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COMPARISON OF BASELINE CHARACTERISTICS AMONG CASES AND CONTROLS TO ASSESS THE LEVELS OF VITAMIN D SERUM

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Abstract:

Background: *According to an idea, a relationship between non-alcoholic greasy liver ailment (NAFLD) and serum nutrient D levels exist. Be that as it may, the unequivocal job and instruments are obscure.*

Objective: *To study serum vitamin D levels in patients with NAFLD was the aim of this study.*

Place and Time of Study: *Services hospital, Lahore from Feb 2018 to Jan 2019.*

Methods: *Analysis of NAFLD depends on ultrasound (U.S) discoveries after prohibition of other potential reasons for NAFLD and interminable liver infections. For this investigation, fifty patients with NAFLD and another 50 solid volunteers without NAFLD or any clinically clear liver ailments were enlisted. Serum 25(OH) nutrient D levels were estimated utilizing ELISA based test.*

Results: *Serum 25 (OH) nutrient D levels is conversely connected with weight list (BMI), all out cholesterol, triglycerides, low thickness lipoprotein (LDL) and age. There is measurably critical diminishing of serum 25(OH) nutrient D levels in patients with NAFLD than those without NAFLD.*

Conclusion: *It is concluded that Serum 25(OH) vitamin D level and NAFLD has inverse relation.*

Keywords: *NAFLD, Fatty liver, Vitamin D.*

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INTRODUCTION:

NAFLD has the probability of movement to non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC). The accurate instrument of NAFLD advancement is obscure. NAFLD is the most widely recognized liver infection in western countries [1]. It is normally connected with insulin obstruction and metabolic syndrome [2-3]. Nutrient D may have a job being developed of immune system ailments and provocative conditions through creation of incendiary cytokines. Nutrient D has numerous capacities in numerous frameworks of the human body, including muscles, bone, heart, gut, liver and safe framework [4-7]. An expanding proof is indicating a nearby connection between 25 (OH) nutrients D and NAFLD. A few examinations found a noteworthy relationship between nutrient D insufficiency and heftiness, metabolic disorder, type 2diabetes and insulin resistance [6-8]. Along these lines, to explore the conceivable relationship among NAFLD and serum 25(OH) nutrient D levels was the point of this examination.

METHODS:

We selected 50 patients with NAFLD (cases), and 50 sound volunteers without NAFLD or any ceaseless liver infection as controls, cross coordinated with patients in age and sex. This investigation is a cross sectional examination. It was carried out in order to examine the relationship among NAFLD and serum 25(OH) nutrient D.

Inclusion criteria:

All the patients selected were 18 years and above. All participants were the patients of NAFLD.

Detailed history, clinical examination and BMI calculation were done for every patient. All subjects (cases and controls) are admitted to Services hospital, Lahore.

Exclusion criteria:

The Patients with Wilson's disease or hemochromatosis were not selected for the study. Patients with renal failure and clinical, radiological or laboratory evidence of chronic liver disease were excluded. Patients who are taking drugs contain or affecting vitamin D level including: pure vitamin D drugs or multivitamins containing vitamin D formulas were expelled. People with hepatitis B or C infection and Alcohol users: daily consumption of more than 20 grams for males and 10 grams for females were not selected. This study does not include children.

Laboratory evaluation:

After a mid-night fasting, laboratory tests such as fasting blood sugar, lipid profile, complete blood count, liver functions (AST, ALT, serum bilirubin, INR, total protein and albumin) and renal function were carried out for all the patients.

Assessment of NAFLD:

Ultrasound examination was finished utilizing a B-mode arched test from 2.5-5 MHz on a Mindray DP-2200 ultrasound machine (Shenzhen, China). Finding of NAFLD was finished relying upon ultrasound imaging of the liver [9]. NAFLD was reviewed on a scale from 0-3 as indicated by Saverymuttu et al [10], where 0 is alludes to nonappearance of NAFLD, 1 mellow, 2 moderate and 3 serious NAFLD. Ultrasound examination was finished by a similar administrator - who was visually impaired for the consequences of research center information and serum 25 (OH) nutrient D levels-for each subject around the same time of clinical examination and lab appraisal.

Vitamin D level:

Serum is isolated. It is put away in - 25°C for couple of days. It is an ELISA based test. By utilizing The Algeria 25-OH Vitamin D3/D2 Test Strip (ORGENTEC Diagnostika GmbH Carl-Zeiss-Straβe 49-51 Mainz – Germany), 25(OH) nutrient D levels were estimated.

Ethical consideration:

Verbal assent was taken from people who took an interest in the examination. Servises hospital-Faculty of drug moral board of trustees endorsed the investigation.

Statistical analysis:

Multivariable direct relapse examination was performed to distinguish free hazard elements of nutrient D. Gauge statistic, clinical, and research center qualities were recorded as numbers and rate for clear cut information and means and standard deviation for constant information. Understudy's T-test was utilized to think about aftereffects of constant factors among gatherings and chi square test for straight out factors. P-esteem thought about noteworthy if < 0.05. Measurable investigation was finished utilizing SPSS form 22 (IBM SPSS Inc., Chicago, US) for windows 10.

RESULTS:

Grade 1 NAFLD was available in 13 patients (26%), grade 2 out of 24 patients (48%), and grade 3 out of 13 patients (26%). Pattern attributes of patients and control were surrendered (Table 1). No noteworthy contrasts among guys and females in serum 25(OH)

nutrient D levels (Table 2). Patients with NAFLD demonstrated higher white platelet tally, BMI, fasting glucose (FBS), ALT, triglycerides and absolute cholesterol levels than controls.

Table 1: Baseline characteristics of cases and controls.

	Cases (*n* = 50) Mean ± SD	**Control (*n* = 50) Mean ± SD**	***P*- value**
Age (years)	49.88 ± 9.126	47 ± 8.769	0.374
Males/Females	27/23	26/24	0.886
BMI (Kg/m²)	26.18 ± 4.03	23.41 ± 2.64	0.045
HB (g/dl)	12.11 ± 1.44	11.99 ± 1.033	0.809
RBCs (106 cells/mm3)	4.64 ± 0.55	4.48 ± 0.64	0.453
WBCs (103/mm3)	7.69 ± 1.92	6.13 ± 1.28	0.019
Platelets (103/mm3)	266.08 ± 74.35	282 ± 59.48	0.533
FBS (mg/dl)	135.28 ± 93.51	93.50 ± 19.89	0.013
ALT (U/L)	54.42 ± 36.79	24.46 ± 10.43	0.008
AST (U/L)	43.97 ± 27.53	27.30 ± 9.32	0.067
Albumin (g/dl)	4.23 ± 0.34	4.02 ± 0.21	0.07
Total protein (g/dl)	6.99 ± 0.67	6.79 ± 0.69	0.409
Total bilirubin (mg/dl)	0.61 ± 0.24	0.51 ± 0.23	0.242
TG (mg/dl)	200.25 ± 140.13	74.70 ± 34.74	0.008
Cholesterol (mg/dl)	215.38 ± 50.81	150.30 ± 29.37	0
LDL (mg/dl)	131.94 ± 33.42	145.12 ± 42.37	0.296
HDL (mg/dl)	54.39 ± 17.41	54.70 ± 15.05	0.959
INR	1.03 ± 0.09	1.06 ± 0.15	0.496
Creatinine (mg/dl)	0.79 ± 0.28	0.77 ± 0.25	0.879
Vitamin D	18.76 ± 14.37	40.36 ± 22.24	0

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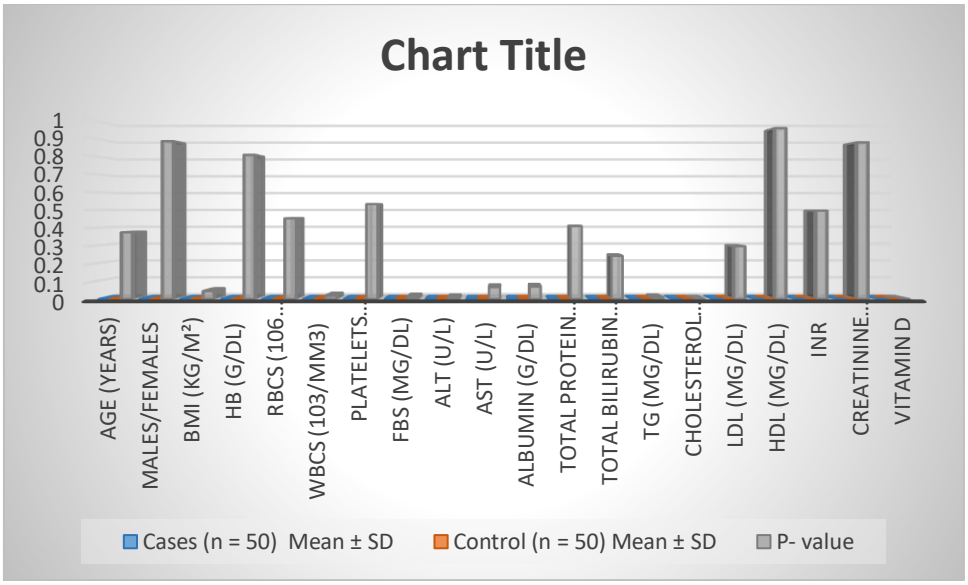


BMI: body mass index, HB: Hemoglobin, RBCs: red blood cells, WBCs: white blood cells, FBS: fasting blood sugar, ALT: alanine transferase, AST: Aspartate transferase, TG: Triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein, INR: international normalized ratio.

NAFLD and 25(OH) vitamin D:

Complete cholesterol levels were conversely connected with serum 25(OH) nutrient D levels and LDL levels were emphatically connected with serum 25(OH) nutrient D levels, generally no different elements influencing serum nutrient D levels. Serum

25 (OH) nutrients D levels were essentially decreased in patients with NAFLD than those without NAFLD (18.76 versus 40.36 p esteem 0.000) (Table 1). No noteworthy distinction in serum 25 (OH) nutrient D levels between patients with grade 1 NAFLD and those without NAFLD. Then again, there are noteworthy contrasts between patients with grade 2 and 3 on one hand and those with grade 1 and 0 then again. Serum 25(OH) nutrient D levels diminished with the expansion in the NAFLD grade (Table 3). Multivariable direct relapse investigation was utilized to evaluate indicators of serum 25 (OH) nutrients D (Table 4).

DISCUSSION:

Patients in our investigation had typical engineered work, with no huge contrasts between NAFLD patients and control bunch in egg whites, INR or bilirubin levels. Along these lines, low dimensions of 25 (OH) nutrients D in NAFLD bunch in our examination isn't because of manufactured inadequacy of nutrient D by the liver.

Table 2: Vitamin D levels in males and females.

Vitamin D (ng/dL)		**Mean ± SD**	***P*- value**
Sex	Female	20.45 ± 16.42	0.234
	Male	24.71 ± 20.27	

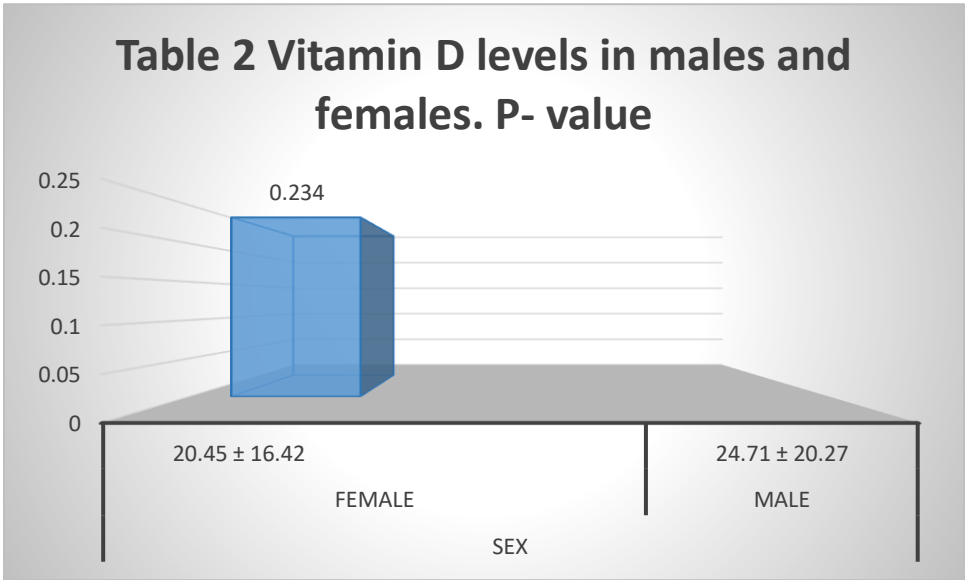
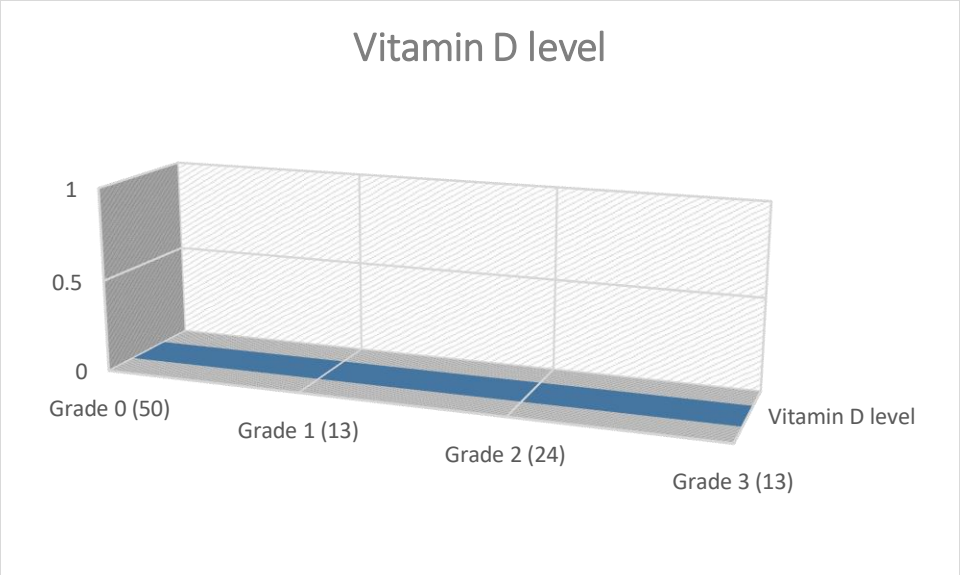


Table 3 Vitamin D levels according to U.S grades of NAFLD.

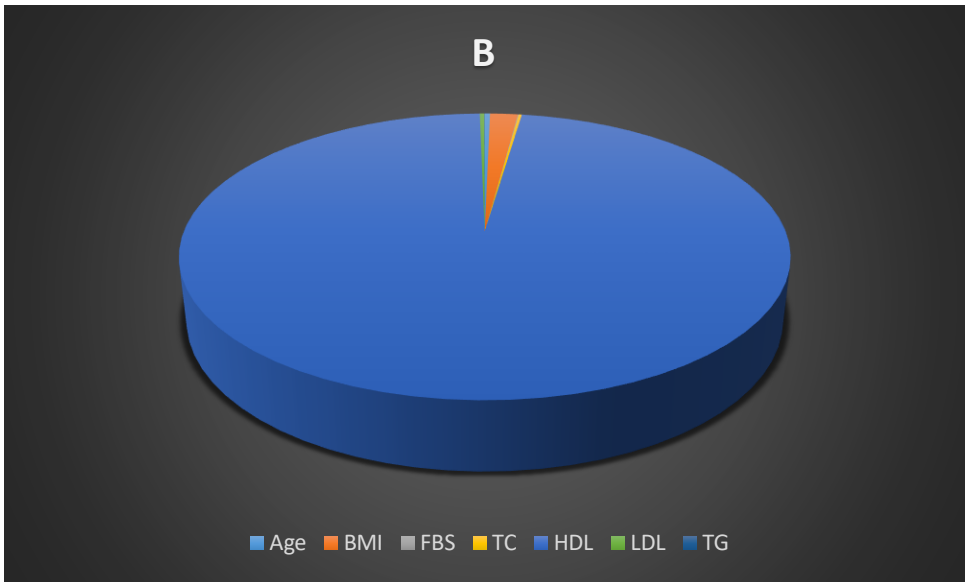
	Grade 0 (50)	**Grade 1 (13)**	**Grade 2 (24)**	**Grade 3 (13)**
Vitamin D level	40.36 ± 22.24 a	35.56 ± 20.83 a	13.39 ± 2.06 b	12.74 ± 5.56 b



a, b: there are significant differences between groups with different letters but not among groups with the same letter.

Table 4 Multivariable linear regression analysis of determinants of vitamin D level, Dependent variable: vitamin D.

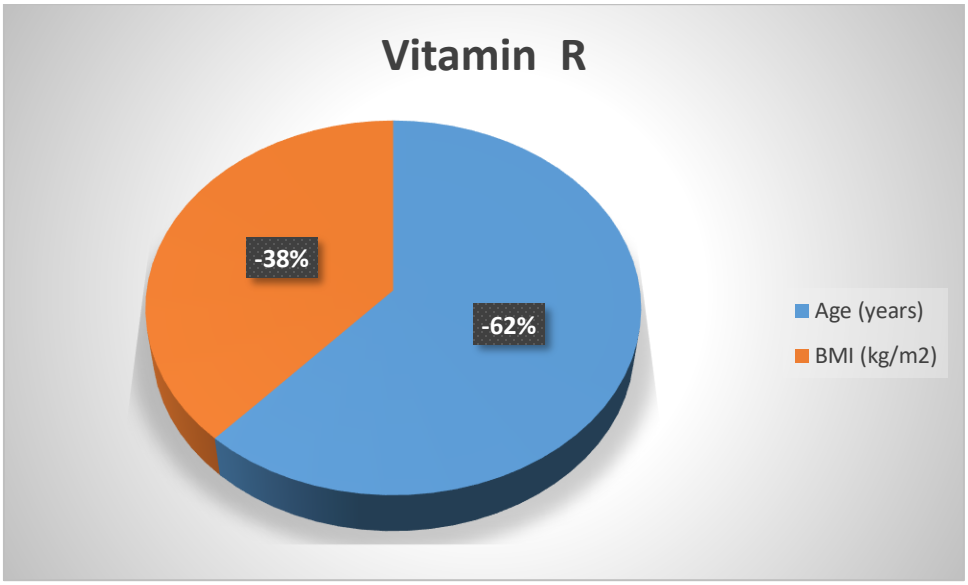
Determinant	**B**	**S. E**	**Beta**	***P*-value**
Age	-0.245	0.284	-0.121	0.395
BMI	-1.274	0.697	-0.281	0.075
FBS	-0.034	0.044	-0.16	0.447
TC	-0.144	0.055	-0.435	0.013
HDL	-71	0.156	-0.067	0.65
LDL	0.203	0.07	0.401	0.006
TG	0.021	0.03	0.157	0.493



BMI: body mass index, FBS: fasting blood sugar, TC: total cholesterol, HDL: high density lipoproteins, LDL: low density lipoprotein, TG: triglycerides

Table 5 correlation between Age & sex and mean vitamin D levels.

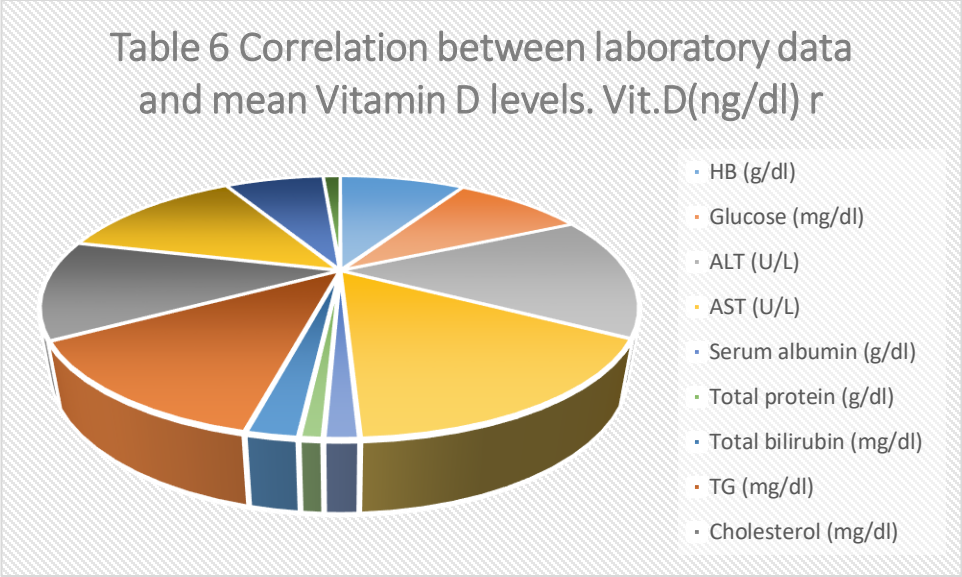
	Vitamin D (ng/dl)	
	R	***P*- value**
Age (years)	-0.3	0.034
BMI (kg/m2)	-0.186	0.01



BMI: body mass index.

Table 6 Correlation between laboratory data and mean Vitamin D levels.

	Vit.D(ng/dl)	
	r	***P*- value**
HB (g/dl)	0.223	0.12
Glucose (mg/dl)	-0.232	0.109
ALT (U/L)	-0.383	0.006
AST (U/L)	-0.409	0.003
Serum albumin (g/dl)	-0.037	0.8
Total protein (g/dl)	-0.025	0.862
Total bilirubin (mg/dl)	0.056	0.7
TG (mg/dl)	-0.324	0.022
Cholesterol (mg/dl)	-0.309	0.029
LDL (mg/dl)	0.329	0.02
HDL (mg/dl)	0.176	0.22
Serum creatinine (g/dl)	-0.031	0.83



HB: hemoglobin, ALT: alanine transferase, AST: aspartate transferase, TG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein is expected to decrease due to synthetic dysfunction of the liver[11]. However, patients in our study had normal synthetic function, with no significant differences between NAFLD patients and control group in albumin, INR or bilirubin levels. So, low levels of 25 (OH) vitamin D in NAFLD group in our study is not due to synthetic deficiency of vitamin D by the liver. Also, vitamin D deficiency may induce NAFLD by impairing hepatic lipid metabolism[12]. Patients with vitamin D deficiency found to have high rates of insulin resistance, metabolic syndrome and inflammatory mediators including IL-4, IL-6 and TNF-α[13,14]. It was reported that vitamin D receptors widely exist in liver tissue with negative association between vitamin D receptors expression and necro-inflammatory grades of NASH[15]. Vitamin D may be sequestrated in the adipose tissue in obese patients[16]. Also, NAFLD patients may have a sedentary life with low sunlight exposure and nutritional imbalance.

Additionally, nutrient D lack may prompt NAFLD by disabling hepatic lipid metabolism [12]. The component by which 25 (OH) nutrients D may prompt NAFLD isn't clear. The liver proselytes' nutrient D to its dynamic structure, 25 (OH) nutrients D, so in liver maladies the 25 (OH) nutrient D levels is required to diminish because of manufactured brokenness of the liver [11]. In our investigation, patients with NAFLD demonstrated altogether diminished serum 25(OH) nutrient D levels than those without NAFLD. Serum 25(OH) nutrient D levels were observed to be logically diminished with the expansion in the ultrasound grades of NAFLD. Nutrient D lack may prompt NAFLD by impeding hepatic lipid metabolism [12]. NAFLD patients may have a stationary existence with low daylight introduction and dietary lopsidedness. Patients with nutrient D lack found to have high rates of insulin opposition, metabolic disorder and fiery middle people including IL-4, IL-6 and TNF-α [13-14]. It was accounted for that nutrient D receptors broadly exist in liver tissue with negative relationship between nutrient D receptors articulation and necro-fiery levels of NASH [15]. Nutrient D might be sequestrated in the fat tissue in stout patients [16]. Barchetta et al [18] 2011, found solid relationship between hypovitaminosis D and NAFLD which was autonomous on age, sex, BMI, lipid profile or glucose level. Chung et al [19] 2016, found that serum nutrient D levels were corresponded contrarily with NAFLD in a portion subordinate way autonomously on known NAFLD hazard factors. Our outcomes are in concurrence with Targher et al [17] 2007 who discovered huge reduction of nutrient D levels in patients with NAFLD than sound controls, which was related with NAFLD histo-pathological highlights. In our examination, nutrient insufficient patients (serum nutrient D under 20 mg/mL[20]) were fundamentally more established in age, more were females, with essentially higher aminotransferases levels than typical nutrient D subjects (serum nutrient D in excess of 30 mg/mL[20]). They additionally have essentially more elevated amounts of triglycerides and absolute cholesterol than subjects with typical dimensions of nutrient D (Table 8). Nelson et al [20] 2016, found that low dimensions of nutrient D are related with high danger of NASH in patients with NAFLD. Our

outcomes are likewise in concurrence with Zhai et al [21] 2016, Wang et al [22] 2016, Yang et al [23] 2017 and Eliades et al [8] meta-examination 2013 who revealed that nutrient D inadequate patients have 1.26-overlay expanded hazard for NAFLD than those with adequate nutrient D. In any case, our outcomes are in opposite with Li et al [24] 2013, who found no noteworthy contrasts between patients with NAFLD and those without NAFLD in serum nutrient D levels. Additionally, Patel et al [25] 2016, Ha et al [26] 2017, De Paula et al [27] 2017 and Park et al [28] 2017 found no relationship between nutrient D levels and NAFLD (Table 7 abridges these investigations).

Table 7 summary of studies results on vitamin D in NAFLD patients.

Study	**NAFLD**		**Controls**	***P*- value**
Targher *et al* 2007	51.0 ± 22		74.5 ± 15	< 0.001
Barchetta *et al* 2011	14.8 ± 9.2		20.5 ± 9.7	< 0.001
Chung *et al* 2016	21.75 ± 7.54		22.02 ± 8.43	0.253
Zhai *et al* 2016	40·05 ± 9.83		40.98 ± 10.80	< 0.01
Yang *et al* 2017	16.63 ± 6.57		18.44 ± 7.30	0.011
Eliades *et al* 2013 meta-analysis	Reported that vitamin D deficient patients have 1.26-fold increased risk for NAFLD than vitamin D sufficient patients			
Li *et al* 2013	22.8 ± 8.4		22.1 ± 8.1	0.21
Patel *et al* 2016	27.9 ± 12.8		27.7 ± 11.9	0.878
Ha *et al* 2017	16.2 ± 6.2		16.2 ± 6.5	0.95
De Paula *et al* 2017	Steatosis	NASH	28.66 ± 8.40	0.55
	27.6 ± 12.49	26.5 ± 7.38		
Park *et al* 2017	21.8 ± 8.7		21.5 ± 8.1	0.151

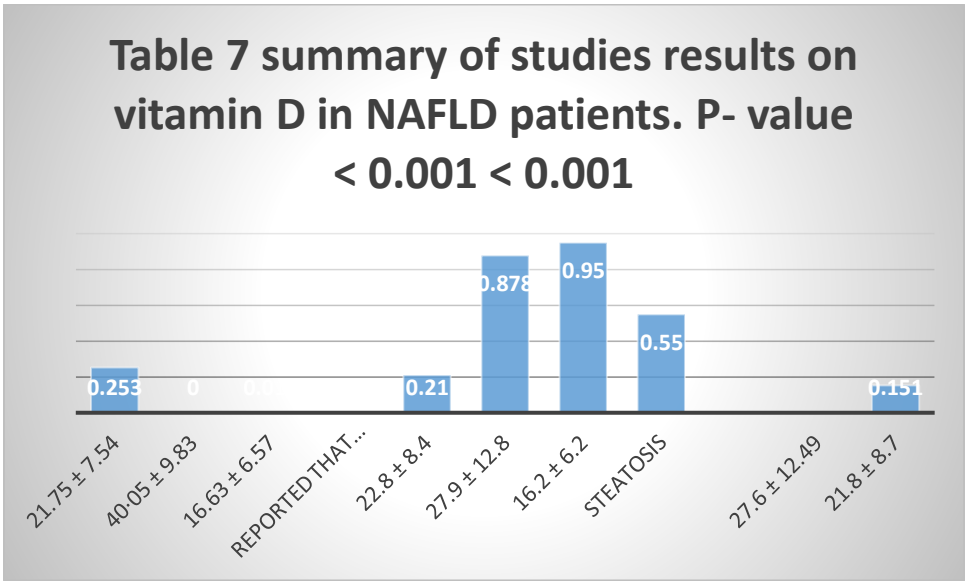
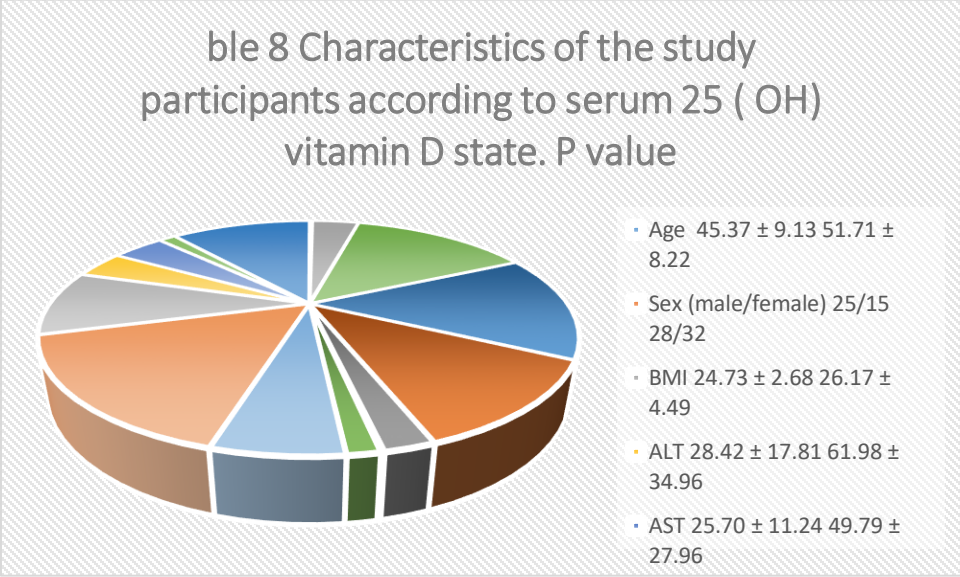


Table 8 Characteristics of the study participants according to serum 25 (OH) vitamin D state.

	NVD	**VDD**	***P* value**
Age	45.37 ± 9.13	51.71 ± 8.22	0.014
Sex (male/female)	25/15	28/32	0
BMI	24.73 ± 2.68	26.17 ± 4.49	0.213
ALT	28.42 ± 17.81	61.98 ± 34.96	0
AST	25.70 ± 11.24	49.79 ± 27.96	0.001
Bilirubin	0.58 ± 0.23	0.59 ± 0.24	0.813
Albumin	4.17 ± 0.26	4.19 ± 0.37	0.856
Total protein	6.90 ± 0.66	6.98 ± 0.69	0.687
FBS	103.00 ± 48.59	140.55 ± 98.92	0.139
TG	112.00 ± 82.00	213.84 ± 148.28	0.009
Total cholesterol	168.84 ± 35.30	222.90 ± 53.37	0
LDL	145.43 ± 32.29	127.92 ± 35.93	0.089
HDL	57.30 ± 16.36	52.71 ± 17.12	0.354
INR	01.04 ± 0.11	1.03 ± 0.10	0.946
Creatinine	0.82 ± 0.25	0.77 ± 0.28	0.53
HB	12.38 ± 1.21	11.90 ± 1.43	0.228
Platelets	284.74 ± 68.66	259.77 ± 72.41	0.234
WBCs	6.76 ± 1.33	7.75 ± 2.12	0.077
RBCs	4.56 ± 0.56	4.64 ± 0.58	0.64



NVD: normal vitamin D, VDD: vitamin D deficiency, BMI: body mass index, ALT: alanine transferase, AST: aspartate transferase, FBS: fasting blood sugar, TG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein, INR: international normalized ratio, HB: hemoglobin, WBCs: white blood cells, RBCs: red blood cells.

These conflicting outcomes among studies might be identified with contrasts in the examined populace, wholesome and ecological components.

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