Beta-2-Microglobulin as a Biomarker In Iraqi Female Patients with Autoimmune Thyroid and Renal Autoimmune Thyroid Diseases

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Abstract:

The present study was performed on 80 female subjects between (30-60) years, who attended the Specialized Center for Endocrinology and Diabetes during the period from April to July; 2011.The subjects were divided into 3 groups : controls , non diabetic autoimmune thyroid patients , and non diabetic autoimmune thyroid patient with renal diseases as complication .

The results showed a significant increase in serum T3,T4 levels in hyperthyroidism patients, and significant decrease in serum T3,T4 levels in hypothyroidism patients ,while a significant difference in serum TSH levels in hyperthyroidism and hypothyroidism patients when compared to control group . The results show also a significant increase in serum antibodies to thyroid peroxidase(anti-TPO) level in both hyperthyroid and hypothyroid patient's when compared to control group. In addition to, there was a significant increase in serum beta-2-microglobulin (β2M) level in thyroid patients and renal thyroid patients compared to control group, while there was no significant increase in serum β2M level in renal thyroid compared to thyroid patient's.

In conclusion, β2M can be used as a biomarker in autoimmune thyroid and renal autoimmune thyroid patient's. In addition to the β2M level in renal thyroid diseases was higher than that in thyroid diseases.

Key words: Autoimmune thyroid disease, β2M , renal diseases , anti-TPO .

Autoimmune thyroid disease (AITD) is a common organ specific autoimmune disorder affecting mostly the middle aged women (30-50 years old). Thyroid autoimmunity can cause several forms of thyroiditis ranging from hypothyroidism (Hashimotos thyroiditis) to hyperthyroidism (Graves Diseases). Both these disorders share many immunologic features and the diseases may progress from one state to other as the autoimmune process changes.Genetic , environmental and endogenous factors are responsible for initiation of thyroid autoimmunity[1].

. Autoimmune thyroid diseases arise when the body attacks its own thyroid gland. When the immune system develops antibodies to thyroid tissue, it treats the thyroid gland as an invader of the body. This causes inflammation of the thyroid gland, which can destroy thyroid tissue over time. The thyroid is responsible for creating two hormones, triiodothyronine and thyroxine. Low levels of these hormones can lead to the symptoms of autoimmune thyroid disease [2].

The human thyroid peroxidase (TPO) is a key enzyme in the synthesis of thyroid hormone. The TPO enzyme helps the reaction which adds iodine to thyroglobulin, a protein necessary to producing the thyroid hormones. TPO function is stimulated by TSH. TPO is a major antigen corresponding to thyroid-microsomal autoantibodies. Anti-TPO auto antibodies are very important to diagnose autoimmune thyroid diseases and also in estimating its clinical course [3]. Thyroid disorders are caused in most cases due to the production of auto-antibodies against different antigens of thyroid tissues. Most important auto-antibodies are that against thyroid peroxidase .Anti-TPO is found in all thyroid autoimmune diseases [4].

Serum beta-2-microglobulin (β2M) was first isolated in 1968 from the urine of patients with Wilson's disease and cadmium poisoning. It has been identified as a low molecular weight protein of 11800 Da. It forms a light chain of class I HLA antigen. It has a 100 amino acid length and is non-covalently associated with a heavy chain of HLA antigens. β2M is found on the surface of all nucleated cells. β2M is filtered by the glomerulus, absorbed and catabolised by the proximal tubules.β2M is excreted in increased amounts in the urine of patients with upper urinary tract infection and connective tissue diseases, such as rheumatoid arthritis and Sjogren's syndrome [5].

Blood B2M levels are elevated in multiple myeloma, chronic lymphocytic leukemia
(CLL), some lymphomas , autoimmune diseases and inflammatory disease . Levels may also be higher in some non-cancerous conditions, such as kidney disease and hepatitis. Normal levels are usually below 2.5 mg/L [6].

Thyroid hormones (TH) are essential for an adequate growth and development of the kidney. Conversely, the kidney is not only an organ for metabolism and elimination of TH, but also a target organ of some of the iodothyronines' actions. Thyroid dysfunction causes remarkable changes in glomerular and tubular functions and electrolyte and water homeostasis. Hypothyroidism is accompanied by a decrease in glomerular filtration, changes in renal morphology such as thickening of the glomerular and tubular basement membranes as well as increased mesangial matrix, hyponatremia, and an alteration of the ability for water excretion[7] .Excessive levels of TH generate an

increase in glomerular filtration rate and renal plasma flow. Renal disease, in turn, leads to significant changes in thyroid function[8].

Thyroid dysfunction causes significant changes in kidney function.Both hyperthyroidism and hypothyroidism affect renal blood flow,GFR,tubular function,electrolytes homeostasis,electrolyte pump function , and kidney structure [9].

Aim of the present study was to assess the relation between the tumer marker (β-2-M) and autoimmune thyroid disease in Iraqi females.

Experimental Part:

Selection of subjects and blood sampling:

Eighty female subjects were enrolled in this study. The age of all studied groups range from (30-60) years. This study was attemted from the Specialized Center for Endocrinology and Diabetes during the period from April to July, 2011.

The subjects divided into three groups:

- Control group (n=20).
- Non diabetic thyroid patients (hyperthyroidism n=20, hypothyroidism n=20).
- Non diabetic thyroid patients with renal diseases, n=20

Renal diseases were (glomerular diseases, inflammation of the urinary tract, ureter stones, urinary crystals).

Five milliliters (5ml) of venous blood were collected from all subjects. Blood samples were transferred into plain tube, allowed to stand for 15 minutes at room temperature, centrifuged at 3500 rpm for 10 minutes.The resulting serum was separated and used for the estimation of T3, T4 , TSH , anti-TPO , β2M .

Methods:

Determination of serum Beta-2-microglobulin:

Beta-2-microglobulin was quantitatively determined in serum by using the Immunometric Enzyme Immunoassay(ELISA) kit from Immuchem [10] .

Highly purified anti-human β2M antibodies are bound to microwells. β2M , if present in diluted serum, bind to the respective antibody.Washing of the microwells removes unspecific components. Horseradish peroxidase (HRP) conjugated anti-human β2M immunologically detects the bound patient β2M forming a conjugate/ β2M / antibody complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end – product . The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of β2M present in the original sample [11].

Determination of serum Anti-TPO :

Anti-TPO is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against thyroid peroxidase (TPO) in human serum or plasma [12]. Principle of method :Highly purified human thyroid peroxidase (TPO) is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidise (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample[13].

Determination of serum T3 , T4 , TSH :

Serum triiodothyronine , thyroxin , and thyroid-stimulating hormone were determinated by VIDAS method which is an automated quantitative test for use on the VIDAS family instruments, for the immunoenzymatic determination T3, T4 , and TSH hormones in human serum using the Enzyme Linked Fluorescent Assay (ELFA). The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA) [14,15].

Statistical Analysis:

All values were expressed as mean ± standard deviation (M±SD). Statistical analysis were performed using student 's T-Test ($p \leq 0.01$) the lowest limit of significance difference between the studied groups [16] .

Results & Discussion:

Levels of triiodothyronine (T3) , thyroxin (T4) , thyroid-stimulating hormone (TSH) , and anti thyroid peroxidase (anti-TPO) in serum of females with autoimmune thyroid diseases (hyperthyroidism and hypothyroidism) and the control groups which expressed as (mean ± SD) presented in table (1).

Table (1) : Diagnostic Parameters (T3, T4, TSH, and anti-TPO) levels (mean ± SD) for control, patients with thyroid diseases (hyperthyroid , and hypothyroid).

Groups / Parameters	Controls No.(20)	Hyperthyroid Patients. No.(20)	Hypothyroid Patients.No.(20)
T3 nmol/L	2.05 ± 0.87	6.27 ± 5.22 □ □	1.18 ± 0.31 □ □
T4 nmol/L	85.30 ± 14.88	204.5 ± 65.3 □	50.07 ± 23.59 □ □
TSH mlu/ ml	2.26 ± 1.02	0.32 ± 0.49 □	25.80 ± 21.14 □ □
Anti-TPO IU/ ml	25.55 ± 12.82	138.12± 33.25 □	111.60 ± 26.41 □

□ □ $P < 0.01$, □ $P < 0.05$

The results revealed a significant elevation in T3 and T4 hormones levels in hyperthyroidism patients , while a significant decrease was found in T3 and T4 levels in serum of hypothyroidism pateints as compared with the control group. The results also show a significant decrease in TSH level in hyperthyroidism patients, and a significant increase in it is level in hypothyroidism patients when compared with control group.The results in agreement with the findings of Dufour D(2007)[17] and Ogedebe H(2007) [18] .A significant elevation in anti-TPO level in hyperthyroidism and hypothyroidism patients comparing with control group,these results were nearly to that obtained by Manorama Swain et al. (2005) [19] ; and Abdelgadir A. et al. (2010)[20] .

The level of serum β-2-M in control, thyroid and renal thyroid patient's groups showed in table (2) .

Table (2) : Mean (±SD) of serum beta-2-microglobulin (β-2-M) level in control , thyroid patients , and renal thyroid patients.

Groups / parameter	Control No.(20)	Thyroid patients No.(40)	Renal thyroid patients No.(20)
β-2-M µg/ml	1.94 ± 0.29	3.73 ± 0.97 □	5.03 ± 1.43 □

□ $P < 0.05$

The results from table (2) showed that there was a significant elevation ($P < 0.05$) in serum β-2-M level in thyroid patients and renal thyroid patients when compared with control group. The results were compatible with that obtained by ZHANG Jinchi (2005) [21] , and TAO Lin (2008) [22] .

In the current study, the elevation in β-2-M level in thyroid patients with kidney diseases was higher than that in thyroid diseases. The elevated levels of serum β_2-microglobulin may reflect the increased metabolism in patients with thyrotoxicosis. Increased levels in active Graves' disease may also partly be caused by immunological activation[23].The increased serum beta 2-microglobulin concentration in thyroid hyperfunction is probably related to metabolic rate, even if autoimmunity might contribute to its overproduction[24].

Beta-2-microglobulin is filtered by the glomerulus, and reabsorbed by the proximal tubular cells where it is metabolized. Its plasma concentration increases with decreasing renal function . $Beta_2$-microglobulin normally is filtered out of the blood by the kidney's glomeruli (a round mass of capillary loops leading to each kidney tubule), only to be partially reabsorbed back into the blood when it reaches the kidney's tubules. In glomerular kidney disease, the glomeruli can't filter it out of the blood, so levels increase in the blood [25]. .

The relation between β-2-M and T3, T4, TSH,and anti- TPO in both hyperthyroid and hypothyroid patient's showed in table (3).

Table (3) : correlation between Beta-2-microglobulin (β-2-M) and the study parameters.

Groups / Parameter	Hyperthyroid Patients (n=20)				Hypothyroid Patients (n=20)			
	T3	T4	TSH	Anti-TPO	T3	T4	TSH	Anti-TPO
β-2-M	NS -0.806	-0.796 *	0.291 *	0.197 *	-0.055 *	-0.036 *	-0.102 *	-0.141 *

* P< 0.05 , NS : no significant

The results revealed a significant positive correlation between β-2-M and TSH,anti-TPO ; and a significant negative correlation with T4,in addition to no significant negative correlation with T3 in hyperthyroid patients group.Table (3) also shows a significant negative correlation between β-2-M with T3,T4,TSH, and anti-TPO in hypothyroid patients groups.

Conclusion:

In conclusion, β2M can be used as a biomarker in autoimmune thyroid and renal autoimmune thyroid patient's. In addition to the β2M level in renal thyroid diseases was higher than that in thyroid diseases.

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بيتا –2– مايكروغلوبيولين كعلامة حيوية لدى النساء العراقيات المرضى بالغدة الدرقية المناعية والمرضى بالغدة الدرقية المناعية المصابات بامراض الكلى .

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الخلاصة :

اجريت هذه الدراسة على 80 عينة من النساء تراوحت اعمارهن ما بين (30-60) سنة.وتمت هذه الدراسة في المركز التخصصي لامراض الغدة الدرقية والسكري للفترة من نيسان الى تموز 2011 ، وقد قسمت العينات الى 3 مجاميع :

مجموعة الاصحاء ، مجموعة مرضى الغدة الدرقية ، مجموعة مرضى الغدة الدرقية المصابين بامراض الكلى كمضاعفات .

تشير نتائج الدراسة الحالية الى زيادة معنوية في مستويات T3,T4 لدى مرضى فرط نشاط الدرقية ونقصان معنوي في مستوىT3,T4 لدى مرضى خمول الدرقية ، بينما يوجد اختلاف معنوي في مستوى TSH لدى مرضى فرط نشاط وخمول الدرقية عند مقارنتهم مع الاصحاء . كما يوجد ارتفاع معنوي في مستوى anti-TPO لدى مرضى فرط نشاط وخمول الغدة الدرقية مقارنة مع

الاصحاء.كما بينت النتائج الحالية ان هناك زيادة معنوية في مستوى β2M لدى مرضى الدرقية ومرضى الدرقية المصابين بامراض الكلى عند مقارنتهم مع الاصحاء، بالاضافة الى وجود ارتفاع غير معنوي في مستوى β2M لدى مرضى الدرقية المصابين بامراض الكلى مقارنة مع مرضى الدرقية.

يمكن الاستنتاج من هذه الدراسة ان β2M هو علامة حيوية لدى مرضى الغدة الدرقية المناعية ومرضى الغدة الدرقية المناعية المصابين بامراض الكلى كمضاعفات ، كما ان مستوى β2M لدى مرضى الغدة الدرقية المناعية المصابين بامراض الكلى يكون اعلى من مستواه لدى مرضى الغدة الدرقية المناعية .

كلمات مفتاحية : امراض الغدة الدرقية المناعية ، بيتا-2-مايكروغلوبيولين ، امراض الكلى ، anti-TPO