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# Expression of circular RNAs ERN1, EYA1, and AKNAD1in Hashimoto Thyroiditis Patients

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**Abstract**

Circular RNAs (circRNAs), a subtype of non-coding RNA, have circular covalently bonded structures. Numerous circRNAs play critical roles in the onset and course of many diseases, including autoimmune thyroid disorders such as Hashimoto thyroiditis (HT). They may also serve as vital new biomarkers for detecting and treating these conditions. Objectives: The current study aimed to assess the expression of the genes hsa_circ_0045272 (ERN1), hsa_circ_0084764 (EYA1), and hsa_circ_000102 (AKNAD1) in HT disease. Methods: One hundred HT patients and one hundred control subjects with matched ages and genders were included in the study. Anti-thyroid peroxidase, antithyroglobulin (anti-TG), and thyroid hormone levels were assessed. Quantitative real-time polymerase chain reaction was utilized for assessing the amounts of circRNA expression in plasma. Logistic regression analysis was conducted to identify the risk factors for HT. Results: The circRNA ERN1 and EYA1 were significantly downregulated in HT patients in comparison with controls ($p<0.001$). However, there was no significant difference between the studied groups regarding AKNAD1 expression ($p=0.204$). Significant positive correlations were observed in the HT group between ERN1 and waist circumference, HDL-C, and anti-TG. Multivariate logistic regression analysis revealed that ERN1 was one of the risk factors for Hashimoto thyroiditis. Conclusion: hsa_circ_0045272 (ERN1) and hsa_circ_0084764 (EYA1) were significantly downregulated in the HT patients, and these circRNAs could be potential biomarkers for HT disease.

**Keywords:** Hashimoto thyroiditis; hsa_circ_0084764; hsa_circ_0045272; hsa_circ_000102; Real-time PCR.

## 1 Introduction:

Hashimoto thyroiditis (HT), often referred to as chronic lymphocytic thyroiditis, is an autoimmune disease only affecting the thyroid gland [1]. The primary pathogenic characteristics comprise lymphocyte infiltration in germinal centers, widespread thyroid gland hypertrophy, thyrocyte atrophy and destruction, and interstitial fibrosis. Thyroglobulin antibody (TGAb) and thyroid peroxidase antibody (TPOAb), which are thyroid autoantibodies, are persistently positive [2]. Numerous factors, including genetic predisposition, environmental factors, and immunological factors, all play a role in the pathogenesis of HT. During the course of the disease, some HT patients experience transitory hyperthyroidism, but the majority eventually experience hypothyroidism. HT is thought to be a frequent cause of hypothyroidism. However, there is currently no complete understanding of the pathophysiology of this condition [3,4].

Messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs) are the two primary categories of ribonucleic acids (RNAs) found in eukaryotic cells. Genomic transcription produces both mRNAs and ncRNAs. Non-coding RNAs (ncRNAs) include extracellular RNAs, circular RNAs, long non-coding RNAs (lncRNAs), and microRNAs (miRNAs). Due to their unclear ability to encode proteins or peptides, these RNAs are known as non-coding RNAs [5]. Although they can affect the expression of other genes in various ways, they regulate gene expression at different levels in numerous diseases, including autoimmune diseases (ADs), and serve as potential therapeutic targets as well as indicators for illness progression and treatment success. In some instances, their modes of action are well understood, and methods for regulating their behaviour are well established. In other instances, their methods are unclear or controversial [6,7].

A family of endogenous ncRNAs having a covalently closed loop structure is known as circular RNAs (circRNAs). Contrary to the characteristics of linear RNA molecules, circular RNA has a special structure that is a covalently closed loop without 5' end caps and 3' poly (A) tails [8]. Generally, "back-splicing" events of the precursor messenger RNAs (pre-mRNAs) result in producing circRNA. These occurrences involve the formation of a 3' phosphodiester link between an upstream 3' splice acceptor and a downstream 5' splice donor [9]. Since circRNAs lack 3' and 5' ends, they are more stable than linear RNAs and are resistant to RNase. CircRNAs can be divided into exon circRNAs, intronic circRNAs, exon-intron circRNAs, intergenic circRNAs, and antisense circRNAs, according to their source [10,11]. It has been discovered that some types of circRNAs play a crucial role in controlling gene expression and transcription by serving as sponges for microRNAs (miRNAs), which interact with RNA binding proteins, and by enhancing mRNA stability by building duplex structures of

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RNA-protein complexes. CircRNA expression patterns show tissue-specific and pathogenesis-related behavior [12]. CircRNAs have been linked to the regulation of the pathogenesis of numerous illnesses, including cancer, cardiovascular conditions, and autoimmune illnesses such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjogren's syndrome. These illnesses may be detected using circRNAs as potential biomarkers [13]. CircRNA expression patterns and functions in HT patients are yet to be fully understood. The current work aimed to assess how the circular non-coding RNAs hsa_circ_000102 (*AKNAD1)*, hsa_circ_0045272 *(ERN1)*, and hsa_circ_0084764 *(EYA1)* contribute to HT pathogenesis.

## 2 Subjects and Methods

### 2.1 Subjects

The Clinical Pathology Department conducted this case-control study between March 2022 and May 2023 in collaboration with the Internal Medicine Department. The current study included 200 participants, who were divided into two groups. Group I consisted of 100 Hashimoto patients who were identified using the European Thyroid Association's diagnostic criteria, which included clinical symptoms, ultrasound findings, measures of thyroid hormones, and thyroid autoantibodies. All HT patients had elevated TSH Hormone and Thyroid peroxidase (TPO) antibodies levels, and they were treated with levothyroxine sodium. Group II consisted of 100 healthy age- and gender-matched individuals who served as controls. There were no thyroid-specific autoantibodies and euthyroid status in the controls. Other thyroid conditions, autoimmune conditions, infections, tumors, and chronic inflammatory conditions were all excluded. This study approved (IRP 3/2022 CPATH5) from the local medical research ethics council and was conducted after obtaining informed consent from all participants.

### 2.2 Methods

Full history-taking and clinical examinations were conducted on all groups. After taking measurements of the subject's weight, height, waist circumference (WC), and the body mass index (BMI) (BMI = Weight in kg/ Height $m^2$) was computed.

#### 2.2.1 Collection and Processing of Samples

Six ml of blood was separated after a sterile venipuncture as follows: 4 ml was placed in a plain tube, and the serum was separated for the AU 680 Beckmann autoanalyzer (Beckmann, USA) to determine the lipid profile and the LDX 800 chemiluminescent immunoassay (Beckman Coulter, USA) to determine thyroid function tests and thyroid autoantibodies. Two ml of blood was delivered in a citrate vacutainer, the plasma was isolated, and it was kept at -80°C until circRNA expression by qRT-PCR was analyzed.

#### 2.2.2 CircRNA Expression

Thermo Scientific's Gene-JET Blood RNA Purification Kit (Thermo Scientific, USA) was utilized for extracting the total RNA from the plasma. The quantities and purity of RNA were determined using the USA-based NanoDropTM 2000 from Thermo Scientific. The cDNA was produced using the Revert Aid First Strand cDNA Synthesis Kit from Thermo Scientific. The reaction was set up in two steps, on ice, with a total volume of 20 µl: 10 µl of RNA, 1 µl of random primer and 1 µl l of nuclease-free water were combined to make a total volume of 12 µl. The samples were then refrigerated on ice after being incubated at 65 °C for 5 minutes. The following components were added to create a final volume of 20 µl: 1 µl of Ribolock RNase inhibitor, 2 µl of 10 mM dNTPs and 1 µl of Revertaid RT were added to 4 µl of 5 X reaction buffer. The following incubation cycles were performed on the 2720 thermal cycler (ABI Systems, Singapore): five minutes at 25°C, sixty minutes at 42°C, and five minutes at 70°C. Before real-time PCR, the cDNA was kept at -20 °C. Thermo Scientific's Maxima SYBR Green qPCR Master Mix (2X) ROX Kit was utilized for conducting real-time PCR. The 20 µl total volume was made up of 1.5 µl of forward and reverse primers **(Table 1)**, 6 µl of cDNA, 1 µl of nuclease-free water, and 10 µl of SYBR green dye. Gene amplification in was completed in three steps using a 7500 real-time PCR system (Applied Biosystems, USA): a denaturation step at 95 °C for ten minutes, then 50 cycles at 95 °C for fifteen seconds, 63 °C for one minute, and 72 °C for one minute, and a final extension step at 72 °C for ten minutes. Melting curves were produced to make sure the RT-qPCR was specific. GAPDH was utilized for calculating the expression of circRNAs using the standard curve approach. Using the $2^{-\Delta\Delta Ct}$ method, normalized to the endogenous housekeeping gene (GAPDH) and compared to the control, the relative expression of genes was measured: ΔCt = Ct target – Ct reference, ΔΔCt = (ΔCt sample –ΔCt control) [14].

**Table 1: Sequences for the studied circular RNAs.**

| Gene name | Primers for each gene |
|---|---|
| 1) hsa_circ_000102 ***(AKNAD1)*** | F: CAACAGTTGGAAGTTGACTG<br>R: GCAGGCACGCCATGATTTT |
| 2) hsa_circ_0045272 ***(ERN1)*** | F: CGGCCTCGGGATTTTTGGAA<br>R: AGCGTATACAGGCTGCCATC |
| 3) hsa_circ_0084764 ***(EYA1)*** | F: GGGGCGAGGTAGAAACTCTC<br>R: TCTGCTGCATCCACCAGTTT |
| 4) *GAPDH* | F: GGGAAACTGTGGCGTGAT<br>R: GAGTGGGTGTCGCTGTTGA |

### 2.3 Statistical Analysis

The IBM SPSS software application, version 20.0, was utilized for data gathering and analysis. Categorical data were displayed as percentages and numbers. The chi-square test was applied to examine the association between the

categorical variables. Student's *t*-test was utilized for comparing two groups for quantitative variables with a regular distribution. To compare two groups for quantitative variables that were not regularly distributed, the Mann-Whitney test was applied. A receiver operating characteristic curve (ROC) was utilized for assessing the markers' diagnostic performance. The 5% level was utilized for determining the significance of the obtained data. Regression analysis was used to identify the risk factors for HT [15].

**3. Results**

The BMI and waist circumference were significantly different between patients and controls ($p<0.001$) **(Table 2)**. The lipid profile, thyroid function tests, and thyroid antibodies showed a highly significant elevation in the HT group compared to controls ($p<0.001$) **(Table 3)**. The *ERN1* and *EYA1* had significantly lower expression in the HT group compared to controls ($p<0.001$). Meanwhile, *AKNAD1* had lower expression in HT patients than in controls, but it did not achieve statistical significance **(Table 4)**.

The ROC curve for *ERN1* revealed that the area under the curve (AUC) was 0.660, where the cutoff value to diagnose HT was <1.21, with sensitivity of 60%, specificity of 56%, positive predictive value (PPV) of 57.7%, and negative predictive value (NPV) of 58.3% **(Figure 1a)**. The ROC curve for *EYA1* demonstrated that AUC was 0.667, where the cutoff value to diagnose HT was <0.923, with sensitivity of 72%, specificity of 62%, PPV of 65.5%, and NPV of 68.1% **(Figure 1b)**. Significant positive correlations were observed between *ERN1* and height, waist circumference, HDL-C, and anti-TG. Moreover, there was a significant negative correlation between *ERN1* and disease duration, TG, and FT3. Meanwhile, there was a significant negative correlation between *EYA1* and FT4 **(Table 5)**. Multivariate logistic regression analysis indicated that *ERN1*, triglycerides, HDL-C, LDL-C, and waist circumference were risk factors for HT development as the disease is multifactorial.

**Table 2: Comparison between the studied groups according to demographic data.**

| | **HT group (n = 100)** | **Controls (n = 100)** | **Test of sig.** | **P** |
|---|---|---|---|---|
| **Age (years)** | | | | |
| Min. – Max. | 18 – 69 | 18 – 59 | | |
| Mean ± SD | 39.22 ± 10.31 | 40.55 ± 125 | t=0.839 | 0.403 |
| Median (IQR) | 39.50 (30 – 45) | 44 (28.50 – 51.50) | | |
| **Gender** | | | | |
| Male | 3 (3%) | 32 (32%) | $\chi^2$= 29.126* | <0.001* |
| Female | 97 (97%) | 68 (68%) | | |
| **Weight (kg)** | | | | |
| Min. – Max. | 50 – 123 | 52 – 94 | | |
| Mean ± SD | 80.61 ± 13.85 | 66.45 ± 7.37 | t=9.027* | <0.001* |
| Median (IQR) | 80 (73 – 89) | 65 (62.50 – 70) | | |
| **Height (cm)** | | | | |
| Min. – Max. | 150 – 183 | 152 – 200 | | |
| Mean ± SD | 162.77 ± 6.86 | 170.12 ± 9.13 | t=6.433* | <0.001* |
| Median (IQR) | 163 (157 – 166) | 168 (164.50 – 172) | | |
| **BMI (kg/m$^2$)** | | | | |
| Min. – Max. | 20.50 – 45.17 | 20.43 – 242 | | |
| Mean ± SD | 30.71 ± 5.48 | 22.91 ± 0.97 | t=14.013* | <0.001* |
| Median (IQR) | 30.10 (26.20 – 33.80) | 23.12 (22.40 – 23.73) | | |
| **Waist circumference (cm)** | | | | |
| Min. – Max. | 70 – 134 | 49 – 97 | | |
| Mean ± SD | 104.20 ± 13.90 | 79.78 ± 11.14 | t=13.710* | <0.001* |
| Median (IQR) | 107 (94 – 113) | 84 (77.50 – 85) | | |
| **Symptoms suggesting hypothyroidism** | | | | |
| Present | 89 (89%) | 0 (0%) | $\chi^2$= 160.360* | <0.001* |
| Absent | 11 (11%) | 100 (100%) | | |

HT: Hashimoto thyroiditis.
IQR: interquartile range.
SD: standard deviation.
t: Student's *t*-test.
p: p-value for comparing the two studied groups.
$\chi^2$: chi-square test.
*: statistically significant at $p \leq 0.05$.

**Table 3: Laboratory results of the studied groups.**

| | HT group (n = 100) | Controls (n = 100) | Test of sig. | P |
|---|---|---|---|---|
| **Total cholesterol (mg/dl)** | | | | |
| Min. – Max. | 128 – 290 | 116 – 198 | | |
| Mean ± SD | 194.63 ± 35.71 | 162.89 ± 23.71 | t=7.405* | <0.001* |
| Median (IQR) | 187 (175 – 213) | 165 (146 – 184) | | |
| **Triglycerides (mg/dl)** | | | | |
| Min. – Max. | 31 – 176 | 48 – 150 | | |
| Mean ± SD | 98.78 ± 37.77 | 115.93 ± 28.22 | U=3385.0* | <0.001* |
| Median (IQR) | 97.50 (66 – 125) | 123 (94 – 138) | | |
| **HDL-C (mg/dl)** | | | | |
| Min. – Max. | 34 – 85 | 45 – 70 | | |
| Mean ± SD | 51.02 ± 12.77 | 54.75 ± 8.21 | U=3496.0* | <0.001* |
| Median (IQR) | 48 (41 – 58.50) | 50 (48 – 64) | | |
| **LDL-C (mg/dl)** | | | | |
| Min. – Max. | 67 – 187 | 60 – 96 | | |
| Mean ± SD | 115.96 ± 27.82 | 77.74 ± 10.64 | t=12.834* | <0.001* |
| Median (IQR) | 112 (94 – 128) | 77.50 (67 – 86) | | |
| **FT3 (ng/dl)** | | | | |
| Min. – Max. | 0.03 – 0.90 | 0.20 – 0.39 | | |
| Mean ± SD | 0.25 ± 0.17 | 0.31 ± 0.04 | 2087.0* | <0.001* |
| Median (IQR) | 0.22 (0.17 – 0.28) | 0.31 (0.28 – 0.35) | | |
| **FT4 (ng/dl)** | | | | |
| Min. – Max. | 0.10 – 0.80 | 0.56 – 1.21 | | |
| Mean ± SD | 0.45 ± 0.18 | 0.91 ± 0.17 | 360.0* | <0.001* |
| Median (IQR) | 0.46 (0.32 – 0.58) | 0.93 (0.83 – 1.06) | | |
| **TSH (mIU/L)** | | | | |
| Min. – Max. | 4.20 – 101 | 0.52 – 5.15 | | |
| Mean ± SD | 37.77 ± 35.74 | 2.73 ± 1.33 | 43.0* | <0.001* |
| Median (IQR) | 14.23 (8.14 – 74.81) | 2.75 (1.85 – 3.84) | | |
| **Anti-TG (IU/ml)** | | | | |
| Min. – Max. | 15 – 1710 | 0.27 – 1.85 | | |
| Mean ± SD | 274.43 ± 328.35 | 0.74 ± 0.33 | 0.000* | <0.001* |
| Median (IQR) | 151 (55.9 – 406) | 0.65 (0.59 – 0.90) | | |
| **Anti-TPO (IU/ml)** | | | | |
| Min. – Max. | 36 – 1440 | 0.95 – 3.85 | | |
| Mean ± SD | 550.23 ± 433.58 | 2.13 ± 0.71 | 0.000* | <0.001* |
| Median (IQR) | 371.50 (225 – 902.5) | 2.05 (1.63 – 2.76) | | |

HT: Hashimoto thyroiditis.
SD: standard deviation.
IQR: interquartile range.
t: Student's *t*-test.
U: Mann-Whitney test.
p: p-value for comparing the two studied groups.
*: statistically significant at $p \leq 0.05$.

**Table 4: Comparison between the studied groups according to the studied circRNA.**

| | HT group (n =100) | Controls (n =100) | U | P |
|---|---|---|---|---|
| **hsa_circ_0045272 *(ERN1)*** | | | | |
| Min. – Max. | 0.09 – 6.54 | 0.15 – 49.44 | | |
| Mean ± SD | 1.49 ± 1.56 | 5.45 ± 9.48 | 3404.0* | <0.001* |
| Median (IQR) | 0.79 (0.39 – 2.31) | 1.80 (0.56 – 6.56) | | |
| **hsa_circ_0084764 *(EYA1)*** | | | | |
| Min. – Max. | 0 – 18.75 | 0 – 19.24 | | |
| Mean ± SD | 1.31 ± 3.34 | 3.54 ± 5.17 | 3334.0* | <0.001* |
| Median (IQR) | 0.41 (0.03 – 1.09) | 1.32 (0.19 – 4.22) | | |
| **hsa_circRNA_000102 *(AKNAD1)*** | | | | |
| Min. – Max. | 0.04 – 36.82 | 0 – 27.89 | | |
| Mean ± SD | 3.25 ± 6.68 | 5.63 ± 8.94 | 4480.0 | 0.204 |
| Median (IQR) | 0.67 (0.34 – 2.77) | 1.09 (0.36 – 4.83) | | |

HT: Hashimoto thyroiditis.
SD: standard deviation.
IQR: interquartile range.
U: Mann-Whitney test.
p: p-value for comparing the two studied groups.
*: statistically significant at $p \leq 0.05$.

**Table 5: Pearson correlations of hsa_circ_0045272 and hsa_circ_0084764 expression levels with clinical and laboratory data in the Hashimoto thyroiditis group.**

| | hsa_circ_0045272 | | hsa_circ_0084764 | |
|---|---|---|---|---|
| | $r_s$ | p | $r_s$ | p |
| **Age (years)** | 0.064 | 0.528 | 0.245 | 0.014* |
| **Weight (kg)** | 0.082 | 0.418 | -0.022 | 0.826 |
| **Height (cm)** | 0.480 | <0.001* | -0.042 | 0.680 |
| **BMI (kg/m$^2$)** | -0.110 | 0.277 | 0.036 | 0.722 |
| **Waist circumference (cm)** | 0.284 | 0.004* | -0.006 | 0.950 |
| **Duration (years)** | -0.260 | 0.009* | -0.043 | 0.670 |
| **Total cholesterol (mg/dl)** | -0.194 | 0.053 | -0.147 | 0.145 |
| **Triglycerides (mg/dl)** | -0.329 | 0.001* | -0.024 | 0.810 |
| **HDL-C (mg/dl)** | 0.304 | 0.002* | -0.161 | 0.110 |
| **LDL-C (mg/dl)** | -0.109 | 0.280 | 0.073 | 0.472 |
| **FT3 (ng/dl)** | -0.267 | 0.007* | -0.092 | 0.363 |
| **FT4 (ng/dl** | -0.037 | 0.716 | -0.231 | 0.021* |
| **TSH (mIU/L)** | 0.028 | 0.784 | -0.018 | 0.859 |
| **Anti-TG (IU/ml)** | 0.318 | 0.001* | -0.112 | 0.269 |
| **Anti-TPO (IU/ml)** | 0.147 | 0.144 | -0.046 | 0.652 |
| **hsa_circ_0084764** | 0.042 | 0.680 | – | – |

$r_s$: Spearman coefficient.
*: statistically significant at $p \leq 0.05$.

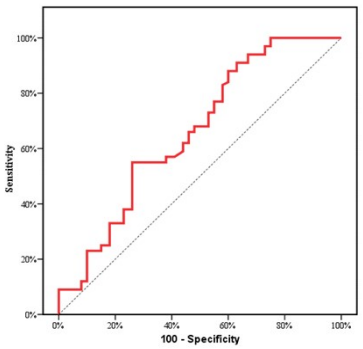


Figure 1 a

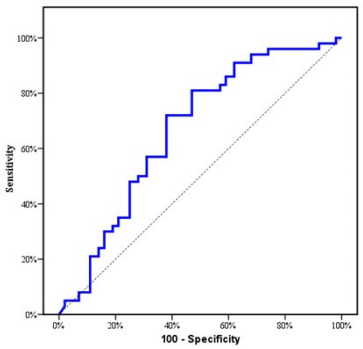


Figure 1 b

**Figure 1: (a) ROC curve for hsa_circ_0045272 (ERN1) to discriminate patients from controls.**
**(b) ROC curve for hsa_circ_0084764 (EYA1) to discriminate patients from controls.**

## 4. Discussion

The most prevalent organ-specific autoimmune condition is Hashimoto thyroiditis. Numerous remarkable immunological and genetic factors affecting HT development have been researched. However, the details of the mechanisms are still unclear [14]. Consequently, it is essential to find novel biomarkers and investigate their roles. One type of ncRNA is circRNAs, which are different from ordinary linear RNAs in that they have a closed circular structure produced by covalent bonding making them resistant to RNA exonuclease breakdown [16]. Furthermore, circRNAs have numerous binding sites for miRNA sponges. They may competitively bind to miRNA, reducing its inhibitory effect on the expression of the target genes [1]. In the present work, three circRNAs were studied, and their expression was evaluated in HT patients. The results illustrated that *ERN1* and *EYA1* were downregulated in the plasma of HT patients compared to controls. However, no significant difference was observed between the studied groups regarding *AKNAD1* expression. The multivariate logistic regression analysis indicated that *ERN1* was considered a risk factor for HT development. According to a study by Li et al., the expression of *ERN1* was decreased in T cells from SLE patients [17]. It was discovered that downregulating *ERN1* in Jurkat cells caused early apoptosis and markedly increased IL-2 production, which are characteristics of the SLE onset. According to another study by Wu et al. [18], one of the most common clinical signs in Graves' disease (GD) patients is thyroid-associated ophthalmopathy (TAO), which has been linked to circRNAs. In order to identify the mRNAs and circRNAs whose expression varied between patients with TAO and the control group, the researchers employed high-throughput RNA sequencing on samples of orbital adipose/connective tissue. They hypothesized that circRNA_14940 (also known as hsa_circ_0084764), by interacting with CCND1 and TNXB to improperly regulate the Wnt signaling pathway, ECM-receptor interaction, and PI3K-Akt signaling pathway, would contribute to the pathogenesis of TAO. However, Sun et al. discovered that *AKNAD1* is a plasma exosomal circRNA that is differently upregulated in individuals with GD [19]. They stated that the circRNA/microRNA/mRNA interaction network indicated the most possible targeted microRNAs for *AKNAD1* and its linked genes. Additionally, their functional studies suggested that the linked genes for *AKNAD1* were involved in immune system activation pathways, such as viral infection and interferon-beta signaling. As far as we are aware, little research has been conducted on circRNA function in HT and other autoimmune thyroid diseases. According to a study by Xiong et al. [1], the thyroid peroxidase antibody serum level was positively correlated with the enhanced gene expression of hsa_circ_0089172. They found that in HT patients miR-125a-3p expression was downregulated and negatively linked with an increase in hsa_circ_0089172 levels. Only a few relevant studies have been published, and the field of study is still in its infancy. The development of new biomarkers and creative methods for HT diagnosis would benefit from the creation of a comprehensive profile of circRNA expression in HT patients.

The expression levels of certain targets are modulated by RNAs through various processes, including chromatin changes, splicing control, and miRNA sponging. RNAs are involved in the pathophysiology of several human disorders. The initiation and development of autoimmune disorders have been linked to changes in the expression of several circRNAs, according to research [20]. CircRNAs may therefore function as immune regulators and potential biomarkers, providing an opportunity for the development of novel therapeutics [21]. Multiple lines of evidence suggested that lncRNAs have primary roles in RA, SLE, multiple sclerosis, and type 1 diabetes [22,23]. Clinical data is mostly utilized for supporting the diagnosis of ADs. Unfortunately, early AD symptoms in many people can be non-specific and suggestive of a wide range of other illnesses [6]. CircRNAs are highly circulating stable molecules that can be found in minimally invasive blood, urine, or saliva samples when using RT-PCR research. This is one of the key benefits of circRNAs as biomarkers. Several circRNAs are variably expressed in distinct thyroid ADs. However, their exact methods of action are yet unknown and need to be confirmed [24]. The molecular basis of these illnesses will be better understood by examining the atypical circRNA expression patterns arising during HT development. Theoretically, this will also support the use of circRNAs as HT diagnostic indicators and

potential therapeutic targets [25]. Our findings present this profile of circRNA expression and its role in the pathogenesis of HT for the first time. The limitation of this study was the sample size, and the results need to be confirmed in large-scale studies.

## 5. Conclusion

hsa_circ_0045272 (ERN1) and hsa_circ_0084764 (EYA1) were significantly downregulated in Hashimoto thyroiditis patients. AKNAD1 had lower expression in HT patients than in controls, however, this did not reach statistical significance. Both ERN1 and EYA1 could be potential Hashimoto thyroiditis diagnostic biomarkers and may be involved in Hashimoto thyroiditis development. ERN1 could be considered as a risk factor for Hashimoto thyroiditis.

## Author Contributions

**Noran T. Aboelkhair:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, supervision, Conceptualization. **Mariam Hemied Raslan Abdu-Rahman:** Methodology, Investigation. **Shaimaa Zewain:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abd El-Monem Abd El-Kader El-Torgoman:** supervision. **Samah El-Ghlban:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, supervision, Conceptualization. All authors have read and agreed to the published version of the manuscript.

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## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Institutional Review Board Statement

The experimental protocol was approved by the Menoufia University Ethics Committee (Approval number: 3/2022 CPATH 5).

**Consent to participate:** Informed consent was obtained from all individual participants included in the study.

**Consent to publish:** Informed consent was obtained to publish the data.

**Data availability:** The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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