Received: 6 June 2016 | Accepted: 6 July 2016

DOI: 10.1111/ijcp.12866

ORIGINAL PAPER

WILEY THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

Effect of Vitamin D and calcium supplementation on ischaemic stroke outcome: a randomised controlled open-label trial

Anu Gupta[1] | Sudesh Prabhakar[1]* | Manish Modi[1] | Sanjay K Bhadada[2] | Mani Kalaivani[3] | Vivek Lal[1] | Dheeraj Khurana[1]

[1]Department of Neurology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[2]Department of Endocrinology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[3]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India

Present address:
*Director Neurology, Fortis Hospital, Mohali

Correspondence
S. Prabhakar, Former Professor and Head, Department of Neurology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.
Email: sudeshprabhakar@gmail.com

Summary

Background and aims: Vitamin D deficiency is a common problem in stroke survivors. Observational studies have reported an association of low vitamin D levels with greater stroke severity, poststroke mortality and functional disability. Randomised clinical trials are lacking. We sought to assess the effect of calcium and vitamin D supplementation in ischaemic stroke survivors with vitamin D deficiency/insufficiency on disability/mortality outcomes.

Methods: In this randomised controlled open-label trial, 73 patients of acute ischaemic stroke were screened for serum 25 hydroxy Vitamin D (25(OH)D) levels. A total of 53 patients with baseline 25(OH)D <75 nmol/L were randomised into two arms. One received vitamin D and calcium supplementation along with usual care (n=25) and the other received usual care alone (n=28). Primary outcome was the proportion of patients achieving a good outcome [modified Rankin Scale score 0–2] at 6 months and all cause mortality at 6 months.

Results: The age (mean±SD) of participants was 60.4±11.3 years, 69.8% were males. The proportion of patients achieving good outcome was higher in the intervention arm (Adjusted OR 1.9, 95% CI 0.6–6.4; *P*=.31). The survival probability was greater in the intervention arm (83.8%, CI 62.4–93.6) as compared with the control arm (59.5%, CI 38.8–75.2; *P*=.049) with adjusted Hazard ratio (HR) of 0.26 (95% CI 0.08–0.9; *P*=.03).

Conclusions: This is the first randomised controlled study assessing the effect of vitamin D and calcium supplementation on ischaemic stroke outcomes and points towards a potential benefit. Findings need to be validated by a larger trial.

1 | INTRODUCTION

Stroke is one of the leading causes of death and disability in the world, South Asian region being the highest contributor (accounting for more than 40% of global stroke deaths).[1] Majority of the survivors are reported to have poor functional outcome.[1]

Vitamin D deficiency has been identified as a common problem in stroke survivors with an estimated prevalence of 71%.[2] Limited mobility with decreased sunlight exposure and malnutrition, frequently observed in patients after stroke, contribute to this deficiency. South Asians are particularly prone, as they have a dark skin tone which acts as a natural sun protectant (dark skin requires at least three to five times longer exposure to make the same amount of vitamin D as compared with white),[3] and are nutritionally poor (vegetarian food habits, lack of food fortification).

Low vitamin D levels in these patients are of concern not only because of musculoskeletal complications[4] but also because the recent prospective observational studies have reported an association of low vitamin D levels with greater stroke severity, functional disability,[5] cerebrovascular death, cardiovascular events and mortality.[6–10] Vitamin D being a neurosteroid with vitamin D receptors widely expressed in neuronal and glial cells, also has a putative neuroprotective role.[11]

Although the observational and experimental literature linking low vitamin D levels with greater morbidity and mortality in stroke patients is alluring, evidence from randomised clinical trials is lacking.

Hence, with this knowledge gap in the background, we planned to assess the effect of vitamin D and calcium supplementation on the outcome of ischaemic stroke in South Asian stroke survivors with vitamin D deficiency, through a randomised clinical trial.

What's known

- Vitamin D deficiency is a common problem in stroke survivors.
- Observational studies have reported an association of low vitamin D levels with greater poststroke disability and mortality.

What's new

- This is the first randomised controlled study assessing the effect of vitamin D and calcium supplementation on ischaemic stroke outcomes.
- It reports a decrease in mortality, and a trend towards improvement in disability with vitamin D and calcium supplementation in ischaemic stroke survivors with vitamin D deficiency.

2 | MATERIAL AND METHODS

2.1 | Study design and recruitment

The study was a randomised controlled open-label trial (December 2011–March 2013) conducted at a tertiary care hospital. 25(OH) D levels were evaluated in patients who satisfied these preliminary inclusion criteria: (i) Presentation within 7 days of onset of first ever ischaemic stroke, (ii) Prestroke modified Rankin Score (mRS) <2, (iii) Age ≥35 years. Patients already on vitamin D and calcium supplementation, those with renal and hepatic impairment and those who underwent thrombolysis were excluded.

Out of 73 patients who satisfied preliminary inclusion criteria, 53 who had vitamin D deficiency/insufficiency [serum 25(OH)D levels <75 nmol/L] were then recruited, and randomised to either receive vitamin D and calcium supplementation along with usual poststroke care or usual care alone. This being a pilot study, we aimed to recruit at least 25 patients in each arm.

All patients had a computed tomography (CT) or magnetic resonance imaging (MRI) brain scan at baseline. The study was approved by the Institute ethics committee and registered with the clinical trials registry India (CTRI; Number – CTRI/2013/02/003440). All subjects gave a written informed consent. The study was performed according to the Declaration of Helsinki and has been reported as per the CONSORT 2010 guidelines.

The serum 25(OH)D levels of the initial 73 patients (who satisfied the inclusion criteria and underwent evaluation for vitamin D levels) were also compared with controls and results have been published previously.[12]

2.2 | Data collection

A questionnaire-based direct interview was used to collect information on disease profile, demographic variables, risk factors and medications used. The Oxford Community Stroke Project classification (OCSP) was used to classify ischaemic stroke subtypes.[13] Patients underwent measurements like blood pressure, serum 25(OH)D, intact parathyroid hormone (iPTH), serum total calcium, inorganic phosphorous, lipid profile, glycosylated haemoglobin (HbA1c), fasting plasma glucose (FPG), renal and liver function tests using standardised methods. Baseline stroke severity was assessed using the National Institute of Health Stroke Scale (NIHSS). Serum 25(OH)D levels were measured by chemiluminescence assay using commercially available kits (Elecsys 2010 system; Roche Diagnostic, Mannheim, Germany). iPTH was measured by immunochemiluminiscence (Elecsys 2010; Roche, Germany, CV=4.3%–7.1%, normal range 15–65 pg/mL). Individuals whose serum 25(OH)D value was less than 50 nmol/L (20 ng/mL) were classified as deficient, 50–74 nmol/L (20–29 ng/mL) as insufficient and ≥75 nmol/L (30 ng/mL) were identified as sufficient.[14] Some of the other disease definitions used are mentioned in Data S1.

2.3 | Randomisation and follow up

Randomisation was done on the basis of a random number generated by computer in a 1:1 allocation ratio. The intervention group received a single intramuscular injection of 600 000 IU cholecalciferol followed by oral cholecalciferol 60 000 IU once a month with one gram elemental calcium daily along with usual poststroke care, the control group received usual care alone. The two groups were followed up for 6 months. Serum 25(OH)D and iPTH levels were repeated at 3 and 6 months. Outcome was assessed using modified Rankin scale (mRS) at 3 and 6 months post stroke. The modified Rankin Scale is an ordinal scale ranging from 0 (no disability) to 5 (severe disability) with a score of 6 allocated to those who die. Some patients who did not visit hospital at 3 or 6 months were interviewed telephonically for outcome assessment, their repeat serum 25(OH)D and iPTH levels were not done. The primary outcomes were (i) Proportion of patients achieving good outcome (mRS 0–2) at 6 months and (ii) All cause mortality in the two arms. The randomisation allocation and outcome assessment was done by a single investigator and was not blinded. The primary outcome was analysed both on intention to treat and per protocol basis. For patients who were lost to follow up, the last available 25(OH)D and iPTH and last recorded mRS was considered as the final value at 6 months.

2.4 | Statistical analysis

Statistical analysis was carried out using Stata 11.0 (College station, TX, USA). Data were presented as number (percentage), mean±SD or

median (minimum–maximum) as appropriate. The baseline categorical and continuous characteristics were compared using chi-square test/Fisher's exact test and student's *t* test/Wilcoxon rank sum test as appropriate. Serum 25(OH)D was compared between the two groups using ANCOVA adjusted for total serum cholesterol levels. The difference in the median iPTH value between the groups was compared using Wilcoxon rank sum test and the change in iPTH values at 6 months from baseline within the groups was compared using Wilcoxon signed rank test. Both intention to treat and per protocol analysis was carried out for the primary outcome (mRS 0–2 at 6 months). The primary outcome was compared between the groups using chi-square test. The results were presented as difference (95% CI). Also odds ratio (OR) (95% CI) was calculated adjusting for total serum cholesterol. The survival probability between the groups was done using Kaplan-Meier curves and tested using log rank test. The hazard ratio (HR) (95% CI) for mortality was calculated using cox proportional hazard model. A $P<.05$ was considered statistically significant.

3 | RESULTS

3.1 | Demographic and risk factor profile

A total of 53 patients who fulfilled the preliminary inclusion criteria and had 25(OH)D levels <75 nmol/L were recruited. The mean age of the cohort was 60.4±11.3 years, 69.8% were males. The detailed clinical profile is summarised in Table S1. After randomisation, the two arms—vitamin D plus calcium-supplemented (n=25) and usual care (n=28) were followed up for 6 months (consort diagram—Fig. 1). Baseline covariates were similar (Table 1) except total serum cholesterol levels which were higher in the supplemented arm ($P=.03$).

3.2 | Follow-up Vitamin D assessment

The serum 25(OH)D levels increased by 47.3 (25.0–69.5) nmol/L in vitamin D plus calcium-supplemented arm ($P<.001$), and by 0.8 (−7.5–8.8) nmol/L in usual care arm ($P=.86$) by 6 months. The between group

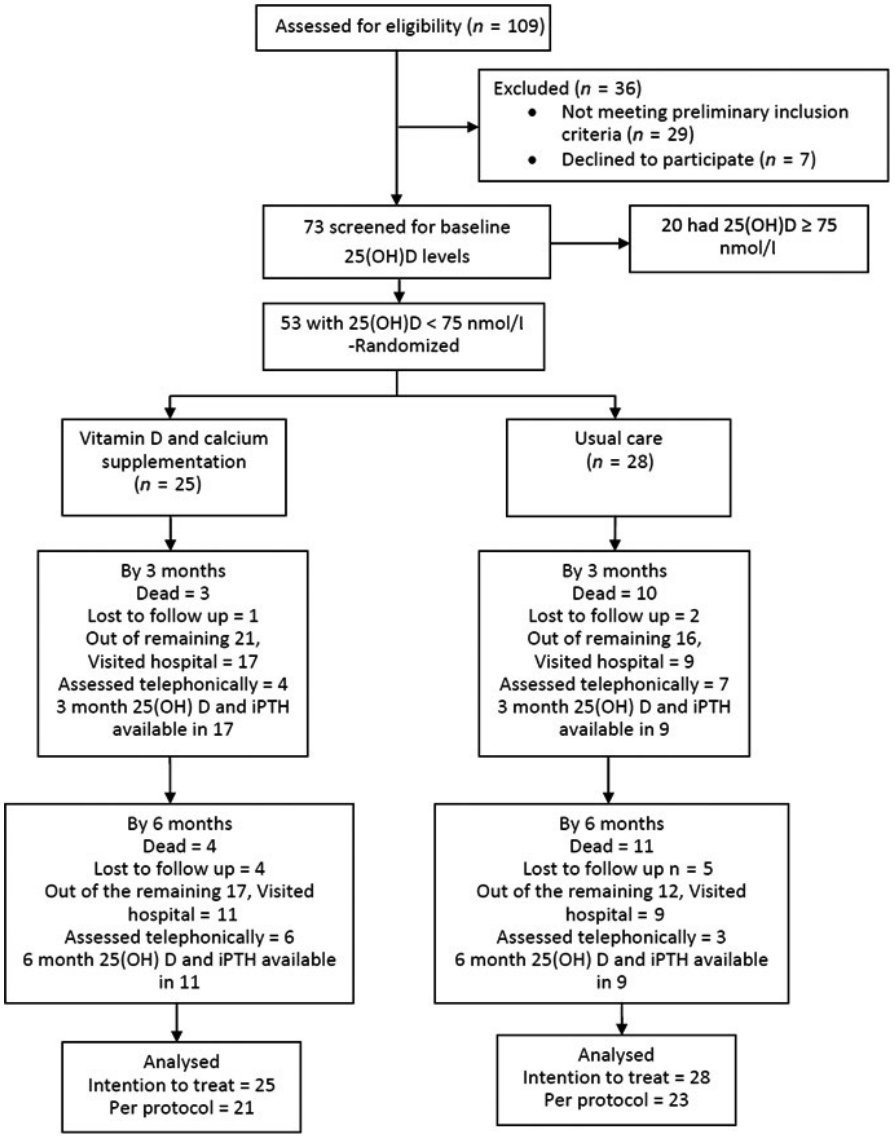


FIGURE 1 Consort diagram showing the trial profile

TABLE 1 Baseline characteristics in the intention to treat population

Parameter	Vitamin D plus calcium (n=25)	Usual care (n=28)	*P* value
Age (years), Mean±SD	61.8±11.5	59.2±11.2	.40
Males, n (%)	17 (68)	20 (71.4)	.79
NIHSS, Median (Range)	12 (3–25)	12 (2–29)	.87
Hypertension, n (%)	22 (88)	23 (82.1)	.71
Diabetes Mellitus, n (%)	16 (64)	11 (39.3)	.07
Smokers, n (%)	3 (12)	7 (25)	.30
Obese, n (%)	15 (60)	15 (53.6)	.64
IHD/CMP, n (%)	7 (28)	7 (25)	.81
AF, n (%)	2 (8)	2 (7.1)	1.00
Drugs, n (%)	3 (12%)	2 (7.1)	.66
Total cholesterol (mmol/L), Mean±SD	4.8±1.5	3.9±1.2	**.03**
LDL (mmol/L), Mean±SD	2.7±1.1	2.1±1.1	.06
HDL (mmol/L), Mean±SD	1.1±0.3	0.9±0.3	.21
Triglycerides (mmol/L), Mean±SD	1.7±0.8	1.5±0.5	.24
HbA1c, Mean±SD	8.0±2.7	7.1±2.1	.16
FBG (mmol/L), Mean±SD	8.0±3.6	6.6±1.8	.13
Vitamin D (nmol/L), Mean±SD	45.0±14.5	42.5±16.8	.60
iPTH (ng/L), Median (Range)	39 (10–167)	43 (19–133)	.97
Calcium (mmol/L), Mean±SD	2.3±0.2	2.3±0.3 (n=23)	.55
Phosphorus (mmol/L), Mean±SD	1.2±0.3 (n=23)	1.1±0.3 (n=23)	.55
OCSP class, n (%)			
LACI	7 (28)	6 (21.4)	.80
TACI	6 (24)	10 (35.7)	
PACI	8 (32)	9 (32.1)	
POCI	4 (16)	3 (10.7)	
Baseline mRS, n (%)			
0–2	1 (4)	4 (14.3)	.36
3–5	24 (96)	24 (85.7)	

and pre–post intervention differences in the serum 25(OH)D levels are summarised in Table S2.

In terms of relative risk, the patients who received usual care were 2.3 times more likely to remain vitamin D deficient/insufficient at 6 months as compared with those who received vitamin D and calcium supplementation (Absolute risk difference with 95% CI: 53% [31%, 74%] and RR with 95% CI: 2.3 [1.4–3.8], *P*<.001, Table S3).

No adverse effects were reported in either of the groups.

3.3 | Follow-up iPTH assessment

In the vitamin D plus calcium-supplemented arm, serum iPTH levels decreased from a median of 39 ng/L at baseline to 34.9 ng/L at 6 months (*P*=.24), while in the usual care arm, serum iPTH levels increased from a median of 43.0 ng/L at baseline to 53.5 ng/L at 6 months (*P*=.003) (Fig. 2). There was significant difference in the iPTH levels between the vitamin D plus calcium-supplemented and usual care arms at 6 months (*P*<.001), which was absent at baseline (*P*=.97) (Fig. 2).

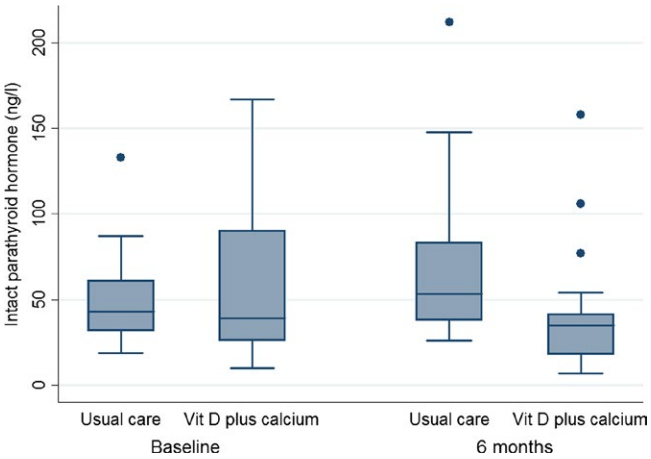


FIGURE 2 Box plot showing iPTH levels between two groups. Usual care: Baseline vs 6 months, *P*=.003. Vitamin D plus calcium: Baseline vs 6 months, *P*=0.24

3.4 | Clinical outcome differences between the randomised arms at 6 months

3.4.1 | Intention to treat basis

At final follow up (6 months), 11 patients (44%) had a good outcome (mRS score between 0 and 2) in the vitamin D plus calcium-supplemented arm and 11 patients (39.3%) had a good outcome in the usual care arm, (Adjusted OR 1.9, 95% CI 0.6–6.4; *P*=.31, Risk difference 4.7% [−21.9–31.3], Table 2).

3.4.2 | Per protocol basis

At final follow up (6 months), 11 patients (52.4%) had a good outcome (mRS score between 0 and 2) in the vitamin D plus calcium-supplemented arm and 10 patients (43.5%) had a good outcome in the usual care arm, (Adjusted OR 2.3, 95% CI 0.6–9.1; *P*=.22, Risk difference 8.9 [−20.5–38.3], Table 2).

The results based on favourable outcome as the end-point (defined by an mRS score of 0–1 at 6 months) are summarised in Table S4.

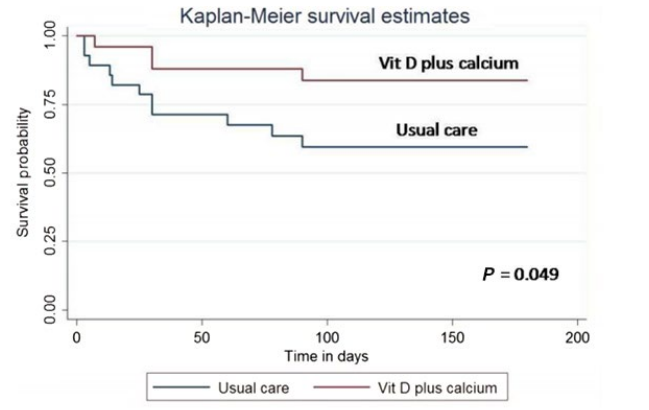
3.5 | Survival analysis and hazard ratio

Overall 15 patients died in the cohort, with four deaths (16%) in the vitamin D plus calcium-supplemented arm and 11 deaths (39.3%) in the usual care arm. The survival probability at 6 months was higher in the vitamin D plus calcium-supplemented arm (83.8%, CI 62.4–93.6) as compared with the usual care arm (59.5%, CI 38.8–75.2), *P*=.049. The hazard of death was 74% less in the vitamin D plus

TABLE 2 Clinical outcomes at 6 months

Outcome (6 months)	Vitamin D plus calcium	Usual care	Difference % (95% CI)	Unadjusted OR/HR with 95% CI	*P* value	Adjusted* OR/ HR with 95% CI	*P* value
Intention to treat	n=25	n=28					
Good (MRS 0-2)	11 (44%)	11 (39.3%)	4.7 (−21.9–31.3)	1.2 (0.4–3.6)	.73	1.9 (0.6–6.4)	.31
Per protocol	n=21	n=23					
Good (MRS 0-2)	11 (52.4%)	10 (43.5%)	8.9 (−20.5–38.3)	1.4 (0.4–4.7)	.56	2.3 (0.6–9.1)	.22
Mortality outcomes	n=25	n=28					
Death	4 (16%)	11 (39.3%)	–	0.34 (0.1–1.1)	.06	0.26 (0.08–0.9)	.03
Survival probability	83.8% (62.4–93.6)	59.5% (38.8–75.2)					
p(log rank)	0.049						

*Adjusted for total cholesterol



Number at risk	30 days	60 days	90 days	180 days
Vit D plus calcium *n* = 25	24	24	21	17
Usual care *n* = 28	22	18	16	12

FIGURE 3 Kaplan-Meier survival estimates

calcium-supplemented arm as compared with the usual care arm (Adjusted HR 0.26, 95% CI 0.08–0.9; *P*=.03 and Unadjusted HR 0.34, 95% CI 0.1–1.1; *P*=.06 – Table 2 and Fig. 3).

4 | DISCUSSION

This study reports the first ever, randomised controlled evaluation of clinical stroke outcomes with vitamin D and calcium supplementation, and points towards a potential benefit, in ischaemic stroke survivors with vitamin D deficiency/insufficiency.

In the present study, patients who received usual care were 2.3 times more likely to remain vitamin D deficient/insufficient at 6 months as compared with those who received supplementation. They also had a significant rise in the iPTH levels at 6 months. Low calcium levels caused by vitamin D deficiency stimulate iPTH secretion resulting in secondary hyperparathyroidism.[15]

The outcome analysis revealed a decrease in mortality with vitamin D and calcium supplementation (Adjusted HR 0.26, 95% CI 0.08–0.9; *P*=.03), and a trend towards improvement in disability (Adjusted OR 1.9, 95% CI 0.6–6.4; *P*=.31).

The previous studies on association of vitamin D deficiency with cerebrovascular deaths, cardiovascular events and all cause mortality have been controversial. Favourable evidence comes primarily from observational studies, with single 25(OH)D measurements (which may not be reflective of a lifetime vitamin D status).[6–10,16] The available randomised controlled studies have not revealed a consistent beneficial effect of supplementing vitamin D on mortality.[17,18] However, these were studies of primary prevention, and not in poststroke patients. They did not do PTH measurements in the supplemented patients to conclude whether the achieved 25(OH)D levels were sufficient to treat or prevent potentially harmful PTH elevations.

The trend towards a better survival probability with vitamin D and calcium supplementation seen in this trial, if real, suggests the following plausible physiological mechanisms. Vitamin D is thought to, protect against various vascular risk factors like hypertension,[19] diabetes,[20] atherosclerosis,[21,22] and exerts antithrombotic,[23] anti-inflammatory[24] and neuroprotective effects.[25,26] Also, calcium and vitamin D supplementation lower parathyroid hormone levels (PTH), and PTH levels have been shown to predict overall cardiovascular mortality.[27] Although, baseline PTH levels were comparable in the two groups, we found significant elevation in PTH levels in the usual care group at the end of 6 months. Hence, whether the evolving secondary hyperparathyroidism contributed to reduced survival probability in the usual care arm, is a question that needs to be answered in further studies.

However, we must acknowledge that residual confounding as a result of baseline differences in the serum cholesterol levels (supplemented arm had higher baseline levels), prevalence of atherosclerosis (which was not evaluated in detail in this study) and, level of outdoor physical activity and dietary habits cannot be ruled out. Olsen et al. have previously shown an inverse relationship between serum cholesterol levels and, ischaemic stroke severity and poststroke mortality.[28]

We did not find a significant difference in the proportion of patients achieving good functional outcome at 6 months after supplementation, but observed a trend towards decrease in disability. Recent studies have shown an association between low 25(OH)D levels and acute ischaemic stroke severity, using the NIHSS [5] and worse functional

outcomes at 3 months using the mRS.[29,30] However, the above studies are of an observational design, done in other ethnic groups and vitamin D deficiency was defined at a different threshold (25–50 nmol/L or 10–20 ng/mL), so difficult to compare with our study.

Vitamin D supplementation has also been shown to improve muscle strength and reduce falls and fractures in elderly poststroke hemiplegics.[31] Our follow up was of a relatively shorter duration, for any translation of these musculoskeletal benefits to improved functional outcomes.

This trial although underpowered, highlights potential benefit of vitamin D and calcium supplementation in improving stroke outcomes, and raises a need for a large randomised controlled trial to prove or reflect its findings. The main strengths of the present study are the randomised controlled design, intention to treat analysis and repeat 25(OH)D and iPTH measurements at 3 and 6 months. The major limitation was that the allocation and outcome assessment was not blinded. Also, follow up was relatively short. Repeat serum 25(OH)D and iPTH levels were not available for all patients, as final outcome for some was assessed telephonically. It is difficult to distinguish between effects of calcium and vitamin D because the intervention combined these ingredients. People with obesity could have required a higher dose to replete their vitamin D status. It was exclusively done on South Asian population, so findings are not generalisable to other races/ethnicities.

To conclude, ischaemic stroke survivors with suboptimal vitamin D status have a high likelihood of remaining vitamin D deficient/insufficient at 6 months post stroke, if not supplemented. This deficiency leads to an evolution towards secondary hyperparathyroidism. There is a decrease in mortality with vitamin D and calcium supplementation, and a trend towards improvement in disability which needs evaluation in a larger trial. Longer follow up may be required to study the effect on morbidity. It would however be interesting to study the evolution trends of secondary hyperparathyroidism and its associations with mortality and morbidity in poststroke patients.

ACKNOWLEDGEMENTS

None.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

Gupta A acquired, analysed and interpreted the data and drafted the manuscript. Prabhakar S, Modi M, Bhadada SK, Lal V and Khurana D contributed to the research design and reviewed the manuscript. Kalaivani M contributed to the analysis and interpretation of the data and reviewed the manuscript. All authors approved the final and submitted version. Authors had access to the study data that support the publication.

DISCLOSURE

None.

REFERENCES

1. Wasay M, Khatri IA, Kaul S. Stroke in South Asian countries. *Nat Rev Neurol*. 2014;10:135–143.
2. Uluduz D, Adil MM, Rahim B, et al. Vitamin D deficiency and osteoporosis in stroke survivors: an analysis of National Health and Nutritional Examination Survey (NHANES). *J Vasc Interv Neurol*. 2014;7:23–28.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930.
4. Sato Y, Asoh T, Kondo I, et al. Vitamin D deficiency and risk of hip fractures among disabled elderly stroke patients. *Stroke*. 2001;32:1673–1677.
5. Daubail B, Jacquin A, Guilland JC, et al. Serum 25-hydroxyvitamin D predicts severity and prognosis in stroke patients. *Eur J Neurol*. 2013;20:57–61.
6. Pilz S, Dobnig H, Fischer JE, et al. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke*. 2008;39:2611–2613.
7. Kilkkinen A, Knekt P, Aro A, et al. Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol*. 2009;170:1032–1039.
8. Bair TL, May HT, Horne BD, et al. Vitamin D deficiency is strongly associated with incident death and cardiovascular disease in a general healthcare population. *Circulation*. 2009;120:S455.
9. Drechsler C, Pilz S, Obermayer-Pietsch B, et al. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. *Eur Heart J*. 2010;31:2253–2261.
10. Anderson JL, May HT, Horne BD, et al. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol*. 2010;106:963–968.
11. Makariou SE, Michel P, Tzoufi MS, et al. Vitamin D and stroke: promise for prevention and better outcome. *Curr Vasc Pharmacol*. 2014;12:117–124.
12. Gupta A, Prabhakar S, Modi M, et al. Vitamin D status and risk of ischemic stroke in North Indian patients. *Indian J Endocrinol Metab*. 2014;18:721–725.
13. Bamford J, Sandercock P, Dennis M, et al. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. 1991;337:1521–1526.
14. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
15. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev*. 2001;22:477–501.
16. Daubail B, Jacquin A, Guilland JC, et al. Association between serum concentration of vitamin D and 1-year mortality in stroke patients. *Cerebrovasc Dis*. 2014;37:364–367.
17. LaCroix AZ, Kotchen J, Anderson G, et al. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. *J Gerontol A Biol Sci Med Sci*. 2009;64:559–567.
18. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ*. 2003;326:469.
19. Pilz S, Tomaschitz A, Ritz E, et al. Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol*. 2009;6:621–630.

20. Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92:2017–2029.
21. Reis JP, von Mühlen D, Michos ED, et al. Serum vitamin D, parathyroid hormone levels, and carotid atherosclerosis. *Atherosclerosis*. 2009;207:585–590.
22. Targher G, Bertolini L, Padovani R, et al. Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. *Clin Endocrinol (Oxf)*. 2006;65:593–597.
23. Goswami R, Mishra SK, Kochupillai N. Prevalence & potential significance of vitamin D deficiency in Asian Indians. *Indian J Med Res*. 2008;127:229–238.
24. Schleithoff SS, Zittermann A, Tenderich G, et al. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*. 2006;83:754–759.
25. Wang Y, Chiang YH, Su TP, et al. Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. *Neuropharmacology*. 2000;39:873–880.
26. Won S, Sayeed I, Peterson BL, et al. Vitamin D prevents hypoxia/reoxygenation-induced blood-brain barrier disruption via vitamin D receptor-mediated NF-kB signalling pathways. *PLoS ONE*. 2015;10:e0122821.
27. Hagström E, Hellman P, Larsson TE, et al. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation*. 2009;119:2765–2771.
28. Olsen TS, Christensen RH, Kammersgaard LP, et al. Higher total serum cholesterol levels are associated with less severe strokes and lower all-cause mortality: ten-year follow-up of ischemic strokes in the Copenhagen Stroke Study. *Stroke*. 2007;38:2646–2651.
29. Daumas A, Daubail B, Legris N, et al. Association between admission serum 25-hydroxyvitamin D levels and functional outcome of thrombolyzed stroke patients. *J Stroke Cerebrovasc Dis*. 2016;25:907–913.
30. Park KY, Chung PW, Kim YB, et al. Serum vitamin D status as a predictor of prognosis in patients with acute ischemic stroke. *Cerebrovasc Dis*. 2015;40:73–80.
31. Sato Y, Iwamoto J, Kanoko T, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis*. 2005;20:187–192.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.