Anti-Thyroglobulin and Anti-Thyroid Microsomal Antibodies in Thyroid Disorders

AL-Naqdy A, Kutty I, AL-Harthi S, AL-Buloshi M , Masoud AL-Maskari

Objective: High titers of antibodies to thyroglobulin (ATA) and thyroid microsomal antigen (ATMA) are the hallmarks of human autoimmune thyroid diseases. The clinical significance of these autoantibodies in other thyroid disorders is still unclear. The aim of this study was to analyze the prevalence and titres of these antibodies in Omani patients (mean age 32, range 5-81 years) with different thyroid disorders. This was done in order to investigate any correlation regarding clinical manifestations that may be unique to patients attending Sultan Qaboos University Hospital (SQUH).

Method: Serum levels of ATA and ATMA in 400 cases involving four groups of thyroid disorders (one hundred each with Hashimoto's disease, Graves' disease, thyroid cancer and goitre) and 100 cases of non-thyroid disorders were studied. The antibodies were tested using a commercial haemagglutination assay (Thymune-T and Thymune-M).

Results: The overall prevalence of ATA or ATMA antibodies with thyroid disease was 47% and in non-thyroid disorders was 8%. The ATA was positive in 27% of all the patients with thyroid disorders compared to only 4% of those in the non-thyroid groups while ATMA was positive in 42% and 8% respectively. Among all patients, ATA and ATMA were positive in 64% of patients with Graves's disease, 81% in those with Hashimoto's, 30% of goiter patients, and 20% of those with thyroid carcinoma. The prevalence according to the age within each group for the three ranges: less than 20 years, between 20-40 years and over 40 years, showed the following results: within Graves were 12, 49 and 39% respectively; in the goitre group: 23, 55 and 22%; in the Hashimotos' group: 18, 54 and 28% and 7, 56 and 37% among the patients with thyroid carcinoma. The female to male ratio prevalence was 68% and 32% in Graves disease, 92% and 8% in Hashimotos', 75% and 25% in thyroid cancer and 88% and 12% in goiter.

Conclusions: This study confirms the prevalence of a high level of thyroid autoantibodies in these Omani patients as in Caucasians, and its correlation to age and gender. It also indicated the importance of screening for ATA and ATMA in non-autoimmune thyroid disorders. Their significance in thyroid cancers needs further elucidation.

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Departments of Microbiology & Immunology and Medicine
College of Medicine
Sultan Qaboos University
Al-Khod 123
Sultanate of Oman.

Thyroglobulin autoantibodies (ATA) and thyroid microsomal antigen (ATMA) are organ-specific antibodies directed against different epitopes of the thyroglobulin molecule or against a component of the smooth endoplasmic reticulum of thyroid cells. The latter antigen was found to be identical or at least in part, to contain thyroid peroxidase (TPO) as a main component and many laboratories now test for this rather than use the classical ATMA assay[1].

Autoimmune destruction of the thyroid gland is believed to be caused by an immune response, both humoral and cell mediated, resulting in tissue damage localized in the thyroid. The presence of thyroid autoantibodies substantially contributes to the pathogenesis of a number of thyroid disorders, such as Hashimoto's thyroiditis, primary myxoedema, Graves' disease, as well as thyroid cancers[2]. They are also present in a smaller percentage of sera from other non-autoimmune thyroid disorders and in patients with pernicious anaemia. Antibody production may be confined to lymphocytes within the thyroid, and sera may be negative. A small number (3%) of people with no evidence of disease may have antibody. Thyroid autoantibodies are found more frequently in females and prevalence increases with age[3].

The prevalence of thyroid antibodies in autoimmune thyroid diseases is greater than that in non-autoimmune thyroid diseases[4]. The role played by the immune system may differ in various conditions. The immune system may either be primarily responsible for the disease process, or secondary to some other diseases, without influencing in the progression or outcome of the disease condition. Several researchers have attempted to identify the mechanism triggering thyroid autoantibody production. Excessive levels of antigen production due to cell destruction resulting from of viral invasion, malignancy, or external irradiation to the thyroid resulting in the overwhelming of the normal low-dose tolerance mechanism[2,5]. Cross-reacting bacterial or viral epitopes eg. *Yersinia enterocolitica*[6] can induce immune responses that may cross-react with a self-antigen having identical confirmation. Abnormalities in immunoregulation, which allow uncontrolled T or B cell immune responses to thyroid antigen, have also been addressed[7,8]. Hereditary or genetic predisposition in autoimmune thyroid diseases is another key factor[9]. Environmental factors could also distort the normal levels. For example, stress or steroids may alter immunoregulation, and the potential role of dietary iodine has also been considered[10,11].

Thyroid autoantibodies in autoimmune thyroid diseases have been reported to range from 1-40% but its prevalence in non-autoimmune diseases is unknown[4]. While the prevalence of thyroid autoantibodies in Caucasians is well known, its prevalence in Omani patients has not been reported.

This study was designed to investigate the prevalence of ATA and ATMA in four groups of patients with thyroid disorders: Hashimoto's thyroiditis, Graves disease, goitre and patients with thyroid neoplasia, and to compare these values. These results were also compared with thyroid antibody titres in patients with non-thyroid disorders.

METHODS

Serum levels of ATA and ATMA in 400 cases involving four groups of thyroid disorders (one hundred each with Hashimoto's disease, Graves' disease, thyroid cancer and goitre) and 100 cases of non-thyroid disorders were studied. The

antibodies were tested using a commercial haemagglutination assay (Thymune-T and Thymune-M).

The commercial assay employed was a passive haemagglutination test (Murex Thymune T and Thymune M). Turkey erythrocytes coated with the thyroglobulin or microsomal antigens are agglutinated by ATA and ATMA yielding an even carpet of cells at the bottom of a microtitre well; settling of the cells into a tight ring or button indicate lack of agglutination. Complement in sera was heat-inactivated at 56°C for 30 minutes prior to testing, to avoid erythrocytes lysis. Positive and negative controls were included in each test. Non-specific reactions were detected by using unsensitised control cells. Sera and erythrocytes were mixed then incubated at room temperature for one hour in the case of ATMA and for 30 minutes in the case of ATA tests. Titres greater than 1:20 (ATA) and greater than 1:100 (ATMA) were regarded as positive.

Figure 1. Percentage of patients positive for ATA and/or ATMA

RESULTS

The overall prevalence of ATA or AMTA antibodies among the 400 Omani patients with thyroid disease was 47%. In the group with non-thyroid disorders, antibodies were positive in 8% (Table 1).

Table 1. **Positive ATA and ATMA in patients with thyroid disorders compared to non thyroid control group.**

	Patients (Thyroid disorders)	Control (Non thyroid)
Number	400	100
ATA	109 (27%)	4 (4%)
ATMA	167 (41%)	8 (8%)
Total	195 (49%)	8 (8%)

The ATA was positive in 27% of all the patients with thyroid disorders compared to only 4% of those in the non-thyroid groups while ATMA was positive in 42% and 8% respectively. Among the 400 patients groups, ATA and ATMA were positive in 64% of patients with Graves disease, 81% in those with Hashimoto's, 30% of goiter patients and 20% of those with thyroid carcinoma.

As shown in Table 2 the age distribution within each group for the three ranges: less than 20 years, between 20-40 years and over 40 years, showed the following results: within Graves were 12, 49 and 39% respectively; in the goitre group: 23, 55 and 22%; in the Hashimotos' group: 18, 54 and 28% and 7, 56 and 37% among the patients with thyroid carcinoma.

Table 2. **Positive and negative ATA and ATMA distribution among different age ranges in patients with four types of thyroid disorders (thyroid neoplasia, goitre, Grave's disease and Hashimoto's thyroiditis**)

		Age (years)	Thyroid Neoplasia	Goitre	Grave's disease	Hashimoto's thyroiditis
ATA	Positive	<20	0	6	7	8
		20-40	6	7	14	30
		> 40	5	3	12	11
	Negative	<20	7	17	5	9
		20-40	50	48	35	25
		>40	32	19	27	17
ATMA	Positive	<20	0	8	12	10
		20-40	9	13	32	33
		>40	11	5	20	15
	Negative	<20	7	15	0	8
		20-40	47	41	17	20
		>40	26	18	19	14

The female to male ratio prevalence was 68% and 32% in Graves disease, 92% and 8% in Hashimotos', 75% and 25% in thyroid cancer and 88% and 12% in goiter. Results are illustrated in Figures 2-5.

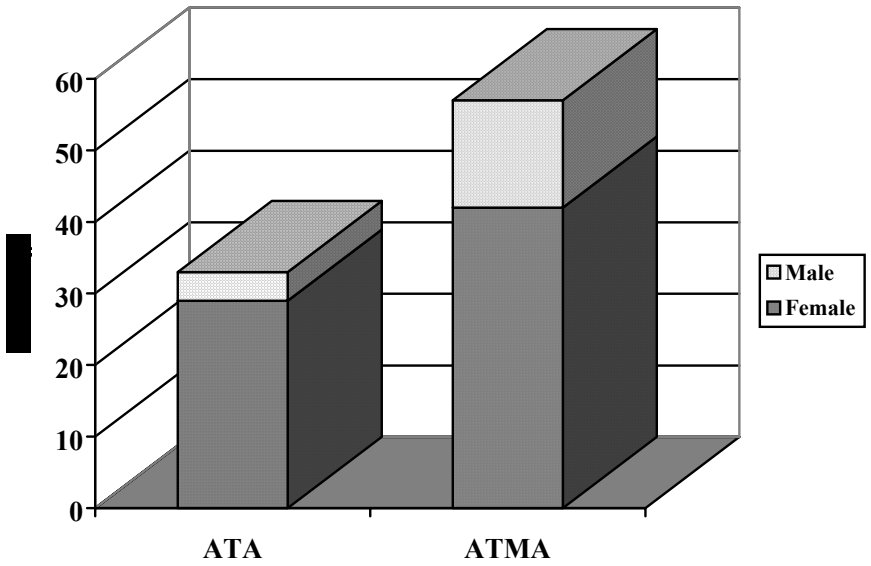

Figure 2. Percentage of patients with Grave's disease positive for ATA/ATMA antibodies according to gender

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Figure 3. Percentage of patients with Hashimoto's disease positive for ATA/ATMA antibodies according to gender

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Figure 4. Percentage of patients with goiter positive for ATA/ATMA antibodies according to gender

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Figure 5. Percentage of patients with thyroid neoplasia positive for ATA/ATMA antibodies according to gender

DISCUSSION

Thyroid autoantibodies are the markers of autoimmunity in autoimmune thyroid diseases. Its prevalence in autoimmune and non-autoimmune thyroid disorders in the Omani population is not known. This study outlines the prevalence of ATA and ATMA among Omani patients who were referred for investigations for suspected cases of immune and non-autoimmune thyroid disorders. The results were compared with those of patients without thyroid disorders. The prevalence and the value of each antibody was analyzed and correlated with gender and age in order to establish the relevance of measuring such antibodies in the diagnosis and prediction of disease progression.

There is no significant difference in the age incidence among the four groups. The thyroid CA group has the lowest number of the younger patients, the increased prevalence of thyroid neoplasia with advanced age, in particular among women, is a well-known observation[12]. The fact that the age range 20-40 constitutes the majority of samples tested as well as those giving positive results, is not necessarily indicative of the nature disease, it may reflect that of the Omani population in general. According to the latest statistics of the Ministry of National Economy- Sultanate of Oman, where this age group constitutes a significant majority of the population.

In addition to age factor, the prevalence and incidence of thyroid disorders is influenced primarily by gender[12]. The current study also demonstrated that the sex ratio in patients with thyroid disorders was biased towards female. In Grave's disease the ratio of male: female was 1:2, in patients with Hashimoto's it was 1:11 and in patients with thyroid carcinomas it was 1:3. The only exception was in the goitre group where the ratio was reversed with a ratio of 7: 1. The reason for this is not obvious and requires further investigation.

The prevalence of anti-thyroid antibodies among patients with thyroid disorders in the current study was greater than in the non-thyroid group and they are in close agreement with previous reports[4,13]. As expected with autoantibody tests, and in particular with thyroid autoantibody testing, the female: male ratio is very high in all cases (except for those suspected of having goitre).

Determination of ATA has been used in conjunction with ATMA to maximize the probability of a positive result in patients with autoimmune thyroid disease. Several studies have suggested that ATA is of less relevance than ATMA in the detection of thyroid disease[14,3]. This study has shown that the presence of ATA was always associated with ATMA, which is consistent with the latter findings. Also, ATA has a lower prevalence and is present at lower titres than ATMA in all groups examined except in Hashimoto's where the majority of patients expressed both autoantibodies. The titre for ATA in these cases was equal to, or higher than ATMA. Autoimmune thyroiditis may demonstrate a response to antigens other than thyroid microsomes.

The overall positivity of the anti-thyroid antibodies tested among the Grave's disease group was found to be higher than that reported by a previous investigator[15]. ATMA, in particular, showed a significantly higher titre in patients with Grave's (this same picture was observed with the patients with Hashimoto's disease).

The highest percentage of positive patients in this study, were in the Hashimoto's thyroiditis group, which may reflect the autoimmune destruction of thyroid glands in those patients. This ratio is relatively high when compared with similar investigations carried out by others[15]. Our data are in accord with those of a study carried out in Kuwait[16] showing that the highest prevalence of positive ATA and ATMA in the Hashimoto's thyroiditis group was among women of the 21-40 age group. This, also, agrees with other findings[17].

The present finding of higher anti-thyroid antibody prevalence among patients with goitre than in the control, group, falls within the range of that described in the literature[18]. The relationship between autoantibodies and goitre have also been studied by other groups[19] and found to be at higher levels compared to healthy controls with the ATMA titer being higher than ATA. All these reports may confirm the autoimmune nature of goitre. Evaluating thyroid autoantibodies in patients with goitre are of particular importance as the presence of these antibodies may indicate that patients will develop an autoimmune thyroid dysfunction.

There was an increased prevalence of thyroid autoantibodies in patients with Carcinoma of the thyroid, though the titres were low, similar findings have been described previously[20]. As with all the organ specific diseases associated with autoantibody production, it is important to determine if the antibodies under investigation are pathogenic or are produced in response to antigens liberated as a result of tissue damage due to causes other than an immunological one. The detection of ATA and ATMA in differentiated thyroid cancers may, therefore, have no intrinsic clinical value. It might indicate either a reaction of the immune system against the tumor cells or the coexistence of a thyroid autoimmune disorder and of a carcinoma. ATA determination, in particular, may be of interest to validate thyroglobulin determinations.

CONCLUSION

A number of conclusions can be drawn from our study. The prevalence of ATA and ATMA in Omani patients with autoimmune and non-autoimmune thyroid disorders is similar to other reported results. This may indicate that there is no race dependant variation. Titres in Hashimotos' were the highest, which confirms the strong autoimmune reaction of this disease. High titres of thyroid autoantibodies were found in thyroid cancer patients. The significance of this is still controversial, but could be of prognostic significance.

Therefore, the screening of young Omani patients for thyroid autoantibodies to predict thyroid disease development worth considering. The prognostic value of thyroid autoantibodies in thyroid cancers need further investigations.

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