Clinical Significance of Anti-neutrophil Cystic Antibodies (ANCA) in Different Types of Autoimmune Hepatitis (AIH)

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Abstract
Background: Autoimmune hepatitis is a long-lasting inflammatory liver ailment of unidentified origin, but it is connected with flowing autoantibodies and extraordinary serum gamma globulin altitudes. Aims: this study was designed to estimate serum concentration of anti-neutrophil cytoplasmic antibodies and its clinical significance in patients with autoimmune hepatitis.

Patients and Approaches: 73 patients (20 male, 53 female) with long-lasting active hepatitis of unknown cause, and 50 healthy individuals (age and sex coordinated) served as control. The scientific considerations of the ailment were evaluated; consisting liver function tests (Transaminases and total bilirubin), autoantibodies including antinuclear antibody (ANA), anti-smooth muscles antibodies (ASMA), anti-liver-kidney microsome-1 antibodies (anti-LKM1), anti-liver cytost antibody-1 (anti-LC1) and anti-soluble liver antigen/liver pancreas (anti-SLA/LP) by indirect immunofluorescence test (IF) and Euro line method. The occurrence of ANCA was identified on alcohol-fixed neutrophils by IIF, a cystolic (C-ANCA) and perinuclear (P-ANCA) staining design was well-thought-out positive ANCA antigenic specificities were investigated by ELISA.

Results: the results of the current study showed 49 (67%) of patients had type 1 AIH, whereas 16 (22%) had type 2, and 8 (11%) had type 3 autoimmune hepatitis. The concomitant positivity of ANCA with high titer was detected solitary in AIH-1 tolerant serum, and absent in the serum of tolerant with type-2 and 3 of the disease. This study revealed that the isolative presence of ANCA represent 21.8% while they coexist with ANA and ASMA in 20% and 57.8% respectively.

Conclusion: it has been concluded that ANCA positivity appears to identify a type 1-AIH with distinct clinical characteristics, more commonly associated with the presence of ASMA.

Keywords: Anti-neutrophil cystic antibodies (ANCA); Autoimmune hepatitis; anti-smooth muscles antibodies (ASMA); antinuclear antibody (ANA)

1. INTRODUCTION
Autoimmune hepatitis (AIH) is a self-perpetuating hepatocellular inflammation of mysterious etiology, characterized by the presence of interface hepatitis on histologic examination, hypergamma globulinenemia, and circulating autoantibodies, which in most cases, respond to immunosuppressive treatment [1,2]. Autoimmune hepatitis accounts for solitary almost 10% of long-lasting hepatitis belongings in the United States, a reduction from formerly described rates that possibly imitates not a accurate alteration in occurrence but well approaches of discovering viral invaders. The pathogenesis of the ailment is one of a hereditarily inclined individual exposed to an ecological mediator that elicits an autoimmune response against hepatocytes antigens [3]. The subsequent immune response yields a necrotizing provocative response that ultimately clues to the obliteration of hepatocytes and improvement of cirrhosis. The aspects close the inherent predilection and the eliciting happenings that clue to the autoimmune response are vague. Autoimmune hepatitis is primarily a disease of young females, though it can happen at any stage and in males or females. Clinical appearances of the ailment concealment a range that spreads from no obvious symptoms to the signs associated hepatic failure. In no obvious symptoms belongings, the ailment may be revealed while irregular serum enzyme planes are exposed through a presentation of monotonous screening assessments. Corticosteroid and immunosuppressive medications are the treatment of choice for this kind of hepatitis. Hepatic transplantation may be the merely treatment for the end-stage ailment [4]. It is eventually accepted that the identification and description of serum autoantibodies have been a powerful strength in the systemic assessment, organization, and analysis of AIH [4,5]. Among the autoantibodies described in this disease are Anti-neutrophil cytoplasmic antibodies (ANCA), these antibodies (Abs) have recognized as a delicate immuno-serologic indicator for sure kinds of universal vacuities, particularly Wegener’s granulomatosis, microscopic polyarthritis and idiopathic crescentic glomerulonephritis. Lately, it has been too defined in tolerant with main sclerosing cholangitis (PSC), long-lasting ulcerative colitis and are detected up to 90% of tolerant with AIH and may be surrogate markers for the disease [6,7]. As a result of extensive studies, it was found that, IgG1 class is predominant in AIH, which distinguishes them from the perinuclear (P-ANCA) in PSC [8]. However, some antinuclear antibodies (ANA) can interfere with ANCA pattern by staining the neutrophil nucleus and cytoplasm; make P-ANCA undistinguishable [9]. Therefore this study was designed to evaluate serum concentration of Anti-neutrophil cystic antibodies (ANCA) and determine its clinical significance in tolerant with autoimmune hepatitis.

2. PATIENTS AND METHODS
2.1 Patients
73 patients (20 male, 53 female) with long-lasting active hepatitis (CAH) of mysterious cause, joining the Teaching Hospital for Gastroenterology and liver disease in a
historical between November 2003 and July 2004 at Ad Diwaniyiah region. Their age ranged between 10-65 years, paralleled with 50 intact persons (age and sex coordinated). Both groups were subjected to serological detection of autoantibodies (ANA, anti-SMA, anti-LKM 1, anti-LC1 and SLA/LP) by IIF and Euro line method. The presence of ANCA was detected on ethanol-fixed neutrophils by IIF, a cytoplasmic (C-ANCA) and perinuclear (P-ANCA) staining pattern was considered positive. Euro immune has provided the upstairs kits corporation, Germany.

2.2 Determination of serum alkaline phosphatase activity
Colorimetric determination of ALP according to Kind and king method [10]. The liberated phenol was measured in the presence of 4-aminocillinpyrine and potassium ferricyanide by using ALP kit supplied by biomerieux.

2.3 Determination of serum transaminase activity
Colorimetric determination of alanine aminotransferase and aspartate aminotransferase activity (ALT&AST) was performed according to the Reitman and Frankel, (1957) [11] using ALT&AST kit supplied by Randox.

2.4 Determination of total Bilirubin in Serum
Quantifiable assessment of direct bilirubin in serum was achieved rendering to DPD method, dichlorophenyl-diazonium-tetrafluoroborate (DPD) to produce the consistent azobilirubin. The absorbance of this dye at 546 nm is straight relative to the total bilirubin level in the sample [12] using TSB kit provided by biomerieux.

2.5 Assessment of serum ANCA
The serum level of ANCA was assessed by Euro Immune ELISA (enzyme-related immunosorbentassay) kit (Germany). 50 microliters all of serum model and analyze diluent were positioned in all well of a ninety six well plate layered with a Lactoferrin (LF) directed to ANCA. This combination was incubated for 2 hours at room temperature, and every well was articulated and eroded 5 times with wash solution. Afterward, fifty μL of substrate solution, which was equipped with identical quantities of steadied hydrogen peroxide (H2O2) and tetramethylbenzidine, was added for a twenty minutes reaction below dim circumstances. The reaction was slaked by the addition of fifty μl stop solution (0.5 N of HCl). Inside thirty minutes, the optical density was assessed at a wavelength of 450 nm by the bio ELISA reader ELx 800 (Molecular Expedient Co., biokit, CA, USA). The serum level of ANCA was measured founded on a standard concentration curve. The association constant (r) of the standard concentration curve was 0.990.

2.6 Statistical analysis
The data were analyzed by using Windows software packages Graphpad Prism v6. Data are stated as mean ± standard error. One way analysis of variance ANOVA analysis was used for the statistical differences between the groups for a measured parameter, Fisher's Exact test was used for correlation between different autoantibodies in studied groups. P values of less than 0.01 were considered to be statistically significant.

3. RESULTS
As it is shown in figure 1, 49(67%) of patients had type 1AIH, whereas16 (22%) had type 2, and 8 (11%) had type 3 autoimmune hepatitis.

The autoantibodies (ANA, ASMA, LKM-1, LC 1, SLA/LP, and ANCA ) were not present in healthy group in comparison to AIH patients, as well as statistical difference (P<0.01) with ASMA in AIH-1 patients sera compared with type-2 and 3 of the disease, as shown in (Table 1). On the other hand, the concomitant positivity of ANCA with high titer were perceived solely in the serum of autoimmune hepatitis tolerant, and with type-2 and 3 of the disease since they represent (65.3%), (figure -2). It has been noticed that, ANA and ASMA exhibit 44.9% and 57.14% respectively. Interestingly, this study showed that the isolative presence of ANCA represent 21.8% while they coexist with ANA and ASMA in 20% and 57.8% respectively.

(The Table 2) shown a significant rise of liver transamiases and total bilirubin in ANCA-positive patients in comparison to ANCA negative patients as well as intact group (P<0.001), ALP stayed regularly 1 to 2 fold rise.

Table 1: Correlation between different autoantibodies in studied groups.

<table>
<thead>
<tr>
<th>Test</th>
<th>AIH Type I (n=49)</th>
<th>AIH Type II (n=16)</th>
<th>AIH Type III (n=8)</th>
<th>Healthy control group (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>ANA</td>
<td>22</td>
<td>44.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ASMA*</td>
<td>28</td>
<td>57.14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ANCA</td>
<td>32</td>
<td>65.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti -LC1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-LKM1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SLA/Lp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The asterisks refer significant difference (P<0.01)

ANA(antinuclear antibody), ASMA(anti-smooth muscle antibodies), ANCA(Anti-Neutrophil Cytoplasmic Antibody), Anti LC1(anti-liver cytosol antibody-1), Anti-LKM1(anti-liver-kidney microsome-1 antibodies), anti-SLA/LP (anti-soluble liver antigen/liver pancreas)
Table 2: The biochemical parameters findings between 3 studied groups.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>ANCA positive (n=32)</th>
<th>ANCA negative (n=17)</th>
<th>Control (n=50)</th>
<th>P amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>79.77±32.9</td>
<td>47.11±21.8</td>
<td>14.44±2.5</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>43-140</td>
<td>29-79</td>
<td>13-20</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>89.88±21.62</td>
<td>49.3±12.1</td>
<td>17.5±3.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>22-163</td>
<td>13-50</td>
<td>11-21</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>147±24.2</td>
<td>172±39.33</td>
<td>81.1±5.66</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>81-190</td>
<td>76-211</td>
<td>61-80</td>
<td></td>
</tr>
<tr>
<td>TSB</td>
<td>9.66±3.2</td>
<td>4.96±1.87</td>
<td>0.5±0.7</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td></td>
<td>0.6-19.0</td>
<td>1.6-7.8</td>
<td>0.3-099</td>
<td></td>
</tr>
</tbody>
</table>

- Numbers are stated as means ± standard error (SE).
- The symbols discuss significant change (P<0.01) according to Tukey's numerous association tests.

ALT(A alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkalcanine phosphatase), TSB (Total serum bilirubin)

4. DISCUSSION

The remark in current work that the mean oldness of the ailment was inferior amongst tolerant with ANCA(-) meanwhile it was revealed designate (28.2 ± 8.44), although it was (43.3 ± 16.1), amongst tolerant with ANCA(+). This outcome was nearly analogous to additional away studies who observed that patients with ANCA(+) were predominantly postmenopausal female [9]. It is well known that the recognition of circulating autoantbodies is probable indication for autoimmune hepatitis and other autoimmune ailments. Recently, new autoantbodies were detected in the serum of patients with AIH, these antibodies known as ANCA, were originally described in patients with certain types of systemic vasculitis, and were well-thought-out adjective indicators of the ailment that strengthened the identification of the disease [13]. Subsequently, ANCA were described as a diagnostic importance because of their frequency in type 1-AIH patients [8]. This study is not exclusive since our data revealed that these Abs represent 65.3% in type 1 of the disease but are inattentive in tolerant with kind 2-AIH. The result obtained from the present study was similar to that reported by many workers [14,15]. Generally, the traditional approaches for the detection of ANCA are the IIF method, herewith method two chief outlines are known, a cystolic (C-ANCA) and a perinuclear (P-ANCA) type [16]. Related to this study it was found that, high titer of P-ANCA is present in the serum of 90.6% of patients with ANCA-positive type 1-AIH whereas (C-ANCA) present in only 9.3%, the result obtained from the present study was similar to that reported by Targan SR et al., who found that P-ANCA was present in 96%of patients with type 1 of the disease[6]. Among the type 1-AIH patients, 65.3% were ANCA (+), 44.9% ANA(+), and 57.14% ASMA(+). Interestingly, these data revealed that ANA antibodies occur in coexistence with P-ANCA in only 20% of cases and the titer of ANCA did not correlate with the titer of ANA. These finding resembled other abroad studies [5,9 ]. On the other hand, 21.8% of patients in this study ANCA was found to be the only marker of AIH, therefore they may be useful to rearrangement tolerant with cryptogenic long-lasting hepatitis by means of consuming AIH. Obviously, the results in this work were in agreement with
the previous studies [17,18] in which ASMA were common in ANCA (+) patients, the positivity of these Abs was observed in 56.2% in the sera of these patients. In addition, this study showed that anti-myeloperoxidase(anti-mPO) and anti-lactoferrin (anti-Lf) Abs were identified in 4(12.5%) whereas, anti-actin Abs were detected in 87.5 % of these patients. Thus, actin seems to represent the major antigenic target. By the obtainability of extremely delicate analyze procedures, certain biochemical checks develop regular laboratory techniques in clinical exercise for diagnostic and predictive drives. Consequently, in the current study biochemical considerations counting AST, ALT, ALP, and TSB were nominated. Our data showed that serum aminotransferase levels were significantly elevated in patients with AIH, while alkaline phosphatase level was 1 to 2 fold elevated and hyperbilirubinemia is present in most patients. In the present work, there was the important connotation of aminotransferases by ANCA positive, meanwhile the current work exhibited that the uppermost level of liver transaminases, in addition to TSB, were detected amongst tolerant with ANCA(+) in contrast to persons with ANCA(-) of the disease. On the other hand, recently several abroad studies confirmed the previous finding in which patients with ANCA display a more severe course of AIH [7], this fact was true when the present study showed that (53.1%) with ANCA(+) associated with the occurrence of relapses, and histologically had more advanced fibrosis or cirrhosis.

5. CONCLUSION
The fore mentioned findings indicate that ANCA positivity appear to identify a type 1-AIH with distinct clinical characteristics, more commonly associated with presence of ASMA and less commonly with ANA and there is no correlation between ANA titer and ANCA titer. Patients are most often postmenopausal female with more severe and advance disease.

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REFERENCES