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# Thyrotropin Binding Inhibiting Antibody (TBIAb) in Graves' Disease

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*To determine the nature of TBIAb in Graves' disease, TBIAb was measured and correlated to various clinical, thyroid functional indices and thyroid autoantibodies.*

*The incidence of TBIAb in untreated Graves' patients, patients in treatment and those in remission was 70.9%, 53.1% and 19.2%, respectively. With respect to clinical indices, there was no correlation between TBIAb and the presence of exophthalmos or periodic paralysis, onset age, diseage duration or sex, but goiter size on initial examination did show significant correlation (r=0.95).*

*Regarding the indices of thyroid function, 20min 99m-Tc uptake (r=0.28), free T4 index (r=0.39) and free T3 index (r=0.40) were well correlated to TBIAb activity. Also Grave's patients with strong antimicrosomal acitivity exhibited a high incidence of TBIAb positiveness.*

Key Words: *TBIAb (Thyrotropin binding inhibiting antibody), Graves' disease, Antimicrosomal antibody*

## INTRODUCTION

The pathogenesis of Graves' disease is largely unknown but the characteristic perturbation of the immune network points to an organ specific autoimmune disorder (1-4). Various methods (3, 5-8) have been developed to detect autoantibodies to the thyrotropin receptor (TRAb) and these autoantibodies are thought to be one of main mechanisms to explain the hyperthroidism in Graves' disease.

Thyrotropin binding inhibiting antibody (TBIAb) is now generally accepted as an autoantibody to the thyrotropin receptor (9, 10) and that at least a protion of this antibodiy can stimulate thyroid function, even though there have been several examples of its nonspecific thyroid membrane binding (11) along with its occurrence in Hashimoto's disease, primary myxedema and other thyroid disorders (12-14).

Clinical correlation of TBIAb to various indices of Graves' disease and its significance in diagnosis and treatment is controversial (15-18). We tried to clarify the nature of TBIAb in Grave's disease especially its correlations to clinical, thyroid functional indices and thyroid specific autoantibodies since TBIAb by itself is a thyroid related autoantibody.

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## MATERIALS AND METHODS

### 1. Subjects

The present study involved 192 patients with Graves' disease (30 males, 87 females, ranging in age from 10-75 years), of whom 117 were untreated, 49 were on antithyroid medication and 26 were in remission for at least 6 months. Thirty two normal controls and 77 patients with Hashimoto's disease were also included.

The diagnosis of Graves' disease was based on the clinical and laboratory features of hyperthyroidism with or without exophthalmos and dermopathy and increased thyroid uptake of 99m-Tc04. Patients who presented a rubbery and/or nodular thyroid, hypo or euthyroid with positive (above 1:100) thyroid microsomal and/or thyroglobulin antibodies were classified as Hashimoto's disease.

## 2. Methods

Only the data obained on the same date and under the same conditions on each patient were include in this study.

Thyroid mass on initial examination which was calculated from the surface area and the long axis of both lobes, was measured from the computerized image of the 99m-Tc-thyroid scan, and was graded into 5 groups (from 15 grams to more than 55 grams: 10 gram scale).

TBIAb was determined using solubilized porcine thyroid membrane (3). 50 ul of Ig G fraction was added to 100 ul of solubilized thyroid membrane (4mg/ml) for 15 min at room temperature. $^{125}$I-TSH in 100 ul of tris-NaCl-BSA (1mg/ml) was added and incubated for another 60 min at 37°C. The volume of the reaction mixture was made up to 0.8ml with tris-NaCl-BSA, then 1000 ul of PEG solution was added. After mixing well, the tubes were centrifuged and the pellet containing receptor bound labelled TSH was counted for $^{125}$I. Determination of nonspecific binding was carried out by replacing soluble receptors with 1% Lubrol solution in the reaction mixture. Results were expressed as percent inhibition of labelled TSH binding calcuated as follows,

$$100 \times \left(1 - \frac{\text{$^{125}$I-TSH specifically bound in the presence of test samples}}{\text{$^{125}$I-TSH specifically bound in the presence of samples from normal pool serum}}\right)$$

Thyroid functiom tests were done by radioimmunoassay (TSH; Abbott, USA, T3RU, T4, T3; Corning, USA) and antimicrosomal antibody, a passive hemagglutination method (Fuji Zoki, Japan).

## RESTULS

### 1. Incidence of TBIAb in Graves' and Hashimoto's diseases (Fig. 1)

The range of TBIAb activity in 32 normal controls was 0% to 19.8% (data below 0% were regarded as 0% inhibition) and we interpreted the data above 20% inhibition as positive.

Among the untreated Grave's patients, 83 of 117 cases had detectable TBIAb activity (70.9%). The incidence of positive TBIAb in Graves' paitents on

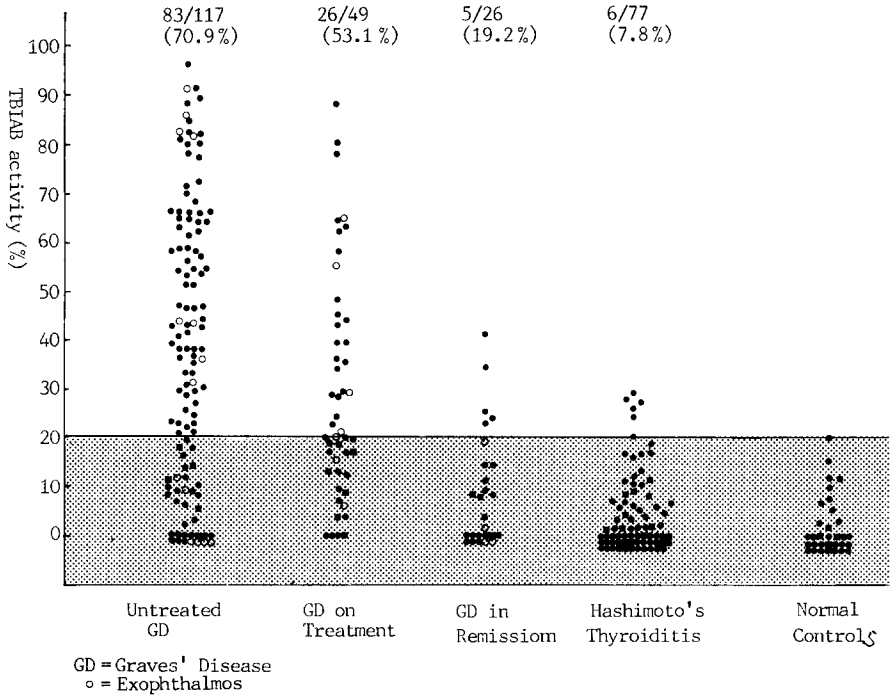


Fig. 1. *Incidence of TBIAb in Graves' and Hashimoto's Disease.*

antithyroid medication regardless of their thyroid function decreased to 53.1% (26 of 49 cases) and of the 26 patients in remission for at least 6 months, 19.1% (5 cases) tested positive.

In Hashimoto's didease, 6 of 77 patients (7.8%) had weakly positive results.

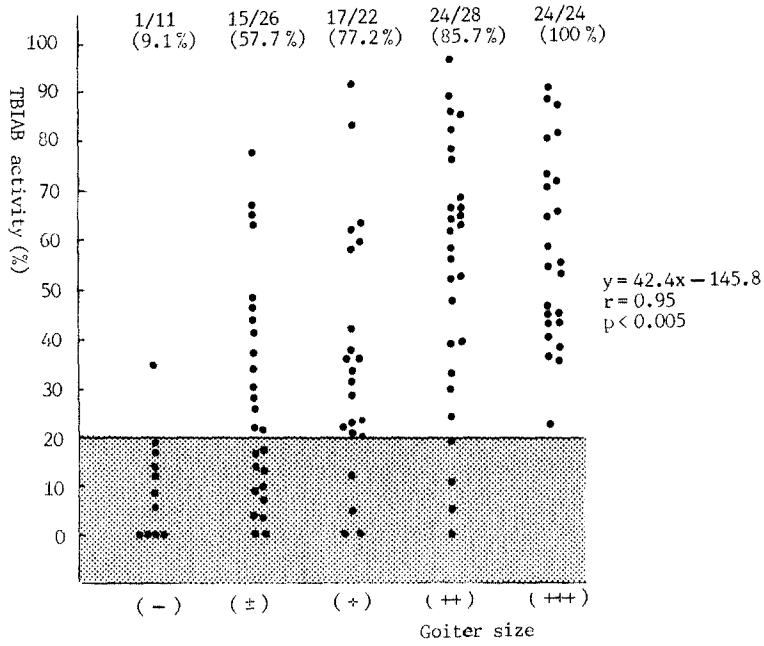


Fig. 2. *Correlation of Goiter size to TBIAb in Untreated Graves' Disease*

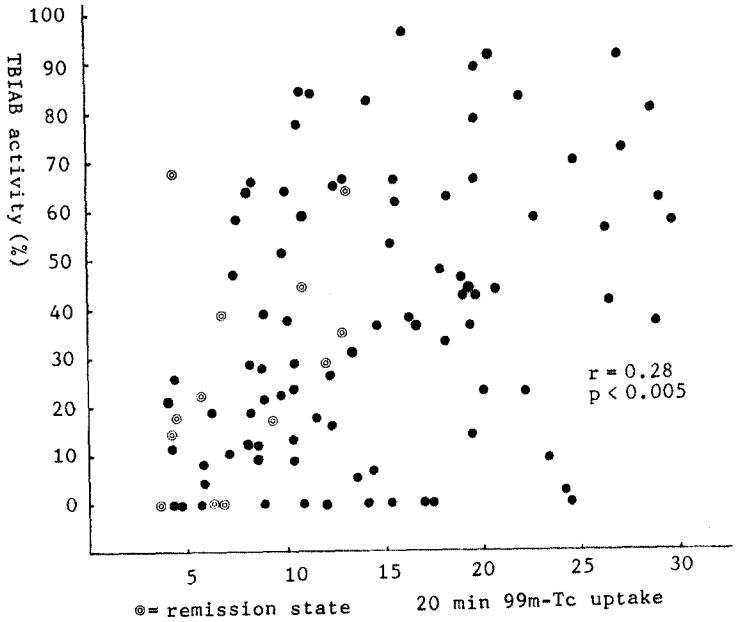


Fig. 3. *Correlation of TBIAb to 20 min 99m-Tc uptake in Graves' Disease*

2. Correlation of TBIAb to Various Clinical Indices.

There were no correlations between TBIAb and age, sex, onset age or disease duration. Also patients presenting exophthalmos and/or dermopathy, or periodic paralysis were no different from patients without them.

But TBIAb and goiter size showed a significant

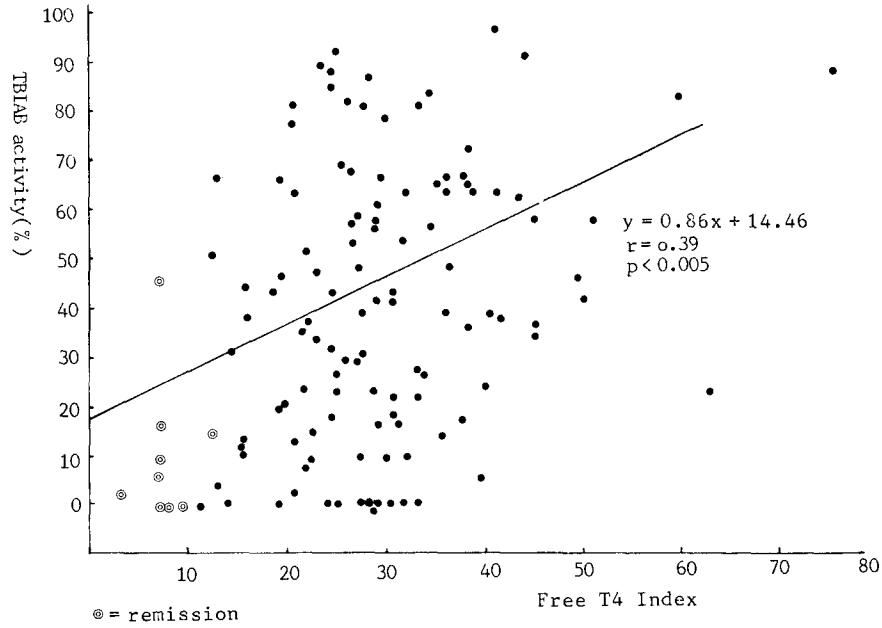


Fig. 4. *Correlation of TBIAb to Free T4 Index*

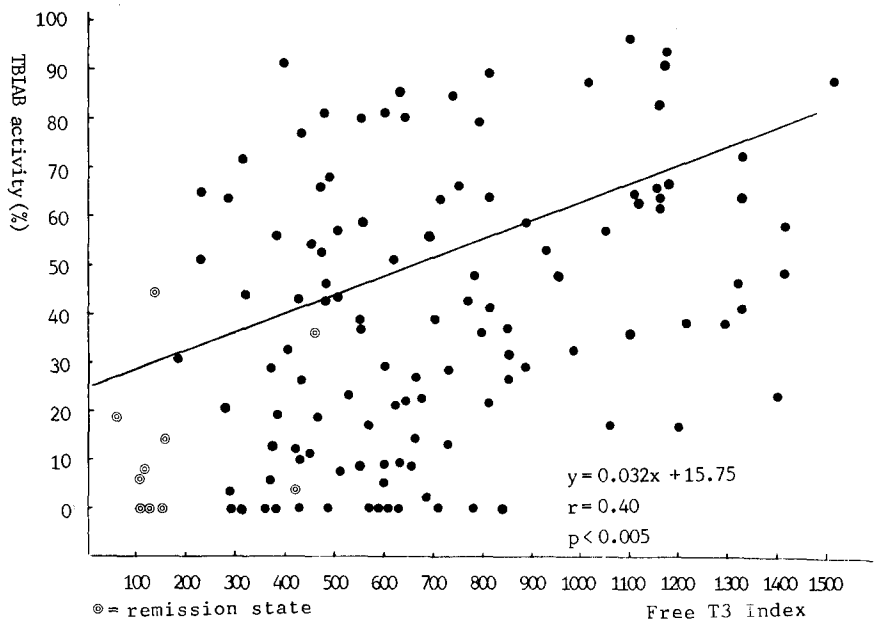


Fig. 5. *Correlation of TBIAb to Free T3 Index*

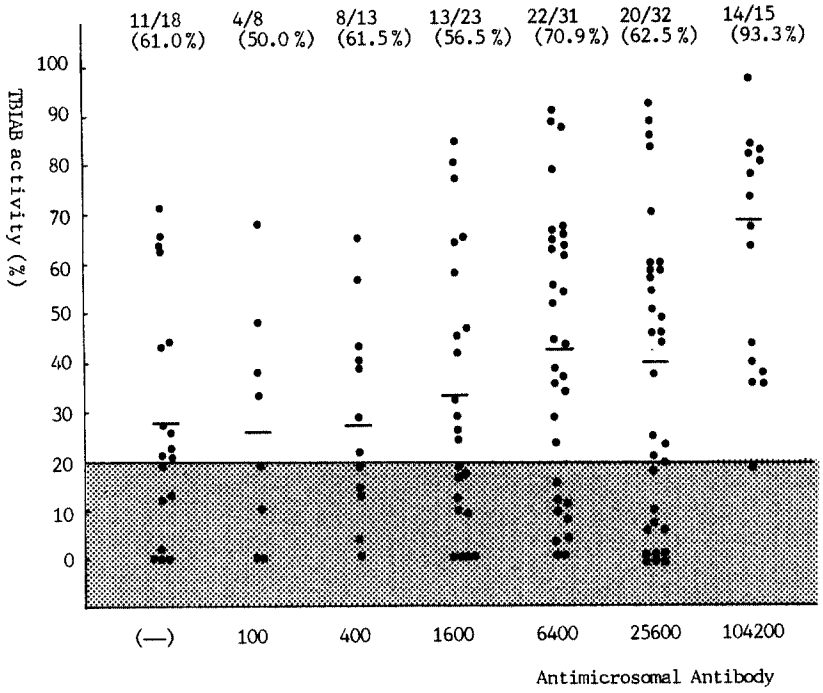


Fig. 6. *Correlation of TBIAb to Antimicrosomal Antibody Activity*

correlation ($r=0.95$, $p<0.005$), as the larger the goiter, the greater the TBIAb activities (Fig. 2).

### 3. Correlation of TBIAb to Various Thyroid Functional Indices

Only the patients who were not on antihyroid medication for at least 6 months were included in these correlations.

In 87 untreated patients and 12 patients in remission, TBIAb activities were significantly correlated with the values of 20 min 99m-Tc-thyroid uptakes ($r=0.28$, $p<0.05$, Fig. 3). More significant correlations were noticed between TBIAb and the free T4 index ($r=0.39$, $p<0.005$, Fig. 4) in 114 new and 9 remission state Graves' patients, and the free T3 index ($r=0.40$, $p<0.005$, Fig. 5) in 115 fresh and 10 in remission. But there was no correlation to thyroglobulin titers.

### 4. Correlation of TBIAb of Antimicrosomal Antibody Activity (Fig. 6)

There were no correlations between TBIAb and the groups who showed rather low antimicrosomal antibody activity. But patients with strongly positive antimicrosomal activity (above 1:102400) had a high TBIAb positiveness and its activity.

Table 1. Correlation of TBIAb to Goiter size and Various Indices of Thyroid Function

| | r | p |
|---|---|---|
| Goiter size | 0.95 | <0.005 |
| Free T3 Index | 0.40 | <0.005 |
| Free T4 Index | 0.39 | <0.005 |
| 99m-Tc uptake (20 min) | 0.28 | <0.005 |

## DISCUSSION

A number of the humoral and cellular mechanisms of Graves' desease have been discorvered.[1-4] Among them, TRAb has received considerable attention because of its presumed action to lead clinical expression as hyperthyroidism. Still, the role of TBIAb in Graves' disease is not clear, except there is general agreement that these antibodies are directed to TSH receptors on follicular cells[9-10] and might be heterogenous[12-13].

In our study we tried to clarify the nature of TBIAb, especially its correlation to clinical, thyroid functional

indices and other thyroid specific autoantibodies.

In untreated Graves' patients, the incidence of TBIAb was 70.9% which decreased to 53.1% in the treated patient group and 19.2% in the remission group. Also we noticed a 7.8% positivity in Hashimoto's disease which has been discussed elsewhere.[16]

Among the clinical indices, remarkable correlation ($r=0.95$) was noticed between TBIAb and goiter size. The correlation might be reasonable since it has been suggested that lympthocytes within the thyroid are the major producer of thyroid autoantibodies,[19] and TBIAb by itself may act as a growth promoting factor of thyrocytes.[13-14] Also TBIAb activity is known to be reduced especially after ablation therapy.[15]

There have been various studies on the correlation of TBIAb and thyroid functional indices.[15-18] Mukhar et al.[15] and Endo et al.[16] found that TBIAb activities correlated only with thyroid uptake of $^{131}I$ or 99m $TcO4^-$ in a rather small number of samples. However, while our data showed low correlation between TBIAb and 99m-Tc-thyrid uptake ($r=0.28$), it revealed a more significant correlation to free T4 and T3 indices ($r=0.39$, 0.40 each) which suggests that at least some portion of these antibodies can stimulate thyroid function in Graves' disease.

On the correlation of TBIAb to other thyroid specific antoantibodies, it was found that the patients with strongly positive antimicrosomal activity (above 1:102400) exhibited a high degree of TBIAb positiveness and also activity, but the patients with low titers of antimicrosomal antibody didn't show any correlation. The first plausible explanation for the relationship is that the patient group with strongly positive antimicrosomal activity included many cases of Hashitoxicosis, a combination disorder of Graves' and Hashimoto's disease, since high titers of autoantibodies and TBIAb positiveness are the prerequisites for diagnosis of Hashitoxicosis. The other explanation, if we assume that the present scale of antimicrosomal antibodies is very narrow in the biological sense, is that the inherent immunlogical network would be quite different among Graves' patients. This is consistent with the data which showed islet cell surface antibodies, as organ nonspecific antibodies in Graves' disease, positive and TBIAb positive group showed a higher percentage of positiveness in antimicrosomal antibodies than those of the Graves' patient groups without ICSA positiveness, which also implies possibly different clincial courses.[20]

In conclusion, TBIAb activities were correlated to goiter size and various thyroid functional indices (Tab. 1), suggesting its role in the manifestation, but other clinical features including exophthalmos didn't have any correlation to regard TBIAb as just one of the immune effector arms in Graves' disease.

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