Palmoplantar pustulosis is characterized by pustule formation in the acrosyringium. Nearly 50% of palmoplantar pustulosis sera produce immunofluorescence of the palmar papillary endothelium from healthy subjects, but also of the endothelium of normal parathyroid gland. With a case-control design the levels of calcium and parathyroid hormone in serum were measured in 60 women with palmoplantar pustulosis and 154 randomly selected population-based control women. One-third of the controls had been smokers, whereas 95% of the cases were or had been smokers. Mean age-adjusted serum calcium was increased in the patients compared with the controls (2.43 vs 2.36 mmol/l; \( p < 0.0001 \)), whereas the parathyroid hormone concentration was suppressed (23.2 vs 31.1 ng/l; \( p < 0.0001 \)). The plasma levels of parathyroid hormone-related protein were normal in patients but there was a strong expression of this protein in the acrosyringium both in palmoplantar pustulosis and control skin. As even a marginal elevation of serum calcium is associated with an increased risk for diabetes, cardiovascular disease and psychiatric disease, we analysed the risk for these disorders in palmoplantar pustulosis patients compared with that in the control group. Both diabetes mellitus and psychiatric disorders were associated with palmoplantar pustulosis patients compared with matched controls. In addition, the expression of PTHrP in palmar papillary endothelium was observed in several organs and studies with PPP sera on non-dermal tissues, IF of the papillary endothelium concentrated at the acrosyringium (the eccrine sweat duct in the epidermis and stratum corneum) (3, 5). Almost half of the PPP sera in our studies induced immunofluorescence (IF) of the papillary endothelium of palmar skin from healthy subjects. When the palmar skin was taken from a smoker, IF occurred both in the endothelium and the acrosyringium (6). Thus smoking might up-regulate the reactivity to PPP sera, possibly by inducing exposure of more antigens or by changing the surface properties of the acrosyringium. In screening studies with PPP sera on non-dermal tissues, IF of the endothelium was observed in several organs and particularly in the parathyroid glands (7).

In view of these findings, as well as a possible increased risk of osteoporosis (4), serum calcium, parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) levels were measured in women with PPP with the aim of determining whether these patients might have an abnormal calcium homeostasis and parathyroid function compared with matched controls. In addition, the expression of PTHrP in palmar skin and in particular in the eccrine sweat gland and duct was studied. PTHrP consists of 34 amino acids and is produced in the parathyroid gland. Its ectopic

Key words: depression; eccrine serum calcium for the high risk for diabetes and depression dermatological disorders. The role of the mildly increased complex disease with an increased risk for several non-

CLINICAL REPORT

Women with Palmoplantar Pustulosis Have Disturbed Calcium Homeostasis and a High Prevalence of Diabetes Mellitus and Psychiatric Disorders: A Case-control Study

Eva HAGFORSEN1, Karl MICHÆLSSON2, Ewa LUNDGREN2, Helena OLOFSSON2, Axel PETERSSON1, Alena LAGUMDZIJA3, Håkan HEDSTRAND1 and Gerd MICHÆLSSON1

Departments of 1Medical Sciences/Dermatology and 2Surgery, University Hospital, Uppsala, Sweden and 3Department of Molecular Medicine, Karolinska Hospital and Institute, Stockholm, Sweden


© 2005 Taylor & Francis. ISSN 0001-5555 DOI: 10.1080/00015550510026587
production in tumour cells is a cause of the hypercalcaemia which may be present in malignant disease. Subsequently, data analysis of comorbidity was carried out by determining the occurrence of diabetes, cardiovascular diseases and psychiatric symptoms in the patients with PPP and controls, as these disorders are known to be more prevalent in individuals with even mildly elevated serum calcium, as reported by Lundgren et al. (8).

MATERIALS AND METHODS

Patients

Sixty consecutive women with PPP (23–76 years old, mean age 55 years) all visiting the dermatology outpatient department at the University Hospital in Uppsala were included in the study which was undertaken in 2001. All patients answered a questionnaire concerning their medical history and medication, smoking habits, sick leave and disability pension. The severity of the PPP varied. Some had 50–100 pustules and erythema and scaling involving the whole palmate surface as well as the palms, whereas others had only a few pustules and mild erythema and scaling.

Four of the patients (smokers) had no lesions at the time of blood sampling, but had shown typical mild–moderate PPP at earlier examination. Eight patients had psoriasis, which was usually mild and localized to the extremities. Two patients were receiving systemic therapy for their PPP (one of them had acitretin 10 mg/day, the other had cyclosporine 1.5 mg/kg body weight per day). Topical treatment of the PPP consisted of emollients and intermittent corticosteroids (irregularly and during a few days when severe flare-ups occurred; no patients received long-term topical corticosteroids) and a few patients were treated intermittently with calcipotriol ointment. None of the patients used calcium and/or vitamin D supplementation. Information about concomitant disease is given in Table I.

Controls

To sufficiently cover the number and age distribution of the cases, control sera for comparison of PTH and calcium were obtained from 154 frequency age-matched women (at least one control per case in each 5-year age group) who were randomly selected from the population register and also participants in two different, previous population-based studies of women examined with dual energy X-ray absorptiometry (DXA) or mammography (9, 10). The former controls (n=56) included women with an age range between 23 and 84 years. The remainder of the controls were participants in the Uppsala mammography screening programme which is offered to all women older than 40 years and 80% of this female population participates. To serve as a control in the present study information on weight and height, smoking habits, presence of diabetes, cardiovascular diseases and psychiatric symptoms, and current and former medication had to be available in addition to the calcium, albumin and PTH values. None of the controls received treatment with systemic corticosteroids or vitamin D or calcium.

Blood samples

Total serum calcium, albumin and PTH were measured by routine methods. Calcium was measured by ortho-cresolphthalein dye binding. The coefficient of variation for calcium is 1–4%. Albumin-modified total serum calcium values were calculated based on deviation of the serum albumin level from the normal means of women of the same age (11): (uncorrected calcium value + 0.019 (42± measured albumin value). With this formula Lundgren et al. (9), in a population comprising 5000 women in Uppsala county, found a mean ± SD calcium value of 2.37±0.09 mmol/l. Intact PTH was measured with the Allegro immunoradiometric sandwich assay (Nichols Institute, San Juan, CA, USA). The coefficient of variation was 6.8%. Values between 10 and 65 ng/l were considered to be within the normal range. The control sera had shown typical mild–moderate PPP at an earlier examination. Eight patients had psoriasis, which was usually mild and localized to the extremities. Two patients were receiving systemic therapy for their PPP (one of them had acitretin 10 mg/day, the other had cyclosporine 1.5 mg/kg body weight per day). Topical treatment of the PPP consisted of emollients and intermittent corticosteroids (irregularly and during a few days when severe flare-ups occurred; no patients received long-term topical corticosteroids) and a few patients were treated intermittently with calcipotriol ointment. None of the patients used calcium and/or vitamin D supplementation. Information about concomitant disease is given in Table I.

Table I. Some data on the medical history and clinical and laboratory variables of the 60 patients with palmoplantaris pustulosis (PPP)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of</td>
<td></td>
</tr>
<tr>
<td>Psoriasis vulgaris</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Coeliac disease/increased duodenal intraepithelial lymphocytes</td>
<td>11 (18)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetes type 1</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Diabetes type 2</td>
<td>15 (25)</td>
</tr>
<tr>
<td>Hypertension/cardiovascular disease</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Manic-depressive disease</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Depression</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Disability pension</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Former</td>
<td>10 (17)</td>
</tr>
<tr>
<td>Current</td>
<td>47 (78)</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
</tr>
<tr>
<td>IgG antibodies to thyroglobulin and/or thyroperoxidase</td>
<td>12 (20)</td>
</tr>
<tr>
<td>IgA antibodies to gliadin (verified coeliac disease included)</td>
<td>11 (18)</td>
</tr>
<tr>
<td>IgA antibodies to tissue transglutaminase</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Raised HbA1C (≥5.3%)</td>
<td>17 (28)</td>
</tr>
<tr>
<td>PPP sera producing endothelial immunofluorescence in healthy palmar skin* (n=59)</td>
<td>26 (44)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>Beta-blockers, ACE inhibitors, calcium-channel blockers</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Glibenclamide, metformin, repagluidine</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Lithium carbonate</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

*See text.

Acta Derm Venereol 85
Skin biopsies – PTHrP expression in palmar skin

Punch biopsy specimens (3 mm in diameter) were taken from normal palmar hypothenar skin (of seven women and one man, non-smokers; and of seven women and one man, all of whom had smoked for many years). Twenty-four specimens from involved palmar PPP skin and eight from non-involved palmar skin in patients with PPP were also obtained. Cryostat sections 6 μm thick were used. Endogenous peroxidase was blocked with 0.3% H2O2 in phosphate-buffered saline for 15 min. The sections were then allowed to react with normal rabbit serum (dilution 1/10) for 10 min to reduce non-specific staining. The polyclonal goat PTHrP (N-19) antibody, dilution 1/50, was incubated for 20 h at 4°C (PTH-rP (N-19): sc-9680, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Biotinylated anti-goat IgG (Vector BA 5000, Vector Lab Inc., Burlingame, CA, USA) diluted 1/200 was used as secondary antibody. Finally the sections were incubated with Vectastain ABC kit. The peroxidase reaction was developed with 3-amino-9-ethylcarbazole. The sections were counter-stained with Mayer’s haematoxylin. The staining pattern and staining intensity (0, +, ++, ++++) of eccrine sweat glands and ducts and epidermis and the contents of the pustules were evaluated on coded sections by the same observer. Pre-adsorption of the polyclonal PTHrP antibody was carried out at 37°C for 6 h (antibody dilution 1/50 and concentration of the peptide (sc-9680) P 10 μg/ml). The pre-adsorbed antibody produced no staining.

Data analysis

The main characteristics of the two variables calcium and PTH are shown by box-and-whisker plots. Both serum calcium, albumin-adjusted calcium and PTH displayed a normal distribution (p > 0.05 estimated by a chi-square test, MedCalc®, version 4.3, Belgium) and they were thus analysed in regression analysis. A possible confounding factor was smoking. The odds ratios more than marginally and they can all also be regarded as either intermediates or not causally associated with the other exposure variables, and therefore these variables should not be considered together in a multivariate model. The medical ethics committee of Uppsala University approved the study and the patients had given their informed consent.

RESULTS

Clinical characteristics

Some anamnestic, clinical and laboratory data of the patients with PPP are given in Table I. Only one of the 11 patients with gluten intolerance had a previously known coeliac disease; the other 10 were identified after screening for antibodies against gliadin/tissue transglutaminase, which was performed in all patients with PPP. Those who were found to have antibodies were further examined with gastroscopy with duodenal biopsies (unpublished data).

Two patients had had diabetes type I since adolescence; one of them also had a previously diagnosed coeliac disease and the other had schizophrenia in addition to severe PPP. Fifteen patients had diabetes type 2 – in all of them with a later onset than the debut of PPP. In seven, a dietary regimen was considered sufficient; eight received oral antidiabetics (Table I).

Two patients had manic-depressive disease. Nine other patients had long-term depressive symptoms including depression, anxiety or insomnia, and one had schizophrenia.

None of the patients had a history of renal disease.

Total serum calcium

As shown in Fig. 1A (crude results) and Table II (age-adjusted results) the mean value for albumin-modified serum calcium in the patients with PPP was highly significantly increased in comparison with that in the controls. The values for unmodified calcium were similar to the modified values (Table II).
albumin value in the PPP and the control group were the same (43 ± 3 and 43 ± 2 g/l). The difference in calcium values was only minimally changed after further adjustment for smoking status or other conceivable co-variates (data not shown). Furthermore, we observed a three times higher relative risk of PPP with each standard deviation increase in albumin-modified serum calcium (multivariate OR 2.76; 95% CI 1.73–4.39).

Patients with PPP with gluten intolerance had significantly lower unmodified and modified calcium than the patients with PPP with no evidence of gluten intolerance (calcium uncorrected 2.35 ± 0.11 vs 2.44 ± 0.08 mmol/l, p=0.0005, unpaired t-test) and albumin-modified calcium 2.36 ± 0.08 vs 2.43 ± 0.07 mmol/l (p=0.0062).

It was not possible to analyse the influence of calcipotriol ointment on the calcium values, as few patients used it regularly.

Serum parathyroid hormone

The mean PTH level in the patients with PPP was significantly decreased (Fig. 1B, crude results; Table II, age-adjusted values). The subgroup with gluten intolerance had a similar mean PTH to the other patients with PPP. The risk of being a PPP patient was halved for each standard deviation increase in serum PTH, independent of serum calcium, smoking status, BMI and co-morbidity (age-adjusted OR 0.45; 95% CI 0.30–0.67). Surprisingly, we found no significant correlation (r=−0.04, p=0.53) between serum calcium and PTH, which is an additional indicator of a disturbed calcium homeostasis. Further, there was no relation between number of pustules and PTH levels or calcium values. The mean PTH value in 14 PPP sera, which produced moderate or strong IF of the palmar endothelium, showed a tendency to be lower than the mean value for the 44 sera giving no or only weak IF (PTH 17.9 ± 11.2 and 27.3 ± 16.9 ng/l respectively,  p=0.05; Mann-Whitney). The mean calcium value was the same in the sera inducing and not inducing IF.

Parathyroid hormone-related protein in plasma

The concentration of PTHrP did not exceed the upper reference level of 2.9 ng/l in any of the 24 samples from the patients with PPP. The mean value was 1.4 ± 0.5 ng/l (95% CI 1.2–1.6). The mean albumin-modified serum calcium value in these samples was 2.53 ± 0.09 mmol/l. There was no correlation between PTHrP, calcium and PTH values.

PTHrP expression in palmar skin

In all specimens there was strong staining of the eccrine sweat ducts and this was most intense in the ducts in the papillary dermis and in the acrosyringium (Fig. 2A, B). The coils displayed weaker staining than the acrosyringium (Fig. 2C). This pattern was observed both in healthy subjects (smokers as well as non-smokers) and in non-involved and involved PPP skin. The site of the strongest staining corresponds to the final target for the inflammation in PPP, which is the acrosyringium. In specimens with a pustule, the staining of the floor of the pustule and also of some unidentified cells in the vicinity of the pustule was stronger than in any other site, whereas the pustule contents were unstained (Fig. 2D, E). Epidermis was also stained, but more weakly than the eccrine sweat ducts. The epidermal staining was strongest in the basal layer. In addition, the papillary endothelium was stained (staining of human dermal endothelial cells was verified on cultured human dermal endothelial cells with IF, results not shown).

PPP, smoking habits and co-morbidity

All but three of the patients with PPP were or had been smokers (95%), whereas only one-third of the controls had ever smoked. Current smoking, compared with never smoking, was associated with a 70 times higher age-adjusted relative risk of having PPP (OR 74.2; 95% CI 20.9–264.2, Table III). Neither in patients nor in controls was there any difference in the mean PTH or calcium value between never or former smokers and current smokers.

| Table II. Mean age-adjusted* serum calcium (crude and albumin-modified) and parathyroid hormone (PTH) levels with 95% confidence intervals in palmoplantar pustulosis (PPP) patients and controls |
|---|---|---|---|---|
| | PPP patients n=60 | Controls n=154 | p values |
| Calcium (mmol/l) | 2.43 (2.41–2.45) | 2.36 (2.34–2.37) | <0.0001 |
| albumin modified | 2.42 (2.40–2.45) | 2.35 (2.33–2.36) | <0.0001 |
| PTH (ng/l) | 23.2 (19.8–26.6) | 31.4 (29.3–33.4) | <0.0001 |

*Age as a continuous variable.
As already shown in Table I, many of the patients with PPP also had non-dermatological disorders. Diabetes mellitus of any type was associated with an age-adjusted OR of 8.7 (95% CI 3.3–22.8) for PPP (Table III). Fifteen patients had diabetes type 2 and two had diabetes type 1, compared with only seven control individuals with diabetes type 2 and none with diabetes type 1. Exclusion of the two patients with diabetes type 1 resulted only in marginally lower OR. Patients with diabetes type 2 had significantly lower PTH than those without diabetes (18.1 ± 16.8 vs 24.5 ± 13.0 ng/l, p=0.05). Mean age-adjusted BMI in patients with PPP (26.4 kg/m²; 95% CI 25.4–27.5) did not differ from that of the controls (25.8 kg/m²; 95% CI 24.9–26.3). There was also no significant difference in mean BMI values in patients with PPP with and without diabetes mellitus (p=0.25).

There was also a substantially increased risk of PPP with psychiatric disorders, mainly depression and anxiety (Table III). The age-adjusted estimate (OR
5.58; 95% CI 2.16–14.40) was attenuated (OR 3.35; 95% CI 1.08–10.30) after further adjustment for smoking status, a variable which in this analysis can be considered as an intermediate. Cardiovascular diseases were no more common among the patients with PPP than among the controls (age-adjusted OR 1.32; 95% CI 0.62–2.79).

DISCUSSION

The results of this study indicate that PPP is associated with a modest but highly significant increase in serum calcium and a significant decrease in the PTH levels in comparison with healthy population-based controls. This finding, which may indicate an abnormal calcium homeostasis, was further strengthened when the subgroup of gluten-intolerant patients with PPP was excluded as they had similar calcium values to the control group and significantly lower mean calcium than the other patients with PPP. Gluten intolerance seems to be more common among patients with PPP (18%; Table I) than in the general population. Thus, in Sweden the prevalence of silent coeliac disease in recent years has been estimated to be less than 1% (14). However, it is not possible in this study to compare the prevalence in the cases and the controls as no screening has been performed in the controls. As coeliac disease has been reported to have a negative influence on intestinal calcium absorption and the calcium homeostasis, this finding seems to be in agreement with previous reports (15).

We have found no studies of any variant of psoriasis in which calcium and PTH levels have been compared with those of healthy subjects. Serum calcium has been measured in clinical trials where topical vitamin D analogues have been used as treatment for psoriasis vulgaris. It is not possible to compare the results of these studies with our results in patients with PPP as no information was given in these studies as to how the calcium values were corrected. Our results indicate that an abnormal calcium homeostasis can be added to the list of anamnestic, clinical and genetic characteristics of PPP.

The extracellular calcium concentration is normally maintained within a narrow range by the calciotropic hormones PTH and 1,25(OH)₂D₃. Alterations in the systemic levels of these hormones or of PTHrP are the major causes of an aberrant extracellular fluid calcium concentration. The most common causes of hypercalcaemia are primary hyperparathyroidism, which was not observed in the present investigation, and malignancy (16). The association between elevated serum calcium and malignancy has been found to be partly attributable to increased levels of circulating PTHrP produced by the tumour cells. Although no increase in the plasma concentrations of PTHrP was found in this study, a rise in calcium levels in PPP due to PTHrP cannot be excluded, as PTHrP is an autocrine-paracrine hormone exerting its effects locally (17) and serum levels might not adequately reflect the local production.

Using immunohistochemistry and in situ hybridisation, keratinocytes (18–20) and endothelial cells (21, 22) have been found to express PTHrP, further cultured endothelial cells have been reported to produce PTHrP (23). In our study we observed that both normal and PPP acrosyringia displayed intense PTHrP staining which was more pronounced than that of the epidermis and endothelium. Furthermore, the floor of the pustule was maximally stained. As palmar and plantar skin have about 600 eccrine glands/cm², compared with 60/cm² in the skin of the back, this might mean that the acrosyringia in palmar-plantar skin could be more important producers of PTHrP than hairy skin.

The inflammation in PPP is exceptionally intense, with – in addition to large numbers of CD4+ T lymphocytes – dense infiltrates of mast cells, many of which are degranulated, and large numbers of neutrophils and eosinophils migrating outwards in the acrosyringium (2, 5). Mast cells contain a number of inflammatory promoters and, in particular TNF-α, etc.
which has been shown to enhance PTHrP-induced hypercalcaemia (24). However, the fact that three of four patients without pustules at the time of blood sampling had calcium levels of 2.60–2.72 mmol/l may indicate other causative factors of elevated serum calcium levels than palmo-plantar inflammation. As our recent findings indicate that 47% of PPP sera also display an endothelial immune reaction in other tissues, in particular in the parathyroid gland (7), there might also be non-dermal mechanisms underlying the increase in serum calcium.

Another inflammatory disease associated with raised serum calcium is sarcoidosis. The probable mechanism causing the raised serum calcium in this disease is overproduction of the active form of vitamin D (1,25(OH)2D3) at sites of granulomatous reactions (25). Admittedly, production of the active form of vitamin D (1,25(OH)2D3) causing the raised serum calcium in this disease is over-producing. As our recent findings indicate that 47% of PPP sera also display an endothelial immune reaction in other tissues, in particular in the parathyroid gland (7), there might also be non-dermal mechanisms underlying the increase in serum calcium.

Our study has some possible limitations. Serum calcium and PTH among controls and cases were measured at different time periods, but the same laboratory setting with continuous internal and external control systems was used for these routine analyses. Recall bias is always a major concern in case-control studies but is not likely to explain the substantial differences observed in prevalence of diabetes mellitus and psychiatric disorders and the possible non-differential misclassification by self-report of these diseases for cases and controls only conservatively biases our estimates. The controls were randomly selected from the population registers of the same county as the cases. Nevertheless, selection bias might be a concern. Although the controls had participated in studies with a population-based setting and a high participation rate, not all eligible controls had participated. As a group, non-participants in any clinical study are less healthy (30), but the differences are of minor order compared with our observed differences.

Further studies are required to understand the mechanisms behind and the relevance of the abnormal calcium homeostasis in PPP. The possible role of vitamin D as a contributory factor needs to be investigated, as well as the role of smoking as a precipitating factor for both the skin lesions and the systemic disease associated with PPP. The results further confirm our recent observations indicating that PPP is a complex systemic disease.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Psoriasis Association, the E Welander and Finsen Foundations, the Swedish Medical Society, the Medical Faculty of the University of Uppsala and the Swedish Research Council.

REFERENCES


Acta Derm Venereol 85


