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Serum Level and Genetic Polymorphism of IL-38 and IL-40 in Autoimmune Thyroid Disease

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Abstract

Autoimmune thyroid disease mainly includes Graves' disease (GD) and autoimmune hypothyroidism (AIH), which is caused by individual genetics, autoimmune dysfunction, and a variety of external environmental factors. Interleukin IL-38 and IL- 40 are involved in a wide range of autoimmune diseases, but little is known about IL-38 and IL-40 expression in autoimmune thyroid disease. This research included 82 female patients with Graves' disease (GD), 78 females with autoimmune hypothyroidism (AIH), and 85 female healthy controls (HC). An enzyme linked immunosorbent assay and sequencing of IL-38 and IL-40 were used to evaluate serum levels and gene polymorphism, respectively. Results showed ~~that~~ significantly lower level of serum IL-38 levels in the GD and AIH groups in comparison with HC group (both p 0.0001). While there were highly significant differences in the GD and AIH groups than in the HC population (both p 0.0001). There was ~~a~~ significant variability in genotype and allele frequencies in the promoter region of *IL-38* and *IL-40* genes between patients with thyroid disease and healthy controls. The IL-38 homozygote genotype GG exhibits a substantial correlation with GD (P=0.000). While the CC genotype of *IL-40* was shown to have a significant correlation with AIH. The findings suggest that serum concentrations of IL-38 and *IL-40* are potential novel diagnostic biomarkers in patients with GD and AIH, and that the homozygotes GG and CC of IL-38 and IL-40, respectively, serve as a potential predisposing factor on GD and AIH development in the Iraqi population.

Keywords: interleukin-38, interleukin-40, Graves' disease, autoimmune hypothyroidism, genetic polymorphism.

المستوى المصلي والتغاير الوراثي للبين ابيضاضين 38 و 40 في مرض الغده الدرقية المناعي الذاتي

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الخلاصة

يشمل مرض الغدة الدرقية المناعي الذاتي بشكل أساسي مرض جريفز (GD) وقصور الغدة الدرقية المناعي الذاتي (AIH) ، والذي ينتج عن الوراثة الفردية ، والضعف المناعي الذاتي ، ومجموعة متنوعة من العوامل البيئية الخارجية. يساهم البين ابيضاض 38− و البين ابيضاض 40 في مجموعة واسعة من أمراض المناعة الذاتية ، ولكن لا يُعرف الكثير عن تعبيرالبين ابيضاضين 38 و 40 في مرض الغدة الدرقية المناعي الذاتي. شمل هذا البحث 82 انثى تعاني من مرض جريفز (GD) ، و 78 انثى مصابًه بقصور الغدة الدرقية المناعي الذاتي (AIH) ، و 85 انثى من مجموعة السيطرة (HC). تم استخدام طريقة الاليزا وتحليل التسلسل للبين ابيضاضين 38و 40 لتقييم مستويات المصل وتعدد الأشكال الوراثي ، على التوالي. أظهرت النتائج انخفاض معنوي في مستوى البين ابيضاض 38 في مصل الدم في مجموعتي GD و AIH مقارنة بمجموعة HC (كلاهما p 0.0001). بينما كانت هناك اختلافات ذات دلالة إحصائية في مجموعات GD و AIH مقارنةً بمجموعة HC (كلاهما p 0.0001). كان هناك تباين كبير في النمط الجيني وترددات الأليل في منطقة البروموتر لجين البين ابيضاضين 38 و 40 بين مرضى الغدة الدرقية ومجموعة السيطرة. اظهر النمط الوراثي لـ لبين ابيضاض 38 متماثل الزيجات GG ارتباطًا وثيقا مع مرضGD (P = 0.000)). بينما تبين أن النمط الجيني CC للبين ابيضاض 40 له علاقة كبيرة مع AIH. تشير النتائج إلى أن تركيز المصل للبين ابيضاضين 38 و 40 هي مؤشرات حيوية تشخيصية جديدة محتملة في المرضى الذين يعانون من GD و AIH ، وأن المتجانسات متماثلة الزيجات GG و CC للبين ابيضاض 38 و 40 ، على التوالي ، تعمل كعامل مهيئ محتمل حول تطويرمرض GD و AIH في المجتمع العراقي.

1. Introduction

Autoimmune thyroid disease, which primarily includes autoimmune hypothyroidism (AIH) and Graves' disease (GD), is fairly common, affecting about 0.2% of men and 2% of women. The disease's etiology appears to be a combination of multiple environmental and genetic influences. Twin studies and the higher disease incidence in families support the contribution of genetic factors. The concordance rate for well diagnosed Graves' disease in monozygotic twins is reported to be 22%, and 0% in dizygotic twins [1]. The cytotoxic T lymphocyte-associated-4 (CTLA-4) gene may also have a role in vulnerability, according to studies on the human leukocyte antigen (HLA) class II genes [2]. However, it is unlikely that any one of these potential genes will add more than 5% to the total genetic susceptibility [3]. Linkage study has recently revealed other possible susceptibility loci on chromosomes X , 14q31 (4), and 20q11 [5]. for detecting susceptibility genes for some diseases, candidate gene studies have proven very effective [6] as well as disease progression causing genes [7] and according to that, the strategy employed in this study.

Cytokines are believed to be implicated in the onset of autoimmune thyroid disease since they are active throughout both the effector phases and the induction of the immunological and inflammatory response. It has been demonstrated that thyroid follicular cells and intrathyroidal inflammatory cells both produce a range of cytokines, such as interleukin-1a (IL-1a), IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12. Interferon-g (IFNg), tumor necrosis factor-a, IL-13, and IL-14[8]. The cytokine network is intricate, with cytokines serving a variety of overlapping and distinct purposes, as well as impacts that can be boosted or suppressed by other cytokines. On the basis of the animal model, cytokine secretion profiles can be classified as pro- or anti-inflammatory, or as T helper cell type 1 (Th1) responses that support cell-mediated immunity (IL-2 and IFNg) or as T helper cell type 2 (Th2) responses that support humoral immunity (IL-4, IL-5, IL-6, IL-10, and IL-13) [9]. A mixed response by both Th1/Th2 is seen in both AIH and GD, despite attempts to categorize autoimmune thyroid disease as a traditional Th1- or Th2-mediated disease [8]. IL-38, a newly discovered cytokine, is located on chromosome 2q13-14.1 [10]. Researchers used a high- throughput cDNA sequencing to identify it in 2001, and

in 2010 they gave it a new name [11]. With a molecular weight of 16.9 kD, 5 exons, and no signal peptide IL-38 was detected. Glycine, proline, and serine are the next most prevalent amino acids, followed by glutamic acid, alanine, and leucine [12]. IL-38 is also a member of the IL-1 family (IL-1F). Different immune cells, such B cells, for instance, release IL-38 [13]. Since IL-38 is a B cell product, it is noteworthy that metabolic disorders, cardiovascular illnesses, and aging are all associated with increased systemic inflammation. In general, human heart, thymus, etc. express IL-38, while tonsil T cells do not [14]. Interleukin 40 (IL-40), a cytokine related to B cell homeostasis and immune response mechanisms, has recently been identified as a B cell-associated cytokine. The C17orf99 gene (chromosome 17 open reading frame 99) encodes a modest (27 kDa) secreted protein that was first identified as this cytokine by Catalan-Dibene et al. in 2017 [15]. IL-40 is one of the few so-called "orphan" cytokines [16] that do not have homologies with any of the well-established cytokine families due to its distinct structural characteristics. Only a few investigations have been done so far on the C17orf99 gene or the IL-40 protein that it produces. It is widely known that only mammals produce C17orf99 or IL-40, and that these expression levels are especially high in the fetal liver, activated B cells and bone marrow [17]. Human B cells produce IL-40 in vitro after being stimulated by anti-CD40 mAb, anti-IgM, and IL-4, and transforming growth factor (TGF)-b1 further increases this production. Additionally, IL-40 was found in multiple human diffuse large B cell lymphoma cell lines [15], and different lymphoma subtypes expressed it differently, according to reports [18]. After being treated with the anti-inflammatory cytokine IL-38, C17orf99 was recently discovered in the co-culture of human respiratory epithelial cells with macrophages to be down-regulated (19). In 2012, [20] presented the linkage between IL-40 and autoimmune inflammation, ~~who~~ named C17orf99 as one of the four autoantigens differentiating those who are healthy from those with autoimmune hepatitis.

Together, IL-40's roles in immune system regulation and B cell homeostasis make it a viable candidate for involvement in the development of autoimmune disorders. IL-40 is a viable candidate for involvement in the etiology of autoimmune illnesses due to its role in the regulation of immunological processes and the maintenance of B cell homeostasis. In this work, we will try to use IL-38 and IL-40 genetic polymorphisms as biomarkers for autoimmune hypothyroidism (AIH) and Graves' disease (GD) in the Iraqi population.

2. Materials and methods

2.1 Study subject

The patient cohort comprised ~~of~~ 160 Iraqi female patients with autoimmune thyroid disease [82 Graves' disease (GD) and 78 autoimmune hypothyroidism (AIH)] obtained from AL-Amal Hospital and Teaching Laboratories in Baghdad Medical City. Medical history, physical examination, laboratory testing, and ultrasound examinations were used to make the diagnosis of autoimmune thyroid disorders [21]. A positive thyroid stimulating hormone receptor antibody (TRAb = anti-TSH), positive anti-thyroid peroxidase antibody (aTPO), and positive anti-thyroglobulin antibody (aTG) are required for the clinical diagnosis of hyperthyroidism in GD. Elevated thyroid hormone levels in the serum and suppressed TSH levels close to zero are also required [22].

2.2 Collection of blood samples

Five milliliters of Samples (patients and control) were taken and the collected blood were put ~~in~~ into two tubes one with EDTA for genetic analysis and the other without EDTA (gel tube) for getting serum after clotted at 4°C for 1 h and centrifuged at 2000 g for 10 min. to measure interleukins concentration.

2.3 Measurement of interleukins concentrations

The obtained serum was stored at –20°C until analysis. Measurements of interleukins (IL-38 and IL-40) in serum samples were performed using ELISA sandwich kits (BT-Lab, China) following the manufacturer's instructions**.**

2.4 Measurements of TRAb, FT3, FT4, TSH, TGAb, and TPOAb in patients (GD and AIH)and control

Using a fully automated immunoassay analyzer, TSH-receptor antibody (TRAb) was discovered (MAGLUMI4000plus; Shenzhen New Industries). A fully automated immunoassay analyzer was used to test FreeT3, FreeT4, thyroid stimulating hormone (TSH), anti-thyroid peroxidase antibody (TPOAb) and anti-thyroglobulin antibody (TGAb) (i-2000; Abbott).

2.5 Extracting DNA

Total genomic DNA from both groups organizations had been collected in tubes with EDTA was extracted by ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA) and DNA was suspended in free distilled water containing DNase/RNase after following the manufacturer's instructions. The purity and concentration of genomic DNA were determined using Nanodrop. The DNA samples were stored at −20 °C until used.

2.6 Determination of SNPs in IL-38 and IL-40 genes

The DNA samples were amplified for SNPs in the promoter region of *IL-38* and *IL-40* genes by using specifically designed primers as shown in Table1. The 20 µl polymerase chain reaction (PCR) was carried out under the PCR cycle conditions specified in Table 2. The PCR products and the ladder marker were separated by electrophoresis on 1.5% agarose gel. After being stained with ethidium bromide dye, the electrophoresis was carried out at 70 V for 2 hours, and the gel was then examined under Gel documentation - UV trans-illuminator. The molecular size of the bands was calculated using a DNA ladder (a 1000 bp DNA ladder). PCR products (778 bp and 740 bp, respectively) were delivered to Macrogen Corporation in Korea for Sanger sequencing using automated DNA sequencers, ABI3730XL, and the data were obtained by email before being analyzed by the clever program.

Table 1: primers sequence of il-38 and il-40 genes

Genes	Sequence (5′→3′ direction)	Product size bp
IL-38	F- AGTGACAGTGACAGACCCAG- R- TGTGCCTCCCTCACAGTTG-	778
IL-40	F-GGCTCCCTTCAGTCTTCAGT- R- CCCGGAACACAGAGAGATGA-	740

Table 2: PCR Program for *IL-38* and *IL-40* genes

Steps	Temperature (˚C)	Time	
Initial Denaturation	**95**	**5 min**	
Denaturation	**95**	**30sec.**	**(35 cycle)**
Annealing	**55**	**30 sec.**	
Extension	**72**	**60 sec**	
Final Extension Step	**72**	**5min**	

*2.7 Statistical analysis***:** Chi-square test was used to compare genotypes for each sample in a significant way. The odds ratio and confidence intervals were used to determine if the

researched factor had a risky or advantageous influence on the groups. Using the direct counting approach, frequencies of the interleukins polymorphism were determined. The SPSS program (version 17.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8.0.0 were used to analyze population differences using the ch2 test and Fisher exact test (San Diego, California USA). The X2 test was used to assess Hardy-Weinberg equilibrium for these polymorphisms within each group. At a 5% level, statistical significance was considered to exist.

3. Results

3.1 Baseline characteristics of participants

The bioclinical and demographic characteristics of patients with GD, AIH and HCs were represented in Table 3. There were no significant differences in ages parameter p>0.05 while the serum level of all clinical parameters (TRAb, FT3, FT4, TSH, TGAb, and TPOAb) showed highly significant differences for HC, AIH, and GD (p < 0.0001).

Table 3: Demographic and bioclinical characteristics of patients with GD, AIH and HCs

characteristics	GD (n = 82) Mean ±SD	AIH(n = 78) Mean ±SD	HC(n = 85) Mean ±SD	p-value	Normal value
Age (year)	41.21±4.29	41.21±4.39	39.55±4.73	0.85 NS	-
TRAb (IU/ml)	15.05±0.98	0.54±0.09	0.54±0.094	<0.0001****	0-1.75
FT3 (pmol/L)	20.95±1.18	4.34±0.21	4.31+0.34	<0.0001****	3.5-6.5
FT4 (pmol/L)	54.81±1.57	14.91±0.56	15.16±0.57	<0.0001****	11.5-22.7
TSH (mIU/L)	0.07±0.016	7.52±0.51	2.59±0.4	<0.0001****	0.55-4.78
TGAb (IU/ml)	658.07±87.48	916.48±94.58	32.20±3.1	<0.0001****	0-60
TPOAb(IU/ml)	833.99±77.25	911.52±93.65	23.54±2.39	<0.0001****	0-60

Note: vs. HCs:; NS: nonsignificant; ****p < 0.0001. Abbreviations: TRAb, TSH-receptor antibody; FT3, free triiodothyronine 3; FT4, free triiodothyronine 4; TSH, thyroid stimulating hormone; TGAb, anti-thyroglobulin antibody; TPOAb, anti-thyroid peroxidase antibody; GD, Graves' disease; AIH, autoimmune hypothyroidism and HC, healthy control.

3.2 Serum concentration of IL-38 and IL-40 in all studied groups

Serum Levels of IL-38 and IL-40 were measured by ELISA technique. IL-38 serum levels in patients with GD (105.98+28.07 pg/ml) and AIH(59.96+2.14pg/ml) were lower than those of HCs(348.37+120.33 pg/ml) (p<0.0001). Also, there were significant differences between the serum level of the patient with GD and AIH (p<0.0001) as shown in Figure 1A. While serum level of IL-40 ~~was~~ showed a higher level in patients with GD (14.66±3.53 ng/l) and AIH (14.53±3.52ng/l) compared with HCs (9.64±3.53ng/l) p <0.0001 but no significant differences was found between GD and AIH (p<0.05) as shown in Figure 1B.

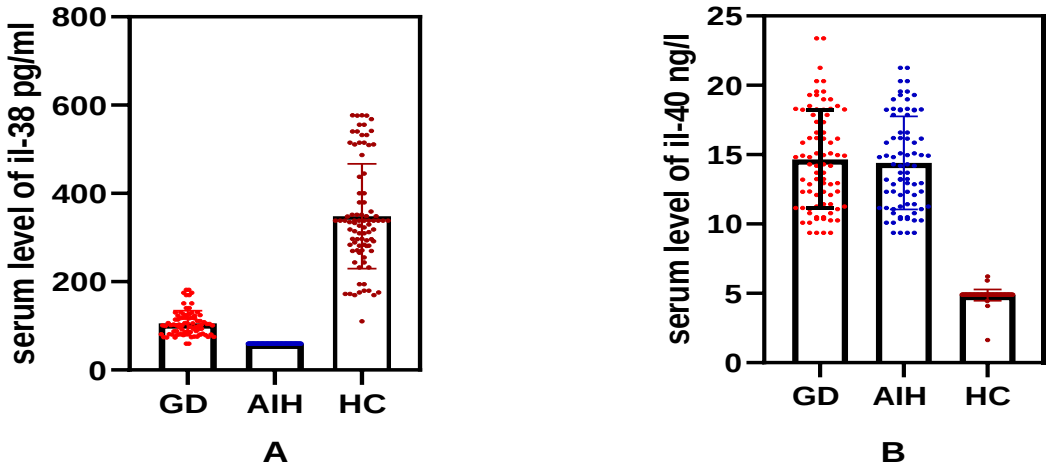

Figure 1: A) Serum level of IL-38. B) Serum level of IL-40. In patients and control groups. GD: Graves'disease, AIH: autoimmune hypothyroidism, and HC: healthy control

3.2 Association of IL-38 and ILl-40 gene polymorphism with thyroid disease

In this case-control study, we examined the distribution of alleles and genotype frequencies of *IL-38 and IL-40* promoter regions among Iraqi patients with autoimmune thyroid disease and healthy controls. Table 4 showed genotypes and allele frequencies in promoter regions of *IL-38 and IL-40* genes. We found novel genotypes of *IL-38* and *IL-40* in the promoter region. There were three genotypes in the promoter region (AA, AG, and GG) and (TT, CT, and CC) of *IL-38* and *IL-40* genes respectively. Our research demonstrates that patients with GD had a higher frequency of the *IL-38* G allele (50% in GD vs. 30.76% in AIH, p = 0.000, OR = 3.36) than healthy controls (20.58%). (Table 4). The autoimmune thyroid illness GD is predisposed to be by allele G, and the GG genotype is associated with a statistically significant 6.0-fold greater risk of GD (p = 0.000, OR = 6.0) and an increased risk of AIH (p = 0.461, OR = 1.50). Patients with GD are more likely to carry it than those with AIH allele G (50% vs. 30.76%, p = 0.000) when comparing the two groups of people with autoimmune disorders. These *IL-38* genotypes were statistically significantly correlated with thyroid illness (GD and AIH). There were no significant differences in Hardy-Weinberg equilibrium (HWE) among groups under study p>0.05 as shown in Table 4.

Table 4: Numbers and percentage frequencies of *IL-38* genotypes, alleles and their Hardy-Weinberg equilibrium (HWE) in GD and AIH patients compared with control groups

gene			GD N= 82 %	p-value OR(95%CI)	AIH N= 78 %	p-value OR(95%CI)	controls N=85 %
IL-38	Genotypes A<G	AA	30(36.58%)	Ref	40 (51.28%)	Ref.	60(70.58%)
		AG	22(26.82%)	0.010* 2.93 (1.24-6.99)	28(35.89%)	0.007** 2.80(1.25 - 6.36)	15(17.64%)
		GG	30(36.58%)	0.000 ** 6.00 (2.43-15.47)	10(12.82%)	0.461 1.50(0.51 - 4.41)	10(11.76%)
	Alleles	A	82 (50%)	Ref.	108 (69.23%)	Ref.	135 (79.41%)
		G	82 (50%)	0.000 ** 3.86(2.32 - 6.45)	48 (30.76%)	0.042* 1.71(1.01 - 2.94)	35 (20.58%)
HWE p-value			0		0.164		0

NS=Non-significant, * significant at p-value ≤ ≤ 0.001, ** significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. Ref. = reference

Results showed in the research groups that patients with GD and AIH are more likely to carry the allele C of the *IL-40* gene in the promoter region. Allele C was discovered in 45.51% of AIH patients (p = 0.000, OR = 2.65), 26.82% of GD patients (p = 0.529, OR = 1.19), and 23.52.6 percent of healthy control subjects (Table 5). According to our findings, having the CC genotype significantly increases the likelihood of thyroid autoimmune disorders (p = 0.000, OR = 4.60). When comparing the AIH patients with the GD patients and the HC groups, there were substantial differences in the frequency of T and C alleles as well as their genotypes (p=0.000). There were significant differences in Hardy-Weinberg equilibrium (HWE) among groups under study p≤ 0.001 as shown in Table 5.

Table 5: Numbers and percentage frequencies of *IL-40* genotypes, alleles, and their Hardy-Weinberg equilibrium (HWE) in GD and AIH patients compared with control groups

gene			GD N= 82 %	p-value OR(95%CI)	AIH N= 78 %	p-value OR(95%CI)	controls N=85 %
IL-40	Genotypes T<C	TT	50(60.97%)	Ref	30 (38.46%)	Ref.	55(64.70%)
		CT	20(24.39%)	0.854 NS 1.10(0.53-2.27)	25(32.05%)	0.040* 2.29(1.03-5.12)	20(23.52%)
		CC	12(14.63%)	0.642 NS 1.32(0.47 - 3.73)	23 (29.48%)	0.000** 4.60(1.95-9.710	10(11.76%)
	Alleles	T	120(73.17%)	Ref.	85(54.48%)	Ref.	130 (76.47%)
		C	44(26.82%)	0.529 NS 1.19(0.70 - 2.02)	71(45.51%)	0.000** 2.65 (1.68 - 4.19)	40(23.52%)
HWE p-value			0.0006 **		0.0018**		0.0014 **

NS=Non-significant, * significant at p-value ≤ 0.05, ** significant at p-value ≤ 0.001, 95% CI: 95% confidence interval. Ref. = reference

4. Discussion

In this investigation, we discovered that serum IL-38 levels were considerably lower in the GD and AIH groups than in the HC group. Principal component analysis demonstrated that GD and AIH may be distinguished from HC by serum IL-38 levels, thyroid-related factors and inflammatory markers. Significantly lower blood IL-38 levels in the HT and GD groups in comparison to healthy control group. According to [23], who is also in agreement with our results, Inhibiting inflammatory signaling allows for a decrease in the secretion of inflammatory mediators by a novel member of the IL-1 family, IL-38. Potential IL-38 receptors include IL-36 receptor, IL-1 receptor 1, IL-1 receptor 10 and IL-38, which show anti-inflammatory activity by linking to these receptors [24]. The importance of IL-38 in autoimmune illnesses is becoming more and clearer from reports.

The focus of some IL-38 research has been on RA, asthma and SLE [25]. However, IL-38's potential contribution to thyroid disease has not yet been looked at. In autoimmune illnesses like GD and AIH, there is an imbalance of inflammatory mediators including IL-35[26], IL-22 [27], and IL-17[28].

According to the findings of our investigation, serum IL-38 levels were lower in GD and AIH than in HC, which is in concaving with anti-inflammatory, IL-38 properties. This conclusion, however, is at odds with previous studies that claim inflammatory diseases like RA [29] and SLE [30] are marked by a high expression of the cytokine IL-38. Patients with Behçet's disease had lower serum IL-38 concentrations, according to Maryam *et al*. [31]. It is possible that variations in the pathophysiology of various diseases contribute to the variances in IL-38 concentrations in AIH and other autoimmune diseases. The major characteristics of AIH include thyroid-related hormone abnormalities, autoimmune antibody production and lymphocytic infiltration. Individuals with GD and AIH had higher concentrations of TGAb and TPOAb, while patients with GD had higher TRAb concentrations (Table 3).

Numerous studies have been published in recent years proving the role of several genes in the emergence of autoimmune thyroid disorders. There are no reports on evaluating the selected gene, IL-38, for the possibility of an autoimmune endocrine illness. In this study, we discovered three genotypes in the IL-38 promoter area (AA, AG and GG). Although there was no significant correlation between the homozygote genotype GG and AIH, there are strong connections between this genotype and GD. A risk factor for developing the condition is the G allele, which is found in people with GD and AIH. This genotype may alter the mRNA expression of the gene and result in low blood levels by afflicting the transcriptional factor of the promoter region.

There has been no prior research on these genotypes in the *IL-38* promoter region. By genotyping 69 SNPs, it was possible to determine in a Korean population, how the IL-1 family of genes (IL1A, IL1B, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL1F10, ILRN) relates to RA. Six (rs7570267, rs3811058, rs315952, rs928940, rs439154 and rs315951) of these 69 SNPs were substantially linked with RA [32-34]. As far as we are aware, as being our study the first one to evaluate the significance of serum IL-40 levels and gene variation for thyroid illness diagnosis. When compared to the HC group, we discovered that the concentrations of serum IL-40 in the GD and AIH groups were greatly significant.

The use of serum IL-40 in thyroid illness has never been studied before. In line with research on other diseases, our study found that IL-40 is implicated in the development of RA. Patients with RA have been found to have local and systemic IL-40 up-regulation, which has been linked to disease activity, autoantibody levels, chemokine levels, and signs of NETosis [35]. This study further demonstrates that the release of the matrix-degrading enzyme MMP-13 and chemokines increased due to extracellular IL-40 by synovial fibroblasts. As recorded by [35] Il-40 has been connected to immunological response and B cell homeostasis. Additionally, RA patients' synovial fluid and serum samples had more IL-40 than OA patients did. IL-40, a systemic autoimmune rheumatic disease characterized by abnormalities and hyperactivity of B cells, was also assessed in the serum of SLE patients [36]. In the promoter region of the IL-40 gene, a unique gene polymorphism was found in this investigation. Three genotypes were discovered in the IL-40 promoter region (TT, CT and CC).

The C allele and CC genotype were found to have a substantial correlation with AIH, however, there was no significant association with GD for the same allele and genotype. Patients with AIH who have the G allele play a crucial role in the disease's development. There have been no prior research on this gene polymorphism in the IL-40 promoter region. The most recent cytokine to be identified is IL-40. A tiny secreted protein (27 kDa) with 265 amino acids, which is primarily expressed by the fetal liver and activated B cells, is encoded by the human genome's C17orf99 gene. While searching the body index of gene expression (BIGE) database, we were interested in this gene [37] for immune system-related uncharacterized genes. Due to its expression pattern (found in the BIGE database) and the existence of a signal peptide, C17orf99 was recognized as a gene of interest.

It is strikingly unrelated structurally to any other cytokine family, suggesting that it probably has a distinct evolutionary history. On a large screen of uncharacterized secreted genes, C17orf99 had been shown to be a potential gene of interest. An estimated 10% of the human genome encodes secreted proteins. Furthermore, because only mammals have gene orthologs, bioinformatics studies led us to hypothesize that C17orf99's activity should be connected to a mammalian-specific immunological function. [38].

5. Conclusions:

This research demonstrates that levels of IL-38 in serum were lower in people with GD and AIH than in the control population, whereas serum levels of IL-40 are greater in people with GD and AIH than in the healthy controls. The homozygote genotype GG of *IL-38* has significant associations with GD but not AIH while CC genotype of IL-40 is associated with AIH but not GD. The results suggest that serum levels of IL-38 and IL-40 are ~~a~~ potential new diagnostic biomarkers in patients with GD and AIH.

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7. Ethical approval

The study was approved by the Research Ethics Committee of College of Science University of Baghdad (Ref.: CSES/0222/0069).

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