Original Research Article

Serum positivity of ANA and ASMA among khat and nonkhat chewers as markers for autoimmune hepatitis type 1

Abstract

Background: Autoimmune hepatitis (AIH) is a necroinflammatory liver disease of unknown etiology. It has been proposed that certain herbs such as black cohosh and dai-saiko might trigger AIH. Khat is an ever green tree whose leaves have been chewed by people in Yemen where AIH is common.

Aims: To measure antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) as serum markers for AIH type1 in healthy people who chew and do not chew khat. It also aimed to determine some other risk factors for ANA and ASMA positivity.

Methods: A total of 100 healthy individuals were enrolled in this study. They were divided into: Daily khat chewers and non-khat chewers. Three ml peripheral blood was withdrawn from each participant. Blood samples were tested for ANA and ASMA using indirect immunofluorescence assay.

Results: The age of khat chewers ranged from 30-60 years with mean age 40.4±7.6 years. Non-khat chewers age ranged from 30-57 years with mean age 39.9±6.2 years. The majority of khat chewers and non-chewers were in age groups 30-39 and 40-49 years old. There is no association between khat chewing and ANA or ASMA serum positivity ($\chi^2 = 0.33$, $P = 0.39$ and $\chi^2 = 1.5$, $P = 0.16$; respectively). However, ANA and ASMA positivity were significantly increased with age of the participants ($\chi^2 = 7.79$, $P = 0.044$ and $\chi^2 = 10.6$, $P = 0.011$, respectively).

Conclusion: khat chewing has no association with ANA and ASMA positivity. Nevertheless, ANA and ASMA positivity has an association with aging.

Key words

Autoimmune hepatitis, antinuclear autoantibodies, anti-smooth muscle autoantibodies, Khat chewing, Yemen

Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease in which the immune system attacks liver cells.[1] AIH is a relatively uncommon disease. It can occur any age but it is most common in young females. It is characterized by elevated levels of serum transaminases, seropositivity for autoantibodies, a histological picture of interface hepatitis and well-response to immunosuppression therapy.[2-5] According to the pattern of autoantibodies in patient's serum, AIH is subdivided into two types. Type 1 AIH is the most common form of AIH, (80% of AIH cases). It is commonly termed as a classic AIH. Type 1 AIH is mainly characterized by circulating antinuclear antibodies (ANA), anti-smooth muscle antibodies (SMA), atypical perinuclear antineutrophilic cytoplasmic antibodies (pANCA), and soluble liver antigen/liver pancreas (SLA/LP).[6-9] Type 2 AIH is a rare disorder and is mainly pediatric; average age is around 10 years. AIH type 2 is characterized by presence of liver/kidney microsomal antibodies type 1, 2 or 3 (LKM-1, 2 and 3) and liver cytosol type 1 (anti-LC-1). [6,9,10]

The etiology of AIH remains unknown but is postulated to be related to an autoimmune process triggered by either genetic predisposition or environmental agents. AIH is strongly associated with human leukocyte antigens (HLA-DR3 and HLA-DR4, HLA-DR6).[11-14] Environmental factors are thought to be the triggering factors for the development of AIH in genetically

susceptible individuals. These environmental factors could be viruses, drugs, chemicals or certain herbs.[15-17]

Khat (Catha edulis) is an herb its leaves have been chewed for centuries by people who live in the Eastern part of Africa and Yemen. The khat leaves chewed daily by a high proportion of the adult population in Yemen for the pleasant mild stimulant effect.[18] Khat has recently been recognized as a triggering agent for a severe form of AIH in young males in Yemen and Somalia.[19-23] Our study aimed to measure serum ANA and ASMA as early markers for AIH type1 in Yemeni healthy people who chew khat.

Subjects and methods

Our study was a cross-sectional study which was conducted for four months from March to June, 2014. It was carried out in Sana'a city, the capital of Yemen. One hundred individuals were enrolled in the study divided into two groups. First group involved 50 individuals who daily chew khat for at least five years. Second group include 50 persons who never chewed khat. According to gender, the two previous groups were subdivided into 25 males and 25 females. Participants were selected randomly from khat or social sessions. Study was approved by Faculty of Medicine and Health Sciences, University of Science and Technology. Written consent was obtained from each participant.

Inclusion criteria

Any apparently healthy individual who was equal or over 30 years, did not complain any signs and symptoms and did not take any medications.

Exclusion criteria

Carriers of hepatitis viruses, pregnant women or females who ingested oral contraceptive pills were excluded from the two study groups.

Data from each individual were collected using predesigned questionnaire. Three ml of venous blood was withdrawn from each person into a plain tube. The sample was allowed to clot at room temperature and was centrifuged at 3500 rpm for five minutes, then serum was separated from each sample into Eppendorf tubes and stored at -20 °C till tested. Serum ANA and ASMA were measured using indirect immunofluorescence assay on HEp2 cells according to manufacturer instructions (Innova, San Diego, USA). The nuclear staining patterns of ANA test were detected on liver and gastric cells. Nuclear staining patterns observed on theses tissues include: homogenous, centromere, speckled, peripheral and nucleolar. Sera were considered as positive for ANA when titers were >1/80 and positive for ASMA when titers were >1/40.

Data analysis:

Statistical data analysis was done using SPSS (version 15). Data were presented as numbers and percentages. Significant association was measured using $\chi 2$ and *P* value tests. P-value less than 0.05 was considered statistical significant.

Results

This study was conducted on 50 khat chewers and 50 non-khat chewers. The age of the khat chewers ranged from 30 years to 60 years with mean age 40.4±7.6 years old. The age of non-khat chewers ranged from 30 years to 57 years with mean age 39.9±6.2 years old.

The prevalence of serum ANA among the study groups was 14%. ANA test was positive in 8(16%) of khat chewers whereas it was positive in 6(12%) of non khat chewers. The difference was statistical non-significant ($\chi^2 = 0.33$, $P = 0.39$). ASMA test was positive in 21 % of the study population. It was positive in 13(26%) of khat chewers whereas it was positive in 8(16%) in non khat chewers ($\chi^2 = 1.5$, $P = 0.16$), table 1.

ANA test was positive in 4(8.3%) individuals at age group 30-39 years old, in 5(12.8%) individuals at age 40-49 years old, and in 5(33.3%) at age 50-60 years old. This difference was statistically significant ($\chi^2 = 7.79$, $P = 0.044$), table 2.

ASMA test was positive in 6(12.5%) individuals at age group 30-39 years old, in 8(20.5%) individuals at age 40-49 years old, and in 7(54%) at age 50-60 years old. The association between ASMA and age was statistical significant ($\chi^2 = 10.6$, P value = 0.011), table 3.

Eight (16%) males had ANA positive results while 6(12%) females had ANA positive tests. The difference in ANA results between both males and females was non-statistically significant ($\chi^2 = 0.33$ and $P = 0.4$), table 4. Seven (14%) males had ASMA positive results whereas 14(28%) females had ASMA positive tests. The difference in ASMA results between both males and females was non-statistically significant ($\chi^2 = 3$ and $P = 0.07$), table 4.

Twenty eight (56%) of khat chewers were washing khat before chewing while 8(16%) did not wash khat or rarely washing khat (14, 28%). Although the majority of ANA test positive did not or rarely wash khat before chewing than those who washed khat, there is no statistical significant association ($\chi2 = 3.76$ and $P = 1.5$). Furthermore, no association was found between khat washing and ASMA positivity ($\chi2 = 0.9$ and $P = 0.65$), table 5.

ANA test was positive in 5(5%) smokers and ASMA test was positive in 6(6%) and 1(1%) who smoked sometimes. The association between ANA and ASMA positivity with smoking was statistical non-significant ($\chi2 = 0.89$, $P = 0.53$; $\chi2 = 0.3$, $P = 0.87$, respectively), table 6.

Discussion

AIH type 1 occurs primarily in adults with a female predominance. Pathogenesis of AIH type 1 is unknown, though both genetic and environmental factors are likely to predispose to the disease. Circulating ANA and ASMA are key to the diagnosis of AIH type 1.[24] Khat chewing is proposed to cause AIH. [25] Hence our study aimed to measure serum ANA and ASMA among khat chewers and non-khat chewers as early markers for liver damage by khat chewing.

This study revealed that chewing khat had no association with increased serum level of ANA or ASMA. Although Riyaz *et al*, 2014,[25] reported khat to be a possible cause of AIH, our findings suggest that khat chewing may have no direct role in the induction of ANA and ASMA production and imply Khat interaction with other factors such as genetic susceptibility and khat abuse in the pathogenesis of AIH. Another explanation is that Khat risks on hepatocytes may be due to other mechanisms such as hepatotoxicity or increase the rate of hepatocytes apoptosis rather than autoantibody production. [26-29]

Our study showed that the positivity of ANA and ASMA tests were increasing by increase age. This result is supported by studies which reported serum ANA prevalence in the general population to be highest in elders.[30-32] Nevertheless, other studies found no association between serum ANA with age.[33-34]

Equal males and females were enrolled in this study. No statistical differences were found between the ANA and ASMA results in both men and women. This finding is in agreement with other study which reported no association between ANAs and gender.[31,33,35,36] In contrast, other studies reported presence of ANA and/or ASMA to be more predominant in females than in males. [30, 34, 35, 37]

Current study demonstrates that smoking had no association with ANA and ASMA positivity. Similar results are reported by other studies.[30,33, 36,38,39] On the other hand, this finding is in disagreement with Karabulut *et al*, 2011, who reported association between ANAs positivity and smoking.[40]

Many khat farmers use pesticides for better khat cultivation which may have harmful effects on liver cells. Serum positivity of ANA was more frequent among participants who did not wash khat; however, the difference was not statistical significant which may be attributed to small number of the studied sample.

Conclusion

Khat chewing has no association with ANA and ASMA positivity. Nevertheless, ANA and ASMA positivity has an association with aging. Therefore, adults over 30 years old should be screened for ANA and ASMA positivity because such individuals are at risk for development of autoimmune diseases.

Limitations of the study

Obstacles which we encountered during the study were difficulty to determine type and amount of khat as well as time for how long chewing.

Acknowledgments

This work has been supported by grants from Almughales Organization

Disclosure

The authors declared no conflicts of interest.

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Table 1: ANA and ASMA test resultsfor khat chewers and non-khat chewers

	Khat chewers	Non-khat chewers	χ^2	OR	95% CI	*P*
ANA test results						
Positive	8 (16%)	6 (12%)	0.33	1.4	0.447-4.367	0.39
Negative	42 (84%)	44 (88%)				
ASMA test results						
Positive	13 (26%)	8 (16%)	1.5	1.84	0.67-4.04	0.16
Negative	37 (76%)	42 (82%)				

Table 2: ANA test results in different age groups

	ANA test results				χ^2	***P***
	Positive		Negative			
	N	%	N	%		
30-39 years (n= 48)	4	8.3	44	91.7	7.79	0.044
40-49 years (n= 39)	5	12.8	34	87.2		
50-59 years (n= 13)	5	38.5	8	61.5		
Total	14	14	86	86		

Table 3: ASMA test results in different age groups

	ASMA test results				χ^2	*P*
	Positive		Negative			
	N	%	N	%		
30-39 years (n= 48)	6	12.5	42	87.5	10.6	0.011
40-49 years (n= 39)	8	20.5	31	79.5		
50-60 years (n= 13)	7	54	6	46		
Total	21	21	79	79		

Table 4: ANA and ASMA positivity among both males and females

	Males		Females		χ^2	*OR*	*95% CI*	*P*
	No.	%	No.	%				
ANA test								
Positive	8	16 %	6	12 %	0.33	1.4	0.447-4.367	0.4
Negative	42	84 %	44	84%				
ASMA test								
Positive	7	14 %	14	28 %	3	1.8	0.687-4.94	0.07
Negative	43	86 %	36	72%				

Table 5: Association of ANA and ASMA with khat washing

	ANA positivity				χ^2	*P*
	Positive		Negative			
	No.	%	No.	%		
Yes	2	4	26	52	3.76	1.5
No	2	4	6	12		
Rarely	4	8	10	20		
	ASMA positivity					
	Positive		Negative			
	No.	%	No.	%		
Yes	6	12	22	44	0.9	0.65
No	3	6	5	10		
Rarely	4	8	10	20		

Table 6: ANA and ASMA test results with smoking

	ANA test results				χ^2	*P*
	Positive		Negative			
	No.	%	No.	%		
Yes	5	5	23	23	0.89	0.53
No	9	9	60	60		
sometimes	0	0	3	3		
	ASMA test results					
	Positive		Negative			
	No.	%	No.	%		
Yes	6	6	22	22	0.3	0.87
No	14	14	55	55		
sometimes	1	1	2	2		