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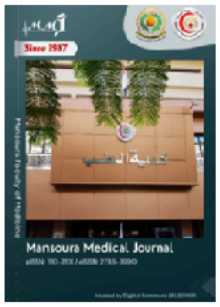

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INTRODUCTION

The antinuclear antibody (ANA) was the first marker to distinguish autoimmune diseases (Vischer,1970) and the ANA test is an excellant 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 reliable diagnostic marker of autoimmune liver disease. It's 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 erythromatosus (SLE) (+ve latex testfor ANA +ve latex for L.E. test).
- 5 patients with juvenile onset diabetes.
- 5 patients with chronic active hepatitis (HB5 Ag -ce).

- 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.

- Sera of all cases were aliquoted and stored At - 20°C

- Demonstration of ANA & AMA antibodies by immunofluorescence.

Immunofluorescence technique:

- Plasma protein Antisera, (& - globulin fraction fluorescein conjugated). Ig polyvalent, IgG, IgM and IgA obtained from Behringe werke.

- F. T. A. control serum positive fluoresein congugated was diluted 1 : 5.

- F. T. A. control serum negative fluorescin conjugated was diluted 1 : 5.

- 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).

- 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 immunglobulin 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 immunofluorscence reaction, Indirect immunofluorescence test were performed according to standard procedures on air-dried, acetone - fixed cryostate 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 ohserved fluorescence was expresaed semi-quantitatively on a O - 4 scale (O - absent , 1 = minimal and 4 = maximal reaotion), 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 :

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

2) Immunoflurescent 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 anaccuracy of 0,1 mm were measured using the Behringe werke measuring Vieiver for immunoanalysis.

Evalution :

- 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 immunoglobulins G, M, A were detected in normal healthy population included in this study and used as control measurments.

Gasses of Rheumatoid arthritis.

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

Cases with systemic lupus erythromatosus.

- 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 immunoflurosence with anti mitochondrial antibody conjugate. The deposits take also fine granular pattern in

all cases (Fig. I).

Cases with Thyrotoxioosis :

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

- The ANA is depoaited in the nuclei of cuboidal cells lining the acini in cases of thyrotoxicosis by means of indirect immunofluorescence with antinuclear antibody conjugate. The deposit take granular Fattern 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 occurance 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 standerd 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 relation ship between the antibodies and the pathogenesis of the disease is unknown. In this study AMA was detected in all cases and this in agrement 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 thyoid stimulating) it ig 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 thyriotoxicosis using the indirect method.

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

Also for routine diagnosia and follow up still serological tegt 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 (I) : 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 arthritig. 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 - 108

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 immunoflurescent method there is only a moderate detec tion 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 diabetea.

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 con centration 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 - 00558	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 noticedincrease in the concentrations of IgG & IgA Nor-partigen method & moderate clumping by immunoiluorescent 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 . sctive hepatitis .	4.45	37.7	1.6
5 - Thyrotoxicosis .	0.488	7	0.425
6 - Behct's syndome .	5.29	19	0.43

From this table IgA concentration was high in caseg of Behcet's syndromes, 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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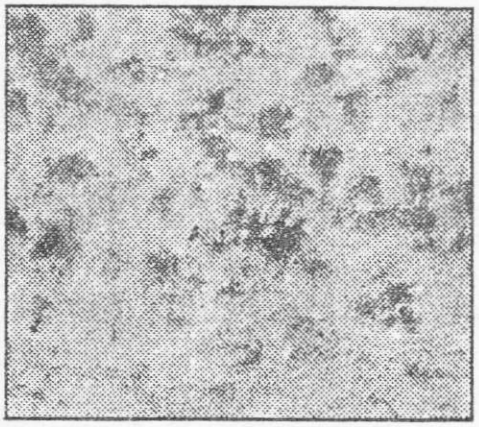

Fig. (1) AMA is deposited in the cytoplasm of hepatocytes of liver in cases of chronic active hepatitis (indirect immunofl - uroscence - Anti mitochondrial antibody conjugate).

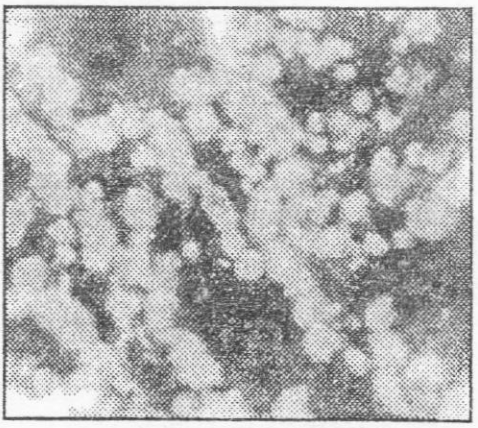

Fig. (2) ANA is deposited in the nuclei of cuboidal cells lining . the acini in ceses of thyrotoxcosis.
(indirect immunofluroscence with antinuclear antibody conjugate).

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الاجسام المناعيه والجسم النووى الفلورنسى فى مرض اضطراب المناعه الذاتيه

اجريت هذه الدراسة على ٢٠ حاله التهاب روماتويد ، ٥ حالات ذئبه احمراريه جهازيه ، ٥ حالات سكر شبابى ، ٥ حالات التهاب كبدى مزمن ، ٦ حالات الغده الدرقيه التسممه ، ١٠ حالات مرض بهسبت 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 التألق المناعى المباشر .

٣ - تعيين المضادات الاجسام النوويه ومضادات الاجسام الميتوكندريه بطريقة التألق المناعى الغير مباشر .

٤ - اخذت عينات نسيجيه طازجه فى حالات التهاب الكبد الوبائى المزمن والتسمم الدرقى وصبغت بصبغة الهيماتوكسلين والايوسين . كما جهزت هذه العينات للفحص بالامينوفلورسنت الغير مباشر لتحديد مضادات الاجسام النوويه ومضادات الاجسام الميتوكندريه فى الانسجه .

وقد وجد أن :

- اختبار التألق المناعى الغير كمباشر لتعيين مضادات الاجسام النوويه والاجسام الميتوكندريه مفيد فى حالات الذئبه الاحمراريه الجهازيه والالتهاب الكبدى المزمن ومرض بهسبت والسكر الشبابى .
- تعيين نسبة الاجسام المناعيه بأى من الطريقتين الانتشار المناعى أو التألق المناعى المباشر لم يعطى فروقا فى الكميه
- الاجسام المناعيه أ نسبتها عاليه فى مرض بهسبت والالتهاب الكبدى المزمن والتهاب الروماتويد .
- الاجسام المناعيه ج نسبتها عاليه فى جميع انواع مرض اضطراب المناعه الزاتيه ماعدا حالات الغده الدرقيه التسممه .
- الاجسام المناعيه م نسبتها عاليه فى حالات السكر الشبابى .
- وجدت مضادات الاجسام النوويه فى نواة الخليه المبطنه للغده الدرقيه فى ٥٠٪ من حالات التسمم الدرقى المفحوصه بالامينوفلورسنت الغير مباشر .
- كما وجدت مضادات المناعه الذاتيه فى الانسجه يلعب دورا هاما فى تشخيص هذه الحالات .