

Association of serum chitotriosidase enzyme levels with 24-base pair duplication variant and autoimmune thyroid disease

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Abstract

Emerging research increasingly highlights the role of chitotriosidase (CHIT1) in mediating inflammatory cascades and autoimmune dysfunction. This study sought to evaluate the association between serum chitotriosidase levels, the 24-base-pair duplication (24-bp dup) variant in exon 10 of the *CHIT1* gene, and the two main subtypes of autoimmune thyroid disease (AITD). The study cohort comprised 45 “treatment-naïve” AITD patients (categorized into Graves’ disease and Hashimoto’s thyroiditis subgroups) alongside 45 healthy euthyroid control subjects. Clinical evaluation, thyroid imaging, and complete hormone profiling (TSH, FT3, FT4), alongside serological biomarkers—thyroid peroxidase antibodies (TPO Ab) and thyrotropin receptor antibodies (TRAb)—were used to diagnose AITD and its subtypes. Serum CHIT1 concentrations were determined via sandwich enzyme-linked immunosorbent assay (ELISA), while the *CHIT1* gene was assessed for the 24-base pair duplication (24-bp dup) polymorphism using polymerase chain reaction (PCR). Serum CHIT1 concentrations were significantly elevated in the AITD group compared to controls ($p < 0.001$), with significant elevations observed in both Graves’ ($p < 0.001$) and Hashimoto’s ($p = 0.015$) subtypes. However, no significant difference was observed in CHIT1 concentration between the two AITD subtypes ($p = 0.994$). Participants heterozygous for the CHIT1 24-base pair duplication exhibited significantly lower serum CHIT1 concentrations than the wild-type homozygotes across all the study population ($p < 0.001$). In conclusion, serum chitotriosidase levels are significantly associated with both autoimmune thyroid disease and the allelic distribution of the CHIT1 exon 10 24-bp dup variant. It should be emphasized that prospective studies with larger cohorts, expanded genotyping studies and appropriate diagnostic performance analyses are needed to establish clinical applicability.

Keywords: Chitotriosidase, AITD, Graves’ disease, Hashimoto’s thyroiditis, 24-base-pair duplication.

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Introduction

Autoimmune thyroid disease (AITD) comprises two main clinical manifestations: hypothyroid

autoimmune thyroiditis (Hashimoto’s disease) and hyperthyroid Graves’ disease, as well as subclinical thyroid dysregulation. The underlying immunomodulatory mechanisms in AITD

pathogenesis are complex and remain incompletely understood, involving multiple environmental, inflammatory and genetic factors that result in immune tolerance dysfunction.¹ Further investigation into these immunomodulatory mechanisms with the genetic predispositions is essential to develop pathophysiological understanding and personalized management of AITD.²

Chitotriosidase enzyme (CHIT1), a glycosyl hydrolase which belongs to the chitinases,³ was originally detected in high levels in the plasma of patients with Gaucher disease, an inflammatory lysosomal storage disease.⁴ Human chitotriosidase chitinolytic effect has a protective role against the chitin-containing organisms engulfed by the macrophages.^{5,6}

Chitotriosidase has a significant role in the activation of the adaptive and innate immune systems.^{7,8} Previous studies focused on chitotriosidase immunomodulatory influence represented in induction of cell-mediated immunity, activation of macrophages, antigen presentation,^{6,9,10} as well as indirect enhancement of immune cells such as eosinophils and T-helper cells^{5,6} through stimulation of collagenase type I, eotaxin and Interleukin-8.^{11,12} Given its involvement in inflammatory signaling pathways and the innate immune system,^{13,14} chitotriosidase role in human inflammatory diseases has been widely investigated.^{15,16}

A common insertion mutation of 24-base pair duplication (24-bp dup) in exon 10 (rs150192398) of the human chitotriosidase (*CHIT1*) gene,¹⁷ introducing defective splicing and deletion of 87 nucleotides in mRNA, is responsible for identified enzyme deficiencies in various populations.^{18,19}

This study investigated the association between serum chitotriosidase enzyme concentration with *CHIT1* gene 24-bp dup variant and their possible implication in autoimmune thyroid disease.

Patients and Methods

Study Cohort

The Clinical Pathology and Internal Medicine Department at Beni-Suef University Hospital

jointly executed the current study. The study population (N = 90) included a control group of 45 apparently healthy euthyroid participants recruited from the Blood Bank and 45 AITD patients, naïve to treatment, enrolled from the Endocrinology Outpatient Clinic, Beni-Suef University Hospital. Patients with autoimmune thyroid disease were further categorized into 22 patients with Graves' disease and 23 patients with Hashimoto's thyroiditis whose diagnosis was based on the American Thyroid Association guidelines (20,21). Exclusion criteria included patients with known diagnosis of diabetes mellitus, multinodular goiter, thyroid carcinoma, chronic liver disease, or renal impairment. Additionally, participants were excluded if they had history of autoimmune, inflammatory, or infectious disorders, or long-term medications known to interfere with thyroid function.

Methodology

Clinical Assessment

Comprehensive clinical assessments were performed for all participants, encompassing physical evaluations alongside detailed medical history. Imaging protocols consisted of thyroid ultrasonography supplemented by color Doppler imaging to evaluate intrathyroidal vascularity and a technetium-99m thyroid uptake scan.

Baseline Laboratory Investigations

Physical evaluations and imaging were paired with baseline laboratory investigations, which included a complete blood count (CBC), serum creatinine, liver function tests and a complete thyroid hormone profile (TSH, FT3, FT4). The presence of autoimmune thyroiditis was evaluated in all patients in the cohort through quantification of circulating titres of thyroid peroxidase antibodies (TPO Ab) and thyrotropin receptor antibodies (TRAb). In summary, serological biomarkers, clinical features, and imaging were used according to the established global standards to diagnose AITD and its subtypes.^{20,21}

Extraction of DNA and Genotyping of the CHIT1 Duplication

High-purity genomic DNA was isolated from peripheral whole blood specimens using the QIAamp DNA Blood Mini Kit (Cat. No. 51104, QIAGEN, Hilden, Germany). Identification of the 24-base-pair (bp) insertion allele within exon 10 of the human chitotriosidase gene (*CHIT1*, rs150192398) was carried out via Polymerase Chain Reaction (PCR) followed by size-fractionation electrophoresis, following methodological principles outlined by Lee, et al. 2007.²²

Oligonucleotide primers targeting the region encompassing the duplication polymorphism were synthesized with the following sequences: forward 5'-GAAGAGGTAGCCAGGCTCTGG-3' and reverse 5'-CTGCCGTAGCGTCTGGATGAG-3'.

Assay mixtures were prepared to a final volume of 10 µL, comprising 1× PCR buffer, 1.5 mM magnesium chloride (MgCl₂), 0.4 mM dNTP mix (Cat. No. 201900, QIAGEN), 0.8 µM of each oligonucleotide, 0.03 U/µL HotStarTaq Plus DNA Polymerase (Cat. No. 203603, QIAGEN), and 15–30 ng of template DNA. Assay reliability was verified by incorporating non-template controls (NTCs) into each amplification run to screen for potential reagent contamination. Thermal amplification was conducted under the following parameters: an initial enzyme activation phase at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and chain extension at 72°C for 45 s; followed by a final extension stage at 72°C for 5 min. Amplicons were electrophoretically size-fractionated on a 3% high resolution agarose gel matrix containing SYBR Safe Gel Stain (Cat. No. S33102, Thermo Fisher Scientific, Waltham, MA, USA) and detected via UV transillumination. Genotypic classification relied on fragment length disparity: the 75-bp product indicated the intact wild-type (Wt) allele, while the 99-bp fragment confirmed the presence of the 24-bp dup variant. Quality control for the genotyping assays was validated through negative controls and re-testing of 5% of random selection of blind duplicate samples, all of which demonstrated 100% concordance.

Quantification of Serum Chitotriosidase 1 by Enzyme-Linked Immunosorbent Assay

Serum chitotriosidase-1 (CHIT1) levels were quantified via a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Cat. No. SBR50420, Assay Genie, Dublin, Ireland) in accordance with the manufacturer's protocol. Briefly, calibrators and serum specimens were added to microplate wells coated with anti-human CHIT1 monoclonal antibody, capturing the target analyte on the solid matrix. After clearing unbound sample matrix via washing, a biotinylated secondary antibody specific to human CHIT1 was incubated in the wells. Excess detection antibody was rinsed away, and horseradish peroxidase (HRP)-labelled streptavidin was introduced to form active enzyme complexes. Following a final wash sequence to remove residual conjugate, tetramethylbenzidine (TMB) substrate was added to initiate colorimetric development, yielding a signal intensity directly corresponding to captured CHIT1 concentration. The reaction was terminated with an acidic stopping reagent, causing a color conversion from blue to yellow, and absorbance was measured spectrophotometrically at a primary wavelength of 450 nm.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation or median with range, depending on distribution. Categorical variables were summarized using frequencies and percentages. To analyze the association between qualitative parameters, Pearson's Chi-square was applied. For two-group comparisons of continuous data, the Student's t-test was utilized for parametric data, while the Mann-Whitney U test was used for non-parametric data. Variations across three groups were evaluated using Analysis of Variance (ANOVA) or the Kruskal-Wallis test for parametric and non-parametric datasets, respectively, followed by post-hoc testing for pairwise evaluation. All statistical assessments were two-tailed, with a *p*-value (<0.05) defining statistical significance. Finally, factors demonstrating significance in the

univariate analysis were entered into a multiple linear regression model to identify independent predictors of chitotriosidase enzyme levels, adjusting for age and gender. Multivariate outcomes were reported as regression coefficients alongside their standard errors.

Results

Descriptive Data of the Study Population:

Descriptive analysis (demographic, clinical, radiological, and laboratory manifestations) of

the studied groups [Graves' disease, Hashimoto's thyroiditis and control] is outlined in Table 1. The total study population comprised ninety participants.

Thyroid function markers (TSH, FT3, FT4), iodine uptake and exophthalmos manifestations showed highly significant differences across the different groups [Graves' disease, Hashimoto's thyroiditis and control]; $p < 0.001$ (Table 1).

Table 1. Illustration of the descriptive data across the participant groups.

	Graves' disease (N = 22)	Hashimoto's thyroiditis (N = 23)	Control (N = 45)	*p-value
Sex (Count, %)				
Male	8 (36.4%)	5 (21.7%)	13 (28.9%)	NS
Female	14 (63.6%)	18 (78.3%)	32 (71.1%)	
Age (years) Mean $\pm$ SD	42.62 $\pm$ 6.98	40.33 $\pm$ 8.38	38.76 $\pm$ 10.41	NS
Clinical findings (Count, %)				
Pretibial myxoedema	3 (13.6%)*	0 (0.0%)	0 (0.0%)	NS
Exophthalmos	19 (86.4%)*	0 (0.0%)	0 (0.0%)	<0.001
Radiological findings				
Thyroid scan				
Iodine uptake (Count, %)				
Increased	21 (95.5%)	0 (0.0%)	0 (0.0%)	<0.001
Normal	1 (4.5%)	0 (0.0%)	45 (100.0%)	
Reduced	0 (0.0%)	23 (100.0%)	0 (0.0%)	
Pattern of uptake (Count, %)				
Diffuse	17 (77.3%)	0 (0.0%)	0 (0.0%)	<0.001
Localized	4 (18.2%)	23 (100.0%)	0 (0.0%)	
Normal	1 (4.5%)	0 (0.0%)	45 (100.0%)	
Laboratory findings				
Creatinine (mg/dL) Mean $\pm$ SD	0.87 $\pm$ 0.22	0.83 $\pm$ 0.22	0.85 $\pm$ 0.25	NS
Thyroid function Median (IQR)				
TSH (μ U/mL)	0.04 (0.49)	6.65 (2.55)	2.8 (1.50)	<0.001
FT3 (pg/mL)	4.00 (5.20)	1.48 (1.03)	2.0 (0.80)	<0.001
FT4 (ng/dL)	4.50 (4.10)	0.48 (0.93)	1.4 (0.50)	<0.001

Normally distributed continuous variables (age and creatinine) were compared using one-way ANOVA, whereas thyroid function results were analyzed using the Kruskal-Wallis test. Categorical variables (sex, clinical findings, and thyroid scan results) were evaluated using Pearson's chi-square test and Fisher's Exact Test. IQR: interquartile range was calculated using the difference between the 75th percentile (Q3) and the 25th percentile (Q1). * $p > 0.05$ is not significant (NS)..

CHIT1 Genotype Distribution among the Studied Groups

The homozygous wild-type genotype (Wt/Wt) was more prevalent (70.8% in Hashimoto's and 71.4% in Graves' subgroups and 62.2% of the control group) than the heterozygous genotype (Wt/24-bp dup). On the other hand, no

homozygous mutant genotype (24-bp dup/24-bp dup) was detected in the study population. No significant difference was detected in the CHIT1 24-bp dup allelic distribution when comparing the control group to the overall autoimmune thyroid disease group ($P = 0.37$; Figure 1A) or its specific subtypes, Graves' and Hashimoto's diseases ($p = 0.67$; Figure 1B).

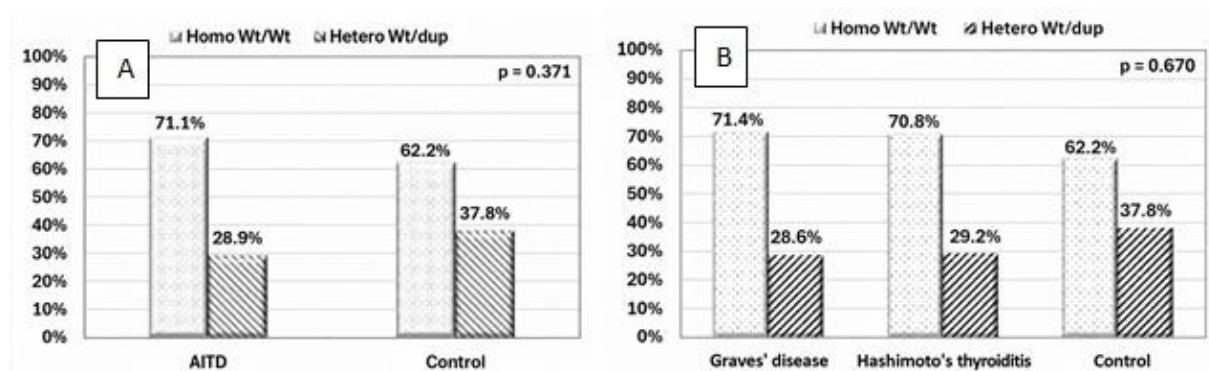


Figure 1. Genotype prevalence among the different studied groups. Diagram [A] illustrates genotype distribution in autoimmune thyroid disease (AITD) & control groups; diagram [B] illustrates genotypic distribution among Graves' disease, Hashimoto's thyroiditis and control groups.

Univariate Analysis of Serum Chitotriosidase Concentration Predictors

Association of Serum Chitotriosidase Concentration with Autoimmune Thyroid Disease and its Subtypes

A significantly higher chitotriosidase concentration was noted in autoimmune thyroid disease ($p < 0.001$; Figure 2A) and its

subtypes [Graves' disease ($p < 0.001$) and Hashimoto's thyroiditis ($p = 0.015$); Figure 2B], compared to the control group.

No statistically significant difference in chitotriosidase concentration was detected when comparing Hashimoto's disease with Graves' disease. ($p = 0.944$; Figure 2B).

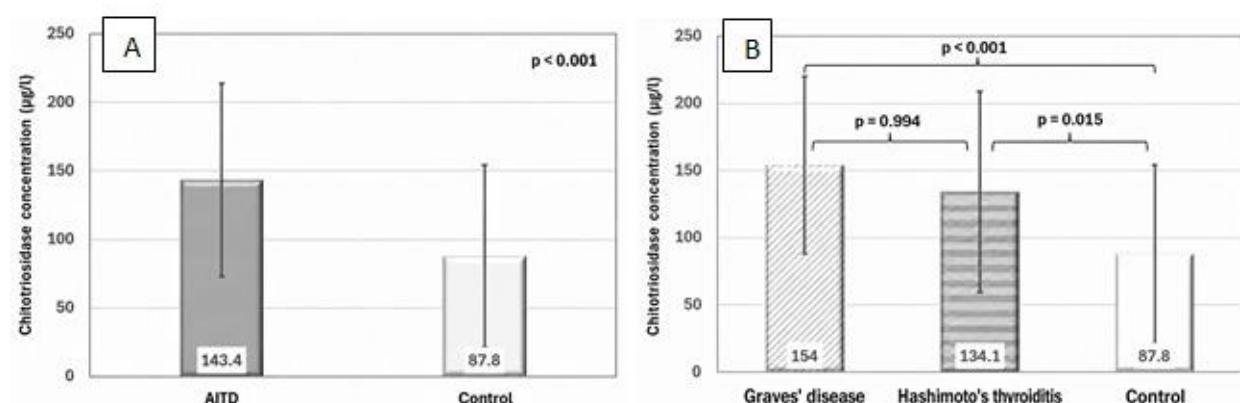


Figure 2. Serum chitotriosidase concentration across the study groups. (A) comparison of Chitotriosidase concentrations between autoimmune thyroid disease (AITD) & control groups; (B) Comparison of chitotriosidase concentrations across Graves' disease, Hashimoto's thyroiditis and control groups.

Association of Serum Chitotriosidase Concentration with *CHIT1* Genotype (24-Base Pair Duplication Variant)

Serum chitotriosidase levels were significantly lower in the heterozygous genotype (Wt/24-bp dup) than the homozygous wild-type genotype (Wt/Wt) across the entire study population ($N = 90$, $p < 0.001$; Figure 3) and within all studied groups; autoimmune thyroid disease ($P < 0.001$), control group ($p < 0.001$), Hashimoto's thyroiditis group ($p = 0.003$), Graves' disease group ($p = 0.002$), (Table 2). Overall, chitotriosidase levels showed significantly lower levels in participants carrying the 24-bp dup variant. No mutant homozygous genotype was detected in the entire study population.

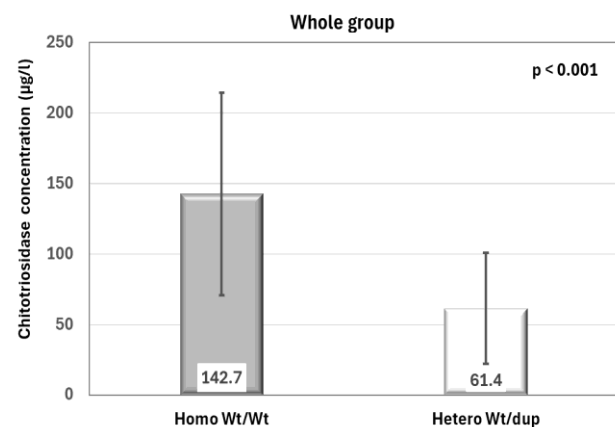


Figure 3. Comparison of chitotriosidase concentration according to *CHIT1* genotype in the total study population.

Table 2. Serum chitotriosidase concentration across the study groups stratified by *CHIT1* genotype.

Chitotriosidase concentration (pg/ml)	Genotype		* <i>p</i> -value
	Homozygous (Wt/Wt)	Heterozygous (Wt/24-bp dup)	
AITD Median (IQR)	171.00 (93.30)	75.00 (56.60)	<0.001
Graves' disease Median (IQR)	178.10 (61.45)	96.85 (60.25)	0.002
Hashimoto's thyroiditis Median (IQR)	165.40 (99.60)	74.00 (32.35)	0.003
Control Median (IQR)	75.00 (91.3)	29.80 (36.4)	<0.001

**p*-value <0.05 is considered significant. Mann-Whitney U test was used to compare CHIT1 concentration according to the genotypes. IQR: interquartile range was calculated using the difference between the 75th percentile (Q3) and the 25th percentile (Q1).

Association of Serum Chitotriosidase Concentration with the Laboratory Parameters

Serum creatinine concentrations showed no statistically significant difference across the studied groups: Hashimoto's thyroiditis, Graves' disease and control group (Table 1, $p = 0.884$). However, a weak, statistically significant correlation was observed between the serum concentration of chitotriosidase and creatinine

($r = 0.22$, $p = 0.035$) in the entire study population ($N=90$).

No correlation was detected between serum chitotriosidase concentration and TPO Ab or TRAb antibody titres in any of the studied groups: Hashimoto's disease ($p = 0.308$ and $p = 0.104$, respectively), Graves' disease ($p = 0.804$ and $p = 0.652$, respectively), and overall AITD ($p = 0.25$ and $p = 0.955$, respectively).

Moreover, serum chitotriosidase concentration showed no significant correlation with the hormone profile in patients with AITD: FT3 ($p = 0.08$), FT4 ($p = 0.13$) and TSH ($p = 0.15$) and in the control group: T3 ($p = 0.45$), FT4 ($p = 0.24$) and TSH ($p = 0.84$).

Multivariate Analysis of Serum Chitotriosidase Predictors

Multiple linear regression was conducted to identify independent predictors of serum chitotriosidase concentration using variables

found to be significant in univariate analysis. The model included autoimmune thyroid disease, age, sex, *CHIT1* 24-bp dup genotype (homozygous wild-type, heterozygous), and serum creatinine as predictor variables, with chitotriosidase concentration as the dependent variable (Table 3). Serum chitotriosidase levels were independently associated with *CHIT1* genotype ($p < 0.001$), autoimmune thyroid disease ($p < 0.001$), and serum creatinine ($p = 0.008$).

Table 3. Multiple linear regression analysis of independent predictors of serum chitotriosidase concentration.

Variables Entered	$B \pm SE$	p -value
Autoimmune thyroid disease (Graves' disease & Hashimoto's thyroiditis)	50.497 ± 12.050	<0.001
<i>CHIT1</i> Genotype (homozygous/heterozygous)	-82.410 ± 12.824	<0.001
Creatinine (mg/dL)	69.908 ± 25.611	0.008
Age (years)	-0.980 ± 0.659	NS
Sex	-5.676 ± 13.159	NS

Dependent Variable: chitotriosidase concentration (pg/mL).

Predictors: Autoimmune thyroid disease, *CHIT1* genotype, Creatinine, Age, Sex.

Note: B, unstandardized regression coefficient; SE, standard error.

$p > 0.05$ is not significant (NS).

Discussion

Chitotriosidase enzyme, produced mainly by activated macrophages, plays a significant role in adaptive and innate immunity.¹⁶ Consequently, many studies^{23,24} explicated chitotriosidase implications in an increasing number of inflammatory based autoimmune diseases.^{16,23,24} Expanding data has been relating immune dysregulation, immune cell infiltration, macrophage activation and chronic inflammation to autoimmune thyroid disease (AITD).² To our knowledge, this is the first study to conduct a joint evaluation of serum chitotriosidase enzyme concentrations and *CHIT1* 24-base pair duplication polymorphism in Graves' disease and Hashimoto's thyroiditis. Serum chitotriosidase enzyme level was significantly elevated in AITD group, Graves' (G group) and Hashimoto's (H group) when each of these groups was compared to the control group; $p < 0.001$, $p < 0.001$ and $p = 0.015$, respectively. However, no significant difference

in the serum chitotriosidase concentration was detected between the two AITD subtypes ($p = 0.944$).

Two studies evaluated chitotriosidase enzyme in thyroid gland disorders. The first one was Ozisik et al, 2023 study,²⁵ whose enrollment included only patients with euthyroid Hashimoto's disease in comparison to ours where both Hashimoto's and Graves' disorders were recruited and no euthyroid state was involved. In agreement to our study, a significant elevation in chitotriosidase enzyme was detected in Hashimoto's patients compared to the control group which was interpreted as an indicator of inflammation.²⁵ The finding that serum chitotriosidase was elevated in treatment-naïve patients might point to the evidence that Chitinases were significantly expressed in the early stages of autoimmune diseases.¹⁶ The second study, by Edral et al, 2008²⁶ demonstrated an increase of chitotriosidase level in a different cohort of

patients with subclinical hypothyroidism who received Levothyroxine. The elevated serum chitotriosidase on achievement of the euthyroid state suggested that levothyroxine therapy might activate macrophage pathways as stated by Edral et al, 2008²⁶

In our series, the different subtypes of AITD expressed no significant statistical discrimination in serum chitotriosidase concentrations. The small sample size may have decreased the statistical power needed to detect the minor variations in serum chitotriosidase concentrations between the two disease subtypes. Nevertheless, Graves' disease and Hashimoto's thyroiditis belong to the same spectrum of autoimmune thyroid disease (AITD) and are considered variants of a common inflammatory process. Furthermore, the literature and evidence-based research support a clear genetic link between these two disorders, which can occur simultaneously within the same individual.^{1, 27, 28} Consequently, this genetic background may help explain the absence of a significant difference in serum chitotriosidase concentrations between Graves' disease and Hashimoto's thyroiditis.

The premise of investigating chitotriosidase in autoimmune thyroid disease (AITD) was grounded in emerging evidence highlighting CHIT1 as an inflammatory biomarker across diverse infectious, inflammatory, and autoimmune conditions.¹⁶ These include sarcoidosis,²³ chronic obstructive pulmonary disease (COPD), bronchial asthma,²⁴ atherosclerosis,²⁹ non-alcoholic fatty liver disease,³⁰ juvenile idiopathic arthritis,³¹ malaria³² and Gaucher disease.⁴ Chitotriosidase-1 (CHIT1) exerts opposing effects depending on its cellular source and the underlying pathology. While Kupffer cell-derived CHIT1 can trigger hepatic fibrosis, the enzyme also confers host defense against chitin-containing pathogens during infection.³³ Internationally, chitinases are increasingly applied as biomarkers—notably for assessing sarcoidosis and fever of unknown origin in Italy and monitoring chronic hepatitis induced fibrosis in China.³⁴ Continued expansion of knowledge in this research field is highly anticipated.¹⁶

The utility of chitotriosidase as an inflammatory marker is limited by genetic variants in the CHIT1 gene that affect enzyme activity and concentration.³⁵ We genotyped the most common variant, a 24-bp dup in exon 10 of *CHIT1* coding gene (rs150192398). In our study cohort, serum chitotriosidase levels were significantly reduced in heterozygous carriers of the *CHIT1* 24-bp dup variant across AITD patients, AITD subtype groups, and healthy controls. These results aligned with previous studies demonstrating that the wild-type homozygotes exhibit the highest mean enzyme concentrations, whereas heterozygous individuals display intermediate levels.^{24, 35} Furthermore, while literature indicates that mutant homozygotes for 24-bp dup (rs150192398) revealed a total deficiency of chitotriosidase enzyme,²⁴ this could not be verified in our study, as no mutant homozygotes were identified, likely due to the limited sample size. Overall, these findings corroborate the association between *CHIT1* 24-bp dup genotype and reduced chitotriosidase enzyme concentration.^{24,35} Relying on chitotriosidase to monitor inflammation in AITD patients without accounting for the *CHIT1* 24-bp dup variant may yield false negative results. Therefore, *CHIT1* genotyping should be integrated into the interpretation of the enzyme measurements, and establishing genotype-dependent chitotriosidase reference ranges is essential to optimize its clinical utility.^{35,36}

In the current study, serum CHIT1 concentration was quantified using ELISA assay. Although ELISA measures the protein concentration rather than the direct enzymatic activity, prior work by, Csongrádi et al, 2021³⁵ supports the reliability of this technique. Specifically, they confirmed a strong association between CHIT1 concentration and enzymatic activity measured via fluorogenic assays ($r^2 = 0.846$, slope = $0.979 \text{ mU}/\mu\text{g}$). Furthermore, Csongrádi et al, 2021.³⁵ demonstrated that *CHIT1* 24-bp dup polymorphism is consistently associated with both CHIT1 activity and concentration.³⁵ These validations justify the use of the ELISA assay measuring serum CHIT1 concentration as a robust and reliable approach.

Allelic distribution of CHIT1 24-base pair duplication polymorphism shows significant variation across different populations according to their ethnicity and geographical locations.³⁵ The association of CHIT1 deficiency and 24-bp dup necessitates the recognition of the allelic frequency, homozygosity and heterozygosity of the 24-bp dup in different populations to verify CHIT1 usage as an inflammatory marker.^{30, 35} In the present study, the 24-bp dup was identified in a heterozygous genotype in 37.8 % of the control population and 28.9% of the AITD group with no significant difference of the genotypic distribution between the two groups ($p = 0.37$). Close heterozygous genotype prevalence (34.4%) was noted in an Egyptian population studied by Elmonem et al, 2016.³⁷ Different allelic frequencies of CHIT1 24-bp dup polymorphism was presented in various studies in many populations; 42.7% in Portuguese population,³⁸ 23% in North America (the USA),³⁹ 20% in Europe (Dutch population),⁴⁰ 32% in South America (Brazilian population),⁴¹ and 58% in Asia (Chinese Han population).⁴² However, this polymorphism was non-existent in black south Africa (in the Republic of Benin) with 1.6% prevalence of CHIT1 deficiency due to the presence of other mutations.⁴³

None of our study populations exhibited homozygosity for the 24-base pair duplication. In contrast, CHIT1 enzyme was completely deficient due to mutant homozygosity in about 6–21% of the Caucasians³⁵ and 5.11% in a previous Egyptian study.³⁷ The small number of populations enrolled in these studies might explain the variation in prevalence of the mutant homozygosity. Cross sectional studies with larger numbers of population are needed to validate the prevalence of mutant homozygosity.

Elmonem et al investigated the association between chitotriosidase enzyme activity and renal function across two studies.^{17, 44} Patients with chronic kidney disease demonstrated mild-to-moderate elevations in chitotriosidase activity.⁴⁴ In addition, chitotriosidase activity was significantly correlated with nephropathy progression, urinary albumin to creatinine ratio and the estimated glomerular filtration rate (eGFR).¹⁷ Consequently, creatinine was included

in the multivariate regression model for the current cohort to evaluate its role as an independent predictor of chitotriosidase concentration.

On multiple linear regression analysis; chitotriosidase concentration was significantly associated with the presence of 24-bp dup polymorphism, autoimmune thyroid disease and creatinine level after adjusting age and gender, p value <0.001 . In other words, chitotriosidase level could be predicted according to CHIT1 genotype & AITD status by linear regression analysis in our study.

While this study excluded patients with known diagnosis of diabetes mellitus, multinodular goiter, thyroid carcinoma, renal impairment, or chronic liver disease, as well as participants with history of autoimmune, inflammatory, or infectious disorders, the potential influence of unmeasured inflammatory factors on serum CHIT1 concentrations represent a source of residual confounding. Additionally, the relatively small sample size represents a notable limitation of our work.

Although CHIT1 has been classified as an innate immune mediator and inflammatory marker,¹⁶ the exact role of CHIT1 in autoimmune thyroiditis and its progression warrants deeper investigation with a larger sample size. Future studies are needed to define CHIT1 intracellular operating dynamics, explicit receptors, interacting proteins, downstream signalling cascades in AITD. Eventually, uncovering these molecular mechanisms might help in detecting novel therapeutic targets and evolving effectual target therapies.

Author Contributions

MN; contributed to the conceptualization and study design, laboratory techniques management, data analysis and manuscript writing/editing and corresponding author. NMS; contributed to sample collection; patients' recruitment, enrollment and clinical assessment, as well as data collection and analysis. KEE and RES; supervised and validated patient eligibility and recruitment, supervised the clinical assessment, and provided mentorship and manuscript revision. HAS; contributed to recruitment, enrollment and clinical assessment of the patients.

Declaration of Conflicting Interests

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Ethical approval

The current study abided by Declaration of Helsinki principles and was approved by Research Ethical committee, Faculty of Medicine Beni-Suef University, (FMU-BSU REC) (Approval No: FMBSUREC/04012023/Aboud).

Informed consent

Written informed consent was acquired from all participants.

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