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ORIGINAL ARTICLE

Hashimoto Thyroiditis genetic expression of purine receptor and immunological correlation of IL-17 and IL-38

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ABSTRACT
Hashimoto thyroiditis is an autoimmune disease that destroys thyroid cells by cell and antibody-mediated immune processes, The pathology of the disease involves the formation of antithyroid antibodies that attack the thyroid tissue, causing progressive fibrosis continuous scientific evidence indicates the involvement of purinergic receptors in various Autoimmune diseases. Their subsequent activation and inhibition leads to various morphological and biochemical changes. In this study P2Y1, P2Y2, and P2Y4 were examined using qRT-PCR to quantify the genetic fold changes. A total of seventy patients fully diagnosed with hashimoto thyroiditis were selected from various hospitals in the middle Euphrates area/ iraq. venous blood was collected aseptically and transferred into a EDTA tubes for serological IL-17, IL-38 using ELIZA techniques. Purinergic receptors P2Y1, P2Y2, P2Y4 were examined using qRT-PCR. The result shows a reduced serum IL-38 levels and significant reduction in purine receptors genes fold change.
Keywords: *Hashimoto, Thyroiditis, Autoimmune, IL-17, IL38, Purinergic receptors*

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INTRODUCTION

Hashimoto thyroiditis is an autoimmune disease that destroys thyroid cells by cell and antibody-mediated immune processes. It is the most common cause of hypothyroidism in developed countries. In contrast, worldwide, the most common cause of hypothyroidism is an inadequate dietary intake of iodine [1,2,3,4]. Autoimmune thyroiditis (AT) refers to the various pathogenetic manifestations of chronic lymphocytic thyroiditis, and it is a major common cause of gained hypothyroidism as well as linked is to a variety of certain autoimmune endocrine as well as non-endocrine diseases[5]. Autoimmune thyroid diseases (AITD) are the most prevalent organ-specific autoimmune diseases (ADs) and affect 2 - 5% of the population with great variability between genders (i.e., women 5–15% and men 1–5%) (2). AITD include Graves' Disease (GD) and Hashimoto Thyroiditis (HT), among others. HT and GD are the major causes of hypothyroidism and hyperthyroidism, respectively [6]. They reflect the loss of immunological tolerance and share the presence of cell and humoral immune response against antigens from the thyroid gland with reactive infiltration of T cells and B cells, autoantibody generation and, subsequently, the development of clinical manifestations [7].

Interleukin 17

Interleukin-17 (IL-17) is a proinflammatory cytokine that is mainly secreted by T helper-17 (Th17) cells in the initial CD4þ T cell subset. IL-17 secretion contributes to epithelial homeostasis, acute inflammatory responses, and B cell stimulation after appropriate stimuli, acting as a bridge between the innate and acquired immune responses. Notably, numerous studies have revealed that IL-17 plays important roles in various diseases, including infectious and autoimmune diseases, cardiovascular disorders, nonalcoholic fatty liver disease, and hematological and solid cancers [8].

Interleukin 38 (IL-38)

Interleukin-1 family (IL-1F) members comprise a total of 11 pro-inflammatory cytokines, including IL-1α, IL-1β, IL-33, IL-18, IL-36α, IL-36β, and 1Ra), IL-36Ra, IL-37, and IL-38 .IL-38, a recently recognized IL-1F member, is extensively expressed by immune cells and plays a crucial role in a diverse array of

inflammatory autoimmune diseases, however, its exact signaling and functional pathway remains poorly understood since its discovery sixteen years ago. IL-38 is considered to be an antagonist similar to IL-1Ra and IL-36Ra, which mainly inhibit Th17 cytokines and are associated with disease severity and treatment, suggesting that IL-38 might represent a potential biomarker for predicting inflammatory autoimmune disease development and the clinical efficacy of inflammatory autoimmune disease therapeutics, As the nature of IL-38 in common inflammatory autoimmune diseases has been well demonstrated, IL-38 has been suggested to be a biomarker for the development of other inflammatory autoimmune diseases [4]

Purinergic receptors

Purine or adenosine receptors (ARs) are a family of G protein–coupled receptors (GPCRs) with 4 subtypes: A1, A2A, A2B, and A3. P2 receptors are sub grouped into the ligand-gated ion channel receptors P2X with 7 receptor subtypes: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7, and P2Y, which are G protein-coupled metabotropic receptors with 8 subtypes: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14 [9]. Purinergic signaling plays a crucial role in such processes of immune system, as immunological responses, inflammation, platelets aggregation, and cytokine production as well as in autoimmune diseases and infections. All types of purinergic receptors are expressed in immune cells. For example, adenosine receptors are expressed on myeloid as well as on lymphoid lineage of immune cells[10,12]

Continuous scientific evidence indicates the involvement of purinergic receptors in various Autoimmune diseases. Their subsequent activation and inhibition leads to various morphological and biochemical change. very little is known about purine receptors and their action in the thyroid gland or in thyroid related diseases, Human thyrocytes also express P2Y1, P2Y2, P2Y4, and P2Y11 mRNAs and application of ATP induces interleukin-6 production and release from these cells, presumably through activation of one of these receptors [12]. Thyrotroph cancer cell lines also express several subtypes of P2XRs and P2YRs. The mRNA transcripts for P2X3, P2X4, and P2X5 subunits are found in rat thyroid FRTL-5 cells, as well as P2Y2, P2Y4, and P2Y6 mRNAs [11].

MATERIAL AND METHODS

A total of seventy patients fully diagnosed with hashimoto thyroiditis were selected from various hospitals in the middle Euphrates area. Subjects were firstly diagnosed by clinicians using ultrasound and referred for further laboratory investigations [16]. A total of thirty two subjects free from any known disease were recruited as control subjects. Subjects involved in this study were fully consenting patients. This study follows the ethical approval obtained by the university of kufa ethical committee 2022. Five ml of venous blood was collected aseptically and transferred into a EDTA tubes for serological IL-17, IL-38 using ELIZA techniques. 1 ml of whole blood was mixed with 1 ml TRIZOL for RNA extraction. Primers and qRT-PCR conditions are showing below [13,14,15].

Primers	Left	Right	Product length (bp)
P2Y1	Ctgtgtggaccccattcttt	Tcgggacagtctccttctga	439
P2Y2	Gagcatcctcaccacctca	gctattccagggttccaggt	634
P2Y4	Gaagaagcagcagaacacca	caaggagtctgcactggtca	319

Step	Temperature	Duration	Cycle(s)
cDNA Synthesis	57°C	55 mins	1
Pre-Denaturation	95°C	7 mins	1
Denaturation	95°C	25 secs	40
Annealing	57 °C - 72°C	60 secs	1
Melt Curve		30 min	

RESULTS

Interleukin 17 and 38

Serological immune parameters play an important role in destruction of thyrocytes in hashimoto thyroiditis. In the current study interleukin 17 and 38 were measured in patient with clinically confirmed hashimoto thyroiditis and compared to control subjects. Patient with hashimoto thyroiditis had a mean IL-17 level of 320 ng/ml with a standard error of 9.354 while control subjects had a mean IL-17 level of 5.2 ng/ml with a standard error of 0.334. Statistical analysis of difference shows a clear Statistical difference ($p<0.005$) between mean IL-17 levels between hashimoto thyroiditis patients and control subjects as shown below in figure (1). Comparison between mean serum interleukin 17 and 38 in patients with hashimoto thyroiditis and control subjects is shown in figure (1). Patient with hashimoto

thyroiditis had a mean IL-38 level of 0.027 ng/ml while control subjects had a mean IL-38 level of 1.58 ng/ml with a standard error of 0.027. Patients with hashimoto thyroiditis also had a mean IL-17 level of 320 ng/ml while control subjects had a mean IL-17 level of 5.2 ng/ml. Statistical analysis of difference shows a clear Statistical difference ($p<0.005$) between mean IL-38 levels and IL-17 in hashimoto thyroiditis patients. The results of serological interleukins indicate a reduction in serum IL-38 and an increase in IL-17.

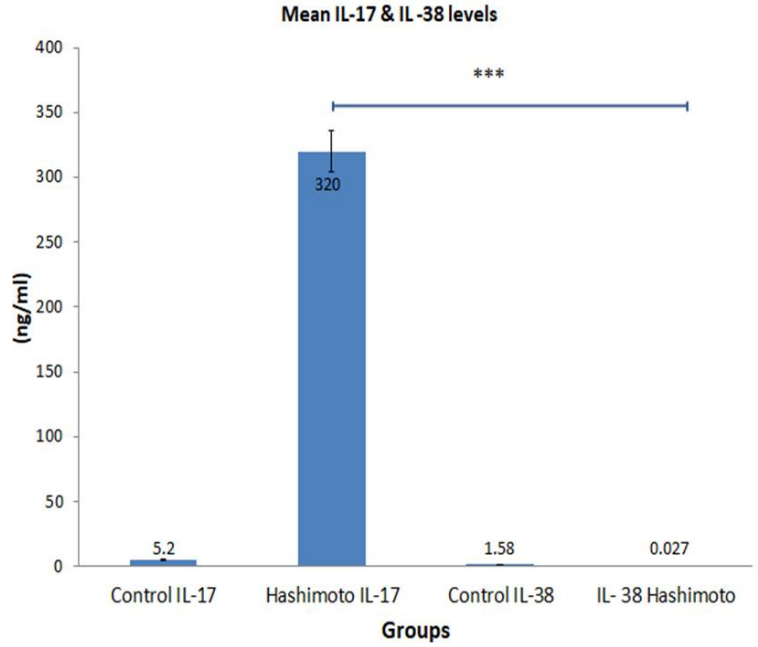

Figure 1: Graphical comparison between mean IL-17 and 38 levels in hashimoto patients and their corresponding control groups. Statistical analysis of difference is shown between each group and their individual control groups. A * is indicative of statistical significance of a p vale of $p<0.005$.

Purinergic receptor gene expression

Noticeable decrease in genetic fold changes in all purinergic receptors compared to their subsequent expression in control subjects. The average gene fold change of P2Y1 in hashimoto patients is 0.412 which is below the average of control subjects indicating a noticeable serological decrease. P2y2 gene fold change is 0.118 in hashimoto patients and 1in control subjects. P2y4 receptor gene fold change is 0.58 in hashimoto patients while control subjects had a gene fold change of 1. Genetic expression of purinergic receptors in hashimoto thyroiditis patients was significantly reduced as shown in figure (2).

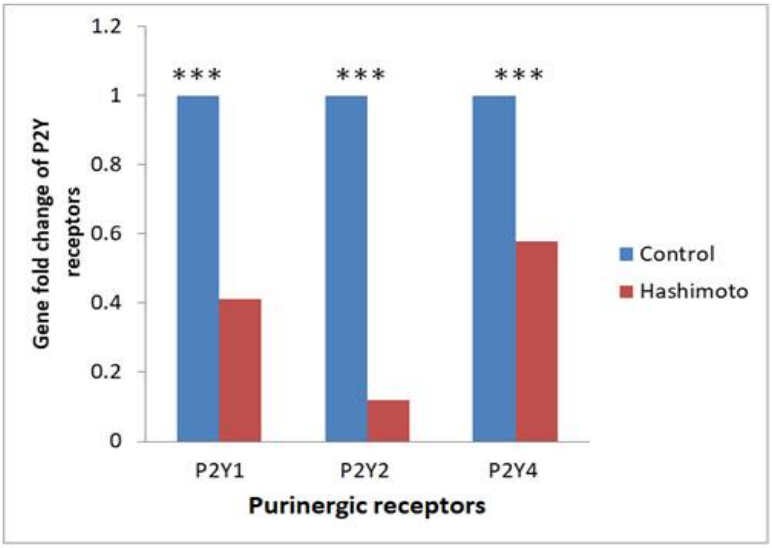

Figure 2: Graphical comparison between gene fold changes of purine receptors (P21, P2Y2, P2Y4) in hashimoto and control groups. Statistical analysis of difference is shown between each group and their individual control groups. A * is indicative of statistical significance of a p vale of $p<0.005$.

DISCUSSION

Significant increase in interleukin 17 in hashimoto thyroiditis patients and a significant decrease in IL-38 indicates a relevant synergistic effect of both interleukin. Due to the lacking scientific evidence on IL-38, this study concludes that IL-38 is significantly reduced in hashimoto thyroiditis patient as an immunological response. More over purinergic receptors which are actively involved in many metabolic and cellular changes in disease including cellular proliferation and differentiation are down regulated in hashimoto thyroiditis patients [17]. This can be due to many reasons including the possibility of P2Y1, P2Y2 and P2Y4 antagonists rise in hashimoto thyroiditis serum. Possible purinergic receptor antagonists include MRS 2179, PPADS and suramin can be released from various cells to inhibit and minimize the effect of purinergic receptors. As a result IL-38 can be a leading cause for the inhibition of purine receptors in hashimoto thyroiditis through the release and chemical inhibition of purine receptors which causes their genetic down regulation in hashimoto thyroiditis. This study shows that IL-17 is actively involved in hashimotothyroiditis while IL-38 is reduced. Quantifiable genetic expression for purinergic receptors is also decreased due to possible involvement of IL-38 reduction in hashimoto thyroiditis [19].

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