IMMUNOGLOBULIN CLASS AND PATTERN OF NUCLEAR FLUORESCENCE IN SOME AUTOIMMUNE DISORDERS

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INTRODUCTION

The antinuclear antibody (ANA) was the first marker to distinguish autoimmune diseases (Vischer, 1970) and the ANA test is an excellent screening directed against any of the constituents of the nucleus (Edmonds, 1985).

Mitochondrial antigen is a lipoprotein situated in the inner mitochondrial membrane (Berg et al., 1967). The mitochondrial antibody test has proved to be a reliable diagnostic marker of autoimmune liver disease. Its diagnostic value in primary biliary cirrhosis has been widely confirmed (Sherloet, 1970).

MATERIAL AND METHODS

Patients :

This study was carried on 51 patients. Cases included the following groups :

- 20 patients with active rheumatoid arthritis (+ve latex test for rheumatoid factor).
- 5 patients with systemic lupus erythematosus (SLE) (+ve latex test for ANA +ve latex for L.E. test).
- 5 patients with juvenile onset diabetes.
- 5 patients with chronic active hepatitis (HBs Ag -ve).

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- 6 patients with primary or secondary thyrotoxicosis.
- 10 patients with Behcet's syndrome (elevated leucocytic count +ve latex for L. E. test).
- 10 normal healthy personnels.
- Phosphate Buffer concentrated pH 7.2 was diluted 1 + 19 with distilled water (PBS).
- Suffered glycerol solution was prepared by adding 1 part of PBS + 4 parts of glycerol.

Procedure :

The contents of each container (Ig polyvalent, IgG, IgM and IgA) were dissolved in one ml. distilled water. The preparations were diluted 1 : 10 with phosphate buffered saline solution (PH 7.2).

Immunofluorescence technique:

- Plasma protein Antisera, (& - globulin fraction fluorescein conjugated). Ig polyvalent, IgG, IgM and IgA obtained from Behringwerke.
- F. T. A. control serum positive fluorescein conjugated was diluted 1 : 5.
- F. T. A. control serum negative fluorescein conjugated was diluted 1 : 5.
- Patients sera were diluted 1 : 6 in (PBS).
- One drop (about 10 ul) of each diluted patients serum and controls was placed on clean slide for each immunoglobulin detection.
- Slides were incubated at 37°C for 3 hours to dry.
- Fixation was done in acetone for 10 min. and dried.
- Slides were carefully rinsed with

PBs twice 5 min. each time in cuvettes

- Slides were incubated for 30 min at 37°C in a moist chamber.
- Slides were rinsed and dried as described before.
- Slides were mounted with buffered glycerol solution and covered with a cover slide.
- Slides were examined under fluorescent microscope at 400 to 600 X magnification.

Tissue biopsies were taken in cases of chronic active hepatitis and thyrotoxicosis and stained with Hx. & E. for routine diagnosis and for immunofluorescence.

- Procedure of immunofluorescence reaction, Indirect immunofluorescence test were performed according to standard procedures on air-dried, acetone - fixed cryostat sections, employing conjugate dilutions of 1/10 and 1/20.

Sections were counterstained with

Evan's blue, diluted 1/10,000 in the conjugate. Sections were examined on a leitz ortholux fluorescence microscope equipped with a pleomopak epifluorescence device, with filter combination. I. Microphotographs were made on a agfachrome 50 L diapositive film. The observed fluorescence was expressed semi-quantitatively on a O - 4 scale (O - absent, 1 = minimal and 4 = maximal reaction), according to the intensity of the staining or the extent of the fluorescence, (El-Dosoky et al., 1984).

- All sera were screened for quantitative analysis of Igs (IgG, IgM, IgA) using :

1) Single radial immunodiffusion with nor-partigen Ig plates obtained from Behring werke.

2) Immunofluorescent technique :

- Single radial immuno diffusion with nor-partigen Ig, both control serum and the specimens to be examined were applied undiluted.

- The volume required per well

was 5 ul (0.005 ml.) using the Behring dispenser 5 ul.

- After introduction of the specimens, the plate was allowed to stand tightly closed at room temperature.
- After expiration of a diffusion period of 18 hours, the diameters of the precipitates to an accuracy of 0,1 mm were measured using the Behring werke measuring Vieiver for immunoanalysis.

Evaluation :

- The diamters in mm were determined. The concentrations in g/L were obtained from the standard graph paper obtained by the manufacturer.
- Normal control ranges of immunoglobulins concentrations were evaluated by the same method for each immunoglobulin.

RESULTS

Normal concentration of immuno-

globulins G, M, A were detected in normal healthy population included in this study and used as control measurements.

Gasses of Rheumatoid arthritis.

RNA was detected by indirect immunofluorescence technique in 7 cases with a percentage of 35%.

Cases with systemic lupus erythematosus.

- ANA was positive in all cases tested .

Case with juvenile diabetes :

AMA waa detected in 3 caaea with a percentage of 60 %.

Case with chronic active hepatitis :

- AMA was detected in 4 cases 80%.

- The AMA is deposited in the cytoplasm of hepatocytes of liver in cases of chronic active hepatitis by means of indirect immunofluorescence with anti mitochondrial antibody conjugate. The deposits take also fine granular pattern in

all cases (Fig. 1).

Cases with Thyrotoxicosis :

- ANA was detected in only 2 cases (33.3 %).

- The ANA is deposited in the nuclei of cuboidal cells lining the acini in cases of thyrotoxicosis by means of indirect immunofluorescence with anti-nuclear antibody conjugate. The deposit take granular pattern in 50 % of cases (Fig.2).

Cases with Behcet's syndrome :

- ANA was detected in 7 cases (70%).

DISCUSSION

Systemic auto-immune diseases are characterized immunologically by the occurrence of auto antibodies and hyper - gamma globulinaemia (Kallenberg et al., 1983).

In our study ANA and AMA were valuable in detecting of S.L.E.chronic active hepatitis , Behcet's syndrome & juvenile diabetes. This is in agreement with Rauch et al (1985) who also tried

to use this as a method of differentiated between S. L. E. and rheumatoid arthritis.

MacFarlane (1985) found that ANA to be present in 30 % of patients with rheumatoid arthritis.

Hughes (1984) stated that standard testing for ANA provides the screening test for S. L. E.

Detection of auto antibodies in tissue often plays a key part in diagnosis. The non-organ specific auto antibodies are found in chronic active hepatitis but the relationship between the antibodies and the pathogenesis of the disease is unknown. In this study AMA was detected in all cases and this in agreement with Paronetto & Popper,(1976),who describe that AMA is one of important parameter in their pathogenesis.

In thyrotoxicosis there is L. A. T. S. (long acting thyroid stimulating) it is an auto antibody it is much prominent in cases of pretibial myxoedema. However, in this work ANA was

demonstrated in tissue of 50 % cases of thyrotoxicosis using the indirect method.

Anther IgG is recently discovered and called human thyroid stimulating immunoglobulin (HTSI) Allison, (1970).

Also for routine diagnosis and follow up still serological test plays the only and reliable tools for diagnosis. In our study IgG was high in all cases of autoimmune diseases except in cases

with thyrotoxicosis. Rosenbaum et al.(1988) concluded that discrete group of IgG is common in connective tissue disease patients.

In our study IgA was high in cases of Behcet's syndrome and chronic active hepatitis and rheumatoid arthritis.

In this study IgM, was high only in cases of juvenile diabetes. In Senaldi et al. (1988) there is IgM reduction in S. L.E.

Table (1) : Nor -partigen method .

Immunoglobulin	Range	Mean
IgA	1.01 - 3.09	2.96
IgG	4.63 - 14.3	9.63
IgM	0.592 - 1.84	1.45

Table (2) : Immunoglobulin concentrations of the arthritic cases.

Immunoglobulin	Immunofluorescent	Nor - partigen Method		
		Range of Conc - entratiopn g/L	Mean Conc - entratiopn g/L	Normal control g/L
IgA	+	1.8 - 2.4	2.1	1.01 - 3.09
IgG	++	16.3 - 22.6	19.1	4.63 - 14.3
IgM	+	0.32 - 0.479	0.425	0.592 - 1.08

From table (2) :It noticed that in the nor-partigen method there is an increase in concentration of IgG and a decrease in concentration of IgM while IgA lies in the normal control range and also in the immunofluorescent method there is only a moderate detection of IgA.

Table (3) : Ig concentration in cases of S/E.

Immunoglobuli Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc . g/L	Mean Conc - g/L	Normal control g/L
IgA	-	0.34 - 0.47	0.42	1.01 - 3.09
IgG	++	1.9 - 24.9	22.6	4.63 - 14.3
IgM	-	0.32 - 0.535	0.479	0.592 - 1.84

From table (3): It noticed a decrease in concentration of IgA and IgM and increase in concentration of IgG by nor-partigen method . By immunofluorescent method, no clumping appeared with IgA or IgM while moderate clumping appeared with IgG.

Table (4) : Ig concentration in cases of juvenile diabetes.

Ig Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc . in g/L	Mean Conc - g/L	Normal control g/L
IgA	-	0.558 - 853	0.777	1.01 - 3.09
IgG	++++	24.1 - 36.8	30.600	4.63 - 14.3
IgM	+++	3.9 - 4.6	4.480	0.592 - 1.84

From Table (4) :It noticed that there is an increase in concentration of IgG & IgM and decrease in concentration of IgA.

Table (5) :Immunoglobulin Goncentration in cases with chronic active hepatitis.

Ig Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc . g/L	Mean Conc - g/L	Normal Range g/L
IgA	++	3.8 - 4.72	4.150	1.01 - 3.09
IgG	++++	26 - 40	37.700	4.63 - 14.3
IgM	+	1.30 - 1.85	1.600	0.592 - 1.84

From Table (5) : It noticed that mean IgG & IgA concentration were higher than normal control but IgM concentration was in the normal range.

Immunoglobulin detection by the immunofluorescent technique showed no difference from nor-partiger method,

Table (6) : Immunoglobulin concentration in cases with thyrotoxicosis

Ig Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc g/L	Mean Conc - g/L	Normal control g/L
IgA	-	0.420 - 0.0558	0.488	1.01 - 3.09
IgG	+	3.7 - 10.2	7	4.63 - 14.3
IgM	-	0.32 - 0.479	0.425	0.592 - 1.84

From Table (6) : It noticed that IgA & IgM concentrations more than normal range while IgG concentration was in the normal range.

Table (7) : Immunoglobulin concentration in cases with Behcet's syndrome.

Ig Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc g/L	Mean Conc - g/L	Normal contro g/L
IgA	++	4.59 - 6.39	5.29	1.01 - 3.09
IgG	++	8.02 - 23.4	19	4.63 - 14.3
IgM	-	0.32 - 0.535	0.43	0.592 - 1.84

From Table (7) : It noticed increase in the concentrations of IgG & IgA Nor-partigen method & moderate clumping by immunofluorescent method of the immunoglobulins while that of IgM was lowered than the normal range.

Table (9) : Ig concentration in different cases with autoimmune diseases.

Type of Autoimmunity	IgA g/L	IgG g/L	IgM g/L
1 - Rh . arthritis .	2.1	19.1	0.425
2 - S . L . E .	0.42	22.6	0.479
3 - J . D . M .	0.777	30.6	4.48
4 - Ch . active hepatitis .	4.45	37.7	1.6
5 - Thyrotoxicosis .	0.488	7	0.425
6 - Behcet's syndrome .	5.29	19	0.43

From this table IgA concentration was high in case of Behcet's syndrome, chronic active hepatitis & rheumatoid arthritis . IgG was high in all cases except in cases with thyrotoxicosis . While IgM was high only in cases of juvenile diabetes.

Table (7) : Immunoglobulin concentration in cases with Behcet's syndrome.

Ig Class	Immunofluorescent Method	Nor - partigen Method		
		Range of conc g/L	Mean Conc - g/L	Normal contro g/L
IgA	++	4.59 - 6.39	5.29	1.01 - 3.09
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IgM	-	0.32 - 0.535	0.43	0.592 - 1.84

From Table (7) : It noticed increase in the concentrations of IgG & IgA Nor-partigen method & moderate clumping by immunofluorescent method of the immunoglobulins while that of IgM was lowered than the normal range.

Fig. (1) AMA is deposited in the cytoplasm of hepatocytes of liver in cases of chronic active hepatitis (indirect immunofluorescence - Anti mitochondrial antibody conjugate).

Fig. (2) ANA is deposited in the nuclei of cuboidal cells lining the acini in cases of thyrotoxicosis. (indirect immunofluorescence with antinuclear antibody conjugate).

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الاجسام المناعية والجسم النوى الفلورنسى فى مرض اضطراب المناعة الذاتية

اجريت هذه الدراسة على ٢٠ حالة التهاب روماتويد ، ٥ حالات ذئبه احمراريه جهازيه ، ٥ حالات سكر شبايى ، ٥ حالات التهاب كبدي مزمن ، ٦ حالات الغده الدريقيه التسمميه ، ١٠ حالات مرض بهسيت كما اخذت ١٠ حالات سليمه واعتبرت كحالات منظمه .

تم عمل التحاليل الاتيه :

- ١ - تعيين كمية المضادات المناعه أ ، ج ، م بطريقة الانتشار المناعى .
- ٢ - تعيين كمية المضادات المناعه أ ، ج ، م بطريقة التآلق المناعى المباشر .
- ٣ - تعيين المضادات الاجسام النوويه ومضادات الاجسام الميتوكوندرية بطريقة التآلق المناعى الغير مباشر .

٤ - اخذت عينات نسيجه طازجه فى حالات التهاب الكبد الوبائى المزمن والتسمم الدرقى وصبغت بصيغة الهيماتوكسلين والاوسين . كما جهزت هذه العينات للفحص بالامينوفلورسنت الغير مباشر لتحديد مضادات الاجسام النوويه ومضادات الاجسام الميتوكوندرية فى الانسجه .

وقد وجد ان :

- اختبار التآلق المناعى الغير كمباشر لتعيين مضادات الاجسام النوويه والاجسام الميتوكوندرية مفيد فى حالات الذئبه الاحمراريه الجهازيه والالتهاب الكبدي المزمن ومرض بهسيت والسكر الشبايى .
- تعيين نسبة الاجسام المناعيه بأى من الطريقتين الانتشار المناعى أو التآلق المناعى المباشر لم يعطى فروقا فى الكميّه
- الاجسام المناعيه أ نسبتها عاليه فى مرض بهسيت والالتهاب الكبدي المزمن والتهاب الروماتويد .
- الاجسام المناعيه ج نسبتها عاليه فى جميع انواع مرض اضطراب المناعة الذاتيه ماعدا حالات الغده الدريقيه التسمميه .
- الاجسام المناعيه م نسبتها عاليه فى حالات السكر الشبايى .
- وجدت مضادات الاجسام النوويه فى نواة الخليه المبطنه للغده الدريقيه فى ٥٠٪ من حالات التسمم الدرقى المفحوصه بالامينوفلورسنت الغير مباشر .
- كما وجدت مضادات المناعة الذاتيه فى الانسجه يلعب دورا هاما فى تشخيص هذه الحالات .