SERUM VITAMIN D AND B_{12} LEVELS IN ALCOHOLIC MALE PATIENTS: A CROSS-SECTIONAL STUDY

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ABSTRACT

INTRODUCTION

Alcohol dependence is a global problem and is rapidly increasing in developing countries. We aimed to analyze the serum levels of vitamin B_{12} and vitamin D in chronic alcoholic patients and their association with parameters of liver function.

MATERIAL AND METHODS

A cross-sectional study was carried out in Universal College of Medical Sciences, Bhairahawa, Nepal from March 2020 to September 2020 on patients visiting the Psychiatric Out Patient Department (OPD) for the treatment of alcohol dependence. The patients were categorized as excessive and moderate drinkers. Serum vitamin B_{12}, vitamin D, and hepatic function parameters were measured.

RESULTS

The median serum vitamin B_{12} and vitamin D levels were 467.8 pg/ml and 24.9 ng/ml respectively. Excessive drinkers had significantly higher B_{12} levels than moderate drinkers. Vitamin B_{12} levels correlated positively with liver function parameters, as well as alcohol amount and duration of consumption. Vitamin D levels were insufficient in 57 (71.25%) of the overall participants.

CONCLUSION

Serum levels of vitamin B_{12} are not affected in patients with alcohol dependence. Alcohol consumption, however, reduces serum concentrations of vitamin D. Vitamin B_{12} concentration is positively associated with liver enzymes and other parameters of liver function.

KEYWORDS

Alcoholism, Liver function parameters, Vitamin B_{12}, Vitamin D.

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INTRODUCTION

Alcohol abuse is a common problem throughout the world and its consumption is rapidly increasing in developing countries including Nepal.[1,2] It has been well documented that alcohol is toxic to the human body and can damage multi organs such as the liver, heart, and pancreas among others.[3]

Levels of serum vitamin B_{12} vary in alcoholic subjects and there exist sex differences in the serum vitamin B_{12} levels.[4] In a study conducted on alcohol cirrhotic patients, Airoldi M et al and Lamber D et al found elevated levels of serum vitamin B_{12} and plasma cobalamin.[5,6]

Some studies have found a positive association between alcohol consumption and vitamin D while other studies have found a negative association between them.[7-11] The present study aimed to investigate the possible relationship between alcohol use and serum levels of these two vitamins along with liver function tests amongst the Nepalese population where alcohol consumption is very common.

MATERIAL AND METHODS

A hospital based cross-sectional study was performed on 80 alcoholic men. The participants included the patients visiting the Psychiatric OPD for the treatment of alcohol dependence without clinical and radiological signs of alcoholic liver disease (ALD). The ethical approval was taken from the Institutional Review Committee of the Universal College of Medical Sciences (UCMS/IRC/020/20) before the study. Patients with a known recent history of jaundice, liver, bone, and renal diseases and participants under drugs that altered serum Ca, P, and uric acid levels were excluded from the study. Both verbal and written consents were taken from the participants. The study duration was from March 2020 to September 2020.

A convenient sampling technique was used and each participant was required to fill a proforma that included their socio-demographic status, amount and duration of alcohol consumed, and laboratory parameters which were filled after measurements. Participants were characterized as excessive and moderate drinkers according to the CDC criteria. Both binge drinkers (≥5 drinks/occasion where one occasion equals 2-3 hours) and heavy drinkers (≥15 drinks/week) were considered as excessive drinkers.[12]

Serum vitamin B_{12} and vitamin D (25-hydroxy cholecalciferol) were analyzed via chemiluminescence assay (Maglumi 2000). Serum AST, ALT, ALP and phosphate levels were measured using the automated analyzer (Humastar 600). Serum bilirubin levels were estimated using a semi-automated analyzer by Jendrassik and Grof's method (Biossay 240+). Serum uric acid and calcium were estimated using colorimetric analysis by modified phosphotungstate assay and ortho-cresolphthalein method respectively.

The reference ranges for the laboratory parameters were considered as per the manufacturer's manual. They were as follows: vitamin B_{12}: 200-1100 pg/ml, vitamin D: 30-100 ng/ml (10-30 ng/ml was considered insufficient and <10 ng/ml was considered deficient), ALT and AST: up to 45 IU/L, ALP: 80-306 IU/L, albumin: 3.5-5.3 gm/dl, total protein: 6-8 gm/dl, total bilirubin: 0.3-1.2 mg/dl, direct bilirubin: 0.1-0.4 mg/dl, indirect bilirubin: 0.2-0.8 mg/dl, uric acid: 4.4-7.6 mg/dl, calcium: 9-11 mg/dl, and phosphate: 3-5 mg/dl.

The data were analyzed using statistical package for social sciences (SPSS) version 16. As the data were non-normally distributed, data were expressed in their median values along with their interquartile ranges. Non-parametric tests like the Mann-Whitney U test, Kruskal-Wallis test, and Spearman's correlation analysis were performed to analyze the data.

RESULTS

A total of 80 alcoholic male participants of 21 to 67 years were included in the study. The participants were categorized as moderate and heavy drinkers according to the CDC criteria.[12] Out of the total, 59 participants were heavy drinkers. The distribution of the study participants is shown in Figure 1.

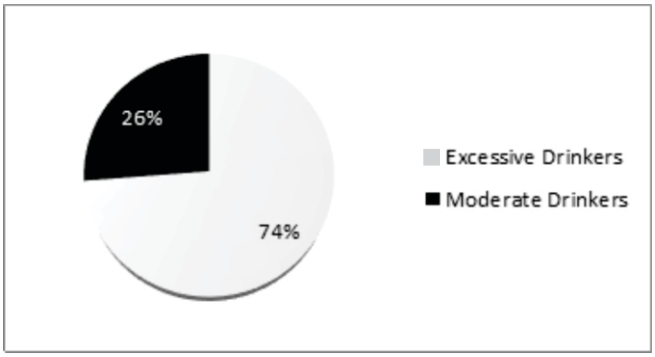

Figure 1. Pattern of drinking in the study participants (n= 80)

Out of the total 80, only 14 patients had serum vitamin B_{12} levels below the reference range of which nine were moderate drinkers. The median serum vitamin D level was found to be insufficient in the study participants (both total and within groups). Out of the total 80 participants, 57 patients had insufficient serum vitamin D levels of which 42 were excessive drinkers. None of the patients were deficient. Serum vitamin B_{12} levels were significantly high in the heavy drinkers as compared to the moderate drinkers. Similarly, serum levels of liver enzymes AST and ALT as well as bilirubin and uric acid were also found to be significantly higher in the heavy drinkers. Serum albumin and total protein

levels were significantly lower in the heavy drinkers (Table 1).

Table 1. Distribution of numerical variables among the study participants and between alcoholic groups

Variables	Reference range	Total population (n = 80)	Excessive drinker (n = 59)	Moderate drinker (n = 21)	*p*-value
Age (years)	N/A	36 (28-45)	39 (35-47)	26 (25-29.5)	<0.001
BMI (Kg/m ^{2})	18-24.9	23.12 (20.79-25.31)	22.89 (20.15-25.10)	24.38 (22.40-25.65)	0.095
Alcohol amount (ml)	N/A	500 (250-1000)	750 (500-1500)	250 (190-300)	<0.001
Alcohol duration (yrs)	N/A	14.5 (8.3-20)	17(11-24)	7 (5.5-11)	<0.001
Vit B_{12} (pg/ml)	200-1100	467.8 (261.3-1957.9)	925 .9 (400.5-2000.0)	258 .4 (143.8-395.8)	<0.001
Vit D (ng/ml)	30-100	24.9 (18.9-32.6)	24.9 (19.2-32.8)	24.1 (16.7-32.6)	0.544
AST (IU/L)	Up to 45	51.5 (30.2-122.0)	81.0 (41.0-154.0)	28.0 (24.5-32.0)	<0.001
ALT (IU/L)	Up to 45	50.5 (36.0-85.7)	64.0 (41.0-112.0)	37.0 (29.0-47.0)	<0.001
ALP (IU/L)	80-306	213.0 (179.5-280.0)	213.0 (176.0-297.0)	216.0 (189.5-263.5)	0.642
Albumin (g/dl)	3.8-5.3	4.7 (4.3-4.9)	4.5 (4.2-4.9)	4.8 (4.6-5.1)	0.004
Total p rotein (g/dl)	6-8	7.8 (7.2-8.0)	7.6 (7.2-7.9)	7.9 (7.6-8.2)	0.035
T. bilirubi n (mg/dl)	0.3-1.2	0.8 (0.5-1.1)	1.0 (0.7-1.3)	0.5 (0.5-0.6)	<0.001
D. bilirubin (mg/dl)	0.1-0.4	0.3 (0.2-0.5)	0.4(0.2-0.7)	0.2 (0.2-0.3)	<0.001
I. bilirubin (mg/dl)	0.2-0.8	0.5 (0.3-0.7)	0.6 (0.3-0.7)	0.3 (0.3-0.4)	0.001
Ca (mg/dl)	9-11	8.9 (8.6-9.6)	8.9 (8.5-9.6)	8.9 (8.6-9.6)	0.565
P (mg/dl)	3-5	3.9 (3.2-4.3)	3.9 (3.1-4.5)	3.9 (3.4-4.1)	0.883
Uric a cid (mg/dl)	4.4-7.6	4.5 (3.7-5.9)	5.0 (3.7-6.1)	3.9 (3.4-5.0)	0.042
SBP (mm/Hg)	120	120 (120-120)	120 (110-130)	120 (120-120)	0.952
DBP (mm/Hg)	80	80 (70-80)	80 (70-80)	80(80-80)	0.189

N/A=Not applicable; Vit D Vitamin D; AST Aspartate transaminase; ALT Alanine transaminase; ALP Alkaline phosphatase; SBP-Systolic blood pressure; DBP-Diastolic blood pressure. Data are expressed as median (25^{th}-75^{th} percentile). *p*-values obtained from Mann-Whitney U test. *p*-values <0.05 considered statistically significant.

Socio-demographic profiles of the study participants were compared between heavy and moderate drinkers. All of the parameters including smoking habits were found to be significantly different between the groups (Table 2).

Table 2. Socio-demographic profile parameters of the study population

Variables		Total (n = 80)	Alcoholic group: Excessive drinkers (n = 59)	Alcoholic group: Moderate drinkers (n = 21)	*p*-value
Type of family	Joint	13	13	0	0.019
	Nuclear	67	46	21	
Marital status	No	22	5	17	<0.001
	Yes	58	54	4	
Education	Illiterate	2	2	0	<0.001
	<10	27	27	0	
	10 to 12	23	23	0	
	Bachelor	21	6	15	
	Masters	7	1	6	
Smoking	No	31	27	4	0.031
	Yes	49	32	17	
Occupation	Private/public office	33	28	5	<0.001
	Student	18	5	13	
	Driver/labor/plumber/farmer	20	20	0	
	Retired	5	5	0	
	Doctor/Engineer	4	1	3	

p–values obtained from chi-square analysis.

Serum vitamin B_{12}, AST, total bilirubin, and direct bilirubin levels had a significant but weak positive correlation with both the parameters. The details are presented in Table 3.

Table 3. Association of numerical variables with amount and duration of alcohol consumption

Variables	Amount of alcohol (ml) ρ	Amount of alcohol (ml) *p*-value	Duration of drinking (years) ρ	Duration of drinking (years) *p*-value
Vit B_{12}	0.506	**<0.001**	0.493	<0.001
Vit D	0.080	0.483	0.183	0.105
AST	0.593	**<0.001**	0.415	<0.001
ALT	0.273	**0.014**	0.027	0.813
ALP	- 0.068	0.548	0.156	0.168
TB	0.509	**<0.001**	0.323	0.003
DB	0.485	**<0.001**	0.355	<0.001
IB	0.446	**<0.001**	0.213	0.057
TP	**-0.205**	0.069	**-0.139**	0.220
Albumin	**-0.285**	**0.010**	-0.199	0.076
Ca	-0.283	**0.011**	-0.058	0.609
P	-0.090	0.425	-0.029	0.798
SBP	-0.099	0.380	0.030	0.790
DBP	-0.145	0.198	0.035	0.761
Uric acid	0.218	0.054	0.156	0.169

Vitamin B_{12} positively correlated with ALT, AST and bilirubin levels significantly. A significant negative correlation of vitamin B_{12} was found with serum albumin, calcium, and phosphate levels. Similarly, Vitamin D levels positively correlated with total and direct bilirubin levels (Table 4).

Table 4. Association of serum vitamin B_{12} and vitamin D levels with liver function and other laboratory parameters

Variables		ALT	AST	ALP	TB	DB	IB	Albumin	TP	Ca	P	Uric acid
B_{12}	ρ	0.335	0.638	0.156	0.471	0.423	0.438	-0.229	-0.126	-0.368	-0.329	0.101
	P	0.002	<0.001	0.168	<0.001	<0.001	<0.001	0.041	0.266	0.001	0.003	0.376
Vit D	ρ	-0.112	0.145	-0.042	0.240	0.268	0.203	-0.130	-0.025	-0.140	-0.031	0.061
	P	0.322	0.200	0.712	0.032	0.016	0.071	0.250	0.824	0.215	0.788	0.594

Vit D-Vitamin D; TP-total protein; TB-Total bilirubin; DB-Direct bilirubin; IB-Indirect bilirubin. ρ=Correlation coefficient. *p*-values obtained from Spearmann's correlation analysis. *p*-values <0.05 considered statistically significant.

DISCUSSION

Chronic alcoholism is a global burden with many socio-economic, nutritional and health hazards.[3,13] We aimed to evaluate the association of vitamin B_{12} and vitamin D levels with parameters of liver function in 80 male subjects. The participants had no clinical or radiological evidence of alcoholic liver disease (ALD) and its complications.The sociodemographic variables were significantly different in the excessive and moderate drinkers showing the risk associated in the participants in concern with these status besides biochemical variables in this study.

The median serum vitamin B_{12} level in our study was 467.8 pg/ml which is within the reference range. The participants were further categorized as excessive and moderate drinkers, and serum vitamin B_{12} level was significantly higher in the excessive drinkers (p<0.001). We found a highly significant and positive correlation of serum vitamin B_{12} levels with the both amount of alcohol consumed and the duration of drinking. In a similar study by Liappas IA et al, the mean serum vitamin B_{12} level was also within the reference range.[3]

Other studies in patients with alcohol dependence have also reported no apparent effect of alcohol consumption in vitamin B_{12} levels.[14,15] In contrast, a study published in Nature in 1969 reported impairment in vitamin B_{12} absorption in alcoholics, with evidence of ultra-structural ileal abnormalities in a subject after ethanol consumption.[16] The modern consensus is that, while serum vitamin B_{12} levels might be increased in patients with ALD, it still does not reflect the real or functional B_{12} status of the body and should be approached with caution.[17, 18] There is evidence of patients with megaloblastic anemia responding to B_{12} therapy despite normal serum levels.[17] There are few possible explanations for this, although all of the hypotheses are merely speculative as of now. Firstly, measurement of vitamin B_{12} by currently available assays is subjected to misinterpretation. Rather, the measurement of holotranscobalamin (HoloTC), the transcobalamin II-cobalamin complex (TC II-cobalamin complex) which is said to be a biologically 'active' fraction of vitamin B_{12}, might reflect a more accurate B_{12} status. Secondly, hepatic damage in ALD leads to defective storage and/or leakage of vitamin B_{12} elevating its level in serum.[17,18] Therefore, interpretation of elevated serum vitamin B_{12} levels in ALD patients as normal might be misleading.

Vitamin B_{12} positively and significantly correlated with serum AST, ALT, and serum bilirubin levels. Serum albumin had a significant negative correlation with vitamin B_{12} levels. These results support the above interpretation of increased B_{12} levels in chronic alcoholics. Similar results were obtained in various studies that included patients with alcohol dependence as well as ALD.[3,15]

Vitamin D is a fat-soluble vitamin and is even supposed to have a protective effect against alcohol-induced liver injury.[13] Nutritional status, sunlight exposure, and alcohol-induced inflammation of absorptive surfaces might play a role in determining the plasma vitamin D levels. In the present study, the median serum vitamin D level (25-hydroxy cholecalciferol) was 24.9 ng/ml, which lies in the insufficient range. Out of the total 80 participants, 57 (71.25%) had insufficient vitamin D levels. No significant association of vitamin D levels with the amount of alcohol consumed, duration of consumption and with liver enzymes. However, a weak positive correlation with total and direct bilirubin levels was observed. In a previous study conducted in Nepal, 91% of the participants had inadequate vitamin D levels, and 64% had truly deficient vitamin D levels.[19] A study by Quintero-Platt G et al also reported vitamin D insufficiency and deficiency in alcoholics which decreased with declined liver function. Similar to the results of the present study, there was no association between vitamin D levels and the amount of ethanol as well as the duration of consumption.[20] A review by Rossi RE et al also suggested decreased vitamin D levels with increased liver damage in alcoholics.[21] In another review by Tardelli VS et al, the authors noted varied association of alcohol intake with vitamin D levels. In the review, some articles noted a positive association of vitamin D with alcohol intake while others reported negative or no association.[22] Further studies with consideration of other variables like geographical variation, nutritional status, and sunlight exposure are recommended to gain a more comprehensive picture.[22]

CONCLUSION

Vitamin B_{12}, Vitamin D, and liver function parameters were analyzed in serum samples of 80 male patients with alcohol dependence. While vitamin B_{12} was within the reference range for most patients, vitamin D levels were markedly insufficient. Vitamin B_{12} was positively associated with liver enzymes and bilirubin levels and negatively associated with albumin levels. Excessive drinkers had high median serum vitamin B_{12} levels than moderate drinkers. Vitamin B_{12} levels significantly rose with prolonged duration of consumption as well as increased alcohol amount.

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