Antithyroglobulin Antibodies in Diabetes Mellitus

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SUMMARY

Tanned red-cell hemagglutination studies for antithyroglobulin antibodies revealed prevalences of 10.1 per cent clinically significant positive tests (titers 1/25 or greater) in a group of 317 euthyroid diabetic patients free of thyroid dysfunction and of 4.0 per cent positives in a control group of 424 nondiabetic, similarly euthyroid individuals. In both groups the positive tests were more common in the females than in the males and were almost all in those of forty years of age and over. Below age forty the prevalences of tests in the diabetic and nondiabetic subjects were almost equal and of low titer. The positive tests occurred most often in the fifty- and sixty-year age group in the diabetic patients and were less frequent in the aged (seventy and over), approaching the levels observed in the corresponding age bracket in the control group. The bulk of the increase in positive tests in the diabetic group was borne by the white female (23.9 per cent) and, to a lesser degree, by the negro female (12.3 per cent) in the fifty to sixty-nine age bracket. The findings do not permit any conclusion that the thyroid gland in diabetics is involved in autoimmune disease. DIABETES *17*:136-40, March, 1968.

In 1961 Pettit et al. reported an increased prevalence of thyroid antibodies in the serum of diabetic children.[1] Positive tests were found in 22 per cent of fifty-eight cases in contrast to 1.1 per cent in 178 euthyroid apparently nondiabetic children and none in fifty-one children from an orphanage. Additional observations two years later by these workers revealed similar results: 17.3 per cent positive tests in 109 juvenile-type diabetics and 21.2 per cent in ninety-nine adult-type diabetics, with an over-all prevalence of 19.6 per cent positive tests in the total series of 208 diabetic patients.[2] On the other hand, Moore et al.[3] studied sixty-five diabetic patients and found that 9 per cent had positive tests, a frequency practically identical with 8 per cent observed in a group of sixty-five normals. Also, Maret and Berthaux[4] reported 32.9 per cent positive instances in eighty-two diabetic patients (of whom seventy-five were women), and 26.3 per cent in 110 normals, all of whom were women.

In recent years there has been a number of reports of high prevalences of antithyroid antibodies in several presumably nonthyroid-linked diseases,[5,6] as well as reports of an unexpectedly high frequency of positive tests in normals.[7-9] Because of these studies, and because of the conflicting reports in diabetes mentioned above, we elected to study thyroid antibodies in diabetic patients with the sensitive tanned red-cell technic[10] with the hope of gaining new information concerning the relation of the thyroid gland to diabetes mellitus.

METHODS

Subjects studied were drawn from metabolic and medical clinics, medical wards and private practice thus providing a reasonably representative sample of the community. All were presumably healthy and free of evidence of thyroid disorder. They were believed to be euthyroid by clinical evaluation and serum PBI level. If there was a suspicion of thyroid abnormality, further studies were made with twenty-four-hour radioactive iodine uptake, BMR, and serum cholesterol determination. The presence of a goiter, even if the patient was euthyroid, was cause for elimination of the subject from the study. Also, efforts were made to exclude diseases such as systemic lupus erythematosus and other collagen disorders known to be associated with increased prevalence of thyroid antibodies.[6,11]

A total of 741 subjects was studied. The diabetic group included 225 females (108 white, 117 negro) and ninety-two males (fifty-nine white, thirty-three negro). The mean age was 50.9 years (range 14-89).

The control group included 250 females (170 white, eighty negro) and 174 males (130 white, forty-four negro). The mean age was 50.6 years (range 14-87).

Sera from venous blood samples were tested with the tanned red-cell (TRC) technic of Fulthorpe et al. using formalinized sheep cells coated with purified human thyroglobulin.*[10] The technic is simple and highly sensitive and is uniquely suitable for the assessment of

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thyroid antibodies to thyroglobulin. Positive titers were determined serially at 1/5, 1/25, 1/250, 1/2500, 1/25,000 and 1/250,000 levels. No titer higher than 1/25,000 was obtained in any patient.

The question of what titer to accept as unequivocally "significant" remains unsettled. It is generally held that a titer of 1/5 is of no clinical significance.[6-9,12-15] In the present study titers of 1/5 often proved evanescent when the tests were repeated at intervals. On the basis of extensive testing performed by us in such thyroid dysfunctions as Hashimoto's disease, myxedema, hyperthyroidism and nontoxic goiter we have come to accept a titer of 1/25 as being abnormal and therefore clinically "significant." Similar criteria have been adopted by a number of investigators.[6-9,15,18] Titers in the magnitude of 1/25 to 1/2500 are moderate by usually accepted standards, and "significant" is not necessarily synonymous with "cytopathogenicity."[8] It has been proposed that positive tests are related to the development of lymphocytic foci in the thyroid gland which, in the great majority of patients, supposedly disappear without residual thyroid damage.[16,17] The verity of this relation remains to be determined, however, because of uncertainty as to what constitutes an abnormal titer.

RESULTS

The percentage of significant titers (1/25 or greater) in the diabetic group was 10.1 per cent, a prevalence exceeding that of 4.0 per cent in the controls ($p < 0.01$, table 1). In the white diabetic females the frequency of such titers was 13.0 per cent and in the control women 7.1 per cent. The percentages for the corresponding negro females were 9.4 per cent and 2.5 per cent. In the male groups similar trends were found, white diabetics and controls being 6.8 and 2.3 per cent respectively, and negro diabetics and controls being 9.1 and 0.0 per cent respectively. The negro diabetic patients (both male and female) appeared to have relatively greater percentages of significant tests, when compared with controls, than did the corresponding white diabetic groups. This is emphasized by the virtual parity of white: negro percentages in the diabetic group (10.8 per cent : 9.3 per cent) when compared with the predominantly white percentage in the control group (5.0 per cent : 1.6 per cent).

TABLE 1

The prevalence of positive TRC tests in diabetic and control subjects

	Number positive	Number negative	Total
Diabetes	32 (10.1 per cent)	285	317
Control	17 (4.0 per cent)	407	424
Total	49	692	741

$\chi^2 = 9.91$ $p < 0.01$

The relations of ages to positive significant titers are shown in figure 1. The prevalences of positive tests appeared higher in both control and diabetic after age forty (figure 2). Unfortunately the number of positive tests in the subjects below age forty was too small to allow statistical analysis.

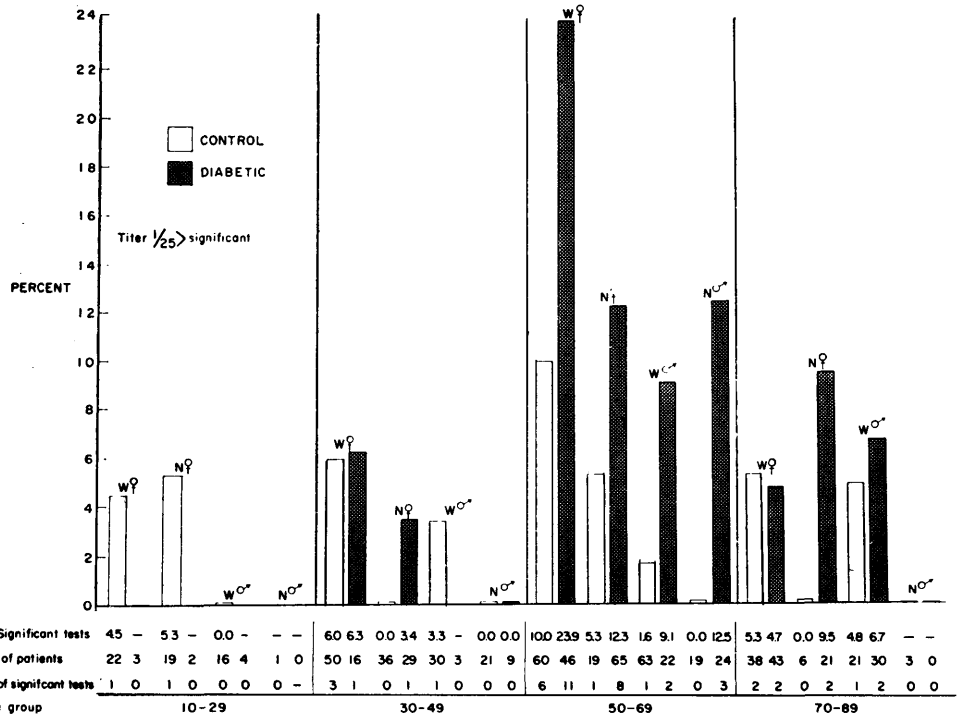
In table 2 the levels of titers are shown according to sex and race. Titers of 1/5 constituted approximately two-thirds (62.2 per cent) of the positive tests in the control group, whereas in the diabetic group they amounted to one-third (33.8 per cent) of those positive. In the control group the percentage of positive tests fell sharply in both females and males as the magnitude of the titers rose. In the diabetic group the bulk of positive titers was 1/25, with a sharp reduction in the number of patients having 1/250 titers.

It is not possible to compare the findings in the control series with those of other similar studies. Several reasons may account for the discrepancies reported.[6,7,12,19] The discriminatory titers selected often have been different, the sensitivity of the tanned-cell technic may vary, the selection and geographic derivation of the clinical material has been variable, and there is increasing evidence of high prevalences of antithyroglobulin titers in several nonthyroid diseases.[3,5,6,11] In particular the studies reported have presented a very wide range of TRC titers; most include all titers (1/5 and greater), others report 1/10 and greater, and still others 1/20 and greater. In the control group of the present study, the 10.6 per cent prevalence of all positive tests (including the 1/5 or nonsignificant titer) agrees closely with the 9.0 per cent reported by Hackett and Beech,[7] 9.0 per cent by Mackay and Perry,[8] 8.2 per cent by Hill[9] and the 6.0 per cent by McGiven.[20]

DISCUSSION

The findings support the original observations of Pettit and Landing[1,2] of an increased prevalence of thyroid antibodies in diabetic patients. In addition, the predominance of significant tests in females over those in males in the present study is in keeping with the recognized increased prevalence of thyroid abnormalities in the female sex. Of interest is the estimated female: male sex ratio of 4:1, established for clinical myxedema in 1948 by Means.[21] There is an unexplained predilection for lymphoid infiltration by the female thyroid

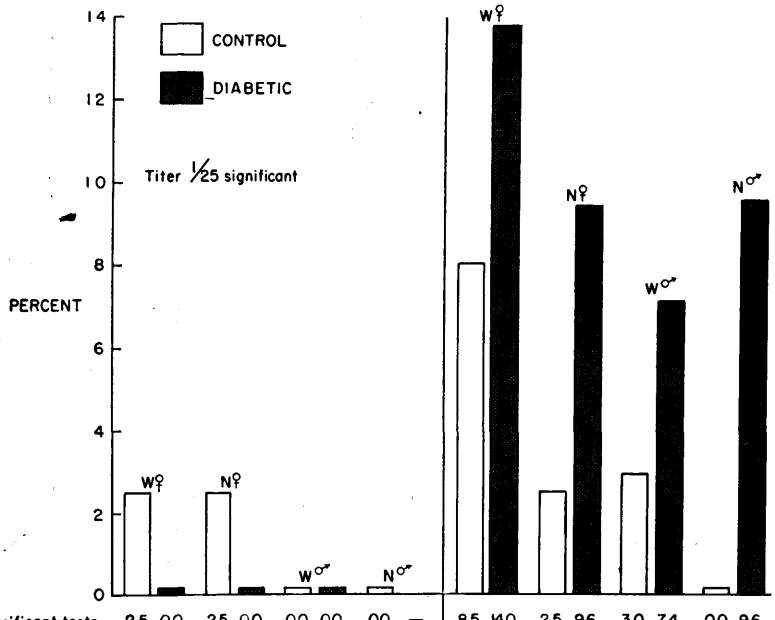
AGE DISTRIBUTION OF SIGNIFICANT TRC TESTS BY SEX AND RACE



	10-29								30-49								50-69								70-89							
% Significant tests	4.5	–	5.3	–	0.0	–	–	–	6.0	6.3	0.0	3.4	3.3	–	0.0	0.0	10.0	23.9	5.3	12.3	1.6	9.1	0.0	12.5	5.3	4.7	0.0	9.5	4.8	6.7	–	–
No. of patients	22	3	19	2	16	4	1	0	50	16	36	29	30	3	21	9	60	46	19	65	63	22	19	24	38	43	6	21	21	30	3	0
No. of significant tests	1	0	1	0	0	0	0	–	3	1	0	1	1	0	0	0	6	11	1	8	1	2	0	3	2	2	0	2	1	2	0	0
Age group	10-29								30-49								50-69								70-89							

FIGURE 1

SIGNIFICANT TESTS WITH RESPECT TO AGE FORTY, BY SEX AND RACE



	Below Age 40								Above Age 40							
% Of significant tests	2.5	0.0	2.5	0.0	0.0	0.0	0.0	–	8.5	14.0	2.5	9.6	3.0	7.4	0.0	9.6
No. of patients	40	8	40	11	31	5	9	2	130	100	40	106	99	54	35	31
No. of significant tests	1	0	1	0	0	0	0	0	11	14	1	11	3	4	0	3
Age group	Below Age 40								Above Age 40							

FIGURE 2

TABLE 2

Distribution of positive TRC tests by sex, race and titer

Titer of TRC	Race	Control group Per cent of positive TRC tests Group	Fe-male	Male	Diabetic group Per cent of positive TRC tests Group	Fe-male	Male
	W	6.7	6.5	7.0	7.0	9.1	3.4
1/5	N	6.3	3.7	—	3.3	1.0	—
	T	6.6	5.6	8.0	5.2	5.1	5.4
	W	3.7	4.1	2.3	7.6	9.1	5.1
1/25	N	0.8	1.2	—	6.0	6.9	—
	T	2.6	3.2	1.7	6.8	7.9	4.3
	W	1.3	2.4	0.0	3.2	4.0	1.7
1/250	N	0.8	1.2	—	3.3	6.5	—
	T	1.2	2.0	0.0	3.2	3.2	3.2
	W	0.3	0.6	0.0	0.6	1.0	0.0
1/2500	N	0.0	0.0	—	0.0	0.0	—
	T	0.2	0.5	0.0	0.3	0.5	0.0

gland.[16] This process reaches a peak in the sixth decade, thereafter falling rapidly as fibrosis of the gland proceeds.[16] In addition, Simmonds in earlier studies found lymphocytic islands in the thyroid gland most often in subjects in the sixth and seventh decades of life,[22] the ages of the present subjects when the prevalence and magnitude of significant tests appeared to be highest in both the diabetic and nondiabetic groups. Hjert and Mogensen's observations have shown gradual disappearance of thyroid antibodies after thyroidectomy.[12] In the aging thyroid there is atrophy, fibrolymphocytic replacement of thyroid parenchyma and decreased blood supply of the aging thyroid.[23] In consequence the gland presents progressively less antigenic tissue, with a tendency thereby to corresponding decreases in thyroid antibody formation, possibly eventually to the point of extinction.[24] Such may be the mechanism responsible for the declining positive antibody tests observed in the aged (seventy or over) of the present subjects.

The biologic significance of elevated antibody titers remains obscure.[12] There is general agreement that thyroid antibodies are nonpathogenic.[20,25,26,27] Actually, the presence of circulating antibody against a certain antigen (extrinsic or intrinsic in origin) may cause immunologically competent cells of the same specificity to be pathogenetically inert.[28] Indeed, the formation of thyroid antibodies could be looked upon as a physiological event.[29] The work of Daniels, Pratt, Roitt and Toorigiani (cited by 29) indicates that in some primates thyroglobulin may continually "leak" from the lymph channels draining the thyroid into the blood. By analogy many normal people aside from those rendered immunologically tolerant to thyroglobulin from contact in uterine life, might be expected to have circulating antibody to thyroglobulin. A propos, Mackay and Burnet[27] assume that many functional stresses facilitate leakage into the circulation of small amounts of thyroid antigen. These small amounts provoke only trivial increases in the activity of corresponding immunologically competent cells, and there is no antibody formation or tissue damage. A large leak of thyroid antigens could provoke only antibody production, provided good homeostasis is maintained. Such a mechanism might explain the occurrence of even high titers of circulating antibody unassociated with tissue damage. Finally, investigations in the fields of cross-reactive antigens[28] and of basement membrane disease of the thyroid gland have indicated some relations of basement membrane change and the presence of antibodies in significant titer.[30] Basement membrane damage is usually associated with lymphocytic and plasma-cell infiltration. The significance of these findings in relation to the present observations remains to be determined. however.

The findings of the present study do not point to the presence of autoimmune disease of the thyroid gland in diabetes mellitus. It was proposed at first that a TRC positive test might be transiently present as a reflection of lymphoid infiltration of the thyroid gland.[17] This concept is now practically discarded as TRC positive tests can be produced experimentally without thyroid gland changes. Indeed, neither lymphocytes nor plasma cells are present in the normal thyroid gland.[28] Goudie et al.[31] noted lymphoid infiltration and Askenasy-cell changes, resembling closely those of early Hashimoto's disease, in 300 thyroids from patients without clinical thyroid disease; noteworthy was the confinement of the gland changes chiefly to females over age sixty (40 per cent involvement) compared with 6 per cent in elderly males. Similar reports[22,32] sugggest the possibility that the present findings possibly represent very early (minimal) participation in autoimmune thyroid disease. Further speculation hereon is not warranted at present, however.

Finally, it has been suggested that the occurrence of certain overlapping traits in diabetes mellitus and in thyroid disease might indicate related genetic mechanisms predisposing to the two conditions.[33-35] But Hales and Hyams offer evidence that hyperglycemia in thyrotoxicosis may be on a basis different from that of diabetes mellitus.[36] The relation of these two conditions to each other as well as the significance of the occurrence of thyroid antibodies remains uncertain.

REFERENCES

[1] Pettit, M. D., Landing, B. H., and Guest, G. M.: Antithyroid antibody in juvenile diabetes. J. Clin. Endocrinol. Metab. *21*:209-10, 1961.

[2] Landing, B. H., Pettit, M. D., Wiens, R. L., Knowles, H., and Guest, G. M.: Antibody and chronic thyroiditis in diabetes. J. Clin. Endocrinol. Metab. *23*:119-20, 1963.

[3] Moore, J. M., Glass, M. B., and Neilson, J. McE.: Antibodies to gastric mucosa and thyroid in diabetes mellitus. Lancet *2*:645-47, 1963.

[4] Maret, L., and Berthaux, P.: Les Anticorps Antithyroglobuline chez les Diabétiques Agés. Rev. Franc. Geront. *11*:7-9, 1965.

[5] Buchanan, W. W., Crookes, J., Koutras, D. A., Melrose, A. G., and Goudie, R. B.: The association of autoimmune thyroiditis and cirrhosis of the liver. Scot. Med. J. 7:22-26, 1962.

[6] Skanse, B., and Nilsson, S. B.: Hypergammaglobulinemia and thyroid antibodies. Acta Med. Scand. *172*:505-12, 1962.

[7] Hackett, E., and Beech, M.: Thyroglobulin antibodies in patients without clinical diseases of the thyroid gland. Lancet *2*:402-04, 1960.

[8] Mackay, I. R., and Perry, B. T.: Autoimmunity in human thyroid diseases. Australasian Ann. Med. *9*:84-92, 1960.

[9] Hill, C. W.: Thyroglobulin antibodies in 1,297 patients without thyroid disease. Brit. Med. J. *1*:1793-96, 1961.

[10] Fulthorpe, A. J., Roitt, I. M., Doniach, D., and Couchman, E.: A stable sheep cell preparation for detecting thyroglobulin auto-antibodies and its clinical applications. J. Clin. Path. *14*:654-60, 1961.

[11] Hijmans, W., Doniach, D., Roitt, I.M., and Holborow, E. J.: Serological overlap between lupus erythematosus, rheumatoid arthritis and thyroid autoimmune disease. Brit. Med. J. *2*:909-14, 1961.

[12] Hjort, T., and Mogensen, E. F.: Thyroid auto-antibodies. Acta Med. Scand. *171*:289-99, 1962.

[13] Doniach, D., Hudson, R. V., and Roitt, I. M.: Human auto-immune thyroiditis: clinical studies. Brit. Med. J. *11*:365-73, 1960.

[14] Hjort, T.: The thyroglobulin pool in the thyroid gland in patients with and without thyroid auto-antibodies. Acta Path. Microbiol. Scand. *58*:429-44, 1963.

[15] Hjort, T., Jeppesen, F., Okholm, R., and Tamler, J.: The prognostic significance of thyroid auto-antibodies in the development of postoperative myxedema. Danish Med. Bull. *10*:159-97, 1963.

[16] Levit, T.: The Thyroid. Edinburgh and London, B. & S. Livingstone, 1954, pp. 16-25, 149-150.

[17] Roitt, I. M., and Doniach, D.: Thyroid auto-immunity. Brit. Med. Bull. *16*:152-58, 1960.

[18] Roitt, M., and Doniach, D.: Human autoimmunizing thyroiditis: serological studies. Lancet *2*:1027-33, 1958.

[19] Blizzard, R. M., Chandler, R. W., Landing, B. H., Pettit, M. D., and West, C. D.: Maternal autoimmunization to thyroid as a probable cause of athyreotic cretinism. New Eng. J. Med. *263*:327-36, 1960.

[20] McGiven, A. R.: Autoimmunity in thyroid disease. New Zeal. Med. J. *63*:21-24, 1964.

[21] Means, J. H.: The Thyroid and Its Diseases, 2nd ed., Lippincott, Philadelphia, 1948, p. 205.

[22] Simmonds, M.: Über Lymphatische Herde in der Schilddruse. Virchow's Arch. Path. Anat. *211*:73-89, 1913.

[23] McGavack, T. H., and Seegers, W.: Status of the thyroid gland after age fifty. Metabolism *8*:136-50, 1959.

[24] Doniach, D., and Roitt, I. M.: Auto-immunity in Hashimoto's disease and its implications. J. Clin. Endocrinol. Metab. *17*:1293-1304, 1957.

[25] O'Gorman, P.: Auto-immune disease. Guy's Hosp. Reports *112*:402-18, 1963.

[26] Sclare, G., and Taylor, G.: Experimental thyroiditis and the placental transfer of auto-antibodies. J. Path. Bact. *82*:29-44, 1961.

[27] Mackay, Ian R., and Burnet, F. Macfarlane: Autoimmune Diseases. Springfield, Illinois, Charles C Thomas, 1963, pp. 63,69.

[28] Burnet, F. M.: The Integrity of the Body. Cambridge, Harvard University Press, 1963, pp. 24, 158, 161.

[29] Assem, E. S. K., Trotter, W. R., and Belyavin, G.: Thyroglobulin and thyroglobulin antibodies in the serum of normal adults. Immunology *9*:21-29, 1965.

[30] Stuart, A. E., and Allan, W. S. A.: The significance of basement membrane changes in thyroid disease. Lancet *2*: 1204-06, 1958.

[31] Goudie, R. B., Anderson, J., and Gray, K. G.: Non-precipitating antithyroid antibodies in hospital patients with asymptomatic thyroid lesions. J. Path. Bact. 77:389-400, 1959.

[32] Hiramoto, R., Engel, K., and Pressman, D.: Tetramethyl rhodamine as immunohistochemical fluorescent label in the study of chronic thyroiditis. Proc. Soc. Exper. Biol. Med. *97*:611-14, 1958.

[33] Althausen, T. L.: The disturbance of CHO metabolism in hyperthyroidism. JAMA *115*:101, 1940.

[34] Perlman, L. V.: Familial incidence of diabetes in hyperthyroidism. Ann. Int. Med. *55*:796, 1961.

[35] Kreines, K., Jett, M., and Knowles, H. C., Jr.: Observation in hyperthyroidism of abnormal glucose tolerance and other traits related to diabetes mellitus. Diabetes *14*:740, 1965.

[36] Hales, C. N., and Hyams, D. E.: Plasma concentrations of glucose, nonesterified faty acid, and insulin during oral glucose tolerance tests in thyrotoxicosis. Lancet *2*:69, 1964.