DETECTION OF MICROSOMAL AND THYROGLOBULIN ANTIBODIES IN AUTOIMMUNE THYROID DISEASES: EVALUATION OF TWO METHODS

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SYNOPSIS

The presence of microsomal and thyroglobulin antibodies was investigated in 120 patients with thyroid disease by the haemagglutination (HA) method (using turkey erythrocytes) or by the immunofluorescence test (IFT). For microsomal antibodies, good correlation was observed between the two methods with the HA test being significantly more sensitive than IFT. On the other hand correlation between HA and IFT was poor for thyroglobulin antibodies. The presence of a 'blocking factor' was investigated and the possible reasons for the poor correlation discussed.

INTRODUCTION

Tests to detect the presence of auto-antibodies to components of the thyroid gland have been shown to be of value in the diagnosis and assessment of autoimmune thyroid diseases (Doniach and Roitt, 1975). Most laboratories measure the presence of antibodies to two thyroid constituents, thyroglobulin (Roitt et al, 1956) and microsomal antigen (Roitt and Doniach, 1958; Belyavin and Trotter, 1959). Thyroglobulin antibodies have been detected by various techniques including haemagglutination (HA) using tanned sheep erythrocytes (Fulthorpe et al, 1961) and by immunofluorescence tests (IFT) (Bigazzi and Rose, 1976). Antibodies to microsomal antigen may be detected by the complement-fixation test (CFT) (Trotter et al, 1957), IFT (Holborrow et al, 1959) or HA using tanned sheep erythrocytes (Fujita and Yamada, 1970; Amino et al, 1976). More recently, a further substantial technological improvement occurred with the development of HA tests using turkey erythrocytes as the carrier particle for the detection of both thyroglobulin and microsomal antibodies (Cayzer, 1978). These tests appear to be more rapid and specific than previous tests (Cayzer, 1978). The recent availability of the new turkey erythrocyte HA tests as a complete kit and the high incidence of autoimmune thyroid disease in the region has prompted us to introduce the tests as a diagnostic service. It would seem, however, that uncertainties still exist regarding the best technique to be used. Tung et al (1974) reported that IFT was more sensitive than HA. Others have shown the two tests to be of comparable sensitivity (Perrin and Bubel, 1974; Amino et al, 1976). It has also been suggested that, for diagnostic purposes, it is necessary to do only the haemagglutination tests (Doniach, 1978; Doniach et al, 1979). Furthermore, the probable presence of a 'blocking factor' or haemagglutination inhibitor (Wilkin et al, 1979) in the sera of patients with autoimmune

thyroid disease has also cast doubt on the reliability of the HA test (Wilkin et al, 1979). The purpose of our study was thus to compare the turkey erythrocyte HA test with immunofluorescence methods for the detection of thyroglobulin and microsomal antibodies.

PATIENTS AND METHODS

Patients. Unselected serum samples from about 120 patients suspected to have thyroid disease were obtained from the clinics and wards of the University Hospital, Kuala Lumpur. Exact data on the incidence of the various types of thyroid diseases in the region is not available but one survey (Menon, 1978) and thyroid function tests (T4, T3 uptake, FTI, TSH) done on the above samples would suggest that a majority of the patients had thyrotoxicosis (Graves' disease). Blood samples were allowed to clot at room temperature and sera, if not immediately tested, were stored at -20°C. For haemagglutination tests sera were inactivated at 56°C for 1 hour.

Turkey Erythrocyte Haemagglutination (HA) Test. Specimens were tested by the recently available commercial haemagglutination kits: 'Thymune-T' for thyroglobulin and 'Thymune-M' for microsomal antigen (Wellcome Reagents Ltd., Beckenham, England). The tests were carried out according to the manufacturers instructions in U-shaped microtitre plates (Cooke Laboratories, Va, USA). For the thyroglobulin HA test serum samples were titrated in doubling dilutions starting at a dilution of 1:10. The microsomal HA test was carried out by making fourfold dilutions of serum starting at a dilution of 1:100.

Immunofluorescence Test (IFT). Sera were also tested by the indirect immunofluorescence technique using thyrotoxic thyroid glands as substrate. Test sera were diluted 1:10 and applied to thyroid sections for 30 mins at room temperature followed by anti-human-Ig/FITC conjugate (Behringwerke, W. Germany) with two 20 mins washes before and after addition of conjugate. Positive sera were further titrated and tested in the same manner. Thyroid sections were used unfixed to detect microsomal antibodies but were fixed for 3 mins in methanol for detection of antibodies to thyroglobulin.

RESULTS

Microsomal antibodies. It was found that 63 sera (53%) were negative by both HA and IFT (Fig. 1). Some sera found to be negative by IFT (23 sera or 19%) were positive by the HA test at titres ranging from 1 : 100 to 1 : 6400 (Fig. 1). Sera found to be positive by IFT at various titres were also positive by HA (28 sera or 24%) and all at significantly higher titres (Fig. 1). Finally, a small number of sera negative by the HA test were positive by IFT at low titres of 1 : 10 or 1 : 20 (Fig. 1).

Thyroglobulin antibodies. Comparison of the two methods to detect antibodies to thyroglobulin showed that 63 sera (52%) were negative by both methods (Fig. 2). A considerable number of specimens (34 sera or 28%) negative by the HA test were positive by IFT at titres ranging from 1 : 10 to 1 : 6400 (Fig. 2). A smaller number (8%) showed the reverse result of being negative by IFT but positive by HA (Fig. 2). The remainder of sera tested (13%) were positive by both methods; those positive at a certain titre by HA were positive at either lower or higher titres by the IFT test and vice versa (Fig. 2). Due to the probable presence of a haemagglutination inhibitor in the sera tested (Wilkin et al, 1979) all sera which were negative by the HA method were inactivated at 56°C for 1 hour (see methods) and were titrated extensively to a final dilution of 1 : 5120 — all remained negative.

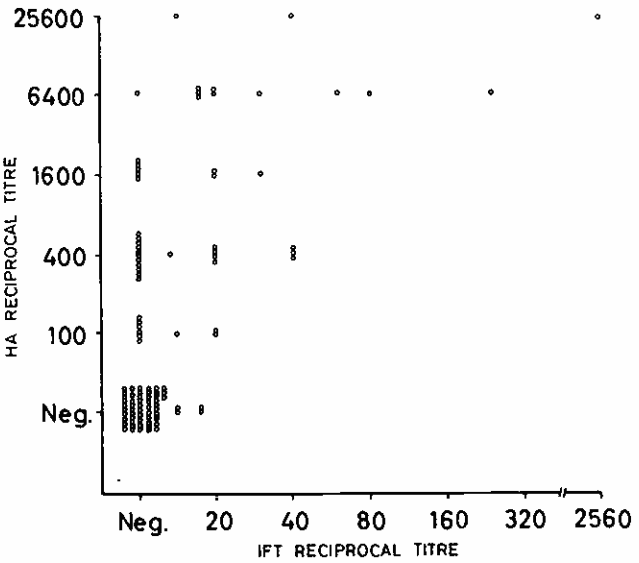

Figure 1. Comparison of the haemagglutination (HA) test and the immunofluorescence test (IFT) to detect microsomal antibodies.

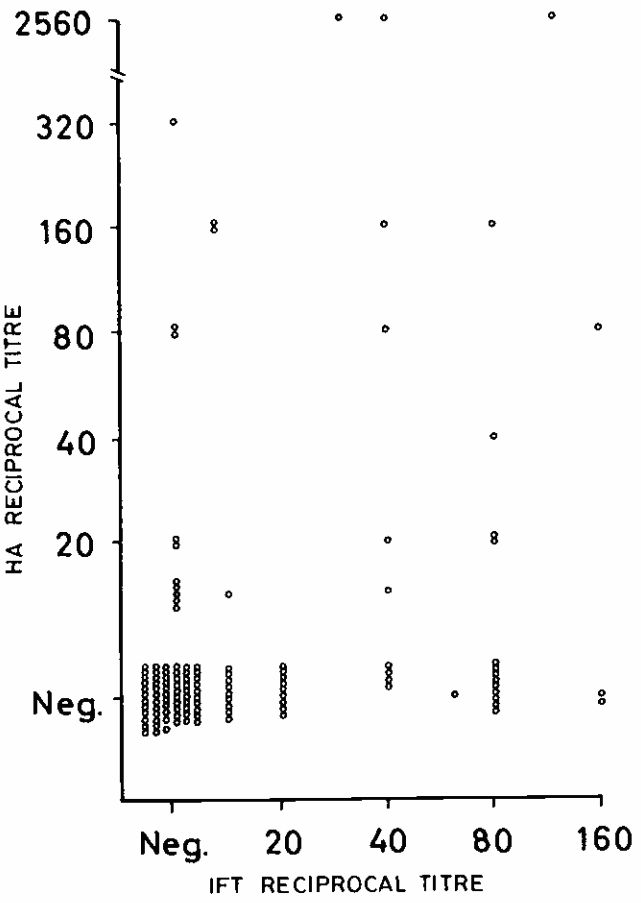

Figure 2. Comparison of the haemagglutination (HA) test and the immunofluorescence test (IFT) to detect thyroglobulin antibodies.

DISCUSSION

Initially developed in the 1960's (Fulthorpe, 1961), Japanese workers developed, in 1970 (Fujita and Yamada, 1970), a haemagglutination (HA) test for thyroid microsomal antibodies and a kit was introduced with sheep erythrocytes as the carrier particle. The test has proved to be sensitive, specific and simple to perform (Bird and Stephenson, 1973; Perrin and Bubel, 1974; Amino et al, 1976). Recently, a further improvement was introduced with the use of turkey erythrocytes as the carrier particle in HA tests for both microsomal and thyroglobulin antibodies (Cayzer, 1978). There are fewer cross-reactions with turkey erythrocytes compared to sheep cells thus making the test more specific and alleviating the need to absorb all sera with control, uncoated cells prior to testing. Additionally, the nucleated turkey erythrocytes settle easily and the test can be read more rapidly, usually in one hour. The lyophilization of the turkey cells also improves shelf life and simplifies transport problems. These advantages, together with the quantitative nature of HA tests have led some laboratories (Doniach, 1978; Doniach et al, 1979) to use these tests exclusively and in preference to the more subjective immunofluorescence test (IFT).

The microsomal HA test kit using turkey erythrocytes was compared with the indirect IFT method in the present study. Good correlation was observed overall between the two methods (Fig. 1). The results also showed that the HA test appeared to be significantly more sensitive than the IFT method as shown by the finding that a significant proportion of sera negative by IFT (27% of all negative sera) were positive by the HA test at titres ranging from 1 : 100 to 1 : 6400. Additionally, sera positive by the IFT method were also positive by HA at significantly higher titres. These results are in agreement with those of Cayzer et al (1978) who showed good correlation between the two methods as well as a 100-fold greater sensitivity of the turkey erythrocyte HA test (Cayzer, 1978). Other studies (Bird and Stephenson, 1973; Perrin and Bubel, 1974; Amino et al, 1976) using sheep erythrocytes as the carrier particle also showed good correlation between the two methods although the sensitivities of the tests were about equal (Perrin and Bubel, 1974; Amino et al, 1976).

Different results, however, were obtained when the HA and IFT methods were used to detect antibodies to thyroglobulin (Fig. 2). Most significant is the finding that a considerable number of sera found to be negative by HA (35% of all negative sera) were positive to various degrees by the IFT method. Additionally, the correlation was poor with respect to the titres obtained for sera which were positive by both tests. Several possible explanations should be considered in relation to these discordant findings. Firstly, sera which were positive by IFT but not by HA may represent 'false-positives'. The indirect nature of the IFT method may result in non-specific staining effects due to the adsorption of serum constituents by the thyroid sections. Alternatively, and as has been suggested by Tung et al (1974), it may be that, for the detection of thyroglobulin antibodies, the IFT test is more sensitive than the HA method. Thirdly, it is also possible that the IFT method is able to detect non-agglutinating antibodies undetectable by the HA test. Alternatively, the nature of the antigen to which the antibody binds in the two tests may be different ie. the two tests independently measure antibodies of different specificity. The last two explanations are unlikely as it implies the presence of a variety of antibodies recognizing different antigenic determinants at different times during the course of disease. Finally, it is also possible that the sera tested, the majority of which come from thyrotoxic patients, contained an intermittent and non-specific inhibitor of haemagglutination as recently reported by Wilkin et al (1979). The presence of such an inhibitor could then result in 'false-negatives'. A similar phenomena has been observed in HA tests for the insulin, anti-insulin (Arquila, 1962) and the growth hormone, anti-growth hormone systems (Grumbach and Kaplan, 1962). It is unlikely, however, that the presence of such a 'blocking factor' is the explanation for the results obtained in the present study as the result remained negative by the HA test despite extensive titration as well as heat inactivation of the sera at 56°C for 1 hour (Arquila, 1962; Amino et al, 1976). It is difficult to determine which of the above possibilities is relevant to the present findings. Many more sera need to be tested and compared and those where a discrepancy was noted should perhaps be tested by a third, more sensitive method such as radioimmunoassay (Mori and Kriss, 1971; Salabe et al, 1972). It would also be useful to determine the exact incidence of thyroglobulin (and microsomal) antibodies in the thyroid diseases prevalent in the region; this study is currently in progress.

In conclusion, it is clear that the turkey erythrocyte HA test for microsomal antibodies is a sensitive, economical and technically simple quantitative assay which may be more convenient than IFT in routine screening procedures for autoantibodies in thyroid disease. The case, however, is not so clear for thyroglobulin antibodies. Clearly more studies are needed before any one method is chosen exclusively. A reasonable solution would be to employ both IFT and HA methods at least until more information becomes available.

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