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Prevalence of Anti-thyroid Peroxidase, and Anti-thyroglobulin in Sudanese Patients with Thyroid Diseases

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ABSTRACT

Background: Autoimmune thyroid disease (AITD) may be characterized by the measurement in serum of antibodies to thyroid peroxidase. Thyroid antibody testing is not routinely available in developing countries, and few studies have measured thyroid antibodies among Arab or Africans. The significance of thyroid autoimmunity in an Arab setting is thus unclear.

Objective: The aim of this study is to measure the thyroid antibodies in patients with different thyroid pathologies, to assess its diagnostic value, especially in autoimmune thyroid disease.

Design: Prospective study

Method: We measured antibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) using an ELISA technique in 208 patients with various thyroid pathologies attending Fedail clinic, and Khartoum teaching hospital in Khartoum, Sudan. Patients were grouped into five categories according to presentation, family history and laboratory investigation.

Results: TPOAb and TgAb were found in 5% and 10%, respectively, of healthy adult controls, in 66.7 % and 27.8% of patients with GD, in 100% and 66.7% of patients with Hashimoto, and in 15.5% and 12.3% of patients with non-autoimmune thyroid disease.

Conclusion: Thyroid autoimmunity is not common in these Sudanese patients, and TPOAb was significantly associated with auto-immune thyroid disease. The clinical utility of these antibody measurements requires further evaluation in a wider Sudanese, Arab, and African population.

INTRODUCTION

A variety of disorder can plague the thyroid gland, including autoimmune disorders, benign and malignant tumors, and goiter (an enlargement of the thyroid which caused by either over- or underproduction of thyroid hormone) {Brown, 2003}.

The search for antibodies that will reliably diagnose, predict and monitor the autoimmune thyroid diseases is a quest that is beset with difficulties. Multiple antigen configurations of thyroglobulin (TG) are produced when it is iodinated resulting in functionally active but immunologically distinct molecules. Thyroid peroxidase (TPO), originally described as thyroid microsomal antigen, is present on the apical surface of thyroid follicular cells and is the antigen most closely involved in cell-mediated cytotoxicity. Multiple B cell reactive epitopes exist each giving rise to different antibodies (Sinclair, 2008).

Thyroglobulin (Tg) antibodies can be present in the serum of patients with

proven thyroid disorders as IgG, IgA or IgM. However, IgA represent an uncommon finding with only low concentrations in positive samples, IgM are more frequent but again at very low concentrations compared to IgG (Delespesse, 1976). This explains why most testing methods detect selectively IgG antibodies. Other groups have shown that these IgG antibodies belong to any of the IgG subclasses (Davies, 1986).

Thyroid peroxidase (TPO) autoantibodies are the hallmark of autoimmune thyroid disease in humans. Formerly known as 'thyroid microsomal antibodies', they are of IgG class and are associated with thyroid destruction and hypothyroidism. TPO is the primary enzyme involved in thyroid hormone synthesis. It is a membrane-bound glycoprotein comprising two identical 100-kD subunits. (McLachlan, 1995, Portmann, 1988).

It is striking that even in families in which several members have antibodies to TPO or Tg, clinically overt AITD is not the rule. This demonstrates that the generation of a B cell immune response to thyroid antigens is not in itself sufficient to cause AITD, and that other tissue-specific responses or immune system factors are also necessary (Vaidya, 2002).

MATERIALS AND METHODS

Five mL of venous blood were collected from thyroid disease patients of the study population by venipuncture, and placed in plain container, after clotting, blood samples were centrifuged for three minutes at 3000 rpm, and serum separated and stored in an Eppendorf tube at -20^{o}C until analyzed for thyroid function test and thyroid antibodies.

Blood specimens also collected from normal Sudanese individuals with no family history of thyroid disease and matching ethnicity and sex. The patients were categorized according to the stock of Sudanese tribes into 11 groups, which represents all the ethnic groups of the Sudanese.

For the determination of the anti-thyroid (anti-Tg, and anti-TPO) antibodies concentrations, ELISA technique used. Diluted serum, negative and positive control sera, and standards were placed in a 96 wells coated plates. Then conjugate, enzyme, and stop-reaction reagent were added to each well. Each assay includes a calibration (six standards) curve, positive control (C+), and negative control (C-). Finally the colored products were read using ELISA reader, getting the results in form of standard curve and concentrations.

RESULTS

The result of anti-TPO antibody of thyroid disease patients and control group was positive in 21.2% (44/208) and 5% (3/60) respectively, p. value (0.011), return to Fig.1.

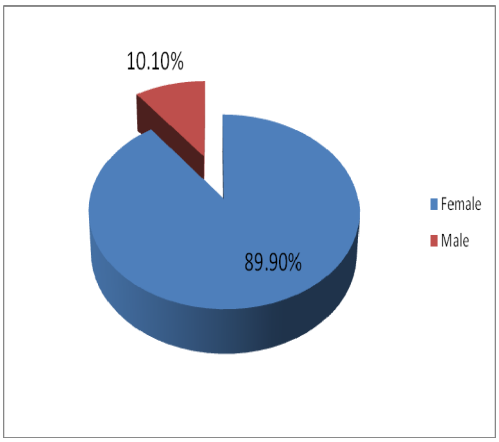

Fig. 1: Proportion of females and males of the patients with thyroid diseases

In Graves disease 66.7% (12/18) of the patients were give positive result of anti-TPO antibody, which is highly significant (0.000) when compared to control group, see (Figs. 2 a& b).

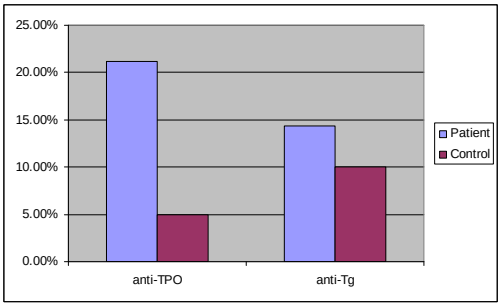

Fig. 2a: Anti-TPO, and anti-Tg antibodies in thyroid disease patients (n=208) and control group (n=60):

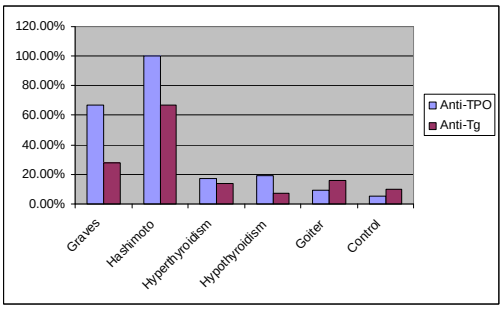

Fig. 2b: Anti –TPO, and anti-Tg antibodies in the different categories of patients with thyroid disease compared to control group.

Anti-Tg antibody result showed no significant difference between thyroid disease patients and control group, p. value (0.528). Anti-Tg antibody was positive in 27.8%, and in 10% of patients and control group respectively, P. value 0.041.

In this study 89.9 % (n=187) of the patients were females, and 10.1 % males (Figure1). The age mean of the patients was 39.3 years (11 – 80), and the age mean at the onset of thyroid disease was 35 years (10 - 74) (Tables 1 & 2).

Table 1: Age of patients and their age at the onset of the thyroid disease

	Age(year)	Age at onset of disease
Mean	39.3	35
Minimum	11	10
Maximum	80	74

Table 2: Family History of the patients with thyroid diseases

Family History	Frequency	Percent	Cumulative %
Yes 1st degree	029	13.9	13.9
Yes 2nd degree	049	23.6	37.5
No	130	62.5	100.0
Total	208		

The control group of normal individuals with no family history of thyroid disease matched the ethnicity, sex and age of the patients. The patients of this study were from 11 different Sudanese tribes. The four tribes, Jalia, Baggara, Johayna, and Nile Nubian were of majority proportion, they represent 56.7 %, 15.4 %, 10.1 %, and 9.1 % of the patients, respectively.

DISCUSSION

The majority of female patients in this study were in the age 20 to 50 years at the onset of thyroid disease; appear to be at risk, due to the effect of thyroid autoantibodies on reproduction. These findings are partly in agreement with those reported by Biassoni *et al*. (1998), who observed in the study among the Bororos a slight but non-significant rise in the prevalence of goiter in females from puberty to 45 years, probably related to an increased need for care during pregnancy and lactation. However, no individual over the age of 45 was found to have goiter. (Biassoni, *et al*. 1998).

It is well known that organ-specific endocrine autoimmunity develops more frequently in women, including autoimmune thyroid disease (AITD) (Barker *et al*, 2005). In this study, the result of anti-TPO antibody was significantly different between patients (n=208) and control group (n=60), with p. value (0.011). 21.2% (n=44) of the thyroid disease patients had positive anti-TPO antibody, while in control group 5% (n=3) positive.

Anti-Tg antibody result showed no significant difference between patients and control group, in patients and control group, positive in 14.4% (n=30) and 10% (n=6), with p. value = 0.528.

The production of anti-TPO is inheritable in an autosomal fashion in women but not in men (Phillips et al, 1991). The occurrence of thyroid autoimmunity, and hence the utility of antibody testing in African patients with thyroid disease, is therefore unclear.

Chronic hypothyroidism is most closely associated with Hashimoto's thyroiditis, all types of thyroiditis may progress to permanent hypothyroidism. This outcome is more likely in patients with higher serum concentrations of

thyroid antibodies or in patients in whom a more severe hypothyroid phase develops. (Elizabeth N, 2003). In this study, patients with Graves' disease, 66.7% (12/18) were give positive result of anti-TPO antibody. All the three patients with Hashimoto's thyroiditis gave positive result (100%) of anti-TPO antibody, while in control group was positive in 5% (3/60) P. value 0.000. Anti-Tg antibody was positive in 27.8% (5/18), and in 66.7% (2/3) of patients with Graves' disease and Hashimoto's thyroiditis respectively, while in control group positive in 10% (6/60), P. value 0.041. When Graves' disease and Hashimoto's thyroiditis considered as autoimmune thyroid disease, anti-TPO antibody was positive in 71.4% (15/21), compared to 5% (3/60) positive in control group, p. value = 0.000. Anti-Tg antibody in patients with autoimmune thyroid disease, was positive in 33.3% (7/21) of the patients, while positive in 10% (6/60) of control group, with P. value = 0.034.

Autoimmune thyroid disease, Hashimoto thyroiditis and Graves' disease, patients produce high levels of thyroid autoantibodies and contain lymphoid tissue that resembles secondary lymphoid follicles. (Armengol MP, 2001). The hypothyroid phase of thyroiditis results from the gradual depletion of stored thyroid hormones (Elizabeth N, 2003).

In non autoimmune thyroid diseases of this study, hyperthyroidism, hypothyroidism, and goiter, anti-TPO antibody was positive in 17.5% (10/57), 19.4% (13/67) and 9.5% (6/63), respectively, while in control group positive in 5% (3/60). Anti-Tg antibody positive in 14% (8/57), 7.5% (5/67), and 15.9% (10/63) respectively, while in control group positive in 10% (6/60).

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ARABIC SUMMARY

معدل انتشار الأجسام المضادة للثايروقلوبيولين و البيروكسديز عند مرضئ الغدة الدرقية السودانيين

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يمكن وصف مرض المناعة الذاتية للغدة لدرقية (AITD) عن طريق قياس الأجسام المضادة للبيروكسيديز في مصل الدم. اختبار الأجسام المضادة للغدة الدرقية ليست متاحة بشكل روتيني في البلدان النامية، والقليل من الدراسات أجريت لقياس الأجسام المضادة للغدة الدرقية بين العرب أو الأفارقة.

الهدف من هذه الدراسة هو قياس الأجسام المضادة الغدة الدرقية في المرضى الذين يعانون من أمراض الغدة الدرقية ، لتحديد قيمتها التشخيصية ، لا سيما في مرض المناعة الذاتية للغدة الدرقية. فى هذه الدراسة تم قياس الأجسام المضادة لثايروجلوبولين (TgAb) وانزيم بيروكسيديز الغدة الدرقية (TPOAb) باستخدام تقنية ELISAعند مرضى الغدة الدرقية (208) الذين يترددون على مركز فضيل، ومستشفى الخرطوم التعليمي في الخرطوم ، السودان. تم تصنيف المرضى في خمس فئات وفقا للعرض والتاريخ العائلي والاختبارات المختبرية.

تم الحصول على النتائج TPOAb و TgAb في 5 ٪ و 10 ٪ ، على التوالي ، من مجموعة العينة الضابطة للبالغين الأصحاء ، وفي 66.7 ٪ و 27.8 ٪ من المرضى الذين يعانون من مرض Grave، في 100 ٪ و 66.7 ٪ من المرضى الذين يعانون من هاشيموتو (Hashimoto)، و15.5 ٪ و 12.3 ٪ من المرضى الذين يعانون من أمراض الغدة الدرقية غير أمراض المناعة الذاتية.

نستخلص من هذه الدراسة أن أمراض المناعة الذاتية للغدة الدرقية نسبتها قليلة في هؤلاء المرضى السودانيين ، وكان TPOAb مرتبطا بشكل كبير مع مرض الغدة الدرقية للمناعة الذاتية. الفائدة التشخيصية لقياس هذه الأجسام المضادة يتطلب مزيدا من التقييم بدراسة أوسع على مستوى السودان والمنطقة العربية و الأفريقية.