

## Research Article

# Serological Auto Antibodies in Health and Liver Disease in a Nigerian Population- A Preliminary Study

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University of Ibadan, Nigeria. Email:otes123@gmail.com**Received:** July 03, 2014; **Accepted:** July 29, 2014;**Published:** July 30, 2014**Abstract**

**Introduction:** Some autoantibodies are useful in the diagnosis of autoimmune liver diseases. There is dearth of information on the prevalence, pattern of autoantibodies in black population of Africans with liver diseases. To the knowledge of the authors, there is no such information among Nigerians. This study determined the prevalence, pattern and significance of serological autoantibodies among patients with liver diseases and apparently healthy individuals in Nigeria.

**Materials and Methods:** The seroprevalence of antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), anti-liver kidney microsomal antibodies (Anti-LKM-1), anti-soluble liver antigen/liver pancreas (Anti-SLA-LP), perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), were determined in age and sex-matched patients with liver diseases and apparently healthy controls using ELISA method over a two-year period. There were one hundred and twenty six patients with liver diseases consisting of 91 (72.2%) males and 35 (27.8%) females) and 82 apparently normal control subjects, made up of 59 (72%) males and 23 (28%) females. Appropriate statistical methods were used to determine Odds ratio, Pearson Chi square and Students't-test. Significant statistical difference was specified at  $p < 0.05$ .

**Results:** The patients consisted of hepatocellular carcinoma (HCC) 77 (61.1%), liver cirrhosis 32 (25.4%), chronic hepatitis 10 (7.9%), acute viral hepatitis 4 (3.2%), alcoholic cirrhosis 1 (0.8%) and primary biliary cirrhosis 2 (1.6%).

Of all the autoantibodies analysed (126 cases and 82 controls), only AMA was significantly higher among cases compared with controls. Antimitochondrial antibodies were present in 76 (60.3%) of the cases compared with 36 (43.9%) controls ( $p < 0.05$ ), while ANA were present in 42 (39.3%) of cases compared with 27 (39.7%) controls ( $p = 0.68$ ). Anti-soluble liver antigen (anti-SLA/LP) and pANCA were absent among cases and controls.

Though few in number, chronic hepatitis had the highest frequency of AMA, being positive in 9 (90%) of the 10 cases, compared to HCC, in which AMA was present in 48 (62.3%).

**Conclusion:** The prevalence of serological autoantibodies was similarly high in both liver diseases and in health, except for AMA. Serum autoantibodies, therefore, appear to be insignificant and insufficient for the diagnosis of autoimmune liver disease in Nigerians. Other parameters should be considered whenever there is a clinical suspicion of autoimmune liver disease among Nigerians.

**Keywords:** Human stem cells; Progenitor;  $\beta$ -cells; Insulin; Differentiation; De-differentiation; Culture; Re-differentiation

## Introduction

Autoantibodies are immunoglobulins that react with normal host proteins and may be physiologic or pathologic [1]. The physiologic autoantibodies, also known as polyreactive antibodies, do not fix complements and are produced by normal humans and animals. They are found in low concentrations in the serum of normal humans of all ages, though commoner in women than men [2]. It is suggested that, physiologic or natural autoantibodies may constitute the antibodies secreted by B cells prior to encountering foreign

antigens [3]. They are usually low affinity IgM isotype, though IgA and IgG isotypes are also found. CD5+ B cells, which represent 10-25% of circulating B lymphocytes, have been found to produce natural autoantibodies [4]. Pathologic autoantibodies on the other hand are produced by CD5- B cells which are usually monoreactive, have high affinity and are typically detectable only in autoimmune individuals [5,6]. The use of ELISA technique to measure the liver-related autoantibodies is unique in that hitherto, the cumbersome indirect immunofluorescence technique with use of rat kidneys was

employed in most studies [7,8]. The use of ELISA, which has been found to be sensitive, specific, objective and rapid would facilitate standardized approach to measurement of autoantibodies and afford comparability of studies globally.

There are several studies on pathological autoantibodies in the Caucasian populations but there is dearth of scientific literature on the prevalence and pattern of autoantibodies in black populations of Africa with liver diseases, and none from Nigeria. This study determined the prevalence, pattern and significance of autoantibodies among patients with liver diseases in Nigeria.

## Materials and Methods

A cross sectional study to determine the seroprevalence of liver-related autoantibodies among age and sex-matched consenting adults with liver diseases who were admitted or seen at the clinic of the hospital and compared to apparently normal controls was carried out at the University College Hospital, Ibadan, Nigeria over a two-year period. The control group was selected randomly and they consisted of volunteering relations of patients, medical, nursing and support staff of the hospital. Ethical approval was sought and obtained from the University of Ibadan/University College Hospital, Institutional Review Board (IRC protocol number: UI/IRC/07/0027).

The patients consisted of hepatocellular carcinoma (HCC) 77 (61.1%), liver cirrhosis 32 (25.4%), chronic hepatitis 10 (7.9%), acute viral hepatitis 4 (3.2%), alcoholic cirrhosis 1 (0.8%) and primary biliary cirrhosis 2 (1.6%), while the mean ages of the cases and the controls were  $47.5 \pm 14.4$  yrs and  $39.6 \pm 16.5$  yrs respectively.

Sera of the consecutive 126 patients consisting of 91 (72.2%) males and 35 (27.8%) females with liver diseases and 82 apparently normal controls consisting of 59 (72%) males and 23 (28%) females, ( $p > 0.05$ ), were analysed for antimitochondrial antibodies (AMA), anti-liver kidney microsomal antibodies (Anti-LKM-1), anti-soluble liver antigen/liver pancreas (Anti-SLA-LP), perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and antinuclear antibodies (ANA were analysed in only 107 liver cases and 67 controls), using qualitative Enzyme-linked Immunosorbent Assay (ELISA) method with kits from AESKU Diagnostics, GmbH, Germany. The AESKU Diagnostics kit consisted of an ELISA plate with 96 microwells, Negative control, Positive control, Cut-off control, Test and Control sera, and Conjugate among others.

Briefly, after incubation of diluted sera (1:101) in microplates coated with specific antigen, patient's antibodies, if present in the specimen, bind to the antigen. Unbound fraction was washed off. Incubated anti-human immunoglobulins conjugated to Horseradish peroxidase (conjugate) reacted with the antigen-antibody complex of the samples in the microplates. Unbound conjugate was washed off. Addition of TMB-substrate generated an enzymatic colorimetric (blue) reaction, which was stopped by diluted acid (colour changes to yellow). The rate of colour formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibody in the patient's sample. The positive samples were those with reading values greater than the value of the Cut-off control, while the negative samples were samples with reading values lower than the Cut-off control.

Other laboratory tests such as liver function tests, prothrombin time, and alphafetoprotein among others were carried out using standard laboratory methods. Specifically, liver function tests were determined with the clinical chemistry autoanalyser (Hitachi 912), using enzymatic method, while alphafetoprotein was carried out by using radioimmunoassay technique. Prothrombin time (INR) was done manually using commercially prepared thromboplastin.

Diagnosis of liver diseases was made by relevant clinical features and laboratory tests for liver function, prothrombin time, alphafetoprotein, ultrasonography and liver biopsy.

Statistical analysis was carried out with SPSS statistical software version 11.0 for windows. Prevalence rates of autoantibodies and other analytes were calculated to reflect the relative frequency of each liver disease. Odds ratio (OR) and ninety five percent confidence interval (95% CI) were calculated. Pearson Chi square test was used to compare proportions while Students't-test was used to compare means. Where numerical values were low, Fischer's exact test and medians were used. Significant statistical difference was specified at  $p \leq 0.05$ .

## Results

The mean levels of bilirubin, gamma-glutamyltransferase, alkaline phosphatase, globulin, albumin, PTR and alpha-fetoprotein were high among some of the liver cases. Higher ALT 123 iu/l vs. 12.2 and AST 196.6 iu/l vs. 24.3 were recorded among cases compared with controls ( $p < 0.01$ ) as shown in Table 1. Among the test subjects, hepatomegaly occurred in 99 (78.6%), ascites in 72 (57.1%) and jaundice in 62 (49.2%).

Only AMA showed significantly higher prevalence among cases compared with controls, as shown in Table 2. Antimitochondrial antibodies were present in 76 (60.3%) of the cases compared to 36 (43.9%) controls ( $p < 0.05$ ), while ANA were present in 42 (39.3%) of cases compared with 27 (39.7%) controls ( $p = 0.68$ ). Anti-soluble liver antigen (anti-SLA/LP) and pANCA were completely absent among cases and controls.

Chronic hepatitis had the highest frequency of AMA, being positive in 9 (90%) of the 10 cases; this was followed by HCC, 48 (62.3%). The only patient with alcoholic cirrhosis was also positive for AMA. Anti-LKM-1 was positive in only one case and control,

**Table 1:** Biochemical and Clinical parameters among subjects with liver disease:

Biochemical parameter	n	Range	Mean (SD)	Median
Total Bilirubin (mg/dl)	126	0-40	9.5 ± 10.2	5.3
ALT (iu/l)	126	0.6- 963	123.4 ± 154	87.5
AST (iu/l)	126	2.5-882	196.6 ± 176.1	139.0
γ-GT (iu/l)	126	27-1009	341.9 ± 300.9	236
Alk Phosphatase (iu/l)	126	26-1226	338.9 ± 267.8	249
Total Protein (g/dl)	126	4.4-10.7	7.9 ± 1.3	6.8
Albumin (g/dl)	126	1.3-4.6	2.8 ± 0.4	3.2
Globulin (g/dl)	126	2.1-7.2	4.9 ± 0.96	4.9
Prothrombin time ratio	126	0.65-3.26	1.51 ± 0.4	1.26
Alphafetoprotein (uk/L)	38	4.5-711.5	125.2 ± 249.2	8.7

NB: Among controls ALT was  $0.32 \pm 3.6$ ; AST was  $0.37 \pm 2.2$ .

**Table 2:** Prevalence of auto antibodies among cases and controls.

	Cases N= 126	Controls N= 82	p-value
ANA	42(39.3)	27(39.7)	0.68
AMA	76(60.3)	36(43.9)	0.02
LKM-1	1(0.8)	1(1.2)	Fischer's exact 1.000
pANCA	0	0	
Anti-SLA/LP	0	0	

as shown in Table 2. The highest positivity for AMA among cases was recorded in the age group 30-39 years in contrast to the controls which was in age group less than 30 years, as shown in Table 3. It also showed that the least occurrence of AMA was found in the age group 70 years or more, in both cases 5(6.6%) and controls 2(5.6%). Anti-LKM was recorded positive in one sample each among cases and controls, and both were below 30 years of age, as shown in Table 3.

The positive ANA and AMA levels were not significantly different among cases compared with the control group, as shown in Table 4, but the positive ANA and AMA among cases were significantly higher than that in the controls, as shown in Table 4. However, comparison of negative pANCA, anti-LKM-1 and anti-SLA/LP levels among cases and controls showed significant difference, with higher values among cases for anti-LKM-1 and anti-SLA/LP, and higher values among controls for AMA and pANCA.

## Discussion

Autoimmune liver diseases are relatively rare in Nigeria, though there is anecdotal evidence that autoimmune disorders do exist [9,10]. Also, there is evidence for existence of autoimmune diseases and serological autoantibodies in Nigeria and indeed in Africa [11,12]. In the sixties, Greenwood [13] postulated that the rarity of autoimmune disorders among Nigerians may be due to the presence of several environmental parasitic antigens stimulating the immune system. His study at the University College Hospital, Ibadan, showed that diseases in which autoimmune processes were thought to be involved were uncommon in Western Nigeria, and suggested that the infrequent occurrence of autoimmune diseases in parts of tropical Africa was related to the immunological disturbance produced by multiple parasitic infections [13].

The spectrum of liver diseases found during the study period suggested that, in the hospital setting, most of the liver diseases seen were HCC (61.1%) and liver cirrhosis (25.4%), with autoimmune liver diseases being uncommon constituting only about 1.6%. This confirms the age long suspicion that autoimmune liver diseases are rare among Nigerians, compared with Caucasian populations. There were no readily available data among other African countries to compare our findings on autoimmune liver diseases with, because there were no published data on autoimmune liver diseases. This study which was carried out over a twenty-four month period showed that males were more afflicted significantly with liver diseases than females in keeping with findings in previous studies [13,14]. This gender difference in prevalence of liver diseases is thought to be multifactorial.

Our study has shown a relatively high prevalence of liver-related autoantibodies among Nigerians but without a correlation with autoimmune liver diseases, as the levels were similar in the various

**Table 3:** Age group distribution of subjects positive for autoimmune markers.

Age grp (yrs)	AMA		Anti-LKM-1		ANA	
	Cases	Control	Cases	Control	Cases	Control
<30	10 (13.2)	15 (41.7)	1 (100)	1 (100)	6(14.3)	8(29.6)
30-39	19 (25)	7 (19.4)	0	0	8(19.0)	5(18.5)
40-49	14 (18.4)	3 (8.3)	0	0	12(28.3)	2(7.4)
50-59	15 (19.7)	6 (16.7)	0	0	9(21.4)	4(14.8)
60-69	13 (7.1)	3 (8.3)	0	0	4(9.5)	2(7.4)
≥70	5 (6.6)	2 (5.6)	0	0	3(7.1)	6(22.2)

NB: Values with percentages in parenthesis.

**Table 4:** Relative strength of positive and negative autoimmune markers among liver cases compared with controls.

	N	Mean±SD	t	P
ANA Positive				
Cases	43	880.3±288.2	-1.6	0.11
Controls	28	1021.1±435.1		
ANA Negative				
Cases	42	305.4±95.8	2.48	0.16
Controls	30	250.3±89.1		
AMA Positive				
Cases	76	1434.7±358.0	0.9	0.35
Controls	36	1369.4±307.9		
AMA Negative				
Cases	50	534.5±220.1	-2.61	0.01
Controls	46	637.3±158.4		
pANCA Negative				
Cases	126	191.69±77.17	-3.10	0.00
Controls	82	226.93±84.49		
LKM-1 Negative				
Cases	125	212.12±88.56	2.65	0.01
Controls	81	178.64±88.23		
SLA Negative				
Cases	126	255.81±93.44	7.16	0.00
Controls	82	170.74±65.89		

liver diseases. Similarly, the frequency of the autoantibodies was high in apparently normal individuals with no history or clinical evidence of liver diseases. It is of note that there was no significant difference in the frequencies of autoantibodies in patients with liver diseases compared to the normal controls. These findings would suggest that autoimmune liver diseases are rare in Nigerians and that autoantibodies are unlikely to have any significant aetiopathogenic role in liver diseases in Nigeria.

In this study, antimitochondrial antibodies (AMA) were the only autoantibodies found to be significantly higher among the patients with liver disease (60.3%) compared with the apparently normal control group (43.9%)  $p < 0.05$ . The enigma of this finding is in the fact that even those who were apparently healthy also had a relatively high percentage of AMA though its presence is supposed to be diagnostic

of primary biliary cirrhosis [15]. Similarly, the lack of significant difference in the levels of ANA and LKM-1 in liver cases compared to controls would suggest that there is a mechanism responsible for erratic production of liver-related autoantibodies in the studied population, regardless of health status of the liver. This is contrary to what obtains among the Caucasians in whom the presence of ANA correlates well with systemic or organ specific autoimmune disease, being the most common autoantibodies in autoimmune hepatitis [16]. In the same population, ANA has been found to be predictive of autoimmune diseases [17,18], while LKM-1 is a useful laboratory tool in the diagnosis of type-2 autoimmune hepatitis, which is commoner among children and young adults [19]. These findings need to be further substantiated by similar studies among blacks.

Some earlier studies in Nigeria, Uganda and other African black populations have noted the ubiquitous presence of serum autoantibodies, and actually postulated a significant relationship between the occurrence of autoantibodies in the sera of Nigerians, and the presence of high levels of malaria antibody together with high levels of IgM. Based on aforementioned findings, Greenwood and his colleagues then concluded that the findings suggested that the speckled antinuclear factor found in African sera may be a cross-reacting antibody to a nuclear component of malaria parasites [13,20,21]. In 1995, Skalskyet al [22], in a study of chronic liver diseases in rural south-west Cameroun found that serum autoantibodies were frequently found and were not correlated with the presence of autoimmune liver disease. The complete absence of anti-SLA/LP in both the test and the control subjects further validates the rarity of type-1 autoimmune hepatitis among our patients with liver diseases, as these autoantibodies had been found to be 100% specific for AIH<sup>23</sup>. Similarly, pANCA were also completely negative in both cases and controls in this study, further substantiating the rarity of autoimmune liver diseases in our cohort of patients with liver diseases. These findings are in contradiction to studies in Caucasian populations [23-26] and in India, a developing country, where they have been found to be useful in the diagnosis of autoimmune liver disease [27] and sometimes used for prognostication [28]. If the postulation of Greenwood<sup>13</sup> about four decades ago, that malaria was responsible for the erratic production of autoantibodies among our population, it would suggest that the battle against malaria is far from over in spite of the huge investment being made into research and pharmacotherapeutics. It is however tempting to postulate that autoimmune diseases will emerge in our population, if and when the scourge of malaria is stemmed.

As a result of our findings, serological autoantibodies may not be sufficient for the diagnosis of autoimmune liver disease in Africans and other parameters may have to be considered whenever there is a clinical suspicion of autoimmune liver disease. In contrast to non-pathologic autoantibodies, which are usually found in the older age groups, AMA in our study was found more in the younger age groups. The significance of this is not yet clear. However, anti-nuclear antibodies on the other hand did not demonstrate any particular pattern of age difference.

The positive ANA and AMA levels were not significantly different among cases compared with the control group, as shown in Table 4, but the positive ANA and AMA among cases were significantly higher than that in the controls, as shown in Table 4. However, comparison

of negative pANCA, anti-LKM-1 and anti-SLA/LP among cases and controls showed significant difference, with higher values among cases for anti-LKM-1 and anti-SLA/LP, and higher values among controls for AMA and pANCA (Table 4). The significance of these findings are however, not immediately clear.

In conclusion, there was no published study on autoimmune liver disease to compare our study with in Nigeria, making this study a pioneering effort in Nigeria and in most countries of Africa. Autoimmune liver diseases appear to be uncommon in Ibadan, Nigeria and, the prevalence of autoantibodies to liver antigens is equally high in individuals with or without liver disease. Antimitochondrial antibodies were significantly higher among cases with liver disease compared to controls. Since most of the patients in this study had hepatocellular carcinoma and liver cirrhosis which are also associated with fibrosis, there may be a link between serum AMA and fibrosis in the liver. It could be reasonably concluded that autoantibodies to liver antigens might be unreliable in predicting autoimmune liver disease in the studied population.

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