HIGH PROTON PUMP INHIBITOR EXPOSURE INCREASES RISK OF CALCINOSIS IN SYSTEMIC SCLEROSIS

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Abstract

Objective

To investigate the association between proton pump inhibitor (PPI) use and the presence and severity of calcinosis in systemic sclerosis (SSc).

Methods

We analysed data from two SSc cohorts from a single centre. Cohort 1 included 199 patients reviewed over 10 years, for whom retrospective data on PPI use and calcinosis were available. Cohort 2 was recruited prospectively and included 215 consecutive patients, who underwent clinical assessment. Outcomes of interest were presence of current calcinosis (CC) or calcinosis at any time (CAT).

Results

The cohort 1 data analysis showed that among patients on standard dose PPIs 20% had calcinosis, while in those on high doses of PPIs calcinosis was present in 39% (p=0.003). Analysis of the data from cohort 2 confirmed these findings, demonstrating that the odds of CAT increased significantly with longer PPI exposure (OR 1.04, 95% CI 1.02-1.06, p<0.001), longer disease duration (OR 1.08, 95% CI 1.05-1.12, p<0.001) and greater age (CAT OR 1.03, CI 1.01-1.05, p=0.010). Multivariable logistic regression showed that higher exposure to PPI remained a significant predictor of calcinosis, with PPI exposure>10 years increasing the risk of CAT over 6-fold, compared to no PPI (OR 6.37, 95% CI 1.92-21.17, p = 0.003) after adjusting for disease duration and antibodies.

Conclusion

We confirm a significant association between high PPI exposure with severity of calcinosis in SSc. Given the clinical impact of calcinosis and reflux in SSc, PPI exposure as a potentially modifiable risk factor for calcinosis requires further evaluation.

Key words

Systemic sclerosis, calcinosis, protein pump inhibitors

Key messages

Retrospective and prospective cohorts demonstrated a significant association between high PPE and calcinosis in SSc patients.

High PPI exposure predicted development of calcinosis independent of disease duration in the prospective study.

Given the impact of calcinosis on SSc these findings warrant further study.

Background

First introduced in 1989, proton pump inhibitors (PPI) remain one of the most prescribed classes of medication in the developed world, notwithstanding emerging evidence of their association with some safety concerns, especially after long-term use (1). These have included reports of nutritional and electrolyte deficiencies (magnesium, vitamin B12), altered gut microbiota (small intestinal bacterial overgrowth, *Clostridium difficile* infection), fractures, cognitive impairment, chronic kidney disease and subacute cutaneous lupus erythematosus (1-3). Interestingly, there have been some reports of effects on mineral bone density, the development of vascular calcification and calcium pyrophosphate dihydrate disease (CPPD) with PPI use (4, 5). However, most of these studies are retrospective observational in nature and there remains a paucity of robust data to support these associations.

In systemic sclerosis (SSc), PPI are the mainstay treatment for gastroesophageal reflux disease (GORD), which affects at least 70% of the patients (6). Calcinosis is the deposition of calcium hydroxyapatite in the subcutaneous tissues. It is a major clinical problem affecting up to half of SSc patients, as well as patients with other connective tissue diseases including dermatomyositis and fasciitis (7, 8). The aetiology of calcinosis in SSc remains unclear, however tissue ischaemia and chronic trauma may be contributory (9, 10).

In SSc, calcinosis appears to be associated with longer disease duration, digital ulceration, acro-osteolysis, anti-centromere and anti-PM/Scl antibodies (8, 10). It is a major source of morbidity for SSc patients and to date there are no proven effective treatments (8, 11).

Objective

To investigate the association between PPI use and osteoporosis in an initial discovery SSc cohort, followed by further evaluation of the association between PPI use and presence and extent of calcinosis in a validation SSc cohort.

Patients and Methods

This study complies with the Declaration of Helsinki. Informed consent was obtained from patients reviewed in our centre which have been approved by the London-Hampstead and the London-Fulham Research Ethics Committees. All patients met the 2013 ACR/EULAR criteria for SSc.

Cohort 1 (discovery cohort)

First, we retrospectively reviewed clinical data of SSc patients from our centre, seen over the preceding decade (cohort 1). The aim was to investigate the relationship between PPI use and osteoporosis. Patients who had had at least one dual energy X-ray absorptiometry (DEXA) scan performed were included in the study. Demographic and clinical characteristics were recorded, including results from the DEXA scans, classified as normal bone density, presence of osteopenia or osteoporosis. Presence of calcinosis clinically was recorded and plain radiographs, where available, were reviewed to confirm calcinosis. PPI treatment prior to DEXA scans was characterized as none, standard dose or high dose PPI (12). Ordinal logistic regression was used to assess the effects of various factors on DEXA results.

Cohort 2 (validation cohort)

As the analysis of the data from cohort 1 indicated a possible association between PPI use and presence of calcinosis, we set up a prospective study to specifically test this hypothesis. Data on disease history and clinical assessment findings were prospectively collected from

consecutive SSc patients during their clinic visits (cohort 2). Patients were asked about history of PPI use, including dose and duration, presence or absence of calcinosis including site(s) involved. Information including immunosuppressive treatments reported by patients was additionally verified through electronic record review. Calcinosis was graded by physician assessment in terms of size (<1cm, ≥1 and ≤3cm, >3cm) and number of body sites involved (1 affected site, 2-3, >3). Where possible, we collected data on plain radiology of clinically affected sites within the preceding 12 months to confirm calcinosis as assessed clinically.

A dose equivalence score was established for PPI use with the following standard doses as defined in cohort 1: lansoprazole 15mg, omeprazole 20mg, pantoprazole 20mg, esomeprazole 20mg and rabeprazole 10mg 12. A total daily PPI equivalent dose was calculated for each patient. To quantify PPI exposure over time we calculated PPI exposure score (PPE) by multiplying the total duration of use in years by the total daily PPI equivalent dose. PPE was categorised into four groups: no exposure, $\leq$ 5 years, >5 and $\leq$10 years and >10 years. Logistic regression was used to assess association between calcinosis and demographic or clinical patient characteristics.

Results

Retrospective analysis of discovery cohort, evaluating the association between PPI, osteoporosis and calcinosis.

Table 1 summarises key clinical characteristics for cohorts 1 and 2. The discovery cohort consisted of 199 SSc patients, 91.5% were female, mean age 57.3 years, 69.4% had limited SSc, and 25.1% had calcinosis. No significant association was found by univariable or multivariable analysis between PPI treatment and osteoporosis. After adjusting for relevant

covariates and potential confounders including age, body mass index, steroids and bisphosphonate treatments, there was a trend towards association between calcinosis and development of osteoporosis (OR 1.67, 95% CI 0.91, 3.06; p=0.096).

Interestingly, there was a strong association between calcinosis and level of PPI use. Among patients who had not received PPIs, calcinosis was present in 12.5%, among those on standard dose PPIs, 20% had calcinosis and among those on high doses of PPIs, calcinosis was present in 39% (p=0.003).

Prospective analysis of validation cohort, evaluating the association of PPI and calcinosis.

We pursued additional analyses to understand the potential association between PPI and calcinosis in a prospective cohort of 215 consecutive patients attending clinical follow-up (cohort 2). Table 1 outlines PPI use and calcinosis characteristics for those subjects. Plain radiographs were requested for those with clinical calcinosis, those with clinically suspected calcinosis and for other indications including arthralgia within 12 months of assessment. These were available for 136 (63.3%) of the patients in cohort 2. This confirmed calcinosis in 55 (79.7%) of the 69 subjects with current calcinosis (CC). For the remaining 14 subjects, calcinosis was present clinically but no radiographs were performed.

Data on size of calcinosis and number of body sites involved for each PPE category were collected. Whilst the majority of patients did not have calcinosis, amongst those that did there were some interesting observations. Notably, all patients with large calcinotic deposits (>3 cm) had exposure to PPI and the majority (73.3%) of these had a PPE>10 years. Similarly, there were numerically more patients with >1 body site involved (n=38) than those with only one body site involved (n=10) in the group with PPE>10 years (n=95). Female patients were more likely to develop calcinosis (36.0% CC and 41.7% CAT) compared

to males (15%, p=0.014 and 25%, p=0.071 respectively). We found no association between CC or CAT and cutaneous subset,overlap syndrome or renal disease. Consistent with reported studies, there was strong association between CC and digital ulcers (OR 3.1, 95% CI 1.5, 6.4; p=0.002).

Univariable data analysis demonstrated that female sex, greater age, longer disease duration, ACA and anti-PMScl antibodies significantly increased the odds of both CC and CAT (Table 2). PPE was also associated with significantly increased odds of both CC and CAT, with 4% increase in the odds of CC or CAT for every year longer exposure to standard dose PPI (OR 1.04, 95% CI 1.02-1.06, p<0.001 for both). Categorisation of PPE revealed that compared to no PPI exposure, the odds of calcinosis development became significantly increased after PPI exposure for over 10 years.

In a multivariable analysis duration of PPI exposure remained a significant predictor of increased risk for calcinosis development after adjusting for disease duration and autoantibody specificity. Compared to no exposure, PPI treatment for over 10 years increased the odds of CAT over 7 fold and the odds of CC nearly 8 fold (Table 2). There was no significant difference in the effect of individual PPIs on CC or CAT after adjusting for duration of PPI exposure.

Fifty three (24.7%) of the subjects were on steroid treatment at the time of clinical assessment. We found no association between calcinosis and current steroid treatment. 109 (50.7%) of the cohort were on immunosuppressive medication at the time of assessment. These included mycophenolate mofetil, methotrexate, azathioprine, cyclophosphamide and biologics. In addition, 55 subjects (25.6%) of the cohort were on hydroxychloroquine (HCQ).

Whilst there was no association between overall immunosuppressive treatment and calcinosis, there was a statistically significant association between HCQ treatment ever and CAT (OR 0.45, 95%CI 0.24, 0.86; p=0.015).

Discussion

This is the first study identifying an association between PPI use and calcinosis in SSc. This result adds to the broader association of tissue calcification reported with vascular disease, and more recently CPPD spectrum diseases. It is noteworthy that our study showed a dose response effect of PPI on calcinosis in contrast to the heterogeneity reported in other studies that evaluated PPI-associated fractures and *Clostridium difficile* infection.

PPI may promote tissue calcification via vascular endothelial cell injury. By binding to and inhibiting dimethylarginine dimethylaminohydrolase, the enzyme that degrades asymmetric dimethylarginine (ADMA), PPIs elevate ADMA levels and it has been shown that plasma ADMA levels correlate with coronary vascular calcification (13). Moreover, PPIs may activate pro-atherogenic pathways via modulation of the chemokine secretory phenotype of senescent coronary endothelial cells (14). Further evidence for vascular injury was demonstrated in another report that Lansoprazole disrupts endothelial lysosomal acidification, enzymatic activity and proteostasis resulting in endothelial senescence (15). However, it is unclear if similar mechanisms may underlie the soft tissue calcification typically associated with SSc.

Although the discovery cohort demonstrated a trend towards association of PPI use and osteoporosis in this retrospective cohort, this association with osteoporosis remains

controversial (16, 17). Notably we identified a potential dose-dependent relationship between PPI and calcinosis in SSc patients. The results were unexpected and led us to seek confirmation of the association in an independent cohort. In our second cohort, 83.7% of scleroderma patients had persistent GORD and 84.7% had been on PPI, with 81.4% being on current PPI therapy. Our rates of GORD are similar to those quoted in other SSc cohorts/literature (18). There are manifold reasons to treat reflux disease in the SSc cohort, including for symptomatic relief of GORD, and to prevent adverse effects of long term acid reflux including strictures, Barrett's oesophagus and the potential for microaspiration-associated epithelial lung injury (18, 19). Often, standard dose PPI are not efficacious enough to adequately control disease symptoms and thus SSc patients are maintained on higher doses. The inverse association between HCQ and calcinosis is noteworthy given the pleotropic effect of HCQ and therefore deserves further evaluation in a prospective study.

There are several proposed, but no validated, classification systems for assessment of calcinosis (8, 20). In our study, we adopted a complementary composite clinico-radiological and time-dependent assessment tool specifically to assess overall burden of calcinosis. We envisaged that this user-friendly tool may be utilised in routine clinical practice.

We are cognizant of the limitations of this study. First, there may be bias in data collection as physicians were asked to indicate site and size of calcinosis present by clinical assessment. Secondly, in the validation cohort recall bias may affect accuracy of the patients' self-reporting on PPI use and calcinosis. However, medical records, clinical assessment and radiology where appropriate were reviewed to reduce this bias. We do not have radiographs available on all patients at sites of calcinosis to confirm the presence and size of calcinosis. Therefore, we may have missed some cases of subclinical calcinosis. For those patients with clinically identified calcinosis, 79.7% had x-rays available of at least one

body site to confirm calcinosis presence and size, improving reliability. Finally, the issue of temporality and slow evolution of calcinosis may affect interpretation of the results. Given the persistence of reflux symptoms in majority of patients with SSc, it behoves the clinicians to continue PPI where necessary accepting that our study does not ascribe causality to the apparent association shown in this study.

Conclusion

We confirm a significant association between PPI exposure with calcinosis in SSc and this observation may conceptually extend the global effect associated with vascular calcification reported in other diseases. These findings if validated in larger independent cohorts, may influence clinical decision in management of severe reflux with judicious use of PPI in particular amongst those at risk of progressive calcinosis.

Disclosures:

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Table 1. Patient and Disease Characteristics

Characteristic	Cohort 1, Number (%)	Cohort 2, Number (%)
Total number	199 (100)	215 (100)
Female	182 (91.5)	175 (81.4)
Age [a] (mean)	57.3 (SD 12.0)	57.4 (SD 13.4)
Disease Duration (mean years)	12.7 (SD 8.6)	13.4 (SD 9.9)
Scleroderma subtype		
Limited	138 (69.4)	141 (65.6)
Diffuse	61 (30.7)	74 (34.4)
Overlap	70 (35.2)	52 (24.2)
Antibody category		
Anti-centromere antibody	55 (28.1)	68 (31.6)
Anti-topoisomerase I antibody	42 (21.4)	55 (25.6)
Anti-RNA polymerase III antibody	11 (5.6)	26 (12.1)
ANA+ ENA -	31 (15.8)	20 (9.3)
Anti-U3RNP antibody	3 (1.5)	11 (5.1)
ANA negative	6 (3.0)	9 (4.2)
Anti-PmScl antibody	18 (9.2)	9 (4.2)
Other antibody [b]	36 (18.1)	25 (11.6)
Calcinosis		
Current	N/A	69 (32.1)
Past	N/A	14 (6.5)
At any time	50 (25.1)	83 (38.6)
Never	149 (74.9)	132 (61.4)
Current Calcinosis body sites, number (%) out of all with current calcinosis		
1 / 2-3 / >3	N/A	17 (24.6) / 28 (40.6) / 24 (34.8)
Current Calcinosis Size, number (%) out of all with current calcinosis		
<1cm / 1-3cm / >3cm	N/A	34 (49.3) / 20 (29.0) / 15 (21.7)
Current Calcinosis location body site/s, number **(%) out of all with current calcinosis**		
Finger	N/A	38 (55.1)
Elbow	N/A	22 (31.9)
Knee	N/A	11 (15.9)
Hand / Wrist / Forearm / Shoulder	N/A	6 (8.7) / 5 (7.3) / 7 (10.1) / 7 (10.1)
Foot / Leg / Buttock	N/A	4 (5.8) / 3 (4.4) / 2 (2.9)
Gastroesophageal reflux disease (GORD)[c]	N/A	180 (83.7)
Proton Pump Inhibitor use		
Current	N/A	174 (80.9)
Past	N/A	8 (3.72)
Never	N/A	33 (15.4)
Mean years on proton pump inhibitor	N/A	14.5 (SD 16.3)

[a] *cohort 1 - age at first DEXA scan, cohort 2 - age at study enrolment;*
[b] *other antibodies include nRNP, Ro, La, Th/To, SL, hnRNP, NOR90, Mi2, Ku.*
[c] *GORD defined as a history of reflux symptoms or a diagnosis of GOR documented in patient notes*

Table 2. Associations between clinical characteristics and calcinosis in the prospective cohort.

	Current calcinosis			Calcinosis at any time		
	Univariable analysis					
	OR/odds	**95% CIs**	**p-value**	**OR/odds**	**95% CIs**	**p-value**
Age, years	1.04	(1.01 , 1.06)	0.003	1.03	(1.01 , 1.05)	0.010
Age = 40 years (Ref.)	0.24	(0.14 , 0.42)		0.37	(0.23 , 0.61)	
Male	0.31	(0.12 , 0.79)	0.014	0.47	(0.21 , 1.01)	0.054
Female (Ref.)	0.56	(0.41 , 0.77)		0.72	(0.53 , 0.97)	
Disease duration, years	1.09	(1.05 , 1.13)	<0.001	1.08	(1.05 , 1.12)	<0.001
At onset (Ref.)	0.14	(0.08 , 0.25)		0.21	(0.12 , 0.36)	
DcSSc	0.77	(0.42 , 1.42)	0.399	1.04	(0.58 , 1.85)	0.899
LcSSc (Ref.)	0.52	(0.36 , 0.73)		0.62	(0.44 , 0.87)	
Overlap	0.72	(0.36 , 1.45)	0.360	0.89	(0.47 , 1.70)	0.725
No overlap (Ref.)	0.51	(0.37 , 0.70)		0.65	(0.47 , 0.89)	
ACA	6.10	(1.91 , 19.52)	0.002	7.28	(2.27 , 23.34)	0.001
ATA	1.13	(0.31 , 4.05)	0.857	1.96	(0.58 , 6.67)	0.280
ARA	2.12	(0.54 , 8.34)	0.283	4.22	(1.13 , 15.73)	0.032
U3RNP	4.79	(0.97 , 23.55)	0.054	4.79	(0.97 , 23.55)	0.054
PMScl	9.58	(1.61 , 56.95)	0.013	9.58	(1.61 , 56.95)	0.013
ANA	1.44	(0.31 , 6.61)	0.641	2.46	(0.59 , 10.29)	0.216
Other antibodies (Ref.)	0.17	(0.06 , 0.50)		0.17	(0.06 , 0.50)	
PPI<=5yrs	1.55	(0.40 , 6.07)	0.528	2.32	(0.70 , 7.70)	0.169
5<PPI<=10yrs	2.06	(0.60 , 7.12)	0.252	2.42	(0.79 , 7.44)	0.124
PPI>10yrs	7.66	(2.50 , 23.43)	<0.001	7.32	(2.61 , 20.54)	<0.001
No PPI (Ref.)	0.13	(0.05 , 0.38)		0.17	(0.07 , 0.45)	
	Multivariable analysis					
	OR/odds	**95% CIs**	**p-value**	**OR/odds**	**95% CIs**	**p-value**
Disease duration, years	1.07	(1.03 , 1.11)	<0.001	1.07	(1.03 , 1.11)	<0.001
Other antibodies (Ref.)						
ACA	9.83	(2.65 , 36.44)	0.001	11.09	(3.03 , 40.51)	<0.001
ATA	2.14	(0.52 , 8.82)	0.292	3.87	(1.00 , 15.00)	0.050
ARA	3.26	(0.71 , 15.07)	0.130	7.65	(1.75 , 33.37)	0.007
U3RNP	8.57	(1.40 , 52.41)	0.020	8.09	(1.37 , 47.88)	0.021
PMScl	23.02	(2.82 , 188.02)	0.003	19.59	(2.54 , 151.04)	0.004
ANA	1.27	(0.24 , 6.77)	0.781	2.55	(0.53 , 12.17)	0.242
No PPI (Ref.)						
PPI<=5yrs	1.74	(0.36 , 8.36)	0.492	2.55	(0.67 , 9.77)	0.171
5<PPI<=10yrs	2.21	(0.51 , 9.63)	0.291	2.45	(0.68 , 8.82)	0.170
PPI>10yrs	7.65	(1.96 , 29.94)	0.003	6.37	(1.92 , 21.17)	0.003
Constant	0.01	(0.002, 0.07)		0.01	(0.002, 0.07)	

Other antibodies include nRNP, antiPR3, Th, Anti SL, NOR90, Mi2, Ku & hnRNP; PPI: Proton Pump Inhibitor Exposure.
Results are based univariable and multiple regression analyses.