Research Article

Association of caspase-3 with thyroid hormone levels in patients with hypothyroidism – A hospital-based study

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Abstract

Introduction: This hospital-based prospective study examined the relationship between thyroid hormone levels and serum levels of caspase-3, a critical marker of apoptosis, in 183 patients diagnosed with hypothyroidism.

Methods: Serum caspase-3 concentrations were measured using a human caspase-3 ELISA kit. Elevated caspase-3 levels indicate increased apoptotic activity.

Results: In the overall cohort, the serum caspase-3 levels were not significantly correlated with the thyroid hormone level. However, among patients with mildly elevated TSH (Group I), caspase-3 levels were inversely correlated with T_3 ($r = –0.319$, $p < 0.05$) and T_4 ($r = –0.377$, $p < 0.001$). Age-stratified analysis revealed that among younger participants (18–40 years), age independently predicted lower caspase-3 levels ($p = 0.027$), suggesting a decline in apoptotic activity with advancing early adulthood. In older individuals (≥41 years), serum T_4 independently and negatively predicted caspase-3 levels ($p = 0.048$). Partial correlation analysis controlling for age rendered the previous associations non-significant, indicating that age was a confounding factor.

Conclusion: The relationship between thyroid hormones and apoptosis appears to be both age- and severity-dependent. While thyroid hormones are inversely associated with apoptotic activity in mild hypothyroidism, age independently influences caspase-3 in younger individuals and T_4 remains a significant predictor in older adults. These findings underscore the complex interplay between aging, thyroid function, and apoptosis, with implications for thyroid-related pathophysiology across the lifespan.

Introduction

Hypothyroidism is characterized by a deficiency of thyroid hormones and can lead to a wide range of metabolic and systemic disturbances, becoming potentially fatal if left untreated [1]. Thyroid disorders affect an estimated 200 million people globally, with nearly 60% remaining undiagnosed. Thyroid dysfunction is often due to inadequate iodine intake, whereas autoimmune thyroid diseases are more prevalent in regions with sufficient or supplemented iodine intake [2]. Primary hypothyroidism is typically diagnosed by elevated serum levels of thyroid-stimulating hormone (TSH), reflecting the high sensitivity of the hypothalamic–pituitary–thyroid axis to subtle fluctuations in circulating thyroid hormones [3]. The condition may result from autoimmune destruction (e.g., Hashimoto's thyroiditis), iodine deficiency, infiltrative diseases, congenital anomalies, or iatrogenic causes such as neck irradiation, thyroidectomy or radioiodine therapy [4,5]. Transient forms such as subacute, postpartum and silent thyroiditis also contribute to intermittent thyroid dysfunction. [6]. The prevalence of hypothyroidism increases with age, especially in countries undergoing demographic transitions toward aging population, projected to reach 1.4 billion elderly individuals by 2030 [7].

Beyond their classical role in metabolism, thyroid hormones are critical regulators of cellular growth, differentiation and development. These effects are mediated via TSH receptor pathways that regulate proliferative or apoptotic outcomes, depending on the cell type and developmental context [8]. Growing evidence has focused on understanding how thyroid hormone imbalances alters intracellular signalling pathways governing cell cycle regulation and programmed cell death [9]. Apoptosis is a tightly regulated process essential for maintaining tissue homeostasis by eliminating damaged or dysfunctional cells. It can be initiated via intrinsic pathways such as mitochondrial cytochrome c release or extrinsic receptor-mediated mechanisms involving tumour necrosis factor (TNF) signalling. Dysregulated apoptosis contributes significantly to the pathophysiology of various diseases, including cancer, where impaired cell death promotes tumour angiogenesis, survival, and invasion [10].

Caspases, particularly caspase-3, play a central role in the execution phase of apoptosis by cleaving key cellular substrates at specific aspartic acid residues [11,12]. Beyond its apoptotic role, caspase-3 has been implicated in tissue differentiation, regeneration, and neurodevelopment, highlighting its broader physiological relevance [13]. Given the multifaceted roles of caspase-3 and the regulatory influence of thyroid hormones on cellular fate, this study aimed to evaluate serum caspase-3 levels in individuals with hypothyroidism and examine their association with TSH concentrations, while assessing the modulatory effects of age and sex on this relationship.

Materials and Methods

This prospective cross-sectional study aimed to assess serum caspase-3 levels in patients with hypothyroidism. The study protocol was approved by the Institutional Ethical Committee (IEC KMC MLR; Approval number- 07/2024/474). Purposive sampling was used employed to recruit 183 adult patients (aged 18–60 years) whose serum TSH levels exceeding 15 mIU/mL. Exclusion criteria were applied to minimize confounding factors that could independently affect thyroid function or apoptotic activity. Patients with malignancies or those undergoing chemotherapy or steroid therapy were excluded, as these treatments can directly alter caspase-3 expression [14-16]. Individuals with autoimmune thyroid disorders, including Hashimoto's thyroiditis, were excluded to avoid variability from immune-mediated apoptosis [17]. Patients with recent surgery, trauma, or acute infections were excluded as these conditions may activate systemic apoptotic pathways unrelated to hypothyroidism [18,19]. Patients with chronic systemic illnesses were also excluded, as such conditions may affect both thyroid status and apoptosis [20].

Patients included in the study were either hospitalized (inpatients) or managed as outpatients at the time of sample collection, with serum samples obtained during routine clinical investigations. Leftover serum samples collected from the Clinical Biochemistry Laboratory were anonymized, coded, and stored at −80°C until further analysis.

Serum caspase-3 levels were quantified using the KRISHGEN Biosystems Human Caspase-3 ELISA Kit (KBH4804), based on the sandwich ELISA principle for serum or plasma. Patient sera, standards and controls were pipetted into microplate wells pre-coated with capture antibody. After incubation, the wells were washed to remove unbound materials. The detection antibody was then added, followed by a substrate solution. The reaction was stopped with sulfuric acid and absorbance was measured at 450 nm using a microplate reader. Caspase-3 concentrations were calculated from a standard curve generated by plotting known concentrations against optical density values. The KRISHGEN Biosystems kit was selected following a comparison with available commercial assays. The kit was chosen for its high sensitivity and specificity for human serum/plasma samples, with a detection range of 0.16–10 ng/mL, which covers the expected physiological concentration of caspase-3 reported in prior studies. It has been previously used in clinical research, ensuring the comparability of findings, and offering acceptable intra and inter assay variability (<8% and <10%, respectively). Local availability and technical support further supported its selection for the study.

The thyroid hormone parameters (serum total T_3, serum total T_4 and serum TSH) corresponding to the selected samples were retrieved from the Laboratory Information Management System (LIMS). The thyroid hormones were measured through

electrochemiluminescence immunoassay (ECLIA) via the Elecsys Thyroid Assay Kit (Roche Diagnostics). The intra-assay CVs for total T_3 ranged from 1.4% to 2.3% and the interassay CVs ranged from 2.2% to 3.3%. For total T_4, the interassay CVs ranged between 3.5% and 7.2%. The intra-assay CVs of TSH were between 0.7% and 3.4% and the interassay CVs were between 1.8% and 11.2%. Duplicate entries were avoided by cross-checking for age, sex and accession number. Only one record per patient was included.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies and percentages, while continuous variables are expressed as mean ± standard deviation (SD), as they are normally distributed. The chi-square test was used to assess associations between categorical variables. Comparisons of continuous variables between two groups were performed through independent t tests, whereas comparisons among more than two groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Pearson's correlation analysis was used to examine the bivariate relationships between serum caspase-3 and other variables in the overall cohort.

To account for potential confounding factors and multicollinearity, multiple linear regression models were constructed with serum caspase-3 as the dependent variable. Continuous predictors (age, serum T_3, serum T_4 and serum TSH) were mean-centered prior to analysis. Owing to high collinearity between T_3 and T_4, two separate regression models were fitted-one including T_3 and the other including T_4. Regression assumptions (linearity, homoscedasticity, independence, and normality of residuals) were assessed. Variance inflation factors (VIFs) were examined to assess multicollinearity, with values <5 considered acceptable. A two-tailed p value <0.05 was considered statistically significant.

Results

The study included 183 participants aged 18-60 years (mean ± SD: 42.64 ± 10.67), with 71% females and 29% males. The participants were classified into three TSH groups (15-20, 21-50 and >51 µIU/mL) to represent increasing biochemical severity of hypothyroidism and to assess whether gradations in TSH elevation are associated with differences in apoptotic activity.

Comparison of biochemical parameters

As expected, the serum T_3 and T_4 concentrations decreased progressively, whereas the TSH concentration increased across groups (all $p < 0.001$). Caspase-3 levels did not differ significantly between the TSH groups ($p = 0.632$) (Table 1).

Table 1: Comparison of age, thyroid function parameters (Serum T_3, T_4 and TSH) and serum caspase-3 levels among the three TSH groups.

Parameters	Group I (n=61)	Group II (n=60)	Group III (n=62)	p value#
	Mean ± SD (Min – Max)			
Age	43.74 ± 10.57 (22.00 – 60.00)	42.23 ± 10.01 (19.00 – 58.00)	41.97 ± 11.43 (18.00 – 60.00)	**0.615**
Serum T_3	0.62 ± 0.14 (0.26 – 0.86)	0.40 ± 0.11a** (0.17 – 0.63)	0.06 ± 0.04 b**, c** (0.02 – 0.21)	**<0.001**
Serum T_4	3.94 ± 0.82 (1.83 – 5.29)	2.72 ± 0.73 a** (1.31 – 4.13)	0.49 ± 0.27 b**, c** (0.14 – 1.52)	**<0.001**
Serum TSH	17.65 ± 1.72 (15.00 – 20.80)	31.62 ± 8.04 a* (21.10 – 50.90)	111.99 ± 44.13 b**, c** (51.90 – 254.00)	**<0.001**
Serum Caspase 3	0.43 ± 0.24 (0.09 – 1.28)	0.38 ± 0.26 (0.10 – 1.24)	0.41 ± 0.27 (0.17 – 1.32)	**0.632**

Abbreviations: T_3 – Triiodothyronine; T_4 – Thyroxine; TSH – Thyroid Stimulating Hormone.

#, Comparison of means across multiple groups was performed using ANOVA.

Intergroup comparisons were assessed using Tukey's post-hoc test.

Superscripts a, b and c indicate specific group comparisons, whereas * and ** denote levels of statistical significance.

a = Comparison between Group I and Group II;

b = Comparison between Group II and Group III;

c = Comparison between Group I and Group III; * $p < 0.05$; ** $p < 0.001$

Gender-based

There was no significant difference in the mean serum T_3, T_4, TSH, or caspase-3 levels between males and females ($p<0.05$ for all) (Table 2).

Table 2: Comparison of age, thyroid function parameters (Serum T_3, T_4 and TSH) and serum caspase-3 levels between sexes and age groups.

Comparison of various parameters between genders			
Parameters	**Males (n=53)**	**Females (n=130)**	**p value**
	Mean ± SD (Min – Max)		
Serum T_3	0.35 ± 0.25 (0.02 – 0.86)	0.37 ± 0.26 (0.02 – 0.87)	0.885
Serum T_4	2.37 ± 1.52 (0.20 – 5.23)	2.38 ± 1.61 (0.14 – 5.29)	0.97
Serum TSH	51.49 ± 43.88 (15.50 – 174.00)	55.29 ± 51.43 (15.00 – 254.00)	0.638
Serum Caspase-3	0.40 ± 0.28 (0.16 – 1.32)	0.41 ± 0.25 (0.09 – 1.28)	0.773
Comparison of various parameters between age groups			
Parameters	**18 – 40 years (n=74)**	**41 – 60 years (n=109)**	**p value**
	Mean ± SD (Min – Max)		
Serum T_3	0.32 ± 0.25 (0.02 – 0.87)	0.38 ± 0.25 (0.02 – 0.86)	0.115
Serum T_4	2.16 ± 1.59 (0.16 – 5.29)	2.52 ± 1.55 (0.14 – 5.23)	0.135
Serum TSH	59.80 ± 50.63 (15.00 – 217.00)	50.38 ± 48.19 (15.30 – 254.00)	0.205
Serum Caspase-3	0.41 ± 0.26 (0.19 – 1.28)	0.41 ± 0.25 (0.09 – 1.32)	0.983

Abbreviations: T_3 -Triiodothyronine; T_4 -Thyroxine; TSH - Thyroid Stimulating Hormone.

Comparison of means between two groups were performed using independent t tests.

Age groups

Participants were stratified into 18–40 and 41–60-year groups to reflect early and later adulthood, given known age-related alterations in thyroid physiology and apoptotic activity.

The serum T_3, T_4, TSH and caspase-3 levels did not significantly differ between the two groups ($p > 0.05$). While slight variations existed in the mean values (e.g., the serum T_3 and T_4 levels were slightly greater in the older group and the serum TSH level was slightly greater in the younger group), these differences were not statistically significant [Table 2].

Correlation analysis

Correlation analysis revealed that serum caspase-3 levels were not significantly related to age or serum T_3, serum T_4, or serum TSH levels among the study participants (all p values > 0.05). In the subgroup analysis, significant negative correlations were observed between caspase-3 and serum T_3 ($r = –0.319$, $p < 0.05$) and between caspase-3 and serum T_4 ($r = –0.377$, $p < 0.001$) in TSH Group I only. No significant correlations were detected in Groups II or III or when the data were stratified by sex. In the 18–40-year age group, age was negatively correlated with caspase-3 ($r = –0.262$, $p = 0.024$) (Table 3).

Table 3: Correlations between serum caspase-3 levels and age, and thyroid function parameters (T_3, T_4 and TSH) in the whole cohort and within subgroups.

In all participants		
Parameters	**Serum Caspase -3**	
	r value	**p value**
Age	-0,028	0,702
Serum T 3	-0,044	0,551
Serum T_4	-0,056	0,451
Serum TSH	-0,001	0,984

Based on TSH Groups			
TSH Groups	**Parameters**	**Serum Caspase -3**	
		r value	**p value**
Group I	Age	0,079	0,546
	Serum T_3	-0,319	<0.05
	Serum T_4	-0,377	<0.001
	Serum TSH	0,014	0,917
Group II	Age	-0,217	0,096
	Serum T_3	-0,038	0,772
	Serum T_4	-0,045	0,734
	Serum TSH	-0,068	0,604
Group III	Age	0,027	0,836
	Serum T_3	-0,061	0,635
	Serum T_4	-0,077	0,553
	Serum TSH	0,011	0,932
Based on Age Group			
Age Group	**Parameters**	**Serum Caspase -3**	
		r value	**p value**
18-40 years	Age	-0,262	0,024
	Serum T_3	-0,024	0,841
	Serum T_4	-0,025	0,829
	Serum TSH	-0,014	0,903
41-60 years	Age	0,145	0,133
	Serum T_3	-0,059	0,542
	Serum T_4	-0,078	0,419
	Serum TSH	0,008	0,937
Based on Gender			
Gender	**Parameters**	**Serum Caspase -3**	
		r value	**p value**
Males	Age	0,218	0,117
	Serum T 3	-0,156	0,266
	Serum T_4	-0,162	0,246
	Serum TSH	0,042	0,767
Females	Age	-0,117	0,184
	Serum T_3	0,002	0,981
	Serum T_4	-0,011	0,9
	Serum TSH	-0,019	0,829

Abbreviations: r-Pearson's correlation coefficient; T_3 – Triiodothyronine; T_4 – Thyroxine; TSH – Thyroid Stimulating Hormone.

When controlling for age in the partial correlation analysis, no significant associations were detected between caspase-3 and serum T_3 ($r = –0.055$, $p = 0.462$), serum T_4 ($r = –0.043$, $p = 0.563$), or serum TSH ($r = –0.002$, $p = 0.979$), indicating that age may have confounded the observed relationships.

Multiple Regression Analysis – Whole Cohort

Collinearity diagnostics revealed that serum T_3 and T_4 were highly correlated, with VIF values > 10 and tolerance < 0.2 when they were entered together in the same model. To avoid instability in the parameter estimates, two separate regression models were performed. Model 1 included age (centered), sex, TSH (centered) and T_3 (centered). Model 2 included age (centered), sex, TSH (centered) and T_4 (centered). In both models, none of the predictors demonstrated a statistically significant association with the serum caspase-3 concentration ($p > 0.05$ for all). The VIF values were less than 3, indicating that collinearity did not bias the estimates after centering. These

results suggest that neither demographic variables nor thyroid hormone levels independently predict apoptotic activity, as measured by serum caspase-3, in this cohort (Table 4).

Table 4: Multivariable linear regression models assessing predictors of serum caspase-3 levels in the whole cohort.

Variable	B	SE	β	t	p value	95% CI (Lower, Upper)	VIF
Model 1 (T_3 included)							
Constant	0,389	0,077	-	5,073	<0.001	0.238, 0.540	-
Age_c	-0,001	0,002	-0,021	-0,274	0,784	-0.004, 0.003	1,03
Sex (Female vs Male)	0,013	0,043	0,022	0,294	0,769	-0.073, 0.098	1,04
TSH_c	0	0,001	-0,094	-0,779	0,437	-0.002, 0.001	2,6
T_3_c	-0,118	0,122	-0,117	-0,965	0,336	-0.358, 0.123	2,61
Model 2 (T_4 included)							
Constant	0,388	0,077	-	5,072	<0.001	0.237, 0.539	-
Age_c	0	0,002	-0,02	-0,259	0,796	-0.004, 0.003	1,03
Sex (Female vs. Male)	0,013	0,043	0,023	0,303	0,762	-0.072, 0.099	1,03
TSH_c	-0,001	0,001	-0,127	-1,027	0,306	-0.002, 0.001	2,76
T_4_c	-0,026	0,02	-0,157	-1,26	0,209	-0.066, 0.015	2,77

Abbreviations: B = Unstandardized coefficient; SE = Standard error; β = Standardized coefficient; CI = Confidence interval; VIF = Variance inflation factor; Age_c, TSH_c, T_3_c, T_4_c = centered variables (original value minus sample mean).

Notes: Dependent variable = Serum caspase-3 (ng/mL).

Model 1: Age_c, sex, TSH_c, T_3_c.

Model 2: Age_c, sex, TSH_c, T_4_c.

No predictor was statistically significant ($p > 0.05$) and all the variance inflation factor (VIF) values were < 3, indicating no problematic multicollinearity.

Multiple Regression Analysis – Age-stratified

When stratified by age group, only age (centered) in participants aged 18–40 years was significantly associated with serum caspase-3, with older individuals showing lower levels (Model 1: B = −0.016, 95% CI −0.032 to −0.001, p = 0.038; Model 2: B = −0.016, 95% CI −0.032 to 0.000, p = 0.045). No other predictors, including sex, TSH, or thyroid hormone concentrations (T_3 or T_4), were statistically significant in either age group (all $p > 0.1$). In the 41–60-year age group, no variables were significantly associated with caspase-3. The variance inflation factors (VIF) for T_3/T_4 and their interaction terms exceeded 25 in the younger group and 90 in the older group, indicating severe multicollinearity and limiting the interpretability of these coefficients (Table 5).

Table 5: Multiple Linear Regression for Predictors of Serum Caspase-3 by Age Group.

Age Group	Variable	B	SE	β	p value	95% CI (Lower, Upper)	VIF
Model 1 (T_3 included)							
18–40 years	Constant	-0,008	0,309	-	0,978	-0.626, 0.609	-
	Sex (Female vs. Male)	0,02	0,077	0,031	0,797	-0.134, 0.174	1,042
	Age_c	-0,016	0,008	-0,415	0.038*	-0.032, -0.001	2,866
	TSH_c	0	0,001	-0,095	0,623	-0.003, 0.002	2,797
	T_3_c	-0,692	0,598	-0,677	0,251	-1.884, 0.500	25,54
41–60 years	Constant	0,535	0,293	-	0,071	-0.046, 1.115	-
	Sex (Female vs. Male)	0,003	0,053	0,005	0,962	-0.102, 0.107	1,025

	Age_c	0,013	0,009	0,248	0,162	-0.005, 0.030	3,302
	TSH_c	-0,001	0,001	-0,153	0,334	-0.002, 0.001	2,662
	T_3_c	0,451	0,943	0,448	0,634	-1.420, 2.322	93,55
Model 2 (T_4 included)							
18–40 years	Constant	-0,015	0,325	-	0,964	-0.663, 0.634	-
	Sex (Female vs. Male)	0,02	0,077	0,03	0,798	-0.134, 0.174	1,042
	Age_c	-0,016	0,008	-0,418	0.045*	-0.032, 0.000	3,121
	TSH_c	-0,001	0,001	-0,104	0,608	-0.003, 0.002	3,048
	T_4_c	-0,109	0,096	-0,662	0,26	-0.301, 0.083	25,32
41–60 years	Constant	0,569	0,312	-	0,071	-0.050, 1.189	-
	Sex (Female vs. Male)	0,003	0,052	0,005	0,956	-0.101, 0.107	1,022
	Age_c	0,014	0,009	0,269	0,144	-0.005, 0.032	3,598
	TSH_c	-0,001	0,001	-0,203	0,208	-0.003, 0.001	2,763
	T_4_c	0,075	0,153	0,452	0,626	-0.229, 0.378	92,21

Abbreviations: B = Unstandardized coefficient; SE = Standard error; β = Standardized coefficient; CI = Confidence interval; VIF = Variance inflation factor; Age_c, TSH_c, T_3_c, T_4_c = centered variables (original value minus sample mean).

Sex coded as 0 = female (reference), 1 = male.

*p < 0.05 indicates statistical significance.

Variance inflation factor (VIF) values >10 indicate severe multicollinearity; values between 5–10 suggest moderate multicollinearity.

Notes: Dependent variable = Serum caspase-3 (ng/mL).

Model 1: Age_c, sex, TSH_c, T_3_c.

Model 2: Age_c, sex, TSH_c, T_4_c.

Discussion

In this study, we examined 183 hospitalized patients with hypothyroidism and elevated TSH levels to assess the serum level of caspase-3, as a marker of apoptosis and its relationship with thyroid hormones. Hypothyroidism is increasingly prevalent in India, particularly among older adults and pregnant women, with a reported global prevalence of 2–4% [21]. Despite national salt fortification, factors such as iodine deficiency, maternal hypothyroidism, consanguinity and environmental exposure contribute to India's high burden of thyroid disease [22,23]. Our cohort reflected the expected female predominance (71%), which is consistent with previous studies [24].

The relationship between TSH levels and aging remains controversial, with prior studies showing increased, decreased, or unchanged TSH concentrations in older adults [25]. Such variability may stem from population differences, methodologies, or age-related changes in hypothalamic–pituitary–thyroid responsiveness [26]. In our study, a slight decrease in TSH levels with advancing age was observed, although thyroid hormone concentrations did not differ significantly between age groups. This finding suggests age-related changes in TSH regulation that may occur independently of peripheral thyroid hormone levels.

Thyroid hormones play a critical role in regulating cellular growth, proliferation, and programmed cell death, primarily through TSH receptor–mediated signalling pathways. These regulatory effects are highly dependent on cell type and developmental context [27]. Experimental evidence has shown that TSH can downregulate autophagy and promote apoptosis in chondrocytes, as well as induce apoptosis in thyroid follicular cells to maintain thyroid gland homeostasis [28,29]. Conversely, TSH may also exhibit anti-apoptotic effects by inhibiting Fas-mediated apoptosis and activating pro-survival signalling pathways such as MAPK/ERK and PI3K/Akt [30,31]. These contrasting actions underscore the complexity of TSH-mediated regulation of cellular fate.

The normal serum caspase-3 level has been reported to be approximately 0.2 ng/mL [32]. In our study, all three TSH-based groups showed elevated caspase-3 levels, reflecting increased apoptotic activity in hypothyroid patients. Contrary to our initial hypothesis, caspase-3 did not differ significantly across TSH groups and was not correlated with TSH, even in patients with severe hypothyroidism (TSH > 51 mIU/mL). These findings imply that TSH, despite being a useful marker of disease severity, does not directly reflect apoptotic activity. This may be related to the dual nature of TSH, which has been shown to promote apoptosis in some contexts (e.g., thyroid follicular cells and chondrocytes) [28,29] and inhibits apoptosis in others through Fas antagonism and pro-survival signalling [30,31].

At the bivariate level, thyroid hormones (particularly T_3 and T_4) were strongly associated with caspase-3 levels. In mild hypothyroidism (Group I), both T_3 ($r = –0.319$, $p < 0.05$) and T_4 ($r = –0.377$, $p < 0.001$) were inversely correlated with caspase-3, suggesting that early reductions in thyroid hormones may be linked to increased apoptosis. These results align with experimental data showing anti-apoptotic effects of thyroid hormones across multiple cell types, including cancer cells, cardiomyocytes, and neurons [33-35]. However, these associations were attenuated after adjustment for age, indicating that age acted as a confounding variable.

Age-stratified analyses provided further insight into these relationships. Among younger adults (18–40 years), advanced age was significantly associated with lower caspase-3 levels, suggesting a physiological decline in apoptotic activity with progression through early adulthood. This observation aligns with existing evidence indicating reduced apoptotic turnover with aging, reflecting lower tissue renewal demands [36]. In this age group, thyroid hormones did not remain independent predictors of caspase-3 once age was accounted for. In contrast, among older adults (41–60 years), age itself was not significantly associated with caspase-3 levels, while serum T_4 emerged as an independent predictor. This finding suggests a shift in the relative influence of age and endocrine factors on apoptotic regulation in later stages of adulthood, with thyroid hormone availability playing a more prominent role.

Collectively, these results highlight the complex and age-dependent interplay between thyroid function and apoptosis. Although caspase-3 levels were elevated across all degrees of hypothyroidism, the determinants of apoptotic activity varied by age group. Age predominated as a regulatory factor in younger individuals, whereas thyroid hormone levels - particularly T_4 - were more influential in older adults, underscoring the context-dependent nature of thyroid hormone - apoptosis interactions.

This study has several limitations. The absence of a euthyroid control group limited the ability to establish baseline caspase-3 concentrations for comparison. Additionally, incomplete clinical information regarding disease duration, aetiology, comorbidities and treatment history may have influenced caspase-3 variability. The possibility of euthyroid sick syndrome in hospitalized patients cannot be entirely excluded; however, this was minimized by restricting inclusion to patients with markedly elevated TSH levels (>15 mIU/mL), supporting the diagnosis of true primary hypothyroidism.

Despite these limitations, the study adds valuable clinical evidence to the understanding of thyroid hormone–apoptosis interactions, emphasizing the importance of age stratification when interpreting apoptotic markers in hypothyroidism. These findings provide a rationale for future mechanistic and longitudinal studies to clarify how thyroid dysfunction and aging jointly influence apoptotic pathways across the lifespan.

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Research Ethics

The research related to human use has complied with all the relevant national regulations and institutional policies and in accordance with the tenets of the Helsinki declaration and has been approved by the authors' Institutional Ethics Committee (IEC KMC MLR 07/2024/474).

Informed consent

The Institutional Ethics Committee approved a waiver of informed consent, as anonymized leftover serum samples obtained during routine clinical testing were used.

Author contributions

The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools

AJE for improving language.

Conflict of interest

The authors state that they have no conflicts of interest.

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None declared.

Data availability

The raw data can be obtained upon request from the corresponding author.

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