

Hypovitaminosis D in Hepatitis C patients and its relation with demographic and laboratory data

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Abstract

Objective: To determine the frequency of vitamin-D deficiency in hepatitis C patients and its relation with demographic and baseline laboratory data.

Method: The cross-sectional study was conducted at the University Institute of Medical Laboratory Technology, Faculty of Allied Health Sciences, The University of Lahore, Pakistan, from April 3 to July 24, 2017, and comprised diagnosed hepatitis C genotype 3 patients aged 18-60 years. Demographic data was collected on a predesigned proforma. Tests included complete blood counts, liver function test, hepatitis C viral load and 25-hydroxy Vitamin-D level. Data was analysed using SPSS 24.

Results: Of the 115 patients, 54(47%) were male and 61(53%) were females. Mean vitamin-D level was 22.3 ± 11.3 . Total 25(21.7%) patients showed normal level of vitamin-D while the level was low in 90(78.3%) patients; 41(35.6%) showed vitamin-D insufficiency and 49(42.6%) vitamin-D deficiency. Significant effect of sun exposure was recorded on patient's vitamin-D level ($p=0.00$). Significantly low hepatitis C viral load was seen in patients with normal vitamin-D ($p=0.026$).

Conclusion: Patients with hepatitis C virus infection had high incidence of hypo-vitaminosis D.

Keywords: Hepatitis C virus, Hepatitis C infection, Acute hepatitis, Chronic hepatitis C, Vitamin D, Vitamin D deficiency, hypo-vitaminosis D in hepatitis C. (JPMA 69: 1637; 2019).

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Introduction

Hepatitis C virus (HCV) infection is a major global concern across the world, infecting approximately 200 million people.¹ It is a major health problem of developing countries and unfortunately Pakistan is an endemic country, sharing huge number of hepatitis C patients. It is estimated that approximately 6% of Pakistan's population is infected with HCV.² Moreover, it is also reported that in Pakistan, overall 69% deaths in intensive care units (ICUs) are primarily related to patients infected with HCV or co-infected with hepatitis C.² This high proportion of morbidity and mortality rate due to HCV indicates serious threats to Pakistan.

Despite the fact that hepatitis C treatment is available and a lot of effort has been made to lessen the high incidence of HCV in Pakistan, the incidence is on the rise. Poor compliance and failure to reduce viral load in treated patients remains an obstacle.³

Many other factors have been studied which may

influence the incidence of HCV infection, its severity and response to anti-viral therapy by the patients.⁴ These factors include age, gender, other liver abnormalities, body's ability to bear anti-viral therapy side-effects, and patient's immunity to fight against the virus. A competent immune system along with anti-viral therapy can completely eradicate viral infection.⁵

Vitamin D is one of those factors that provide immunity to fight against many infectious agents. Vitamin D regulates transcription of over 200 genes involved in cell proliferation and differentiation, immune-modulation, inflammation and fibrogenesis. It has been suggested that vitamin D and its metabolites have significant association with hepatitis C infection.^{5,6} Vitamin D ameliorates the necro-inflammatory process and inhibits liver fibrosis, and, subsequently, its deficiency could contribute to the advancement of chronic hepatitis.^{6,7}

Pakistan is a developing country and a major proportion of health budget is spent on efforts to curb hepatitis C infection. Strategies must be developed to understand risk factors and to explore the predictors that can be beneficial in the treatment of HCV. It is needed to

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establish if any association is present between viral actions and host reactions based on serum 25-hydroxy Vitamin-D (25[OH]D) level.

The current study was planned to assess the frequency of hypo-vitaminosis D in hepatitis C patients and to find out its relation with demographic and laboratory data.

Patients and Methods

The cross-sectional, descriptive study was conducted from April 3 to July 24, 2017, at the University Institute of Medical Laboratory Technology (UIMLT), Faculty of Allied Health Sciences (FAHS), The University of Lahore, Lahore, Pakistan.

After approval from the institutional review committee, sample size was calculated using Open-Epi calculator while assuming 80% power at a 5% confidence level by using insufficiency of vitamin D level in hepatitis C patients as reported earlier.⁸ Diagnosed chronic hepatitis C genotype 3 infected patients aged 18-60 years who visited the Nawaz Sharif Social Security Hospital in Lahore during the study period were included. Those taking vitamin D supplementation, having hepatitis B or human immunodeficiency virus (HIV) co-infection, liver carcinoma, renal abnormality, alcoholics and intravenous (IV) drug users were excluded.

After obtaining written informed consent from the patients, demographic data, including age, gender, eating habits, average sun exposure time between 10am and 3pm and wearing veil (hijab) by females while going outdoor, was recorded on a pre-designed proforma. History was taken for possible routes of transmission by asking questions about risk-associated procedures and practices.

Weight of the patients in kilograms (Kg) and height in inches was noted and was used to calculate body mass index (BMI). The five categories of obesity as defined by the World Health Organization⁹ based on BMI were used in this study. These categories include underweight BMI<18.5, normal range (BMI=18.5-22.9), over weight (BMI23-24.9), obese (BMI=25-29.9) and obese II (BMI>30). Two groups of patients were made on the basis of their age. These groups included 18-40 years and 41-60 years ago.

Viral ribonucleic acid (RNA) isolation, HCV quantitation and HCV genotyping were done by using a commercial kit (Gene-Proof diagnostics). Baseline blood samples of

selected patients were collected using sterile technique in ethylenediaminetetraacetic acid (EDTA) containing vacutainer and plain tube without additive. EDTA sample was used to perform total leukocyte count (TLC), haemoglobin (Hb) level, red blood cells indices and platelets count on Sysmex KX-21 semi-automated haematology analyser. Clotted sample was used to perform renal function tests (RFTs), total bilirubin level, alanine transaminase (ALT) level, aspartate transaminase (AST) levels on Microlab-300 semi-automated chemistry analyser (Merck) whereas 25(OH)D level was performed using special chemistry analyser.

TLC between $4-11 \times 10^9/L$ and platelets count between $150-450 \times 10^9/L$ were considered normal in both genders, whereas Hb between 12-16g/dl and 13.5-17.5g/dl were considered normal in females and males respectively. Total bilirubin level <1.0mg/dl whereas ALT and AST levels up to 40 IU/L were considered normal. Vitamin D level >30 ng/ml (75nmol/l) was considered normal, while levels between 20-29.9 ng/ml (52-72 nmol/l) was labelled insufficient and <20ng/ml (50 nmol/l) was defined as vitamin D deficiency.

Data was analysed using SPSS 24. Categorical variable were expressed as frequencies and percentages, whereas continuous variables were expressed as mean $\pm$ standard deviation (SD). Chi-square test, independent sample t-test and Pearson correlation were used to analyse data. $P < 0.05$ was considered statistically significant.

Results

Of the 115 patients, 61(53%) were females with a mean age of 44.2 ± 10 years, and 54(47%) were males with a mean age of 39.28 ± 11.4 . Overall mean age of the sample was 41.9 ± 11.0 years.

History of blood transfusion and surgical procedures of the sample was noted (Table 1). Of the 54 males, 36(66.7%) were in the habit of getting shaved at some barber shop, whereas all the 61(100%) females reported

Table-1: Transfusion history in male and female patients.

	Male n (%)	Female n (%)	p-value
Transfusion of blood	6 (11.1)	23 (37.7)	0.001*
Surgical procedure	12 (22.2)	36 (59)	0.000*
Dental Procedure	22 (40.7)	37 (60.7)	0.033*
Family Member infected with Hepatitis C virus	16 (29.6)	19 (31.1)	0.860
History of hospital admission	19 (35.2)	38 (62.3)	0.004*

* Statistically significant

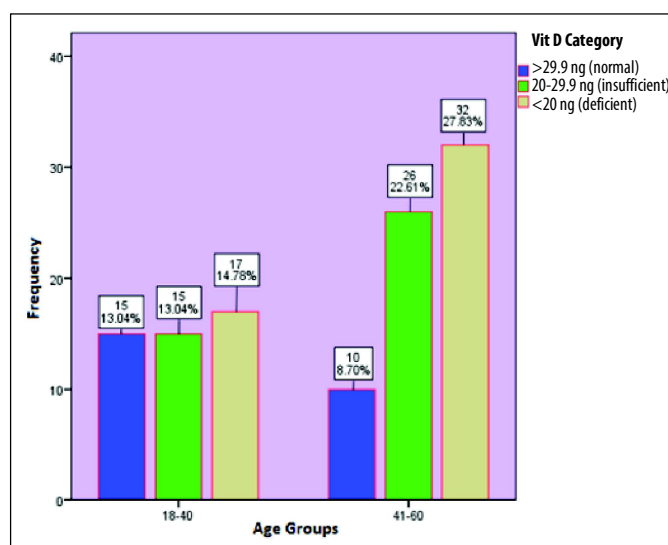


Figure: Vitamin-D status in age groups.

having got their ear and nose pierced from local shops with unreliable sterilised equipment. There was no patient with a history of IV drugs or tattoos. All male patients (100%) reported having got circumcised by some barber under uncertain hygienic conditions, while all patients in the sample reported IV or intramuscular (IM) medicine injections at some stage of life due to sickness.

Overall, 104(90.6%) patients had presented with complaint of fatigue, 43(37.4%) nausea, 93(80.9%) muscular pain, and 94(81.7%) had pain in joints and bones. Also, 44(38.3%) patients had recurrent fever, especially at the end of the day. No significant difference was found between clinical symptoms of patients with normal and low vitamin D levels ($p>0.05$).

Anaemia was the most common haematological abnormality 57 (49.6%), followed by thrombocytopenia 20(17.4%), leucocytosis 10(8.7%) and leukopenia 4(3.5%). Moderate and mild thrombocytopenia was observed in 1(0.9%) and 19(16.5%) patients respectively. Out of 57 anaemic patients, 54(94.7%) presented with mild anaemia compared to 3(5.3%) moderate anaemia patients. Overall, 12(10.4%) patients showed raised total bilirubin level, 74(64.3%) demonstrated raised serum glutamate-pyruvate transaminase (SGPT) levels and 80(69.6%) had raised serum glutamic oxaloacetic transaminase (SGOT) levels.

Mean vitamin D level of the patients was 22.3 ± 10.9 ng/ml. It was 22.4 ± 10.4 ng/ml in males and 22.4 ± 11.3 ng/ml in

Table-2: Lab Findings of patients with Normal and Low Vitamin-D level.

	Vitamin-D Status		p-value
	Normal ≥ 30.0 ng/ml	Below than normal ≤ 29.9 ng/ml	
Viral load IU/ml	969143 ± 1603558	3188021 ± 7186756	0.026*
TLC (x 10 ⁹ /L)	7.6 ± 2.7	7.2 ± 2.5	0.480
HB (g/dl)	12.3 ± 1.7	12.7 ± 1.5	0.809
MCV (fl)	82.3 ± 3.3	83 ± 3.6	0.275
MCH (pg)	26.8 ± 1.4	27 ± 1.4	0.569
Platelets (x10 ⁹ /L)	207 ± 68	219 ± 74	0.528
Urea (mg/dl)	28.1 ± 4.1	28 ± 5	0.080
Creatinine(mg/dl)	0.8 ± 0.1	0.8 ± 0.1	0.350
T.Bil. level (mg/dl)	0.8 ± 0.3	0.7 ± 0.3	0.297
ALT (IU/L)	62 ± 36	67 ± 41	0.399
AST (IU/L)	64 ± 38	71 ± 45	0.405

* Statistically significant, TLC: Total leukocyte count, HB: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, ALT: Alanine transaminase, AST: Aspartate Transaminase, T.Bil. Total bilirubin.

females. Overall, 25(21.7%) patients showed normal levels of vitamin D in their blood whereas low levels of vitamin D were found in 90(78.3%) patients. Out of these 90 patients, 41(35.6%) demonstrated vitamin D insufficiency and 49(42.6%) patients showed vitamin D deficiency (Table 2).

Low level of vitamin D was slightly, but not significantly, more frequent in males ($p=0.561$).

Vitamin D deficiency and insufficiency were seen in the group aged 41-60 years but the difference was not statistically significant ($p=0.088$) (Figure).

Overall mean BMI was 25.5 ± 4.4 (range: 16-35.4). The mean BMI in males was 25.1 ± 4.4 and 25.9 ± 4.0 in females. Negative correlation was found between BMI and vitamin D level ($r= -0.048$).

Overall mean viral load was 2705656 ± 6458263 IU/ml. Mean viral load in patients with normal vitamin D level was 969143 ± 1603558 IU/ml and in vitamin D insufficient and deficient patients, it was 3188021 ± 7186756 IU/ml. Significantly low viral load was seen in patients with normal vitamin D level ($p=0.026$).

Of the total, only 2(1.7%) patients said they used to consume fish regularly while 17(14.8%) said they had it occasionally. Also, 7(6.1%) patients reported regular intake of eggs and 10(14.8%) had them occasionally. IN terms of milk, 57(49.6%) patients used it on a regular basis, while 17(14.8%) used it occasionally. There was no significant correlation between vitamin D level with intake of fish, eggs or milk ($p>0.05$).

Only 7(13%) male patients, due to their outdoor nature

of job, reported getting more than half an hour of sun exposure every day. Mean Vitamin D level of patients having <30 minutes sun exposure between 10am and 3pm was 21.4 ± 10.2 whereas in patients with >30 minutes sun exposure, the mean vitamin D was 37.54 ± 9.9 ($p=0.001$).

Of the 61 female patients, 8(13.1%) reported wearing Abaya as part of their attire on a regular basis when going outdoors. Out of these 8 females, 1(12.5%) had normal vitamin D level whereas vitamin D insufficiency and deficiency was found in 2(25%) and 5(62.5%) patients respectively. Out of the 53(87%) females who did not wear any additional clothing, 13(24.5%) had normal vitamin D level, 17(32.1%) were vitamin D insufficient, and 23(43.4%) had deficiency. There was no significant difference between vitamin D levels of females wearing Abaya / Hijab and those not using any special clothing ($p=0.576$).

Discussion

Hepatitis C is a major healthcare problem in Pakistan. Despite the availability of its treatment, reducing burden of chronic hepatitis C is a challenge.⁸ Vitamin D could be an important mediator of immunity against HCV. Vitamin D controls a variety of genes directly or indirectly related to immune function, cellular proliferation, differentiation, apoptosis and angiogenesis. Subsequently, its deficiency results in less active immune response against these infections.⁹

A study reported that deficiency of serum 25(OH)D also contributed to the failure of treatment. Low serum 25(OH)D levels at baseline were associated with failure to achieve sustained virological response (SVR) in HCV following treatment with pegylated interferon and ribavirin.⁶

In the present study, overall low vitamin D levels were found in 78.3% patients vitamin D deficiency in 42.6% and insufficiency in 35.6% patients. Similar findings have been reported previously.^{6,10,11} Results of the current study, however, contradict the findings previously reported in which overall low vitamin D level was found in 90% patients; deficiency in 31% and insufficiency in 59% patients.⁸

The present study was conducted in the summer months of April-July which are reported to affect vitamin D status directly.^{12,13} This indicates that may be hypo-vitaminosis

D in HCV patients is under-reported in the present study and the level of vitamin D in these patients could be even lower if their levels be tested in the winter season.

It is interesting that patients possessing normal vitamin D level had significantly low circulating HCV viral loads in their blood compared to patients having low vitamin D levels. Similar results have been reported previously.¹⁴ These results strengthened the hypothesis that if vitamin D status of HCV patients is corrected, it will improve immune response of patients which will subsequently result in achieving SVR followed by therapy.

Reported data also suggests that BMI is reversely proportional with vitamin D level in blood. Persons with high BMI usually possess high fat content which serves as a reservoir of fat-soluble vitamin D of body, resulting in low levels of circulating vitamin D. This excessive pooling of circulating vitamin D and very slow release of deposited vitamin D results in hypovitaminosis D.¹⁵ In the present study, this negative relation of BMI with vitamin D is reflected by the presence of significant negative correlation between these two in chronic hepatitis C (CHC) patients.

In this study, relatively high frequency of vitamin D deficiency was found in patients aged >40 years. Aging affects vitamin D status by reduced absorption of vitamin D in intestines, diminished expression of vitamin D receptors, lessened production via skin, impaired renal production of 1,25(OH)₂D and deficiency of vitamin D substrates.¹⁶ Thus, patients with high BMI as well as with age >40 years having HCV infection must be dealt with more cautiously regarding vitamin D status. It is suggested to test vitamin D status at all stages of hepatitis C-associated liver disease as with progression of disease, vitamin D deficiency increases as well.¹⁰

Idea of supplementation with vitamin D during progression of the disease as well as throughout the course of the treatment is also proposed as vitamin D halts advancement of fibrosis and it is reported to improve achievement of SVR in hepatitis C patients.

The current study's limitation is the absence of control group.

Conclusion

Vitamin D and HCV viral load were found to be inversely proportional to each other. Age and BMI both affected vitamin D status. Significant effect of sun exposure was

recorded on vitamin D level. No significant correlation of vitamin D level was found with either diet or attire while going outdoors. Anaemia and thrombocytopenia were the most common haematological abnormalities in CHC patients whereas raised ALT and AST levels were also common.

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