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RESEARCH ARTICLE

STUDY ON CRYPTOGENIC CHRONIC LIVER DISEASE HAVING ELEVATED ALKALINE PHOSPHATASE LEVELS WITH SPECIAL REFERENCE TO PRIMARY BILIARY CIRRHOSIS AND ANTI MITOCHONDRIAL ANTIBODY (AMA) IN INDIAN POPULATION -- A CASE –CONTROL STUDY

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ABSTRACT

Background: The anti-mitochondrial antibody (AMA) is frequently tested in cryptogenic cirrhosis (CC) with elevated alkaline phophatase (ALP) levels. Like other group of chronic liver disease of known etiology may , on the course of time, lead to decompantion , liver failure and Hepatocellular carcinoma, Cryptogenic liver disease has similar course which need to be evaluated early.
Aims: To determine how frequently AMA test is positive in the above mentioned clinical situation and to identify the discriminating value of clinical features in patient who tested positive in both study groups.
Setting and design: Tertiary care hospital based retro-spective case control study.
Materials and Methods: This was a retrospective study where 120 patients with chronic liver disease for whom the anti mitochondrial test (AMA) was done because of a raised alkaline phosphatase (ALP) formed the population from which cases and controls were drawn. 10 patients who tested AMA positive served as cases and 20 subjects that were randomly chosen from the AMA negative group served as controls.
Results: AMA was rarely positive (about 8.33%) in this group of cryptogenic chronic liver disease with high ALP values in Indian. The only discriminating investigations are a high Total Bilirubin, and activated Partial Thromboplastin Time in cases which are AMA positive. The liver biopsy of one patient having AMA negative found to have features of Primary biliary Cirrhosis in our study.
Conclusion: We need to do liver biopsy in the early part of the disease to ascertain the pathogenetic mechanism of liver injury in the crypto group, because in the advanced stage we hardly find the accurate sample for histopathologic evaluation. This is not an argument for discarding the AMA test in Indians but it may throw light on the evolution of this disease.

INTRODUCTION

Cryptogenic chronic liver disease is defined as chronic liver disease the aetiology of which is unknown after exhaustive investigations. The etiology in 5-10 % of cases with cirrhosis, despite reasonably extensive investigations, remains unresolved. The AMA is reported to be more than 90-95% sensitive and more than 90-95 % specific in patients with Primary biliary Cirrhosis (PBC). There are about 5% of patients who are negative for AMA but have the other features of PBC. The first question we asked is how frequently in this study population of chronic liver disease with elevated alkaline phosphatase was this test positive. If the test was positive in the majority it would mean that testing need not be done as the pre-test possibility would be high.

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If on the other hand the test was rarely positive biopsy would need to be done more often. The second query was to find out if the population that tested positive for AMA was in any way different from those who tested negative. If similar then the implications are that the test is falsely negative in the majority of cases. If different then the pristine disease is probably very rare in this country. The former conclusion can be arrived at only after more intense investigation. These issues may appear to be unrelated it is in the realm of chronic liver disease of unknown aetiology.

Aim

- To determine how frequently the Anti mitochondrial antibody test is positive in patients with chronic liver disease with high serum alkaline phosphatase
- To identify the discriminatory value of clinical features in patients who tested positive for AMA from those with a negative test.

MATERIALS AND METHODS

Study design: Retrospective case-control study.

Study population and sample size

This was a retrospective study where 120 patients with chronic liver disease for whom the anti mitochondrial test (AMA) was done because of a raised alkaline phosphatase (ALP) either at presentation or on initial follow up formed the population from which cases and controls were drawn. 10 patients who tested AMA positive (and this was the entire AMA positive group of the 120 cases) served as cases and 20 subjects that were randomly chosen from the AMA negative group served as controls. In order to do this we used the following method of identifying the total AMA positive population. The Study was conducted in Christiana Medical College and Hospital.

The records of the serological laboratory between January 2010 and October 2014 were scrutinized and all those for whom an AMA test was requested were chosen for inclusion. All of these had chronic liver disease with an elevated serum alkaline phosphates level. All subjects who tested positive for the AMA were included as cases. Of those in this population who had elevated alkaline phosphatase but tested negative for AMA a sample of subjects was drawn to act as controls. This was random but was intentionally skewed towards the female gender to match the gender ratio of the cases.

A random selection of 20 test negative controls was heavily skewed towards the male sex. Therefore a selection of 2 males and 18 females were made randomly from gender separate groups. The next stage was examining the records and investigations of both these groups at admission. The following steps were undertaken.

1) The clinical findings and investigational reports of the cases were compared with those in the controls to look for any significant differences.

2) The clinical findings on presentation that were recorded for the study were the presence of jaundice, ascites and pedal oedema. Other clinical findings were not included in the comparative analysis. Encephalopathy and spontaneous bacterial peritonitis was recorded if it was the reason for admission. A past history of either was disregarded.

Among the investigations, the indices of liver function, haematological parameters such as haemoglobin and platelets, ESR and Anti nuclear antibody (if available) were compared in the two groups. Further the ratio such as SGOT/SGPT was also compared. Other investigations such as imaging and endoscopy were not included in the study

MATERIALS AND METHODS

Descriptive statistics like mean and SD were presented for normally distributed continuous variables and median with interquartile range for non-normally distributed continuous variables. The results between the two groups were compared for statistically significant difference. For the categorical variables chi-square test was used. For continuous variable with normal distribution, t- test was used. For continuous variable with non-normal distribution, Mann-Whitney's U test was used. P value of <0.05 was considered significant. The statistical analysis was done using SPSS software for windows version 16.

RESULTS

AMA was requested for 120 patients during the period between January 2010 to October 2014. Out of total 120 patients 95 were females and 25 were males. The age range was between 18-70Years (Mean ± SD=43.63 ± 13.11) Only 10 patients were tested positive for AMA and rest were negative. In the Positive group were nine Females and one Male; age ranges from 18 – 70 years (Mean ± SD=43.8 ± 13.63).

Alkaline Phosphatase levels

The range of ALP levels for all 120 cases was 56 to 1266 IU/L with mean ± SD=303.83 ±267.34IU/L; ALP value for AMA positive cases (n=10) range 132 -1266 IU/L with a median value =267.50 IU/L and a mean ± SD=368.40 ± 340.08 IU/L. ALP for Controls group (n=20) range from 133 to 853IU/L with a median value =204.50 IU/L and mean ± SD = 294.06 ± 189.78).

Characteristics of patients

There were no significant differences in age, sex and MELD score between AMA positive and AMA negative groups.

Table 19

Characteristics of patients.

	Study group AMA(+)	Control group AMA (-)	*P value*
No of patients.	N=10	N=20	-
Age in years.	18-70 (Mean=38)	18-62 (Mean=42.7)	ns
Sex	M=1/F=9	M=2/F=18	ns
MELD	8-10	7-11	ns

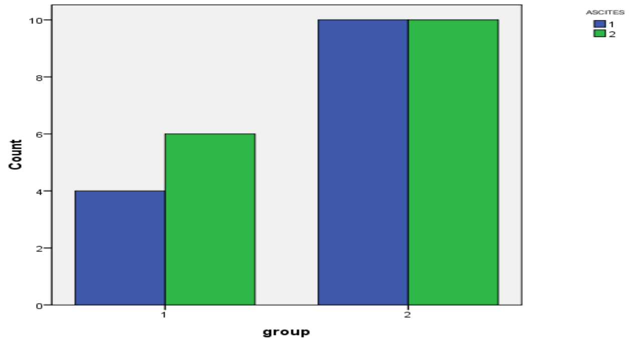
Clinical Features

Ascites: 4(40%) patients in group -1 and 10(50%) patients in group -11 were having ascites, rest were negative both clinically and radiologically.

Table 20

ASCITES	Yes	No	Total
Group -1(AMA+)	4(40%)	6(60%)	10(100%)
Group-2(AMA-)	10(50%)	10(50%)	20(100%)

As cites

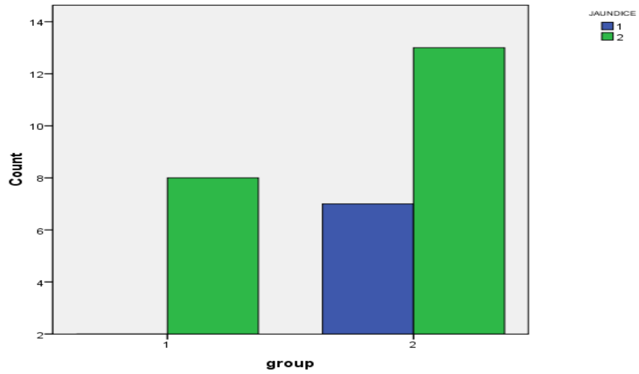


Jaundice: 2(20%) patients from gr-1 and 7(35%) from gr-11 were having Jaundice, this was not statistically significant.

Table 21

Jaundice	Yes	No	Total
Group-1(AMA+)	2(20%)	9(90%)	10(100%)
Group-2(AMA-)	7(35%)	13(65%)	20(100%)

Jaundice

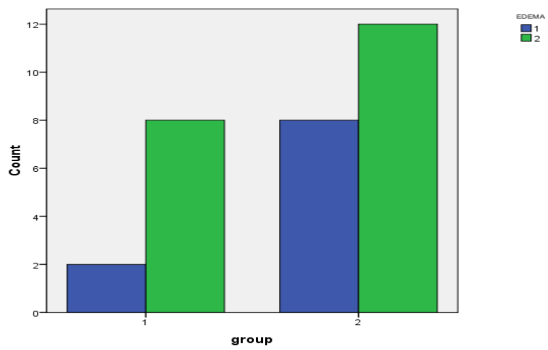


Edema: Pedal edema was seen in 2(20%) patients in gr-1 and 8(40%) patients in gr-2, this was not statistically significant.

Table 22

Edema	Yes	No	Total
Group-1(AMA+)	2(20%)	08(80%)	10(100%)
Group-2(AMA-)	8(40%)	12(60%)	20(100%)

Edema



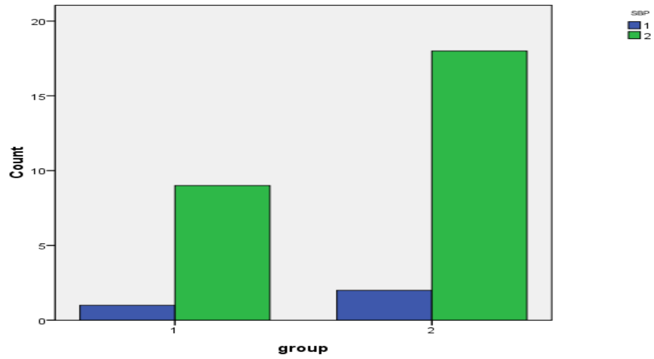
Complications

Spontaneous bacterial peritonitis: There were 1(10%) in gr-1 & 2(10%) in gr-11 having SBP, this was not statistically significant.

Table 23

SBP	Yes	No	Total
Group-1(AMA+)	1(10%)	9(90%)	10(100%)
Group-2(AMA-)	2(10%)	18(90%)	20(100%)

SBP

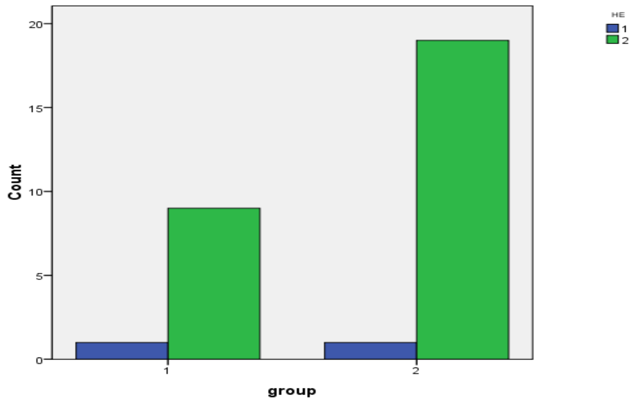


Hepatic encephalopathy: 1(10%) patient from group-1 and 1(05%) patient from group-2 developed hepatic encephalopathy, this was not statistically significant.

Table 24

HE	Yes	No	Total
Group-1(AMA+)	1(10%)	9(90%)	10(100%)
Group-2(AMA-)	1(05%)	19(95%)	20(100%)

Hepatic encephalopathy

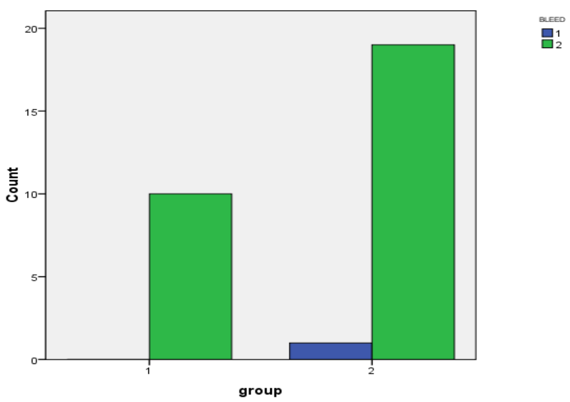


Bleed: There was 1(05%) patient who had esophageal variceal bleed in gr-2 but there was no episode of bleed in gr-1, this was not statistically significant.

Table 25

Bleed	Yes	No	Total
Group-1(AMA+)	0	10(100%)	10(100%)
Group-2(AMA-)	1(05%)	19(95%)	20(100%)

Bleed



Composite

Table 20-25

Indices	AMA positive	AMA negative	s/s[p<0.05]
Ascites	4(40%)	10(50%)	ns
Jaundice	2(20%)	7(35%)	ns
Edema	2(20%)	8(40%)	ns
SBP	1(10%)	2(10%)	ns
HE	1(10%)	2(10%)	ns
Bleed	0	1(10%)	ns

s/s=statistically significant, ns= not significant SBP=spontaneous bacterial peritonitis, HE= hepatic encephalopathy.

Investigation and Laboratory parameters

The two groups did not show significant difference for most of the parameters except TB and aPTT (Ref to Table-21).

Table 26

	Group-1(AMA+)		Group-2(AMA-)		Mean differ.
	Mean	± SD	Mean	± SD	
TB	2.3	3.4	5.555	7.8	3.255 **
DB	1.18	2.13	3.525	5.945	2.345
Alb:	3.33	0.636	3.135	0.701	-0.205
Glo:	4.31	0.735	3.83	1.128	-0.28
SGOT	97.6	65.501	168.4	278.683	70.8
SGPT	75.8	55.838	135	314.547	59.2
ALP	360.4	346.176	265.55	210.654	-94.85
PT	12.63	5.81	13.56	2.08	0.93
aPTT	23.16	16.366	35.87	5.648	12.71 **

** significant at P<0.05
TB= Total bilirubin, DB=Direct bilirubin,Alb= albumin, Glo=globulin, ALP=alkaline phosphatase, PT= prothronbin time, aPTT= activated partial thromboplastin time.

Independent samples t-Test

For all non normally distributed data a Mann Whitney test was done for the various parameter to see if there is any statistically significant difference between the two groups and was found to be significant only for TB and APTT at P<0.05.

Other features

ANA: ANA were positive 50% in both the groups, there was no significant statistically.

Liver biopsy and histology

Liver biopsy were available on 3 out of 10 AMA positive and on 11 out of 20 AMA negative patients. The rest did not undergo liver biopsy. In the AMA positive group, none of them had characteristics of Primary biliary cirrhosis. In AMA negative patients, biopsy findings were similar ; however one patient had features suggestive of primary biliary cirrhosis and another had granulomas on histology (ref. table-22 &23).

Table 27

Parameters	Groups(AMA+And AMA-)	N	Mean	SD	t-Value	df
ALB	1	10	3.38	0.561	0.959	0.346
	2	20	3.14	0.701	1.034	0.312
GLOBULIN	1	10	4.310	0.7355	0.128	0.899
	2	20	4.255	1.2344	0.152	0.881
PT	1	10	12.630	5.8071	-0.647	0.532
	2	20	13.560	2.0775	-0491	0.634
HB	1	10	10.410	2.1605	-0.759	0.454
	2	20	11.060	2.2369	-0.768	0.452
TC	1	10	6480	2766.386	-0.702	0.489
	2	20	7360	3437.778	-0.756	0.458
SGOT/SGPT	1	10	1.3962	0.47843	-O.546	0.589
	2	20	1.5176	0.61377	-0.594	0.558

HB= hemoglobin, TC= Total count

Table 28

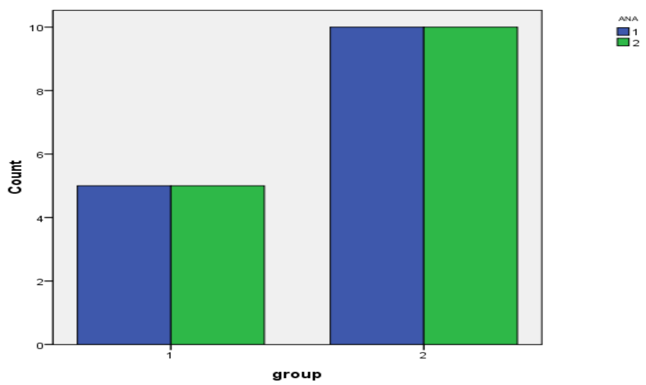
Test Statistics[b]

	MELD	TB	DB	SGOT	SGPT	ALP	APTT	PLATELATE
Mann-Whitney U	73.500	54.000	62.000	100.000	95.500	80.000	49.000	99.000
Wilcoxon W	128.500	109.000	117.000	310.000	305.500	290.000	104.000	154.000
Z	-1.170	-2.028	-1.680	.000	-.198	-.880	-2.245	-.044
Asymp. Sig. (2-tailed)	.242	.043	.093	1.000	.843	.379	.025	.965
Exact Sig. [2*(1-tailed Sig.)]	.248[a]	.044[a]	.100[a]	1.000[a]	.846[a]	.397[a]	.024[a]	.983[a]

a. Not corrected for ties.

b. Grouping Variable: GROUP

Ana Positivity



DISCUSSION

Cryptogenic liver disease in the late stages is often overlooked because the condition requires transplantation and this mode of therapy is widely available in the West (1,2,3,). However this is not always possible in poorer societies. There are new treatments that are emerging which may rescue people with advanced disease (4,5,6). The best example is the success that new therapies have had in such conditions such as HBV infection. It is possible that in the future we may similarly be able to treat other conditions in late stages without resorting to liver transplantation. It is therefore important to study patients who are passed off as cryptogenic disease only on the grounds that the serological markers of viral infection and a history of

Comparison of USG abdomen and liver histology between the AMA positive and AMA negative groups (table 29&30):

Table 29

USG/ Liver Bx

AMA+(n=10)		AMA- (n=20)	
USG abdo.	**Liver bx**	**USG abdo.**	**Liver bx**
1)Coarse,Shrunken & vr+	**-**	**1)Coarse&vr+**	**-**
2)Irregular&surfae nodularity	**-**	**2)Coarse**	**Micronodular cirrhosis**
3)Coarse&lobulatd	**Sinusoidal congestion& pericellular fibrosis**	**3)Mildly Coarse & enlarged**	**Moderate periportal inflammation with periportal & bridging fibrosis**
4)Coarse	**Bridging fibrosis, nudularity &focal bile duct damage**	**4) Coarse &vr+**	**-**
5)Coarse & lobulated	**-**	**5) Coarse &vr+**	**Focal portal inflammation &fibrosis**
6)Coarse	**-**	**6)Early CLD**	**Chronic cholestasis.**
USG abdo.	**Liver bx**	**USG abdo.**	**Liver bx**
7)Coarse , nodular & vr+	**-**	**7)Nodular& vr+**	**-**
8)vr+, coarse &nodular	**-**	**8) Coarse &vr+**	**Granulomatous inflammation with periportal bridging fibrosis**
9)Coarse	**9) early focal bridging fibrosis&moderate periportal inflammation.**	**9) Early CLD**	**-**
10)Shrunken, coarse& nodular		**10)Coarse &nodular**	**-**
		11) Coarse &irregular margin	**-**
		12) Shrunken, coarse	**Cirrhosis with focal activity.**

Table 30. (USG / liver biopsy in AMA – ve patients):

USG abdomen	Liver bx
13)Shrunken coarse& Micronodular cirrhosis	Micronodular cirrhosis
14)Coarse & irregular surface	Portal inflammation with destruction &disappearance intrahepatic bile ducts , abnormal bile duct proliferation, fibrosis & cirrhosis and f/s/o primary biliary cirrhosis.
15) Coarse & irregular surface	Cirrhosis with mild steatosis
16) Coarse & irregular surface	-
17)Diffusely Coarsing & irregular surface	Bridging fibrosis with nodularity & mild inflammation
18)Coarse & irregular surface	-
19) Coarse & shrunken	Early focal bridging fibrosis & moderate periportal inflammation
20) Coarse & shrunken	-

alcohol abuse is absent (7,8,9,10). The studies on those with high Alkaline phosphatase levels showed that the AMA test was very rarely positive (11). The majority of patients who tested positive were women who also were positive for the ANA test. Therefore the conclusion that can be drawn is that PBC is either a rare disease or that it is falsely negative in many of our patients (12). This has to be proven by other means such as by examining histology in this group (13,14). The group who tested positive for AMA was not different from those that were negative either on the basis of clinical or investigational findings, which makes a case for a large population of occult PBC in this population. These studies make a case for liver biopsies in advanced liver disease. This and other investigations and studies in cryptogenic liver disease may prepare us for the therapies that may appear in the future. Anti-mitochondrial antibody is considered the serological hallmark of primary biliary cirrhosis (PBC), but may be missing in our population.

Conclusion

AMA was rarely positive (about 8.33%) in this group of cryptogenic chronic liver disease with high ALP values in Indian. The clinical picture and investigational results were not different from AMA negative controls. The only discriminating investigations are a high Total Bilirubin, and activated Partial Thromboplastin Time in cases which are AMA positive. The liver biopsy of one patient having AMA negative found to have features of Primary biliary Cirrhosis in our study. We need to do liver biopsy in the early part of the disease to ascertain the pathogenetic mechanism of liver injury in the crypto group, because in the advanced stage we hardly find the accurate sample for histopathologic evaluationThis is not an argument for discarding the test in Indians but it may throw light on the pathogenetic mechanisms.

Abbreviations

TB= Total bilirubin, DB=Direct bilirubin, Alb= albumin, Glo=globulin, ALP=alkaline phosphatase, PT= prothronbin time, aPTT= activated partial thromboplastin time. AMA-Anti-Mito-chrondrial antiboby, ANA-Anti neuclear antibody, MELD-Model of end stage liver disease.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Dr K C Das, Dr.Sumeet david, and Dr. George Kurian, involved in the clinical assessment, study, and writing this case control study,All authors read and approved the final manuscript.

Consent

Full written consent was received for the manuscript to be published.

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