Glucose Tolerance, C-Peptide Response to Glucagon and Thyroid and Adrenal Functions and Their Relationship to Organ-specific Auto-antibodies in Children with Vitiligo.

ASHRAF T. SOLIMAN, MD, ABDUL RAOUF AL-SUWAID, MD*,
HANI GAMIL, MD*, MOHAMMED MAGDI, AND
MAURICE G ASFOUR, MD, MUSCAT, SULTANTE OF OAMN.

From: Department of Pediatrics and Endocrinology, Royal Hospital and Department of Dermatology, Nahda Hospital, Muscat, Sultanate of Oman.*

SUMMARY

We studied the prevalence of vitiligo in 560 patients with diabetes mellitus, 500 adults with non-insulin dependent diabetes mellitus (NIDDM) and 60 children with insulin dependent diabetes mellitus (IDDM), and investigated the thyroid and adrenal functions, glucose tolerance and C-peptide response to intravenous (i.v.) glucagon in 15 children with vitiligo and 20 age-matched normal controls.

The prevalence of vitiligo was higher in patients with IDDM (3.33%) compared to those with NIDDM (0.6%). This confirms the anticipated clinical association between IDDM and vitiligo. The prevalence of circulating auto-antibodies was slightly higher in children with vitiligo compared to the control group. Endocrine tests revealed that only one child (6.6%) with vitiligo had glucose intolerance with low C-peptide response to i.v. glucagon (peak <1.5 ng/ml), his serum was negative for insulin antibody. One patient had moderately high titer of insulin-antibody, however, his glucose tolerance and C-peptide release after glucagon were normal. One patient had moderately high circulating thyroid microsomal antibody. His FT4 and TSH concentrations were normal. Basal and ACTH-provoked cortisol concentrations were normal in all the studied children.

Renal and hepatic functions and hematologic parameters were within normal in all patients with vitiligo and their serum was negative for kidney/liver microsomal antibody, glomerular antibody, kidney basement membrane, antibody and gastric parietal-cell antibody.

Introduction

Vitiligo is a relatively common disease that affects between 1 and 2% of the general population. It is more common in dark races,

however, it spares no racial group.[1] The clinical association between vitiligo and many organ-specific auto immune diseases including Hashimoto's thyroiditis, Graves' disease, idiopathic adrenal insufficiency, insulin dependent diabetes mellitus, chronic active hepatitis, atrophic gastritis, and pernicious anemia suggests that vitiligo may also have an auto immune etiology.[2-5] Similarly, the increased prevalence of auto antibodies in patients with vitiligo[4,6] and amelioration of the disease after Prednisolone therapy support this hypothesis of auto immune aggression.[7]

In order to clarify the relation/association between vitiligo and endocrine abnormalities, if any, we investigated some endocrine, hepatic and renal functions, and the presence of organ-specific and non-specific auto antibodies in 15 children with vitiligo. In addition, we studied the prevalence of vitiligo in 60 children with IDDM and 500 adults with NIDDM.

Patients and Methods

Five hundred and sixty diabetic Omani patients (60 children with IDDM and 500 adults with NIDDM who attended consecutively the Diabetic outpatient Clinics of El-Nahda and Royal Hospital, Muscat, Oman were studied for the prevalence of vitiligo that was ascertained by two investigators.

On the other hand, 20 Omani patients with vitiligo (8 children and 12 adults) who attended consecutively the Dermatology Clinic of El-Nahda Hospital were studied for their endocrine functions and the presence of auto antibodies. The duration of the disorder ranged from 6 weeks to 12 years, and its extent was very variable. There was a wide spectrum of activity of the disease that extended from complete spontaneous repigmentation to rapid progressive depigmentation. Forty age and sex matched normal subjects served as the control group. Informed consent for the testing procedures was obtained from all the patients and parents of all children before including in the study.

All children were examined thoroughly with emphasis on family history of auto immune and allergic disease, and their anthorpometric data, including the body mass indices, were recorded. These data are summarized in Table 1. Following an overnight fast (8-h), venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 a.m. for determination of complete blood count, serum creatinine, albumin, bilirubin, alanine transferase (ALT), alkaline phosphatase (ALP), and calcium and phosphorus concentrations. Standard indirect immunofluorescent techniques were used to detect antinuclear factor,[8] antithyroglobulin antibody,[9] thyroid microsomal antibody,[10] gastric parietal cell antibody,[11] antimitochondrial antibody,[12] smooth muscle antibody,[13] glomerular basement membrane antibody, brush border antibody, reticulin antibody, kidney/liver microsomal antibody, and insulin antibody. Another serum sample was kept frozen at -20^{o}C until analyzed for free thyroxin (FT4), TSH, and cortisol by radio immunoassay (RIA).

Table 1. Anthropometric data of children with vitiligo and controls.

	Vitiligo n=15		Controls n=20	
	Mean	±SEM	Mean	±SEM
Age (year)	7.5	1.4	8.1	1.3
Weight (kg)	35	5.7	32	3.5
BMI kg/m2	19.3	1.4	18.1	0.9
BMI-SDS	-0.11	0.13	0.2	0.9
Height SDS	-0.87	0.17	-0.25	0.07

BMI = body mass index.

After obtaining the basal samples, a standard oral glucose tolerance test was performed, and samples of blood obtained before, and 1 and 2 hours after the oral glucose load (1.75g glucose/kg with maximum dose =75g glucose) for estimation of serum glucose using glucose oxidase method. On the next

morning and after an 8-h overnight fast, 250 ug/m2 ACTH (Tetracosatin) was injected intravenously and blood samples obtained before and 60 minutes after the injection for estimation of cortisol concentrations by radio immunoassay.[14] On the third morning and after an overnight fast, 15 ug/kg (maximum dose = 1mg) of glucagon was injected i.v. and blood samples obtained before and 5 and 10 minutes after the injection for determination of C-peptide concentrations by RIA.[15]

Results

Table 2 shows the auto-antibody results. There was a small increase in the prevalence of circulating organ-specific auto-antibodies concentrations in the vitiligo group (6.6%, n=1) positive for insulin antibody, and (6.6%, n=1) positive for thyroid microsomal antibody, compared with the normal controls.

Table 2. Results of auto-antibody tests.

	Vitiligo	Controls
Number	15	20
Mean age	7.5	8.1
Range (yr)	(4-15)	(5-16)
Female	8	10
Male	7	10
Thyroglobulin AB	0	0
Thyroid Microsomal AB	1- +++	1- +
Insulin AB	1-++	0
Gastric Parietal cell AB	0	0
Anti-nuclear AB	0	0
Mitrochondrial AB	0	0
Smooth muscle AB	1-++	0
Kidney/liver microsomal AB	0	0
Glomerular BM AB	0	0
Glomerular brush border AB	0	0
Reticulin AB	0	0

AB = antibody, + = positive

Table 3 summarizes the biochemical and hematologic data of the two groups. Renal, hepatic, and hematologic parameters were within the normal range for all the study cases.

Table 3. Biochemical and hematological data of patients and controls.

	Vitiligo n=15		Controls n=20	
	Mean	±SD	Mean	±SD
Urea mmol/L	3.6	1.1	3.2	1
Creatinine umol/L	51.8	10.6	48.7	8.7
Calcium mmol/L	2.2	0.23	2.1	0.07
PO4 mmol/L	1.4	0.2	1.4	0.4
ALP IU/L	181	78	161	64
Bilirubin umol/L	10.1	2.3	15.5	1.7
Albumin g/L	45.5	10.5	39.5	1.3
Globulin g/L	32	3.4	30	1
ALT IU/L	15	2.7	27	1.3
Hb gm/dl	12.8	1.14	12.8	0.4
Hct	0.39	0.03	0.38	0.01
MCV fl	74.3	7.7	72.4	3.3

PO4 = phosphorus, Hct = hematocrit, MCV = mean cell volume SD = standard deviation

The hormonal and glucose data of the two groups are shown in table 4. Impaired glucose tolerance was noted in one child with vitiligo (BG values were 5.6, 10.5, and 8.6 before and 1 and 2 hours after the glucose load respectively) who had defective C-peptide release in response to glucagon (peak C-peptide = 1.4 ng/ml), while none of the controls had glucose intolerance. This patient did not have detectable insulin antibody in his serum. Another child with vitiligo was positive for serum insulin antibody but had normal glucose

tolerance and normal C-peptide response to glucagon (C-peptide concentrations = 5.9 and 4.6 ng/ml at 5 and 10 minutes after i.v. glucagon respectivley). One child with vitiligo had high titer of circulating thyroid microsomal antibody with normal serum FT4 and TSH concentrations (FT4 = 19.4 pmol/L and TSH = 2.5 mIU/mL). Both basal (8 am) cortisol concentration and cortisol response to ACTH stimulation were normal in all the study cases.

Discussion

The clinical association between vitiligo and organ-specific auto immune diseases including IDDM, Grave's disease, idiopathic adrenal failure and pernicious anemia has been noted in many studies.[2-6] These findings suggest that vitiligo also has an auto immune etiology. We studied some of the endocrine functions as well as renal, hepatic and hematologic parameters in relation to the presence of circulating organ-specific and non-specific antibodies in 15 children with vitiligo as well as in 40 normal age-matched controls.

The prevalence of serum auto-antibodies, both organ-specific and non-specific, in our children with vitiligo was slightly higher than that for the control group. In agreement with this finding, many studies showed that less than 20% of patient with vitiligo have some circulating auto-antibodies. [3,6,15-17]

One patient (6.6%) with vitiligo had abnormal glucose tolerance and defective C-peptide response to glucagon. This patient did not have detectable insulin antibody in the serum. The one patient with moderately high titer of insulin antibody had normal glucose tolerance and normal C-peptide secretion after i.v. glucagon. It appears that in children with vitiligo, deficient insulin secretion might be present without elevation of insulin-antibody. Although the prevalence of circulating insulin antibody was higher in the vitiligo group in comparison to the normal group, the presence of insulin antibody in the serum was not associated with abnormal glucose tolerance and / or C-peptide release. High thyroid microsomal antibody titer associated with normal thyroid function was noted in one vitiliginous child but in none of the controls. This might denote increase incidence of auto immune thyroid disease in these children. Basal and ACTH-provoked cortisol concentrations were normal in all patients with vitiligo.

Table 4. Hormonal and glucose data of patients and controls.

	Vitiligo n=15		Controls n=20	
	Mean	**±SEM**	**Mean**	**±SEM**
FBG mmol/L	5.2	0.13	4.5	0.1
BG (2-h) umol/L	6.2	0.3	5.5	0.5
C-pep (b) ng/ml	1.9	0.4	1.9	0.25
C-pep (p) ng/ml	4.3	0.52	4.3	0.45
FT4 pmol/L	16.6	0.15	17.4	0.2
TSH uIU/ml	1.8	0.06	1.6	0.07
Cortisol (b) pmol/L	468	46	475	22
Cortisol (p) pmol/L	1006	107	1062	70

FBG = fasting blood sugar, C-pep = C-peptide, (b) = basa, (p) = peak.

All the children with vitiligo had normal hepatic and renal functions and their serum was negative for kidney/liver microsomal antibody, glomerular antibody, and basement membrane antibody. None had evidence of anemia or detectable serum gastric-parietal cell antibody.

Of the 500 NIDDM patients, 3 patients had vitiligo (0.6%), and of the 60 children with IDDM, two cases had vitiligo (3.3%). This confirms the anticipated clinical association between IDDM and vitiligo. The prevalence of

vitiligo in NIDDM (0.7%) was no higher than reported in the non-diabetic population. [18]

In conclusion, vitiligo is associated with IDDM, but not with NIDDM, as well as with auto immune thyroid disease. This gives further weight to the theory that vitiligo is an auto immune disease.

References

1.FITZPATRICK TB, EISEN AZ, WOLFF K, FREEDBERG IM, AUSTEN KF, eds. Dermatology in General Medicine, 3rd ed. Library of Congress and Cataloging in publishing data, MacGraw Hill, USA, 1987.

2.LERNER AB, NORDLUND JJ, Vitiligo - What is it? Is it important? J Amer Med Assoc 1978; 239:1183-7.

3.CUNLIFFE WJ, HALL R, NEWELL DJ. Thyroid disease and auto immunity. Brit J Dermatol 1968; 80:11-19.

4.HERTZ KC, GAZZE LA, KIRKPATRICK CH. Auto immune vitiligo. Detection of antibodies to melanin-producing cells. N Eng J Med 1977; 294:634-7.

5.GOULD IM, GRAY RS, URBANIAK SJ. Vitiligo in diabetes mellitus. Br J Dermatol 1985; 113:153-155.

6.BROSTOFF J, BOR S, FEIWELL M. Auto antibodies in patients with vitiligo. Lancet 1969; II:177-180.

7.THOMAS PS. Prednisolone causing amelioration of both vitiligo and chronic active hepatitis. Br J Hosp Med 1987; 38 (5):464-66.

8.BECK JS. Variations in the morphological patterns of "auto immune" nuclear fluorescence. Lancet 1961; I:1203-5.

9.FULTHORPE AJ, ROITT IM, DONIACH D. A stable sheep cell preparation for detecting thyroglobulin auto-antibodies and its clinical applications. J Clin Pathol 1964; 14:654-56.

10.HOLBOROW EJ, BROWN PC, ROITT IM. Cytoplasmic localization of "complement fixing" auto-antigen in human thyroid epithelium. Br J Exper Pathol 1959; 40:583-86.

11.ADAMS JF, GLEN AIM, KENNEDY EH. The histological and secretory changes in the stomach in patients with autoimmunity to gastric parietal cells. Lancet 1964; I:401-4.

12.GAUDIE RB, MAC SWEEN RNM, GOLDBURG DM. Serological and histological diagnosis of primary biliary cirrhosis. J Clin Pathol 1966; 19:527-9.

13.FOREST MG. Adrenal steroid deficiency states. In : Brook CGD, ed. Clinical Pediatric Endocrinology, 2nd ed. London : Blackwell, 1989; 368-406.

14.FABER OK. Beta-cell function and diabetes control in insulin-dependent diabetes mellitus. Acta Endocrinol 1985; 110 (suppl):73-78.

15.JOHNSON GD, HOLBOROW EJ, GLYNN LE. Antibody to smooth muscle in patients with liver disease. Lancet, 1965; II:878-80.

16.WOOLFSON H, FINN OA, MACKIE RM ET AL. Serum anti-tumor antibodies and auto-antibodies in vitiligo. Brit J Dermatol 1975; 92;395-400.

17.HOWITZ J, SCHWARTIZ M. Vitiligo, achlorhydria and pernhicious anemia. Lancet 1971; 1:1331-33.

18.DAWBER RPR. Vitiligo in maturity-onset diabetes mellitus. Brit J Dermatol 1968; 80:275-279.

Correspondence:
Ashraf T Soliman MD
Chief, Pediatric Endocrinology
Royal Hospital, P.O.Box 1331
Muscat, Sultanate of Oman.