Original Article

C3 and C4 complement components in autoimmune hepatitis.

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Summary:

Background: *. Autoimmune hepatitis (AIH) is an unresolving inflammation of the liver of unknown cause associated with interface hepatitis on histological examination,hypergammaglobulinemia and presence of circulating autoantibodies.*
Antibody-mediated tissue injury might be responsible for tissue injury in AIH therefore; a number of studies have been focused on the complement system in these patients .
The aim of the study is to estimate the level of complement (C3 and C4) in different types of Autoimmune hepatitis (AIH).
Methods: *The study was performed on 73 Iraqi patients with autoimmune hepatitis (AIH), attending the teaching hospital for gastroenterology and liver disease in a period between November 2003 and July 2004. : Anti- soluble liver ANA , SMA,LKM-1,and LC-1 Abs were detected using Enzyme-Linked Immunosorbent Assay (ELISA) technique . Anti-SLA/LP was detected using the Euroline method.*
Quantitation of serum C3 and C4 of the study groups were carried by single radial immudiffusion (SRID) test. Equal volume
Results*: Significant differences was observed between AIH patients and healthy control group in the mean level of C3 and C4. In addition, level of C4 in the sera of patients with type 2 was lower than those of type 1 and 3.*
Conclusion:*It was conclude that patients with AIH do not have abnormal catabolism of complement or increased level of cleavage products of C3, whereas, the depression of complement level may reflect decreased hepatic synthesis.*

Introduction: -

Autoimmune hepatitis (AIH) is an unresolving hepatocellular in- flammation of unknown cause that is characterized by the presence of periportal hepatitis on histologic examination, serum liver-associated autoantibodies and hypergammaglobulinemia(1,2).

It is generally accepted that, antibody-mediated tissue injury might be responsible for tissue injury in AIH therefore; a number of studies have been focused on the complement system in these patients (3). However, the amount of complement does not necessarily indicate the amount of their participation in disease process since their serum level depends on synthesis and catabolism (3, 4). Generally it was found that decreased levels of C3 and C4 are associated with SLE, chronic hepatitis and various autoimmune disorders (5, 6). In addition, the serial estimation of serum complement may provide a useful marker for disease progression and therapeutic monitoring (7).As a result of extensive studies, several investigators reported that patients with AIH have lower than normal amount of complement, especilly for type-2 (8, 9).

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Patients and Methods: -

Patients : a seventy three patients (20 male, 53 female) with chronic active hepatitis (CAH) of unknown cause, attending The Teaching Hospital for Gastroenterology and liver disease in a period between November 2003 and July 2004.Their age raged between 10-57 years, compared with 50 healthy individuals (age and sex matched). Both groups were subjected to serological detection of ANA, SMA LKM-1, LC-1, and SLA/LP Abs by IIF and Euro line method.

Laboratory investigation: -

ANA , SMA,LKM-1,and LC-1 Abs were detected using Enzyme-Linked Immunosorbent Assay (ELISA) technique used human IgG Fc as the antigen coated the microwells plate and isotype-specific horse antibodies coupled to radish peroxidase; result were expressed as the optical density. Anti-SLA/LP was detected using the Euroline method. The test kit contains test strips coated with parallel lines of antigens, which have been purified by affinity chromatography.
Euro immune has supplied the above kits company, Germany.

Quantitation of serum C3 and C4 of the study groups were carried by single radial immudiffusion (SRID) test. Equal volume (5µl) of the reference sera and test samples were added to wells in an agarose gel containing monospecific antisera .The sample was diffused readily through this gel, and substance being assayed (antigen) form a precipitin ring with the monospecific antisera. Ring diameters were measured following 48hrs. Incubation at room temperature (18-25 C°), the diameter of the precipitating ring was calibrated using measuring viewer. Unknown concentration were determined from the reference curve, and expressed as mg/dl. Biomaghreb has supplied the C3 and C4 kits company, Tunisia

Results:-

As it is shown in table 1, 49(67%) of patients had type 1 whereas, 16 (22%) had type 2, and 8 (11%) had type 3-AIH.

Table 1: The frequency distribution of AIH types.

AIH types	No	%
Type I- AIH	49	67.0
Type II- AIH	16	22.0
Type III- AIH	8	11.0
Total	73	100%

On the other hand there was significant difference in the mean age of patients with different types of AIH, since patients with type 2-AIH are more younger 27.2±9.44 than those with type 1(34.82±10.7) and 3 (31.25±6.52).

There were 53 females and 20 males with female to male ratio 2.6:1.

The autoantibodies (ANA, SMA, LKM-1, LC-1, and SLA/LP) were not detected in healthy control group just in few no. in comparison to AIH patients. Concerning the autoantibodies that present in type 1-AIH, our data revealed that ANA and SMA represent 30.14% and 42.47% respectively, whereas LKM-I, and LC-1 Abs (marker of type 2) exhibit 16.44% and 13.7% respectively, while SLA/LP Abs exhibit 100% of patients with type 3 .(Figure-1)

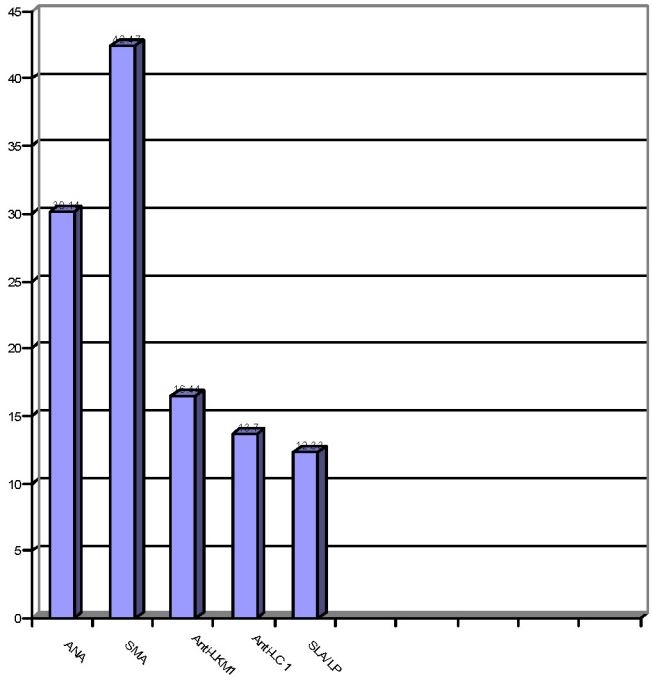

Figure 1: Frequency distribution of autoantibodies among AIH patients.

Significant differences was observed between AIH patients and healthy control group in the mean level of C3 and C4 as demonstrated in table 2 (P<0.001), while no significant differences in the mean of C3, and C4 level between different types of the disease.

Interestingly, this study showed that level of C4 in the sera of patients with type 2 was lower than those of type 1 and 3 though statistically not significant.

Serum complement concentration (µg/dl)	AIH-I (n=49)	AIH-II (n=16)	AIH-III (n=8)	Healthy control group (n=50)
C3	53.57±24.38 (11.9 – 114.9)	68.5±20.1 (22.5 – 114.6)	63.49±29.45 (22.5 –120.6)	128.68±27.27 (88.3-193)
C4	17.08±7.56 (5.9 – 40.9)	12.842±3.54 (2.8 – 18.2)	18.93±4.064 (9.3 – 49.9)	31.18±5.351 (20.4-40.0)

Table 2: Serum complement concentrations (mg/dl)level between 3 types of AIH comparing to healthy control .

- Highly significant for C3 and C4 comparing to healthy control (P<0.001)
- Not significant comparing to different types (P>0.05).

Discussion:-

It is generally accepted that type 1 is the most common form of AIH, however; in the present study type 1-AIH showed to represent 67% of the

cases while type 2 and 3 represented 22% and 11% respectively, whereas. These findings are in contrast with abroad studies who revealed that type-1 represent 80%. While type 2 and 3-AIH represent 4% and 3% respectively (10, 11). To our knowledge we couldn't compare these results with other studies carried in Iraq, simply because it is the first time that this disease have been studied in details.

These discrepancies in AIH type's frequency may be attributed to different environmental events and genetic background, which influence the etiology and pathogenesis of the disease.

The detection of serum autoantibodies is presumptive evidence for AIH and other autoimmune disorders. Therefore, when compared with autoantibody profile of healthy control, AIH – associated ANA, and SMA, exhibited (30.14% vs. 6.0%, and 42.47% vs. 8.0%) respectively while, the concomitant positivity of LKM-1, LC-1, and SLA/LP were observed only in AIH patients sera, and never in the sera of healthy control. The prevalence showed in the present study for autoantibodies are substantially in line with previous reports (12, 13, 14), which used the same IFA screening dilution employed here.

Complement components C3 and C4 were observed to be decreased in sera of AIH patients in comparison to the healthy controls. These findings supported by abroad studies, which showed that patients with AIH do not have abnormal catabolism of complement or increased level of cleavage products of C3, whereas, the depression of complement level may reflect decreased hepatic synthesis (3, 15).

Hence it is very logic to see in this study as well as other studies that decreased level of serum complements C3 and C4 were evidently noticed. Strikingly, in this work the level of C4 in patients with type 2 was lower than their level in type 1, and 3-AIH though it is non significant, these finding could probably due to genetic factors. Since several abroad studies declared that type 2-AIH were associated with C4 A gene deletions (16,17).

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