Effect of vitamin D supplementation on various parameters in non-alcoholic fatty liver disease patients

Mazhar Hussain[1*], Javed Iqbal[2], Saeed Akhtar Malik[3], Akbar Waheed[4], Shazia Shabnum[5], Lubna Akhtar[1] and Hira Saeed[2]

[1]Department of Pharmacology, Sheikh Zayed Medical College, Rahim Yar Khan, Pakistan
[2]Department of Medicine, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, Pakistan
[3]Department of Radiology, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, Pakistan
[4]Department of Pharmacology, Islamic International Medical College, Riphah International University, Islamabad, Pakistan
[5]Department of Dentistry, Sheikh Zayed Medical College/Hospital, Rahim Yar Khan, Pakistan

Abstract: There are still no FDA approved drugs for NAFLD so far. Vitamin D may be a good therapeutic option for NAFLD patients due to its insulin sensitizing and anti-inflammatory properties. The purpose is to investigate the effect of oral vitamin D supplementation on various parameters in NAFLD patients. In this double blind randomized placebo controlled trial, 109 patients of NAFLD diagnosed by abdominal ultrasound and liver enzymes were divided into two groups for treatment with oral capsule of vitamin D3 50,000 IU and capsule placebo weekly for a period of 12 weeks. Anthropometric, chemical, metabolic and inflammatory parameters were assessed pre and post treatment by using SPSS 16. After 12 weeks oral treatment with vitamin D , its level increased significantly in vitamin D group from 12.5±4.2 to 24.5±3.8 ng/ml p =0.003 vs placebo group. This rise was further accompanied by decrease in HOMA-IR (4.56±1.6 to 3.26± 1.8 p=0.003) liver enzymes (i.e. ALT: 72.±17.6 to 54.5±14.5 IU/L p=0.04; AST: 68±14.5 to 46.± 10.5 p =0.002) serum CRP 3.25±0.68 to 2.28±0.44 mg/L p =0.06 and increase in serum adiponectin 8.56 ±1.12 to 10.44±2.35 mg/L p =0.03 as compared to placebo group. However non significant changes were observed in both groups in terms of body weight, BMI, and serum lipid profiles. Vitamin D supplementation not only improved its own status but also caused a significant amelioration in metabolic, chemical and inflammatory parameters in NAFLD patients. So it should be consider as an adjunctive therapy in NAFLD patients.

Keywords: Vitamin D, dyslipidemia, NAFLD, HOMA-IR, aminotransferases, CRP.

INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is one of the most common progressive liver disorders that usually start as benign disease. NAFLD is usually reversible but if it cannot control appropriately, it progresses to non alcoholic steatohepatitis (NASH) and ultimately to hepatic fibrosis and then to liver cirrhosis. While on the other hand hepatocellular carcinoma and cardiovascular diseases are its disastrous complications (Younossi *et al*., 2016). In recent years NAFLD is spread like an epidemic in developing countries like Pakistan with a prevalence rate of about 30% (Ashtaris *et al*., 2015). Life style modifications and urbanization are one of its route causes while obesity, metabolic syndrome, type 2 diabetes mellitus, dyslipidemia and hypertension are its associated risk factors that pose an enormous burden clinically as well as economically (Milic *et al*., 2014). There is strong need to control NAFLD as it is predicted to be the one of the most common cause of liver transplantation in future in both developed and developing countries (Byrne *et al*., 2014).

Life style modifications which compromise of weight loss, exercises and dietary plans are usually the first step in the management of NAFLD. Besides life style modification various drugs have been tried with disappointing results up till now. Even no single licensed dug therapy is approved for NAFLD patients so far, which is still a major challenge in medical field (Centis *et al*., 2013; Darb *et al*., 2016).

In recent years a lot of work has been done on vitamin D beyond its role in bone mineral homeostasis. Its low level in the body is mainly due to decrease milk intake, limited Sun exposure and increase body mass that predisposes the population to various disease risk (Hilger *et al*., 2014). In addition to their primary location such as bone, intestine and kidney, vitamin D receptors also present in brain, liver, muscle, immune and endocrine system. The adequate level of vitamin D in the body not only protects against various musculoskeletal disorders but also against obesity, metabolic syndrome, diabetes mellitus, infertility, infections, CNS, cardiovascular, respiratory, autoimmune and malignant disorders (Pludowski *et al*., 2013).

The purpose of choosing vitamin D in this study was that vitamin D has strong potential to revert NAFLD associated pathological changes. First vitamin D reduces insulin resistance a key metabolic abnormality in NAFLD patients (Von *et al*., 2010). Second Vitamin D has strong immune modulatory, anti-inflammatory, anti-fibrotic and

**Corresponding author:* e-mail: mazharhussain214@gmail.com

anti-oxidant properties. The role of inflammation and oxidative damage in the pathogenesis and complications of NAFLD is already well understood. That's why drugs such as silymarin, vitamin E and pentoxyfyline that possess potent anti- inflammatory and anti-oxidant potential use in NAFLD with positive results (Eliades and Spyrou, 2015).

Finally more than 70% of the NAFLD patients are obese with deranged lipid profile, (Younossi *et al*., 2016). Low level of vitamin D has strong association with obesity and deranged lipid profiles in various studies review (Prasad and Kochhar, 2016).

In present study we investigated the effects of oral vitamin D supplementation on body weight, BMI, insulin resistance, dyslipidemia, hepatic enzymes, CRP and adiponectin in NAFLD patients**.**

MATERIALS AND METHODS

A double blind randomized placebo controlled trial of 12 weeks duration was conducted at Sheik Zayed Medical College/Hospital at its medical OPD after getting ethical committee permission from institutional review board. Initially 210 patients with BMI > 28 were screened at the medical outdoor based on clinical presentations of vague upper abdominal distress, indigestion, dyspepsia and generalized body weakness. Out of which 140 patients were enrolled in the study following inclusion and exclusion criteria. The inclusion criteria include randomized selection based upon age, sex, BMI>28, fatty liver on sonographic findings, moderate increase in hepatic enzymes with altered serum lipid profile. The exclusion criteria include pregnant & lactating women, smokers, type 2 diabetes, hypertension, chronic hepatitis B & C infection, alcoholic liver disease, chronic liver disease, decompensated liver disease and hepatocellular carcinoma. Patents with any history of cardiac, renal and thyroid disorders were excluded. Those patients whose ultrasound and liver enzymes were extreme abnormal worked for hereditary & auto immune diseases of liver were also excluded from study.

In addition detailed history was taken about drugs that causes hepatic injury and those drugs which usually given in NAFLD like pioglitazone, metformin, vitamin E and silymarin. A written informed consent was gain from all participants and study perspectives were clearly explained to all patients before enrollment. A total of 109 patients were randomly divided in to interventional (55) and placebo (54) group respectively. A computed generated number was given to each patients based upon randomization. Group A was given capsule Vitamin D3 50,000 IU orally weekly for a period of 12 weeks while Group B was given capsule placebo with similar color, packing, weight, shape, duration but it constitute liquid paraffin as an active agent. Patients were asked to bring the capsule vial back during follow up visit in order to assess the compliance of drug. Life style modifications such as restriction of high carbohydrates and fat diet with increase physical activity were advocated to all patients as standard care of NAFLD.

Fatty liver changes were graded from I to III based upon the echogenicity of liver parenchyma, hepato-renal contrast, blurring of intra hepatic vessel border and diaphragm analyzed on high resolution abdominal ultrasound machine by an experienced radiologist. Patients, radiologist, investigators and laboratory staff were blinded to study plan throughout the study period.

Body weight and height was measured by wearing light clothes by digital weighing balance and microtoise respectively. Body mass index (BMI) was measured by standard formula weight in kg divided by square root of the height in meters. 10ml blood was drawn from cubital vein after an overnight fasting of 12 hour pre and post treatment in order to analyze blood sugar, liver enzymes, lipid profiles, serum insulin, homeostasis model assessment-insulin resistance (HOMA-IR), serum adiponectin and CRP. Diabetic patients were ruled out at start of the study by doing blood sugar test by glucose oxidase per oxidase method. A semi-automated clinical chemistry analyzer was used to analyze serum lipid profiles and liver enzymes by using spectrophotometeric principle. Insulin concentration was detected by immunoassay analyser using insulin kit. HOMA-IR was calculated by the following equation fasting Glucose (mmol/L) $_{x}$ insulin (mU/L) /22.5. The hs-CRP was measured by turbid metric immune assay method (BioSystems Co, Barcelona, Spain) while serum adiponectin was analyzed with enzyme linked immunosorbant assay (ELISA) with adipogen kit Korea.

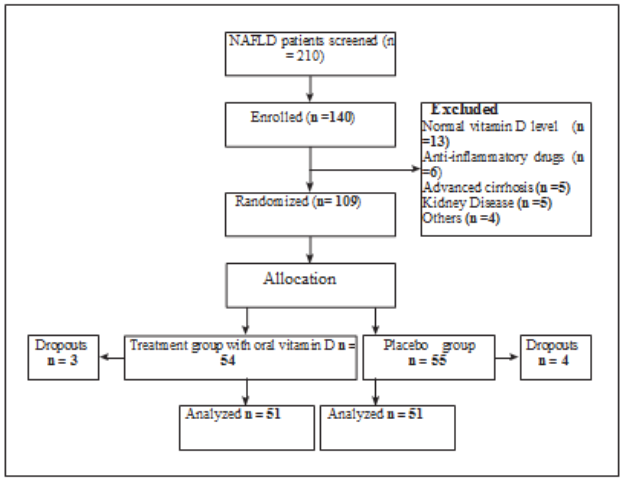
STATISTICAL ANALYSIS

All numeric data was analyzed by using statistical package for social sciences (SPSS-16). Values were expressed as mean ± standard deviation. Differences among interventional and placebo groups at baseline were assessed by t-test. In order to compare the changes from baseline to 12 weeks with in each group paired t-test and between groups t-test or Mann-Whitney U-test was used respectively. $P<0.05$ was considered to be statistically significant. A while comparison changes from Seeing interventional/placebo group ratio of 1,1, an active reduction of ALT in 50% in interventional group and 10% in placebo group anticipated dropout rate of 10% we enrolled 44+10. A sample size (35 per group) was calculated to detect a difference of serum LFTS level over 5 IU/L with 90% power and 5% significance. The sample size was increased to 40 per group to accommodate anticipated dropout rate.

RESULTS

A total of 109 patients were allocated for treatment, 54 in the interventional and 55 in placebo group. Overall safety and tolerability profile of vitamin D supplementation was quite good; however two patients in the interventional group complaint of severe allergy while one patient in interventional and four patients in the placebo group cannot complete the study because of loss of follow up. So, 102 patients completed the study and 51 patients were analyzed in each group. A flow chart of patient's recruitment for placebo controlled trial has shown in (table 1). The difference regarding body weight, BMI, serum lipid profile, liver enzyme and fatty liver grading on abdominal ultrasound among two groups were not statistically significant (table 2). After 12 weeks oral treatment with vitamin D, its level increased significantly in vitamin D group from 12.5±4.2 to 24.5±3.8 ng/ml p =0.003 vs placebo group. This rise was further accompanied by decrease in HOMA-IR (4.56±1.6 to 3.26± 1.8 p=0.003) liver enzymes (i.e. ALT: 72.±17.6 to 54.5±14.5 IU/L p=0.04; AST: 68±14.5 to 46.± 10.5 p =0.002) serum CRP 3.25±0.68 to 2.28±0.44 mg/L p =0.06 and increase in serum adiponectin 8.56 ±1.12 to 10.44±2.35 mg/L p=0.03 as compared to placebo group. However non significant changes were observed in both groups in terms of body weight, BMI, and serum lipid profiles (table 3).

Table 1: Flow char of patient's recruitments for placebo controlled trial



DISCUSSION

Our study showed that oral supplementation with vitamin D3 capsule at a dose of 50,000IU weekly for a period of 12 weeks significantly improved HOMA-1R, serum aminotransferases, C-reactive protein (CRP) and serum adiponectin. However vitamin D failed to improved body weight, BMI and serum lipid profiles in NAFLD patients. As insulin resistance is one of the hallmarks in the pathogenesis of NAFLD and the drug which improves insulin sensitivity and reduces insulin resistance such as metformin and pioglitazone showed their effectiveness in various experimental and clinical studies (Ozturk and Kadayifci, 2014). Vitamin D also improves insulin secretion and sensitivity and reduces insulin resistance via regulation and expression of insulin receptors in beta cells of pancreas, liver and peripheral tissues (Alvarez and Ashraf, 2010). In this study vitamin D supplementation significantly reduced insulin resistance which is measured by HOMA-IR.

Studies revealed that both dyslipidemia and obesity are strongly correlated with NAFLD (*Younossi* et al., 2016). Although in our study vitamin D supplementation for a period of 12 weeks failed to improve obesity and serum lipid profile significantly that may be due to short study duration. However we observed that vitamin D supplementation significantly increased the level of adiponectin. Adiponectin is adipokines whose biosynthesis is significantly altered in various obesity related conditions in which NAFLD is one of them. Similar to our study, a randomized controlled trial showed that vitamin D failed to improve obesity but it improved leptin to adiponectin ratio and HOMA-IR significantly (Belenchia *et al*., 2013). While in another study high level of vitamin D reduces the risk of NAFLD independently irrespective of obesity and metabolic syndrome (Rhee *et al*., 2013). So increasing the level of adiponectin by vitamin D will be good potential for NAFLD patients.

One of the pathgnomic mechanisms in NAFLD and its related complication is ongoing oxidative stress and inflammation. Vitamin E and Silymarin which is used in NAFLD management has also anti-inflammatory and anti-oxidant properties (Centis *et al*., 2013). There is marked increase in the level of CRP in NAFLD patients as compared to healthy control. Moreover CRP is an independent predictor of severity of histological features in terms of steatosis, necroinflammation and fibrosis in NAFLD patients after adjusting important confounders such as metabolic syndrome and insulin resistance (Targher, 2006). In this study vitamin D supplementation caused a significant reduction of inflammation by reducing c-reactive protein, The proposed mechanism by which vitamin D reduces inflammation and oxidative stress through its anti-inflammatory, anti-oxidant and anti-fibrotic properties by reduction of various inflammatory and oxidative cytokine such as resistin, CRP, IL-4, IL-6 and TNFα, type 1 collagen in hepatocyte (Eliades and Spyrou, 2015; Abramovitch *et al*., 2011).

The data about studies on vitamin D supplementation and its association with NAFLD and NASH are quite controversial. In one perspective a meta analysis of 17 cross sectional and case control studies showed that low

Table 2: Baseline characteristics of patients (N= 109) with NAFLD

Baseline parameters	Vitamin D (n=54)	Placebo (n=55)	P-value
Age(years)	27±1.7	29±19	0.88
Gender (M/F)	34/20	36/19	0.82
Body weight(kg)	85±14.5	87 ±17.5	0.74
BMI (Body Mass index kg/m^2)	28.6±1.8	29.2 ±1.2	0.15
Blood pressure Systolic (mmhg)	115±8.2	110±6.6	0.03
Blood pressure Diastolic (mmhg)	80.5±7.4	78±9.4	0.04
Blood sugar fasting(mg/dl)	85 ±16.5	89±14.5	0.78
Fatty liver grading (1/2/3)	54(13/32/9)	55(12/35/8)	0.29

Values are presented ± standard deviation
t-test between two groups

Table 3: Comparison of the changes from baseline to end point within and between groups' after treatment with Vitamin D and Placebo

Variables	Vitamin D (n-51)		P value*	Placebo (n-51)		P value*	P value$^+$
	Baseline	End Point		Baseline	End Point		
Body weight(kg)	85±14.5	82.5±10.5	0.002	87±12.6	85.5±13.7	0.02	0.60
BMI(kg/m^2)	28.6±1.8	27.7±0.9	0.001	29.2 ±1.2	28.1±1.1	0.000	0.42
HOMA-IR	4.56±1.6	3.26± 1.8	0.002	4.32±2.25	4.25±2.78	0.26	0.003
TC (mg/dl)	254±18.8	250±20.5	0.75	245± 25.5	244±27.5	0.31	0.62
TG(mg/dl)	182±23.5	175±16.5	0.171	175±19.6	170±20.8	0.424	0.88
LDL-C(mg/dl	145±16.8	143±20.4	0.316	155±17.5	152±16.5	0.69	0.47
HDL-C(mg/dl)	35.5±7.6	37.8±8.2	0.548	34.6±6.5	35±9.5	0.461	0.46
ALT(IU/L)	72.±17.6	54.5±14.5	0.001	76±18.6	74±12.2	0.63	0.04
AST(IU/L)	68±14.5	46.± 10.5	0.002	64.5±14.6	60±11.0	0.86	0.02
Vitamin D (ng/ml)	12.5±4.2	24.5±3.8	0.001	15.4±2.82	17.5±3.5	0.629	0.00
hs-CRP(mg/L)	3.25±0.68	2.28±0.44	0.005	3.48±0.72	3.11±0.65	0.517	0.06
Adiponectin(mg/L)	8.56 ±1.12	10.44±2.35	0.002	8.95±1.68	8.44±1.72	0.148	0.03

P value* (comparison within groups) P value$^+$ (comparison of changes of each variable between the two groups)

level of vitamin D has strong association with NAFLD that might play its role in the development of NAFLD. Even its low level not only correlates NAFLD but also its progression and severity in the form of NASH in both children and adults (Eliades *et al*., 2013). On the other hand a systematic review and meta analysis of seven randomized control trials concluded that vitamin D supplementation did not improve body weight, HOMA-IR, liver enzyme and lipid profile in patients of NAFLD (Tabrizi *et al*., 2017).

There were limited studies conducted on vitamin D in order to observe its effects on various parameters NAFLD patients. Barchetta *et al*. (2016) revealed that vitamin D therapy did not improved any metabolic, chemical, cardiovascular and radiological parameters in NAFLD patients, however this study was conducted in type 2 diabetic patients for 24 weeks and most of the parameters were not disturbed too much in contrast to our study.

Another study conducted by Sharifi *et al.,* 2014 demonstrated that oral treatment with vitamin D significantly reduced inflammatory and oxidative markers such as CRP and MDA (malondialdehyde) in NAFLD patients for 16 weeks period. In our study vitamin D also lower CRP and increased adiponectin but failed to improved body weight, lipid profile similar to this studies. Foroughi *et al.* (2016) conducted study on diabetic patients, in which vitamin D supplementation improved insulin resistance (HOMA-IR) and fasting blood sugar but failed to affect HOMA-B and insulin level in patients of NAFLD. Our study showed similar result in sense that it also improved HOMA-IR but it was conducted on non-diabetic patients which further explains that vitamin D had independent effect on insulin resistance in NAFLD patients. Another study demonstrated that vitamin D supplementation with calcium has additive beneficial effect on various biochemical parameters in NAFLD patients (Amiri *et al.,* 2016).

While on the other hand; high dose of vitamin D supplementation for a period of 24 weeks has no beneficial effect on insulin sensitivity and liver histology in non cirrhotic patients of NASH, which is more advanced form of NAFLD (Kitson *et al*., 2015). The cause of these controversies may be due to potential

confounders such physical activity, vitamin D and Ca intake, dietary energy intake, sun exposure. There is strong need for further clinical trial of vitamin D supplementation in NAFLD patients after adjusting these potential confounders.

The main strength of our study was that we tried to adjust confounders such as study conducted in winter, non diabetics, physical activity, diet, have reduced level of vitamin D which favors meta analysis of 17 studies in which vitamin D low level has strong association with NAFLD. Second strength of our studies was that we excluded all those patients who are taking any anti-inflammatory drugs such as statins, NSAIDS. Finally we carefully noticed about duration of physical activity, sun exposure and calcium supplements.

CONCLUSION

Vitamin D supplementation not only improved its own status but also caused a significant amelioration in metabolic, chemical and inflammatory parameters in NAFLD patients.

REFERENCES

Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Ben Tov A, Brazowski E and Reif S (2011). Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. *Gut*., **60**: 1728-1737.

Alvarez JA and Ashraf A (2010). Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. *Int. J. Endocrinol*., 351385. doi: 10.1155/2010/351385.

Amiri HL, Agah S, Azar JT, Hosseini S, Shidfar F and Mousavi SN (2016). Effect of daily calcitriol supplementation with and without calcium on disease regression in non-alcoholic fatty liver patients following an energy-restricted diet: Randomized, controlled, double-blind trial. *Clin. Nutr*., **36**(6): 1490-1497.

Ashtaris S, Pourhoseingholi MA and Zali MR (2015). Non alcoholic fatty liver disease in Asia: Prevention and planning. *World J Hepatol*., **7**(13): 1788-1796.

Barchetta I, Del Ben M, Angelico F, Di Martino M, Fraioli A, La Torre G, Saulle R, Perri L, Morini S, Tiberti C and Bertoccini L (2016). No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *BMC Med*., **14**(1): 92.

Belenchia AM, Tosh AK, Hillman LS and Peterson CA (2013). Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: A randomized controlled trial. *Am. J. Clin. Nutr*., **97**(4): 774-781.

Byrne CD and Targher G (2015). NAFLD: A multisystem disease. *J. Hepatol*., **62**(1): S47-S64.

Centis E, Marzocchi R, Suppini A, Grave RD, Villanova N and Hickman IJ (2013). The role of life style change in the prevention and treatment of NAFLD. *Curr. Pharm. Des*., **19**: 5270-5279.

Darb D, Potrillo-Sanchez P and Cusi K (2016). Pharmacological management of non alcoholic fatty liver disease. *Metabolism*, **65**(8): 1183-1195.

Eliades M and Spyrou E (2015). Vitamin D: a new player in non-alcoholic fatty liver disease? *World J. Gastroenterol.*, **21**(6): 1718.

Eliades M, Spyrou E, Agrawal N, Lazo M, Brancati FL, Potter JJ, Koteish AA, Clark JM, Guallar E and Hernaez R(2013). Meta analysis: Vitamin D and non alcoholic fatty liver disease. *Aliment. Pharm. Ther*., **38**(3): 246-54.

Foroughi M, Maghsoudi Z and Askari G (2016). The effect of vitamin D supplementation on blood sugar and different indices of insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD). *Iranian J. Nurs. Midwifery Res*., **21**(1): 100.

Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, Pierroz DD, Weber P and Hoffmann K(2014). A systematic review of vitamin D status in populations worldwide. *Br. J. Nutri*., **111**(1): 23-45.

Kitson MT, Pham A, Gordon A, Kemp W and Roberts SK (2016). High-dose vitamin D supplementation and liver histology in NASH. *Gut*., **65**(4): 717-788.

Milic S, Lulic D and Stimac D. Non alcoholic fatty liver disease and obesity: Biochemical, metabolic and clinical presentations (2014). *World J. Gastroenterol*., **20**(28): 9330-9337.

Ozturk ZA and Kadayifci A (2014). Insulin sensitizers for the treatment of non alcoholic fatty liver disease. *World J. Hepatol*., **6**(4): 199206.

Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, Shoenfeld Y and Lerchbaum E (2013). Vitamin D effects on musculo skeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality- a review of recent evidence. *Autoimmun. Rev*., **12**(10): 976-989

Prasad P and Kochhar A (2016). Interplay of vitamin D and metabolic syndrome: A review. *Diabetes Metab. Syndr*., **10**(2): 105-112.

Rhee EJ, Kim MK, Park SE, Park CY, Baek KH, Lee WY, Kang MI, Park SW, Kim SW and Oh KW (2013). High serum vitamin D levels reduce the risk for nonalcoholic fatty liver disease in healthy men independent of metabolic syndrome. *Endocr. J*., **60**(6): 743-752.

Sharifi N, Amani R, Hajiani E and Cheraghian B (2014). Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocr. J*., **47**(1): 70-80.

Tabrizi R, Moosazadeh M, Lankarani KB, Akbari M, Heydari ST, Kolahdooz F, Samimi M and Asemi Z (2017). The effects of vitamin D supplementation on metabolic profiles and liver function in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. *Diabetes Metab. Syndr*., **2**: 975-982.

Targher G (2006). Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. *J. Hepatology*., **45**(6): 879-881.

Von Hurst PR, Stonehouse W and Coad J (2010). Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient a randomised, placebo-controlled trial. *Br. J. Nutr*., **103**(4): 549-55.

Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L and Wymer M (2016). Global epideminiology of non alcoholic fatty liver disease- meta-analytic assessment of prevalence, incidence and outcomes. *Hepatolgy*, **64**(1): 73-84.