ORIGINAL ARTICLE (APMC – 556) DOI: 10.29054/APMC/18.556

Prevalence of Vitamin D Deficiency in Hepatitis C Patients

Lubna Aftab, Sadia Falak, Aftab Islam, Nabeela Faisal, Muhammad Faisal Bilal Lodhi, Sara Muzaffar

ABSTRACT

Objective: The objective of this study was to compare the vitamin D levels between HCV negative individuals and HCV positive patients. **Study design:** Randomized cross-sectional study. **Settings:** Dar-us-Shifa clinic, Faisalabad. **Duration:** 20 August, 2017 to 20 February 2018. **Methodology:** The patients were divided into two classes; control group comprised of 23 individuals who were sero-negative for anti-HCV antibody and 51 patients were hepatitis C RNA-PCR positive. HCV RNA–PCR was detected by ARTUS ® HCV QS-RGQ V1 by QIAGEN GmbH on Rotor –Gene Q5 Plex – MDx. Vitamin D levels were measured by chemiluminescence. SPSS version 20 was used for statistical analysis. **Results:** Out of 74 patients, both male and female, 53(71.6%) patients had Vitamin D deficiency irrespective of HCV sero-negative or positive. 40(78.6%) of chronic hepatitis C patients had Vitamin D deficiency.18(35.3%) of CHV patients, had severe vitamin D deficiency, 14(27.5%) had moderate vitamin D deficiency, 8(15.7%) had mild deficiency. 13 of HCV negative participants had mild to moderate vitamin D deficiency too. Mean vitamin D level of control group was 30.4 ng/mL (normal)and 25.08 ng/mL in HCV patients. **Conclusion:** Most of the patients having chronic hepatitis C suffer from vitamin D deficiency or insufficiency. The mean vitamin D value was in the optimal range in HCV negative individuals, in the suboptimal range or deficient in the HCV positive patients. The results were non-significant.

Keywords: Vitamin D, hepatitis C, liver disease.

Corresponding Author
Prof. Dr. Muhammad Faisal Bilal Lodhi
Professor of Surgery, Unit-I
FMU / Allied Hospital, Faisalabad-Pakistan
Contact: +92 333-6508406
Email: faisalbilallodhi@gmail.com

Submitted for Publication: 07-11-2018 *Accepted for Publication: 06-12-2018*

Article Citation: Aftab L, Falak S, Islam A, Faisal N, Lodhi MFB, Muzaffar S. Prevalence of Vitamin D Deficiency in Hepatitis C Patients. APMC 2018;12(4):303-6.

INTRODUCTION

Vitamin D deficiency is a global issue approximately affecting one billion individuals worldwide. Vitamin D deficiency is also prevalent in Pakistan in spite of adequate sunlight exposure all the year around. About 53.5% of our population has vitamin D deficiency, 31.2% have vitamin D insufficiency and 15.3% have normal vitamin D levels.[1] Vitamin D is one of the fat-soluble vitamins and can be called a hormone because of its endogenous synthesis. Vitamin D is not widely distributed in nature but fish, eggs and milk may be a good source. The absorption of vitamin D from gut occurs in the presence of bile acids followed by uptake by chylomicrons to the bloodstream.[2] Endodermal synthesis of vitamin D requires the presence of ultra violet B radiation present in sunlight. The precursor of vitamin D synthesis in skin is 7-dehydrocholestrol. The activation of vitamin D occurs in two step hydroxylation reactions; first hydroxylation at 25th carbon atom occurs in liver, this is one of the reasons vitamin D deficiency is related to chronic liver disease. Next hydroxylation occurs at 1st carbon of the steroid in proximal convoluted tubules of kidneys in the presence of enzyme alpha 1 hydroxylase (a member of cytochrome P 450). This leads to the formation of activated form 1,25 $(OH)_2$ vitamin D, also known as calcitriol.[3] Calcitriol can bind and activate specific nuclear receptors known as VDRs. VDRs have wide distribution in human body and are not only limited to the nuclei of many cells that are involved in the calcium and phosphate metabolism but also are widespread in other tissues. A few examples include the presence of VDR in epidermal keratinocytes, activated T cells, cells presenting antigens and phagocytes. Genomics have proven that vitamin D regulates hundreds of genes in many cells and tissues scattered all over the human body.[4,5] In general it regulates calcium levels and bone formation and resorption. However, vitamin D affects other systems also. These effects include cellular production and immune modulation involving T cells particularly. The extra-skeletal effects have strong correlation with pathophysiology and management of infectious diseases, cardiovascular abnormalities, autoimmune disorders, degenerative diseases and numerous types of cancers. The vitamin D-VDR complex in combination of retinoid X receptors forms transcription factors which in turn regulate gene expression. The transportation of vitamin D to site of action or storage site (adipose tissues) via circulation is mediated by carrier proteins made in liver. These carrier proteins are of two types; DBP (vitamin D binding protein) and albumin. 80% of vitamin D is transported and stabilized by these liver proteins. To evaluate the vitamin D status of a person, the most reliable test is to determine the level of serum 25-OH cholecalciferol because it has a longer half-life (20 days).[6] These studies lead us to compare the status of this important vitamin in HCV negative individuals and HCV positive patients. Hepatitis C virus is a rapidly spreading disease affecting about 130-150 million people worldwide. Many of HCV carriers progress to cirrhosis of liver and even to hepato-cellular carcinoma and death. An estimated 80 million people are suffering from HCV induced chronic liver disease globally. The low and middle income

countries are found to be more vulnerable. There are six genotypes of HCV which differ in geographic distribution and response to therapy.[7] Approximately 27% of cirrhosis and 25% of hepatocellular carcinoma occurring worldwide is reported in patients of chronic hepatitis C.[8] Various assessment systems used to analyze the extent of liver damage have proven a corresponding decrease in vitamin D levels with increasing liver damage.[9]

METHODOLOGY

Study Design: Randomized cross-sectional study.
Settings: Dar-us-Shifa clinic, Faisalabad.
Duration: 20 August, 2017 to 20 February 2018.
Methods: Participants were aged between 20-60 years, had no other known chronic illness and no vitamin D supplementation for last six months. The participants were divided into two classes;

- Control group comprised of 23 individuals who were sero-negative for anti-HCV antibody on the basis of screening test using an enzyme immunoassay by Abbott HCV PHA, Abbott Diagnostic.
- 51 patients with hepatitis C RNA-PCR positive on real time RT PCR-Qualitative test.[10]

Serum 25-OH-Vitamin D:

25-OH-Vitamin D serum concentrations were evaluated in all patients by enhanced chemiluminescence method, using Vitros ECi immunodiagnostic system and a dedicated reagent (Johnson & Johnson).[11] This test uses an immunoassay design to measure vitamin D_2 and D_3.

Statistical analysis:

Standard statistical analysis for biochemical assays was done on SPSS version 20. Correlation was found between patients and controls. This was followed by comparison between serum vitamin D levels with controls and cases using ANOVA. Mean ± standard deviation, range and variance were also calculated. Results were considered significant if p value was < 0.05.

RESULTS

For classification of participants with Vitamin D deficiency they were categories with severe deficiency, deficiency, insufficiency, sufficiency and toxicity group according to level of serum 25(OH) D levels as presented in Table 1.

Table 1: Range of serum 25(OH) D levels and their classification

<= 20.0ng/mL	Severe deficiency
20.1 - 25.0ng/mL	Deficient
25.1 - 30.0ng/mL	Insufficiency
30-100 ng/mL	Sufficiency
Greater than 400 ng/mL	Toxicity

To compare the vitamin D levels between HCV negative individuals and HCV positive patients, this study indicates that serum 25 OH-D levels in patients of HCV induced liver disease are close to being statistically significant (p-value = 0.067) from the patients of control group (Table 2).

Table 2: Relationship between HCV and Serum 25(OH) cholecalciferol

Serum 25(OH) cholecalciferol			HCV		Total
			Negative	Positive	
Serum 25(OH) Cholecalciferol	<= 20.0	Count	2	18	20
		% within Serum 25(OH)D	10.0%	90.0%	100.0%
		% within HCV	8.7%	35.3%	27.0%
	20.1 - 25.0	Count	6	14	20
		% within Serum 25(OH)D	30.0%	70.0%	100.0%
		% within HCV	26.1%	27.5%	27.0%
	25.1 - 30.0	Count	5	8	13
		% within Serum 25(OH)D	38.5%	61.5%	100.0%
		% within HCV	21.7%	15.7%	17.6%
	30.1+	Count	10	11	21
		% within Serum 25(OH)D	47.6%	52.4%	100.0%
		% within HCV	43.5%	21.6%	28.4%
Total		Count	23	51	74
		% within Serum 25(OH)D	31.1%	68.9%	100.0%
		% within HCV	100.0%	100.0%	100.0%

Chi-square = 7.172^{NS}; p-value = 0.067NS = Non-significant ($p>0.05$)

Out of 74 subjects, approximately half of the participants of control group had serum 25 OH-vitamin D in the optimal range. Suboptimal levels were seen in some participants of the HCV positive group while other participants of HCV positive group presented quite decreased serum 25 OH-vitamin D. The results showed that only 21 participants had serum 25-OH vitamin D levels in the optimal range that is above 30 ng /mL.47.6% of these were HCV negative and 52.4% were HCV positive. In 53 participant (71.6%) serum 25-OH- D levels were less than 30ng/mL, 40 (78.6%) of these were chronic hepatitis C patients. 18(35.3%) of chronic hepatitis C sufferers, had severe vitamin D deficiency, 14(27.5%) had moderate vitamin D deficiency, 8(15.7%) had mild deficiency. 13 were HCV negative participants which had had suboptimal vitamin D levels in this study. Among these13; 5 had mild, 6 had moderate and 2 had severe vitamin D deficiency.

Comparison for HCV RNA PCR QL and Serum 25(OH) D level also indicates non-significant relationship with HCV RNA PCR positive or negative respondents and level of 25 (OH) D as shown in Table 3 and Figure 1.

Table 3: Comparison for HCV RNA PCR QL regarding Serum 25(OH) D

HCV RNA PCR QL	N	Mean ± SD	SE	t-value	P-value
Negative	23	30.413 ±10.4287	2.1745	1.720^{NS}	.090
Positive	51	25.088 ±13.0701	1.8302		

NS = Non-significant (p>0.05)

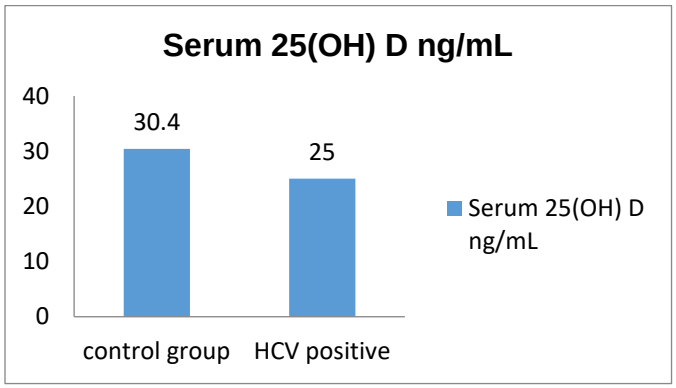

Figure 1: Mean values of serum 25(OH) cholecalciferol levels in two groups

Association between the groups included in the study regarding vitamin D levels indicated that there is insignificant relationship between the two groups and vitamin D levels as presented Table. 3 Presenting analysis of variance table for serum 25(OH) D regarding different groups.

Table 3: Analysis of variance table for serum 25(OH) D regarding different groups

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	639.487	2	319.744	2.113^{NS}	0.128
Within Groups	10744.014	71	151.324		
Total	11383.502	73			

NS = Non-significant (p>0.05)

DISCUSSION

Hepatitis C virus (HCV) is a scrupulous factor of hepatic disease and one of the leading health issues worldwide. Hepatitis C sufferers range up to approximately 175 million globally. Annually 3 to 4 million new patients with HCV are diagnosed. The problem remains endemic in Pakistan. Statistically 5.3% of Pakistani population is suffering from HCV.[12,13]

This study indicates that serum 25 OH-D levels in patients of HCV induced liver disease did not vary significantly from the patients of control group. This was because some of the HCV negative individuals were vitamin D deficient. A number of factors can be responsible for suboptimal vitamin D levels in HCV negative individuals including racial differences, BMI and dietary factors.[14] 90% of patients with severe vitamin D deficiency were HCV sufferers. Thirty-four participants had moderate vitamin D deficiency. Of these 11 were HCV RNA PCR negative and might be having insufficiency of vitamin D due to some other reason. The number of participants with normal range of vitamin D were 21, 10 of these were HCV RNA PCR negative and rest were positive. 2 participants not having chronic hepatitis C infection were vitamin D deficient, 11 patients with hepatitis C induced liver disease had normal vitamin D levels. According to some studies liver dysfunction associated with cirrhosis leads to a state of hypovitaminosis D and vitamin D deficit is not the etiology of liver cirrhosis. A number of studies are in support of the hypothesis that vitamin D deficiency is prevalent in liver diseases including viral hepatitis.[15-18] Vitamin D deficiency is also not significant in the compensated phase of liver cirrhosis and vitamin D deficiency worsens with the progression of liver disease.[19] Many studies show a strong relationship between vitamin D deficiency and advanced liver disease.[20,21] However, relationship between vitamin D and viral hepatitis needs to be further investigated. Inconsistency of results is seen with regard to the impact of vitamin Don HCV infections in some studies.[22-24] These studies have shown non-significant association of serum vitamin D levels with HCV induced liver disease.

CONCLUSION

From the above-mentioned results and discussions, it is concluded that Vitamin D deficiency is prevalent in our population irrespective of HCV. Vitamin D deficiency is seen in some of the HCV positive patients. This is attributed to decreased vitamin D absorption, activation, transportation and storage. Thus, a vicious cycle develops as lowered vitamin D decreases the immunity and increases the viral load. Early detection of HCV by screening test helps in improvement of treatment outcome. Vitamin D supplements can improve treatment outcome. Lifestyle modifications based upon increased exposure to sunlight must be advocated to improve the vitamin D status of the general population.

REFERENCES

1. Riaz H, Finlayson AE, Bashir S, Hussain S, Mahmood S, Malik F et al. Prevalence of Vitamin D deficiency in Pakistan and implications for the future. Expert Rev Clin Pharmacol. 2016;9(2):329-38.
2. Konstantakis C, Tselekouni P, Kalafateli M, Triantos C. Vitamin D deficiency in patients with liver cirrhosis. Ann Gastroenterol. 2016;29(3):297–306.
3. Gutierrez JA, Parikh N, Andrea D. Classical and emerging roles of vitamin D in hepatitis C virus (HCV) infection. Semin Liver Dis. 2011;31(4):387-98.
4. Ross AC, Taylor CL, Yaktine AL, Del V, Heather B. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. The National Academic Press (Washington, D.C). 2011:15-20.
5. Zittermann A and Gummert JF. Non-classical Vitamin D Actions. Nutrients. 2010;2(4):408–25.
6. Anty R, Tonohouan M, Ferrari PP, Piche T, Pariente A. Low Levels of 25-Hydroxy Vitamin D is independently associated with

the risk of bacterial infection in cirrhotic patients. Clin Transl Gastroenterol. 2014;5(5):56-7.
7. Amitabh BS and Anthony DH. A Public Health Approach to Hepatitis C Control in Low- and Middle-Income Countries. PLOS Medicine. 2015;12(3)1575-85.
8. Miriam JA. Epidemiology of hepatitis C virus infection. World Journal of Gastroenterology. 2007;13(17):2436-41.
9. Zhao X, Li J, Jia J. Vitamin D serum level is associated with Child-Pugh score and metabolic enzyme imbalances, but not viral load in chronic hepatitis B patients. Medicine (Baltimore). 2016;95(27):3926-32.
10. Cobb B, Heilek, G, Vilchez RA. Molecular diagnostics in the management of chronic hepatitis C: key considerations in the era of new antiviral therapies. BMC Infect Dis. 2014;14(5):8-10.
11. Glendenning P, NobleJ, Taranto, Musk A, McGuiness M, Goldswain P, et al. Issues of methodology, standardization and metabolite recognition for 25-hydroxyvitamin D when comparing the Dia Sorin radioimmunoassay and the Nichols Advantage automated chemiluminescence protein-binding assay in hip fracture cases. Annals of Clinical Biochemistry. 2003;40:546–51.
12. Munir S, Saleem S, Idrees M, Tariq A, Butt S, Rauff B et al. Hepatitis C Treatment: current and future perspectives. Virol J. 2010;1(7):296-9.
13. Idrees M and Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis. 2008;8(1):69-72.
14. Agunova Z, Porojnicu AC, Lindberg F, Hexeberg S, Moan J. The Dependency of vitamin D status on body mass index, gender, age and season. Anticancer res. 2009;29(9):3713–20.
15. Baur K, Mertens JC, Schmitt J. Combined effect of 25-OHvitamin D plasma levels and genetic vitamin D receptor (NR 1I1) variants on fibrosis progression rate in HCV patients. Liver International. 2012;32(4):635-43.
16. Peterlik M and Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Invest. 2005;35(5):290-304.
17. Stokes CS, Volmer DA, Grunhage F, Lammert F. Vitamin D in chronic liver disease. Liver International. 2013;33(3):338-52.
18. Kitson MT and Roberts SK. Delivering the message: the importance of vitamin D status in chronic liver disease. J Hepatol. 2012;57(4):897-90.
19. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Science. 2010;55(9):2624-8.
20. Heuman DM, Mihas AA, Habib A. MELD-XI: a rational approach to “sickest first” liver transplantation in cirrhotic patients requiring anticoagulant therapy. Liver Transpl. 2007;13(1):30-7.
21. Miroliaee A, Toosi N, Khalilzadeh O, Alireza E, Abdollahi A, Mazloumi M. Disturbances of parathyroid hormone–vitamin D axis in non-cholestatic chronic liver disease: a cross-sectional study. Hepatol Int. 2010;4(3):634–40.
22. Hoan NX, Tong HV, Song LH, Meyer CG, Velavan TP. Vitamin D deficiency and hepatitis viruses-associated liver diseases: A literature review. World J Gastroenterol. 2018;24(4):445-60.
23. Corey KE, Zheng H, Mendez-Navarro J, Delgado-Borrego A, Dienstag JL, Chung RT. Serum vitamin D levels are not predictive of the progression of chronic liver disease in hepatitis C patients with advanced fibrosis. PLoS One. 2012;7(2):2714-25.
24. Duarte MP, Farias ML, Coelho HS, Mendonça LM, Stabnov LM, do Carmo d Oliveira M, etal. Calcium-parathyroid hormone-vitamin D axis and metabolic bone disease in chronic viral liver disease. J Gastroenterol Hepatol. 2001;16(9):1022–7.

AUTHORSHIP AND CONTRIBUTION DECLARATION

AUTHORS	Contribution to The Paper	Signatures
Dr. Lubna Aftab Demonstrator, Biochemistry University Medical & Dental College, Faisalabad	Worked out almost all the technical details and performed the experiments	
Dr. Sadia Falak Assistant Professor, School of Pharmacy The University of Faisalabad, Faisalabad	Helped in interpreting the results and worked on the manuscript	
Dr. Aftab Islam Medical Practitioner Dar-us-Shifa Clinic, Faisalabad	Encourage to investigate and helped in data collection	
Dr. Nabeela Faisal Senior Demonstrator, Biochemistry University Medical & Dental College, Faisalabad	Contributed to the final version of manuscript	
Prof. Dr. Faisal Bilal Lodhi Professor of Surgery, Unit-I FMU / Allied Hospital, Faisalabad	Contributed to the critical revision of the article	
Dr. Sara Muzaffar WMO Saman Pindi Gujrat	Proof reading and formatting