



Association between khat chewing and high viral load in chronic hepatitis B infection

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ABSTRACT

Background and aims: chronic hepatitis B infection (CHB) is a worldwide health threat that has moderate to high prevalence in Yemen. There is accumulating evidence of the hepatotoxicity of khat, which is commonly and habitually chewed in Yemen and elsewhere, but little is known in CHB. This study aimed to examine the association between khat chewing and hepatitis B virus (HBV) serum DNA level (viral load) in such cases. **Methods:** Between January and December 2016, a cross-sectional study was carried out on consecutively consenting 210 CHB participants (khat chewers and non-chewers) attending the hepatology clinics of two major hospitals in Sana'a city. After a structured interview, blood samples were collected for the determination of HBV markers and HBV load. **Results:** the study population, residing different cities in Yemen, had a median age of 36y (range = 20-72) with a 2.9:1 male to female ratio. Viral load was significantly ($P < 0.001$) higher among khat chewers than non chewers: median (Interquartile range) = 1308 (531.5-5916.5) vs 177 (60-627) IU/ml. Khat chewing was observed, via multivariate logistic regression analysis, to be significantly ($p < 0.001$) and independently associated with a clinically significant high HBV load (> 2000 IU/ml) even after adjustment for the studied socio-demographic and laboratory factors. **Conclusion:** This study suggests khat chewing, especially on a daily basis, as a potential risk factor for high viremia and, therefore, be prudently discouraged in CHB.

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1. Introduction:

Chronic hepatitis B virus infection (CHB) is a global as well as a local public health threat with

a 3.5% worldwide prevalence and estimated 849,024 deaths in 2015 alone [1]. In Yemen, although improving after the introduction of the HBV vaccine in 2000, CHB is still endemic, with an estimated overall HBsAg sero-prevalence of

1.8–5.8% [2–5]; a prevalence of 10.8% in pregnant women [6], and 18–24% in cases of chronic liver disease [7–9].

The critical role of HBV load in CHB cases cannot be overemphasized. It has been considered the cardinal predictor of disease progression and was linked to necroinflammation and the development of liver fibrosis and cirrhosis, as well as hepatocellular carcinoma (HCC), hence its exceptional importance for both treatment as well as follow-up decisions.

making [10–14]. Moreover, baseline HBV load has been an independent prognostic factor for sustained virologic response to nucleotide analogues [15], and high viremia constitutes a risk factor for transmission, including from the mother to child [16], that requires special attention and therapy [14]. Furthermore, high HBV load has been associated with higher morbidity and mortality from chronic liver disease and HCC, and it predicts recurrence of HCC after curative resection [17, 18].

On the other hand, considered an illicit drug in many countries, the khat plant (*Catha edulis*Forsk) is increasingly cultivated and therefore abundantly available in Yemen and east Africa and is chewed by millions, mainly where it is cultivated but also in Australia, Europe, and the United States [19]. In Yemen, chewing khat has been a widely accepted social norm for decades, but with increasing popularity and an estimate of 3–4-hour-daily chewing sessions by 32–90% and 9–50% of adult males and females, respectively [20–23]. While several studies have studied the acute and chronic hepatotoxic effect of khat and its association with the development of chronic liver disease and cirrhosis [24–26], data are scarce regarding the potential deleterious combination of khat chewing and CHB. In fact, no study has specifically examined the effect of khat chewing on HBV load. Hence, our study mainly aimed to explore the potential association

of khat chewing with HBV load (viremia) in CHB.

2. Participants and Methods

Study design & setting: An observational cross-sectional study was carried out on consecutive 210 participants with CHB, 50% of whom were khat chewers, who attended the hepatology clinics in Athawra General Hospital and the Hospital of the University of Science and Technology (Sci & Tech.) in Sana'a, Yemen, in the period from January to December 2016. The study was approved by the ethics and scientific committees of the Faculty of Medicine, Sana'a University.

Participants: eligible participants were consenting adults (≥ 18 years old) who had CHB, defined as hepatitis B surface antigen-HBsAg positivity for at least the past six months [27], with positive anti-HBc IgG, and had serum HBV-DNA more than 25 international units per ml (IU/ml). Participants with HCV, HIV, autoimmune hepatitis, cirrhosis, HCC, or other terminal illness and those on antiretroviral treatment were excluded from this study. Participants with informed consent were interviewed, and demographic and clinical data were collected via an interview and a questionnaire. Blood samples were collected after the interview.

Laboratory testing: The qualitative presence of HBc-IgG antibodies (together with HBsAg, an objective indication of chronicity) and HBeAg (hepatitis B e-antigen) were determined via the COBAS® e 411 analyzer (Rosch Diagnostics GmbH, Germany) using Electro-Chemiluminescence Immunoassay (ECLIA) per the manufacturer's instructions. Quantitative assessment of serum HBV-DNA was performed with the High Pure System (HPS) for DNA extraction and real-time polymerase chain reaction (RT-PCR) using the COBAS®TaqMan® 48 Analyzer (Rosch Diagnostics GmbH, Germany) that has a lower detection limit of 25 IU/ml. Serum viral load of HBV in patients was classified as high

(>2000 IU/ml) and low (\leq 2000 IU/ml) based on the clinical significance described earlier [28].

Statistical analysis:

The statistical package of social science program (SPSS) version 21 (IBM®, Armonk, NY, USA) was used for data analysis.

First the khat chewing and non chewing participants were compared using univariate analyses: Mann Whitney U test for continuous variables and Chi square test for categorical variables; factors associated with khat chewing were examined via univariate logistic regression. Significance and independence of association between khat chewing and hepatitis B viral load was studied with all variables included (socio-demographic, recruitment hospital and laboratory) using logistic regression model after checking for multicollinearity between independent variables. In order to investigate the potential dose-response relationship between intensity of khat chewing and high viral load, participants were classified into three groups based on the number of khat chewing days per week. To evaluate the combined effect of khat chewing and smoking, participants were classified into four groups: non chewers and non smokers, chewers and nonsmokers, non chewers and smokers, chewers and smokers. Percentages cited in tables are those of columns. All p values were two sided; and a p value $<0.05\%$ was considered significant.

3. Results

Baseline characteristics of study participants

As shown in Table 1, the 210 Yemeni CHB participants had a median age of 36 years, with the age group 30-39y dominating (42.2%) the sample population. They were predominantly males (male:female ratio = 2.9), nonsmokers (84.2%), and predominantly residing in Sana'a (32%) but also coming from other cities [Table 1]. The overall HBeAg positivity rate was 16%, and 28.1% of all

CHB participants had a clinically significant viral load greater than 2000 IU/ml.

The vast majority of chewers (85.7%) chew khat on a daily basis [Table 3]. Among khat chewers, khat chewing was significantly more frequent in the age group 30-39y ($p = 0.017$), followed by the 40-49y group when compared to younger participants.

Comparison between CHB khat chewers and non-chewers

CHB Khat chewers were significantly more likely to be males ($p = 0.013$) with a 4.5:1 male to female ratio and were more likely to smoke cigarettes ($p < 0.001$). HBV load varied significantly between khat chewers and non-chewers and tended to be higher in the former ($p < 0.001$) [Figure 1A, Table 1]. Chewers and non-chewers, however, did not vary significantly with respect to age distribution, residence, recruitment hospital, or HBeAg status [Table 1].

Factors associated with high hepatitis B viral load

Univariate and multivariate logistic regression analysis for factors associated with clinically significant HBV load (>2000 IU/ml) in CHB participants is shown in table 2. The analysis revealed that HBeAg positivity ($p < 0.001$), khat chewing ($p = 0.001$) and cigarettes smoking ($p = 0.025$) were significantly associated with high HBV load after adjustment for other factors like age, sex, residence and recruitment hospital. In addition, frequency of khat chewing showed a significant (p trend < 0.001) linear association with high HBV load [Figure 1B]. The association was especially significant ($p < 0.001$) among daily khat chewers, with an odd ratio (OR) of 7.1 [Table 3].

However, unlike other significantly associated variables, adjusting for frequency of khat chewing pushed the association of high viremia with smoking to the margin of significance ($p = 0.05$). The p value was 0.049 when age, as a continuous variable, replaced the grouped

variable in the model (data not shown). Such a shift in significance is likely due to the lack of smokers in the group of 1-3-days-per-week chewers and presence of only two smokers who never chew khat, none of whom had high HBV load. Analysis of the combined effect of smoking and khat chewing, nonetheless, showed that

smoking increased the odds of having high viral load by at least 11. Of note, there was insufficient statistical evidence that age (as a continuous or as a grouped variable) or other studied variables were associated with high HBV load.

Table (1): Baseline socio-demographic and lab characteristics of all chronic hepatitis participants according to khat chewing status

Criterion	All (n=210)	Non chewers (n=105)	Chewers (n=105)	Univariate analysis [†]	
	n (%)	n (%)	n (%)	OR (95% CI)	P
Age, year, median(IQR)	36 (15)	35 (17)	37 (15)		0.167
Age groups					0.085
20-29	50 (23.8)	32 (30.5)	18 (17.1)	1	
30-39	89 (42.4)	38 (36.2)	51 (48.6)	2.39 (1.17, 4.87)	0.017
40-49	26 (12.4)	11 (10.5)	15 (14.3)	2.42 (0.92, 6.39)	0.073
≥50	45 (21.4)	24 (22.9)	21 (20)	1.56 (0.68, 3.54)	0.292
Sex					0.012
Female	54 (25.7)	35 (33.3)	19 (18.1)	1	
Male	156 (74.3)	70 (66.7)	86 (81.9)	2.26 (1.19, 4.3)	0.013
Smoking					<0.001
No	177 (84.3)	103 (98.1)	74 (70.5)	1	
Yes	33 (15.7)	2 (1.9)	31 (29.5)	21.57 (5, 92.9)	<0.001
Residence					0.983
Sana'a	67(31.9)	35 (33.3)	32 (30.5)	1	
Ibb	35 (16.7)	16 (15.2)	19 (18.1)	1.3 (0.57, 2.94)	0.532
Taiz	27 (12.9)	14 (13.3)	13 (12.4)	1.01 (0.42, 2.48)	0.973
Aden	22 (10.5)	11 (10.5)	11 (10.5)	1.1 (0.42, 2.87)	0.855
Shabwa	13 (6.2)	5 (4.8)	8 (7.6)	1.75 (0.52, 5.9)	0.367
Raimah	11 (5.2)	7 (6.7)	4 (3.8)	0.63 (0.17, 2.34)	0.485
Hadramawt	11 (5.2)	6 (5.7)	5 (4.8)	0.91 (0.25, 3.28)	0.887
Almahweet	8 (3.8)	4 (3.8)	4 (3.8)	1.1 (0.25, 4.74)	0.905
Lahj	6 (2.9)	3 (2.9)	3 (2.9)	1.1 (0.21, 5.81)	0.916
N/A	10 (4.8)	4 (3.8)	6 (5.7)	1.64 (0.42, 6.34)	0.473
Hospital					0.153
Athawra	78 (37.1)	34 (32.4)	44 (41.9)	1	
Sci & Tech.	132 (62.9)	71 (67.6)	61 (58.1)	0.66 (0.38, 1.17)	0.154
Viral Load (IU/ml), median (IQR)	565 (3413)	177 (567)	1308 (5385)		<0.001
Viral load Groups					<0.001
Low (≤2000 IU/ml)	151(71.9)	88 (83.8)	63 (60)	1	
High (>2000 IU/ml)	59 (28.1)	17 (16.2)	42 (40)	3.45 (1.8, 6.61)	<0.001
HBeAg					1
Negative	176 (83.8)	88 (83.8)	88 (83.8)	1	
Positive	34 (16.2)	17 (16.2)	17 (16.2)	1 (0.48, 2.1)	1

[†]P values in continuous variables (age and viral load) were based on Mann-Whitney U test. P values for categorical variables (**in bold**) were based on Chi-Square test. The crude odds ratio (OR) and p value for the rest of categorical variables are based on univariate logistic regression for subset analysis of khat chewers. IU/ml: international units per ml of serum. HBeAg: Hepatitis B virus e antigen. IQR: interquartile range. N/A: not available.

Table (2): Factors associated with high viral load in CHB participants in Sana'a, Yemen

	HBV load		Statistical analysis			
	Low (n=151)	High (n=59)	Univariate [†]	Multivariate [‡]		
	n (%)	n (%)	OR (95% CI)	B (95% CI)	SE	OR (95% CI)
HBeAg						
-ve (n=176)	145 (96)	31 (52.5)	1	1		1
+ve (n=34)	6 (4)	28 (47.5)	21.8 (8.3, 57)***	3.7 (2.7, 6.4)**	2.4	39.2 (11.8, 130)***
Khat chewing						
No (n=105)	88 (58.3)	17 (28.8)	1	1		1
Yes (n=105)	63 (41.7)	42 (71.2)	3.5 (1.8, 6.6)***	1.7 (0.8, 3.4)**	1.3	5.7 (2.1, 15.5)**
Smoking						
No (n=177)	135 (89.4)	42 (71.2)	1	1		1
Yes (n=33)	16 (10.6)	17 (28.8)	3.4 (1.6, 7.3)**	1.1 (0.2, 2.4)*	0.6	3 (1.2, 8)*
Age (years)						
20-29 (n=50)	35 (23.2)	15 (25.4)	1	1		1
30-39 (n=89)	71 (47)	18 (30.5)	0.6 (0.3, 1.3)	-0.1 (-1.3, 1.1)	0.8	0.9 (0.3, 2.7)
40-49 (n=26)	16 (10.6)	10 (16.9)	1.5 (0.5, 3.9)	0.1 (-1.6, 1.9)	0.9	1.1 (0.3, 4.4)
≥50 (n=45)	29 (19.2)	16 (27.1)	1.3 (0.6, 3)	0.5 (-0.7, 1.9)	0.6	1.7 (0.5, 5.6)
Sex						
Female (n=54)	36 (23.8)	18 (30.5)	1	1		1
Male (n=156)	115 (76.2)	41 (69.5)	0.7 (0.4, 1.4)	-0.4 (-1.5, 0.8)	0.6	0.7 (0.3, 1.9)
Residence						
Sana'a (n=67)	54 (35.8)	13 (22)	1	1		1
Other cities (n=143)	97 (64.2)	46 (78)	1.9 (0.9, 3.9)	0.9 (-0.2, 2.6)	0.7	2.4 (0.9, 6.2)
Hospital						
Athawra (n=78)	54 (35.8)	24 (40.7)	1	1		1
Sci. & Tech. (n=132)	97 (64.2)	35 (59.3)	0.8 (0.4, 1.5)	-0.3 (-1.2, 0.7)	0.5	0.8 (0.3, 1.7)

[†]Univariate association analysis resulted in unadjusted odds ratio (OR) based on logistic regression with single variable.
[‡]Multivariate statistics were based on logistic regression model for high viral load prediction adjusted for all socio-demographic and recruitment-site variables included. Low HBV load: ≤2000 IU/ml; High HBV load: >2000 IU/ml. B: Beta coefficient; SE: standard error of B coefficient and 95% confidence interval (CI) were based on 1000 bootstraps. ORs were rounded to first decimal point. *p<0.05; **p<0.01; ***p<0.001. Logistic model characteristics: Hosmer and Lemeshow p=0.33, Nagelkerke R²= 0.486; -2log-likelihood=162.935.

Table (3): Logistic regression models with khat chewing frequency or combined khat chewing-smoking variables

	HBV load		Multivariate analysis			
	Low (n=151)	High (n=59)				
	n (%)	n (%)	B (95% CI)	P	OR (95% CI)	P
Khat chewing frequency[†]						<0.001
No (n=105)	88 (58.3)	17 (28.8)	1		1	
1-3 days/week (n=13)	10 (6.6)	3 (5.1)	0.75 (-18.3, 2.5)	0.3	2.1 (0.3, 14)	0.4
7 days/week (n=92)	53 (35.1)	39 (66.1)	2 (1, 3.7)	0.001	7.1 (2.4, 20.7)	<0.001
Smoking (yes vs no)	16 (10.6)	17 (28.8)	0.98 (-0.18, 2.3)	0.08	2.7 (1, 7.2)	0.05
Logistic regression with combined effect of khat chewing and smoking[‡]						
Khat chewing-smoking						
No khat, No smoke (n=103)	86 (57)	17 (28)	1		1	
+ Khat, No smoke (n=74)	49(32.5)	25 (42.4)	1.66 (0.8-3, 3.5)	0.001	5.3 (1.9, 14.5)	0.001

No Khat, + smoke (n=2)	2 (1.3)	0 (0)	-18.4 (-19.2, -16.4)	0.004	0.000 [§]	0.99
+ khat, +smoke (n=31)	14 (9.3)	17	2.8 (1.8, 4.7)	0.001	17 (5, 57.4)	<0.001

† Logistic regression with khat chewing frequency (showing the effect on smoking variable) adjusted for all other variables: grouped age, sex, smoking, HBeAg status, residence and recruitment hospital. ‡ Adjusted for all other variables. B: Beta coefficient and 95% confidence interval (CI) were based on 1000 bootstraps. § OR was not calculated due to low number of cases in the low viral load group and lack of cases in the high load group. OR (odds ratio) numbers were rounded to first decimal point. Low HBV load: ≤2000 IU/ml; High HBV load: >2000 IU/ml.

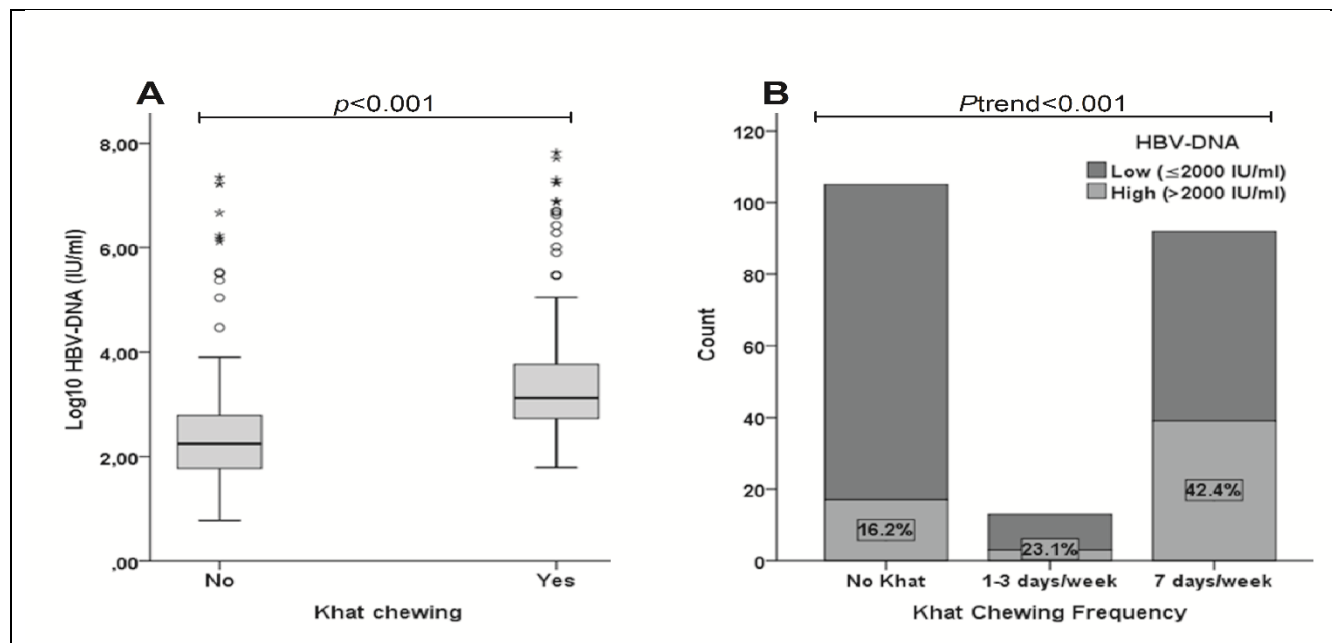


Figure (1): A: Box and whiskers plot of log10 serum hepatitis B virus (HBV) DNA (in international units per ml -IU/ml) in chronic hepatitis B khat chewers and non chewers showing a significant difference between the two groups according to Mann Whitney U test. **B:** Stacked graphs of number & percentage of chronic hepatitis B participants according to their khat chewing frequency (in days per week) and their serum viral load (high (>2000 IU/ml), low (≤2000 IU/ml)). Chi square test was used for linear trend.

4. Discussion

The importance of viral load in CHB is well recognized, and our study examined HBV load in relation to khat chewing. The current study found a highly statistically significant association between khat chewing and clinically significant high viral load in CHB cases. Khat chewing maintained an independent association after adjustment for age, sex, smoking, and HBeAg status. Such association was especially significant among

participants chewing khat on a daily basis, who constituted the vast majority of chewers in our sample. Daily khat chewers were 7.1 times more likely to have HBV load >2000 IU/ml than non chewers.

While the association of high HBV load with HBeAg sero-positivity found in this study has actually been well established [1; 14], the association with cigarette smoking in this study was less straight forward because of the low number of smokers who do not chew khat (only

two), none of whom had high viral load. Our study, with such a limitation to investigate smoking nonetheless, showed that smoking had at least an additive effect of smoking to chewing on the viral load. Three studies have reported viral load in relation to smoking. Chook et al. [29] did not find a significant association in a recent cohort study investigating the protective effect of coffee drinking. However, in two studies focusing on fibrosis and HCC risk factors in male cohorts with special reference to smoking, Xiong et al. [30] did not find a significant difference in viral load between smokers and nonsmokers unless propensity score matching was undertaken, while Wang et al. [31] provided evidence of the association of smoking with high viremia and went further to suggest smoking-induced viremia as a mediatory for HCC development.

In agreement with the present study, Chook et al. [29] found neither gender nor age to be significantly associated with high viral load in Malaysia while investigating the effect of coffee drinking on HBV load. Furthermore, apparently, characteristics of khat chewers, found in this study among CHB participants, did not differ from those in the healthy general population. For instance, a significant association between khat chewing and cigarette smoking was found in Ethiopian students [32] and Yemeni residents in the UK [33]. In addition, the higher prevalence of khat chewing among males than females and the relation to age have also been previously reported [22; 34; 35].

Our study contrasted the report of Sallam et al. [5], who did not find HBeAg positive cases in their sample from Taiz, but we found eight from the same city. This could simply be explained by the fact that HBeAg positivity is largely related to the stage of infection and to the development of precore mutants [14].

Although the exact mechanism of interaction between khat, which contains many different alkaloids and other compounds [36], and HBV

infection is largely unknown, the picture is further complicated by the potential contamination of khat with organophosphorus compounds that are heavily used during cultivation and are capable of causing liver damage [37-39]. There is accumulating evidence that khat can adversely affect the liver in animal models [40; 41] and could be associated with acute and chronic liver disease in humans [24; 25; 42-44]. Some previous cell culture work [45; 46] proposes the generation of reactive oxygen species (oxidative stress) and induction of liver cell apoptosis, at least in part, as an explanatory mechanism for the nature of the liver insult. Autoantibodies, found in some cases [47], suggested autoimmune hepatitis as another mechanism, but such a hypothesis was not supported in a recent case-controlled study [26]. On the other hand, the association of khat chewing with high viremia, which is indicative of viral replication, might be explained by the potential adverse effect of khat on the immune system or its components addressed before, albeit mostly, in animal models [48-51].

Two limitations of this study should be taken into consideration when interpreting the results and making generalizations. First, the study depended on a single-point testing of HBV DNA level in the sera of CHB participants, which makes long-term temporal relations less evident. Secondly, the study was not all-inclusive of factors that might affect viral load, like the recently reported coffee drinking [29] and possibly the effect of the duration of infection beyond the six-month cutoff mark. However, according to the E-value calculation [52], an unmeasured covariate/confounder should have an $OR \geq 4.21$ -fold with both khat chewing and high HBV each in order to explain away the present association between the latter two, which is not the case for coffee drinking. Hepatitis D virus and HBV genotype were not investigated in this study under the assumptions that coinfection with hepatitis D virus is very rare in Yemen, according to previous studies from various regions in Yemen [2; 53] and that HBV type B is

the most dominant genotype and no significant difference in viral load vs. the rare genotype A was observed [54; 55]. Alcohol drinking, illegal in Yemen and assumed to have low prevalence, was not observed here but was not associated with high viremia in a recent cohort study either [31].

5. Conclusion

This study highlights the association of khat chewing with HBV load and suggests khat chewing as a potential risk factor for clinically significant high viremia. Together with previous studies of khat's harmful effects, it underpins the necessity to discourage khat chewing by healthcare professionals, especially in CHB cases. Further studies to investigate such an association and its mechanism would be worthwhile.

6. References

- [1] World Health Organization. WHO Global Hepatitis Report. Geneva: WHO, 2017. www.who.int/publications/i/item/9789241565455.
- [2] Al-Nabehi BAH, Al-Shamahy H, Saeed WSE, Musa AM, Hassan AM, Khalil EAG. Sero-Molecular Epidemiology and Risk Factors of Viral Hepatitis in Urban Yemen. *International Journal of Virology* 2015; 11(3):133-138. DOI: [10.3923/ijv.2015.133.138](https://doi.org/10.3923/ijv.2015.133.138).
- [3] Bawazir AA, Parry CM, Hart CA, Sallam TA, Beeching N, Cuevas LE. Seroepidemiology and risk factors of hepatitis B virus in Aden, Yemen. *J Infect Public Health* 2011; 4(1):48-54. DOI: [10.1016/j.jiph.2010.11.003](https://doi.org/10.1016/j.jiph.2010.11.003).
- [4] Gacche RN, Kaid AM. Epidemiology of viral hepatitis B and C infections in Ibb city, Yemen. *Hepat Mon* 2012; 12(7):460-462. DOI: [10.5812/hepatmon.6140](https://doi.org/10.5812/hepatmon.6140).
- [5] Sallam TA, Raja'a YA, Bahaj S, Al Shami AM, Lu M, Roggendorf M et al. Hepatitis B virus carrier rate, prevalence and susceptibility and impact of immunization program among households in the city of Taiz, Yemen. *Vaccine* 2012; 30(37):5564-5568. DOI: [10.1016/j.vaccine.2012.06.008](https://doi.org/10.1016/j.vaccine.2012.06.008).
- [6] Murad EA, Babiker SM, Gasim GI, Rayis DA, Adam I. Epidemiology of hepatitis B and hepatitis C virus infections in pregnant women in Sana'a, Yemen. *BMC Pregnancy Childbirth* 2013; 13:127. DOI: [10.1186/1471-2393-13-127](https://doi.org/10.1186/1471-2393-13-127).
- [7] Bajubair MA, Elrub AA, Bather G. Hepatic viral infections in Yemen between 2000--2005. *Saudi Med J* 2008; 29(6):871-874. PM:18521468.
- [8] Thabit AM, Al-Moyed KA, Al-Balushi MS, Hasson SS, Sallam TA. Occult hepatitis B virus among chronic liver disease patients in Yemen. *Asian Pacific Journal of Tropical Disease* 2012; 4-6. DOI: [10.4236/aim.2022.123008](https://doi.org/10.4236/aim.2022.123008).
- [9] Guneid AM, Gunaid AA, O'Neill AM, Zureikat NI, Coleman JC, Murray-Lyon IM. Prevalence of hepatitis B, C, and D virus markers in Yemeni patients with chronic liver disease. *J Med Virol* 1993; 40(4):330-333. PM:8228926.
- [10] Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; 130(3):678-686. DOI: [10.1053/j.gastro.2005.11.016](https://doi.org/10.1053/j.gastro.2005.11.016).
- [11] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295(1):65-73. DOI: [10.1001/jama.295.1.65](https://doi.org/10.1001/jama.295.1.65),
- [12] Mendy ME, Welzel T, Lesi OA, Hainaut P, Hall AJ, Kuniholm MH et al. Hepatitis B viral load and risk for liver cirrhosis and hepatocellular carcinoma in The Gambia, West Africa. *J Viral Hepat* 2010; 17(2):115-122. DOI: [10.1111/j.1365-2893.2009.01168.x](https://doi.org/10.1111/j.1365-2893.2009.01168.x).
- [13] Zhou JY, Zhang L, Li L, Gu GY, Zhou YH, Chen JH. High hepatitis B virus load is associated with hepatocellular carcinomas development in Chinese chronic hepatitis B patients: a case control study. *Virol J* 2012; 9:16. DOI: [10.1186/1743-422X-9-16](https://doi.org/10.1186/1743-422X-9-16).
- [14] EASL. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; 67(2):370-398. DOI: [10.1016/j.jhep.2017.03.021](https://doi.org/10.1016/j.jhep.2017.03.021).
- [15] Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. *J Hepatol* 2008;(49):634-651. DOI: [10.1016/j.jhep.2008.07.013](https://doi.org/10.1016/j.jhep.2008.07.013).
- [16] Lee LY, Lee GH, Mattar C, Saw S, Aw M. Maternal HBeAg positivity and viremia associated with umbilical cord blood hepatitis B viremia. *Pediatr Neonatol* 2019; 60(5):517-522. DOI: [10.1016/j.pedneo.2019.01.002](https://doi.org/10.1016/j.pedneo.2019.01.002).
- [17] Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. *Am J Gastroenterol* 2006; 101(8):1797-1803. DOI: [10.1111/j.1572-0241.2006.00647.x](https://doi.org/10.1111/j.1572-0241.2006.00647.x).

- [18] Yang T, Lu JH, Zhai J, Lin C, Yang GS, Zhao RH et al. High viral load is associated with poor overall and recurrence-free survival of hepatitis B virus-related hepatocellular carcinoma after curative resection: a prospective cohort study. *Eur J Surg Oncol* 2012; 38(8):683-691. DOI:[10.1016/j.ejso.2012.04.010](https://doi.org/10.1016/j.ejso.2012.04.010).
- [19] World Health Organization. WHO Expert Committee on Drug Dependence. *World Health Organ Tech Rep Ser* 2006; (942):i, 1-i, 4. PM:17373571.
- [20] Numan N. Exploration of adverse psychological symptoms in Yemeni khat users by the Symptoms Checklist-90 (SCL-90). *Addiction* 2004; 99(1):61-65. PM:14678063.
- [21] Al Mugahed L. Khat chewing in Yemen: turning over a new leaf. *Bull World Health Organ* 2008; 86(10):741-742. DOI:[10.2471/blt.08.011008](https://doi.org/10.2471/blt.08.011008).
- [22] Numan N. The Green Leaf: Khat. *World J Med Sci* 2012; 7(4):210-223. DOI:[10.1111/j.1360-0443.2004.00570.x](https://doi.org/10.1111/j.1360-0443.2004.00570.x).
- [23] Al Abed AA, Sutan R, Al Dubai SA, Aljunid SM. Family context and Khat chewing among adult Yemeni women: a cross-sectional study. *Biomed Res Int* 2014; 2014:505474. DOI:[10.1155/2014/505474](https://doi.org/10.1155/2014/505474).
- [24] Mahamoud HD, Muse SM, Roberts LR, Fischer PR, Torbenson MS, Fader T. Khat chewing and cirrhosis in Somaliland: Case series. *Afr J Prim Health Care Fam Med* 2016; 8(1):e1-e4. DOI:[10.4102/phcfm.v8i1.1124](https://doi.org/10.4102/phcfm.v8i1.1124).
- [25] Orlien SMS, Sandven I, Berhe NB, Ismael NY, Ahmed TA, Stene-Johansen K et al. Khat chewing increases the risk for developing chronic liver disease: A hospital-based case-control study. *Hepatology* 2018; 68(1):248-257. DOI:[10.1002/hep.29809](https://doi.org/10.1002/hep.29809).
- [26] Orlien SMS, Ahmed TA, Ismael NY, Berhe N, Lauritzen T, Gundersen SG et al. High Seroprevalence of Autoantibodies Typical of Autoimmune Liver Disease in Eastern Ethiopia: Is Chewing of Khat (*Catha edulis*) a Triggering Factor? *Can J Gastroenterol Hepatol* 2018; 2018:4980597. DOI:[10.1155/2018/4980597](https://doi.org/10.1155/2018/4980597).
- [27] World Health Organization. Guidelines for the prevention care and treatment of persons with chronic hepatitis B infection. Geneva: WHO, 2015. ISBN: 978 92 4 154905 9.
- [28] EASL. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; 50(2):227-242. DOI:[10.1016/j.jhep.2008.10.001](https://doi.org/10.1016/j.jhep.2008.10.001).
- [29] Chook JB, Ngeow YF, Tee KK, Lee JWT, Mohamed R. Increased Coffee Intake Reduces Circulating HBV DNA and HBsAg Levels in HBsAg-Negative Infection: A Cohort Study. *Viruses* 2019; 11(9). DOI:[10.3390/v11090808](https://doi.org/10.3390/v11090808).
- [30] Xiong M, Li J, Yang S, Zeng F, Ji Y, Liu J et al. Impacts of cigarette smoking on liver fibrosis and its regression under therapy in male patients with chronic hepatitis B. *Liver Int* 2019; 39(8):1428-1436. DOI:[10.1111/liv.14108](https://doi.org/10.1111/liv.14108).
- [31] Wang YH, Chuang YH, Wu CF, Jan MC, Wu WJ, Lin CL et al. Smoking and Hepatitis B Virus-Related Hepatocellular Carcinoma Risk: The Mediating Roles of Viral Load and Alanine Aminotransferase. *Hepatology* 2019; 69(4):1412-1425. DOI:[10.1002/hep.30339](https://doi.org/10.1002/hep.30339).
- [32] Gebrie A, Alebel A, Zegeye A, Tesfaye B. Prevalence and predictors of khat chewing among Ethiopian university students: A systematic review and meta-analysis. *PLoS One* 2018;13(4):e0195718. DOI:[10.1371/journal.pone.0195718](https://doi.org/10.1371/journal.pone.0195718).
- [33] Kassim S, Croucher R, al'Absi M. Khat dependence syndrome: a cross sectional preliminary evaluation amongst UK-resident Yemeni khat chewers. *J Ethnopharmacol* 2013; 146(3):835-841. DOI:[10.1016/j.jep.2013.02.011](https://doi.org/10.1016/j.jep.2013.02.011).
- [34] Ageely HM. Prevalence of Khat chewing in college and secondary (high) school students of Jazan region, Saudi Arabia. *Harm Reduct J* 2009; 6:11. DOI:[10.1186/1477-7517-6-11](https://doi.org/10.1186/1477-7517-6-11).
- [35] Mahfouz MS, Rahim BE, Solan YM, Makeen AM, Alsanosy RM. Khat Chewing Habits in the Population of the Jazan Region, Saudi Arabia: Prevalence and Associated Factors. *PLoS One* 2015;10(8):e0134545. DOI:[10.1371/journal.pone.0134545](https://doi.org/10.1371/journal.pone.0134545).
- [36] Halbach H. Medical aspects of the chewing of khat leaves. *Bull World Health Organ* 1972; 47(1):21-29. PM:4538902.
- [37] Al-Mhbashi H, Al-Shoba K. The Potential Toxicity of Organophosphorus Compounds on Yemeni Qat- Farmers and Consumers. *Sana'a University Journal of Medical Sciences (SUJMS)* 2014; 6(1):117-124.
- [38] Engidawork E. Pharmacological and Toxicological Effects of *Catha edulis* F. (Khat). *Phytother Res* 2017; 31(7):1019-1028. DOI:[10.1002/ptr.5832](https://doi.org/10.1002/ptr.5832).
- [39] Karami-Mohajeri S, Ahmadipour A, Rahimi HR, Abdollahi M. Adverse effects of organophosphorus pesticides on the liver: a brief summary of four decades of research. *Arh Hig Rada Toksikol* 2017; 68(4):261-275. DOI:[10.1515/aiht-2017-68-2989](https://doi.org/10.1515/aiht-2017-68-2989).
- [40] 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. DOI:[10.1016/s0378-8741\(02\)00223-4](https://doi.org/10.1016/s0378-8741(02)00223-4).
- [41] A, Abdulla MA, Al Mamary M, Noordin MI, Abdelwahab SI, Alabsi AM et al. Toxicological

- Features of *Catha edulis* (Khat) on Livers and Kidneys of Male and Female Sprague-Dawley Rats: A Subchronic Study. *Evid Based Complement Alternat Med* 2012; 2012. DOI:[10.1155/2012/829401](https://doi.org/10.1155/2012/829401).
- [42] Brostoff JM, Plymen C, Birns J. Khat--a novel cause of drug-induced hepatitis. *Eur J Intern Med* 2006; 17(5):383. DOI:[10.1016/j.ejim.2005.12.010](https://doi.org/10.1016/j.ejim.2005.12.010).
- [43] Chapman MH, Kajihara M, Borges G, O'Beirne J, Patch D, Dhillon AP et al. Severe, acute liver injury and khat leaves. *N Engl J Med* 2010; 362(17):1642-1644. DOI:[10.1056/NEJMc0908038](https://doi.org/10.1056/NEJMc0908038).
- [44] 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. DOI:[10.1111/j.1478-3231.2010.02228.x](https://doi.org/10.1111/j.1478-3231.2010.02228.x).
- [45] 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):389-395. DOI:[10.3892/ijmm.2013.1394](https://doi.org/10.3892/ijmm.2013.1394).
- [46] Mohan S, Abdelwahab SI, Hobani YH, Syam S, Al Zubairi AS, Al Sanousi R et al. *Catha edulis* Extract Induces H9c2 Cell Apoptosis by Increasing Reactive Oxygen Species Generation and Activation of Mitochondrial Proteins. *Pharmacogn Mag* 2016; 12(Suppl 3):S321-S326. DOI:[10.4103/0973-1296.185732](https://doi.org/10.4103/0973-1296.185732).
- [47] S, Imran M, Gleeson D, Karajeh MA. Khat (*Catha Edulis*) as a possible cause of autoimmune hepatitis. *World J Hepatol* 2014; 6(3):150-154. DOI:[10.4254/wjh.v6.i3.150](https://doi.org/10.4254/wjh.v6.i3.150).
- [48] Ketema T, Yohannes M, Alemayehu E, Ambelu A. Evaluation of immunomodulatory activities of methanolic extract of khat (*Catha edulis*, Forsk) and cathinone in Swiss albino mice. *BMC Immunol* 2015; 16. DOI:[10.1186/s12865-015-0072-5](https://doi.org/10.1186/s12865-015-0072-5).
- [49] House RV, Thomas PT, Bhargava HN. Comparison of immune functional parameters following in vitro exposure to natural and synthetic amphetamines. *Immuno pharmacol Immunotoxicol* 1994; 16(1):1-21. DOI:[10.3109/08923979409029897](https://doi.org/10.3109/08923979409029897).
- [50] Al Habori M, Al Mamary M. Long-term feeding effects of *Catha edulis* leaves on blood constituents in animals. *Phytomedicine* 2004; 11(7-8):639-644. DOI:[10.1016/j.phymed.2003.06.004](https://doi.org/10.1016/j.phymed.2003.06.004).
- [51] Bin-Jalilah I, Dallak M, Al-Hashem FH, Nwoye LO, Sakr HF, Jamil A et al. Derangement of hemopoiesis and hematological indices in Khat (*Catha edulis*) - treated rats. *African Journal of Biotechnology* 2014; 13(2):349-355. DOI:[10.5897/AJB2013.13373](https://doi.org/10.5897/AJB2013.13373).
- [52] VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med* 2017; 167(4):268-274. DOI:[10.7326/M16-2607](https://doi.org/10.7326/M16-2607).
- [53] Sallam TA, Tong CY, Cuevas LE, Raja'a YA, Othman AM, Al Kharsa KR. Prevalence of blood-borne viral hepatitis in different communities in Yemen. *Epidemiol Infect* 2003; 131(1):771-775. DOI:[10.1017/s0950268803008653](https://doi.org/10.1017/s0950268803008653).
- [54] Sallam TA, William Tong CY. African links and hepatitis B virus genotypes in the Republic of Yemen. *J Med Virol* 2004; 73(1):23-28. DOI:[10.1002/jmv.20056](https://doi.org/10.1002/jmv.20056).
- [55] Sallam TA, Tong CY. Two distinct types of hepatitis B virus core promoter variants in Yemeni blood donors. *J Med Virol* 2002; 68(3):328-334. DOI:[10.1002/jmv.10207](https://doi.org/10.1002/jmv.10207).