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Circulating Interleukin-1 and Its Association with Thyroid Function and Autoantibodies in Hashimoto Thyroiditis: A Case–Control Study

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Abstract

Background

Interleukin-1 (IL-1) may contribute to inflammatory pathways in Hashimoto thyroiditis, but disease-associated elevation does not necessarily indicate a relationship with thyroid biochemical abnormalities within affected patients.

Objective

To examine associations between circulating IL-1 and thyroid markers in Hashimoto cases and compare IL-1 concentrations with controls.

Methods

This was a case-control study of 67 cases and 67 controls in Karachi, Pakistan. 100% of the participants were female. In each case, Spearman's and exploratory age-adjusted partial correlations were performed between the within-case values for TSH, T4, T3, anti-thyroglobulin, and anti-microsomal (AM) antibodies. Holm correction was performed on each family of 5 tests. In between-group comparisons, the Mann–Whitney U test, MDS, and MDWIDI were performed. Confidence intervals derived by the bootstrap and age-adjusted median regression were used to aid interpretation.

Results

Median IL-1 was 52.06 [45.59–136.03] in cases and 30.00 [15.59–62.65] in controls, reported in provisional ng/L units pending assay verification. The distributions differed (Ucase = 3404.5; $p = 2.46\times10^{-7}$). Probability of superiority was 0.758 (95% CI: 0.668–0.839), and the median difference was 22.06 ng/L (95% CI: 14.12–39.41). The age-adjusted median difference was 22.38 ng/L (95% CI: 15.55–40.34). No within-case association survived Holm correction. TSH showed the largest adjusted coefficient (partial ρ = 0.231; 95% CI: −0.009–0.443; Holm p = 0.312); other adjusted coefficients ranged from − 0.055 to 0.036.

Conclusion

Recorded IL-1 was higher in cases, without statistically significant within-case thyroid-marker associations.

Introduction

Hashimoto thyroiditis (HT) is an organ-specific autoimmune disease that involves failure of immune tolerance, an infiltration of lymphocytes, and damage to follicles, which may lead to impaired thyroid hormone production. Although euthyroid and hypothyroid presentations share immune features based on immune activation and hormonal disturbance, the two are separate components of the disorder [1, 2]. Thyroid antibodies are useful markers for establishing the diagnosis of an autoimmune disease, but antibodies cannot completely describe clinical heterogeneity or functional impairment [3, 4].

Pathophysiology of Hashimoto's thyroiditis consists of interactions between genetic predisposition, environmental factors, immunological disturbances, and inflammatory mechanisms. Activated T-lymphocytes, B-lymphocytes, macrophages, and other immunological cells mediate the injury to thyroid follicles using cellular and humoral immune responses [4, 5]. It has been found recently that inflammation markers and cytokine signaling pathways may have some role in the process of activation of immune response and destruction of thyroid tissue [6, 7].

Since inflammatory pathways are biologically based, they are useful for the analysis of measurements other than those which are based on traditional thyroid functions. Hashimoto thyroiditis involves a critical immune imbalance between pro-inflammatory T helper 17 (Th17) cells and regulatory T (Treg) cells [8, 9]. An increased ratio of Th17 to Treg cells in both peripheral blood and thyroid tissue drives chronic inflammation and tissue destruction [10]. Single-cell analyses and immunological studies in autoimmune thyroid microenvironments frequently highlight the presence of IL-1 expression. So, all these observations position interleukin-1 (IL-1) within a wider immune system network and do not make it a single marker of thyroid dysfunction [11].

Clinical studies illustrate both the promise and uncertainty of circulating inflammatory measurements. Increased levels of circulating IL-1β were also reported in Pakistani HT patients compared to controls by Siddiq et al. [12], and elevations of IL-17 and IL-23 were reported by Bayraktar et al. [13]. However, group differences should not take the place of the ability to trace thyroid abnormalities in patients. Likewise, it is not possible to readily extrapolate the relationships of antibodies with TNF-α to IL-1 [14]. Clinically, there are conflicting reports regarding studies of the inflammatory markers in blood cells [6, 15].

Considering these previous studies, this study was designed to look at the level of IL-1 in the serum of 67 cases of HT and 67 controls with blood samples collected in Karachi. The main objective of the study was to determine within-case associations with T3, T4, TSH, anti-thyroglobulin, and anti-microsomal antibodies. Moreover, the supporting objectives were to compare IL-1 between groups and explore age-adjusted associations. The contribution is the analytical separation of disease-associated elevation from biochemical relationships within cases, addressing a clinical question which cannot be answered by tissue expression or group comparisons alone.

Research Methodology

Study design and participants

This case-control study investigated associations between circulating interleukin-1 (IL-1) and thyroid biomarkers. Participants were recruited at PNS Shifa Hospital, Karachi, Pakistan, between January 2025 and June 2026. Sample processing and biochemical analyses were performed at the Multidisciplinary Research Laboratory.

The study included 67 clinically diagnosed Hashimoto thyroiditis cases and 67 healthy controls with ages recorded in the study dataset. Cases were identified through clinical history, examination, and laboratory assessment, including TSH > 4.2 mIU/L and elevated anti-thyroglobulin and anti-microsomal antibodies (anti-thyroglobulin antibodies > 116 IU/mL, and anti-microsomal antibodies > 13.8 IU/mL).

In this study, adult women were recruited to participate. The women who were clinically diagnosed with Hashimoto thyroiditis (HT) through their medical history, physical examination, and laboratory tests, TSH level > 4.2 mIU/L, serum T3 and serum T4 level measurements, and elevated levels of anti-thyroglobulin antibody and anti-microsomal antibody were considered cases. The participants who had HT levels within laboratory reference intervals and no known autoimmune disease were taken as controls. Both study groups (cases and controls) did not differ with respect to exclusion criteria, which extended to having a history of diabetes mellitus, hepatitis B or C, rheumatoid arthritis, hepatocellular carcinoma, colorectal carcinoma, or treated thyroid dysfunction (including thyroxine therapy). To minimize the heterogeneity determined by sex-related differences in immune and thyroid physiology, recruitment was limited to female participants. The participant-selection process requires confirmation from the recruitment log.

Sample size

The sample-size calculation used the Kelsey method in OpenEpi Version 3 for an unmatched case-control comparison. Inputs consisted of a two-sided significance level of 0.05, power of 85%, control-to-case ratio of 1:1, a 40% (hypothetical) control exposure rate, and a desired odds ratio of 0.30. The software-derived case-exposure proportion was equal to 16.67%, and the number of participants in each group was 67. In this calculation, the exposure was defined as a dichotomous binary, rather than as the correlated outcome in the within-case model for IL-1.

Sample collection and biochemical measurements

Fasting venous blood was collected under aseptic conditions. Serum was separated by centrifugation at 3000 rpm for 10 minutes, aliquoted, and stored at − 80°C until analysis. IL-1 was measured using a commercial human interleukin-1 ELISA kit (E0077Hu). Concentrations were calculated using recorded optical densities and a standard curve and reported in provisional ng/L units. The assay's detection and quantification limits, analytical range, replicate procedures and quality-control results remain unverified against the applicable laboratory documentation.

Hormones T3, T4, and TSH were quantified with the use of enzyme-linked immunosorbent assay (ELISA) and chemiluminescence methods, while anti-thyroglobulin and anti-microsomal antibodies levels were determined using the chemiluminescence method. Reported reference range levels were 0.8-2.0 ng/mL for T3 and 0.8–1.8 ng/dL for T4 (assay details unverified). Optical density was used to calculate analyte concentrations.

Statistical analysis

Analyses used Python with NumPy 2.5.4, SciPy 1.18.1, pandas 3.1.0, and statsmodels 0.15.0. Mean ± sample standard deviation was used for continuous measurements, whereas median [Q1-Q3 quartiles] and minimum-maximum were used for the summary of non-continuous measurements. Linear interpolation was used to find quartiles. During the discussion of demographic characteristics, no significance testing was performed because these characteristics were the same for both instances. Histograms, normal Q-Q plots, individual point boxplots, and empirical cumulative distribution plots were examined. The Shapiro-Wilk tests were not used automatically as a method-selection rule but were used to aid in providing distributional evidence. No imputation was performed in the reported analyses, and extreme observations were retained.

The principal association analyses used the 67 cases only. The Spearman correlation was used to evaluate associations between recorded IL-1 concentration and T3, T4, TSH, and two antibodies. The partial correlations between age-adjusted ranks of IL-1 and markers, using an intercept and age ranks, were performed. Ties were given mean rankings. Two-sided unadjusted Spearman p-values were asymptotic; partial-correlation p-values used a t approximation with 64 degrees of freedom. The paired participant bootstraps used 10,000 resamples for both computational seeds, 20261102 and 20261104, that were specified in the construction of the marginal 95% confidence intervals. Age and rank were reordered within each resample.

For comparison between case-control ILs, a two-sided Mann-Whitney U test with tie correction and continuous correction was used. The 10,000 independent within-group bootstrap resamples were used to estimate probability of superiority, rank-biserial correlation, and sample median difference with seed 20261102. An age-adjusted median quantile regression was performed. For this, the additive group indicator and continuous age centered around the mean were used; a percentile confidence interval was used (2,000 within-group resamples, and seed 20261103). Linear programming was used for fitting quantile regression.

An alternate log-linear model utilized the same predictors, given HC3 robust covariance and a t-based approach, with 131 residual degrees of freedom. The exponentiated group coefficient was reported as a geometric mean ratio and as percentile intervals based on 800 within-group bootstrap resamples of seed 20261103. The residual plots and residual Shapiro-Wilk testing, Breusch-Pagan testing, and leverage and Cook's distance were explored. Observations with Cook's distance greater than 4/n were

deleted during the construction of the sensitivity analysis, and hypothetically lower concentration limits of 1 and 10 ng/L were used in the working concentration units.

Exploratory IL-1–age correlations were also analyzed separately by group using 10,000 paired bootstrap resamples and 19,999 within-group permutations (seeds 20261106 and 20261107). Absolute Spearman coefficients and a plus-one correction were used for permutation p-values. The two samples of permutation testing were corrected using Holm. All confidence intervals are based on observed measurements and do not account for uncertainty in the assays. Analytical extensions developed after data inspection or reviewer feedback were treated as exploratory. The association families were considered statistically significant if they yielded 2-sided Holm-adjusted p-values < 0.05.

Results

The participants with Hashimoto's had higher recorded levels of IL-1 than did the controls. Median IL-1 was 52.06 ng/L [Q1–Q3: 45.59–136.03] in cases and 30.00 ng/L [15.59–62.65] in controls (Table 1; Table 3). The Mann-Whitney independent sample comparison also suggested there was a difference in the distribution and ranks of recorded concentrations (U for cases = 3404.5; complementary smaller U = 1084.5; asymptotic p = $2.462751496926631 \times 10^{-7}$). The probability of superiority was 0.758 (95% CI [0.668, 0.839]), with ties receiving half weight. The corresponding rank-biserial correlation was 0.517 (95% CI [0.337, 0.679]), and the sample median difference was 22.06 ng/L (95% CI [14.12, 39.41]) (Table 4). Moreover, the distributions overlapped substantially (Fig. 1). It was noted in this study that IL-1 concentrations were right-skewed in both groups, and departures from normality remained after natural logarithmic transformation (Table 5; Figs. 2–3).

Table 1
Demographic and biochemical characteristics of Hashimoto thyroiditis cases and controls

Variable	Cases (n = 67)	Controls (n = 67)
Age, years	39.69 ± 11.73	40.69 ± 11.99
T3	2.00 [1.61–3.00]	2.50 [2.00–3.00]
T4	11.00 [9.00–12.00]	10.00 [10.00–11.00]
TSH	12.00 [8.95–18.85]	2.50 [1.33–3.50]
Anti-thyroglobulin	131.00 [123.00–158.50]	45.00 [23.50–103.50]
Anti-microsomal	45.00 [22.00–55.15]	5.00 [2.50–5.60]
Final IL-1	52.06 [45.59–136.03]	30.00 [15.59–62.65]

Age is mean ± SD; laboratory measurements are median [Q1–Q3]. Each variable has 67 observations per group. Quartiles use linear interpolation.

Table 3
Complete descriptive statistics for age and biochemical measurements in Hashimoto thyroiditis cases and controls

Variable	Cases n = 67 mean ± SD; median [Q1–Q3]; range	Controls n = 67 mean ± SD; median [Q1–Q3]; range
Age (years)	39.69 ± 11.73; 40.00 [30.00–48.00]; 20.00–60.00	40.69 ± 11.99; 41.00 [32.00–51.00]; 20.00–60.00
T3 (unit and total/free type unverified)	2.37 ± 0.92; 2.00 [1.61–3.00]; 1.06–4.40	2.40 ± 0.62; 2.50 [2.00–3.00]; 1.08–3.00
T4 (unit and total/free type unverified)	10.65 ± 2.50; 11.00 [9.00–12.00]; 5.00–16.50	10.50 ± 1.04; 10.00 [10.00–11.00]; 5.50–15.00
TSH (mIU/L)	20.09 ± 19.46; 12.00 [8.95–18.85]; 4.68–100.00	2.43 ± 1.07; 2.50 [1.33–3.50]; 0.32–3.90
Anti-thyroglobulin (IU/mL)	140.40 ± 21.39; 131.00 [123.00–158.50]; 118.00–189.00	59.21 ± 41.03; 45.00 [23.50–103.50]; 0.00–115.00
Anti-microsomal (unit unverified)	40.89 ± 19.89; 45.00 [22.00–55.15]; 14.00–85.00	4.92 ± 2.94; 5.00 [2.50–5.60]; 0.50–12.00
Final IL-1 (ng/L)	127.40 ± 141.77; 52.06 [45.59–136.03]; 1.18–511.77	61.60 ± 83.19; 30.00 [15.59–62.65]; 0.59–320.00

Table 4
Between-group comparison of circulating IL-1 concentrations and corresponding effect estimates

Supporting estimate	Value	95% CI	p
Mann–Whitney U case / smaller U	3404.5 / 1084.5	Not applicable	$2.462751496926631 \times 10^{-7}$
Probability of superiority	0.758	0.668 to 0.839	Not separately tested
Rank-biserial correlation	0.517	0.337 to 0.679	Not separately tested
Unadjusted sample median difference ng/L	22.06	14.12 to 39.41	Not separately tested
Age-adjusted conditional median difference ng/L	22.38	15.55 to 40.34	Not calculated

The left panel displays individual observations and boxplots on a logarithmic vertical axis; the right panel displays empirical cumulative distributions on the original concentration scale. Each group includes 67 participants.

Table 5
Shapiro–Wilk tests of raw and natural log-transformed IL-1 concentrations by study group

Group and scale	Shapiro W	p
Case raw	0.68431	1.02×10^{-10}
Case ln	0.85235	1.24×10^{-6}
Control raw	0.64755	2.09×10^{-11}
Control ln	0.94399	0.0046

Panels display histograms and normal Q–Q plots on both scales, individual observations with boxplots for cases and controls, and the empirical cumulative distribution among controls.

Among the 67 cases, no clear monotonic relationships were observed between IL-1 and the thyroid markers. After age adjustment, partial Spearman coefficients were − 0.055 for T3 (95% CI [− 0.308, 0.207]), − 0.034 for T4 (95% CI [− 0.264, 0.197]), 0.231 for TSH (95% CI [− 0.009, 0.443]), 0.036 for anti-thyroglobulin (95% CI [− 0.200, 0.270]) and 0.010 for anti-microsomal antibodies (95% CI [− 0.225, 0.243]). None of the five Holm-adjusted p-values was below 0.05 (Table 2B; Fig. 4). These results are based on an alternative question to the case-control comparison: do higher levels of IL-1 identify patients who are having biochemical changes or severity of Hashimoto thyroiditis?

Table 2
B. Age-adjusted partial Spearman correlations between circulating IL-1 and thyroid markers among Hashimoto thyroiditis cases

Marker	Age-adjusted partial rho [95% CI]	Adjusted p / Holm p
T3	-0.055 [-0.308, 0.207]	0.66 / 1.00
T4	-0.034 [-0.264, 0.197]	0.785 / 1.00
TSH	0.231 [-0.009, 0.443]	0.062 / 0.312
Anti-thyroglobulin	0.036 [-0.200, 0.270]	0.774 / 1.00
Anti-microsomal	0.010 [-0.225, 0.243]	0.933 / 1.00

Unadjusted analyses yielded similar results. TSH had the largest positive coefficient, although its interval included zero (ρ = 0.230; 95% CI [− 0.012, 0.441]; unadjusted p = 0.06059; Holm-adjusted p = 0.30294). The remaining unadjusted correlation coefficients ranged (− 0.065 to 0.022, Table 2A). In general, the overall pattern above showed little difference with age adjustments made (Tables 2A and 2B, Fig. 4). Substantial IL-1 variation remained at individual thyroid-marker values, without a clear monotonic pattern (Fig. 5). Both the age-adjusted and unadjusted comparisons were considered as separate groups of 5 associations each; the age-adjusted associations were used in the extended analysis to comprise the main group of associations, and the unadjusted associations were taken to also provide some

background information (Table 2A and 2B). The 95% confidence intervals are marginal and not adjusted for multiple testing.

Table 2
A. Unadjusted Spearman correlations between circulating IL-1 and thyroid markers among Hashimoto thyroiditis cases

Marker	Unadjusted rho [95% CI]	Unadjusted p / Holm p
T3	-0.042 [-0.303, 0.220]	0.733 / 1.00
T4	-0.065 [-0.290, 0.165]	0.602 / 1.00
TSH	0.230 [-0.012, 0.441]	0.061 / 0.303
Anti-thyroglobulin	0.022 [-0.208, 0.254]	0.857 / 1.00
Anti-microsomal	-0.017 [-0.245, 0.215]	0.894 / 1.00

The supporting age-adjusted log-linear model estimated a geometric mean ratio of 2.527 (HC3, t-based 95% CI [1.698, 3.761]; $p = 9.38076508 \times 10^{-6}$). The interval [1.707, 3.739] was obtained with a separate within-group bootstrap. The model estimated an age-adjusted geometric mean ratio of 2.53 for cases versus controls. This is in the same direction as the rank comparison, but these are different indicators of the properties of the concentration distributions and cannot be substituted for each other (Tables 4 and 6B; Fig. 6). Residual diagnostics showed departures from normality and clustering, warranting caution in interpreting the log-model inference. The Shapiro–Wilk test showed some non-normality in the residuals ($W = 0.92192$; $p = 9.88135086 \times 10^{-7}$) and the Breusch–Pagan test revealed no significant evidence of heteroscedasticity ($LM = 1.54102$; $p = 0.46278$). The diagnostic plots (Fig. 6) also showed the presence of a significant number of residual tail departures and clustering. Estimates of variance are improved when using HC3 (covariance) when there is heteroscedasticity, but it is not an adjustment to compensate for model misspecification or departures from the standard residuals distribution. It should then make sense of the age-adjusted median regression, which gave an estimated difference in the conditional median of 22.38 ng/L with a stratified bootstrap 95% CI of [15.55, 40.34] (Table 4). After omitting influential observations in a sensitivity analysis, the probability of superiority increased from 0.758 to 0.783, and the adjusted median difference increased from 22.38 to 25.50 ng/L (Table 6A). The adjusted geometric mean ratio changed from 2.53 (95% CI [1.70, 3.76]) to 2.47 (95% CI [1.78, 3.43]) (Table 6B).

Table 6
B. Sensitivity analyses of the age-adjusted IL-1 geometric mean ratio under observation-omission and hypothetical concentration-floor scenarios

Scenario	Adjusted GMR [HC3 CI]
All recorded values	2.53 [1.70, 3.76]
Omit Cook flags	2.47 [1.78, 3.43]
Floor below 1 ng/L	2.49 [1.69, 3.67]
Floor below 10 ng/L	2.31 [1.65, 3.22]

Panels display residuals versus fitted values, a residual normal Q–Q plot, and Cook's distance versus leverage.

Similarly, limited adjustment was made to estimates for supporting groups when making the hypothetical concentration floor adjustment. A flooring value under 1 ng/L did not affect the probability of superiority (0.758); however, a flooring value under 10 ng/L resulted in a probability of superiority of 0.759. In both scenarios, the sample median difference for the sample remained 22.06 ng/L and the adjusted sample median difference 22.38 ng/L (Table 6A). The geometric mean ratio that was adjusted for the 2 floors was 2.49 at the 1-ng/L level and 2.31 at the 10-ng/L level (Table 6B). The major interpretation of this is that IL-1 concentration was higher in cases in comparison to controls (Tables 2A, 2B and 4). This separation weakens the biological argument that the group contrast might be due to inflammatory variation associated with disease, but the measure taken does not define IL-1 as a good index of current thyroid hormone disturbance or antibody titers. The TSH interval [− 0.009, 0.443] is compatible with little association or a moderate positive association (Table 2B). The lack of association between the concentrations observed and the uncertain associations do not support a diagnostic threshold, incremental diagnostic utility, prognosis or treatment responsiveness (Tables 2A, 2B and 4).

Table 6
A. Sensitivity analyses of between-group IL-1 rank and median differences under observation-omission and hypothetical concentration-floor scenarios

Scenario	n case/control	Superiority	Median difference	Adjusted median difference
All recorded values	67 / 67	0.758	22.06	22.38
Omit Cook flags	65 / 61	0.783	22.06	25.50
Floor below 1 ng/L	67 / 67	0.758	22.06	22.38
Floor below 10 ng/L	67 / 67	0.759	22.06	22.38

Discussion

The significantly higher levels of IL-1 in the case group are consistent with the finding of Siddiq et al. [12], which showed higher circulating IL-1β in Pakistani patients with HT. Not analytically interchangeable, but this does suggest an investigation of an accompanying inflammatory difference in the context of HT. They used a cytometric bead assay, which does not appear to be the same as our ELISA, and the specific IL-1 analyte in our assay remains unverified. It is not appropriate to compare absolute concentration directly. The lack of resolved assay units and quantification limits also precludes interpretation of what may be considered a clinically validated biomarker.

The uncertain within-case relationships are consistent with Bayraktar et al. [13], who reported higher IL-17 and IL-23 levels in all HT groups and no correlations among the variables they measured in the hypothyroid group. While these were other mediators, elevated inflammatory mediators do not necessarily track thyroid biochemistry within individual patients. With a positive TSH value, our correlations with negligible and moderate were still valid. There was no significant age adjustment that aided in clarifying the timing of associations, and multiplicity correction further lent support to the interpretation of the cautions and did not support the claim that there are no known biological relationships.

The results of the antibody against the thyroid glands presented here contrast with Li et al. [14], who found a positive linkage between the thyroid antibody and cytokines (TNF-α and IFN-γ) in HT patients. This is not necessarily a conflict between inflammatory pathways and populations but rather a difference between them and IL-1. Patient factors such as clinical characteristics also play a role: Baş Aksu et al. [4] reported that antibody-positive patients had different antibody levels, and the need for treatment differed. However, antibody-based selection could mean our cases are less diverse, and other clinical differences (such as how long the disease has been present and illness state) could yet account for the different associations.

The other inflammatory markers also have inconsistent clinical patterns. Related findings were reported by Bozdag and Gundogan Bozdag [15], who reported an increase in inflammatory parameters in all, but not differentiated thyroid functional subgroups of HT. In contrast, there were no statistically significant differences in neutrophilic to lymphocytic ratio (NLR) and platelet to lymphocytic ratio (PLR) found between HFD and NWD groups of obese euthyroid participants in the study by Resber et al. [6]. The results of both studies cannot confirm or refute our cytokine results, since neither measured the levels of IL-1. In these comments, they warn against confusing systemic inflammation as a homogeneous response mechanism with a parameter that always rises with dysfunction of the thyroid gland.

There is mechanistic supporting evidence for this dissociation. Fang et al. [8] described peripheral Th17/Treg alterations, while Zhang et al. [9] identified IL-1β-expressing immune cells in thyroid tissue. These results suggest cell-to-cell interactions but do not prove that there is a given threshold of serum level to indicate an intrathyroidal injury. Circulating association is thus not an exclusion criterion for tissues. According to Wrońska et al. [11], several immune pathways are involved in HT; here no

differences between tissue-level functions are measured, and accordingly no hypothesis of a compartment is made.

The study therefore provides additional context information on the group separation along with ambivalences at the intra-case level. These findings do not establish diagnostic accuracy, prognostic value, or monitoring utility. Limited precision, incomplete assay documentation, and female-only sampling restrict generalizability. Sensitivity analyses cannot compensate for measurement error, although extremes have been verified. Repeated measurements of IL-1 could be correlated with thyroid function and clinical outcomes. Future follow-up studies should confirm the measurement used (analyte, units), and future studies could shed light on treatment and disease activity. Such a method would lead to a better relationship between inflammatory biology and clinical heterogeneity found in the current literature about HT [1, 11].

Conclusion

In this study, circulating levels of IL-1 were significantly elevated in Hashimoto women compared to controls, and there was substantial overlap between the groups. This difference was confirmed by rank-based comparisons and by a similar direction of the age-adjusted median difference. The results suggest an inflammatory contrast between the cases studied and the controls. There were no monotonous relationships of IL-1 with the hormone levels of T3, T4, TSH, anti-thyroglobulin and anti-microsomal antibodies in the case group after multiple testing and after age adjustment. However, the TSH confidence interval remained compatible with little or moderately positive association. These results should therefore be interpreted as limited evidence of biochemical tracking, rather than proof that IL-1 is unrelated to thyroid autoimmunity.

The distinction between group differences and within-case relationships is the central contribution of this investigation. Increased concentrations within affected participants do not imply either that IL-1 is a tool to help determine disease severity or that it can predict clinical outcomes. These applications would need to be validated in different clinically defined populations by suitable longitudinal designs.

Abbreviations

HT
Hashimoto thyroiditis
IL-1
interleukin-1
TSH
thyroid-stimulating hormone
ELISA
enzyme-linked immunosorbent assay
CI

confidence interval
HC3
heteroscedasticity-consistent covariance estimator.

Declarations

Author Contribution

Iram Saddiqa Aamir led the investigation, performed IL-1 ELISA assays, and drafted the manuscript. Sheikh Abdul Saeed supervised the research and critically reviewed the manuscript. Shazia Shakoor supervised the statistical analysis and finalized the results. Pervaiz Asghar provided clinical supervision and contributed to data collection and analysis. Hina Moazzam contributed to data collection and analysis. Sehar Khan performed IL-1 ELISA assays. All authors reviewed and approved the final manuscript and agree to be accountable for the integrity and accuracy of the work.

Data Availability

The datasets generated and analyzed during this study are not publicly available due to participant confidentiality and ethical restrictions but may be obtained from the corresponding author upon reasonable request, subject to institutional approval.

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Figures

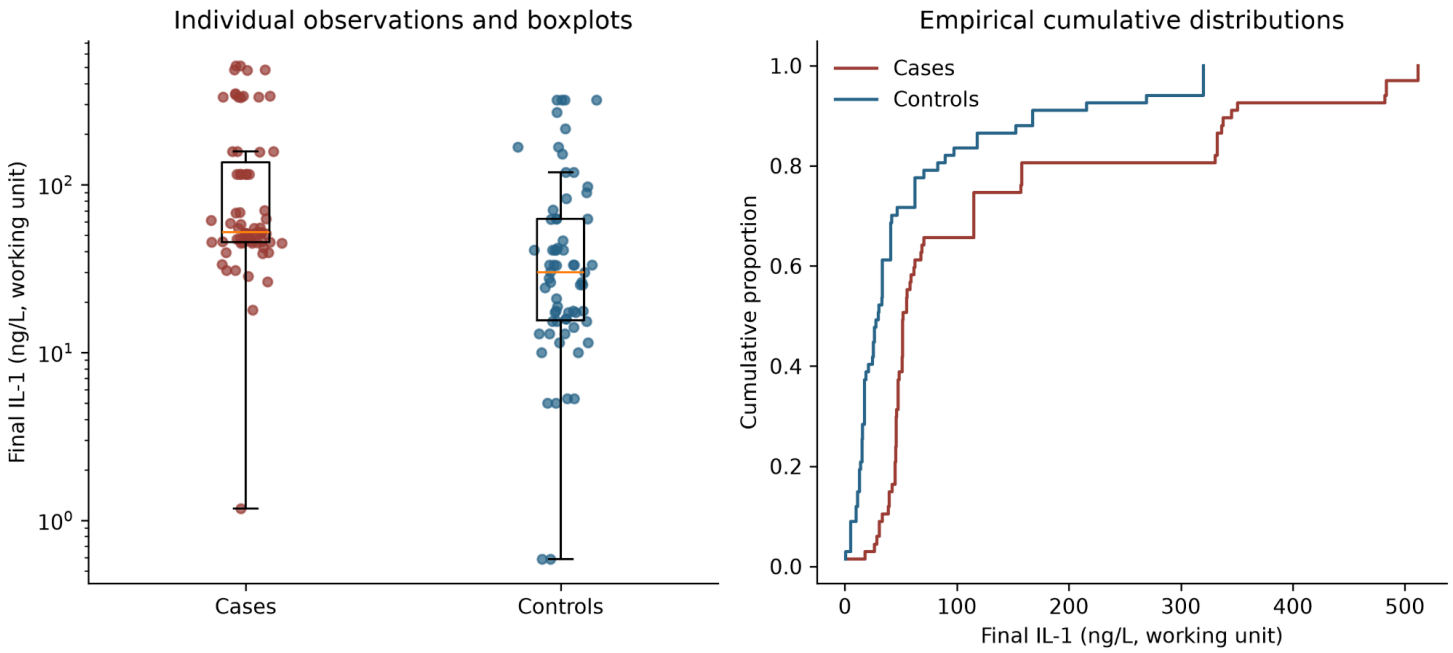


Figure 1

Distribution of circulating IL-1 concentrations in Hashimoto thyroiditis cases and controls.

The left panel displays individual observations and boxplots on a logarithmic vertical axis; the right panel displays empirical cumulative distributions on the original concentration scale. Each group includes 67 participants.

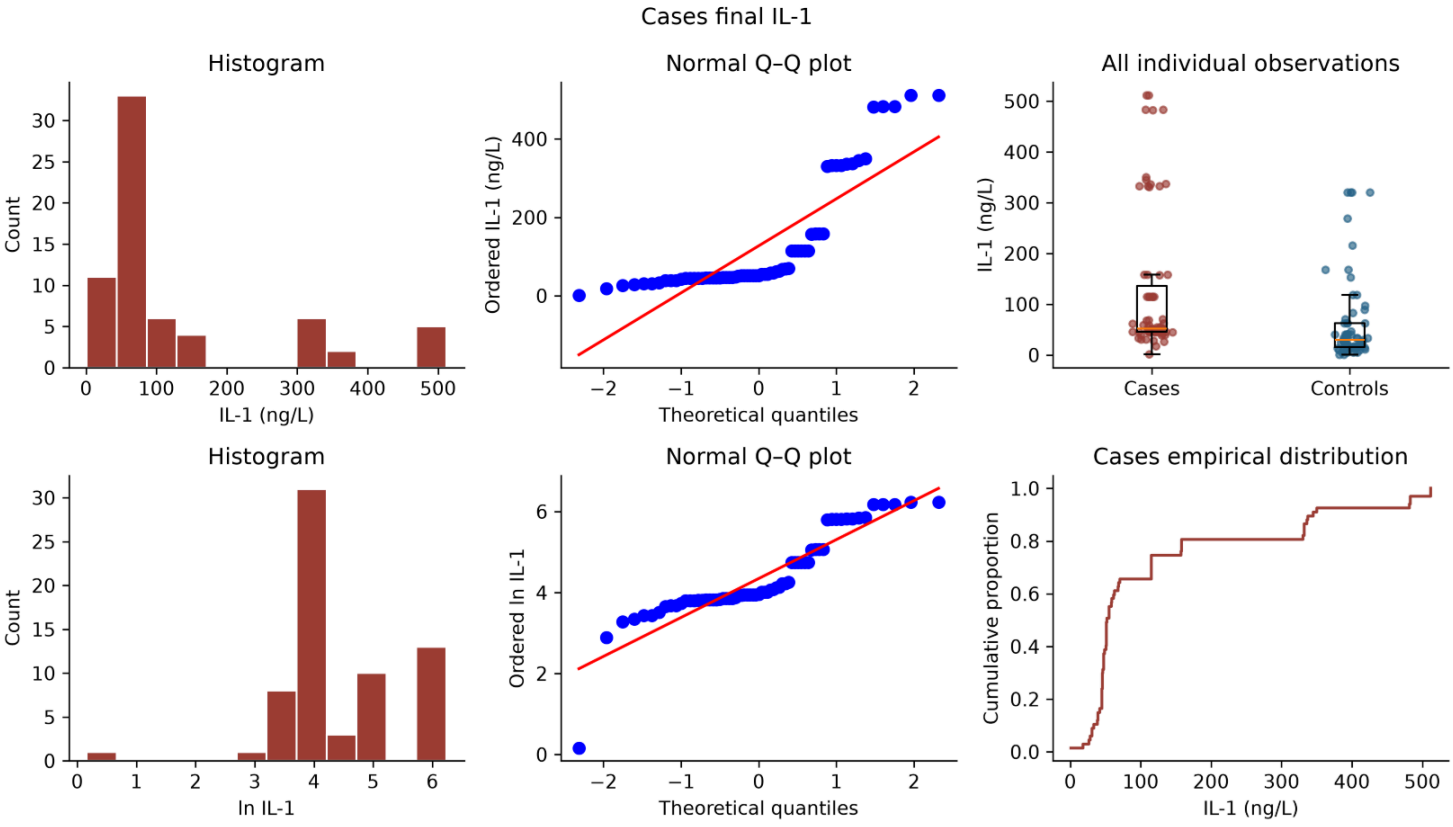


Figure 2

Distributional assessment of raw and natural log-transformed IL-1 concentrations among Hashimoto thyroiditis cases. *Panels display histograms and normal Q–Q plots on both scales, individual observations with boxplots for cases and controls, and the empirical cumulative distribution among cases.*

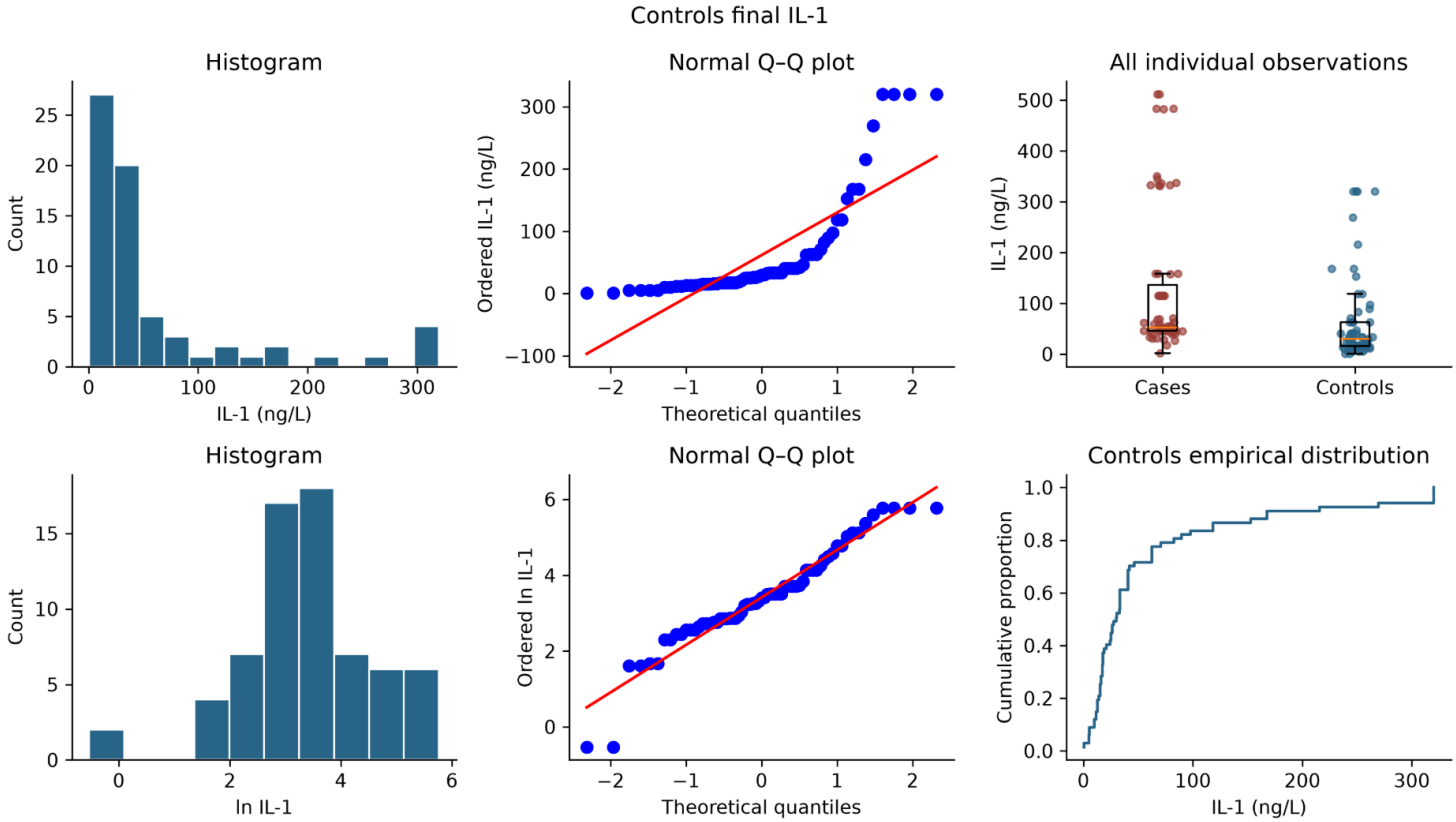


Figure 3

Distributional assessment of raw and natural log-transformed IL-1 concentrations among controls. *Panels display histograms and normal Q–Q plots on both scales, individual observations with boxplots for cases and controls, and the empirical cumulative distribution among controls.*

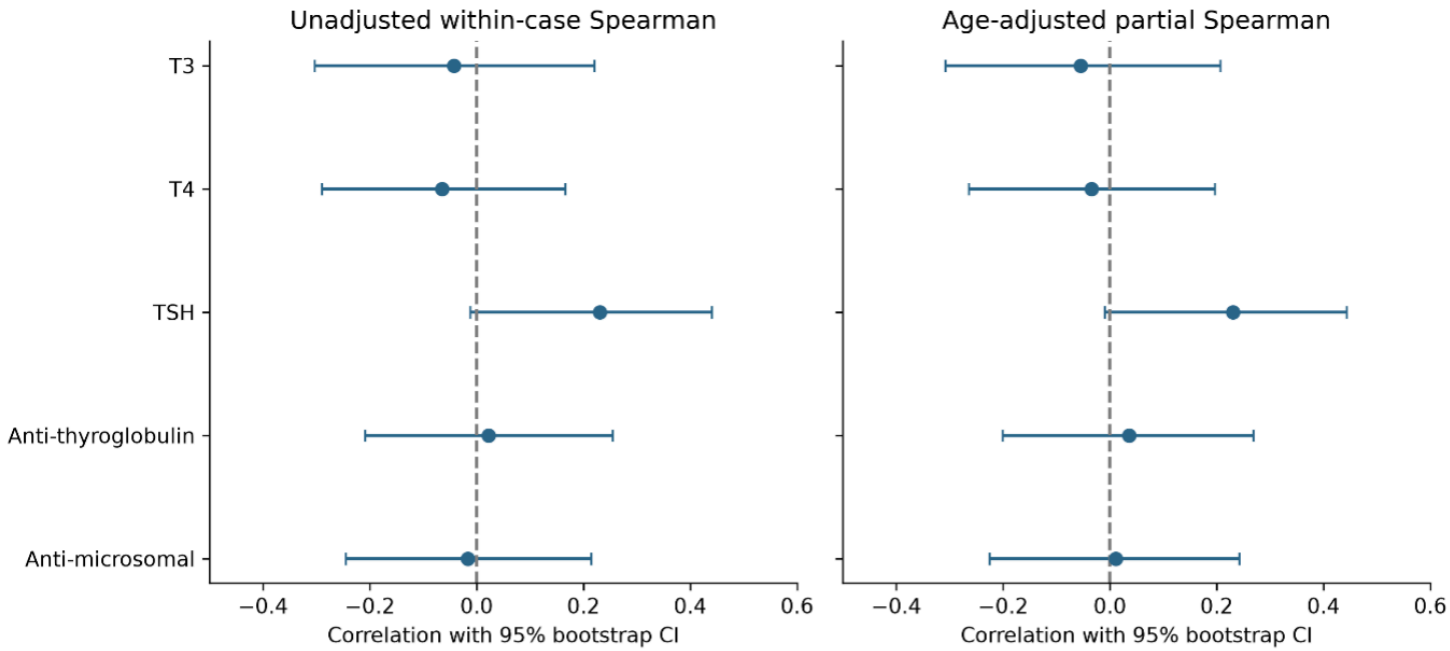


Figure 4

Unadjusted and age-adjusted associations between circulating IL-1 and thyroid markers among Hashimoto thyroiditis cases.

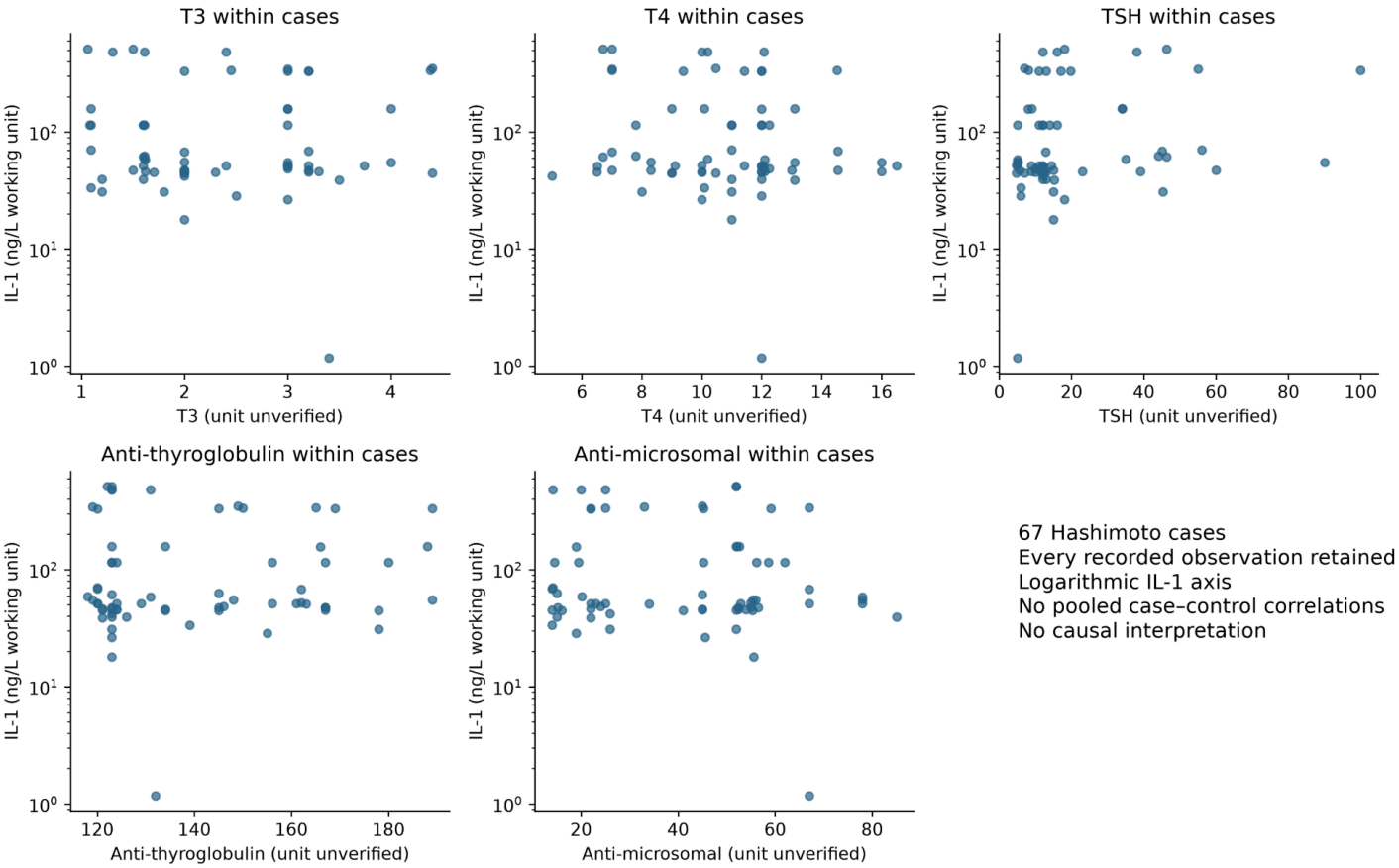


Figure 5

Scatterplots of circulating IL-1 concentrations against thyroid markers among Hashimoto thyroiditis cases. *Each point represents one participant (n = 67). IL-1 concentrations are displayed on a logarithmic vertical axis. Points may overlap where values are tied.*

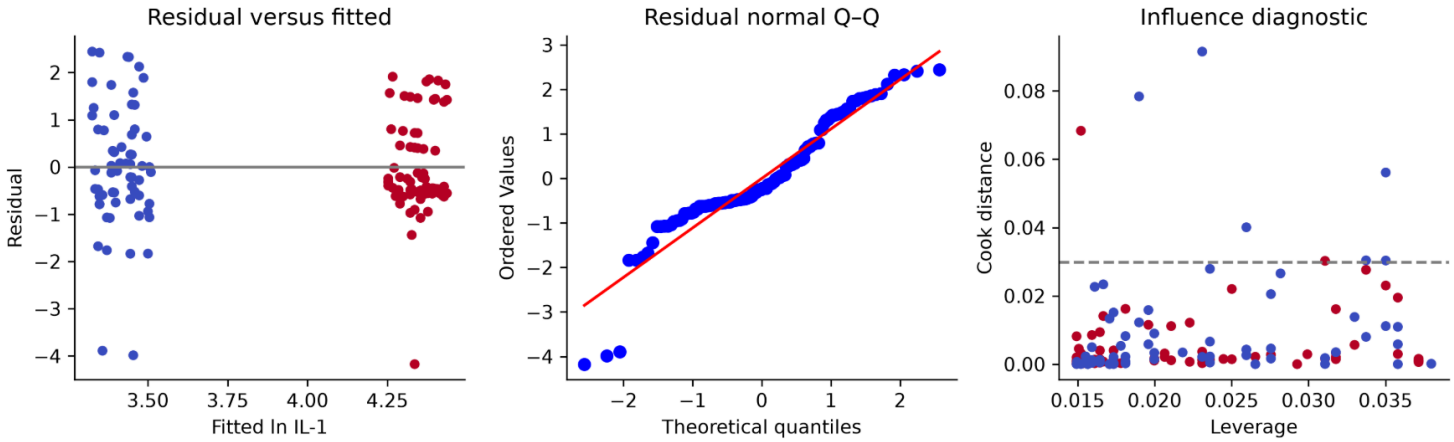


Figure 6

Residual and influence diagnostics for the age-adjusted log-linear model of IL-1 concentrations. *Panels display residuals versus fitted values, a residual normal Q–Q plot, and Cook's distance versus leverage.*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DatafileDIS.sav
- datasheet.xlsx
- IL1RESULTS.xlsx
- GraphsDIS.xlsx
- Output1DIS.spv