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Possible Roles of Micro RNA-142-5p and Claudin-1 in Development of Hashimoto's Thyroiditis in Iraqi Patients

Khaled Nather Taha[1*], Farhan Abood Risan[2], Abdulameer Jasim Mohammed[1]

[1] College of Health and Medical Techniques - Baghdad, Middle Technical University, Baghdad, Iraq.

[2] Institute of Medical Technology \ Mansour, Middle Technical University, Baghdad, Iraq.

[*] Corresponding author E-mail: kaled.nather@gmail.com

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Abstract

Background: Micro RNA 142-5p may one day be employed as diagnostic or therapeutic targets in the therapy of autoimmune diseases. The expression of microRNA142-5p in serum from newly diagnosed patients with Hashimoto's thyroiditis has only been studied in a few tissue cases. In addition, elevated microRNA142-5p in tissue is associated with reduced Claudin1 tissue expression in thyrocyte HT patients. This study tries to evaluate these findings in serum of HT before starting treatment. Methods: One hundred-twenty subjects enrolled in this study, divided into 40 newly diagnosed HT patients before treatment are classified into (Euthyroid HT, Subclinical HT, and Overt HT) based on thyroid function test presentation, 40 non-immune hypothyroidism, and 40 healthy controls. The possible mechanisms of microRNA-142-5p in Hashimoto's thyroiditis were investigated by measuring serum MicroRNA 142-5p by quantitative RT-PCR, in addition, to measuring serum Claudin1 by sandwich ELISA. Results: At the time of presentation, the outcome of the tests revealed that 12.5 percent of HT patients had euthyroidism, 30 percent had overt thyroiditis, and 57.5 percent had sub-clinical thyroiditis. miR-142-5p, that's been found to be expressed at significant levels in HT patient serum. Moreover, the ELISA test demonstrated serum Claudin1 was no significant difference between the studied groups. Conclusions: Our data suggest that micro RNA 142-5p may play a role in HT development and pathogenesis through an as-yet-unidentified mechanism, even though serum Claudin1 is not significantly affected in the serum of HT. Chemical thyroid function characteristics at the time of HT manifestation in adults are mostly influenced by the age of the patients and gender.

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Keywords: Autoimmune Diseases; Hashimoto's Thyroiditis; Micro RNA; miR-142-5p; Claudin 1.

1. Introduction

Hashimoto's thyroiditis (HT) was discovered around 1912 by the Japanese doctor effort Hakaru Hashimoto [1] Because of its prevalence in recent years, it has become the first and most prevalent organ-specific AID [2]. In the field of medicine, Hashimoto's thyroiditis is still the most widespread type of hypothyroidism (primary type) and is associated with the progression of thyroid cancer and thyroid lymphoma. Thus, Understanding the pathogenesis of HT is crucial. For more than a century, the actual source of the immunological reaction was unknown, Lymphocyte infiltration with damage to thyroid follicular cells show thyrocyte was damaged with a variety of cell-mediated and humoral mediated responses. There is significant progress occurring in identifying numerous key genes and environmental variables associated with the development of HT [3]. Unfortunately, few documents concern the functional non-coding sequences associated with HT, especially Micro RNA 142-5p. Micro RNA are tiny, non-coding RNAs with approximately22 nucleotides which have recently been found to be a new type of gene expression regulator. Micro RNA 142-5p are tiny RNAs that attach to the three loci (UTRs) of target messenger RNAs and then either destroy them or stop them from being made, Micro RNA 142-5p are variably expressed in autoimmune illnesses, according to growing evidence, MicroRNA control might have an impact on whether they are in the process of developing or being prevented [4], including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS) primary type, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), multiple sclerosis (MS), and ulcerative colitis (US). Until recently, there were only a few studies that found dysregulated microRNA expression in HT patients' PBMC and serum [5,6], Only two pilot studies employed 10 specimens of fine-needle aspiration (FNA) biopsies found that three Micro RNA 142-5p were dysregulated in HT [7] as well as 21 specimens obtained using laser capture micro-dissection [8]. We are concerned that the results were not entirely representative, most likely due to the unequal distribution of the distinctive lesions collected from FNA biopsies, as well as the fact that they have a limited number of Micro RNA 142-5p and several participants.

Nomenclature & Symbols			
TPO Ab	Anti-thyroperoxidase autoantibodies	NS	Non–Significant
Tg Abs	Anti-thyroglobulin autoantibodies	HT	Hashimoto's thyroiditis
SPSS	Statistical Package for the Social Sciences	OV	Overt HT
SC	Subclinical HT	NIHT	Non-immune hypothyroidism
EU	Euthyroid HT	US	Ultrasonography

The serum of the HT-associated Micro RNA 142-5p remains undetected like this study design, it is crucial to understand the expression MicroRNA at different stagesof HT. Thyrocytes are therefore unusual from traditional endocrinocyte in that they have an exocrine role as well as a polarized, phenotype of epithelial cells, which is characteristic of exocrinocyte. The follicle, composed of mono epithelium and just a middle cavity in which thyroglobuline is stored and iodothyronine is synthesized, is the primary component of the thyroid. The junctional complex of thyroid follicular cells consists, in part, of tight junctions (TJ) and adherence junctions (AJ), which encircle the cells close to their lumenal (apical) pole and limit paracellular permeability. As for other epithelial linings, a tight barrier between the extracellular compartments, the lumen, and the extrafollicular space, is critical to normal thyroid function, because it promotes cell polarity and the establishment of trans-epithelial solute gradients of, for instance, iodide and thyroglobulin. Conversely, destruction of the para-cellular barrier would challenge thyroid function and, in the context of autoimmunity, might facilitate the exposure of normally secluded auto-antigens, Tg in the follicular lumen, and TPO in the apical plasma membrane, to the immune system, Claudin1 is the major protein of junction [9]. For these reasons, we used 120 serum samples from newly diagnosed HT patients to examine the levels of Micro RNA 142-5p and serum Claudin1 in HT patients, non-immune hypothyroidism, and healthy controls.

2. Materials and Methods

2.1. Study design

In this study, Between April 2020 and June 2020, one hundred twenty participants were divided into groups: 40 newly diagnosed HT and 40 having non-immune hypothyroidism as negative immune control, and the remaining 40 are age, sex-matched controls. The following criteria were used to select participants: (1) age 18 years or older; (2) thyroid autoantibodies (TPO Abs) and /or autoantibodies (Tg Abs) positive; (3) hypoechogenic on U/S thyroid patterns consistent with autoimmune thyroid disease (at the time of recruitment) and (4) thyroid enlargement U/S. exclusion criteria included: (1) history of thyroidectomy, (2) pregnant or on contraceptive pills, (3) morbid obesity, (4) under thyroid treatment. This research was conducted in Educational Laboratories/Medical City (Baghdad Governorate). The serum concentrations of TSH, T3, T4, FT3, and FT4 were determined using chemiluminescent immunometric assays, and the levels of anti-TPO Abs as well as anti-Tg Abs) were determined using the Elisa kit (Germany company Aeskuliza). Values more than 60 or 180 IU/ml, respectively, were considered positive by the aforementioned methodologies. Examinations of the thyroid using ultra-sonography for the measurement of echo-genicity were always performed by an expert using high-resolution ultra-sonography devices [10] measuring thyroid enlargement using the United State reference values for thyroid volume as a guideline for their findings. Clinical records of patients and/or specific questionnaires were used to reconstruct information regarding their family's history of thyroid disease, autoimmune disorder, and clinical status at the time of diagnosis. The Ethics Committee of Educational Laboratories/Medical City has given their approval for the project. microRNA extracted by trizol reagent then stored and measure levels of serum MicroRNA 142-5p later by PCR, serum Claudin1 detected by (sunlong) sandwish ELISA kit. Diseases or conditions such as acute (e.g., pancreas inflammation), long-standing inflammatory disease (e.g., RA, DM, and polymyalgia), endocrine disorders requiring treatment (other than HT), previous myocardial infarction, and history of malignancies were all exclusion factors for HT patients in this study. If hemolytic was visible, serum samples were ruled out. As a result, three specimens from the diseased group and two samples from the healthy control group were removed.

2.2. Micro RNA Isolation and qPCR

According to the manufacturer's recommendations, micro RNA was obtained using the miRNeasy Serum/Plasma Basic Kit (Qiagen, Germany). After centrifugation, RNA was rinsed from the columns by adding 20 _L RNase-free water to the columns. The separated micro RNA was kept at 80 _C for a short time. A kit (Qiagen, Germany) was used to create complementary DNA, and then qPCR was done in duplicates. Interplate calibration was used for all qPCRs, and the mean of cycle threshold (Ct) readings was computed. Fold change was used to calculate the levels of expression of selected micro RNA under study [20].

2.3. Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 25.0. (Armonk, NY: IBM Corp). The findings are presented in the following way: means and standard deviations, median, as applicable. The Student's t-test (normal distribution) or the Mann - Whitney U test (nonparametric) was used to make comparisons across groups, as applicable. Pearson's correlation analysis was used and the significance level for this study was established at 0.05 percent.

3. Results

Table 1 summarizes the gender distribution for the 120 participants diagnosed with HT. At the time of presentation, test findings revealed that 12.5 percent of patients had euthyroidism comprise from 60% female and 40% male, 30 percent had overt hypothyroidism including 92% female and 8% male, and 57.5 percent had sub-clinical hypothyroidism consisting from74 % female and 26% male, Non-immune hypothyroidism comprise 70% female with 30% male, from above information we detect female gender was the most frequently gender category in studied groups.

Table 1 Frequencies and percentages of gender in different studied subgroups

Gender	Subgroups Hashimoto's Euthyroid	Hashimoto's subclinical hypothyroidism	Hashimoto's overt hypothyroidism	Non-immune hypothyroidism	Healthy control	Chi p value
Female	3 (60%)	17 (74%)	11 (92%)	28 (70%)	23 (57%)	0.220
Male	2 (40%)	6 (26%)	1 (8%)	12 (30%)	17 (42%)	
Total	5 (100%)	23 (100%)	12 (100%)	40 (100%)	40 (100%)	

Table 2 Show the HT patients' median age of 39 (30 - 50) was greater than the control group's 34 (26 – 38) and non-immune hypothyroidism 34 (25.75- 50.5). Table 3 demonstrated TSH, T3, T4, FreeT3, Free T4, anti-Tg Abs, and anti-TPO Abs variables were shown to be strongly associated with stage of HT and among other studied groups with a highly significant difference among studies groups.

Table 2 Summary statistics of Age variable by groups

	Groups	No.	Median (1st Q- 3rd Q)	P value
Age	Hashimoto	40	39.00 (30.00- 50.0)	.008
	Non-immune. hypothyroidism	40	34.00 (25.75- 50.5)	
	Controls	40	34.00 (26.00- 38.0)	

Table 3 Distribution of thyroid function profile with immunological markers in different Hashimotos disease presentation status

	HT/SC	HT/EU	HT/OV	Control	NIHT	Pr > F(Model)
TSH mIU/L	8.68 b	3.93 ab	9.77 b	1.85 a	6.91 b	< 0.001
T3 nmol/L	1.44 a	1.40 a	1.20 a	1.42 a	1.22 a	< 0.001
T4 nmol/L	69.50 b	64.90 ab	55.00 a	93.10 c	64.80 ab	< 0.001
FT3 pmol/L	3.42 bc	3.40 bc	2.66 a	3.77 c	3.22 b	< 0.001
FT4	11.25 b	11.28 b	9.32 a	15.38 c	9.38 a	< 0.001
Anti TPOIU/ml	369.03 b	691.52 c	679.91 c	3.19 a	5.82 a	< 0.001
Anti TgIU/ml	24.64 ab	26.69 ab	29.99 ab	14.39 a	30.87 b	0.03

Table 4 reveal the median and 1st Q- 3rd Q main effect of groups on Claudin_1 ng/ml was not significant and the differences in Claudin-1 ng/ml among the groups were all similar, F (2, 114) = 1.63, p = .201, and Table 5 reveal the comparisons of the serum CLDN1 between all sub-groups under this study with a non-significant difference also.

Table 4 Median and interquartile ranges of serum Claudin 1 among the studied groups

Markers	Groups	Median (1st Q- 3rd Q) n = 40	P value*	ηp2
Claudin 1 ng/ml	Hashimoto	10.295 (8.185- 13.155)	.201	0.03
	NIHT	10.52 (9.123- 11.13)		
	Control	9.70 (8.543- 10.30)		

*Results for Significance Testing against the levels of groups using F-Tests (ANOVA)

Table 5 Comparisons of the serum CLDN1 among studied sub-groups

	HT/SC	**HT/EU**	**HT/OV**	**CONTROL**	**NIHT**	**PR > F(MODEL)**
CLDN 1 NG/ML	12.08 a	12.27 a	13.04 a	9.66 a	10.94 a	0.26

We found that the correlation between MicroRNA142-5p expression level and Claudin-1 was not significant. In both groups of hypothyroidism patients (Hashimoto's thyroiditis vs non-immune hypothyroidism as appears in Table (6).

Table 6 Spearman correlation results between MicroRNA142-5pfold and Claudin-1

Groups	Combination of variables	rs Coefficients	95% CI	n	Sig.
Hashimoto	MicroRNA142-5p-Claudin-1ng/ml	rs -0.31	[-0.72, 0.26]	14	p.281
NIHT	MicroRNA142-5p-Claudin-1ng/ml	rp -0.28	[-0.72, 0.32]	13	p.350

4. Discussion

The age median of the studied groups was matched with control, as previous studies mentioned that approximately five percent of the total the world's population is affected by autoimmune illnesses. The age at onset for each type of disease varies widely. so the results of the present

study were in agreement with another Iraqi study like that of Hanfush [11] and another foreign study by Peretianu who had found that thyroid disorder is a common occurrence in middle age [12]. The findings of this study also accord with those of Manji *et al*., (2006), who reported that the highest ages of HT incidence were in the 4th to 6th decade of AITDs [13]. Other demographic characteristics (female prevalence) differed considerably between groups. The current findings are consistent with those of Mahmood *et al*., (2006), who found that out of 2425 people with thyroid problems, 2135 (88.04 percent) were females and 290 (11.96 percent) were men in Iranian research [14, 15]. According to other studies, the majority of thyroid disorders at the time of HT start with a hypothyroid state [16, 17, 18]. Overt hypothyroidism and sub-clinical hypothyroidism were the most common thyroid dysfunctions identified upon diagnosis in the current investigation [19, 21]. Clinical and biochemical involvement were both a lot worse in the individuals with overt HT presentation in the current series, as described recently in another research study [22]. These findings could illustrate why sub-clinical hypothyroidism has been the most popular common thyroid function pattern according to our research sample at the start. A concomitant role of environmental elements, on the other hand, cannot be ruled out. In reality, since 1991, dietary iodine prophylaxis with fortified iodine salt has been widely advocated throughout the Iraqi region. There is a growing understanding that dysregulated microRNA expression arises in multiple autoimmune disorders [23], Micro RNA 142-5p linked with the disease has an important pathogenic function in the pathophysiology of autoimmune disorders [24,25]. A result of our findings reveals that MicroRNA 142-5p revealed dysregulated level in HT (P value less than 0.05, fold change more than 2). Other studies show the majority of altered micro RNA 142-5p, including micro RNA -142, micro RNA -146, micro RNA -223, micro RNA -150, micro RNA - 155, and micro RNA -21, had been linked to several autoimmune disorders and reported to regulate immune responses [26,27]. This could be because the thyroid gland is being assaulted by a variety of cell- and antibody-mediated immunological mechanisms, as well as leukocyte invasion. These findings suggest that HT serum microRNA142-5p levels could be put under focus for enhancement sensitivity and specificity, this observation is compatible with the microarray analysis results, Nodular nodule, and papillary thyroid cancer with or without HT were compared. These findings indicate that micro RNA 142-5p is intimately related to HTAs a result, micro RNA 142-5p may play a role in HT. micro RNA 142-5p have recently attracted a lot of attention as a unique class of possible laboratory test for diagnosis or monitoring of autoimmune illnesses despite its exciting role in cancer [28,29]. In this study, although we verify that serum microRNA-142-5p expression could be detected in serum samples from patients with HT vs. non-immune hypothyroidism and healthy controls, additional investigations with larger serum sample sizes are required to corroborate micro RNA 142-5pexpression in the serum of HT studied groups. microRNA with addition to their stability in the circulation, research has shown that circulating Micro RNA 142-5p are good biomarkers for cancer diagnosis since they are tissue-specific and often dys-regulated in cancer [30]. As a result of these findings, micro RNA 142-5p is expected as a target for HT [31]. Recent research reveals it has been established that micro RNA -142-5p, in conjunction with micro RNA -142-3p, are 2 transcripts of the has- micro RNA -142 locus [32], according to reports, hematopoiesis - specific micro RNA 142-5p [33]. Thus, researchers have concentrated on the dys-regulated and immune-modulation role in autoimmune disease-associated PBMC [34]. Recent investigations, however, have demonstrated that microRNA-142-5p is expressed in the hippocampus [35] also proliferating involuntary muscle involuntary myocyte of the vascular smooth muscle [36]. Furuse *et al*. were the first to isolate Claudin-1 from liver chicken and define its role in TJ [37]. The number of CLDN family is a significant indicator for proteins which are the most crucial elements of TJ [38]. Furthermore, Claudin1, which is transcribed by the CLDN1 gene, was substantially abundant in normal thyrocyte but decreased in HT-damaged thyrocyte CLDN1. Transcription is downregulated in autoimmune disorders, according to more evidence [39], and this disagrees with our result. This may be due to the Claudin-1 tissue expression in the previous study not linearly correlated with serum Claudin-1 with no explained reason yet. This study discovered that micro RNA 142-5p does not affect serum CLDN1 expression in the current investigation *in vivo*. In contrast to another.

In a prior study utilizing ISH analysis, cells with lower CLDN1 expression typically showed elevated micro RNA 142-5p expression in HT tissue, that's been undetectable in normal thyroid epithelial cells. These mutually exclusive patterns of expression substantiate the notion that CLDN1 is a powerful micro RNA 142-5p target gene *in vivo* [40]. The statistically sufficient sample size of this study is one of its strongest features also all participants were selected from a central laboratory in Baghdad, the research population comes from many places to this center and is most likely representative of the Iraqi population.

5. Conclusion

Thyroid gland problems are a serious public health concern, and a large number of people are at risk of developing an autoimmune thyroid illness. Middle-aged people were the most commonly affected by autoimmune thyroid disorders, with females being the more likely candidates for the diseases, implying that a sex-biased illness condition and age preference existed. For the first time, we measure the serum MicroRNA142-5p expression of HT and found up-regulated expression. Interestingly, The Claudin1 protein in serum was not identified as a direct or even indirect target of micro RNA -142-5p, Our findings suggested that microRNA-142-5p may play a role in the etiology of HT and might be used as a good marker in future research.

Clinical significance

Self-polymerized resins are frequently utilized in dentistry, particularly in the field of maxillofacial rehabilitation. The mechanical properties of such material must be satisfactory. The application of TiO_2 increased greatly the acrylic resins 'flexural strength.

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