Introduction: Statin-associated muscle symptoms (SAMS) can lead to medication non-adherence among statin users. There is a complex relationship between SAMS, vitamin D and low-density lipoprotein cholesterol (LDL-C). The objective of this study was to evaluate the relationship between vitamin D, LDL-C and occurrence of SAMS. Methods: This was a cross-sectional study in patients using statins. Thorough patient histories were taken, a clinical examination was conducted and SAMS were recorded. Levels of vitamin D, creatine phosphokinase (CPK) and LDL-C were measured. These parameters were compared amongst statin users with SAMS and those without SAMS. Levels of vitamin D and LDL-C were converted into percentiles and their relationship with SAMS was evaluated in terms of odds ratio. Receiver operating characteristics (ROC) were drawn, taking vitamin D and LDL-C as predictors of SAMS. Results: A total of 121 statin users were enrolled in this study. Thirty-eight patients (31.4%) presented with SAMS. Significantly lower levels of serum vitamin D were observed amongst statin users with SAMS compared with those without SAMS (19.8 ± 9.67 ng/mL versus 25.0 ± 14.6 ng/mL; 95% confidence interval -10.4 to -0.07; P=0.04). With vitamin D levels less than or equal to 5th, 10th and 25th percentile, the chances of occurrence of SAMS were significantly higher, but not at the 50th percentile (corresponding vitamin D level of 20.21 ng/mL). LDL-C did not show any conclusive relationship with SAMS. ROC curves showed a significant discrimination for vitamin D levels, but not for LDL-C. Conclusion: Statin users with low levels of vitamin D are at increased risk of developing SAMS. However, LDL-C status of statin users failed to predict any meaningful association with SAMS. Given the small sample size of this study, these results should be regarded as preliminary.

Keywords
Atorvastatin, creatine phosphokinase, myalgia, rosuvastatin

Disclosures: Harsheen Kaur, Jagjit Singh, Jeet Ram Kashyap, Ravi Rohilla, Harmanjit Singh, Shivani Jaswal and Rajiv Kumar have no financial or non-financial relationships or activities to declare in relation to this article.

Acknowledgement: This project was initiated under the Indian Council of Medical Research’s short term studentship programme, which is for sensitising undergraduate medical students towards research. This work was presented as a research poster at Pharmacology 2018, the annual meeting of the British Pharmacological Society held in London, UK, 18-20 December 2018.

Review Process: Double-blind peer review.

Compliance with Ethics: All procedures were followed in accordance with the responsible committee on human experimentation and with the Helsinki Declaration of 1975 and subsequent revisions, and informed consent was received from the patient involved in this study. Ethical approval was received from the Institutional Ethics Committee vide letter No. IEC/2018/97.

Authorship: The named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Access: This article is freely accessible at touchENDOCRINOLOGY.com © Touch Medical Media 2020.

Received: 7 May 2020
Accepted: 23 June 2020
Published Online: 6 October 2020

Citation: European Endocrinology. 2020;16(2):137–42

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Support: No funding was received in the publication of this article.

Statin-associated muscle symptoms (SAMS) may lead to medication non-adherence among statin users. SAMS include myalgia, myositis and rhabdomyolysis (rarely seen). According to a recent study conducted in India, about 22% of statin users are affected by SAMS. Many studies have been conducted to establish a correlation between SAMS and levels of vitamin D and creatine phosphokinase (CPK; a marker of muscle damage), but the exact mechanism is not yet clear. It has been documented that statins can affect vitamin D levels; low-density lipoprotein cholesterol (LDL-C) is a vitamin D carrier and statins, by reducing LDL-C, could decrease vitamin D levels. However, to complicate this, some statins and vitamin D are metabolized by the CYP3A4 enzyme; hence by competing for binding with CYP3A4, statins could increase levels of vitamin D. While there is evidence that vitamin D supplementation can reduce myalgia, most clinical studies have produced mixed results regarding the relationship between SAMS and vitamin D levels. Hence there is a complex relationship between SAMS, vitamin D and LDL-C levels. Moreover, studies evaluating the relationship between LDL-C levels, vitamin D levels and SAMS are currently limited. The main objective of this study was to establish the relationship between SAMS and levels of vitamin D and LDL-C.

Methods
Study design, study setting and ethics

This single-centre, cross-sectional, comparative, observational study was carried out from 26 June 2018 to 28 September 2018, in the Departments of Cardiology, Pharmacology and Biochemistry of the Government Medical College and Hospital, Chandigarh, India, after obtaining permission from the Institutional Ethics Committee (vide letter No. IEC/2018/97). The study was carried out according to the
principles of Good Clinical Practice and the Declaration of Helsinki. Male and female patients visiting the cardiology outpatient department who had been prescribed statins, irrespective of the indication, were included in the study. However, those with onset of myalgia before commencement of statin treatment, traumatic injuries, fever, viral or bacterial infections, arthritis of any type and other conditions presenting with muscular pain, and those who were not willing to give written informed consent, were excluded.

Eligible patients attending the cardiology outpatient department of our institute, fulfilling the inclusion criteria and having none of the exclusion criteria, were approached. The objective and the methodology were explained to the participants and, if they agreed to participate, written informed consent was obtained. A thorough medical history was taken (details pertinent to personal information, disease and medicines and other treatment modalities, concomitant diseases and medications, duration of statin use) and a clinical examination was conducted, after which, individual SAMS were recorded in case-report form. In order to conduct laboratory investigations, 5 mL of blood was withdrawn from each patient in aseptic conditions.

Biochemical estimations

Relevant laboratory investigations, including vitamin D levels, CPK levels, and LDL-C levels, were performed for all patients. Vitamin D was estimated by a fully automated chemiluminescence system (ADVIA Centaur XP, Siemens, Munich, Germany) using the specific kit. This estimation is based on competitive immunoassays as described by Thienpont et al. Vitamin D levels were classed as deficient (<20 ng/mL), insufficient (20–30 ng/mL) and sufficient (>30 ng/mL). CPK estimation was done using a Roche Modular P800 chemistry analyser. The principle and the method have been described in detail by Pisani et al. An enzymatic colorimetric assay on the Roche Modular P800 chemistry analyser. The principle and the method have been described in detail by Pisani et al.

Results

Demographic characteristics of the participants

The predicted sample size required for this study was 246; however, due to the institutional and funding constraints and the limited period for

**Table 1: Comparison of various parameters between statin users with and without statin-associated muscle symptoms**

| Parameter                        | Statin users with SAMS n=38 | Statin users without SAMS n=83 | p value |
|----------------------------------|-----------------------------|--------------------------------|---------|
| Age, years                       | 57.24 ± 10.61               | 56.65 ± 11.40                 | 0.789 (t-test) |
| Weight, kg                       | 65.37 ± 10.57               | 64.93 ± 13.25                 | 0.857 (t-test) |
| Height, meters                   | 1.63 ± 0.07                 | 1.61 ± 0.09                   | 0.422 (t-test) |
| BMI, kg/m²                       | 24.55 ± 4.08                | 24.79 ± 4.81                  | 0.789 (t-test) |
| Duration of statin use, median days (range) | 180 (3–1,620)             | 45 (1–1,800)                  | <0.010* (Mann–Whitney U test) |
| Number of concomitant drugs      | 5.26 ± 1.70                 | 5.41 ± 1.76                   | 0.669 (t-test) |
| Number of patients taking fibrates | 2                           | 0                             | 0.097 (Fisher exact test) |
| Number of patients with history of smoking | 9                           | 22                            | 0.741 (Chi square test) |
| Number of patients with history of alcohol intake | 9                           | 21                            | 0.848 (Chi square test) |
| Number of patients with family history of CAD | 2                           | 17                            | 0.033* (Fisher exact test) |
| Mean dose of atorvastatin, mg/day | 30.32 ± 11.96               | 29.58 ± 12.83                 | 0.785 (t-test) |
| Mean dose of rosvastatin, mg/day  | 12.86 ± 4.88                | 12.73 ± 4.67                  | 0.956 (t-test) |
| Serum vitamin D levels, mg/mL     | 19.81 ± 9.67                | 25.05 ± 14.67                 | 0.047* (t-test) |
| CPK levels, IU/L                  | 149.63 ± 119.94             | 97.36 ± 46.94                 | 0.001* (t-test) |
| LDL-C levels, mg/dL               | 86.82 ± 71.50               | 83.77 ± 46.83                 | 0.781 (t-test) |

*Significant value defined as p<0.05.

Data are presented as mean ± standard deviation unless stated otherwise.

BMI = body mass index; CAD = coronary artery disease; CPK = creatine phosphokinase; LDL-C = low-density lipoprotein cholesterol; SAMS = statin-associated muscle symptoms.
Participants had muscular pain affecting predominantly the lower limbs, was 1.4 months (range 0.13–23.77 months [4–713 days]). Twenty-four use was 6.62 months, and the median time of onset of muscular pain was 0.13 months (range 0.13–23.77 months). Twenty-eight of the 32 patients who developed muscular pain (28 with myalgia and 4 with myositis) were male and 15 were female patients. The difference in occurrence of SAMS among male and female statin users was not found to be statistically significant (p=0.458). Twenty-eight participants were found to have SAMS (p=0.458). Twenty-eight of the 32 patients who developed muscular pain (28 with myalgia and 4 with myositis) were male and 15 were female patients. The difference in occurrence of SAMS among male and female statin users was not found to be statistically significant (p=0.877). Thirty-one (30.1%) atorvastatin users and seven (38.9%) rosuvastatin users were found to have SAMS (p=0.877). Twenty-eight of the 32 patients who developed muscular pain (28 with myalgia and 4 with myositis) were male and 15 were female patients. The difference in occurrence of SAMS among male and female statin users was not found to be statistically significant (p=0.877). Thirt...
percentiles, the chances of occurrence of SAMS were significantly raised by 12.42, 3.52 and 4.80 times compared with those with vitamin D levels more than the 5th, 10th and 25th percentile cut-offs, respectively (Table 4). Various percentile cut-off values of vitamin D and their relationship with SAMS are shown in Table 4. However, similar cut-off percentiles for LDL-C did not show any conclusive relationship with SAMS (Table 5). ROC curves were drawn with vitamin D and LDL-C as predictors and SAMS as a dependent variable. With vitamin D levels of ≥17.2 ng/mL, the sensitivity and specificity of predicting SAMS were 50% and 26.5% (area under curve [AUC] 0.350, p=0.008; Figure 2), respectively. With respect to LDL-C, AUC was 0.471 (p=0.605).

Multivariate analysis
We did not find any significant relationship between the risk of occurrence of SAMS (dependent variable), vitamin D (OR 0.95, 95% CI 0.91–1.01, p=0.083), LDL-C (OR 1.0, 95% CI 0.99–1.01, p=0.959), age (OR 1.0, 95% CI 0.97–1.04, p=0.754), gender (female OR 1.63, 95% CI 0.64–4.15, p=0.308) or BMI (OR 0.97, 95% CI 0.88–1.06, p=0.543). However, there was a significant association between risk of SAMS and CPK levels. Each unit increase in CPK levels led to a 1% increase in the risk of developing SAMS (OR 1.01, 95% CI 1.003–1.019, p=0.007).

Discussion
This study was undertaken to establish a relationship between SAMS and levels of vitamin D and LDL-C. The prevalence of SAMS among the statin users enrolled in this study was 31.4%. In a recent study conducted in India, Singh et al. reported the occurrence of SAMS in 22% of geriatric statin users. In another Indian study, Mulchandani et al. reported myalgia in 32% of statin users. In the present study, significantly higher levels of CPK (a marker of muscle injury) were observed among patients with SAMS compared with those without SAMS. This further strengthens the association of statin use and likelihood of occurrence of SAMS.

The observed mean onset of myalgia pain after statin use in our study (6.6 months) was similar to that of a study by Hansen et al., which reported 6.3 months; however, we observed an earlier, shorter range of onset: 4 days to 2 years, compared with 1 week to 4 years. Significantly lower levels of vitamin D were observed among patients who presented with SAMS compared with those who did not (Figure 1); a finding that is consistent with previous studies. However, when we separated patients into groups based on their vitamin D status as deficient (<20 ng/mL), insufficient (20–30 ng/mL) or sufficient (>30 ng/mL), there was no significant difference between the number of patients in each group with respect to the occurrence of SAMS. Taylor et al. also found that vitamin D deficiency or insufficiency and changes in vitamin D levels during statin therapy did not predict the occurrence of SAMS.

As the current study was conducted in India, and many Indian people have insufficient levels of vitamin D, we converted their levels into percentiles, which revealed that the chance of occurrence of SAMS was significantly increased in those with vitamin D levels less than or equal to the 5th, 10th and 25th percentiles. This association was not significant at a percentile cut-off level of 50, which corresponds to vitamin D levels of ≥20.12 ng/mL. This gives a meaningful conclusion that as vitamin D levels increase, there is less likelihood of the occurrence of SAMS.
Figure 2: Receiver operating characteristic curve for various predictors taking statin-associated muscle symptoms as dependent variable

![ROC Curve](image)

Hence, vitamin D deficiency may exacerbate SAMS in statin users. A study by Riche et al. also found that deficient levels of vitamin D are significantly associated with SAMS, and that patients with vitamin D levels >20 ng/mL had a lower risk of SAMS compared with those with vitamin D levels of ≤20 ng/mL. In a study by Bittner et al., 49% of atorvastatin users at baseline and 56% at 1 year, had vitamin D deficiency, and myalgia was present in 8.3% of vitamin D-deficient subjects. Similar results were recently reported by the Pennisi et al., who observed a significant relationship between vitamin D deficiency and SAMS.

In the present study, no association was found between the occurrence of SAMS and LDL-C levels. This is in agreement with the findings of a study by Piantanida et al. who reported no significant difference in LDL-C levels among patients with vitamin D levels <10 ng/mL, 10–20 ng/mL and ≥20 ng/mL.23 The average LDL-C levels in our study were found to be ≤20 ng/mL. In a study by Bittner et al., 49% of atorvastatin users at baseline and 56% at 1 year, had vitamin D deficiency, and myalgia was present in 8.3% of vitamin D-deficient subjects. Similar results were recently reported by the Pennisi et al., who observed a significant relationship between vitamin D deficiency and SAMS.

The data regarding the association of levels of vitamin D and LDL-C with SAMS are somewhat mixed. Our study attempted to explore this relationship; however, our conclusion must be regarded with caution due to the small sample size and cross-sectional nature of our study. As most of the population in the Indian subcontinent are deficient in vitamin D, assessment of vitamin D as a major predictor of SAMS requires subdivision into smaller values (percentiles) to provide a clear picture among those who have a higher degree of vitamin D deficiency. We highlighted the occurrence of SAMS among patients with the lowest levels of vitamin D in an Indian population that is already vitamin D deficient.

The limitations of this study include a small sample size, which was due to the time constraints, as the project was to be completed within a restricted period of 3 months, and reduced availability of vitamin D kits due to funding constraints. Another limitation includes the cross-sectional nature of the study, as a prospective study in the assigned duration for completion and submission of the project was not feasible. The chemiluminescence method of vitamin D estimation, used in this study, has a drawback of over-reporting vitamin D deficiency due to poor traceability to the reference method (mass spectrometry). Another limitation of the study is the measurement of vitamin D in patients with dyslipidaemia. As vitamin D is a lipophilic substance and is particularly vulnerable to the presence of other lipids, especially triglycerides, in the serum or plasma sample, this can change the ability of the binding agent to associate with vitamin D in the sample; this can produce variability in the measurement.

**Conclusion**

We can conclude that statin users with low levels of vitamin D are at increased risk of developing SAMS; this risk decreases with higher levels of vitamin D. However, due to the observational nature of this study and other limitations, further randomised interventional studies are needed to verify these findings. LDL-C status of statin users failed to predict any meaningful association with SAMS. Further large-scale prospective studies are required to investigate the exact relationship between these parameters.

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