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Immunological Assessment of Human Adenosine Deaminase Activity in Iraqi Female With Thyroid Autoimmune Disease

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ABSTRACT

Background: Human Adenosine deaminase is an essential enzyme for modulating the bioactivity of thyroid hormones, and It is important for the maturation and differentiation of lymphocytes, although its clinical importance in thyroid diseases have yet to be identified. **Objective:** The aim of the current study is to determine the Adenosine deaminase concentration in healthy controls, and in autoimmune thyroid diseases such as Graves' Disease, and Hashimoto's Thyroiditis.

Patients and methods: A total of 183 serum specimens of 103 female patients with autoimmune thyroid diseases and 80 healthy control groups were included in this study and collected from the Baghdad Medical City, Iraq. Quantitative Human Adenosine Deaminase ELISA kits were used to estimate concentration of serum Adenosine deaminase.

Results: There were a highly significant differences between thyroid patients and controls regarding TSH, T4 and adenosine deamainase serum levels (P-value 0.000), while no significant differences were seen with T3. On the other hands, There was a significant difference in adenosine deaminase protein level of autoimmune thyroid diseases patients in compared with controls (8.39±4.74 vs. 6.99±2.411, P<0.05). The results also indicate that both of age and BMI shows significant differences in patients compared with controls (42.63±12.97 vs. 38.94±12.82, P<0.05).

Conclusion: There were a highly significant differences between thyroid patients and controls in TSH, T4, Anti-TPO and anti-TG, except T3.

Keywords: Adenosine deaminase, Thyroid, Autoimmune Diseases, Graves' Disease, Hashimoto's Thyroiditis, ELISA, Case control study, Baghdad Medical City.

INTRODUCTION

Autoimmune thyroid diseases (AITDs) are the most frequent organ-specific autoimmune disorders (ADs), affecting around 2-5% of the population. There is a large gender disparity between those affected, with 5–15% of women affected compared to 1% of men. Autoimmune thyroid disease is also referred to as AITD, according to **Leemans *et al.*** [(1)]. AITD manifests itself in a variety of ways, two of which being Hashimoto Thyroiditis (HT) and Graves' Disease (GD). They both represent a loss of immunological tolerance and share the existance of cellular and humoral immune responses against thyroid gland Ags, includes the reactive infiltration of B and T cells, the generation of autoantibodies, and then the manifestation of clinical symptoms. These are the most common factors that contribute to hypothyroidism and hyperthyroidism, respectively, are HT and GD [(2)].

The use of particular drugs, having a low birth weight, and being exposed to an excessive amount of iodine are all environmental risk factors that might lead to the development of AITD. There is a correlation between the use of tobacco products, high levels of stress, and exposure to radiation and the development of this illness [(3)]**.** The presence of A history of thyroid disease in the family is considered to be one of the most significant risk factors. that contribute to an increased chance of getting thyroid disease. If you have a female family within the first degree who has thyroid disease, your risk is somewhat increased (mother, sister, or daughter). According to **Bonnema and Hegedüs** [(4)], a history of goiter in one's family, particularly throughout childhood, can lead to the development of a benign goiter.

The enzyme adenosine deaminase, also known as ADA, is found in every human tissue despite its polymorphic nature. In addition to regulating the levels of adenosine and serving as a general indicator of the immune system in cells, ADA is necessary for the differentiation and maturation of T lymphocytes. This is the case even though ADA also plays a role in managing these levels. In addition to this, it acts as a broad indication of cellular immunity. In addition, ADA accelerates the irreversible transformation of adenosine into inosine, which is a critical step toward maintaining adequate amounts of adenosine. This mechanism is necessary because levels of adenosine could potentially go out of control [(5)]. **Kaya *et al*.** [(6)] Several autoimmune diseases, including psoriasis [(7)], autoimmune hepatitis [(8)], inflammatory bowel disease [(9)], and rheumatic disease [(7,8,9)], have been found to be associated with high serum concentrations [(10)]. In the meantime, a higher level of ADA enzymatic activity was found in the peripheral white blood cells of GD patients and in the monocytes of HT patients [(11)]. Insurgents led by **Karbownik *et al.*** [(12)]. As a consequence of this, the levels of ADA in the serum have the potential to act as an indicator for the monitoring and evaluation of GD conditions. In addition, the antithyroid drug propylthiouracil (PTU), which is administered orally, was found to significantly reduce epidermal ADA activity and alleviate psoriatic plaques in

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patients who suffered from psoriasis. This discovery was made possible by the fact that PTU is an antithyroid medication [13]. In light of these data, we came up with the hypothesis that PTU, which blocks the action of ADA, would be an effective medication for the treatment of hyperthyroidism. As a consequence of these findings, we came to the conclusion that ADA would make an excellent therapeutic target for GD. As far as we can tell, there has been no clinical investigation into the potential connection between blood ADA levels and GD. This is the best information we have.

The aim of the current study is to determine the Adenosine deaminase concentration in healthy controls, and in autoimmune thyroid diseases such as Graves' Disease, and Hashimoto's Thyroiditis.

PATIENTS AND METHODS

A case-control study was conducted from November 2021 to Jully 2022. A total of 103 serum samples of female patients with AITD were included in this study them age ranging between 11 and 74 years old, were collected from the Baghdad Medical City Baghdad, Iraq, corresponding to 80 healthy controls them age ranging between 15 and 68 years old.

Study population: Inclusion criteria in the patients group were females with thyroid diseases. Whereas exclusion criteria were patients with other type of hormonal disease, and patients receiving chemotherapy. Control grouphad no thyroid diesses or other type of hormonal disease.

At the initial examination, the patient's medical history is taken to determine whether or not the patient has any risk factors for developing an autoimmune thyroid disease. The patient questioned about symptoms of the disease such as changes in weight and energy, or changes in menstrual or fertility cycles.

The general physical exam typically includes measurement of vital signs, pulse and respiration rate, as well as palpation of the thyroid gland.

During the local physical exam, examination of the neck for swelling and inspects the thyroid for any physical abnormalities.

Anthropometrics are a type of measurement used to monitor the physical changes in an individual over time, in order to assess overall health, physical growth and condition, as well as body size and composition. These measurements are commonly used to track changes in people suffering from chronic illnesses such as AITD. In order to accurately measure anthropometrics in people suffering from AITD, measurements should be taken of five areas of the body: height, weight, skinfold thickness, body circumference, and lean body mass.

Laboratory tests in order to confirm a diagnosis of an autoimmune thyroid disease. Common blood tests include thyroid stimulating hormone (TSH) to measure hormone levels, T3 and T4 to measure levels of the two major thyroid hormones, antithyroglobulin antibody (TGAb) and thyroperoxidase (TPO) to measure the presence or absence of an autoimmune response, and other tests depending on the individual case.

The Human Adenosine Deaminase (ADA) ELISA Kit was utilized to perform the Sandwich-ELISA analysis on all of the serum samples in order to determine the levels of Human Adenosine Deaminase (ADA). Procedure provided by the manufacturer (SL0061Hu/Sunlongbiotek/China). An ELISA kit specifically designed for human adenosine deaminase (ADA) was utilized in order to determine the levels of ADA that were present in human serum samples.

An anti-ADA antibody has been used to cover the microplate in a previous step. In the relevant wells of the microplate, either standards or samples were combined with the antibody that was specified. After that, an antibody that was specific for ADA was added to each well of the microplate, and it was incubated after being treated with horseradish peroxidase (HRP). Washing gets rid of any loose components. In each well, the TMB substrate solution was thereafter added. Only the wells that contain ADA and HRP-conjugated anti-ADA antibodies will look blue and then change yellow when the stop solution is added. These wells will be the only ones. The spectrophotometric analysis at 450 nm was used to determine the optical density (OD).

Ethical considerations:

This study was ethically approved by the Institutional Review Board of the College of Science, University of Baghdad. Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means and SD, and independent sample t-test was used for comparison between groups. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

There was a significant difference in adenosine deaminase level of patients in compared with controls (**Table 1**).

Table (1): The mean of adenosine deaminase protein in AITD patients and controls.

Study Groups		N	Mean	Std. Deviation	*P*-value
Adenosine Deaminase	**Patient**	103	8.394	1.747	**0.017****
	Control	80	6.997	1.411	

The patients with AITD were classified into two types; Hashimotus's and Garves's diseases. There are no significant differences in ages of patients with Hashimotus disease compared with patients with Graves' disease. Moreover, the result was showed that no significant difference in level of adenosine deaminase among disease types of AITD, as shown in **Table 2**.

Table (2): The correlation of AITD types in patients with ages and adenosine deaminase.

Thyroid Disease		N	Mean	Std. Deviation	*P*-value
Ages	Hashimotus	46	44.587	1.707	0.163^{Ns}
	Graves	57	41.053	3.803	
Adenosine Deaminase	Hashimotus	46	8.076	1.675	0.526^{Ns}
	Graves	57	8.653	1.481	

As shown in **Table 3** body mass index (BMI) was estimated. A high significant difference in BMI of patients was observed in compared with heathy control (*P* <0.0001).

Table (3): Means of BMI parameter in AITD patients and control groups.

Study Groups		BMI				*P*-value
		Low	Normal	Over-weight	Obesity	
Patient	No.	22	32	27	8	**0.0001 HS**
	%	24.7%	36.0%	30.3%	9.0%	
Control	No.	0	7	22	2	
	%	0.0%	22.6%	71.0%	6.5%	

The result shows in **Table 4** that there is a significant difference in age of patients compared with control group with (*P* <0.05).

Table (4): Means of age parameter in AITD patients and control groups.

Age (years)	AITD Patients	Controls	*P*-value
Count	103	80	**0.0460****
Mean	42.63	38.94	
Standadrd devision	12.97	12.82	

There were a highly significant differences between thyroid patients and controls in TSH, T4 and adenosine deamainase (ADA) serum levels (P-value 0.000), while no significant differences were seen with T3 as shown in **Table 5**.

Table (5): The mean values of studied immunological parameters between study groups.

Study Groups		Mean	Std. Deviation	P-value
TSH	Patient	5.3864	1.13886	0.001^{HS}
	Control	2.0176	00666	
T4	Patient	8.8928	1.48253	0.001^{HS}
	Control	7.4037	1.11375	
T3	Patient	1.2390	01217	0.610^{NS}
	Control	1.1897	0.18197	
ADA	Patient	8.39484	1.747099	0.017**
	Control	6.99790	1.411931	

On the other hand, the the mean values for studied immunological parameters were also evaluated between the two types of thyroid disease, Hashimotus and Graves in addition to age as shown in **Table 6**. In which significant differences were seen with all of them except with Adenosine Deaminase (ADA) (P-value 0.526).

Table (6): The mean values of studied immunological parameters between Thyroid diseases types (only patients).

Thyriod Disease		Mean	Std. Deviation	P-value
Ages	Hashimotus	44.587	1.707	0.163^{NS}
	Graves	41.053	3.803	
TSH	Hashimotus	10.972	1.454	**0.001^{HS}**
	Graves	0.798	0.193	
T4	Hashimotus	6.700	1.607	**0.001^{HS}**
	Graves	10.631	2.099	
T3	Hashimotus	0.865	0.205	**0.001^{HS}**
	Graves	1.547	0.161	
ADA	Hashimotus	8.076	1.675	0.526^{NS}
	Graves	8.653	1.481	

DISCUSSION

The results of the current study indicates that the serum level of adenosine deaminase was higher in AITD than healthy controls. These findings agreed with **Lu *et al.*** [14] who reported that Patients diagnosed with Graves disease had significantly elevated serum ADA levels compared to healthy controls, and researchers found a strong correlation between elevated serum ADA levels in GD patients and robust thyroid function.Patients with Graves' disease who have untreated hyperthyroidism are

at risk for a variety of problems, including but not limited to: diet and exercise, osteoarthritis, thromboembolism, cardiac arrhythmia, bone fracture, vascular dysfunction, and congestive heart disease [15]. Because of an increase in intracellular ATP and oxygen consumption, as well as a dysfunction in the mitochondrial respiratory chain, the hypermetabolic state of hyperthyroidism leads to an imbalance in redox balance and an increase in peripheral tissue reactive oxide species (ROS) generation [16]. In a prospective investigation of 21 GD patients, it was discovered that thyroid function and oxidative stress indices were connected, and that restoring euthyroidism significantly reduced oxidative stress indexes [17].

As a result, the pathophysiological basis for the emergence of GD-related problems may be an increase in oxidative stress. By giving the rodents levothyroxine, Baldissarelli and his associates were able to produce a model of hyperthyroidism in rats. They found that ADA activity in hyperthyroid rat platelets was considerably higher than in controls, and that ADA levels in hyperthyroid rat platelets were positively correlated with ROS levels [18]. The levels of ROS and ADA in the blood of patients with a range of illnesses have been linked, according to numerous research [19].

Additionally, it has been demonstrated in numerous studies that reducing ADA levels greatly lowers the quantity of ROS generated in vivo [20]. The ability of ADA to accelerate T cell growth and differentiation is the mechanism underlying the relationship between these two phenomena [6]. **Erkilic *et al.*** [21] found that activated T-cells can release inflammatory cytokines, which in turn increases the activation of neutrophils and macrophages and finally leads to an excess ROS generation. As a result, it's conceivable that ADA serves as the link between GD problems, oxidative stress, and hyperthyroidism.

According to **Geffner *et al.*** [22] the first patient with ADA deficit and thyroid dysfunction was identified (1993). They recounted the case of a young girl who had an ADA deficiency when she was 8 years old and who developed hypothyroidism about 2 years later [22]. An incremental rise in total body mass is frequently the first sign of hypothyroidism. Pharmaceuticals are used in the majority of hypothyroidism treatments, including those for autoimmune illnesses. These therapies aim to restore a sufficient thyroid hormone supply in order to normalize TSH levels. Research indicates Even after the hypothyroidism state has been achieved, 82% of treated women still have body overweight, and 35% of them are obese (thyroid hormones and TSH levels normalization within the laboratory limits).

This remains the case even after one has reached euthyroidism [23]. Both obesity and the autoimmune disorder known as Hashimoto's disease are inflammatory conditions that affect the body. Interestingly, elimination diets and their potential anti-inflammatory benefits and clinical improvements because these disorders are characterized by persistent inflammation with low-grade and an excess pro-inflammatory cytokines production such as IL-6 and TNF-alpha. Because of these characteristics, we believe that elimination diets may help patients with these disorders.There is evidence, both from experiments and from clinical studies, that persistent inflammation can result in an increase in the amount of water that is retained by the body and in the extracellular space [24]. This effect can also be seen in persons who have Hashimoto's illness, which is characterized by an accumulation of water in the glycosaminoglycans of connective tissue that ultimately results in subcutaneous edema. Both obesity and hypothyroidism are common clinical conditions that have been shown to have a strong connection between them. The significance of the association has grown in recent years as a direct result of the remarkable rise in the prevalence of obesity across the globe. Patients frequently believe that obesity is a direct result of having thyroid dysfunction. According to a recently proposed notion, variations in thyroid-stimulating hormone (TSH) could be the consequence of being overweight. According to findings from recent studies, the hormone leptin, which is produced by adipocytes, appears to be the primary mediator in the connection between obesity and thyroid autoimmunity [25]. Autoimmune thyroiditis affects girls at a higher rate than males, and the incidence of the condition during childhood and adolescence rises with increasing age. When compared to the prevalence of euthyroid goiter, the frequency of overt and subclinical hypothyroidism at the time of diagnosis is comparable [26]. The bulk of thyroid disorders that are age-related and gender-related are caused by hypothyroidism, which is more prevalent in older women and is ten times more prevalent in females than in males [27].

CONCLUSION: Based on the findings, there is a significant difference in adenosine deaminase level of patients in compared with controls. No significant differences in level of adenosine deaminase among disease types of AITD was estimated. There were a highly significant differences between thyroid patients and controls in TSH, T4, Anti-TPO and anti-TG, except T3

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