ORIGINAL ARTICLE

Effect of Vitamin D Supplementation on Lipid Profile Among Patients with Metabolic Syndrome: Randomized Controlled Trial in Tertiary Care Setting, Rawalpindi

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ABSTRACT

Objective: To evaluate the effect of vitamin D supplementation on lipid parameters in patients with metabolic syndrome

Study Design: Randomized controlled trial.

Place and Duration of Study: This study was conducted at the Department of Medicine, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, from April 2024 to July 2024.

Methods: Adult patients satisfying the National Cholesterol Education Program Adult Treatment Panel III criteria for metabolic syndrome were included. Exclusion criteria included current use of statins, use of vitamin D supplements during the last 6 months, hypothyroidism, pregnancy, chronic kidney disease stages 3- 5, nephrotic syndrome, and cholestasis. Patients in the intervention arm were given Vitamin D3 in two oral doses of 200000 IU each, taken at weeks 0 and 4, in addition to usual medications. Patients in the control arm received only usual medicines. Serum total cholesterol, triglycerides, high-density lipoproteins, and low-density lipoproteins were measured at baseline and after eight weeks.

Results: There were 78 patients aged 49.41± 11.84 years. Median high-density lipoprotein levels increased by 0.09 mg/dL in the intervention group, as compared to a decrease of 0.02 mg/dL in the control group (*P*=0.03). This change was significant in patients with sufficient vitamin D levels as baseline, but not amongst those with vitamin D deficiency. Administration of vitamin D was not associated with a substantial change in total cholesterol, triglycerides, or low-density lipoproteins.

Conclusion: In adults with metabolic syndrome, administration of vitamin D supplements is associated with a beneficial effect on serum high-density lipoproteins.

Keywords: *Cardiometabolic Risk Factors, Dyslipidemia, Lipids, Metabolic Syndrome, Vitamin D.*

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Introduction

Cardiovascular disease is a leading cause of death all over the world. It is estimated to have been responsible for 20.5 million deaths in 2021, more so in lower income countries.[1] Ischaemic heart disease (IHD) happens to be the commonest non-communicable disease related to premature deaths in a vast majority of the countries. The incidence of IHD has been steadily increasing with time. It was previously estimated in 2020 that the prevalence rate would increase from 1655 to 1845 per 100000 population over a course of 10 years.[2] This rampant growth means that achieving a one-third reduction in premature mortality from non-communicable diseases, as envisioned by sustainable development goals set by the United Nations, might not be possible.[3] Metabolic syndrome (MetS) is a conglomeration of different factors that predispose affected individuals to a higher risk of IHD, both individually and as a group. Depending on the definition used, it is present in 30- 40% of adults,

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predisposing them to a higher risk of adverse outcomes.[4] Central to the pathophysiology of this process is the role of proinflammatory cytokines such as tumor necrosis factor alpha, interleukin 6, and resistin released from the adipose tissue.[5] The resultant insulin resistance and endothelial dysfunction contributes to a metabolic milieu that threatens vascular health. Vitamin D, traditionally associated with bone health and calcium metabolism, has for some time been recognized to have pleotropic effects, including a desirable impact on the suppression of inflammation. A lot of evidence exists to prove that vitamin D deficiency is associated with an increased risk of IHD.[6] However, quite strangely, clinical trials consistently fail to show a mortality benefit associated with vitamin D replacement.

High serum low-density lipoprotein (LDL) and triglyceride (TG) levels, as well as low serum high-density lipoprotein (HDL) levels, are a hallmark of MetS. Dyslipidemia is the most common risk factor for IHD.[7] Targeting abnormal lipid fractions, primarily through the use of statins, is thus one of the cornerstones in primary prevention of IHD. Vitamin D replacement could also play a contributory role. There is observational data to show an inverse relationship between serum Vitamin D and LDL levels.[8] However, evidence from clinical trials does not consistently point in favour of or against the use of vitamin D as a modulator of blood lipids. These differences could stem from different protocols used for vitamin D replacement across studies, as well as from participants' baseline serum 25-hydroxyvitamin D levels. Moreover, local data is also sparse. We therefore planned this study to evaluate the effect of vitamin D supplementation on lipid parameters in patients with MetS. The results would help determine whether such a practice should be used routinely.

Methods

This two-arm parallel group randomized controlled trial was carried out at the Department of Medicine, Pak Emirates Military Hospital, Rawalpindi from April 2024 to July 2024. The study protocol was developed in accordance with the CONSORT 2010 guidelines for randomized controlled trials, which were the most recent at the time of trial initiation. Ethical approval was obtained from the Ethics Review Committee of Pak Emirates Military Hospital, Rawalpindi on dated February 2024, vide letter no: A/28/ERC/594/23, and was later registered at the Iranian Clinical Trial Registry on 14th April 2024, vide trial id no: IRCT20240215061017N1 prospectively. The criteria for inclusion in this trial were adult patients (aged ≥19 years) of either gender who satisfied the National Cholesterol Education Program Adult Treatment Panel III criteria for MetS. Exclusion criteria included the current use of lipid-lowering drugs, use of vitamin D supplements during the last 6 months, hypothyroidism, pregnancy, chronic kidney disease stages 3-5, nephrotic syndrome, and cholestasis. Sample size calculation was done with the EpiTools Sample size calculator for the detection of differences between two means with different variances. Farag HA et al. previously reported reductions in LDL of 13.7 ± 43.8 and -8.3 ± 30.9 with vitamin D and placebo, respectively.[9] Using these figures, a minimum sample size of 74 patients was determined to give a power of 80% for the detection of significant differences between the two groups.

Patients visiting the outpatient clinics were selected using convenience sampling and were screened against the inclusion and exclusion criteria listed above. Consent was obtained from all participants.

Blood pressure, height, weight, and waist circumference were recorded for all patients during the first visit, using standard techniques. Blood samples were collected for biochemical analysis. Serum total cholesterol, TGs, and HDL levels were measured with a Roche Cobas C 501 Analyzer (Roche Diagnostics International Ltd, Germany) using photometric transmission. The same machine also provided the calculated LDL values. Vitamin D levels were measured with the Roche Cobas e411 Analyzer (Roche Diagnostics International Ltd, Germany) using chemiluminescent immunoassay. Online computer-generated sequences were used for block randomization, and this allocation was concealed in opaque envelopes. Patients in the intervention arm were given Vitamin D3 in two oral doses of 200000 IU each, taken at weeks 0 and 4. This was in addition to the usual medications. Patients in the control arm received only the usual medicines. Serum total cholesterol, TGs, HDL, and LDL were measured at baseline and again at 8 weeks. Informed consent was taken from all participants after explaining the study

protocol and addressing their queries in detail.

Data was analysed with SPSS 24 on an intention to treat principle. Mean/standard deviation or median/interquartile range were used to express continuous data with normal or skewed distributions, whereas frequencies and percentages were used for categorical variables. Changes in different lipid fractions over the course of eight weeks were compared between the two arms using an independent samples Mann-Whitney U test. Statistical significance was set at $P<0.05$.

Results

The flow of patients through different stages of this study is shown in Figure 1. A total of 78 patients were included in the final analysis. These included 53 (67.95%) males and 25 (32.05%) females, with a mean age of 49.41± 11.84 years. Their baseline characteristics are shown in Table 1. Most of these parameters were the same in both groups, except for blood pressure and waist circumference. Median levels of different lipid fractions at the start and end of the study period have been compared in Table 2. It can be seen that vitamin D therapy was associated with a greater increase in serum HDL levels as compared to no intervention (0.09 mg/dL rise in the intervention group and 0.02 mg/dL fall in the control group; $P=0.03$). The results remained the same when the analysis was carried out separately for patients with baseline vitamin D deficiency. However, among patients with adequate levels of vitamin D, supplementation was associated with a beneficial effect on HDL levels. (Table 3). There was no difference between the two groups in changes in other lipid fractions.

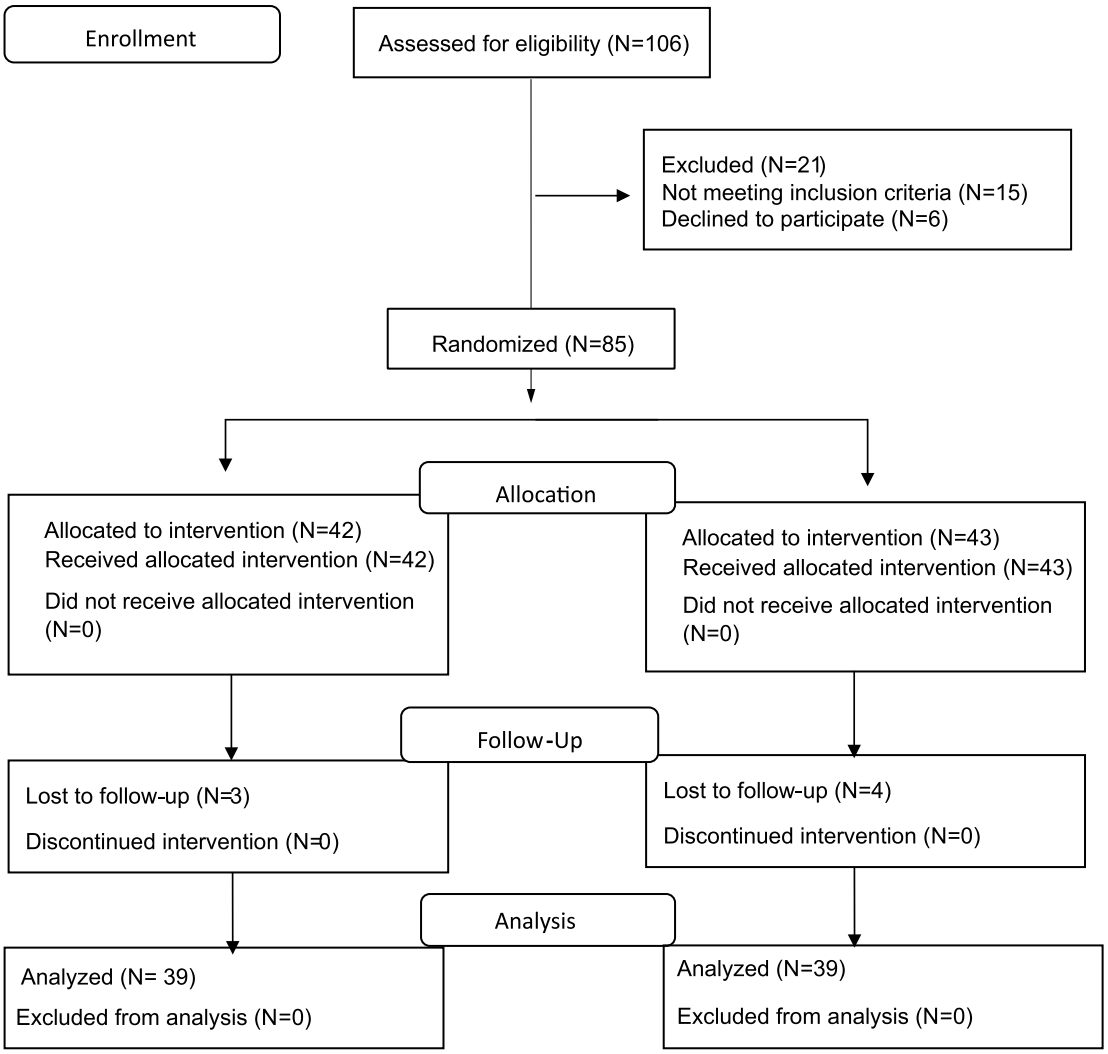

Fig.1: Consort flow diagram

Table 1: Baseline characteristics

Parameter	Total population	Intervention group	Control group	*Mann Whitney U* Test	*P* -value
Height (cm)	170.85± 6.01	171.12± 5.70	170.58± 3.65	705.500	0.578
Weight (kg)	81.68± 11.59	81.36± 11.56	82.00± 11.76	446.000	0.667
Body mass index (kg/m^2)	28.06± 4.26	27.84± 4.11	28.27± 4.45	730.500	0.764
Waist circumference (cm)	91 (42.75- 102)	99 (86- 104)	86 (42- 97)	446.000	0.002
Systolic blood pressure (mmHg)	136.92± 15.77	141.36± 15.01	132.49± 15.43	496.00	0.008
Diastolic blood pressure (mmHg)	84.56± 2.81	87.85± 8.88	81.28± 7.90	486.50	0.006
Serum 25-hydroxy vitamin D (ng/mL)	75.38 (51.22- 94.33)	74.21 (51- 90.01)	76.54 (54.37-97.70)	741.00	0.719
Serum total cholesterol (mmol/L)	4.38 (3.40- 5.09)	4.32 (3.39- 5.02)	4.43 (3.40- 5.23)	724.500	0.719
Serum triglycerides (mmol/L)	2.75 (1.92- 4.02)	2.80 (1.85- 3.64)	2.63 (2.07- 4.13)	726.500	0.734
Serum high-density lipoproteins (mmol/L)	0.93 (0.84- 1.17)	0.93 (0.82- 1.22)	0.93 (0.85- 1.11)	757.00	0.972
Serum low-density lipoproteins (mmol/L)	3.21 (2.42- 3.99)	3.01 (2.17- 4.00)	3.21 (2.79- 3.98)	682.00	0.433
Fasting plasma glucose (mmol/L)	7.85 (7.00- 10.45)	8.50 (7.00- 10.00)	7.80 (6.90- 10.86)	677.000	0.644
HbA_{1c} (%)	7.19 (6.50- 9.43)	8.50 (6.60- 9.80)	6.90 (6.40- 8.60)	611.500	0.136

Table 2: Changes in different lipid fractions compared between the two arms

Parameter	Intervention group Baseline	After 8 weeks	Decrease	Control group baseline	After 8 weeks	Decrease	*Mann Whitney U* Test	*P*-value
Total Cholesterol	4.32 (3.39-5.02)	4.44 (3.90- 4.95)	0.04 (-0.47- 0.27)	4.43 (3.40-5.23)	4.44 (3.84- 5.29)	-0.07(-1.22- 0.29)	688.00	0.469*
Triglycerides	2.80 (1.85- 3.64)	2.20 (1.78- 3.14)	0.30 (0.03-0.81)	2.63 (2.07-4.13)	2.77 (2.08- 3.35)	0.00 (-0.09- 0.76)	590.00	0.088*
HDL	0.93 (0.82- 1.22)	1.10 (0.92- 1.21)	-0.09 (-0.024-0.00)	0.93 (0.85-1.11)	0.92 (0.82- 1.18)	0.02 (-0.04- 0.10)	467.50	0.003*
LDL	3.01 (2.17- 4.00)	2.99 (2.21- 3.37)	0.13 (-0.26- 0.45)	3.21 (2.79-3.98)	3.10 (2.44- 3.58)	0.00 (-0.15- 1.07)	758.50	0.984*

**Statistical significance for comparison of the decrease between the two groups*

HDL: High-Density Lipoproteins, LDL: Low-Density Lipo proteins

Table 3: Reduction in lipid fractions compared on basis of baseline vitamin D status

Vitamin D status	Parameter	Intervention group	Control group	*Mann Whitney U* Test	*P*-value
Deficient	Total cholesterol	0.02 (-0.45- 0.19)	-0.11 (-0.47- 0.12)	164.500	0.474
	Triglycerides	0.26 (-0.22- 0.62)	-0.01 (-0.09- 0.07)	136.500	0.133
	HDL	0.06 (-0.27- 0.04)	0.02 (-0.05- 0.05)	134.500	0.118
	LDL	0.11 (-0.25- 0.70)	-0.04 (-0.20- 0.10)	155.00	0.325
Sufficient	Total cholesterol	0.08 (-0.48- 0.30)	-0.03 (-1.47- 0.49)	178.00	0.736
	Triglycerides	0.36 (0.08- 1.15)	0.19 (-1.19- 2.03)	163.00	0.448
	HDL	-0.11 (-0.24- 0.00)	0.03 (-0.12- 0.13)	106.500	0.019
	LDL	0.13 (-0.38- 0.21)	0.29 (-0.11- 1.17)	143.500	0.191

HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins

Discussion

Vitamin D deficiency is considered to be endemic in both developed and developing countries.[10] According to a systematic review, Pakistan is the worst affected south Asian country, with a prevalence of 73%.[11] Whereas this generally correlates with overall health status, a cause-and-effect relationship with many diseases has not been firmly established. This randomized controlled trial was done to determine the impact of vitamin D supplementation on lipid parameters. It demonstrated a benefit of vitamin D supplementation on high density lipoproteins in adult patients with MetS. This effect was observed over 8 weeks in patients with adequate baseline vitamin D levels, but was absent in patients who were deficient in Vitamin D to begin with. There was no change in other lipid fractions after vitamin D replacement as compared to the control group.

Results equitable to those documented in this study have previously been reported from Saudi Arabia.[12] Amongst 120 healthy individuals with hypovitaminosis, administration of vitamin D was associated with an increase in serum HDL, but no change occurred in any of the other lipid fractions. It is worth noting that the absence of a control group in this study made it difficult to control for confounders.

Results from most past studies are contradictory to ours. In a study by Jabeen S et al. on 40 patients with acute coronary syndrome, a single 200000 IU dose of vitamin D was associated with a reduction in total cholesterol and LDL levels, as well as an increase in HDL levels. However, there was no significant effect on serum triglycerides.[13] Miao J et al. compared two different vitamin D replacement regimens in an RCT involving 289 patients at a high risk of hypertension.[14] A higher dose of vitamin D (4000 IU daily) was associated with an increase in serum TGs, as compared to lower doses (400 IU daily) for six months.

In a study of 28 patients with type 2 diabetes mellitus and vitamin D deficiency, much lower cumulative doses of vitamin D3 were used for replacement. Whereas serum total cholesterol levels decreased, there was no impact on other lipid fractions, including TGs, HDL, and LDL.[15] However, this study did not have a control group and the authors were not sure if a larger sample size would provide the same results. Amongst two different groups of patients with type 2 diabetes mellitus, vitamin D replacement at 4000 IU per day for 16 and 48 weeks, respectively, had no effect on lipid profile.[16,17] In a clinical trial involving 53 overweight females in Iran, vitamin D given at a dose of 50000 IU per week for six weeks did not affect the serum lipid parameters.[18] The results remained identical in an Indian trial involving 121 prediabetic females, who were given slightly higher doses of vitamin D (60000 IU weekly) for eight weeks.[19]

In a randomized controlled trial, Ponda MP et al. randomized 150 vitamin D-deficient patients to receive 50000 IU of vitamin D3 weekly for 8 weeks or placebo.[20] All of these patients had an increased risk of cardiovascular disease. With a cumulative dose similar to that used in our study, no significant changes were observed in any lipid fraction. The authors did not provide any explanation for the lack of effect.

We believe that a possible explanation for the lack of

response to vitamin D supplementation in our patients is failure to achieve adequate vitamin D levels. However, this might not be the only reason since Ponda MP et al. documented normalization of vitamin D levels in their patients at the end of the study period, without a meaningful change in lipid parameters.[20] We did not check for serum vitamin D levels at the end of the study period for a couple of reasons. Firstly, the data analysis was pre-designed to follow an intention-to-treat principle, so that checking compliance with treatment was not a goal in this study. Secondly, we did not use a very high dose for a very long time, and thus the risk of vitamin D toxicity during this study was deemed to be very low. Thirdly, testing for serum vitamin D is very costly and cannot be done repeatedly because of limited financial resources. Results from an Iranian longitudinal study are reassuring in this regard. 345 women with varying baseline serum vitamin D levels received vitamin D3 supplementation at 50,000 IU once a month for 12 months. None of them had markedly elevated vitamin D levels during the study period. Even amongst patients with adequate levels at baseline, the levels did not reach above 50 ng/mL, suggesting that toxicity might not be that common.[21]

We carried out a subgroup analysis for patients with normal or low serum 25(OH) vitamin D levels at baseline, not reported in many of the studies that have been referenced in the preceding paragraphs. This helped us understand the impact of basal vitamin D levels on the biochemical outcomes evaluated in this study.

This was a short-term study. Although HDL levels improved with vitamin D supplementation, we are unsure how this change could have affected long-term clinical outcomes, particularly adverse cardiovascular outcomes. We did not check serum calcium levels periodically or monitor patients for significant adverse events. Changes in serum high-sensitivity C-reactive protein could have served as a surrogate marker of vitamin D's anti-inflammatory activity, but we did not evaluate this aspect.

Based on our observations in this study, we suggest longer-duration trials. This would help better assess the toxicity of vitamin D3 and could also capture data on important clinical outcomes related to changes in lipid fractions.

Conclusion

Administration of vitamin D was associated with an increase in serum HDL levels over the eight-week study period. This effect was seen in vitamin D-replete patients, but not in patients with vitamin D deficiency. There were no changes in total cholesterol, LDL, and TGs associated with this intervention.

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Conflict of Interest: The authors declare no conflict of interest

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Author Contributions

AH: Conception and design of the work, manuscript writing for methodology design, and investigation

ARA: Data acquisition, curation, and statistical analysis, writing the original draft, proofreading, and approval for final submission

MB: Validation of data, interpretation, and write-up of results, revising, editing, and supervising for intellectual content