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 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 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 (HB5 Ag -ce).

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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 personnals.

- 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 Behringer werke.

- F. T. A. control serum positive fluorescein conjugated was diluted 1 : 5.

- F. T. A. control serum negative fluorescein 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 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 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 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 lgs (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

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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 Behringe werke measuring Vliever for immunoanalysis.

Evaluation:
- The diameters 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 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 was detected in 3 cases 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 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 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 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 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.

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

Also for routine diagnosis and follow up still serological tests play 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 Behçet'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.**

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>1.01 - 3.09</td>
<td>2.96</td>
</tr>
<tr>
<td>IgG</td>
<td>4.63 - 14.3</td>
<td>9.63</td>
</tr>
<tr>
<td>IgM</td>
<td>0.592 - 1.84</td>
<td>1.45</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Immunofluorescent</th>
<th>Nor-partigen Method</th>
<th>Normal control g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of Concentration g/L</td>
<td>Mean Concentration g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>+</td>
<td>1.8 - 2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>IgG</td>
<td>++</td>
<td>16.3 - 22.6</td>
<td>19.1</td>
</tr>
<tr>
<td>IgM</td>
<td>+</td>
<td>0.32 - 0.479</td>
<td>0.425</td>
</tr>
</tbody>
</table>

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.**

<table>
<thead>
<tr>
<th>Immunoglobulin Class</th>
<th>Immunofluorescent Method</th>
<th>Nor-partigen Method</th>
<th>Normal control g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of conc. g/L</td>
<td>Mean Conc. g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>-</td>
<td>0.34 - 0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>IgG</td>
<td>++</td>
<td>1.9 - 24.9</td>
<td>22.6</td>
</tr>
<tr>
<td>IgM</td>
<td>-</td>
<td>0.32 - 0.535</td>
<td>0.479</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Ig Class</th>
<th>Immunofluorescent Method</th>
<th>Nor - partigen Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of conc. in g/L</td>
<td>Mean Conc - g/L</td>
<td>Normal Range g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>++</td>
<td>3.8 - 4.72</td>
<td>4.150</td>
</tr>
<tr>
<td>IgG</td>
<td>++++</td>
<td>26.40</td>
<td>37.700</td>
</tr>
<tr>
<td>IgM</td>
<td>+</td>
<td>1.30 - 1.85</td>
<td>1.600</td>
</tr>
</tbody>
</table>

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-partigen method.

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Table (6) : Immunoglobulin concentration in cases with thyrotoxicosis

<table>
<thead>
<tr>
<th>Ig Class</th>
<th>Immunofluorescent Method</th>
<th>Nor - partigen Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of conc g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>-</td>
<td>0.420 - 0.0558</td>
</tr>
<tr>
<td>IgG</td>
<td>+</td>
<td>3.7 - 10.2</td>
</tr>
<tr>
<td>IgM</td>
<td>-</td>
<td>0.32 - 0.479</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Ig Class</th>
<th>Immunofluorescent Method</th>
<th>Nor - partigen Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range of conc g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>++</td>
<td>4.59 - 6.39</td>
</tr>
<tr>
<td>IgG</td>
<td>++</td>
<td>8.02 - 23.4</td>
</tr>
<tr>
<td>IgM</td>
<td>-</td>
<td>0.32 - 0.535</td>
</tr>
</tbody>
</table>

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.

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

From this table IgA concentration was high in cases 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.

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

<table>
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<th>Ig Class</th>
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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.

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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).
REFERENCES


Volume 21, 1991
الجسم المناعي والجهاز النروبي الفلورنسي في مرض اضطراب المناعة الذاتي

أجريت هذه الدراسة على 20 حالة التهاب روماتويدي ، 5 حالات ذبحة احمراره جهازيه ، 5 حالات سكر شبايبي ، 5 حالات التهاب كبدي مزمن، 6 حالات الغد الدرقي التسليمية ، 10 حالات مرض ببسيت كما
أخذت 10 حالات سليمة وأعتبرت كحالات منظمة.

ثم عمل التحاليل الآتية:

1 - تم تعيين كميات مضادات المناعة أ، ج، م بطريقة الاختبار المناعي.
2 - تم تعيين كميات مضادات المناعة أ، ج، م بطريقة التأقلم المناعي المباشر.
3 - تم تعيين مضادات الأجسام النروبي ومضادات الأجسام الميتوكندرية بطريقة التأقلم المناعي الفيبر مباشر.
4 - اخذت عينات تسجيحية طازجة في حالات التهاب الكبد الوبائي المزمن والتسليم الدرقي وربما بصفة الهيماتوكسيلين والايرزين. كما جهزت هذه العينات للفحص بالامينوفلورست الفيبر مباشر

لتحديد مضادات الأجسام النروبي ومضادات الأجسام الميتوكندرية في الأنسجة.

وقد وجد أن:

- اختبار التأقلم المناعي الفيبر ك مباشر لتعيين مضادات الأجسام النروبي وال الأجسام الميتوكندرية مفيد.
- في حالات الذبحة الاحمراره جهازيه والالتهاب الكبدي المزمن مرض ببسيت والسكر الشبايبي
- تعيين نسبة الأجسام المناعي بأي من الطرقتين الاختبار المناعي أو التأقلم المناعي المباشر لم يعطى

فرصا في الكهرباء.
- الأجسام المناعية أ نسبتها عالية في مرض ببسيت والالتهاب الكبدي المزمن والتهاب الروماتويدي
- الأجسام المناعية ج نسبتها عالية في جميع أنواع مرض اضطراب المناعة الذاتي ماعدا حالات الغد
- الدرجة التسليمية.
- الأجسام المناعية م نسبتها عالية في حالات السكر الشبايبي.
- وجدت مضادات الأجسام النروبي في نواة الخليل البطني للغد الدرقيه في 50٪ من حالات التسليم
- الدرجة المحفزة بالامينوفلورست الفيبر مباشر.
- كما وجدت مضادات الأجسام الذاتي في الأنسجة يلعب دورا هاما في تشخيص هذه الحالات.

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