Kala azar one of the diseases that play role in autoimmune Thyroid diseases

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الخلاصة:

إن أمراض المناعة الذاتية للدرقية عبارة عن اضطرابات تحدث في الغدة الدرقية هذه الاضطرابات ناتجة عن خطأ الجهاز المناعي في عملية الاستجابة ويذلك فانه يستهدف خلايا وأنسجة الجسم نفسه. ولغرض إثبات الدور الذي يلعبه مرض كالا أزر في أمراض الغدة الدرقية الناتجة عن أمراض المناعة الذاتية (المرض المناعي للثايروكلوبيولين AITG والمرض المناعي الناتج عن اضطرابات تحدث في الغدة الدرقية نتيجة اختلال الجهاز المناعي للثايروويد بيروكسيديز AITPO) لهذه الدراسة، قد تم التحقق في (50) عينة من المرضى المصابين في مرض كالا أزر (رجال ونساء وأطفال) وكانت أعمارهم تتراوح اقل من (1– 15) سنة. أخذت العينات من مختبر الصحة المركزي. تضمنت مجموعة السيطرة (25) شخص من الأصحاء وقورنت مع مجاميع الدراسة من خلال الجنس والعمر. أجري اختبار فحص طفيلي اللشمانيا دونوفاني في المرضى بواسطة Dip stick rk39، بينما قيست اختبارات أمراض المناعة الذاتية للدرقية (AITG و AITPO) في نفس عينات المرضى بواسطة طريقة التفلور المناعي (IFAT).

كانت النتائج تشير إلى أن المرضى كانوا يعانون من تضخم الطحال والكبد ونقصان في أعداد كريات الدم البيضاء ونسبة الهيموكلوبين. وكانت نتائج التفلور المناعي تشير إلى أن (32) مريض كانوا يعانون من أمراض المناعة الذاتية للغدة الدرقية وبنسبة 64% (المرض المناعي للثايروكلوبيولين والمرض المناعي للثايربيروكسيديز) من جميع الأشخاص التي تضمنتها مجموعة الدراسة: 40% من المرضى يعانون من المرض المناعي الذاتي للثايروكلوبيولين(20 من اصل50 مريض) و24 % يعانون من المرض المناعي للثايروويد بيروكسيديز. وان نسبة المرضى الذين يعانون من المرض المناعي الذاتي للثايوكلوبيولين + المرض المناعي الذاتي للثايروبيروكسيديز) بصورة متصاحبة كانت (11,22%). لقد كشفت الدراسة بان هناك فورقات معنوية ($P < 0.01$) ضمن المرضى الين يعانون من المرض المناعي الذاتي للثيروكلوبيولين والمرض المناعي الذاتي للثيروبيروكسيديز) مقارنة مع بقية المجاميع.

إن الهدف من الدراسة هو كشف الدور المؤثر لمرض كالا أزر في أمراض المناعة الذاتية للغدة الدرقية.

Abstract

Autoimmune thyroid diseases (AITD) are disorders of thyroid gland caused by an immune system defect known as autoimmunity. In autoimmunity the immune system errs in its response and targets the body's tissues and cells. To confirm the possible role of Kalaazar disease in complications of autoimmune thyroid diseases (autoimmune thyroglobulin AITG and autoimmune thyroid peroxidase AITPO in this study, 50 patients (male and female) were investigated afflicted with Kalaazar disease, their ages of (< 1-15) years. They were taking from Central Public Health Laboratory .The control group consisted of 25 healthy subjects comparable for age and sex of study groups. The detection of ***Leishmania donovani*** parasite test was done by using dipstick rK39, whereas autoimmune thyroid diseases (AITG and AITPO) tests estimated in the same patients by using immunofluorescent method (IFAT). The results indicated that patients were suffering from spleenomagaly, hepatomegaly and decreased in white blood cells and hemoglobin percents. The results of IFAT tests showed that 32 patients were evaluated to have AITD with percent 64 % (AITG and AITPO) of all the subjects enrolled in this study: 40% of the patients have AITG (20 out of 50 patients) and 24% have AITPO (12 out of 50 patients). And the percent of (AITG +AITPO) with accompanying was (11, 22%). The study showed there was a highly significant differences ($p<0.01$) among patients with AITG, AITPO comparable with other groups.

The aim of this study was to detect the effective role of Kalaazar disease in autoimmune thyroid diseases.

Key words: Autoimmune thyroid diseases, Kalaazar disease.

Introduction:

Autoimmune diseases are the result of an individual's immune system reacting to self constituents, whatever the specific nature of the autoimmune response, highly specific reactivity of antibodies and/or T-cells is directed against external cell- surface structures, internal cytoplasmic or nuclear constituents, or against secreted products produced by cell different organs [1].

Thyroid antibodies are a type of auto antibodies. Auto antibodies are antibodies that target specific proteins that make up the body's tissues and cells. There are several types of auto-antibodies that target the thyroid gland; these include antibodies directed against thyroglobulin (TG), thyroid peroxidase (TPO), thyroxin (T4), triiodothyronin (T3), thyrotropin (thyroid stimulating hormone or TSH) and TSH receptor[1,2,3].

Autoimmune thyroid diseases are caused by infiltration of the thyroid by lymphocytes. Interestingly the lymphocytic infiltration of the thyroid can result either in destruction of the thyroid cells, resulting in an under active thyroid (a disease called Hashimotos disease), or in stimulation of the thyroid, resulting in

an overactive thyroid (a disease called Grave's disease) [4, 5]. Thyroid diseases are estimated to affect as many as 10 percent of the population, and affect women seven times more of ten than men. They are frequently found in families where there are other autoimmune diseases [6].

Thyroglobulin antibodies (TgAbs) are circulating immunoglobulins directed against different epitopes of the thyroglobulin molecule. Thyroid microsomal antibodies (TPOAbs) are circulating immunoglobulins directed against a component of the smooth endoplasmic reticulum of thyroid cells. Resently, microsomal antigen was found to be identical or at least to contain as main component thyroid peroxidase (TPO) [7]. Detectable levels of TG Abs and/or TPO Abs are mainly associated with thyroid autoimmune disorders and with thyroid cancers but low concentrations are also found in a significant percentage of the normal population [8, 9, 10].

Infectious agents have been implicated in the pathogenesis of variety of autoimmune diseases included the autoimmune thyroid diseases [11]. Auto-antibodies found in sera from patients with leishmaniasis include rheumatoid factors, anti-Sm, anti-RNA, anti-SSA, and anti-SSB[12,13]. ***Visceral leishmaniasis*** (VL) may present with cytopenias along with the formation of many auto antibodies and rarely with presence of mixed cryoglobulinemia, Type II, resulting an auto immune disease [14]. Another study had presented a patient with (VL) who was diagnosed as having systemic lupus erythrematosis (SLE) [15, 16].

Leishmaniasis (Kala azar) is a disease which the clinical diversity reflects a complex interplay between the virulence of the infecting species and the host's immune response. This form of disease exhibits a helper T-cell subtypes 1 (TH1) immune response, with interleukin 2, interferon gamma and interleukin 12 as the prominent cytokines that induce disease resolution [17]. Kala azar is the most sever form of the disease which is untreated, has a mortality rate of almost 100%. It is characterized by irregular bouts of fever substantial weight loss, swelling of the spleen and liver and anemia. Although people are often bitten by sand flies infected with Leishmania protozoa, most do not develop to the disease. However, among persons who are immunosuppressed (as a result of the advanced HIV infectious immunosuppresors treatment for organ transplants, have auto logical malignancy, autoimmune disease), cases quickly evolve to full clinical presentation of sever leishmaniasis [17, 18, 19, 20], and in a study in Athens University medical school had reported 2 new cases of leishmaniasis involving patients with rheumatic disease who received anti tumor necrosis factor (anti.-TNF) agents and this study discussed the implications of leishmaniasis in the setting of anti.TNF therapy, which is an association with in increased risk form opportunistic infection [21]. In another study related with (SLE) announced, the

missed recognition of leishmania infection in a lupus patients was lead to death ,since both the omission of a specific parasite treatment and the increase of the immunosuppressive therapy, in conviction of a lupus flare, accelerate a fatal outcome [22] .

Material and methods

The study include 50 patients (male and female) which was suspected with Kalaazar disease of age (< 1-15) years and 25 healthy blood donors taken as a healthy control group. The group suspected with Kalaazar disease was subjected to the following: Determination of leishmanai donovani in serum by using dipstick rK39 Rapid Immunochromatographic Strip assay kits (InBios, U.S.A.) applied as the leaflet kit, and estimation of specific auto-antibodies IgMAG (profile) for thyroid gland by using Indirect Immunofluorescence Test (IFAT). The method named BIOCHIP Mosaic from UROIMMUN Company (Germany) applied as the leaflet kit.

1- Kalaazar detect rapid test:

A- Principle:

The Kalaazar detect for VL is rapid immunochromatographic assay. It is qualitative test based on immunoassay for the detection of antibodies to VL in human serum. The membrane is pre-coated with novel recombinant VL antigen (rK39) on the test line region and chicken anti-protein A on the control line region. During testing serum sample reacts with the dye conjugate (protein A-colloidal gold conjugate) which has been pre-coated in the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with recombinant VL antigen on the membrane and generates red line. The presence of this red line indicates a positive result.

B- Procedure:

- Allow the sera and buffer to reach the room temperature prior to testing.
- Add 20 micro liters of sera to the test strip in the area beneath the arrow.
- Place the test strip into test tube, or well of 96 well tissue culture plate so that the end of the strip is facing downward as indicated by the arrows on the strip.
- Add 2-3 drops 150 micro liters of chgase buffer solution provided with this test kit.
- Read the result in 10 min.

2- Indirect Immunoflorescent Antibody Test (IFAT) for the detection of anti-thyroid gland in serum:

A- Principle:

The test kit is designed exclusively for the invitro determination of humane auto-antibodies in serum or in plasma. The determination can be performed qualitatively or quantitatively. Frozen sections of Monkey thyroid gland covering the reaction areas of a BIOCHIP Slid are incubated with diluted patient samples. If the reaction is positive, specific antibodies of classes IgA,

IgG and IgM attached to the thyroid gland antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

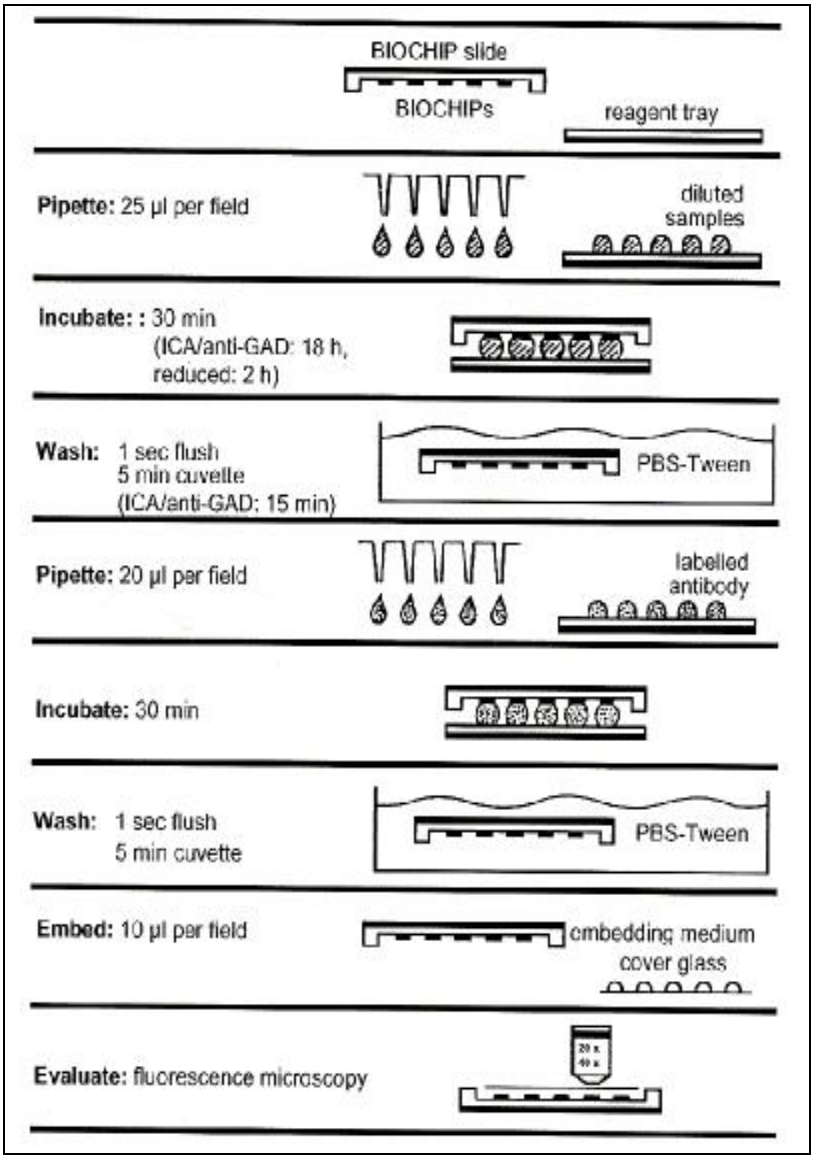
B- Procedure:

- Serum or plasma was diluted at a ratio 1:10 in PBS-Tween.
- 25 ML of diluted samples were added to reaction field of the reagent tray.
- BIOCHIP slides fitted into the corresponding recesses of the reagent tray and incubated for 30 min at room temperature.
- The BIOCHIP slides rinsed with a flush of PBS-Tween and immersed immediately in a cuvate containing PBS-Tween for at least 5min.
- 20 ML of fluorescein-labelled anti-human globulin was added to each reaction field of a clean reagent tray by using stepper pipette.
- BIOCHIP slide removed from cuvate and dried with a paper towel and immediately put into the recesses of reagent tray. Incubation for 30min at room temperature.
- BIOCHIP slide rinsed with a flush of PBS-Tween by using a cuvate with PBS- Tween for at least 5min with shaking then BIOCHIP slide was counterstained with diluted drops of Evan blue.
- 10 ML of embedding medium was added per reaction field.

C- Calculation of results:

BIOCHIP slide were examined under HOX-Magnification of a fluorescent microscope. Their dark green staining identified positively labeled cells.

Titer plane technique as follow :



Steps explained Biochips Technique

Statistical Analysis:

Comparison of paired data from the groups of subjects was done using T-test (t), while correlations between groups were analyzed using person correlation coefficient (r) formula. Statistical tables including observed frequencies with their percentage. SPSS and Microsoft Excel Programs were used for T-test and correlation coefficient calculations respectively [23]. And the validity of AITPO test from AITG test was done by ROC Curve.

Results and Discussion:

All the patients with Kalaazar disease had classic clinical features include high fever, hepatomegaly and spleenomegaly. Major laboratory tests showed pancytopenia (decreased in white blood cells 3000-4000 cu.mm and

haemoglobin range 6.5-9 g/dl). The results of AITDs (AITPO and AITG) were present with specific auto-antibodies with titer of 1:10 by IFAT test. The demographic study showed that there were non significant differences (P>0.05) in gender of patients afflicted with Kalaazar disease ; the male in both healthy control group (18.72%) and patients group (32.64%) were represented with high frequent than female with control group (7,28%) and patients group (18,36%), as noted in (Table-1), and a non significant differences (P>0.05) was showed between the age groups /years of studied group with (1-5 years) increase number and percent in both control (14,56%) & patients (28,56%), as referred in (Table -2). The results showed that the studied groups of leishmaniasis cases consisted of positive AITPO 24% (12 out of 50 patients) and positive AITG (20,40%) at the same patients comparison with controls with a highly significant differences (P<0.01), as referred in (Table-3 and 4) respectively. And the percentage of (AITG +AITPO) with accompanying was (11, 22%) of all the cases of visceral leishmaniasis, as showed in (Table -5).

Gender		Studied Group		Total
		Healthy Control	Patients	
Male	N	18	32	50
	%	72.0	64.0	66.7
Female	N	7	18	25
	%	28.0	36.0	33.3
Total	N	25	50	75
	%	100.0	100.0	100.0

	Value	df	P-Value
Chi-Spuare	.480	1	0.488 NS

Table-1: Sex distribution of visceral leishmaniasis Patients enrolled in the study.

Age groups/Year		Studied Group		Total
		Healthy Control	Patients	
<1	N	4	6	10
	%	16.0	12.0	13.3
1-5	N	14	28	42
	%	56.0	56.0	56.0
6-10	N	7	13	20
	%	28.0	26.0	26.7
11-15	N		3	3
	%		6.0	4.0
Total	N	25	50	75
	%	100.0	100.0	100.0

	Value	df	P-Value
Chi-Spuare	1.725	3	0.631 NS

Table-2: Age distribution of visceral leishmaniasis patients Enrolled in the study.

Thyroid Peroxidase (TPO)		Studied Group		Total
		Healthy Control	Patients	
Positive	N		12	12
	%		24.0	16.0
Negative	N	25	38	63
	%	100.0	76.0	84.0
Total	N	25	50	75
	%	100.0	100.0	100.0

	Value	df	P-Value
Chi-Spuare	7.143	1	0.008 NS

Table-3: The percentage of anti-Thyroid peroxidase antibodies in sera of visceral leishmaniasis patients and control group.

Thyroglobulin (Tg)		Studied Group		Total
		Healthy Control	Patients	
Positive	N		20	20
	%		40.0	26.7
Negative	N	25	30	55
	%	100.0	60.0	73.3
Total	N	25	50	75
	%	100.0	100.0	100.0

	Value	df	P-Value
Chi-Spuare	13.636	1	0.0002 HS

Table-4: The percentage of anti-Thyroglobulin antibodies in sera of visceral leishmaniasis patients and control group.

Thyroid Peroxidase (TPO)		Thyroglobulin (Tg)		Total
		Posative	Negative	
Positive	N	11	1	12
	%	22.0	2.0	24.0
Negative	N	9	29	38
	%	18.0	58.0	76.0
Total	N	20	30	50
	%	40.0	60.0	100.0

	Value	df	P-Value
Chi-Spuare	17.562	1	0.00 HS

Table-5: The percentage of (AITPO+AITG) antibodies with accompanying in sera of visceral leishmaniasis patients.

The validity of AITPO test from AITG test was:
Sensitivity= 55%
Specificity =96.67%
Accuracy= 80%
As referred in table (6) and figure (1) by ROC Curve.

Validity tests	%
Sensitivity	55
Specificity	96.67
Accuracy	80

Table-6: The validity of AITPO test from AITG test by ROC Curve

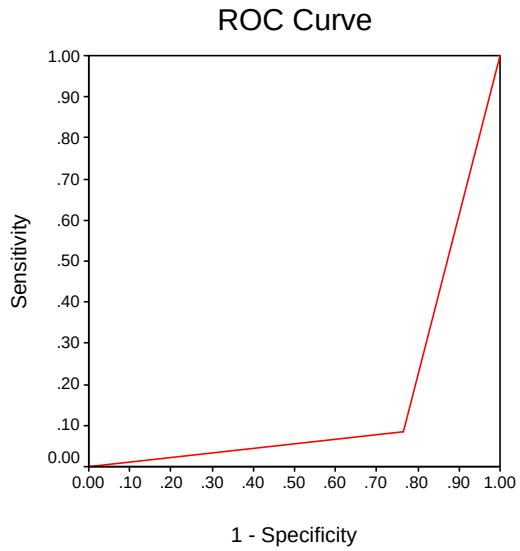


Figure-1: The validity of AITPO test from AITG test by ROC Curve

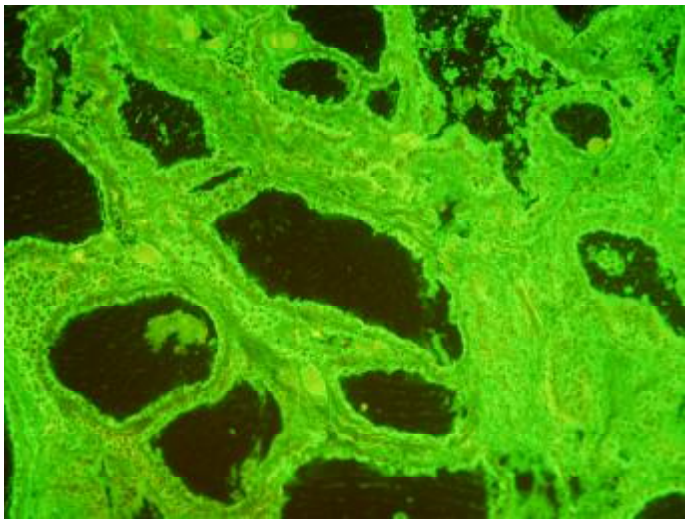


Figure-2: Immunofluorescent of anti –Thyroid peroxidase Abs by IFAT (Dark green staining identified positively labeled anti-Thyroid Gland Abs) HOX-Magnification.

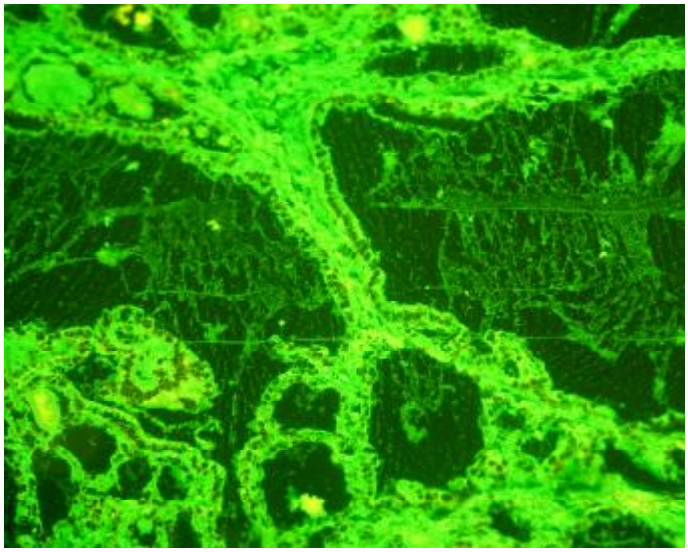


Figure-3: Immunofluorescent of Anti - (Thyroglobulin + Thyroperoxidase) Abs with accompanying by IFAT (Dark green staining identified positively labeled Anti-Thyroid Gland Abs) HOX-Magnification

Infectious agents have been implicated in the pathogenesis of variety of autoimmune diseases namely, rheumatic fever, SLE, myasthenia gravis, IDDM, Sjogren, syndrome and autoimmune thyroid diseases. This study discusses the pertinent data relating to the role of infecting organisms in the development of autoimmune thyroid diseases, by which infection could trigger thyroiditis [11]. This may be related to the strategies encoding leishmania antigens [24]. And /or the involvement in this immune response of CD5+B-1 B cells that are committed to produce multispecific autoantibodies, including IgM-RF, after a weak T-cell interaction [25] . The importance of polyclonal B-cell activation for the genesis and occurrence of auto-antibodies in VL is discussed by Bohm, et al. [26]. However it is known that RF-B lymphocytes can capture immune complex and efficiently present its processed antigenic peptides for T lymphocytes [27]. Thus, RF-B cells could play an important role in the altered immune response verified in VL, including a strong auto-antibody immune response and an exuberant synthesis of antileishmanial antibodies. This could produce larger sized immune aggregates, improving parasite opsonization through Fc receptors on macrophage surface and spreading leishmania infection [28]. While another suggestion that antibodies are induced by molecular mimicry with parasitic antigens rather than by polyclonal B cell activation [13]. Recent experimental evidence suggests that parasites can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment growth and reproduction. The benefit for parasites of hormonal exploitation is so great that they evolved structures similar to the steroid and protein hormone receptors expressed in upper vertebrates that can bind to the hormonal metabolites synthesized by the host [29]. This study had found a

relationship between AITD (TG and TPO) and VL with a specific differences ($P<0.01$) especially in gender with age of (< 1-5) years as show in (Table -2) and figures (2, 3). This phenomenon may be related to the accumulation of antigen presenting dendretic cell (DC) and macrophage (M) in the thyroid gland followed by thyroid autoimmune reactivity, occurs in this intrathyroidal DC accumulation coincides with enhanced growth rate and metabolism of the thyrocytes, suggesting that both phenomena are related. There is a hypothesis that DC known of their super accessory regulators of thyrocyte proliferation and hormone secretion) in other endocrine systems. The clear inhibition of thyrocyte growth by splenic DC demonstrates the regulatory role DC in endocrine systems. Proinflammatory cytokines such as IL-1B and IL-6 are important mediators in this regulation [30]. The dual role of antigen DC represents a link between the immune and endocrine system and may be explain the understanding of the initiation of the thyroid autoimmune reaction and thyroid autoimmune phenomena seen in iodine deficiency [30, 31, 32, 33]. The accumulation and cluster formation of DC and M in the thyroid gland [10, 13, 14], and this accumulation occurs prior to thyroid auto-antibodies formation and prior to the influx large numbers of T and B cells. The accumulation of DC and M are not only acting as APC and effector's cells in host defense ,but also as cells involved in morphogenesis (wound healing and matrix repair). Effects of IL-6 and IL-1B on thyrocytes growth and function, these cytokines inhibit differentiated function such TPO expression, thyroglobulin releasing iodine uptake, and T3 secretion [30, 34, 35]. In a study mentioned that a cascade of gene-inductive events mediating inflammation elimination of the invading organism and induction of T-cell memory against reinvasion. Nrump I, a gene originally identified as Ity/Lsh Bcy for its role in controlling ***S. typhimurium, Leishmania donovani*** and ***Mycobacterium bovies*** infections in mice, regulates this cascade. The structure of the Nrump I protein might relate to its function and might mediate enhanced resistance to infection but cause susceptibility to autoimmune diseases[36].

In conclusion, an increased AITPO and AITG is an autoimmune finding in VL that deserves future studies to elucidate their possible involvement in VL parasitic, AITD and immunopathogenesis.

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