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Correlation between Gene expression of Long Non-coding RNA (XLOC_I2_006631& ENST00000425150) and Central Obesity Phenotype in Hashimoto's Thyroiditis Patients.

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Abstract

Background: Hashimoto thyroiditis (HT) is the commonly occurring autoimmune disorders. It arises due to genetic predisposition, X chromosomal inactivation patterns influenced by environmental factors, and microbiome composition, resulting in an imbalance in self-tolerance mechanisms. Long non-coding RNAs (LncRNAs) are a novel family that attracted attention. It controls genes expression via alteration of chromosome conformation, variation of transcription, splicing, stability and accessibility of mRNAs, and post-translational alteration. LncRNAs have been associated with pathophysiology of various autoimmune and inflammatory diseases such as HT. Obesity and hypothyroidism are two frequently occurring clinical disorders that have been tightly connected. A novel viewpoint suggests that alterations in thyroid-stimulating hormone (TSH) may be related to obesity rather than thyroid disease.

Purpose of the study: This study aimed to evaluate the expression of two lncRNAs (XLOC_I2_006631 and ENST00000425150) in peripheral blood of patients with HT, as well as their correlation with central obesity and laboratory characteristics.

Method: Expression levels of lncRNAs (XLOC_I2_006631 and ENST00000425150) were determined by quantitative real-time reverse transcription polymerase chain reaction from 114 healthy individual serve as controls (patients and age and gender matched).

Results: lncRNA XLOC_I2_006631 expression level was statistically significant increase in HT patient ($P < 0.001$). While lncRNA ENST00000425150 expression level was statistically significant decrease in HT patients ($P < 0.001$). Two lncRNAs were correlated significantly with serum levels of TgAb, TPOAb, waist circumference and body mass index as central obesity phenotype vital sign.

Conclusion: The expressions levels of XLOC_I2_006631 and ENST00000425150 were significant, and correlate with central obesity phenotype and laboratory characteristics in in HT patients. Suggesting that these lncRNAs could be a potential biomarker for HT.

Keywords: gene expression, Hashimoto thyroiditis, Long noncoding RNAs, Real time-PCR, Obesity

1. Introduction

Hashimoto's thyroiditis (HT) is defined as a chronic, organ-specific autoimmune disorder. It's also called chronic autoimmune lymphocytic thyroiditis **(1).** The main pathological features are production of autoantibodies that attack the healthy thyroid and deposition of T lymphocytes in the thyroid parenchyma, which lead to advanced thyroid gland damage, thyrocyte atrophy, and diffuse gland enlargement that finally progresses to hypothyroidism **(2).** In HT, helper T cells specific for thyroid antigen are stimulated to start the antithyroid immune response. After initiation of helper T cells, they stimulate B cells to release auto-antibodies against thyroid tissues to plasma **(3).** Although the actual etiology and pathogenesis of HT are complex and not yet clearly identified, it accepted that epigenetic factors, environmental circumstances, and genetic components are interact to cause the disease **(4).**

The incidence of HT is more in females, and in countries with iodine deficiency. The clinical manifestation of HT is characterized by recurrent attacks of hyperthyroid, followed by euthyroid

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phase then ending as persistent hypothyroidism **(5).** Increased generation of anti-thyroglobulin antibodies (TgAb) and anti-thyroperoxidase antibodies (TPOAb) are a hallmark of laboratory funding. Furthermore, some patients have excessive levels of the hormone thyrotropin **(6).**

Autoimmune disorders, such as HT are affecting an increasing number of people. Globally, at the same time, the prevalence of obesity has significantly increased, obesity can directly affect the activation of inflammatory mechanisms that affect the thyroid in genetically predisposed individuals **(7).** A substantial correlation exists between obesity and persistent low-grade inflammation. Patients who are obese are more likely to develop hypothyroidism and thyroid autoimmunity, indicating a pathogenetic relationship between inflammation, autoimmune, and obesity **(8).** Anti-thyroperoxidase antibodies (TPOAbs) and hypothyroidism are two conditions that obesity is linked to increasing risk of developing **(9).**

Another significant element of the potential reciprocal relationship between the thyroid immune mechanism and obesity is thyroid hormone. In certain cellular/clinical situations, the indirect effect of thyroid hormone on immune response may be mediated by a mix of both genetic and nongenomic effects. These mechanisms are particularly significant in obese individuals, who often exhibit decreased T4 levels **(10&11).**

Long non-coding RNAs (lncRNAs) are a one of transcripts, about two hundred nucleotides in size, it has an expression level lower than mRNAs coding protein. According to genomic a proximity to genes coding for protein lncRNAs are categorized into sense, antisense, bidirectional, intronic and intergenic **(12).** LncRNAs have various regulation mechanisms depending on whether they are found in the cytoplasm or the nucleus. Theses mechanisms have been identified that affect gene translation, whole gene networks, post-transcriptional events, transcription, and other related processes **(13).**

According to previous studies, lncRNAs are important in the pathophysiology of various disorders, such as cancer**(14),** cardiovascular diseases**(15)**, nervous system disease **(16)**, autoimmune disorders as multiple sclerosis **(17),** rheumatoid arthritis (RA) **(18)**, systemic lupus erythematosus (SLE) **(19)**, central obesity **(20&21)** and autoimmune thyroid disease **(22).**

However, in HT the exact role of lncRNAs is not elucidate yet. Therefore, the goal of the current research was evaluating the expression of long non-coding RNAs XLOC_I2_006631 and ENST00000425150 in patients with HT, as well as their correlation with anthropometric parameters, central obesity, and laboratory characteristics.

2. Experimental

Methods

From January 2020 to April 2022, retrospective case-control study was carried out with the cooperation of the departments of Clinical Pathology and Internal Medicine in Egypt. Two hundred and twenty-eight volunteers were registered in this study who were separated into two groups. **Group I (Case group):** 114 HT patients diagnosed based on criteria of European Thyroid Association (ETA), It comprised clinical manifestations, ultrasonography, thyroid-stimulating hormone (TSH), and thyroid antibodies (TPO-Ab) levels. Levothyroxine sodium was administered to all HT patients who had elevated TSH and TPO-Ab levels. Age of patients range from 55-68 years to avoid hormonal influence between males and females in premenopausal age.

Group II (Control group): 114 healthy volunteers of age and gender matched with HT patients served as the control group. The healthy controls had normal thyroid function, no thyroid-specific autoantibodies, no history of thyroid dysfunction or other autoimmune disorders or malignancies. None of the participants were using immunosuppressive medications and none of them displayed any overt clinical signs of infection.

All members in this work handed written informed approval, and Research Ethics Committee in the Faculty of Medicine presented its consent and approval upon the research (INTM3 5/2022).

From all participants; full history was taken and subjected to clinical examination and laboratory investigations.

Clinical examinations included: blood pressure, height, weight, and waist circumference (WC) were measured. The body mass index (BMI) was calculated; Standard weight is described as $18.5 \leq BMI < 24 kg/m^2$, while overweight as BMI ≥ 24 kg/m^2, and obese as BMI ≥ 28 kg/m^2. Central obesity was classified as having a waist circumference of > 85 cm in women and ≥ 90 cm in men (23) (Hales et al., 2018)

Laboratory investigations:

Samples collection: four ml of blood was obtained and divided into Tube A; two ml of blood were conveyed to an EDTA tube for a genetic analysis. Tube B; two ml were put into a plain tube then centrifuged for 20 minutes at 3500 rpm.; the serum used for assay of fasting blood glucose, 2 hours postprandial, lipid profile and thyroid function test. Laboratory tests: By chemiluminescent immunoassay LDX-800 system (Beckman Coulter, Indianapolis, USA) thyroid hormones and antibodies which include: free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone

(TSH), and thyroid antibodies such as anti-thyroglobulin-Ab (TgAb) and anti-thyroperoxidase-Ab (TPOAb) were measured. Fasting blood glucose (FBG), 2-hours postprandial (2-Hpp), kidney functions (urea and creatinine), lipid profile (total cholesterol, triglycerides, high-density cholesterol (HDL-C), and low-density cholesterol (LDL-C)) were assayed with Beckman Coulter AU680 analyzers (Beckman Coulter, Indianapolis, USA). Then estimated glomerular filtration rate was calculated

RNA extraction and real time -PCR

Gene-JET Blood RNA Purification Kit (Thermo Scientific, Carlsbad, California, USA) was applied to freshly extract the total RNA from venous blood. RNA The concentrations and purity were assessed using the NanoDropTM 2000 (Implen NanoPhotometer™ N60 UV/VIS spectrophotometer, Germany). The RNA extract was kept frozen at minus 80°C till the reverse transcription procedure. The Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Carlsbad, California, USA) was used for cDNA synthesis. The reaction was prepared on ice in two phases with net volume of 20 µl: 12 µl of a total volume was created by mixing 10 µl of RNA with 1 µl of hexamer primer and 1µl of nuclease-free water. At 65 °C sample tubes were incubated for five minutes. Then a total volume of 20 µl was reached by mixing the aforesaid mixture with 4 µl of 5 X reaction buffer, 1µl of Ribolock RNase inhibitor, 2 µl of 10 mM dNTPs, and 1 µl of Revertaid RT. The following incubation cycles were performed on the 2720 thermal cycler (ABI Systems, Singapore): five minutes at 30°C, sixty minutes at 45°C, then five minutes at 70°C. Prior to the real-time PCR step, the cDNA was kept at 20°C. Real-time PCR was carried out by SensiFASTTM SYBR Lo-ROX Kit, USA. The 20 µl total volume was made up of 1.5µl of forward and reverse primers (Table 1), 6µl of cDNA, 1 µl of nuclease-free water, and 10µl of SYBR green dye. For amplification, 3 steps were preformed: a preliminary denaturing step at 95 °C for ten minutes, 55 cycles at 95 °C for fifteen seconds, 65 °C for one minute, and 75 °C for one minute, and a finishing extension step at 72 °C for ten minutes. The fluorescence detection and data processing were finalized by the 7500 (Applied Biosystems, USA). Using the 2 Ct method, the relative expression of genes was detected and standardized to the endogenous housekeeping gene (Beta Actin) before being in comparison with the control. ΔCt = Ct target – Ct reference, ΔΔCt = (ΔCt sample –ΔCt control) (23).

Table (1): Sequences for genes

Gene name	primers for each gene
1) XLOC_I2_006631	Forward (F: GAA TGC CAG AAA CTG CTA CCC), Reverse (R: CTG CCC TCT TTG CCC ACT).
2) MECP2	Forward (F: TCG CTC TAA AGT GGA GTT GATT), Reverse (R: TGG GCT TCT TAG GTG GTT TC).
3) ENST00000425150	Forward (F: ATC TCA TTA GTG GAC GCC G), Reverse (R: AGC ATC ACC GAA AGG AAG C).
4) Beta Actin	Forward (F: CAC GAA ACT ACC TTC AAC TCC), Reverse (R:CAT ACT CCT GCT TGC TGA TC)

Statistical Analysis

IBM SPSS software package copy 20.0. (Armonk, NY: IBM Corp) was used to analyzed data. Percentages and numbers and were used to represent categorical data. Chi-square test was applied to describe qualitative data. For continuous data, Kolmogorov- Smirnov test was used to assess normality. Distributed data were expressed as range, mean, standard deviation, median and interquartile range (IQR). For normally distributed data Student t-test was applied. While, for skewed data Mann Whitney test was used. Spearman coefficient was used to correlate between skewed quantitative variables. The distinguishing performance of the markers was assessed using the receiver operating characteristic curve (ROC),a performance and aera under the curve of not less than 50% is considered satisfactory, while area under the curve and performance of about 100% achieves the best results (24)

3. Results

3.1. General Characteristics of the Study Population.

228 subjects were enrolled in this study, who were separated into two groups. Group I: 114 HT patients. Group II: 114 healthy volunteers as the control group. The mean patients age were 39.74 ± 10.73 years and for controls were 39.47 ± 13.13years ($p = 0.869$). Among them, 25 (21.9 %) were men and 89 (78.1 %) were women with Statistically significant ($p \leq 0.05$). The mean disease duration was 5.37 ±5.90 year (Table 2a).

When anthropometric parameters were evaluated, the patient group with HT had higher body weight, BMI, and WC than control group, in both women and men. Where both women and men with HT disease showed high statistically significantly in their weight and BMI ($p < 0.05$), while women patients had high statistically significantly differences ($p < 0.001$) in their WC cut off values. (Table 2 b).

Table (2a): Comparison between the studied groups according to demographic data

Parameters	Control (n = 114)	Cases (n = 114)	Test of Sig.	P value
Age (years)			t= 0.166	0.869
Min. – Max.	55 – 68	55 – 68		
Mean ± SD.	49.47 ± 13.13	49.74 ± 10.73		
Median (IQR)	48.0 (44 – 59)	49.5 (45 – 60)		
Gender			χ^2 = 1.852	0.174
Male	34 (29.8%)	25 (21.9%)		
Female	80 (70.2%)	89 (78.1%)		
SBP (mmHg)	100 – 120	100 – 165	t = 7.664*	<0.001*
Min. – Max.	108.1 ± 8.40	121.1 ± 16.09		
Mean ± SD.	110 (100 – 120)	120 (110 – 120)		
Median (IQR)				
DBP (mmHg)			t = 5.807*	<0.001*
Min. – Max.	70 – 90	70 - 100		
Mean ± SD.	74.52 ± 5.32	80.22 ± 9.03		
Median (IQR)	70 (70 – 80)	80 (70 – 80)		
Duration of disease (years)				
Range	----------	0.25 – 23.0	-----	-----
Median (IQR)		3 (1 – 7)		
Symptoms suggesting				
Hypothyroidism	----------	108 (94.7%)	----------	----------
Autoimmunity	----------	45 (39.5%)	----------	----------

SBP: systolic blood pressure; **DBP:** diastolic blood pressure; **IQR:** Inter quartile range; **SD:** Standard deviation**; t:** Student t-test; χ^2**:** Chi square test; **p**: p value for comparing between the two studied groups**; *:** Statistically significant at $p \leq 0.05$

Table (2b): Comparison studied between the two groups according to anthropometric measurement in male and female

Parameters	Male			Female			
	Control (n = 34)	Cases (n = 25)	T p	Control (n = 80)	Cases (n = 89)	T	p
Weight (kg)	54 – 71	56 – 105	7. <0.001*	52 – 71	50 – 123	10.460*	<0.001*
Min. – Max.	65 ± 4.94	81.88 ± 9.82	89	63.75 ± 5.35	80.06 ± 13.58		
Mean ± SD.	65(61 – 70)	82(77 – 88)	4*	64 (60 – 69)	80 (73 – 87)		
Median (IQR)							
Height (cm)	150 – 184	160 – 179	0. 0.501	152 – 183	150 – 182	2.119	0.036*
Min. – Max.	166.3 ± 7.45	167.5 ± 5.53	67	165.9 ± 7.03	163.6 ± 7.23		
Mean ± SD.	166 (163 – 168)	168 (163 – 170)	7	166(161.5	–164 (157 – 168)		
Median (IQR)				168)			
BMI (kg/m^2)	20.97 – 25.40	19.84 – 41.02	6. <0.001*	20.23	– 20.55 – 45.18	11.959*	<0.001*
Min. – Max.	23.50 ± 1.13	29.26 ± 4.01	98	25.77	29.96 ± 5.20		
Mean ± SD.	23.71 (23 – 24)	28.96(27.8–31.5)	0*	23.15 ± 1.29	29.07(26.2–33.2)		
Median (IQR)				23.23 (22.2 –			
				24)			
WC (cm)			2. 0.041*				
Min. – Max.	64 – 78	61 – 127	15	49 – 78	60 – 134	10.620*	<0.001*
Mean ± SD.	72.38 ± 3.85	80.64 ± 18.85	8*	74.01 ± 5.80	96.53 ± 19.05		
Median (IQR)	73 (70 – 76)	70 (69 – 95)		75 (74 – 77)	97 (84 – 110)		

BMI: body mass index: **WC:** waist circumference; **IQR:** Inter quartile range: **SD:** Standard deviation: **t:** Student t-test: **p**: p value for comparing between the two studied groups: *: Statistically significant at $p \leq 0.05$

Laboratory data of study individuals were categorized in (Table 3 and 4); There were apparent high significant differences in TSH, FT3, FT4, TPOAb, TgAb, Total cholesterol, HDL-C, TG, LDL-C levels between HT patients and healthy control ($P < 0.001$). (Fig. 1). TPOAb positivity was linked to a higher risk of central obesity in both men and women ($P < 0.05$), consistent with the positive correlation between TPO and metabolic parameters (Fig. 2). TPOAb positive was significantly associated with obesity, central obesity, and hyperlipidemia in study participants ($P < 0.05$). The TPOAb-/TgAb-positive group had significantly higher systolic and diastolic blood pressures, as well as 2-hpp test results, than the TPOAb-/TgAb-negative group.

lncRNAs expression profiles. Expression patterns of lncRNA in the HT patients were significantly diverse from those in controls when measured using a cutoff of a two-fold expression difference. Expression levels of lncRNAs XLOC_I2_006631, MECP2 and ENST00000425150 in the blood of 114 HT patients and 114 healthy controls were evaluated by qRT-PCR (Table 5).

Table (3): Laboratory data of studied groups

Parameters	Control (n = 114)	Cases (n = 114)	Test of Sig.	P
FT3 (N: 2.5-4.10 pg/ml)				
Min. – Max.	2.50 – 4.07	0.70 – 3.70	U=1898.0*	<0.001*
Mean ± SD.	3.15 ± 0.41	2.16 ± 0.83		
Median (IQR)	3.14 (2.8 – 3.5)	2.20 (1.6 – 2.7)		
FT4 (N:0.61-1.12ng/dl)				
Min. – Max.	0.56 – 1.21	0.10 – 1.12	U=1096.5*	<0.001*
Mean ± SD.	0.93 ± 0.16	0.53 ± 0.23		
Median (IQR)	0.95 (0.8 – 1.1)	0.52 (0.4 – 0.7)		
TSH (N: 0340-5.60 mIU/L)				
Min. – Max.	1.09 – 5.15	4.2 – 142	U=26.500*	<0.001*
Mean ± SD.	3.09 ± 1.08	38.40 ± 37.27		
Median (IQR)	2.88 (2.2 – 4.1)	15.70 (8 – 75)		
Anti- TG (N:0-4 IU/ml)				
Min. – Max.	0.25 – 2.52	10 – 1700	U=0.0*	<0.001*
Mean ± SD.	0.95 ± 0.53	267 ± 269		
Median (IQR)	0.84 (0.6 – 1.1)	158 (84.7 – 401)		
Anti-TPO (N:0-9 IU/ml)				
Min. – Max.	0.19 – 3.85	37 – 1540	U=0.0*	<0.001*
Mean ± SD.	1.84 ± 0.81	533 ± 427		
Median (IQR)	1.72 (1.1 – 2.5)	363 (205 – 845)		

FBS: fasting blood sugar; **2hpp:**2-hour post prandial; **TG:** triglycerides; **HDL-C**: high density lipoprotein cholesterol; **LDL-C:** low density lipoprotein cholesterol; **eGFR:** estimated glomerular filtration rate; SD**:** Standard deviation; **IQR:** Inter quartile range; **t:** Student t-test; **U:** Mann Whitney test; **p:** p value for comparing between the two studied groups; ***:** Statistically significant at $p \leq 0.05$

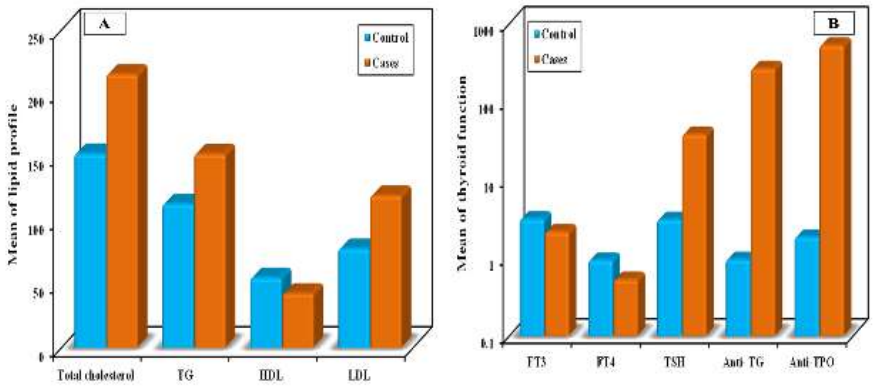

Figure (1): (A)Comparison between the two studied groups according to lipid profile (B) Comparison between the two studied groups according to different parameters

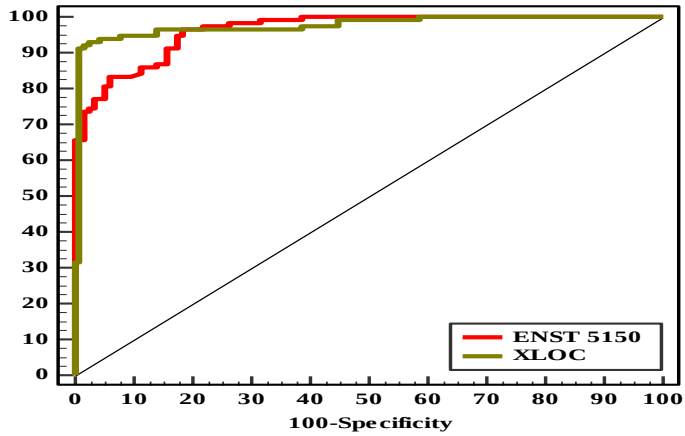

Figure (2): ROC curve for ENST 5150 and XLOC to discriminate patients from control

Table (4): Comparison between the studied groups regarding to thyroid functions

Parameters	Control (n = 114)	Cases (n = 114)	Test of sig.	P
FBG (N:70-100 mg/dl)				
Min. – Max.	70 – 95	70 – 98	t=0.708	0.480
Mean ± SD.	84.10 ± 8.25	83.35 ± 7.63		
Median (IQR)	85 (80 – 100)	83 (76 – 93)		
2-hpp (N< 140 mg/dl)				
Min. – Max.	127 (100 – 138)	128 (87 – 170)	t=2.412*	0.017*
Mean ± SD.	124.9 ± 10.47	128.6 ± 12.65		
Median (IQR)	127 (120 – 133)	128 (121 – 135)		
Urea (N:5-21 mg/dl)				
Min. – Max.	10 – 40	7 – 70	U=6013.0	0.330
Mean ± SD.	25.64 ± 8.98	24.82 ± 9.58		
Median (IQR)	26 (18 – 34)	24 (18 – 32)		
Creatinine (N: male 0.9-1.3 &female 0.6-1.1 mg/dl)				
Min. – Max.	0.55 – 1.20	0.60 – 1.10	U=6148.0	0.481
Mean ± SD.	0.89 ± 0.12	0.88 ± 0.12		
Median (IQR)	0.90 (0.8 – 1)	0.90 (0.8 – 1)		
eGFR (N:90-120 ml/min/1.73m^2)				
Min. – Max.	74.12 – 131.7	76.24 – 124.5	t=0.384	0.701
Mean ± SD.	105.0 ± 12.17	105.6 ± 11.18		
Median (IQR)	104.3 (96.8 – 113.5)	106.4 (98 – 114.6)		
Total cholesterol (N:<200 mg/dl)				
Min. – Max.	115 – 170	148 – 310	t=14.888*	<0.001*
Mean ± SD.	152.8 ± 17.9	215.4 ± 41.2		
Median (IQR)	164 (138 – 167)	206.5 (186 – 243)		
TG (N: 50-150 mg/dl)				
Min. – Max.	48 – 140	72 – 231	t=8.890*	<0.001*
Mean ± SD.	113.1 ± 23.9	152.6 ± 41.0		
Median (IQR)	117 (92 – 136)	160 (119 – 175)		
HDL-C (N:40-60 mg/dl)				
Min. – Max.	45 – 75	23 – 50	t=13.615*	<0.001*
Mean ± SD.	55.06 ± 7.78	42.86 ± 5.57		
Median (IQR)	55 (48 – 60)	44 (40 – 48)		
LDL-C (N: < 100 mg/dl)				
Min. – Max.	59 – 99	67 – 214	t=11.982*	<0.001*
Mean ± SD.	77.91 ± 10.85	119.8 ± 35.66		
Median (IQR)	76.5 (68 – 87)	112 (92 – 143)		

FT3: free triiodothyronine; **FT4:** free thyroxine; **TSH:** thyroid stimulating hormone; **TgAb:** anti-thyroglobulin-Ab; **TPOAb:** anti- thyroperoxidase -Ab; **SD:** Standard deviation; **IQR:** Inter quartile range; **t:** Student t-test; **U:** Mann Whitney test; **p:** p value for comparing between the two studied groups; ***:** Statistically significant at $p \leq 0.05$

Table (5): Gene expression of the studied groups

Genes	Controls (n = 114)	Cases (n = 114)	Test of sig	p
XLOC_I2_006631				
Min. – Max.	0.0026 – 8.4888	0.9094 – 14.7517	U= 332.0*	<0.001*
Mean ± SD.	1.0279 ± 0.8347	6.3336 ± 3.9625		
Median (IQR)	1.0088 (0.6136 – 1.2950)	5.3041 (2.8617 – 10.245)		
MECP2				
Min. – Max.	0.0002 – 1.4748	0.0063 – 6.9459	U= 763.0*	<0.001*
Mean ± SD.	0.6079 ± 0.4432	2.9363 ± 1.7302		
Median (IQR)	0.5474 (0.2256 – 1.0154)	2.4585 (1.7399 – 4.1759)		
ENST00000425150				
Min. – Max.	0.4259 – 1.5470	0.0023 – 0.9912	U= 479.0*	<0.001*
Mean ± SD.	0.9841 ± 0.2475	0.3232 ± 0.2419		
Median (IQR)	1.0265 (0.8540 – 1.1324)	0.2674(0.1107 – 0.5210)		

IQR: Inter quartile range; **SD:** Standard deviation; **U:** Mann Whitney test; **p:** p value for comparing between the two studied groups; ***:** Statistically significant at $p \leq 0.05$

HT patients showed significantly greater levels of XLOC_I2_006631 and MECP2 compared to healthy controls ($P < 0.001$). While healthy controls showed considerably greater levels of ENST00000425150 compared to HT patients ($P < 0.001$).

The ROC curve has been designed to calculate a cut-off value of genes amongst groups for determine if the expression pattern of these lncRNAs would be beneficial as possible biomarkers for HT. The expression of the XLOC_I2_006631 had the best

performance at a cutoff value >1.6751, (AUC = 0.974, 95% CI=0.954 – 0.995, p < 0.001), sensitivity of 93.86% and specificity of 92.11%. For ENST00000425150 expression, the best cutoff value was ≤0.751, (AUC = 0.963, 95% CI=0.944 – 0.983, p < 0.001), with sensitivity of 96.49% and specificity of 81.58% (Table 6)

Table (6): Validity of XLOC_I2_006631 and ENST00000425150 in discrimination patients from controls

Parameters	AUC	p	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
XLOC_I2_006631	0.974	<0.001*	0.954 – 0.995	>1.6751	93.86	92.11	92.2	93.7
ENST00000425150	0.963	<0.001*	0.944 – 0.983	≤0.751#	96.49	81.58	84.0	95.9

AUC: Area Under a Curve; **p** value: Probability value; **CI**: Confidence Intervals; NPV: Negative predictive value; PPV: Positive predictive value; ***:** Statistically significant at p ≤ 0.05; **#**Cut off was choose according to Youden index

Regarding to correlations between gene's expression and levels of thyroid profile, a significant positive correlation between XLOC_I2_006631 and the serum levels of FT4 (r=-0.272; P=0.003; Fig: 4A), TSH (r=0.188; P=0.045), TgAb (r=0.314; P=0.001; Fig:4B) and TPOAb (r=0.504; P<0.001; Fig:4C) had been detected. Only a significant positive correlation between the expression of MECP2, ENST00000425150 and TgAb (r=0.220; P=0.019; Fig:5A& r=-0.291; P=0.002; Fig:5B), TPOAb (r=0.196; P=0.037; Fig:5C.& r=-0.438; P<0.001; Fig:5D) had been observed. A positive correlation was found between the transcription levels of XLOC_I2_006631 and MECP2 (r=0.531; P <0.001; Fig:4D). While a negative correlation was detected between the transcription level of XLOC_I2_006631 and the transcription levels of ENST00000425150 (r=-0.201; P 0.032; Fig:5E). Nevertheless, there was no correlation between MECP2 and ENST00000425150 transcription levels had been observed (r=0.044; P= 0.638)

Moreover, the Univariate and Multivariate Linear regression analysis for the different parameters affecting ENST 0000045150 for HT patients group showed that waist circumference had high significant difference p <0.001 (univariate liner regression Unstandardized Coefficients B 95%C. I; 0.040 (0.019 – 0.062) and (p= 0.055 (multivariate liner regression Unstandardized Coefficients; 0.025 (-0.001 – 0.051).

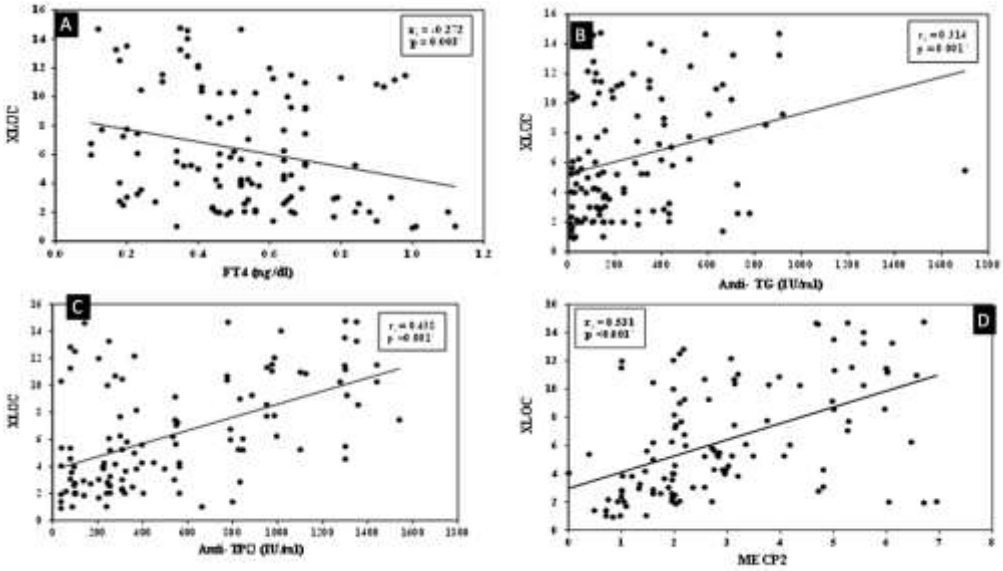

Figure 3: Correlation between XLOC_I2_006631 and thyroid profile in HT patients; (A): significant positive correlation between XLOC and FT4;(B) significant positive correlation between XLOC and anti-TG;(C): significant positive correlation between XLOC and Anti-TPO;(D): significant positive correlation between XLOC and MECP2.

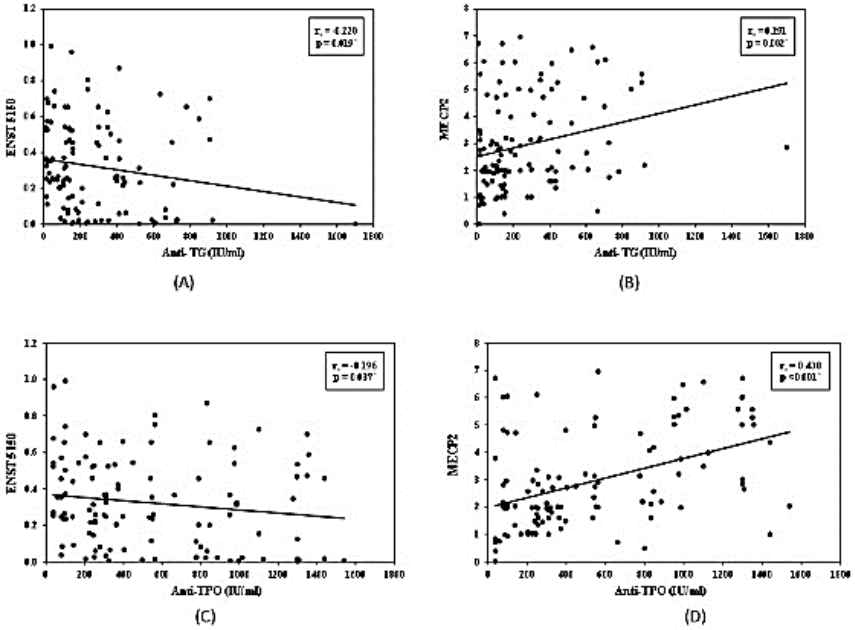

Figure 4: Correlation between the expression of MEcp2, ENst 5150, Xloc,TgAb and TPO Ab ; (A): Correlation between Anti- TG and ENST 5150 in cases group. (B)Correlation between Anti- TG and MECP2 in cases group. (C) Correlation between Anti- TPO and ENST 5150 in cases group (D)Correlation between Anti- TPO and MECP2 in cases group.

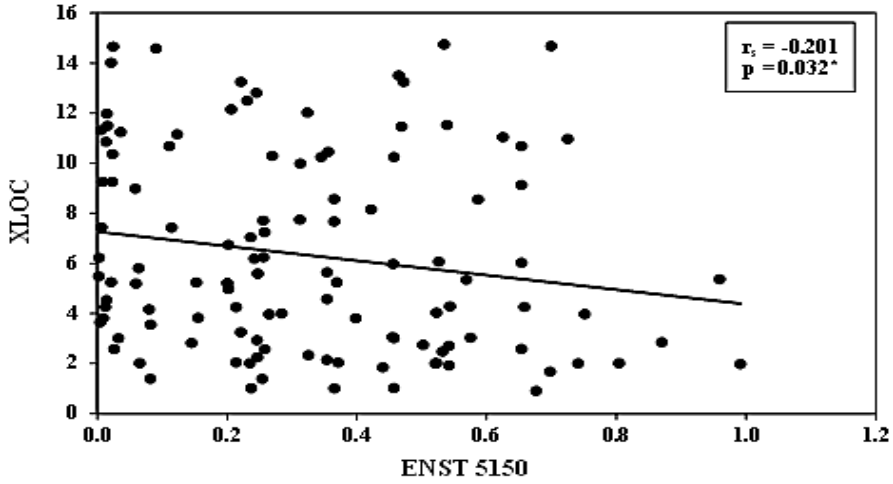

Figure 5: Correlation between XLOC_I2_006631and ENST00000425150in cases group

4.Discussion

LncRNAs, which comprise the majority of human non-coding transcriptome, are crucial immunological checkpoints thought to be implicated in the aetiology of autoimmune diseases. It has been widely studied in the field of cell differentiation and genomic imprinting **(26).** LncRNAs are widely distributed in body fluid, in plasma they are highly stable. LncRNAs contribute to inflammation in autoimmune disorders through releasing of inflammatory molecules such as TNF-a, IL-6, IL-8, IL-1b and IFN-I making them a potentially useful as biomarkers for a variety of disorders **(27).**

The diagnosis of HT is challenging since it can be mistaken for other thyroid diseases. Therefore, the HT must be diagnosed using a combination of clinical symptoms, ultrasound scans, and laboratory investigations including thyroid function tests, and autoantibodies **(28).**

In current research, the exploring two inter-linked diseases, where correlations between genes expression in thyroid autoimmunity and central obesity among HT patients were studied. This study was predicated on the discovery that obesity is linked to chronic low-grade inflammation, which can trigger pathogenic processes and launch the autoimmune cascade.

Two hundred and twenty-eight volunteer were included in this study, 114 HT patients and 114 healthy volunteers, age and gender matched with patients serve as control.

Two-thirds of HT patients were overweight (n= 77; 67%), with more than half having central obesity. The overweight was significantly higher among HT than controls. However, women showed a more prevalence of abdominal obesity than men. This finding was constant with **song et al., (9)** who revealed a clear link between obesity and HT (OR = 1.91, 95% CI 1.10–3.32, P = 0.022). Also, in previous study of thyroid antibodies, by **Zynat et al., (29)** observe that obesity was associated with TPOAb positive patients (OR = 1.93, 95% CI 1.31-2.85, P = 0.001) but not with positive TGAb.

In this study total cholesterol, TG and LDL-C were statistically significant high in HT Patients (p <0.001), with observation that high TSH levels were associated with dyslipidemia. This was explained by **Cengiz et al., (30)** that thyroid hormones inhibit the activity of numerous important enzymes in lipid metabolism, resulting in substantially altered lipoprotein composition and transport in thyroid disorders. Also, **Lei et al., (31)** confirmed that sever hypothyroidism leads to hypercholesterolemia and high LDL-C values. Hyperlipidemia in hypothyroidism is caused by a reduced LDL-C receptor count and impaired LDL-C excretion in the liver.

In this study lncRNA-XLOC_I2_006631 was overexpressed and statistically significantly (P < 0.001) higher in HT patients than controls. Also, its expression level and the blood levels of FT4, TSH, TgAb and TPOAb were significantly positive correlated. These results were agreed with ***Peng et al.,*** **(32)** who observed that XLOC_I2_006631 was overexpressed and was statistically significant in HT patients with p value =0.0051. Similarly, a significant positive correlation was observed between of XLOC_I2_006631 levels and TPOAb. Nevertheless, there was no correlation between XLOC_I2_006631and TgAb serum levels. These findings recommended that lncRNA-XLOC_I2_006631 expression level may be related to development of HT.

MECP2 is one of the methyl-CpG-binding domains (MBD) in addition, it is considered as a pleiotropic DNA protein binding that is mainly linked to methylated cytosine-phosphate-guanine (CpG) and adjusts the expression of several methylation-sensitive genes, such as T lymphocytes upregulate CD 70 **(32) ,** Foxp3 **(33) ,** secreted frizzled-related protein 4 **(34)** and patched 1**(35).**

Methylated DNA and histone deacetylase-containing structures, could be interact with MECP resulting in pair different genomic suppression methods containing histone deacetylation and DNA methylation **(36) .** Furthermore, MECP 2 is able to promote the transcript of genes through linking to unmethylated CPG DNA or joining with CAMP-response component linking protein 1 in promoters **(37).**

Treg is one of the possible mechanisms in pathogenesis of HT. The main signaling pathway of Treg cells is Foxp3 **(38).** MECP2 is a main component of the upstream CpG-rich enhancer in the Foxp3 gene that is methylated in CD 4+T cells, stimulated CD 4+Tcells and peripheral Treg cells **(39).** Initiation of demethylation of CpG site by a DNA methyl-transferase inhibitor triggers the upstream Foxp3 enhancer to stimulate Foxp3 overexpression. Treg cells had a crucial role in the pathophysiology of autoimmune diseases through regulating activation of autoreactive CD 4+T cells and preserving immune homeostasis **(40)**.

In autoimmune thyroid diseases MECP2 was a crucial risk factors for AITDs, suggesting their potential roles in disease pathogenesis. Consequently, it was proposed that MECP2 might be involved in the aetiology of disease **(41).** Furthermore, MECP2 was anticipated to be the possible target gene of lncRNA-XLOC_I2_00663 **(32).**

Based on above data, the expression level of MECP2 mRNA was assayed. MEPC2 was upregulated and statistically significantly (P < 0.001) higher in patients with HT than controls. A significant positive correlation between the expression of MECP2, TgAb and TPOAb were detected. Moreover, a positive correlation was observed between XLOC_I2_006631 expression level and the transcription levels of MECP2. These results were in agreement with **Peng et al. (32)** who found that; the HT patients had higher levels of MECP2 mRNA expression than healthy controls. Additionally, there was a positive correlation between serum levels of MECP2 and TPOAb. On the other hand, there was no association between the levels of MECP2 and TgAb in HT patients. Moreover, there was a strong positive correlation between the transcription levels of MECP2 and the elevated levels of lncRNA XLOC I2 006631. These findings propose that lncRNA-XLOC_I2_006631 could be contribute to the etiology of HT by inducing the expression of MECP2.

In HT stimulation of nuclear factor κB (NF-Kb)/ mitogen-activated protein kinase (MAPK)/ signaling pathway increase level of IL-1β which encouraged the expression of myeloid-related protein 14 (MRP14) in thyroid follicular cells (TFCs). The regulation of MRP14 in TFCs was necessary for IL-1β to promote the release of different chemokines, So, IL-1β aggravates inflammatory prosses in thyroid gland by the MAPK and NF-κB pathway **(42).** Several studies documented that lnc ENST00000425150 was associated with etiology of autoimmune diseases as SLE **(43**) and RA through MAPK pathway **(44).**

There was on enough data about the association of lnc ENST00000425150 with HT. so the expression level of lnc ENST00000425150 was assayed in this work and data analysis revealed that lnc ENST00000425150 in the PBMCs was downregulated and significantly decreased in HT patients ($P < 0.001$) compared with control. Also, negative significant correlation between ENST00000425150 and thyroid autoantibodies were detected. These outcomes agreed with those of **Zhang et al., (45)** who indicated that lnc ENST00000425150 level was dramatically reduced in RA patient ($P < 0.001$) compared to controls. There were significant associations of lnc ENST00000425150 levels with C-RP and ESR in RA patients ($P = 0.0147$& $P = 0.022$). In SLE **Wang et al., (46)** stated that the expression levels of Lnc ENST00000425150 were statistically reduced ($Z=-6.016$, $P < 0.001$) and its expression level were correlated with elevated ESR. Also, **Wu et a., (19)** found that lnc ENST00000425150 were significantly elevated in SLE patients than control. However no significant deference in the levels of lnc ENST00000425150 was found in the RA and primary Sjögren's syndrome (pSS) patients.

In current study, there was significant correlation between ENST00000425150, WC as central obesity indicator ($p<0.001$) and TG ($p=0.003$), this was in agreement with **Sufianov et al (45)** who demonstrated that LncRNAs regulate adipogenesis, lipid mobilization, and adiponectin secretion, and play an important role in the pathophysiology of obesity. LncRNAs also have a role in the regulation of metabolic disorders by expressing tiny peptides that influence lipid droplet formation. LncRNAs can also interact with miRNAs to influence lipid droplet binding proteins and/or other regulators of lipid droplet formation.

Moreover, **Zhang et al., (46) and Wang et al., (47)** documented that lncRNAs modulate adipose tissue and have a significant impact on lipid homeostasis. Also, LncRNAs have been found to correlate with obesity-associated inflammatory diseases, So. it could potentially serve as biomarkers for medicines targeting dyslipidemias in HT patients.

In the current study the diagnostic utility of lncRNA-XLOC_I2_006631 and lnc ENST00000425150 in HT were examined. AUC, sensitivity and specificity for XLOC_I2_006631 were 0.974, 93.86% and 92.11% respectively. While for ENST00000425150 AUC, sensitivity and specificity were 0.963, 96.49% and 81.58%. These findings indicated that lnc XLOC_I2_006631 and lnc ENST00000425150 could serve as possible biomarkers that, when combined with thyroid autoantibodies, and could be improve the diagnose HT.

So, this research specified that lncRNAs (XLOC_I2_006631 and ENST00000425150) may be associated with pathogenesis of HT through regulation of MECP2 and MAPK signaling pathway respectively.

5. Conclusions

In conclusion, the expression of lncRNAs (XLOC_I2_006631 and ENST00000425150) may serve as diagnostic indicator for HT. Also, correlated with waist circumference, BMI, and laboratory characteristics, suggesting that these lncRNAs could be a potential biomarker for HT.Our study has several strengths. First, limited Egyptian studies about the association of central obesity with thyroid autoimmunity Egyptian patients, were accessible. We examine the association of TPOAb and TgAb with obesity as well as the expression of lncRNAs (XLOC_I2_006631 and ENST00000425150), However, more studies are very important for confirm these findings, recognition of long-term relationships between lncRNA levels with various clinical variables, disease activity and effects of treatment. Also, to exclude the probable effect of environmental factors and ethnic diversity.

Ethics Approval:

This study has received approval from the Ethics Committee of menoufia university ID number: (5/2022 INTM3), The research has been carried out in strict adherence to the ethical standards and guidelines set forth by this committee. Further documentation and information supporting this exemption are available upon request.

Conflicts of interest

"There are no conflicts to declare".

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