AUTO ANTIBODIES AND IMMUNOHISTOCHEMICAL STUDIES IN ALOPECIA AREATA

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ABSTRACT

Background:

Alopecia areata (AA) is a common and distressing disorder of unknown etiology. The aim of the present work was to investigate some immunological factors that might have a role in the aetiopathogenesis of AA. Autoantibody and immunohistochemical studies were made.

Methods:

Eighty seven patients with AA and 20 controls were studied for the detection of circulating antibodies against thyroid, gastric, smooth muscle, mitochondrial, ovarian, testicular and vascular tissue. Lymphocyte subsets, Langerhans cells, macrophages, histiocytes and HLA-DR were studied by immunohistochemical techniques.

Results:

Our results showed that AA patients have positive autoantibodies in the following percentages, antitesticular antibodies (38.4%), antinuclear antibodies (9.2%), antithyroid thyroglobulin and antithyroid microsomal antibodies (8%), anticardiolipin (6.9%), antismooth muscle antibodies (5.7%) and antiparietal cell antibodies (4.6%). T4, T8, B lymphocytes, Langerhan cells, macrophages and histiocytes were increased in the perifollicular cellular infiltrations. There was also an increased incidence of HLA-DR.

Conclusions:

Our findings regarding the autoantibodies studied and the perifollicular cellular infiltrate support the view that immunological factors play a role in alopecia areata.

INTRODUCTION :

Alopecia areata is a common worldwide dermatological disease of unknown etiology(1). Genetic(2), endocrine(3), psychological(4) and more recently auto-immune mechanisms have been implicated. Evidence in support of an immunological etiology includes lymphocytic infiltration around the hair bulbs(5), the favorable response to intralesional or systemic steroids(6), the association with auto-immune diseases(7), the increased levels of a variety of auto-antibodies(8) and a decreased number of circulating T cells(9-10-11).

In an attempt to explore a possible role for immunological abnormality in the condition this study reports the immunohistochemical features of the skin lesions and the occurrence of a number of circulating auto- antibodies in patients with alopecia areata,

PATIENTS AND METHODS :

Patients & controls :

Eighty seven patients (76 adults and 11 children) and twenty controls were studied. The patients included 73 male and 14 female (33 Qatari and 54 other nationalities), aged 2 to 56 (mean 29.6) years in which the duration of AA ranged from 1 month to 20 years (the majority less than 1 year) and included alopecia areata (92%), alopecia totalis (4.6%) and alopecia universalis (3.4%)

The controls were 20 normal male volunteers aged 20 to 45 years.

Methods :

1 - Clinical evaluation :

Detailed personal and family histories of AA and auto-immune diseases were obtained from the patients and controls together with a thorough physical examination.

2 - Immunological investigations :

These included the determination of auto-antibodies and peripheral T-lymphocytes (CD4 and CD8). The auto-antibodies tested were anti-smooth muscle (ASMA), anti-thyroglobulin (ATG), anti-thyroid microsomal (AMC), anti-parietal cell (APCA), anti-mitochondrial (AMA), anti-cardiolipin (ACA), anti-nuclear (ANA) and anti-testicular or anti-ovarian.

Techniques(12) and sources of reagents were as follows : ASMA , ANA , AMA and APCA were tested by an indirect fluorescent technique (Fluorokit , Incstar Corp. , Stillwater , USA); ATG and AMC, indirect agglutination (Fujirebio Inc, Tokyo, Japan); ACA , Sandwich ELISA (Cheshire Diagnostic Lim-

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ited , England); CD4 and CD8 , Flow cytometry (Coulter Corp., U.S.A.)

Anti-testicular and antiovarian antibodies were determined by James Shanks Pathology Services (JSPS) London , England.

3 - Histopathology & Immunohistochemistry :

Paraffin-embedded sections of the skin biopsies were stained by haematoxyline and eosin. Paraffin-embedded and/or frozen sections were stained by peroxidase antiperoxidase (PAP)(13) using monoclonal antibodies for pan-leukocytes antigen (LCA), pan [T-Lymphocytes [T cells, T cell helper/inducer (T4), T cell suppressor/cytotoxic (T8)], pan B lymphocytes, HLA-DR, Alpha-1- antitrypsin α1-AT)) and Alpha-1-antichymotrypsin α1-ACT).

4 - Cytogenetic study :

A chromosomal study on 20 randomly selected AA patients was made to investigate their peripheral blood culture by G-banding technique(14).

5 - Other investigations :

Routine hematological, chemistry and hormonal assays included fasting blood sugar level, serum iron, zinc, thyroxin (T4), tri-iodothyronine (T3), thyroid stimulating hormone (TSH) and testosterone levels.

RESULTS :

1 - Clinical :

Of the 87 cases of AA, 76 were adults (older than 12 years) and 11 were children. Seventy one patients (80.9%) showed an alopecia on the scalp and 16 patients (19.1%) had lesions on the face. Alopecia lesions were multiple (58.6%), single (33.4%), totalis (3.4%) and universalis (4.6%). The recurrence of AA was reported in 28 cases (32.2%). Twenty three (26.5%) cases had nail changes (21 nail pitting and 2 nail dystrophy).

Twenty eight cases (32.2%) had an association with other diseases. In addition many had a family history of AA and/or other diseases.(table 1).

2 - Circulating auto antibodies :

One or more circulating auto-antibodies were detected in 43 patients (49.4%). Eleven patients (12.6%) had two auto-antibodies and two patients (2.3%) had three auto-antibodies. Only two (10%) of the controls had one circulating auto-antibody.

The circulating auto-antibodies and their relation to the age, sex and nationality of the patients are listed in table (2).

3 - Pathology :

a - Light microscopy :

In the 74 biopsies taken, the only abnormality noticed in the epidermis was a mild degree of focal spongiosis in 30 (40%) of the biopsies. Degenerative changes were present in the dermal connective tissue around the hair papilla in 48 (65%). Mononuclear inflammatory cell infiltrates were present around capillaries and hair follicles in 52 (70%) consisting mostly of lymphocytes with a few macrophages and histiocytes.

b - Immunohistochemistry :

The most striking feature was a strong expression of HLA-DR, predominantly in the inflammatory cells (Fig. 1).

In 68 (91.9%) biopsies both T-lymphocytes and B-lymphocytes (Fig. 2) were present (approximately 2/3 T-lymphocytes and 1/3 B lymphocytes). The T-lymphocytes showed a predominant T8 (Fig.3) over T4. Macrophages were present and stained for both α1-AT and α1-ACT. (Fig.4) There was no obvious increase in the number of Langerhans cells as judged by dandretic cells in the epidermis stained for S-100 protein. However, dermal dandretic cells appeared slightly increased in number especially amongst the inflammatory cells.

4 - Cytogenetic study :

There was no chromosomal abnormality detected in the peripheral blood of 20 patients (15 males and 5 females) using G-banding technique.

5 - Other investigations :

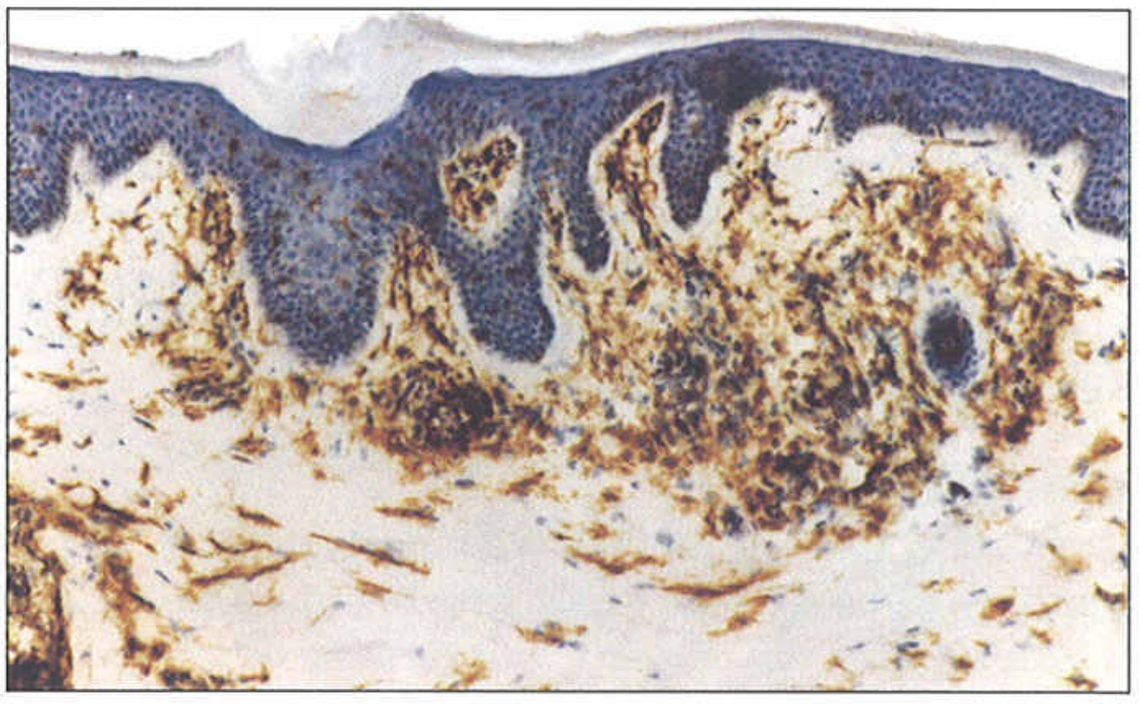

Fig. 1 :Strong staining of dermal cellular infiltrates for HLA-DR antigen PAPX12.

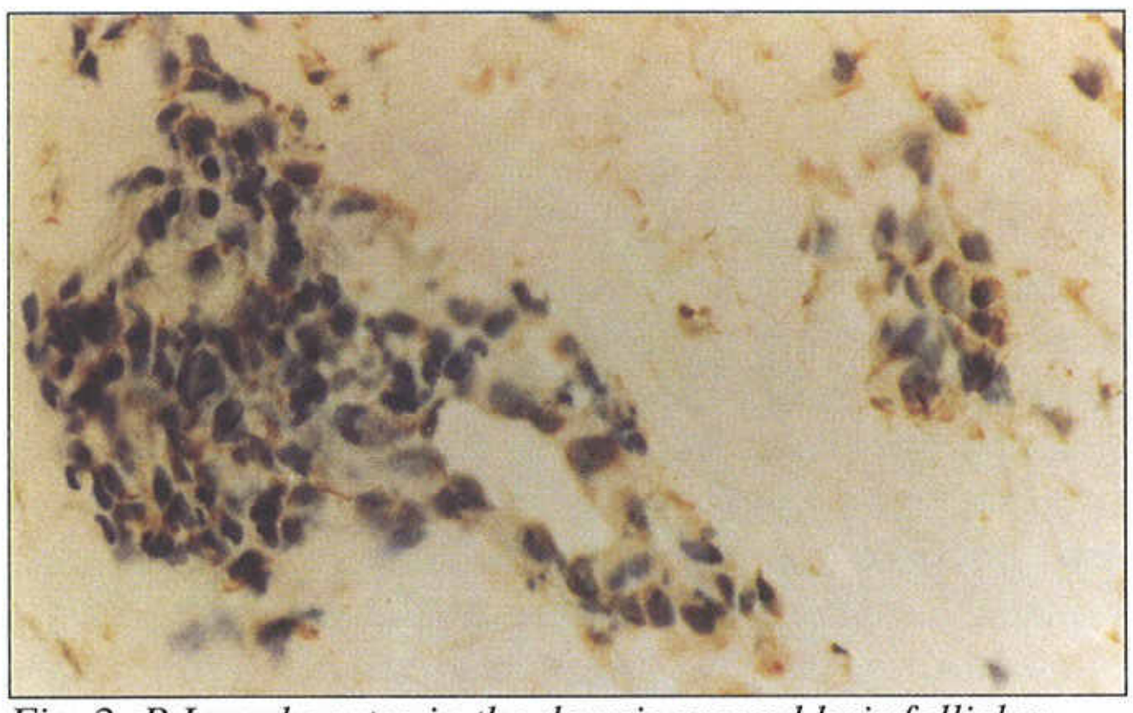

Fig. 2 :B Lymphocytes in the dermis around hair follicles PAPX50.

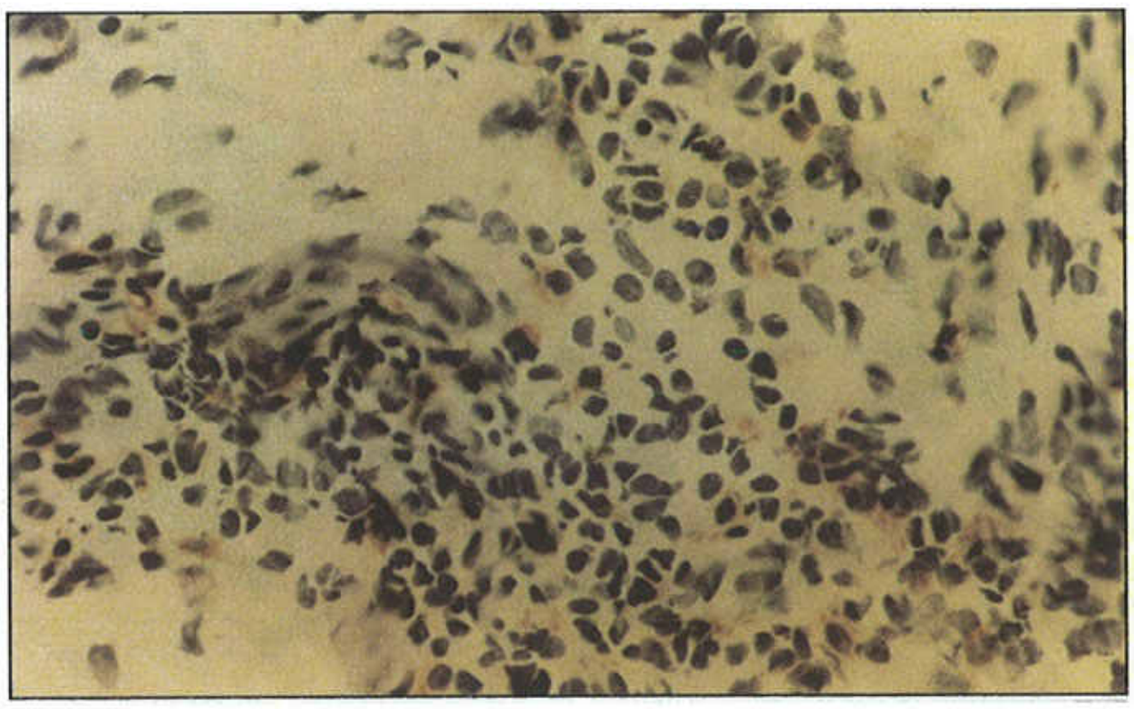

Fig. 3 :Suppressor / cytotoxic T- Lymphocytes (T8) in the dermis PAPX50.

All the patients and controls were subjected to extensive routine hematological analysis, blood chemistry (including BUN, creatinine, urea, TG, albumin, cholesterol, Ca, P, sugar level and liver function tests) and hormonal analysis. Some of the important findings are listed in

table (3).

A significant correlation was found for serum zinc levels and AA in comparing patients and controls (P = 0.0203) without any significant relationship to sex, age, nationality, anti-testicular antibodies or other laboratory parameters.

DISCUSSION :

In recent years, there have been a number of reports suggesting a possible role for immunological mechanisms in the pathogenesis of alopecia aerata(15). These suggestions have been based on clinical and laboratory evidence such as the occurrence of concomitant autoimmune disease in patients and their relatives(16), a decrease in the proportion of circulating T-cells or their subgroups and reduced T-cell function(17).

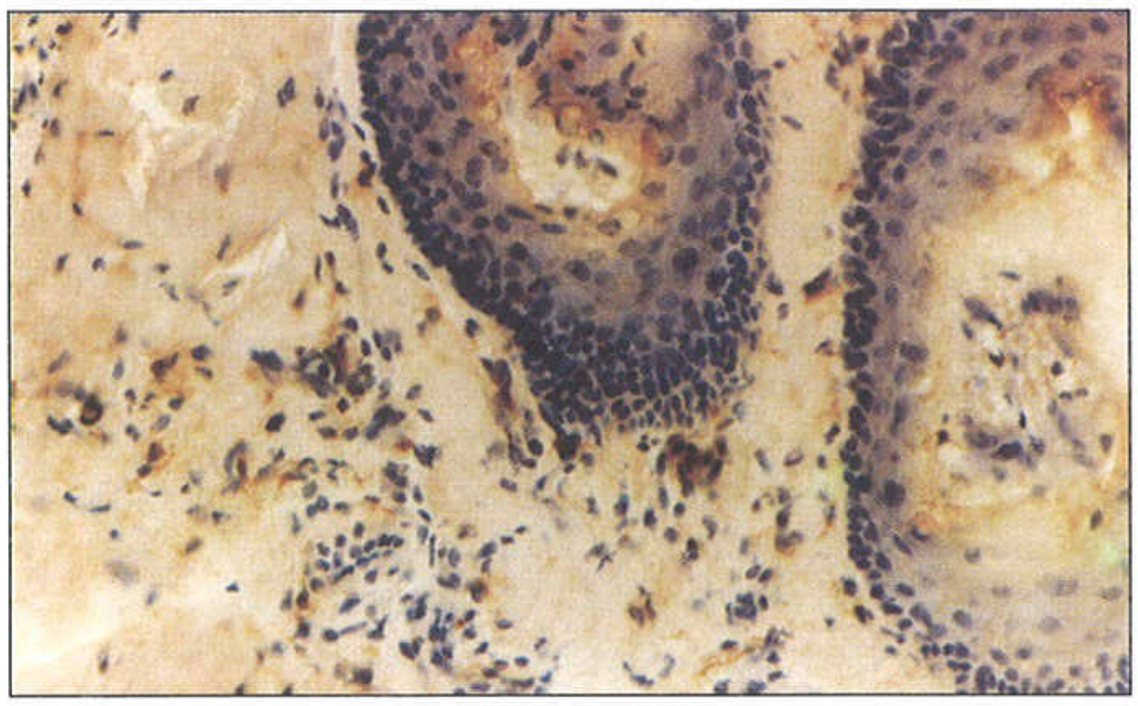

Fig. 4 :A few Histiocytic cells in the dermis have stained for alpha -1- antitrypsin PAPX50.

Auto-antibodies against thyroid antigen, smooth muscle, gastric parietal cell and antinuclear antibodies have all been reported in alopecia areata(11 , 18 , 19). However, most of these cases have not shown clinical evidence of autoimmune diseases.

Almost half of our 87 AA patients, 43 (49.4%), had at least one and occasionally two or three circulating auto antibodies. We also found a high incidence (43.7%) of a family history of AA in our patients compared to 10-20% reported in the literature(16 , 20 , 21). This unusually high incidence may be related to a tendency to marry within a few families in the State of Qatar, and may give some credence to the possibility of AA occurring in families as an autosomal dominant trait.

We did not find any chromosomal abnormalities in 20 of the cases studied by G-banding technique. However, this technique by itself does not exclude the possibility of genetic abnormalities.

We found high serum zinc levels in a significant proportion (P = 0.002) of our patients especially in males with a recurrent form of AA. This is in contrast to the findings of Ead (1981)(23) and Lutz (1991)(23) who found low serum zinc levels and noticed an improvement in their patients after zinc therapy. The high serum zinc levels in our patients may reflect the zinc content of food eaten by people in Qatar possibly supporting the view of Chandra (1984)(24) who believed that an excessive intake of zinc impaired the immune response.

The occurrence of certain antibodies in AA patients in other series are compared with our results in table (4). Although some studies have found an increased occurrence of auto-antibodies in AA patients compared to the normal population(25,26,27,28,29,30), other studies have failed to find such an increase. (31,32, 33).

The occurrence of circulating auto-antibodies may be simply an epiphenomena and not necessarily the cause of auto-immune disease unless substantiated by other clinicopathological evidence.

However, the clinical evidence of auto-immune disease in our patients together with the high incidence of circulating auto-antibodies, some changes in the subpopulation of T-lymphocytes in biopsies and an over expression of HLA DR antigen support the possible significance of immunological mechanisms and auto-immunity in AA.

Future studies are likely to focus on identifying the antigenic target of the immunological reaction directed against hair follicles in patients with AA.

Table 1 : Associated diseases and positive family history in 87 cases of alopecia areata

Associated diseases	In patients		In families	
AA	87	(100%)	38	(43.7%)
Diabetes mellitus	06	(6.9%)	27	(31.1%)
Atopic dermatitis	05	(5.8%)	19	(21.8%)
Bronchial asthma	02	(2.4%)	09	(10.3%)
Vitiligo	03	(3.4%)	07	(8.1%)
Urticaria	02	(2.4%)	07	(8.1%)
Psoriases	03	(3.4%)	04	(4.6%)
Lichen planus	01	(1.1%)	-	
Thyroid diseases	02	(2.4%)	08	(9.2%)
Others*	06	(6.9%)	-	

* included Melasma, androgenetic alopecia and photosensitivity

Table 2 : Circulating auto-antibodies in 87 patients with alopecia areata

	Patients	Correlation			
Serum auto antibodies	NO %	Patients & controls	Age*	Sex*	Nationality*
Smooth muscles	5 (5.8%)	S (P:0.00032)	NS	NS	NS
Parietal	4 (4.6%)	S P:0.0021)	NS	S(P:0.042)	NS
Mitochondrial	0 -	NS	-	-	-
ANA	8 (9.2%)	S (P:0.0004)	S(P:0.026)	NS	NS
Thyroglobulin	1 (1.1%)	NS	NS	NS	NS
Microsomal	6 (6.9%)	S (P:0.0005)	NS	S(P:0.05)	NS
Testicular +	28 (38.4%)	S (P:0.0034)	NS	-	NS
Ovarian ++	0 -	NS	-	-	-
Cardiolipin	6 (6.9%)	S (P:0.0005)	NS	NS	NS
All auto antibodies	43 (49.4%)	S (P:0.002)	NS	NS	NS

* Comparison of positive and negative cases
\+ In 73 male patients only
++ In 14 female patients only

S Significant correlation
NS Non significant correlation

Table 3 : Summary of blood chemistry, hormonal assay and subpopulation of T Lymphocytes.

Serum level	High N° %	Low N° %	Normal N° %	Total patients	Control (N°=20)	%
Blood sugar	6 6.9	0 0.0	81 93.1	87	0	0
Iron level	2 2.4	5 6.8	80 90.7	87	0	0
T4	1 1.1	0 0.0	86 98.9	87	0	0
T3	1 1.1	0 0.0	86 98.9	87	0	0
TSH	1 1.1	3 3.5	83 95.4	87	0	0
Testosterone	5 6.8	10 13.7	58 79.5	73	0	0
Zinc level	32 36.8	0 0.0	55 63.3	87	2 High	10
CD4	1 1.1	0 0.0	86 98.9	87	0	0
CD8	0 0.0	0 0.0	100	87	0	0
T4/T8	0 0.0	0 0.0	100	87	0	0

CD4 Helper T cell
CD8 Suppressor T cell

Table 4 : Auto-immune antibodies in patients with alopecia areata. A comparison of different studies.

	ANA (%)	*ATHA (%)	ASMA (%)	APCA (%)	ACA (%)	ATESA (%)	AMA (%)	OVA (%)
Main et al. (1975)	0	17	21	8	-	-	0	0
Friedman (1981)	4	30	4	15	-	-	0	-
Brown et al. (1982	0	30	-	-	-	30	-	0
Galbraith et al. (1984)	2	38	0	0	-	-	0	0
Korky et al. (1984)	-	28	0	5	-	-	0	0
Temmerman et al (1985)	8	14	2	4	-	-	0	-
Milgraum et al (1987)	18	24	16	5	-	-	0	-
Lutz et al. (1988)	3	10	5	1	-	-	0	0
De Waard et al. (1989)	13	12	23	5	-	-	0	0
Schenk et al. (1989)	28	14	-	-	-	11	0	4
Present study	9.2	8	5.8	4.6	6.9	38.2	0	0

* ATHA : antithyroid antibodies included ATG and AMC

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