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ORIGINAL ARTICLE

The Role of DICER and DROSHA Gene Expression in Assessment of Hashimoto Thyroiditis

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ABSTRACT:

Background: Hashimoto thyroiditis is autoimmune condition that destroys thyroid follicular cells by immune-mediated mechanisms through the development of anti-thyroid antibodies and T lymphocytes activation , which causes ongoing fibrosis of the thyroid. Recently, studies have revealed that miRNAs control different biological activities, as cell division, inflammation and immune reaction. DICER and DROSHA are classes of RNAase III protein family that are vital for formation of most miRNAs**.** The aim of the work is to evaluate the role of DICER and DROSHA gene expression in the diagnosis of Hashimoto Thyroiditis (HT) and its adverse cardiovascular effects.

Methods: DICER and DROSHA gene expression were assessed in 48 HT patients and 24 control subjects by quantitative Real -Time PCR at Zagazig Faculty of Medicine, Egypt. The results were correlated with anti TPO-antibodies, lipid profile and cardiovascular effects.

Results: There is significant decrease in both DICER and DROSHA gene expression in HT patients than control groups. The DICER and DROSHA expression are negatively correlated with anti -TPO antibodies. Regarding DICER expression level, ROC curve showed AUC 0.977, with 93.8 % sensitivity and 91.7 % specificity to diagnose HT. As well, DROSHA ROC curve showed AUC 0.965 with 87.5 % sensitivity and 83.3 % specificity to assess cardiovascular effects of HT.

Conclusion: This study highlights the potential role of DICER and DROSHA gene expression levels in the early diagnosis and pathogenesis of Hashimoto's thyroiditis (HT), particularly in relation to cardiovascular complications. Hence, they provide new insight for early management to prevent such complications.

Keywords: DICER; DROSHA; Hashimoto thyroiditis; cardiovascular effects.

INTRODUCTION

Hashimoto's thyroiditis (HT) is also called chronic lymphocytic thyroiditis. Hiroshi Hashimoto described the disease as characterized by marked lymphocyte infiltration and fibrosis in some follicular cells of the thyroid [1]. Hashimoto's thyroiditis is a member of the greatest prevalent thyroid disorders [2], and its incidence is steadily rising. The incidence in females is 5-10 times greater than that of males, as well it rises with age. The severity of HT is variable among cases. Some Cases with HT acquire hypothyroidism at a younger age or maintain a euthyroid state until old age. However, the severity of this disease is very difficult to expect at their diagnosis [3].

MicroRNAs (miRNAs), have arisen as significant controllers of gene expression. Disorders in the levels of miRNAs are associated with different autoimmune disorders involving HT. Alteration in particular miRNAs expression in HT cases has been linked with autoimmune disorders and thyroid hormone synthesis.

Besides, miRNAs have been proved to regulate gene expression via interaction with nuclear chromatin and some transcription factors. Their function in HT pathogenesis is a progressing field of research [3]. Two thousand or more miRNAs exist in the human genome, and a significant amount of protein-coding pairs to miRNAs [4]. Recently, researches have revealed that miRNAs control several biological activities, such as cell division , inflammatory reaction and immune response [5–9]. Dicer and Drosha are members of RNAase III protein family that are essential for the biosynthesis of most miRNAs which are copied as primary miRNAs (pri-miRNAs) [10]. They are handled by DROSHA and then DICER into around 60–70 nucleotide called (pre-miRNA),then mature miRNA [11,12]. The levels of Dicer and Drosha expression were proved to correlate with the occurrence of many cancers, for example cutaneous melanoma [13], ovarian cancer [14], carcinoma of the cervix [15], and cancer breast [16]. But the control and the precise pathogenesis of these molecules in these pathologies are yet blurred [17]. The use of DICER and DROSHA to assess cardiovascular effects of HT has not been investigated yet. This study aimed to investigate the use of DICER and DROSHA expression level to help for early diagnosis and pickup of Hashimoto thyroiditis cases and its cardiovascular effects .This may aid endocrinologists and cardiologists in proper early management of HT and avoidance of its cardiovascular events.

Aim of the work to evaluate the role of DICER and DROSHA Gene Expression in the pathogenesis and diagnosis of Hashimoto Thyroiditis and its adverse cardiovascular effects.

METHODS

Research Subjects

Between February 2025 and June 2025, this case-control study was performed at Zagazig Faculty of Medicine Medical Biochemistry and Clinical Pathology Departments, Egypt. In this study Forty eight (48) Hashimoto thyroiditis (HT) cases were collected for the research at the Internal Medicine Department along with 24 healthy volunteers as a control group. The sample size was calculated using Open Epi software with confidence level 95% and power 80%. HT patients had thyroid swelling and positive serum anti TPO-antibodies. The control group was of the same age and gender but with negative anti-TPO antibodies. All participants were subjected to thorough history taking, family history ,serum samples withdrawal for gene expression. Thyroid and lipid lab profiles were obtained from patients′ sheets. A written consent was gained from all applicants prior to the study. The patient group was subdivided based on the presence or absence of adverse cardiovascular effects (hypertension, bradycardia, hypercholesterolemia) into 2 subgroups: group IIa included HT without adverse cardiovascular effects and group IIb included HT patients with adverse cardiovascular effects. The adverse cardiovascular effects were assessed clinically and biochemically , and they included bradycardia (heart rate less than 60 Bpm), hypertension (systolic blood pressure › 140 mmHg and or diastolic blood pressure › 90 mmHg) and hypercholesterolemia (LDL › 100mg/dl) .Any patient with a history of thyroid malignancy , treatment of hyperthyroidism, or radioactive iodine therapy was excluded from the study .The study was applied in accordance with Declaration of Helsinki . The study was approved ethically by the Zagazig University institute review board with approval number (ZU-IRB # 1025 /29-1-2025).

RNA extraction

4 ml of venous blood samples were obtained for real-time PCR investigation of plasma DICER and DROSHA levels. RNA extraction was through using QIA amp RNA blood Mini Kits from Qiagen, Germany. The steps were applied following the directions of the kit in an environment free of contamination.

Production of cDNA

Then, the extracted RNA was reverse transcribed and amplified using RT2 qPCR Primer Assay kit, Cat. no.330001 from QIAGEN, Germany. The cDNA was transferred to a –20°C freezer.

Amplification for gene expression values

The amplification was made in a mixture including 5µL of the cDNA, 1 µl of every primer, 10 µL RT2 SYBER Green PCR Master Mix and 10 µL distilled H2O. The amplification was applied by Real time Cycler (Strata gene Mx3005P) qPCR as stated by the next procedure; initial start step 95ºC for ten min followed by 40 rounds of 95ºC for fifteen seconds, and finally 60ºC for one minute. GAPDH was used as internal reference gene, while DICER and DROSHA were used as target genes. The scale of variation of the gene expression level detected in cases compared to controls was assessed by the $2^{-}\Delta\Delta Ct$ method.

Statistical Methods

Collected data were noted and tabulated. They were analyzed using SPSS version 26. One way ANOVA test and Chi-square test were tested to assess the statistical difference among groups. Statistical Significance was defined as (p value <0.05). The Roc curve was implemented to assess the validity of DICER and DROSHA gene expression to diagnose HT as well as to diagnose adverse cardiovascular events.

RESULTS

There is significant difference between the three groups concerning family history as 87.5% of studied group that have Hashimoto thyroiditis with cardiovascular events have positive family history and 37.5% of Hashimoto thyroiditis without cardiovascular events have positive family history (Table 1).

There is statistically significant difference between studied groups regarding systolic blood pressure(SBP), diastolic blood pressure (DBP), heart rate and LDL levels with higher SBP and DBP were reported in both groups that have Hashimoto thyroiditis disease. Regarding the heart rate it was significantly lesser in patients with Hashimoto thyroiditis than control group. Levels of LDL were higher in the two groups that have Hashimoto thyroiditis than control group LDL levels (Table 2).

There is significant difference between studied groups concerning TSH, FT4 besides Anti TPO antibodies levels with higher Anti TPO antibodies and TSH levels were reported in both groups that have Hashimoto thyroiditis disease. Levels of FT4 were lower in the two groups that have Hashimoto thyroiditis than control group FT4 levels (Table 2 and Figure1).

There is statistically significant difference between studied group regarding DICER expression level and DROSHA expression level tests with lower levels were detected in both groups that have Hashimoto thyroiditis without CV events and group that have Hashimoto thyroiditis with CV events in comparison with the control group (Table 3 and Figure 2).

There is a significant positive correlation between DICER Expression level and DROSHA expression level (Table 4). There is a significant negative correlation between DICER Expression level, DROSHA expression level and anti TPO antibodies (Figure 3).

DICER expression level at the level of 0.98 was 93.8% sensitive and 91.7% for prediction of Hashimoto thyroiditis with overall accuracy 93.1%. They also show that DROSHA expression level of 0.98 was 93.8% sensitive and 91.7% specific for prediction of Hashimoto thyroiditis with overall accuracy 93.1% (Table S1and fFgure S3).

Roc curve shows that DICER expression level at the level of 0.67 was 95.8% sensitive and 91.7% specific for prediction of Hashimoto thyroiditis with adverse cardiovascular events

with overall accuracy 93.75%. They also show that DROSHA expression level at the level of 0.54was 87.5 % sensitive and 83.3% specific for prediction of Hashimoto thyroiditis with adverse cardiovascular events with overall accuracy 85.42% (Table S2 and figure S4)

Table (1): Basic characteristics of studied groups

Items	Studied groups			f	P value
	Group I	Group II a	Group II b		
Age Mean ± SD	38.5 ± 7.69	38.04 ± 7.8	38.25 ± 7.84	0.021	0.979
	N (%)	N (%)	N (%)	X^2	P value
Sex					
Male	8 (33.3%)	9 (37.5%)	8 (33.3%)	0.123	0.941
Female	16 (66.7%)	15 (62.5%)	16 (66.7%)		
Family history Positive	2 (8.3%)	9 (37.5%)	21 (87.5%)	31.163	<0.001*

(f) One Way ANOVA test, (X^2) chi square test

Table (2): comparing blood pressure, HR , LDL and thyroid function tests between studied groups

Items	Studied groups			F	P value	Post Hoc
	Group I	Group IIa	Group IIb			
Systolic BP Mean ± SD	112.25±6.88	129.67 ± 6.9	165.63±11.85	226.63	<0.001*	P1<0.001* P2<0.001* P3<0.001*
Diastolic BP Mean ± SD	69.58±6.37	71.83±9.27	112.58±10.84	172.69	<0.001*	P1=0.391 P2<0.001* P3<0.001*
HR Mean ± SD	82.42±7.28	74±5.28	53.25±7.44	119.06	<0.001*	P1<0.001* P2<0.001* P3<0.001*
LDL Mean ± SD	89±13.9	125.83±5.29	209.29±31.85	224.33	<0.001*	P1<0.001* P2<0.001* P3<0.001*
TSH Mean ± SD	1.65±0.98	7.36± 1.42	15.62±4.05	-5.810	<0.001*	P1<0.001* P2<0.001* P3<0.001*
FT4 Mean ± SD	1.38 ± 0.24	0.76±0.11	0.312±0.09	252.139	<0.001*	P1<0.001* P2<0.001* P3<0.001*
Anti TPO antibodies Mean ± SD	8.8± 0.078	184.92±72.78	440.04±138.29	-5.986	<0.001*	P1<0.001* P2<0.001* P3<0.001*

(f) One Way ANOVA test, P1 group I Vs group IIa, P2 group I Vs group IIb, P3 group IIa Vs IIb

Table (3): DICER expression level and DROSHA expression level between the studied groups

Items	Studied groups			f	P value	Post Hoc
	Group I	Group IIa	Group IIb			
DICER expression level Mean ± SD	1±0.009	0.775± 0.098	0.446±0.098	286.236	<0.001*	P1<0.001* P2<0.001* P3<0.001*
DROSHA expression level Mean ± SD	1±0.013	0.642±0.15	0.33±0.098	257.824	<0.001*	P1<0.001* P2<0.001* P3<0.001*

Table (4) Correlation between DICER Expression levels, DROSHA Expression level and Anti TPO antibodies in Hashimoto thyroiditis group

Items		DICER Expression level	DROSHA Expression level
DROSHA Expression level	r	0.739	-------
	P value	<0.001*	-------
Anti TPO antibodies	r	-0.628	-0.567
	P value	<0.001*	<0.001*

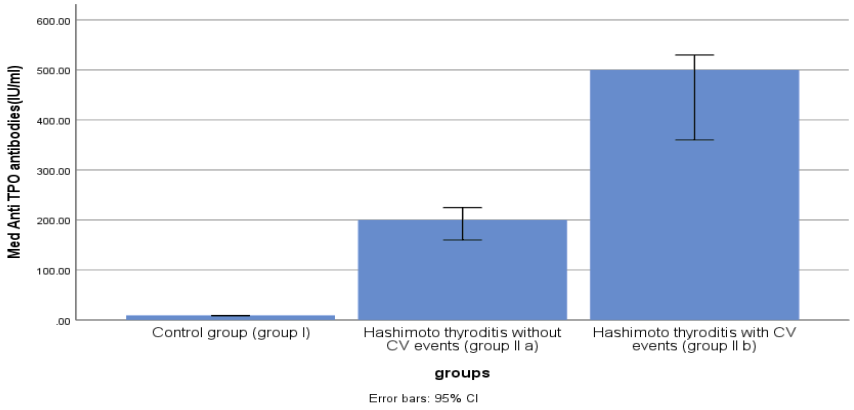

Figure (1): bar chart illustrating median Anti TPO between the studied group

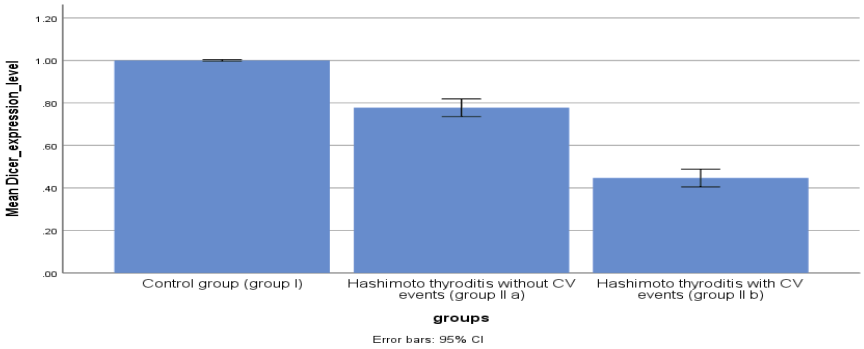

Figure (2): bar chart illustrating mean DICER expression between the studied group

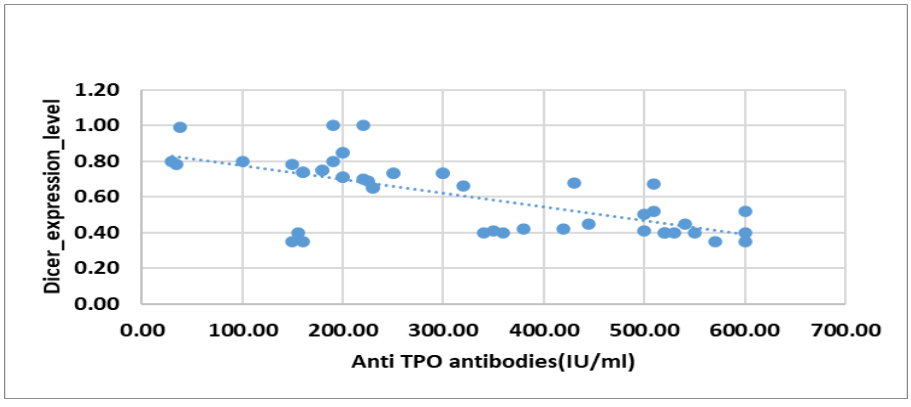

Figure (3): scatter diagram negative correlation between DICER Expression level in and anti TPO antibodies.

DISCUSSION

Hashimoto thyroiditis (HT)is named chronic autoimmune thyroiditis .HT is an autoimmune disease that destroys thyroid follicular cells by immune-mediated mechanisms [18].

It is known for the development of anti-thyroid antibodies and T-lymphocyte triggering, which produce ongoing fibrosis of the thyroid, this autoimmune disease is the most frequent etiology of hypothyroidism in affluent countries [19]. Patients with Hashimoto thyroiditis exhibit different thyroid function levels involving euthyroidism, subclinical hypothyroidism, overt hypothyroidism, and even temporary hyperthyroidism [20].

Women are more likely to have Hashimoto's thyroiditis, which becomes more common as they become older. The illness is more often seen in nations with less iodine deficit [19,21]. Hashimoto thyroiditis can happen by itself or as part of autoimmune polyglandular syndrome (APS) [22]. Graves' disease affects some people who may develop Hashimoto thyroiditis and the other way around. This would suggest a same etiology for these conditions but varied clinical manifestations [23,24].

A family of "small noncoding RNAs," microRNAs (miRNAs) are essential for many biological processes including cell proliferation/differentiation and death since they control gene expression after transcription [25]. Comprising multiple enzymes, the multi-step process of miRNA biogenesis features two key players: ribonucleases DROSHA and DICER [26]. In the nucleus, DROSHA cleaves the primary miRNA transcript into precursor miRNA. This precursor miRNA is then converted by DICER into a double-stranded molecule that includes the mature miRNA guide strand and the passenger strand in the cytoplasm [27].

Alterations in the expression of these enzymes have been implicated in various autoimmune diseases and inflammatory disorders, for example systemic lupus erythematosus and rheumatoid arthritis [28,29]. However, their role in autoimmune thyroid disorders, particularly HT, remains underexplored. Hence, this study aimed to study early diagnosis of Hashimoto Thyroiditis and to evaluate the role of DICER and DROSHA Gene Expression in the pathogenesis and diagnosis of Hashimoto Thyroiditis.

In the current study, there was a statistically significant difference regarding family history, as 87.5% of the group with Hashimoto's thyroiditis and cardiovascular events had a positive family history, compared to 37.5% in the group with Hashimoto's thyroiditis without cardiovascular events.

These findings highlight a significant association between positive family history and the presence of cardiovascular complications in cases with Hashimoto's thyroiditis, signifying that genetic predisposition may have a role not only in the pathogenesis of the disease itself but also in its extra-thyroidal manifestation.

These findings are in line with a large-scale population-based study conducted by **Kim et al.,** [30], which reported that individuals with an affected first-degree relative (FDR) had a 6.5-fold increased risk of developing Hashimoto's thyroiditis compared to those without an affected FDR. The risk was especially elevated among twins (IRR: 102.71), followed by siblings, mothers, and fathers. Interestingly, the familial risk was higher in males and significantly elevated in younger age groups, suggesting a strong genetic component, particularly in early-onset disease.

Our results also further supported by **Kust et al.,** [31], which directly demonstrating that there is genetic predisposition for the development of HT in patients with positive family history of the disease (43.59% of patients with positive family history developed HT themselves).

Additionally, **Thomsen et al.,** [32], in a nationwide Swedish study involving over 25,000 HT patients, reported a familial standardized incidence ratio (SIR) of 4.75 for HT, which was markedly higher when both a parent and a sibling were affected (SIR = 22.06). These findings indicate a strong heritable component in

the development of HT. Additionally, the study found significant associations between HT and other autoimmune diseases within families, further suggesting a shared autoimmune genetic susceptibility.

Overall, these converging findings underscore the importance of detailed family history assessment in the diagnostic workup of thyroid disorders. Early identification of individuals at higher genetic risk may aid in closer monitoring and potentially earlier intervention, especially for preventing systemic complications such as cardiovascular involvement.

Our findings indicated a statistically significant variation in heart rate, LDL levels, SBP and DBP across the studied groups. Both Hashimoto's thyroiditis groups had greater SBP and DBP than the control group. Although LDL levels were far higher in the two Hashimoto groups compared to controls, those with Hashimoto's thyroiditis also had notably lower heart rates, which might suggest cardiovascular problems linked with thyroid dysfunction.

These results support a prospective cohort study by Anwar et al. [33], which found that unusual thyroid hormones have a major influence on cardiovascular parameters including heart rhythm, blood pressure, and lipid profile. Although hyperthyroidism was especially linked to arrhythmias (OR = 4.3, $p < 0.001$), the research revealed a strong correlation between hypothyroidism and both hypertension (OR = 3.2, $p = 0.001$) and dyslipidemia (OR = 2.9, $p = 0.003$). They also found sinus bradycardia in hypothyroid patients and sinus tachycardia in hyperthyroid individuals. These results support the information suggesting that thyroid dysfunction, especially in subclinical forms, may predispose individuals to significant cardiovascular changes, hence underlining the need of early detection and comprehensive cardiovascular screening in patients with thyroid problems.

Furthermore, our findings agree with those of Chen et al. [34], who examined the risk of coronary heart disease (CHD) in HT patients using a cohort study included 4660 matched controls and 1165 newly diagnosed HT patients. Adjusted hazard ratio (HR) of 1.44 (95% CI: 1.05–19.99) showed that HT patients were far more likely to acquire coronary heart disease (CHD) than non-HT persons. Adjusted HRs were 2.06 (95% CI: 1.46–2.92) and 1.83 (95% CI: 1.31–2.55) respectively revealed greater risk for those with hypertension or hyperlipidemia. This risk remained even after considering comorbidities. These findings highlight the need of evaluating cardiovascular risk in people with Hashimoto's thyroiditis.

Our findings revealed a statistically significant difference between the groups under research on TSH, FT4, and Anti-TPO antibody levels given increased TSH and Anti-TPO antibody levels identified in both groups with Hashimoto's thyroiditis. Furthermore, FT4 levels were much lower in both Hashimoto's thyroiditis groups relative to the control group.

Our findings are consistent with those of Gvianishvili et al., [35], who discovered a distinct relationship between increasing TSH and Anti-TPO antibody levels and the beginning of cardiovascular issues. These results reinforce our own by implying that high TSH and Anti-TPO antibodies in Hashimoto's thyroiditis patients could contribute to the onset of cardiovascular problems.

These results agree with earlier studies. TAN ÖKSÜZ et al. [36]. proposing that certain pathophysiological processes seem to be significantly influenced by thyroid hormones on the cardiovascular system. They underlined also that clinical cardiovascular symptoms may result from even subclinical types of thyroid disease, thereby reinforcing the notion that thyroid dysfunction, even in its milder forms, may cause cardiovascular problems.

Furthermore, our results agree with Kawasaki et al., [37], who looked at the correlation between thyroid volume (TV) and thyroid hormone status among a large population of euthyroid Hashimoto thyroiditis (HT) patients. Their findings showed that HT patients with higher TV tended to show decreased FT4. Our results suggest that the pathophysiology of

Hashimoto thyroiditis and its potential cardiovascular effects may be significantly influenced by changes in thyroid hormone levels, particularly a decrease in FT4.

A statistically significant difference in the expression levels of DICER and DROSHA between the groups under research was found when we compared the Hashimoto's thyroiditis group without cardiovascular events and the group with cardiovascular events to the control group. These findings might imply that the downregulation of DICER and DROSHA expression in individuals with Hashimoto's thyroiditis could impair endothelial function and immunological control, hence possibly raising their susceptibility to cardiovascular issues. Disturbed microRNA biogenesis might therefore imply a mechanical relationship between thyroid autoimmunity and cardiovascular risk.

Our findings agree with previous research by Saeki et al. [17]. on the expression patterns and genetic polymorphisms of DICER and DROSHA in individuals with autoimmune thyroid diseases (AITDs), including Graves' disease (GD) and Hashimoto's thyroiditis (HD). They discovered that AITD sufferers had significantly decreased expression levels of both genes when compared to healthy controls, suggesting that the beginning of autoimmune thyroid disorders might be caused by disrupted microRNA processing. Furthermore, their results showed that DROSHA expression was much lower in patients with severe forms of HD, hence confirming the relationship between disease severity and downregulation of the microRNA biogenesis machinery.These findings support the hypothesis that altered expression of essential microRNA processing enzymes like DICER and DROSHA might be responsible for immunological dysregulation and perhaps the cardiovascular issues related to Hashimoto's thyroiditis.

Among people with Hashimoto's thyroiditis, our research revealed a significant negative correlation with anti-TPO antibodies and a significant positive correlation with DICER and DROSHA expression levels. Reduced expression of microRNA-processing enzymes might indicate a possible function in increasing autoimmune activity. Furthermore, DICER and DROSHA expression revealed positive correlations with FT4 and heart rate and negative ones with TSH, LDL, and diastolic blood pressure. These new links imply that damaged miRNA biogenesis might cause metabolic and cardiovascular changes in Hashimoto's thyroiditis in addition to immunological dysregulation.

Furthermore, when examining the relationship between clinical parameters across the combined groups—Group I: healthy controls and Group IIa: Hashimoto's thyroiditis patients without cardiovascular events—and DICER and DROSHA expression levels, comparable correlation findings were noted, suggesting that these associations continue across both groups and imply a possible larger role of changed miRNA biogenesis in Hashimoto's thyroiditis.

To date, there is limited literature addressing these specific correlations, and our study may be among the first to report such associations, especially in the context of both thyroid autoimmunity and cardiovascular risk. However, several previous studies have highlighted the general role of microRNAs in autoimmune thyroid diseases (AITDs). For instance, **Zhou et al.,** [38]. reported that reduced DICER and DROSHA expression may lead to decreased levels of miRNAs such as miR-27b, miR-let-7f, miR-21, and miR-98, which are involved in immune regulation and autoimmunity.

Moreover, **Zhou et al.,** [39]. demonstrated that Dicer-deficient mice develop uncontrolled autoimmune disease. Taken together, these findings suggest that downregulation of DICER and DROSHA genes in AITD patients may impair miRNA production, thereby increasing susceptibility to autoimmune thyroid conditions.

In addition, Dicer is an important ribonuclease involved in the biogenesis of miRNAs **Frezzetti et al.,** [40]. reported that the development of the thyroid gland was not affected by the absence of Dicer through using thyrocyte-specific Dicer knockout mice, but Dicer

knockout resulted in severe hypothyroidism. Furthermore, Dicer inactivation also increased the expressions of Tg and decreased the expressions of cell adhesion proteins in the thyroid cells, such as Cdh16 and Cdh1 [40].

Taken together, our results provide novel insights into the interplay between microRNA biogenesis enzymes and thyroid autoimmunity. The observed correlations suggest that downregulated DICER and DROSHA expression may serve as molecular bridges linking immune dysregulation, hormonal imbalance, and cardiovascular risk in patients with Hashimoto's thyroiditis.

Our study assessed the validity of DICER and DROSHA expression levels as biomarkers for predicting Hashimoto's thyroiditis and associated adverse cardiovascular events. Regarding the prediction of Hashimoto's thyroiditis from the control group, both DICER and DROSHA expression level at 0.98 demonstrated 93.8% sensitivity, 91.7% specificity, and an overall accuracy of 93.1%. Furthermore, for predicting adverse cardiovascular events in Hashimoto's thyroiditis patients, DICER expression level at 0.67 displayed 95.8% sensitivity and 91.7% specificity. DROSHA expression level at 0.54 showed 87.5% sensitivity and 83.3% specificity (Table 6). These findings suggest that both DICER and DROSHA can serve as potential biomarkers for early diagnosis and prediction of both Hashimoto's thyroiditis and cardiovascular events in these patients.

This is, to the best of our knowledge, the first study to explore this dual predictive capability, although previous studies explored the role of these biomarkers individually in autoimmune diseases including studies by [38-40].

CONCLUSION

This study revealed a significant down regulation of both DICER and DROSHA in HT patients, especially those with adverse cardiovascular events, suggesting their involvement in disease progression. Moreover, strong correlations were observed between these gene expressions and several clinical and biochemical parameters, including anti-TPO antibodies, thyroid hormone levels, lipid profile, and cardiovascular indicators. By predicting both the presence of HT and related cardiovascular risks, DICER and DROSHA showed great sensitivity and specificity, hence highlighting their possible use as diagnostic biomarkers.

Recommendations

Future research should include bigger, more varied populations to confirm these results across several demographics. Screening for DICER and DROSHA expression levels may be considered as part of early diagnostic protocols for HT, especially in patients at risk for cardiovascular complications. Moreover, the potential therapeutic targeting of miRNA processing pathways should be further explored as an avenue for novel HT treatments.

Conflict of interest: The authors declare that they have no competing interest.

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Table (S1): Validity of DICER expression level, DROSHA Expression level to predict Hashimoto thyroiditis disease from control group

	AUC	95% Confidence Interval	Cut off	Sensitivity	Specificity	Accuracy	P value.
DICER Expression Level	**0.977**	**0.944 - 1.00**	**0.98**	**93.8%**	**91.7%**	**93.1%**	**<0.001***
DROSHA Expression Level	**0.969**	**0.929-1.00**	**0.98**	**93.8%**	**91.7%**	**93.1%**	**<0.001***

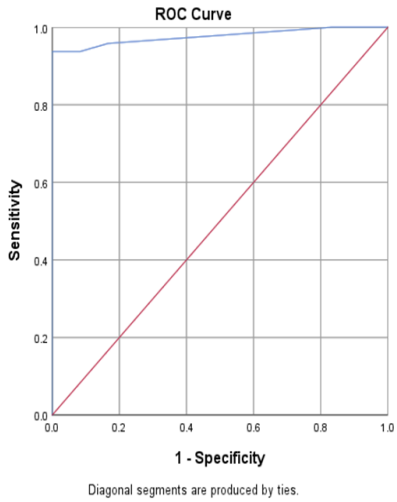
AUC=Area under curve

Table (S2): Validity of DICER expression level, DROSHA Expression level to predict Adverse Cardiovascular events in Hashimoto thyroiditis patients:

	AUC	95 % Confidence Interval	Cutoff	Sensitivity	Specificity	Accuracy	P value
DICER Expression Level	**0.980**	**0.960-1.00**	**0.67**	**95.8%**	**91.7%**	**93.75%**	**<0.001***
DROSHA Expression Level	**0.965**	**0.921-1.00**	**0.54**	**87.5%**	**83.3%**	**85.42%**	**<0.001***

AUC=Area under curve

(A



(B)

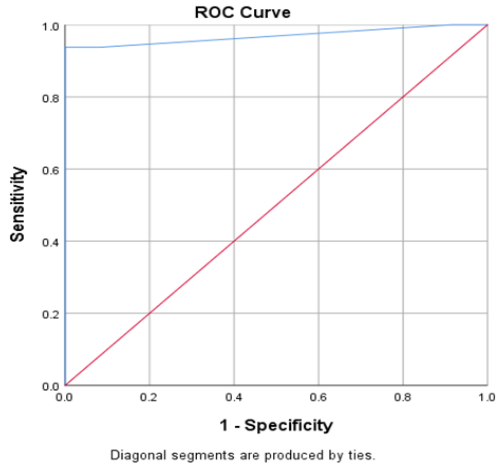
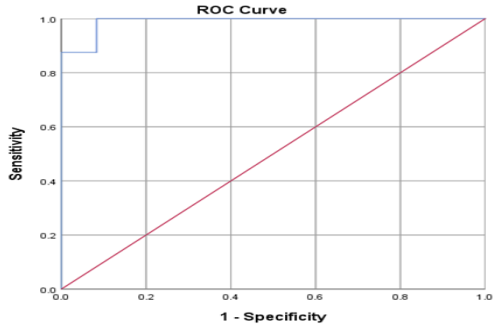


Fig. (S3): Roc curve illustrating validity of DICER expression level in (A) and DROSHA expression level in (B) for diagnosis of Hashimoto thyroiditis

(A



(B)

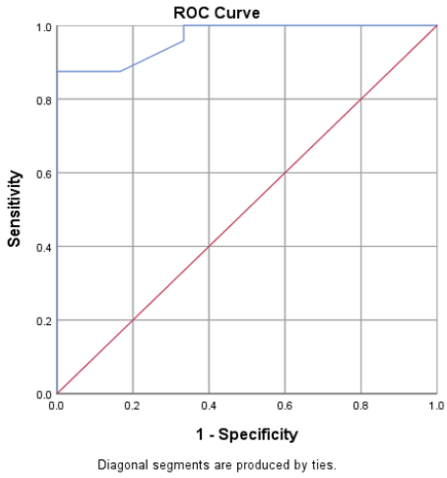


Fig. (S4): Roc curve illustrating validity of DICER expression level in (A) and DROSHA expression level in (B) for prediction of adverse cardiovascular events between Hashimoto thyroiditis groups

Citation

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