The prevalence of Thyroid Microsomal and Thyroglobulin Autoantibodies in Libyan Patients with Graves’ Disease and Idiopathic Myxoeidea

A. S. M. Giasuddin, I. A. Shaafie, A. Abusedra, M. M. Ziu


The prevalence of thyroid microsomal autoantibodies (TM-ab) and thyroglobulin autoantibodies (TG-ab) was studied in Libyan patients with Graves’ disease (GD) and idiopathic myxoeidea (MX). There were 98 cases of GD (68 Caucasians + 30 Blacks); 25–40 years of age; 10 males (7 Caucasians + 3 Blacks) and 88 females (61 Caucasians + 27 Blacks) and 100 cases of MX (72 Caucasians + 28 Blacks); 35–55 years of age; 12 males (9 Caucasians + 3 Blacks) and 88 females (63 Caucasians + 25 Blacks). As a control group (CG), 300 healthy Libyans were included in the study (220 Caucasians + 80 Blacks); 20–55 years of age; 40 males (30 Caucasians + 10 Blacks) and 260 females (190 Caucasians + 70 Blacks). The prevalence of the autoantibodies in GD (Caucasians + Blacks) (TM-ab: 68%, TG-ab: 51%) and MX (Caucasians + Blacks) (TM-ab: 75%, TG-ab: 66%) was significantly higher compared with CG (Caucasians + Blacks) (TM-ab: 9.0%, TG-ab: 3.6%) (p<0.00001). When the subjects were segregated into Caucasians and Blacks, it was observed...
that the prevalence of both autoantibodies was significantly lower in GD (Blacks) (TM-ab: 47%, TG-ab: 26%) and MX (Blacks) (TM-ab: 57%, TG-ab: 50%) compared with GD (Caucasians) (TM-ab: 78%, TG-ab: 62%) (p = 0.002 or 0.001) and MX (Caucasians) (TM-ab: 82%, TG-ab: 71%) (p = 0.05 or 0.01) respectively. The prevalence of 9.0% and 3.6% for TM-ab and TG-ab respectively in healthy Libyans, CG (Caucasians + Blacks), was comparable with the reports from other parts of the world. However, CG (Blacks) seemed to have lower prevalence for both the autoantibodies compared with CG (Caucasians) (TM-ab: 2.5% vs 11.3%, p = 0.03; TG-ab: 1.2% vs 4.5%, p = 0.32). This finding of dissociation in the prevalence of TM-ab and TG-ab between Blacks and Caucasians was taken as evidence in support of the hypothesis that immunogenetic control of autoimmune thyroid diseases is different in Blacks and Caucasians.

Despite the presence of various mechanisms for immunological tolerance to self components, the human body has been unable to avoid the generation of lymphocytes which react with its own constituents. These autoreactive lymphocytes react with self antigens under special circumstances leading to production of autoantibodies and activated T lymphocytes which contribute towards the pathogenesis of autoimmune diseases. Since the finding of anti-thyroglobulin antibodies in the serum of patients with Hashimoto’s thyroiditis in 1956, autoantibodies to different thyroid constituents have been described and found to be associated with autoimmune thyroid diseases (AITD). The prevalence of AITD and circulating thyroid autoantibodies have been reported to vary widely among different populations. Some recent reports have suggested that the prevalence of thyroid autoantibodies, particularly anti-thyroglobulin (TG-ab) and anti-thyroid microsomal (TM-ab), vary greatly among Caucasians, Blacks and Indians.

The presence of TM-ab and TG-ab has been reported to have diagnostic and clinical implications in patients with AITD as well as in the normal population. A literature survey has indicated that no work on thyroid autoantibodies has been reported in Libyans except a retrospective study on the pattern of thyroid diseases. The present study was therefore undertaken to determine the prevalence of TM-ab and TG-ab in Libyan patients with Graves’ disease and idiopathic myxoedema as well as in healthy Libyans as controls.

**Patients and Methods**

**Patients**

Ninety-eight patients with Graves’ disease (GD) in the age group 25–42 years including 10 males (7 Caucasians and 3 Blacks) and 88 females (61 Caucasians and 27 Blacks), and 100 patients with idiopathic myxoedema (MX) in the age group 35–55 years including 12 males (9 Caucasians and 3 Blacks) and 88 females (63 Caucasians and 25 Blacks) were studied.

The GD-patients presented with features of thyrotoxicosis, the most common being symptoms of nervousness (99%), palpitations (90%), weight loss (85%) and signs of diffuse goitre (100%), tachycardia (100%) and exophthalmos (65%).

The MX-patients presented with variable clinical features — the most important being impalpable thyroid gland (95%), weakness (98%), lethargy (92%), dry skin (94%), oedema of the eyes (89%), constipation (62%) and hypersensitivity to cold (80%).

Three hundred healthy Libyans (blood donors and volunteers) in the age group of 20–55 years including 40 males (30 Caucasians and 10 Blacks) and 260 females (190 Caucasians and 70 Blacks) were included in the study as a control group (CG). The patients with GD and MX as well as the CG were segregated into Blacks and Caucasians based on colour, physical features and family history. The serum total triiodothyronine (TT3), total thyroxine (TT4), free thyroxine (FT4) and thyroid stimulating hormone (TSH) were estimated to confirm the clinical classification of patients and subjects into GD, MX and CG. Thyroid scans could not be done due to non-availability of this facility. All the patients and controls were indigenous Libyans hailing from areas of iodine sufficiency.

**Serum specimens**

Blood was taken from the antecubital vein of each subject in the fasting state and the serum separated was dispensed into various aliquots and kept frozen at −70 °C until analysed.

**Radioimmunoassay (RIA) of hormones**

The serum TT3 and TT4 were estimated by using RIA-Kits 125I-T3-COATRIA’ and 125I-T4-COATRIA’ respectively of bioMerieux, France. The serum FT4 and TSH were measured by Amerlex-RIA-Kits of Amersham, England. These RIA-Kits were based on the principle of competitive inhibition. Controls at three levels were used with each assay. The inter and intra-assay coefficients of variation were less than 10%.

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**Date submitted: 07.10.89.**

**Date accepted: 12.11.90.**
Table 1
The percentage distribution of autoantibodies (TM-ab and TG-ab) in patients and control subjects and their statistical analysis

<table>
<thead>
<tr>
<th></th>
<th>TM-ab</th>
<th></th>
<th>TG-ab</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative</td>
<td>Total</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>GD (Caucasians + Blacks)</td>
<td>67 (68)</td>
<td>31</td>
<td>98</td>
<td>50 (51)</td>
</tr>
<tr>
<td>GD (Caucasians)</td>
<td>53 (78)</td>
<td>15</td>
<td>68</td>
<td>42 (62)</td>
</tr>
<tr>
<td>GD (Blacks)</td>
<td>14 (47)</td>
<td>16</td>
<td>30</td>
<td>8 (26)</td>
</tr>
<tr>
<td>MX (Caucasians + Blacks)</td>
<td>75 (75)</td>
<td>25</td>
<td>100</td>
<td>65 (65)</td>
</tr>
<tr>
<td>MX (Caucasians)</td>
<td>59 (82)</td>
<td>13</td>
<td>72</td>
<td>51 (71)</td>
</tr>
<tr>
<td>MX (Blacks)</td>
<td>16 (57)</td>
<td>12</td>
<td>28</td>
<td>14 (50)</td>
</tr>
<tr>
<td>CG (Caucasians + Blacks)</td>
<td>27 (9)</td>
<td>273</td>
<td>300</td>
<td>11 (3.6)</td>
</tr>
<tr>
<td>CG (Caucasians)</td>
<td>25 (11.3)</td>
<td>195</td>
<td>220</td>
<td>10 (4.5)</td>
</tr>
<tr>
<td>CG (Blacks)</td>
<td>2 (2.5)</td>
<td>78</td>
<td>80</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 226.91, \text{ df} = 5, p < 0.00001, \]
\[ \text{Somer's D} = 0.463 \]

\[ \chi^2 = 201.662, \text{ df} = 5, p < 0.00001, \]
\[ \text{Somer's D} = 0.429 \]

Somer's D is explained in Ref. 12.

Table 2
The statistical comparison of various groups of patients and control subjects by applying \( \chi^2 \) test on relevant 2 x 2 contingency subtables obtained from Table 1

<table>
<thead>
<tr>
<th></th>
<th>TM-ab</th>
<th></th>
<th>TG-ab</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \chi^2 )</td>
<td>p</td>
<td>Somer's D</td>
<td>( \chi^2 )</td>
</tr>
<tr>
<td>GD (Caucasians + Blacks)</td>
<td>144.32</td>
<td>&lt; 0.00001</td>
<td>0.61</td>
<td>127.63</td>
</tr>
<tr>
<td>vs CG (Caucasians + Blacks)</td>
<td>171.97</td>
<td>&lt; 0.00001</td>
<td>0.66</td>
<td>183.32</td>
</tr>
<tr>
<td>MX (Caucasians + Blacks)</td>
<td>1.074</td>
<td>0.30</td>
<td>-0.07</td>
<td>3.42</td>
</tr>
<tr>
<td>vs CG (Caucasians + Blacks)</td>
<td>9.41</td>
<td>0.002</td>
<td>-0.31</td>
<td>10.26</td>
</tr>
<tr>
<td>GD (Caucasians) vs GD (Blacks)</td>
<td>6.61</td>
<td>0.01</td>
<td>-0.25</td>
<td>3.85</td>
</tr>
<tr>
<td>MX (Caucasians) vs MX (Blacks)</td>
<td>3.94</td>
<td>0.03</td>
<td>0.125</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\( p > 0.05 \) (significant); \( p > 0.05 \) (not significant). Somer's D is explained in Ref. 12.

Detection of autoantibodies to thyroid microsomal antigen (TM-ab) and thyroglobulin (TG-ab)
The serum TM-ab and TG-ab were detected by Thymune-M and Thymune-T Haemagglutination Kits respectively of Wellcome Reagents Ltd, England. The antibody titres of \( \leq 1:1600 \) and \( \leq 1:20 \) were considered negative and the antibody titres \( \geq 1:6400 \) and \( \geq 1:40 \) were considered positive for TM-ab and TG-ab respectively according to the criteria of the assay kits.

Statistical analysis
The statistical significance of the prevalence of TM-ab and TG-ab was evaluated by a \( \chi^2 \) statistic as described by Steel & Torrie\(^{11} \) using a statgraphics package program in a microcomputer (Model: IBM-XT). Further comparisons between different groups of patients and control subjects were made by applying the \( \chi^2 \) test on the relevant 2 x 2 contingency subtables.

Results
The serum thyroid hormone levels which were used to confirm clinical classification of subjects into CG, GD and MX were as follows (mean ± SD): TT3 (nmol/l)−CG: 1.68 ± 0.49; GD: 7.06 ± 3.55; MX: 0.62 ± 0.44; TT4 (nmol/l)−CG: 113 ± 26; GD: 250 ± 70; MX: 21.2 ± 14.2; FT4 (pmol/l)−CG: 15.2 ± 5.1; GD: 40 ± 10; MX: 3.7 ± 1.6; TSH (mIU/ml)−CG: 3.1 ± 1.8; GD: 0.46 ± 0.42; MX: 48.6 ± 12.2.

Thyroid scans could not be done as this facility was not available. The prevalence of TM-ab and TG-ab and the results of their overall statistical analysis are shown in Table 1. The results of further statistical analysis by \( \chi^2 \) tests on relevant 2 x 2 contingency subtables are shown in Table 2. The various possibilities to explain the fact that five of the patients (GD: 2 Caucasians, 1 Black; MX: 1 Caucasian, 1 Black) were negative for both autoantibodies are discussed below.
Discussion
The prevalence of TM-ab and TG-ab in our patients with GD (Caucasians + Blacks) and MX (Caucasians + Blacks) was significantly higher compared with CG (Caucasians + Blacks). The higher prevalence of TM-ab and TG-ab in MX (Caucasians + Blacks) was, however, not statistically different from those in GD (Caucasians + Blacks). Regarding the normal population, the prevalence of TM-ab and TG-ab has been reported to vary from 6 to 26% and 2 to 15% respectively in different populations. Our findings of 9.0% and 3.6% prevalence for TM-ab and TG-ab respectively in healthy Libyans, CG (Caucasians + Blacks), were within these ranges. When the patients (GD, MX) as well as the control group (CG) were segregated into Caucasians and Blacks, it was found that the prevalence of both TM-ab and TG-ab was significantly lower in Blacks compared to respective Caucasians (Table 2). This observation was supported by the findings reported from other geographical regions of the world. Thus, our findings of dissociation in the prevalence of TM-ab and TG-ab have provided evidence in support of the hypothesis that the immunogenetic control of AITD is different in Blacks and Caucasians.

Genetic differences including those in the HLA-system may be responsible for this differential susceptibility to autoimmune response in Blacks and Caucasians. All our subjects studied were indigenous Libyans hailing from areas of iodine sufficiency and using dietary iodized salt as a routine. Thus, it was unlikely that iodine intake could have played a role in the expression of autoimmune thyroid diseases in our patients.

Although the immunopathological roles of TM-ab and TG-ab are well known, the absence of both the autoantibodies in five of our patients (GD: 2 Caucasians, 1 Black; MX: 1 Caucasian, 1 Black) was difficult to explain. It is possible that these patients with GD might have TSH-receptor autoantibodies whose prevalence is reported to be 90–100% in a recent study. However, the possibility also exists that these patients might have other circulating autoantibodies of immunopathological importance.

Alternatively, some of these patients might have a non-secretory variety of AITD. More sophisticated assay methods for the characterization and quantitation of other autoantibodies and an in vitro culture technique for lymphocytes prepared from the thyroid gland are needed to evaluate these possibilities in our patients.

Acknowledgements
The authors wish to thank the staff of the Department of Statistics, University of Garyounis, Benghazi, for helping with the statistical analysis.

References
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