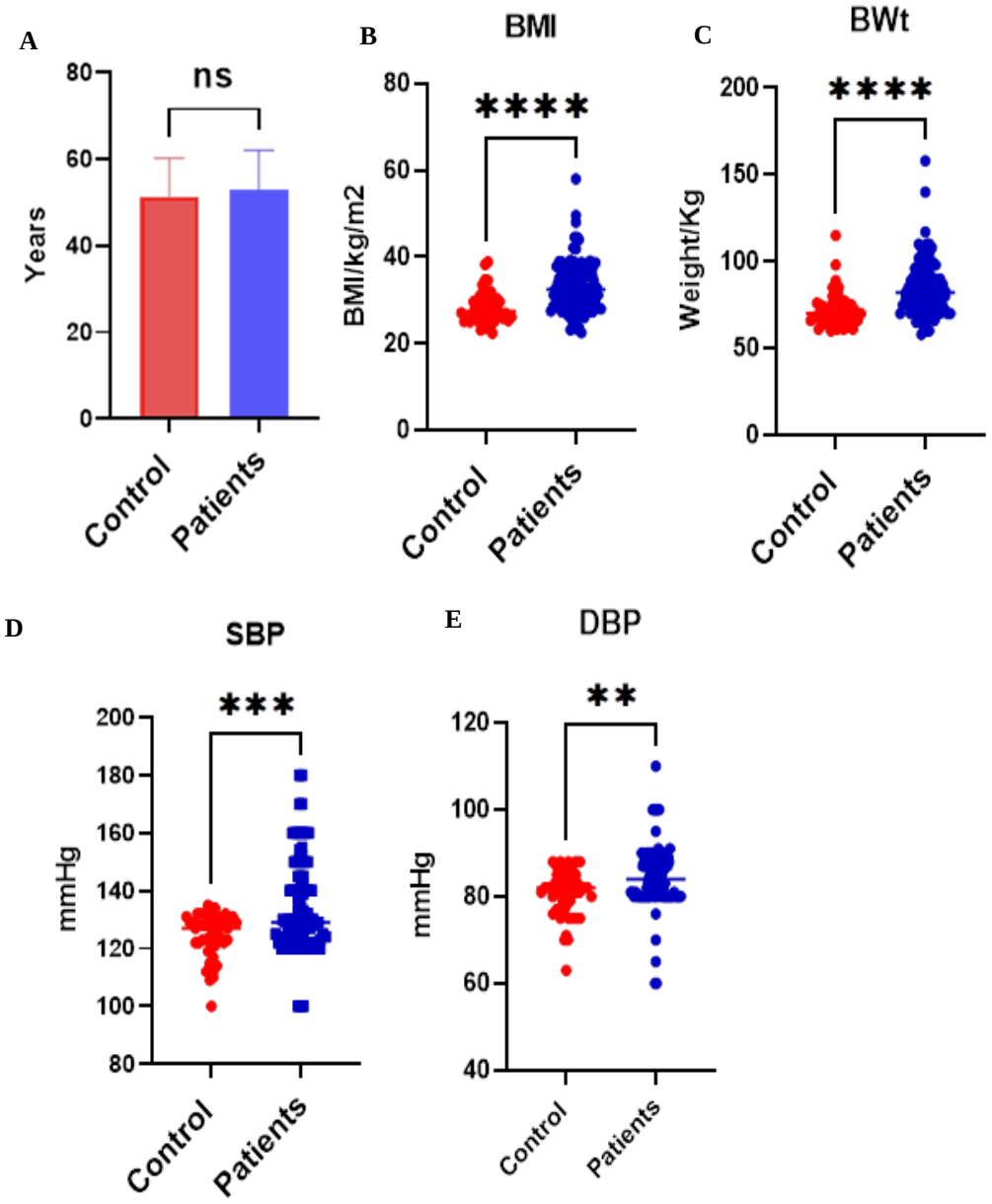

Figure 1: Demographic Analyses of The Study Participants. **A)** Refers to The Difference of The Age Means Between Control and Patients. **B)** Refers To Body Mass Index BMI. **C)** Demonstrates Differences of The Body Weight/Kg Means Between Control and Patients. **D)** Refers to The Differences of The Systolic Blood Pressure SBP Mmhg Between Control and Patients. **E)** Refers to The Differences of The Diastolic Blood Pressure SBP Mmhg Between Control and Patients. * P<0.01, ** P<0.001, *** P<0.0001, Ns=Non-Significant.

3.2. Analyzing of Age, BMI and Comorbidities in Patient and Healthy Control Groups

Table 2 compares different variables between control healthy people (N=50) and patients with hypothyroidism (N=100). it spans a broader age range in patients, up to 89 years, which may indicate disease prevalence or diagnoses in older age. Interestingly, hypothyroidism patients showed a significantly higher proportion of obese individuals (68%) compared to the control group (30%), and a higher prevalence of Diabetes Mellitus (DM) (37% in patients vs. 16% in controls). The majority of patents under the category of 1-6 years treatment, followed by 34% with 7-12 years, while 11% of the patients defined under the category of more than 12 years duration of treatment.

Table 2: Distribution of Variables in Control and Patient Groups

Parameters	Categories	Control N (50)	Patients N (100)
Age (Year), (N)%	40-49	(22), 44	(35), 35
	50-59	(16), 32	(38), 38
	60-69	(12), 24	(22), 22
	70-79	-	(4), 4
	80-89	-	(1), 1
BMI/ (kg/m^2), (N)%	Underweight	-	-
	Normal	(5), 10	(5), 5
	Overweight	(30), 60	(27), 27
	Obese	(15), 30	(68), 68
Duration of treatment (Year), (N)%	1-6	-	(55), 55
	7-12	-	(34), 34
	13-19	-	(6), 6
	20-25	-	(5), 5
DM, (N)%	Yes	(8), 16	(37), 37
	No	(42), 84	(63), 63
Data was presented as number of patients (N) and percentage.			

3.3. Analyzing Thyroid Hormones and Metabolic Markers in Patients and Healthy Control Population

Patients exhibit a marked increase in TSH (4.300 ± 4.708 μU/ml) compared to the control group (2.130 ± 0.8125 μU/ml, $p<0.0015$), indicating potential thyroid dysfunction. While FT3 levels is slightly elevated in patients (5.450±0.997 ng/ml) compared to controls (5.108±0.979 ng/ml), the differences are statistically significant ($p<0.0483$). FT4 is significantly lower in patients (13.38±2.283 pmol/L) than in controls (14.24±1.901 pmol/L, $p<0.0229$), indicating potential disruptions in free thyroxine regulation. Moreover,

patients exhibit higher insulin levels (16.90±9.827 µU/ml) and fasting plasma glucose (117.5±42.49 mg/dl, p<0.0001 and p<0.0044, respectively), suggesting impaired glucose metabolism. Surprisingly, the HOMA-IR index is significantly lower in patients (0.9815±3.186, p<0.0001), indicating potential compensator mechanisms or nuanced metabolic interactions as described in Table 3.

Table 3: Plasma Thyroid Hormone and Metabolic Markers in Patients and Control Groups**.**

Parameters	means±SD		P<0.05	95% CI
	Control N=50	Patients N=100		
TSH, µU/ml	2.130 ± 0.8125	4.300±4.708	<0.0015	0.8421 to 3.497
TT3, ng/ml	1.726± 0.4442	1.772±1.753	0.8546	-0.234 to -0.0214
FT3, pmol/L	5.108±0.979	5.450±0.997	0.0483	0.05292 to 0.6709
TT4, nmol/L	141.8±14.56	138.3±24.71	0.3480	-11.06 to 3.921
FT4, pmol/L	14.24±1.901	13.38±2.283	<0.0229	-1.280 to 0.3973
Insulin, µU/ml	9.023±4.019	16.90±9.827	<0.0001	5.012 to 10.74
FPG, mg/dl	99.70±12.86	117.5±42.49	<0.0044	5.639 to 29.96
HOMA-IR	2.120±5.166	0.9815±3.186	<0.0001	2.133 to 3.959

TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone, FPG=fasting plasma glucose. Data presented as mean±SD, P value≤0.05 considered significant.

3.4. Comparing Blood Lipid Profile in Patients and Healthy Control Participants

Table 4 represents a comprehensive of plasma lipid profiles between healthy control and hypothyroidism patients. Hypothyroidism patients exhibit substantially elevated total cholesterol (184.93±42.299 mg/dl) and triglyceride (141.91±63.952 mg/dl) levels compared to the control group (160.28±29.449 mg/dl and 111.22±34.797 mg/dl, respectively), indicating dyslipidemia and altered lipid metabolism in the patient cohort. Clearly, VLDL levels are significantly lower in patients (28.15±13.136 mg/dl) than in controls (33.81±11.799 mg/dl), suggesting potential differences in lipid composition or metabolism. While LDL levels are modestly elevated in patients (110.08±35.414 mg/dl) compared to controls (101.91±24.445 mg/dl), and HDL levels show no significant difference.

Table 4: Plasma Blood Lipid Profiles in Patients and Control Groups

Parameters	means±SD		P<0.05
	Control N=50	Patients N=100	
Cholesterol	160.28±29.449	184.93±42.299	0.001
TG	111.22±34.797	141.91±63.952	0.002
HDL	48.14 ±12.884	50.46±11.213	0.257
LDL	101.91±24.445	110.08±35.414	0.145
VLDL	33.81±11.799	28.15±13.136	0.011
Data presented as mean±SD, P value≤0.05 considered significant.			

3.5. Duration of L-Thyroxin Treatment

Table 5 shows the distribution of L-thyroxin treatment across different time intervals in hypothyroidism patients. The data shows that the majority of patients fall within the 1-5 years duration category, constituting 42% of the total. The second most prevalent duration range is 6-10 years, encompassing 44% of the patients. A smaller proportion of patients have been on L-thyroxin treatment for longer periods, with 8% in the 11-15 years range, and 3% each for 16-20 years and 21-25 years.

Table 5: Represents Duration of L-Thyroxin Treatment/Year

Duration of treatment/ (years)	Patients%
1-5	42
6-10	44
11-15	8
16-20	3
21-25	3
Data was presented as percentage.	

3.6. Plasma Hormone Levels Following L-Thyroxine Treatment

Table6 demonstrates the plasma hormone levels according to the duration of L-thyroxin treatment. The data represented as Mean ±SD for different hormones: TSH, TT3, FT3, TT4, and FT4. The data suggesting that the levels of all five hormones change over time with L thyroxin treatment. TSH levels decrease over time, while TT3, FT3, TT4, and FT4 levels increase over time. The changes in hormone levels are generally small, but they are statistically significant.

Table 6: Represents Plasma Hormone Levels According to the Duration of L Thyroxin Treatment, Mcg /Year

Duration of treatment (years)	TSH Mean ±SD	TT3 Mean ±SD	FT3 Mean ±SD	TT4 Mean ±SD	FT4 Mean ±SD
1-5	5.817±5.912	1.634±0.2191	5.475±0.8589	134±26.45	13.01±2.487
6-10	3.647±3.546	1.573±0.2129	1.573±0.2129	138.7±23.06	13.61±2.151
11-15	1.913±1.140	1.544±0.1111	5.621±0.9615	144.5±26.55	14.07±1.796
16-20	1.08±1.195	7.54±9.929	5.44±0.8073	146.5±11.48	12.86±1.985
21-25	2.223±1.348	1.477±0.125	5.293±0.7422	165.8±9.362	14.01±3.081
Data was presented as Mean ±SD, SD refers to standard deviation of the mean.					

3.7. Plasma TSH Levels Across L Thyroxin Treatment Durations

Table 7 demonstrates Long-term use of L-thyroxine led to a trend towards lower thyroid-stimulating hormone (TSH) levels, but statistically significant differences were only observed between individuals who had been on L-thyroxine for 6-10 years and those treated for 21-25 years; the latter group showed slightly lower levels. TSH levels in all other treatment time periods were comparable to those of individuals receiving treatment for 1-5 years. These results indicate that the observed decrease in TSH levels may not be consistent across treatment periods.

Table 7: Tukey's Multiple Comparisons Test of Plasma TSH Levels According to the Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	2.170	-0.5817 to 4.921	0.5212
11-15 vs. 1-5	3.904	-1.016 to 8.825	0.1721
16-20 vs. 1-5	4.737	-2.885 to 12.36	0.3372
21-25 vs. 1-5	3.594	-4.029 to 11.22	0.6945
11-15 vs. 6-10	1.734	-3.168 to 6.637	0.6470
16-20 vs. 6-10	2.567	-5.044 to 10.18	0.6868
21-25 vs. 6-10	1.424	-6.187 to 9.035	0.9501
16-20 vs. 11-15	0.8330	-7.802 to 9.468	0.9958
21-25 vs. 11-15	-0.3103	-8.946 to 8.325	>0.9999
21-25 vs. 16-20	-1.143	-11.56 to 9.271	0.9930
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.8.Plasma TT3 levels across L thyroxin treatment durations

Tukey's multiple comparisons test was run to analyze the values of plasma TT3 levels across L-thyroxin treatment/year. A comparison of various treatment duration groups is shown in each row, with the mean difference in TT3 levels, a 95% confidence interval, and an adjusted p-value provided. In some comparisons, such as 16–20 years vs. 1–5 years, statistically significant differences (adjusted p-value <0.05) are noted, indicating significant differences in TT3 levels between individuals with these particular durations of thyroxine treatment as described in the Table 8.

Table 8: Tukey's Multiple Comparisons Test of Plasma TT3 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-0.06085	-0.9339 to 0.8122	0.9997
11-15 vs. 1-5	-0.09006	-1.651 to 1.471	0.9998
16-20 vs. 1-5	5.906	3.488 to 8.325	<0.0001
21-25 vs. 1-5	-0.1571	-2.576 to 2.261	0.9998
11-15 vs. 6-10	-0.02920	-1.585 to 1.526	>0.9999
16-20 vs. 6-10	5.967	3.552 to 8.382	<0.0001
21-25 vs. 6-10	-0.09629	-2.511 to 2.319	>0.9999
16-20 vs. 11-15	5.996	3.256 to 8.736	<0.0001
21-25 vs. 11-15	-0.06708	-2.807 to 2.673	>0.9999
21-25 vs. 16-20	-6.063	-9.368 to -2.759	<0.0001
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.9.Plasma FT3 Levels Across L thyroxin Treatment Durations

Table 9 shows a significant variation in free triiodothyronine (FT3) levels were not observed between any groups when different L-thyroxine treatment durations (1–25 years) were analyzed, indicating that FT3 may not be significantly impacted by treatment duration, at least not during the investigated timeframe.

Table 9: Tukey's Multiple Comparisons Test of Plasma FT3 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	3.902	3.508 to 4.297	<0.0001
11-15 vs. 1-5	-0.1460	-0.8513 to 0.5593	0.9783
16-20 vs. 1-5	0.03524	-1.057 to 1.128	>0.9999
21-25 vs. 1-5	0.1819	-0.9108 to 1.275	0.9904
11-15 vs. 6-10	-4.048	-4.751 to -3.346	<0.0001
16-20 vs. 6-10	-3.867	-4.958 to -2.776	<0.0001
21-25 vs. 6-10	-3.720	-4.811 to -2.629	<0.0001
16-20 vs. 11-15	0.1812	-1.057 to 1.419	0.9941
21-25 vs. 11-15	0.3279	-0.9099 to 1.566	0.9473
21-25 vs. 16-20	0.1467	-1.346 to 1.640	0.9988
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.10. Plasma TT4 Levels Across L-Thyroxin Treatment Durations

The plasma TT4 levels Tukey's multiple comparisons test in Table 10 reveals varying lengths of L thyroxin treatment per year. rows denote a distinct comparison between two duration groups and includes the adjusted p-value, the corresponding 95% confidence interval, and the mean difference in TT4 levels. Notably, after correcting for multiple comparisons, the adjusted p-values are used to assess whether the observed differences are statistically significant. Given that all adjusted p-values in this dataset are greater than 0.05, it is possible that there are no appreciable variations in mean TT4 levels amongst the treatment duration groups that are being compared. Comparing 16-20 years of treatment to 1-5 years revealed an average difference of -12.51 in plasma TT4 levels. However, the wide range of uncertainty (-53.29 to 28.26) and the high adjusted p-value (0.9122) suggest that this difference is not statistically meaningful.

Table10: Tukey's Multiple Comparisons Test of Plasma TT4 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-4.702	-19.42 to 10.02	0.8997
11-15 vs. 1-5	-10.43	-36.75 to 15.89	0.8036
16-20 vs. 1-5	-12.51	-53.29 to 28.26	0.9122
21-25 vs. 1-5	-31.75	-72.52 to 9.028	0.2000
11-15 vs. 6-10	-5.731	-31.95 to 20.49	0.9733
16-20 vs. 6-10	-7.810	-48.52 to 32.90	0.9835
21-25 vs. 6-10	-27.04	-67.76 to 13.67	0.3506
16-20 vs. 11-15	-2.080	-48.27 to 44.11	>0.9999
21-25 vs. 11-15	-21.31	-67.50 to 24.88	0.7003
21-25 vs. 16-20	-19.23	-74.94 to 36.47	0.8712
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.11. Plasma FT4 Levels Across L-Thyroxin Treatment Durations

In most comparisons that represented in the Table 11 (1-5 years vs. 6-10, 11-15, 16-20), the length of L-thyroxine treatment did not significantly affect FT4 levels; however, potential trends of higher FT4 in the 21–25-year group and lower FT4 in the 16–20-year group compared to reference (1-5 years) call for additional research using larger datasets to confirm and explore influencing factors.

Table 11: Tukey's Multiple Comparisons Test of Plasma FT4 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-0.6024	-1.114 to 2.069	0.7428
11-15 vs. 1-5	-1.061	-3.646 to 2.047	0.7532
16-20 vs. 1-5	0.1476	-6.333 to 2.486	>0.9999
21-25 vs. 1-5	-0.9990	-1.813 to 7.006	0.9497
11-15 vs. 6-10	-0.4587	-4.113 to 1.559	0.9852
16-20 vs. 6-10	0.7500	-6.804 to 2.001	0.9821

21-25 vs. 6-10	-0.3967	-2.284 to 6.521	0.9984
16-20 vs. 11-15	1.209	-6.120 to 3.871	0.9367
21-25 vs. 11-15	0.06208	-1.600 to 8.391	>0.9999
21-25 vs. 16-20	-1.147	-1.505 to 10.54	0.9730
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.12. Study the Correlation Between Thyroid Hormones and Metabolic Parameters

The heatmap in the Fig.4, display correlation coefficients and p-values for a range of treatment-related parameters. Among the noteworthy correlations is the one that shows a positive relationship between Insulin and Fasting Blood Glucose (FBG)—that is, higher Insulin levels are associated with higher FBG levels. Furthermore, there is a positive correlation between the length of treatment and the Body Mass Index (BMI), suggesting a relationship between higher BMI and longer treatment periods. On the other hand, a negative correlation has been observed between BMI and Thyroxine (TT4) levels, indicating a negative relationship between higher BMI and lower TT4 levels. Additionally, the data show a positive correlation between TT4 levels and treatment duration, suggesting that longer treatment may have an impact on higher levels of Thyroxine. Notably, there is no discernible relationship between age and treatment duration. The aforementioned results highlight the intricate connections between thyroid hormones, metabolic parameters, and treatment duration, underscoring the diverse range of clinical associations.

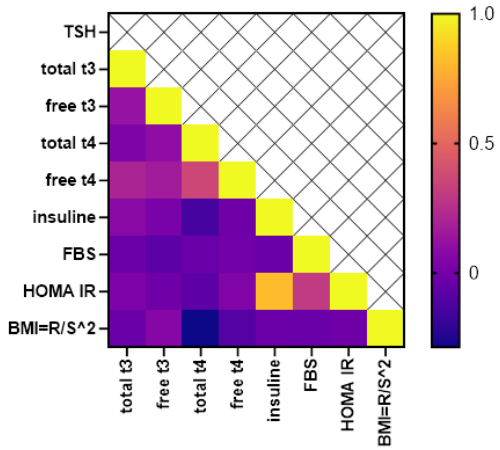

Figure 4: Heat Map Correlation Matrix of the Analysis of Health Parameters in Treatment: Thyroid Function, Insulin Levels, Blood Sugar, And BMI.

3.13. Study Treatment Related Correlations: Thyroid Hormones, Metabolic Factors and Duration in the Clinical Context

Table 12 presents the correlation coefficient (r values) and corresponding P values for various L-thyroxine treatment parameters, including TSH, FT4, Insulin, FBG, TT3, FT3 and TT4. The correlation coefficient (r) indicates the strength and direction of the relationship between two variables. The p-values help determine the statistical significance of these correlations. Clearly, we found a statistically significant positive correlation between BMI and the duration of treatment (r = 0.277, p = 0.005**), indicating that individuals with a longer treatment duration tend to have a higher BMI. Additionally, a significant negative correlation is observed between TT4 and BMI (r = -0.2865, p = 0.0039), suggesting that higher BMI is associated with lower levels of Thyroxine. The data also indicates significant positive correlations between Insulin and FBG, and a notable negative correlation between TT3 and FBG.

Table 12: Treatment-Related Correlations: TSH, Free T4, Insulin, FBS, BMI, Duration, and Age

Parameter	Value	FBG	Insulin	HOMAIR	BMI	Duration of treatment	Age
TSH	r	0.006	- 0.024	- 0.48	0.277	- 0.227	- 0.085
	P value	0.954	0.810	0.637	0.005**	0.023*	0.399
FT4	r	0.007	- 0.016	0.045	- 0.102	0.076	0.073
	P value	0.942	0.874	0.656	0.312	0.454	0.469
Insulin	r	-0.0321		0.812	-0.0257	-0.05789	0.0423
	P value	0.7509		0.0001***	0.7993	0.5672	0.6760
FBG	r		-0.0321	0.306	-0.0314	-0.1583	-0.01965
	P value		0.7509	0.002**	0.7564	0.1157	0.8461
TT3	r	-0.0273	0.07177	0.03486	-0.0305	0.1688	-0.0632
	P value	0.7868	0.4779	0.7306	0.7630	0.0431	0.5320
FT3	R	-0.07560	0.01750	-0.01412	0.06097	-0.00252	0.1153
	P value	0.4548	0.8628	0.8891	0.5468	0.9801	0.2534
TT4	R	-0.03350	-0.1444	-0.07232	-0.2865	0.2314	0.0122
	P value	0.7408	0.1517	0.4746	0.0039	0.0206	0.9034
r refers to the Pearson correlation coefficient, P<0.05 considered significant.							

4. Discussion

There were no statistical differences in age between patients with hypothyroidism and healthy control population (1.360 ± 1.588 years, p-value = 0.3932), but significant differences in weight (13.22 ± 2.470 kg, $p < 0.0001$), Body Mass Index (BMI) (4.872 ± 0.9133 kg/m², $p < 0.0001$), systolic blood pressure (9.210 ± 2.361, $p < 0.0001$), and diastolic blood pressure (3.610 ± 1.152, $p < 0.0021$). These results suggest that various factors such as lifestyle, diet, and genetics might contribute. Furthermore, we compared health parameters between a control group of healthy individuals (N=50) and patients with hypothyroidism (N=100). The hypothyroidism patients showed a wider age range up to 89 years, possibly indicating disease prevalence or diagnoses in older age. Interestingly, there is a significantly higher proportion of obese individuals (68%) in the hypothyroidism group compared to the control group (30%), and a higher prevalence of DM (37% in patients vs. 16% in controls. The majority of patients receive treatment for 1-6 years, then 34% receive treatment for 7–12 years, and 11% receive treatment for more than 12 years. These results point to possible connections between age, DM, and obesity and hypothyroidism; however, more investigation is required to validate these connections and fully comprehend their implications. These findings align with previous publications in which scientists suggested that the frequency and incidence of hypothyroidism rise with people aged, and with women experiencing a two-fold higher prevalence than men according to a population-based study in the Piedmont Region, Italy (Caputo et al., 2020). In general, there are a limited study that explored the effects of hypothyroidism on specific demographic groups (Kumar and Gupta, 2021; Natarajan and Prakash, 2015). Thus, the results of this study regarding the significant variations in blood pressure, BMI, and weight among patients with hypothyroidism may offer insightful new information to the field.

Hypothyroidism patients showed a significant difference in the biochemical parameters comparing to the control group. A significant increase in the plasma patients TSH comparing to the healthy control people, indicating potential thyroid dysfunction. Additionally, elevated levels of hypothyroidism patient's plasma FT3 plasma levels indicated hyperactivity of thyroid biological activity. Unlikely, FT4 was dramatically lower in the patients, signaling potential disruptions in free thyroxine regulation. The observed higher insulin levels and fasting plasma glucose in patients imply impaired glucose metabolism. Remarkably, patients have a lower Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, suggesting compensatory pathways or complex metabolic interactions. Interestingly these findings agree with the other studies in which they reported that increase in TT3 and FT3 plasma levels in hypothyroidism patients. furthermore, there appears to be a strong positive relationship between serum TG and serum TSH/FT3-FT4, while FT4 showed a negative association (Forero-Saldarriaga et al., 2020; Huang et al., 2022; Paczkowska et al., 2020; Selmer et al., 2012). According to the American Thyroid Association, patients who are hypothyroid will have an

elevated T3 level. In some individuals with a low TSH, only the T3 is elevated and the FT4 or FT3 is normal (Forero-Saldarriaga, Puerta-Rojas and Correa-Parra, 2020; Paczkowska et al., 2020).Recently, scientists have found that children in the upper baseline TSH tertile showed higher concentrations of FT3, FT3/FT4 ratio, and TSH, which agree with our data for increased TSH and FT3 levels in patients with hypothyroidism (Carreras-Badosa et al., 2023). These results highlight the intricate relationship between thyroid function and metabolic parameters and hypothyroidism. Contextual factors, such as genetic and lifestyle factors, are important, though, and drawing conclusions about causality based only on correlations found in this study should be done with caution. However, the findings of this study suggest a link between higher insulin levels, elevated fasting plasma glucose, and impaired glucose metabolism in patients, and also indicates signs of potential compensatory mechanisms through a lower HOMA-IR index. These findings are novel and provide significant value in increasing our knowledge of this area. Patients with hypothyroidism showed dramatic elevated of total cholesterol and triglyceride levels compared to the control group, indicating dyslipidemia and altered lipid metabolism. Interestingly, VLDL levels are significantly lower in patients than in controls, suggesting potential differences in lipid composition or metabolism. LDL levels are modestly elevated in patients compared to controls, while HDL levels show no significant difference. These findings align with recent studies in which scientists reported that hypothyroidism significantly associated with blood lipid alterations mainly concerning total cholesterol and LDL (Paczkowska et al., 2020; Ramulu et al., 2016). Additionally, these data agree with previous studies suggested that perturbations in the actions of T3 and T4 influence the normal metabolic pathways (Duntas and Brenta, 2018; Tan et al., 1998). Recently, scientists have found that TSH was high in 5.2% of the 49.5% of patients who were diagnosed with hyperlipidemia, which strongly agree with the findings of this current study (Iqbal et al., 2022a, 2022b, 2021). Hence, these data represent valuable insight in to the relationship between hypothyroidism and lipid profiles.

A trend toward reduced plasma TSH levels with L-thyroxine use. Those who received treatment for 6-10 years and those who received treatment for 21-25 years showed statistically significant differences, with the latter having slightly lower TSH levels. However, TSH levels in other treatment periods were similar to those who received treatment for 1-5 years, indicating that the observed decrease over time is not consistent across treatment periods. Similarly, scientists suggested that Scientists recognized the crucial role of TSH in FT3 conversion, and more studies have shown that temporarily stopping oral levothyroxine increases plasma TSH levels and alters FT3/FT4 ratios significantly within three days (Carlwe et al., 2013; Duntas and Jonklaas, 2019). This suggests a direct correlation between TSH levels and the conversion of FT3 and FT4. The lower TSH levels observed in hypothyroidism patients undergoing therapy across treatment duration may be attributed to the TSH-lowering effect of the treatment.

Plasma TT3 levels are examined across L-thyroxine treatment durations using Tukey's multiple comparisons test. The results show statistically significant differences in some comparisons, such as 16–20 years vs. 1–5 years, indicating significant variations in TT3 levels for these particular treatment durations as described previously. The FT3 levels for various L-thyroxine treatment durations (1–25 years) and does not reveal any significant variation between groups. This suggests that FT3 may not be significantly affected by treatment duration within the investigated timeframe. Multiple comparison test indicates that plasma TT4 levels did not change statistically among the treatment duration groups that were being compared. Not surprisingly, these data agree with various studies, firstly, scientists have explored the effects of thyroid hormone therapy on the level of quality of life and thyroid related symptoms in patients with hypothyroidism, with intervention duration ranging from 3 to 18 months. More studies investigated the duration of L-thyroxine therapy and its impact on thyroid function status and metabolic pathways aiming to optimize levothyroxine therapy (Emerson, 2018; Feller et al., 2018; Hennessey, 2017; Johnson, 2019; Skelin et al., 2018).

5. Conclusion

Our study reveals that a significant difference between patients with hypothyroidism and healthy controls, indicating potential lifestyle and genetic influences. patients with hypothyroidism showed disrupted thyroid function, with elevated plasma TSH and FT3 levels, and lower FT4 levels, alongside dyslipidemia characterized by increased total cholesterol and triglycerides. Trends in plasma TSH levels during L-thyroxine therapy suggest treatment's impact on thyroid hormone regulation. While significant variations occur in plasma TT3 levels across treatment durations, FT3 and TT4 levels remain relatively stable. Hence these data unmask the complexity between hypothyroidism, metabolic pathways and treatment outcomes, necessitating for further research for optimizing treatment strategies and improve patient outcomes.

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