

AUTOIMMUNE HEPATITIS AMONG AUTOIMMUNE LIVER DISEASE IN BASRAH SOUTH OF IRAQ INCIDENCE AND DIAGNOSIS

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ABSTRACT

Autoimmune hepatitis is necroinflammatory disease, it can be divided into two types, Diagnostic features include biochemical evidence of transaminitis, elevated IgG and positive autoantibodies. the study aimed to estimate AIH among Autoimmune Liver Disease and it is clinical feature, the results exhibited that out of 218 patients diagnosed as a autoimmune disease during the period from January 2012 to December 2012 forty patients (29 Female and 11 Male) age ranged (11-45 years) diagnosed as a autoimmune Liver disease patients. The level of AMA, ANA, SMA, LKM-1, SLA and total IgG are measured for patients and

twenty five Healthy controls volunteers (19 female and 6 Male) the result exhibited SLA is more frequent among the patients (50%) while LKM-1 was (7.5%), patients reported significant elevation of IgG level (2619.34+408.29) mg/as compared with healthy control (1127.6+204.25) mg/l $p \leq 0.05$. Simplified score system was applied for the patients diagnosis, only 25(62.5%) of patients are collected ≥ 7 (defined AIH) were the others 14 (35%) collected < 6 and only one patient (3%) collected 6 score (properly AIH). Biomarker study of AIH patients exhibited SLA antibody is a sole marker in the AIH patients it is presented in 20(80%) , the study exhibit positive correlation between anti SLA and ALT enzyme , sex distribution of the patients exhibited the frequency of disease in the female to male approximately 3:1 were female represented 76%. The age group 20-29 (48%) was more effected with disease. According to the auto antibodies detected patients classified into two type of disease Type-1 represented (88%) while Type-2 was (12%), the clinical finding showed that the Type-2 was more severe than Type-1.

INTRODUCTION

Autoimmune hepatitis. (AIH) is a chronic, progressive necroinflammatory disease putatively caused by loss of tolerance to hepatic autoantigens (Czaja, 2007). The current concept of pathogenesis involves initiation by environmental triggers in persons with immunogenetic predisposition reflected by strong human leukocyte antigen (HLA) class II haplotype associations, loss of immunological tolerance to liver autoantigens, generation of an unregulated T-cell-mediated immune attack against those autoantigens, and production of non-species-specific autoantibodies (Manns *et al.*, 2010).

Only few studies have examined the epidemiology of AIH. In a study conducted in a Norwegian population, Boberg *et al.* (1998), found a mean annual incidence of 1.9 cases per 100,000 people per year with a prevalence of 16.9 cases per 100,000 people. Other study conducted in a Spanish population by Primo *et al.* (2004) reported a mean annual incidence of 0.83 cases per 100,000 inhabitants in the population aged more than 14 years with a prevalence of 11.6 cases per 100,000. The disease is considerably less frequent in Japan, where the incidence is between 0.08 and 0.15 cases per 100,000 people per year (Nishioka *et al.*, 1998), while in Brazil it accounts for 5-10% of liver diseases in children (Cançado & Porta, 2000). The annual incidence reported to be 1.9 cases per 100,000 in Norway with prevalence 17 cases per 100,000 (Bobreg *et al.*, 1998), the incidence of disease in New-Zealand is 2 cases per 100,000 person (Ngu *et al.*, 2010). In Brazil it is 5-10% of liver disease in children (Cancado & Porta, 2000) and it is 2.6% of liver transplantation in Europe (Milkiewicz, 1999) and it is about 5.9% of liver transplantation in the national Institutes of health liver transplantation Database (Wisner *et al.*, 2003). In general most other previous data were collected before the first international Autoimmune disease Hepatitis Group Scoring System that is led to lack of optimization in disease diagnosis (Jonson, 1993; Alvarez, 1999). In Iraq there are no data about incidence and prevalence of Autoimmune hepatitis and thus it is impossible to compare Iraqi data with other country so the current study focused on diagnosis and estimate the incidence of autoimmune hepatitis among autoimmune liver disease by Auto antibodies detection, Haematological status evaluation, Biochemical investigation and Simplified scoring applying.

PATIENTS AND METHODS

From 218 autoimmune hepatitis patients forty patients (29 Female and 11 Male) aged between (11-45 years) are selected who are diagnosed as autoimmune liver disease were

collected from three main hospital in Basra (Al-Sader teaching ,Almawany and General Basrah Hospital) from January 2012 to December 2012 and twenty five Healthy controls volunteered (19 female and 6 Male) are included in the study matched with patients sex and age, all are without autoimmune disease or allergy, Four types of viral antibodies were screened HAV ,HBsAg ,HCV and HEV done according to rapid test CTK Biotech, The blood collected by EDTA tubes and run by RUBY for hematological investigation , ALT ,AST and Total Bilirubin was measured by Biolyzer Lichtenfels (Germany) , Enzyme-Linked Immunosorbant Assay (ELISA) was used for autoantibodies detection AMA, ANA,SLA and LKM-1 in both patients and healthy control, Indirect immunoflourescent technique used in SMA detection , Immunodiffusion Plate (RID) technique is used for measurement of IgG concentration ,finally simplified score system applied for cases diagnosis.

RESULTS

Viral screening test: Patients exhibited negative results for all 4 types of anti-viral antibodies, used that enhance the susptibility of patients to effect with autoimmune disease by excluding one of main cause of hepatitis.

Autoantibodies Detection: The level of AMA,ANA,SMA ,LKM-1 and SLA are measured for patients with chronic liver disease and healthy control , the results shown SLA was more frequent among the antibodies, it is detected in 20(50%) patients, SMA 17(42.5%) , ANA 12(30%) , LKM-1 detected in 3(7.5%) AMA presented 4(10%) were all negative of healthy control (figure 1)

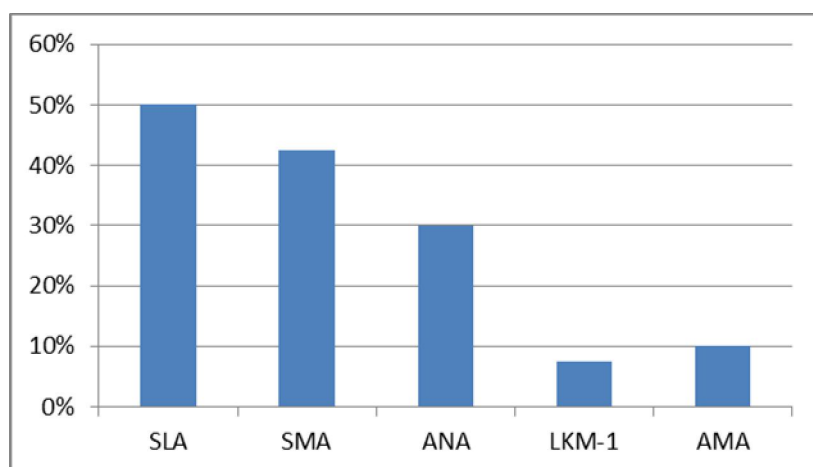
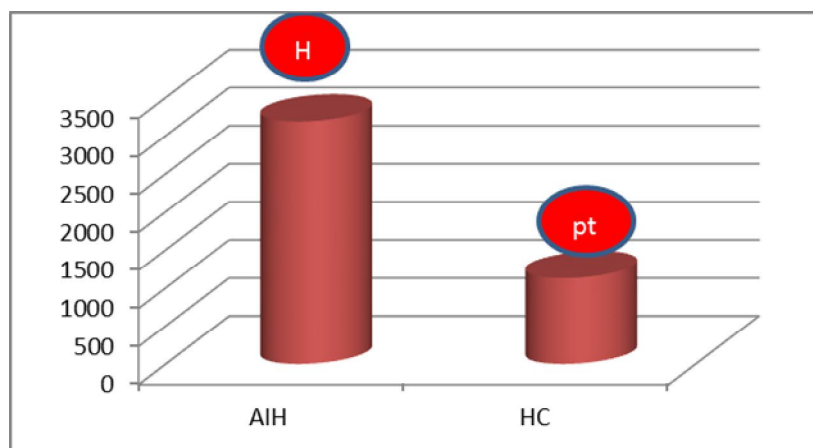


Figure (1): The Frequency of Autoantibodies among Patients with Chronic Liver Disease

Evaluation of Total Immunoglobulin G

Immunodiffusion Plate (RID) technique is used for measurement of IgG concentration in serum, for both patients and control, the study exhibited an increase of total IgG concentration among patients as compare with healthy control figure (2), patients serum reported mean of IgG (2619.34 ± 408.29) mg/l were the mean concentration among healthy control was (1127.6 ± 204.25) mg/l with significant difference $p \leq 0.05$



Pt.: patients, HC.: Healthy control, Normal value: WHO 870-1690 mg/dl

Figure (2): The Mean of Total IgG Concentration among Patients with Chronic Liver Disease and Healthy Control.

Autoimmune Hepatitis Diagnosed According To Score System

Simplified score system was applied for the diagnosis of patients, depending on the biomarker results the study exhibited 25(62.5%) of patients are collected ≥ 7 (defined AIH) were the others 14 (35%) collected < 6 and only one patient (3%) collected 6 score (properly AIH) figure (3), all patients collected below ideal score are excluded from the study

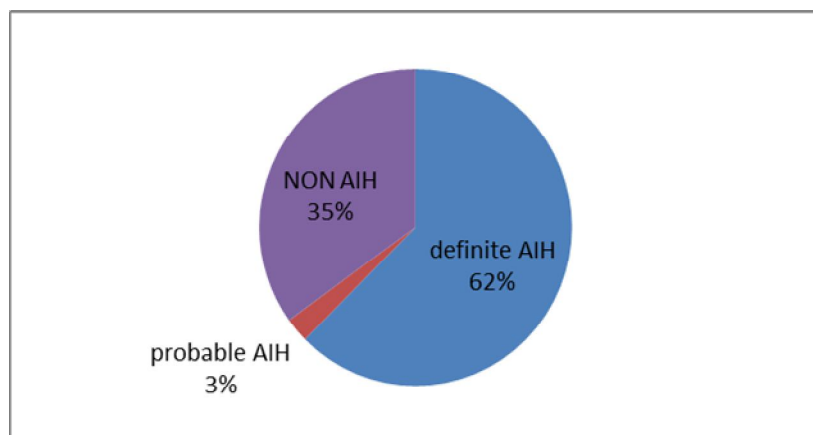


Figure (3): Distribution of Patients with Chronic Liver Disease According To Simplified Score System

Frequency of Autoantibodies among AIH Patients

Among 25 patients diagnosed with AIH according to simplified score system, SLA antibody presented in 20(80%) and 17(68%) 11(44%), 3(12%) for SMA, ANA and LKM-1 respectively figure (4).

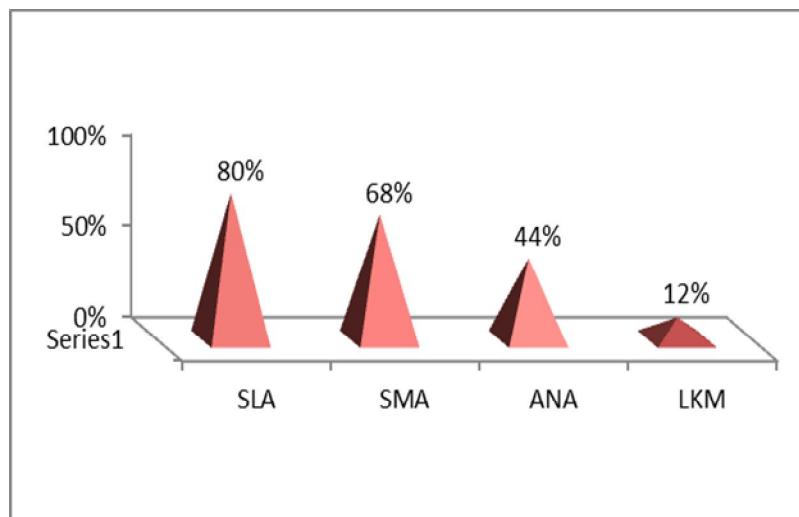


Figure (4): Frequency of Auto Antibodies among AIH Patients

Patients Distribution According of Sex

The study exhibit the frequency of disease in the female to male approximately 3:1 were female represented 76% Figure (5)

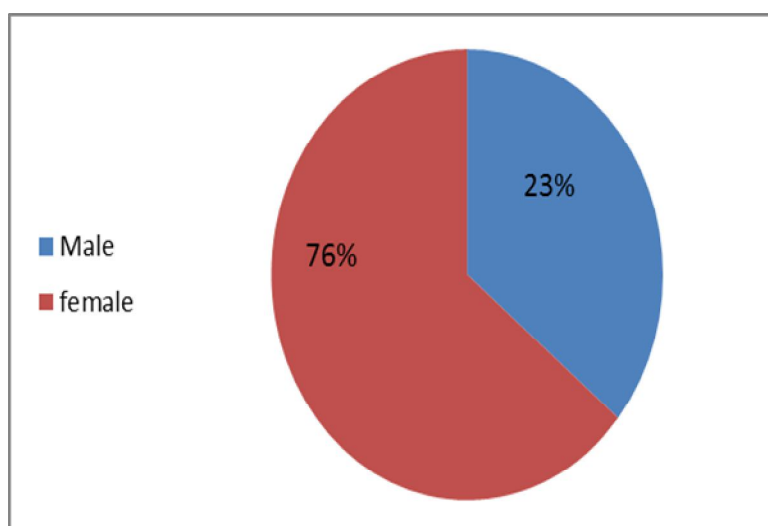


Figure (5): Frequency of Disease According Sex

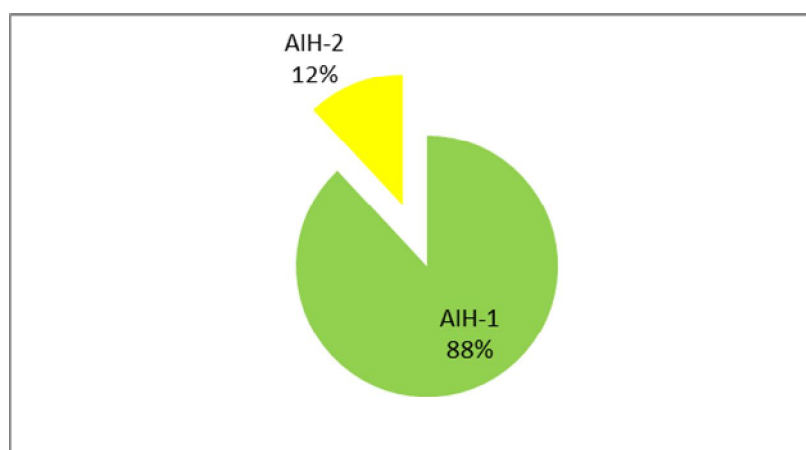
Distribution Aih Patients According To Age Groups

The disease was more frequent in the age group 20-29 (48%) with ratio female to male 5:1 while both age group 10-19 and 30-39 exhibit same frequency of disease (24%) with the same ratio female to male 2:1, the age group 40-49 only 1 female (4%) table (1)

Table(1) :Distribution of AIH And Healthy Control According To Age Group And Sex

Age	AIH (n=25)		Healthy control (n=20)	
	NO	%	NO	%
<20	6	24%	2	10%
20-29	12	48%	8	40%
30-39	6	24%	6	30%
40-49	1	4%	4	20%
MEANS ± SD	25.28 ± 8.35		29.71± 6.50	
RANGE	11- 48		10-44	
SEX	AIH (n =25)		Healthy control (n=20)	
	NO	%	NO	%
FEMALE	19	76%	13	65%
MALE	6	24%	7	35%
TOTAL	25	100%	20	100%

Classification of AIH Patients: According to the type of autoantibody, AIH patients are classified into two types, patients exhibit positivity to antismooth muscles antibody and /or antinuclear antibody classified as type -1 autoimmune hepatitis were positive for LKM- 1 classified as type -2 AIH, In the current study type -1 AIH presented 22(88%)of total autoimmune hepatitis while 3(12%) are type -2 figure(6).

**Figure 6 Classification of AIH According To Type of Disease**

Clinical Presentation in Two Type of Disease

The study exhibit variation of symptoms among patients in the two types of the disease (Type-1 and Type -2 AIH). patients with type-2 AIH exhibited more severe symptoms than type-1 AIH patients table (2). In both types jaundice reported more frequent than other

symptoms then fatigue and loss of appetite while asymptomatic was 18.36% in patients with type -1 AIH where no patient exhibited no symptoms among type-2 AIH

Table (2): Clinical Presentation among Patients with Two Type of AIH

Symptom	AIH-1 n(22) N %	AIH-2 n(3) N %
Asymptomatic	4 (18.63)	0
Jaundice	16 (72.72)	4(100)
Fatigue	10 (45.45)	2 (66.6)
Fever	5 (22.7)	1 (33.3)
Loss of appetite	7 (31.8)	2 (66.6)
Vomiting	2 (9)	1 (33.3)

Autoantibody Associated With Type -1autoimmune Hepatitis

The study of autoantibody pattern in the dominant type (Type-1) in the current study showed that SMA detected alone in 17(77.2%)while 10(45.4%) of patients was positive to ANA , both SMA and ANA are reported in 5(22.7%) of AIH patients . SLA detected in 19(86.3%) of type -1 AIH Figure (7)

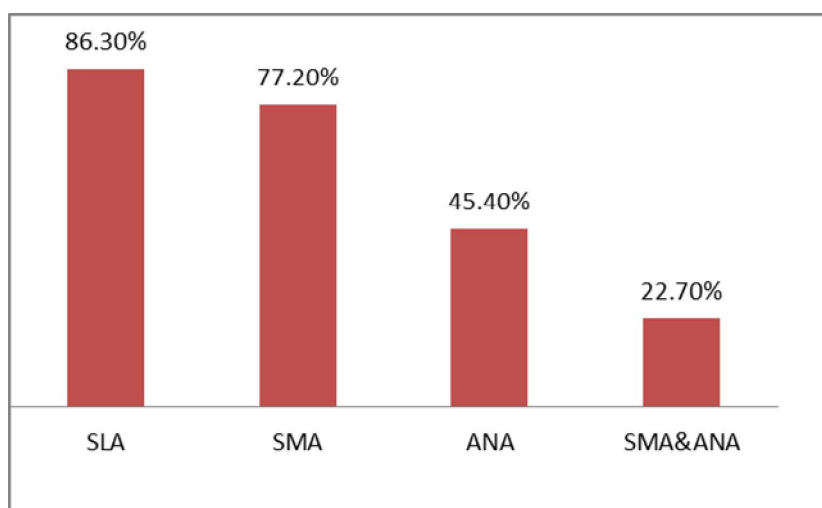


Figure (7): Percentage of Autoantibodies Among Type-1 AIH

Biochemical Analysis

Biolzyer Lichtenfels machine is used for measurement of liver enzyme ,the analysis exhibited that there is a significant elevation of both AST and ALT enzymes were (261.24±33.43)U/l, (249.68±152.6)U/l respectively Table(3) with elevation of alkaline phosphates (161.72 ±33.43)U/l and total bilirubin (3.228 ± 2.25)U/l as compared with control persons.

Table (3): Liver Enzymes Value among Patients and Healthy Control

Biochemical Parameter	AIH(n=25)	HC(n20)	P- Value
ALk	161.72 \pm 33.43	79.35 \pm 8.3	0.000
ALT	249.68 \pm 152.6	39.4 \pm 9.2	0.000
AST	261.24 \pm 147.5	29.45 \pm 5.2	0.000
TBL	3.228 \pm 2.25	0.557 \pm 0.31	0.002

P-value is significant at level 0.05 determined By T-test

Hematological Finding among Aih Patients

The hematological panel between patients and healthy control exhibit no more variations in some parameters and significant difference in others. AIH patients presented with high level of ESR (mean 57.32 \pm 19.49) ,Lymphocyte (mean 26.7432 \pm 13.01303) and low level of hemoglobin (10.04 \pm 0.89) and WBCs count (3.464 \pm 0.9) as compared with healthy control Table (4)

Table (4) Evaluation of Blood Count among Patients and Healthy Control

Parameter	AIH 25 \pm SD	Healthy cont. 20 \pm SD	P Value
HB	10.04 \pm 0.89	13.04 \pm 2.24	0.03
ESR	57.32 \pm	17 \pm 9.4	0.000
Platelet	255 \pm 30	254.4 \pm 1.9	2.34
WBCs	3.464 \pm 0.9	5.795 \pm 9.2	0.02
Lymphocyte	26.7 \pm 13	23.2 \pm 10.2	0.007
Monocyte	6.72 \pm 2.0	7.15 \pm 1.7	0.04
Neutrophile	64.17 \pm 1.7	67.03 \pm 2.8	0.23
Eosinophil	1.43 \pm 0.32	2.06 \pm 0.4	0.43
Basophil	0.92 \pm 0.5	0.51 \pm 0.7	2.29

P-value is significant at level (0.05) it determined by T –test

DISCUSSION

The study exhibited the percentage of AIH among chronic liver disease is higher than percentage that reported by Zyniya and Takahashi (2012) were reported the incidence of AIH is 20% of chronic liver disease and 23% of chronic liver disease was reported in North America (Czaja, 2006) the increase of ratio of AIH patients in the current study may be it caused by selection cases by include the patients with high suspicion of autoimmune hepatitis, or it is resulted from small population included in the current study .Patients whose exhibited LKM-1 positive are collected high score (10) as compared with the negative LKM-1 patients Four patients (10%) are positive for AMA excluded from the study because Antimitochondrial autoantibodies (AMA) are known to be a hallmark of primary biliary

cirrhosis (PBC), Serum AMA are detected in more than 90% of patients with PBC so it is very valuable in the diagnosis of PBC (Gershwin *et.al* , 2000), that plays role in reducing score of diagnosis. Although Antimitochondrial antibodies (AMA) a hallmark of PBC it may occasionally be present in patients with autoimmune hepatitis (Mieli - Vergani *et.al* ,2009) Other 11 seronegative patients are excluded from the study in spite of some studies mentioned that seronegative individual may express conventional antibodies later (Ashima *et.al* ,2011) or those patients may have a signature of auto antibodies that still undiscovered so the study forced to exclude those group of patients to prevent miss diagnosis, studies suggested that the detection of low level of antibodies enhance the diagnosis Of autoimmune hepatitis if other characteristic feature are also present (Gregorio *et.al* ,1997; Czaja 2005). The current study reported dominance of disease among female and this finding is it is consistent with all studies such as Fallatah & Akbar (2010) were female represented 75.7% among autoimmune hepatitis patient and another study demonstrate the dominance of female in autoimmune hepatitis patients in percentage 70-80% (Fairweather *et.al* ,2008) , and studies exhibited the female effected with disease 3.6 time more male (Manns & Vogel ,2006). the predominance of AIH among female may due to the effect of hormonal difference which activate Th2 and subsequently enhance autoantibodies production, The evidences for the influence of sex hormones on autoimmune diseases include the changes in disease severity during pregnancy and during specific periods of the menstrual cycle. It has been suggested that estrogens can enhance the immune response while androgens and progesterone suppress it (Fairweather *et.al* ,2008) female produce higher Th2 cytokine responses than male that lead to increase of antibodies titer in female (Giron, *et.al* ,2000) many reports indicate that sex hormones receptors are expressed by different population of innate an adaptive immunity cells ,B cells have been shown to express both estrogen (Benten, 2002) estrogen receptor found also in monocyte and macrophage (Bebo *et.al*.1999) that reflected the role of sex hormones in immune system function , castration of male mice make the onset of the disease similar to that in female mice (Gubbel,2008).

Obviously the peak of the disease in current study is presented in the young patients (20-29), this results is differ from study conduct in china where the peak of disease was 31.3% among elderly patients as compared with young patients were 9.6% Miline *et.al*(2014) in addition to the study done in Japan were the incidence of the disease among elderly patients was 25% (Czaja & Carpenter, 2006) , from other hand the result is consistent with study done in Saudia Arabia were AIH account 66.6% among the patients 20-29 year (Fallatah *et.al* ,2010) and it

is agreed with study conducted in Baghdad where the age 30-39 exhibit more frequency of AIH case (Alnaaiem,2009) obviously the age distribution simulate the distribution of the studies done in regional area at the same time it differ From age distribution of other area like china and Japan that may reflect the fact of role of race in the AIH , incidence of hepatitis viral infection in both different area and even the nature of different disease in both area and medication regime may cause difference in the presence of disease according to age. some of studies demonstrated that elderly patients presented with more asymptomatic clinical feature as compared with young patients (Miyake *et.al* ,2007) , so the result of current study suggested the elderly patients may be misdiagnosed that lead to reduce the number of AIH patients among elderly age group, in addition the study depend on diseased persons only, the screening of community for liver disease may ply role in the increase of cases d The presence and pattern of autoantibodies are important elements in establishing the diagnosis ,by making the differential diagnosis with other immune mediated hepatobiliary diseases, and in identifying the Type-1 or 2 variant (Manns & Vergani, 2009) .The result in current study exhibited variation of auto antibodies and reflex the dominance of Type-1 of disease among patients that cosistant with Gupta *et.al*, (2001) were type -1AIH was reported 88,9% and another study Type-1 reported 92.2% from total AIH cases (Choudhuri *et al*, 2005) and 80% (Yeoman *et.al* ,2010) , from other hand the results are slightly different from study conduct in Bagdad were type -2 AIH was 22%(ALObeydi,2007) , the small population sample in current study may deviate the result from the exact ratio but in general this result is in line with all studies which provide the dominance of type-1 AIH such as Czaja (2008) , type-2 AIH exhibited more severe symptoms than type-1 AIH patients both types jaundice reported more frequent than other symptoms then fatigue and loss of appetite while asymptomatic was 18.36% in patients with type -1 AIH where no patient exhibited no symptoms among type-2 AIH , in general type -2 AIH is more acute than type-1 AIH (Krawitt , 2006), the study represented asymptomatic patients in ratio lower than Manns *et.al* (2010) study where reported asymptomatic 25-34% , the percentage of jaundice and fatigue it is consistent with study done by Milin *et.al*,(2014) who reported that jaundice is more than 70% of patients with AIH , fever is detected in type-1 and type-2 AIH 22.7% and 33.3% respectively it is higher than recent registration of fever among AIH patients were reported 10%(Milin *et.al*,(2014) , this finding in the current study may result from two reasons either the small population sample studied or patient suffering in addition to AIH disease Infection to other disease due to the continuous use of immunosuppressive drugs , studies exhibited that clinical presentation of AIH can be in the form of asymptomatic liver disease with

abnormal liver test results (Fallatah *et.al* ,2010; Makol *et.al* ,2011),from other hand a total of 26–49% of all AIH cases present as acute hepatitis (Strassburg & Manns ,2009) it is characterized by right upper-quadrant abdominal pain, fatigue and jaundice (Floreani *et.al* ,2006) .The current study found that AIH Type-2 patients have a higher rate of acute hepatitis symptomatically as compared to Type-1 patients that is consistent with Zachou *et.al*, (2004) who reported more than 45% of Type-2 AIH develop severe type of disease.

Study pattern of autoantibodies in type -1 AIH exhibited SLA presented in high ratio (86.3%) among AIH patients in current study it is detected in both Type-1- and type-2 AIH, this antibody is considered as a highly specific marker for AIH -1 undepend on the antigen used for their detection such as native cystolic antigen (Wies *et.al* ,2000) or recombinant 50k Da protein identified as tRNP (ser) sec antigen (Araiso *et.al* ,2008) .This antibody is strongly associated with diagnosis of AIH and is rarely present in other liver disease such as drug induced hepatitis (Volaine *et.al* ,2009),. SLAs are mainly identified in patients who are also positive for ANAs and either SMAs or LKM-1. However, SLAs are also detected in isolation in up to 20% of AIH cases (Invernizzi *et.al* ,2007) ,therefore SLA detection is an important component of the AIH diagnosis . ANAs represent in AIH disease in 13–15% of the patients (Mehendiratta *et. al*, 2009). The mechanism leading to the production of ANA in autoimmune hepatitis is not clear, though it has been related to the release of nuclear components following hepatocyte injury and/or to a loss of B cell tolerance to several nuclear components (Manns *et al*, 1989). SMA represented 77.2% in current study that slightly consistent with Muratori *et.al*,(2002) were reported SMA detected in 80% of AIH –type1, were it is represented 67% in AIH type -1 in Badghdad (Alnaaiem 2009) ,the detection of this antibodies is an evidence of AIH therefore when compared with autoantibody profile of healthy control the current study exhibit detection of SMA,ANA and SLA in the patients serum while control persons exhibit no auto antibodies . were serum of autoimmune hepatitis patients exhibited increase of liver enzyme the increase of those enzyme in the blood of patients is more specific indicator of liver damage because the enzyme are found in liver cells (hepatocyt) which is contact with blood supply, AST and ALT can leak to blood if hepatocyte damaged , ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere (Paul & Giboney, 2005) from other hand SLA positivity can effect ALT increasing. Increase level of ALT indicate Inflammation or damage to cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver ALT into the

bloodstream(Sleisenger & Fordtran's, 2010) , anti-SLA appears to be highly specific for the diagnosis of AIH, and its detection at the time of diagnosis identifies patients with a more severe disease and a worse outcome ,The antibody is reactive against a transfer ribonucleo protein (tRNP^{(ser)sec}) involved in the transport of selenocysteine, (Volkman *et.al* ,2010). Hematological study of patients exhibited increase of blood parameters as compared with healthy control such as Erythrocyte Sedimentation Rate (ESR) although ESR is a nonspecific marker of Inflammation and is affected by other factors, the results must be used with other clinical findings in the diagnosis (Kushner *et.al* ,2009), obviously the increase of ESR level among AIH patients reflex the presence of disease and persistence of Inflammation (Vajpayee *et.al* ,2011). Although both patients and health control reported lymphocyte within normal value ,AIH represented significant increase as compared with healthy control that may reflex the role of lymphocytes in AIH which are mainly involved, The lymphocytes attack the liver cells (hepatocytes) which causes Inflammation and damage , in current study most AIH patients are anemic as compared with healthy control , that it is cosistant with studies done on chronic disease were anemia is a sign of such disease (Zarychanski & Houston,2008). Inflammation promotes the production of white blood cells. Where Bone marrow produces both whites blood cells and red blood cells from the same precursor stem cells. Therefore, the up regulation of white blood cells causes fewer stem cells to differentiate into red blood cells(Nemeth & Ganz ,2006).. This effect may be an important additional cause for the decreased erythropoiesis and red blood cells. Eosinophi and Basophil exhibit no significant difference between healthy and patients that indicated there is no role for those cells in the disease so a usual role remains limited to allergies and the immune response to parasites.

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