IJMS
Vol 30, No 1, March 2005

Original Article

Anticardiolipin Antibodies and Immunoglobulin M and A in Graves' Disease

H. Mostafavi

Abstract

Background: Graves' disease is an autoimmune disease, characterized by the presence of antibodies directed to TSH receptor or nearby regions as well as antibodies to double strands DNA (dsDNA) anticardiolipin and nuclear antibodies. This study evaluated anticardiolipin and rheumatoid factor, such as IgA and IgM antibodies in patients with Graves' disease.

Patients and methods: Anticardiolipin and rheumatoid factor were measured in sera of 84 patients (29 male, 55 female) with evidence of Graves' disease and 41 healthy individuals (15 male, 26 female) with negative history of hyperthyroidism and other autoimmune diseases.

Results: Mean level of anti cardiolipin antibody (ACLA) in patients and control groups were 0.192±0.11 and 0.087±0.200 optical density (OD) respectively. The level of IgM-Rhematoid factor (IgM-RF) of patients and healthy control groups was the same, whereas the mean IgA-RF levels in patients was significantly lower than control group.

Conclusion: Anticardiolipin level in different studies showed various results which may be due to genetic backgrounds. Lower level of IgA-RF may also be due to environmental factors, which stimulate specific lymphocytes that producing this type of antibodies.
Iran J Med Sci 2005; 30(1): 6-9.

Keywords • Anticardiolipin antibody • Rheumatoid factor • Graves' disease

Introduction

Graves' disease is an autoimmune thyroid disease associated with the production of antibodies against TSH receptors or areas close to the receptors leading to stimulation of thyroid cells and excessively producing thyroid hormones, with clinical presentation of hyperthyroidism, and other specific evidences of graves' ophthalmopathy and dermopathy with their own suggested pathogenesis.[1,2] In recent years anti-phospholipid antibodies have gained attention, because they are frequently associated with thrombosis, recurrent abortion and thrombocytopenia. Besides, disease specific autoantibodies, other reactive autoantibodies, with both organ and non-organ specific autoantigens, have been found in autoimmune thyroid disease.[3] Other serological autoimmune phenomenon has also been observed including increased serum DNA binding and positive antinuclear antibodies.[4]

Endocrine Research unit,
Nemazee Hospital,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Correspondence:
Habibollah Mostafavi MD,
Endocrine Research unit,
Nemazee Hospital,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel/Fax: +98 711 6261089
Email: ha_mostafavi@yahoo.com

Table 1: Comparison of mean ACLA in Optical density units (OD) level in healthy subjects and patients of different age and sexes.

Gender	Age (yr)	Control (OD)	Patient (OD)
Male	15-29	0.162±0.063	0.289 ±0.185
	30-44	0.224±0.067	0.191±0.079
	≥45	0.222±0.051	0.236±0.116
Female	15-29	0.241±0.124	0.190±0.128
	30-44	0.162±0.066	0.178 ±0.054
	≥45	0.258±0.29	0.191±0.053
Total		0.200 ±0.087	0.192 ±0.11

Table 2: Comparison of mean IgM-RF in Optical density units (OD) in control and patients of different ages and sexes.

Gender	Age (yr)	Control (OD)	Patient (OD)
Male	15-29	0.384±0.231	0.215 ±0.131
	30-44	0.435±0.191	0.275±0.173
	≥45	0.396±3	0.305±0.316
Female	15-29	0.396±0.268	0.383±0.158
	30-44	0.378±0.234	0.358 ±0.207
	≥45	0.416±386	0.449±0.416
Total		0.360 ± 0.234	0.332 ± 0.219

Moreover, antibodies to double strands DNA(dsDNA), a highly specific marker for systemic lupus erythematous, have also been detected in Graves' disease.[5] Masuko L et al. have shown an association between autoimmune thyroid disease and Rheumatic diseases.[6]

The objective of the present study was to evaluate antiphospholipid antibodies in Graves' disease, and to find whether there is any association between rheumatologic disease and autoimmune thyroid diseases. This was accomplished by measuring the rheumatoid factors (IgA and IgM) using the same method as of Masuko L et al.[6]

Patients and Methods

Among 102 patients referring to the Endocrine Research unit of Nemazee Hospital, affiliated with Shiraz University of Medical Sciences, Shiraz, Iran, 84 patients (29 male and 55 female) with Graves' disease were selected. The selection criteria included: 1) Clinical hyperthyroid with diffuse thyroid enlargement and elevated T4, reisn-T3 uptake (RT3U), thyroid radio active iodine uptake (RAIU), and suppressed TSH level. 2) Evidences of hyperthyroidism both on clinical and laboratory, in conjunction with increased T4, T3-rup, T3 with suppressed TSH and evidences associated with Graves' ophthalmopathy confirmed by CT scan, or ultrasonography. 3) Graves' opthalmopathy confirmed by CT scan or ultrasonography, which demonstrate swelling of extraocular muscles or blunted TRH test.

Prior to any treatment, 5 ml of blood was withdrawn and its serum prepared for the measurements of anticardiolipin antibody (ACLA) and rheumatoid factor (RF) using Elisa method. The values of ACLA and RF (reported in optical density units, OD) were compared with those of 41 age-matched normal individuals (15 male and 29 female), who were euthyroid from clinical and laboratory standpoints and had negative history of hyperthyroidism.

Statistical analysis

Data are presented as mean±SD. Statistical analysis was performed using one way analysis of variance, Pearson coefficients, Chi-square or Fisher's exact tests, and $P<0.05$ considered as significant.

Results

The mean ages of male and female patients with Graves' disease were 38.2±12.1 and 34.01±13.4 years, and those of healthy control individuals were 37.3±11.9 and 33.5±10.2 years, respectively. In patients, hyperthyroidism was confirmed by measuring the levels of hormones. The values were as follows: TSH: 0.2±0.33 µu/ml (normal range 0.3-3.8 µu/ml), T4: 20.2 ± 5.3µg/ml (normal range: 4-12 µg/ml), T3-RUP: 33±11% (normal range: 26-34%), RAIU-2hr= 46±18% (normal range: 5-20%) and -24hr= 67±20% (normal range: 20-55%), T3= 460±205 ngm/dl (normal range: 80-200 ngm/dl).

There was no significant difference between levels of ACLA in patients and healthy subjects. There were significant differences among the mean ACLA levels in both gender of different ages as well as of patients and control groups (table 1). The mean level of IgM-RF, as well as IgM-RF, in patients and control subjects were not statistically different in both males and females (Table 2). As shown in table 3, however, in patients, the mean IgA-RF level was significantly lower than of healthy subjects ($P<0.001$).

The levels of IgA-RF was significantly different between male patients with Graves' disease and healthy control subjects in the age-matched ranges of 15 to 29 and 30 to 44 years, respectively, whereas it was statistically similar in ages ranging 45-years or more (Table 3). However, the IgA-RF levels of female patients and healthy control subjects were statistically different only at ages ranging between15 to 29 years (Table 3).

Discussion

Graves' disease is an autoimmune disease associated with activation of β–lymphocytes and production of different autoantibodies, including antibodies specific to thyroid peroxi-

Table 3: Comparison of mean IgA-RF in Optical density units (OD) of healthy subjects (control) and patients of different gender and age groups.

Gender	Age (yr)	Control (OD)	Patient (OD)
	15-29	0.229±0.110	0.095±0.84
Male	30-44	0.216±0.112	0.097±0.056
	≥45	0.076±0.057	0.065±0.030
	15-29	0.228±0.301	0.092±0.075
Female	30-44	0.134±0.062	0.091±0.075
	≥45	0.220±0.101	0.108±0.093
Total		0.180±0.177	0.072±0.087*

*= P<0.0001

dase, thyroid binding globulin, and TSH.[6] Moreover, the disease is associated with increased levels of autoantibodies like ANA, or antibody to DNA, which are non specific for thyroid.[7] This study, therefore, evaluates the presence and significance of antiphospholipid antibodies and IgM-RF and IgA-RF in patients with Graves' disease.

In recent years, several studies have been done for these nonspecific antibodies and diverse results have been obtained. In the present study the mean levels of anticardiolipin antibody (ACLA) in patients and control healthy subjects were not statistically different, which was similar to the findings of Diez et al.[8] Paggi et al reported that in patients with Graves' disease, 4 of 14 had an IgM-ACLA level more than cutoff point, and 2 had positive IgG and IgM-ACLA.[3] They emphasized that non of their patients had increased levels of IgG and IgM-ACLA, did not show manifestations of antiphospholipid syndrome.[3] In fact, thromboembolic phenomena, thrombocytopenia and recurrent abortion never occurred during their 5-year observation of patients.[9] Three to four-year follow up of these patients did not show any clinical evidences in favor of thromboembolic phenomena as well, which confirms our negative ACLA results.

The conflicting results in various studies may be due to different genetic backgrounds, because several HLA types have been reported in different populations; for example HLA-B8 and HLA-DR3 in South African blacks,[9] HLA-A2 and HLA-DRB1*0501 in Japanese and HLA-B46 and HAL-DR9 in Chinese population.[10] Lupus patients with positive ACLA also have shown differences in HLA. HLA-DR7 was seen more in Italians, whereas HLA-DR4 was more in British population. Finally the negative ACLA level in patients participated in this investigation may, in fact, be due to HLA differences in the study population, which needs to be confirmed by HLA study.

IgM-RF levels of our patients and control individuals of different age groups had significant statistical difference. Our findings are similar to that of Tektonidou who measured rheumatoid factor by latex method.[7] In Graves' disease the titer of IgA-RF was significantly lower than of control subjects. Highton showed that IgA-RF titer was high in most of healthy people.[11] The reason for high IgA-RF titer is that its production depends on specific lymphocytes, which need mitogen stimulation in order to produce IgA.[11] The increase of IgA-RF may indicate a chronic inflammatory process, although it may not be the single cause for increased IgA-RF in the healthy population.[11] A precise evaluation of patients may confirm a chronic stimulating factor (mainly infectious) which may justify the lower level IgA-RF in our Gravies' patients. The other possibility is that the genetic background of our patients might be different from population of other countries. However, this possibility is not supported by Highton study.[10]

Conclusion

Non specific immunologic factors in control group and Graves' disease patients were the same, whereas IgA rheumatoid factor was statistically lower in patients than of control group.

Acknowledgment

Sincere thanks are given to the people of department of immunology of Shiraz medical school for doing the immunological tests.

References

1 Smith TJ. The Putative role of fibroblasts in the Pathogenesis of Graves' disease: evidence for the involvement of in insulin like growth factor-1 1receptor in fibroblast activation. *Autoimmunity* 2003; 36: 409-15.

2 Ajjan RA, Weetman AP. New understanding of the role of cytokines in the pathogenesis of Graves' ophthalmopathy. *J Endocrinol Invest* 2004; 27: 237-45.

3 Paggi A, Caccavo D, Ferri GM, et al. Anitcardiolipin antibodies in autoimmune thyroid disease. *Clin Endocrinol* 1994; 40: 329-33.

4 Ligier S, pham CD, watters AK, et al. Purpura fulminant and anticardiolipin antibodies in a patient with Graves' disease. *Scand j Rhematol* 2002; 31: 371-3.

5 Nakayama T, Yamamoto T, Kanmatsuse K, Kokubun S. Graves' disease associated with anticardiolipin antibody positivity and acquired protein S deficiency. *Rheumatol Int* 2003; 23: 198-200.

6 MasukoHongo K, kato T. The association between autoimmune thyroid diseases and

rheumatic diseases: *Nippon Rinsho* 1999; 57: 1873-7.
7 Tektonidou MG, Anapliou M, Vlachoyiannopoulos HM. Presence of systemic autoimmune disorders in patients with autoimmune thyroid diseases. *Ann Rheumatic Dis* 2004; 63: 1159-61.
8 Diez JJ, Dofforno RA, IgLesias P, et al. Anticardiolipin antibodies in autoimmune thyroid disease. *J Clin Lab Immunol* 1993; 40: 125-34.
9 Omar MA, Hammond MG, Desai RK, et al. HLA class I and II antigens in South African blacks with Graves' disease. *Clin immunolpathol* 1990; 54: 98-102.
10 Farid NR, Bear JC. The human major histocompatibility complex and endocrine disease. *Endocr Rev* 1981; 2: 50-86.
11 Highton J, Hessian PA, Small B, et al. An assessment of the diagnostic value of quantitative measurement of IgA rheumatoid factor. *J Rheumatol* 1985; 12: 854-8.