



RESEARCH ARTICLE

SERUM POSITIVITY OF ANA AND ASMA AMONG KHAT AND NONKHAT CHEWERS AS MARKERS FOR AUTOIMMUNE HEPATITIS TYPE 1

Arwa M Othman¹, Eman A Hamzah², Jamil A Almughales³, Abdullah Al-Mikhlafy⁴

¹Department of Microbiology, Faculty of Medicine and Health Sciences, Sana'a University.

²Aulaqi Specialized Labs, Sana'a, Yemen.

³Faculty of Medicine, King Abdul-Aziz University, Jeddah, Saudi Arabia.

⁴Community Medicine Department, University of Science and Technology, Sana'a, Yemen.

Article Info:



Article History:

Received: 2 June 2017
 Reviewed: 8 July 2017
 Accepted: 23 August 2017
 Published: 15 September 2017

Cite this article:

Othman AM, Hamzah EA, Almughales JA, Al-Mikhlafy A. Serum positivity of ANA and ASMA among Khat and non Khat chewers as markers for autoimmune hepatitis type 1. Universal Journal of Pharmaceutical Research 2017; 2(4): 19-23.

<http://doi.org/10.22270/ujpr.v2i4.R5>

*Address for Correspondence:

Arwa M Othman, Department of Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen, Tel: 00967 774486417
 E-mail: arwaothman@hotmail.com

Abstract

Objectives: 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. Aim of present study was 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 Three ml peripheral blood was withdrawn from each participant.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 chewer's 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). Khat chewing has no association with ANA and ASMA positivity. Nevertheless, ANA and ASMA positivity has an association with aging.

Conclusion: Study concludes that Khat chewing has no association with ANA and ASMA positivity. Nevertheless, ANA and ASMA positivity has an association with aging.

Keywords: Antinuclear autoantibodies, anti-smooth muscle autoantibodies, autoimmune hepatitis, Khat chewing, Yemen.

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease in which the immune system attacks liver cells¹. 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 auto antibodies, a histological picture of interface hepatitis and well-response to immunosuppression therapy²⁻⁵. 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 herbs¹⁵⁻¹⁷. 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 effect¹⁸. Khat has recently been recognized as a triggering agent for a severe form of AIH in young males in Yemen and Somalia¹⁹⁻²³. Current study aimed to measure serum ANA and ASMA as early markers for AIH type1 in Yemeni healthy people who chew khat.

SUBJECTS AND METHODS

Current 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, and 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 these 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 χ^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.

Table 1: ANA and test results for khat chewers and non-khat chewers.

Results	Khat chewers	Non-ASMA 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.

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.

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		

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 ($\chi^2=3.76$ and $p=0.15$). Furthermore, no association was found between khat washing and ASMA positivity ($\chi^2=0.9$ 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 ($\chi^2=0.89$, $p=0.53$; $\chi^2=0.3$, $p=0.87$, respectively), (Table 6).

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

Test	Males		Females		χ^2	OR	95% CI	p
	N	%	N	%				
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.

Result	ANA positivity				χ^2	P
	Positive		Negative			
	N	%	N	%		
Yes	2	4	26	52	3.76	0.15
No	2	4	6	12		
Rarely	4	8	10	20		
ASMA positivity						
Result	Positive		Negative		χ^2	P
	N	%	N	%		
	Yes	6	12	22		
No	3	6	5	10		
Rarely	4	8	10	20		

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 keys to the diagnosis of AIH type 1²⁴. Khat chewing is proposed to cause AIH²⁵. Hence current 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.*,²⁵ reported khat to be a possible cause of AIH, current 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 production²⁶⁻²⁹. Current 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 elders³⁰⁻³². Nevertheless, other studies found no association between serum ANA with age³³⁻³⁴. 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}.

Table 6: ANA and ASMA test results with smoking.

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

On the other hand, this finding is in disagreement with Karabulut *et al.*, who reported association between ANAs positivity and smoking⁴⁰. 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.

CONCLUSIONS

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 current study were associated with the obstacles which we encountered during the study it was difficult to determine type and amount of khat as well as time for how long chewing.

ACKNOWLEDGEMENTS

This work has been supported by grants from Almughales Organization.

AUTHOR'S CONTRIBUTION

Othman AM: writing, review, and editing, data curation. **Hamzah EA:** writing, review and editing, data curation. **Almughales JA:** formal analysis, writing, review, and editing. **Al-Mikhlafoy A:** writing, review, and editing, investigation, data curation, conceptualization. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST

None to declare.

REFERENCES

- Krawitt EL. Autoimmune hepatitis. *The New England J medicine* 2006; 354(1):54-66.
- Czaja AJ. Diverse manifestations and evolving treatments of autoimmune hepatitis. *Minerva Gastroenterol Dietol* 2005; 51:313-333.PMID: 16282960
- Bruguera M. Autoimmune hepatitis In: Shoenfeld Y, Cervera R, Gershwin E. *Diagnostic criteria in autoimmune diseases*. USA, Human Press 2008; 287- 290. <https://doi.org/10.1016/j.jaut.2009.02.007>
- Czaja AJ. Special clinical challenges in autoimmune hepatitis: the elderly, males, pregnancy, mild disease, fulminant onset, and nonwhite patients. *Semin Liver Dis* 2009; 29:315-330. <https://doi.org/10.1055/s-0029-1233530>
- Czaja AJ. Diagnosis and Management of Autoimmune Hepatitis: current status and future directions. *Gut Liver* 2016; 10(2):177-203.<https://doi.org/10.5009/gnl15352>
- Homborg JC, Abuaf N, Bernard O, *et al.* Chronic active hepatitis associated with antiliver/kidney microsome antibody type 1: A second type of autoimmune hepatitis. *Hepatology* 1987; 7(6):1333-1339. <https://doi.org/10.1002/hep.1840070626>
- Volkman MD, Zentgraf H. SLA/LP/tRNP((Ser)Sec) antigen in autoimmune hepatitis: identification of the native protein in human hepatic cell extract. *J Autoimmun*. 2010; 34(1):59-65. <https://doi.org/10.1016/j.jaut.2009.07.005>
- Krawitt EL. Discrimination of autoimmune hepatitis: autoantibody typing and beyond. *J Gastroenterol* 2011; 46 Suppl 1:39-41.<https://doi.org/10.1007/s00535-010-0324-3>
- Liberal R, Vergani D, Mieli-Vergani G. Update on Autoimmune Hepatitis. *J Clin Transl Hepatol* 2015; 3(1):42-52. <https://doi.org/10.14218/JCTH.2013.00015>
- Malik TA, Saeed S. Autoimmune hepatitis: a review. *JPMA. The J Pakistan Medical Association* 2010; 60(5):381-387. <https://doi.org/10.5812/hepatmon.13598>
- Oliveira LC1, Porta G, Marin ML, Bittencourt PL, Kalil J, Goldberg AC. Autoimmune hepatitis, HLA and extended haplotypes. *Autoimmun Rev* 2011; 10(4):189-93. <https://doi.org/10.1016/j.autrev.2010.09.024>
- Hassan NA, Siddiqui AR, Abbas Z, Hassan SM, Soomro GB, Mubarak M, Anis S, Muzaffar R, Zafar MN. Clinical profile and HLA typing of autoimmune hepatitis from Pakistan. *Hepat Mon* 2013; 13(12):e13598. <https://doi.org/10.5812/hepatmon.13598>
- Kaur N, Minz RW, Anand S, Saikia B, Aggarwal R, Das A, Thapa BR, Chawla YK. HLA DRB1 alleles discriminate the manifestation of autoimmune hepatitis as type 1 or type 2 in North Indian population. *J Clin Exp Hepatol* 2014; 4(1):14-8. <https://doi.org/10.1016/j.jceh.2013.12.002>
- Baharlou R, Faghihi-Kashani A, Faraji F, Najafi-Samei M, Setareh M, Zamani F, Tajik N. HLA-DRB1 alleles of susceptibility and protection in Iranians with autoimmune hepatitis. *Hum Immunol* 2016; 77(4):330-335. <https://doi.org/10.1016/j.humimm.2016.01.007>
- Gershwin EM, Vierling JM, Manns MP. *Liver immunology principles and practice*. USA, Human Press. 2007; 263-276.
- Guzman G, Kallwitz E, Wojewoda C, Chennuri R, Berkes J, Layden T, Cotler S. Liver injury with features mimicking autoimmune hepatitis following the use of black cohosh. *Case Rep Med* 2009; 91-98. <https://doi.org/10.1155/2009/918156>
- Béland K, Lapierre P, Alvarez F. Influence of genes, sex, age and environment on the onset of autoimmune hepatitis. *World J Gastroenterol* 2009; 15(9):1025-1034. <https://doi.org/10.3748/wjg.15.1025>
- Hassan NA, Gunaid AA and Murray -Lyon IM: Khat (*Catha edulis*): health aspects of khat chewing. *Health J*. 2007; 13(3): 206-218.PMID: 17687845

19. Chapman MH, Kajihara M, Borges G, *et al.* Severe, acute liver injury and khat leaves. *The New England J Medicine* 2010; 362(17):1642-1644.
<https://doi.org/10.1056/NEJMc0908038>
20. Fallatah HQ at chewing and autoimmune hepatitis: true association or coincidence. *Middle East J Family Medicine* 2010; 8:6-9.
<https://doi.org/10.4172/2332-0877.1000337>
21. Peevers CG, Moorghen M, Collins PL, Gordon FH, McCune CA. Liver disease and cirrhosis because of khat chewing in UK Somali men: a case series. *Liver Int* 2010; 30(8):1242-1243.
<https://doi.org/10.1111/j.1478-3231.2010.02228.x>
22. Patanwala IA, Burt M, Bassendine, M, Hudson. Khat associated end stage chronic liver disease: A case report. *J Medical Cases* 2011; 2(3):104-106.
<https://doi.org/doi:10.4021/jmc168w>
23. Yildiz H, Komuta M, Monsalve C, Starkel P, Lefebvre C. To chew or not to chew: that's the question. *Acta Clin Belg* 2016; 71(3):187-189.
<https://doi.org/10.1002/hep.29809>
24. Manns MP, Lohse AW, Vergani D. Autoimmune hepatitis—Update. *J Hepatol.* 2015; 62(1 Suppl):S100-111.
<https://doi.org/10.1016/j.jhep.2015.03.005>
25. Riyaz S, Imran M, Gleeson D, Karajeh MA. Khat (*Catha edulis*) as a possible cause of autoimmune hepatitis. *World J Hepatol* 2014; 6:150-154.
<https://doi.org/10.4254/wjh.v6.i3.150>
26. Al-Habori M, Al-Aghbari A, Al-Mamary M, Baker M. Toxicological evaluation of *Catha edulis* leaves: a long term feeding experiment in animals. *J Ethnopharmacol* 2002; 83(3):209-217.
[https://doi.org/10.1016/S0378-8741\(02\)00223-4](https://doi.org/10.1016/S0378-8741(02)00223-4)
27. Al-Mamary M, Al-Habori M, Al-Aghbari AM, Baker MM. Investigation into the toxicological effects of *Catha edulis* leaves: a short term study in animals. *Phytother Res* 2002; 16(2):127-132.
<https://doi.org/10.1002/ptr.835>
28. Abid MD, Chen J, Xiang M, Zhou J, Chen X, Gong F. Khat (*Catha edulis*) generates reactive oxygen species and promotes hepatic cell apoptosis via MAPK activation. *Int J Mol Med* 2013; 32(2):389-95.
<https://doi.org/10.3892/ijmm.2013.1394>
29. Ramzy I, Abdelbary M, Abdelhafez H, *et al.* The effect of chronic khat chewing on liver enzyme levels (a Yemenian study). *The Egyptian J Internal Med* 2013; 25 (1):37-41.
30. Satoh M, Chan EK, Ho LA, *et al.* Prevalence and socio demographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum* 2012; 64(7):2319-2327.
<https://doi.org/10.1002/art.34380>
31. Sidhom O, Laadhar L, Zitouni M, *et al.* Spectrum of autoantibodies in Tunisian psychiatric inpatients. *Immunol Invest* 2012; 41(5):538-549.
<https://doi.org/10.3109/08820139.2012.685537>
32. Selmi C, Ceribelli A, Generali E, Scirè CA, *et al.* Serum antinuclear and extractable nuclear antigen antibody prevalence and associated morbidity and mortality in the general population over 15 years. *Autoimmun Rev* 2016; 15(2):162-166.
<https://doi.org/10.1016/j.autrev.2015.10.007>
33. Bonarius HP, Brandsma CA, Kerstjens HA, *et al.* Antinuclear autoantibodies are more prevalent in COPD in association with low body mass index but not with smoking history. *Thorax* 2011; 66(2):101-107.
<https://doi.org/10.1136/thx.2009.134171>
34. Li Q, Karp D, Quan J, *et al.* Risk factors for ANA positivity in healthy persons. *Arthritis Research Therap* 2011; 13:R38. <https://doi.org/10.1186/ar3271>
35. Njemini R, Meyers I, Demanet C, J Smitz, Sosso M, Mets T. The prevalence of autoantibodies in an elderly sub-Saharan African population. *Clin Exp Immunol* 2002; 127:99-106.
<https://doi.org/10.1046/j.1365-2249.2002.01713.x>
36. Kakhkaya N, Akmeden A, Topbaş M. Determination of anti-nuclear antibody seroprevalence in adult age groups in Trabzon Province. *Balkan Med J* 2013; 30:343-344.
<https://doi.org/10.5152/balkanmedj.2013.8125>
37. Hayashi N, Koshiba M, Nishimura K, *et al.* Prevalence of disease-specific antinuclear antibodies in general population: Estimates from annual physical examinations of residents of a small town over a 5-year period. *Mod Rheumatol* 2008; 18(2):153-160.
<https://doi.org/10.1007/s10165-008-0028-1>
38. Eriksson C, Kokkonen H, Johansson M, *et al.* Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. *Arthritis Res Ther* 2011; 13(1):R30.
<https://doi.org/10.1186/ar3258>
39. Young KA1, Terrell DR, Guthridge JM, *et al.* Smoking is not associated with autoantibody production in systemic lupus erythematosus patients, unaffected first-degree relatives, nor healthy controls. *Lupus* 2014; 23(4):360-369. <https://doi.org/10.1177/0961203314520838>
40. Karabulut G, Kitapcioglu G, Inal V, *et al.* Cigarette smoking in primary Sjögren's syndrome: positive association only with ANA positivity. *Mod Rheumatol* 2011; 21(6):602-607. <https://doi.org/10.1186/s13075-017-1255-7>