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INVESTIGATIVE REPORT

Density of *Demodex folliculorum* in Perioral Dermatitis

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The role of *Demodex folliculorum* in perioral dermatitis is not satisfactory explained. Our purpose was to assess the density of *D. folliculorum* in perioral dermatitis and evaluate the relationship of the mite count to previous therapy with topical steroids. A standardized skin surface biopsy of the chin was performed in 82 female patients with perioral dermatitis and in 70 control female subjects. Patients who received previous topical steroid therapy had a significantly higher mite density than the patients who had received no topical steroids ($p<0.001$). In the latter group of patients, the mite density did not differ significantly from that of the control group ($p=0.629$). Mite density increased significantly with the length of treatment with topical steroids ($p<0.001$). Our results suggest that increased density of *D. folliculorum* in perioral dermatitis is a secondary phenomenon, associated with topical steroid therapy. *Key words: perioral dermatitis; Demodex folliculorum; topical steroids; skin surface biopsy*.

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Perioral dermatitis (PD) is a chronic, recurrent dermatosis of the perioral region occurring mainly in young and middle-aged women. Its aetiology is unclear but is thought to be multifactorial. Intolerance to cosmetics, especially frequent use of moisturizing creams, fluoride toothpaste, oral contraceptives, hormonal and gastrointestinal disturbances, ultraviolet provocation and topical steroids have been proposed as possible causes of this disease (1, 2). A variety of microbial agents, including yeasts and bacteria, as well as mites (*Demodex folliculorum*), have been suspected to play a pathogenic role (2).

D. folliculorum is a normal inhabitant of human facial skin in adults (3, 4), therefore its pathogenicity is a matter of quantity (5). The role of *D. folliculorum* in PD has received comparatively little attention. In the studies reported so far, either histological or scarification techniques were employed (6–10). It is still unclear to what degree and under what conditions the mites might contribute to this disease. The role of *D. folliculorum* has been investigated more extensively in patients with rosacea (10–14). Some of these studies were performed with the use of skin surface biopsy (11–13). A significantly increased density of *D. folliculorum* was found in patients with papulopustular rosacea (8, 11, 12) and steroid-induced rosacea (10, 12). It has been suggested that a density of *Demodex* $>5/cm^2$ with the standardized skin surface biopsy could be regarded as pathogenic (11).

Topical steroids are known to play an important part in the pathogenesis of PD (1, 2, 9, 15). They can provoke or aggravate clinical manifestations of the disease, but they are not its only cause. They are still frequently prescribed to patients with PD, probably because of mistaken diagnosis. Little is known about their effect on skin microflora in this disease (16).

To our knowledge, the influence of topical steroids on mite density in PD has not been assessed with the use of standardized methods. In the present study, we investigated the density of *D. folliculorum* in PD with the use of skin surface biopsy, introduced by Marks & Dawber (17), in a standardized skin area, as described by Forton & Seys (11). We were particularly interested in the influence of topical steroids on mite density in this disease.

PATIENTS AND METHODS

Patients

Eighty-two female patients with PD (mean age 40.2 years, range 18–55; SD 14.7) were investigated prospectively. All patients were referred to the Outpatient Department of Dermatovenereology of the University Medical Centre, Ljubljana by general practitioners over a period of 1 year. Typical PD was present in all patients. Erythematous papular rash was primarily distributed in the perioral region and around the nasolabial folds, sparing a small area around the lip margins. Seventy women with healthy facial skin formed the control group (mean age 43.7 years, range 21–56; SD 13.6). The difference in age between the patients and the control subjects was insignificant ($p=0.128$). Only women between 18 and 56 years of age were enrolled in the study in order to exclude the possible influence of sex and to minimize the impact of age on mite density. All study participants were informed about skin surface biopsy and gave their consent for the investigation.

Of the 82 patients with perioral dermatitis, 53 had been treated with various topical steroids before their visit to the dermatology department (SPD group). Mometasone was used in 27 patients, methylprednisolone in 7 and betamethasone in 3. In 16 patients, the treatment consisted of different steroids

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Table I. Demodex folliculorum *prevalence (positive samples) and density in the groups studied*

		Mite positivity		Mite density (n/cm^2)					
Group	n	n (%)	p value	Median	Range	Q1	Q3	Mean ± SEM	p value
PD all	82	51 (62.2)	<0.001	2	0–29	0	5	3.23 ± 0.55	<0.001
SPD	53	40 (75.5)	<0.001	2	0–29	1	7	4.64 ± 0.78	<0.001
NSPD	29	11 (37.9)	NS	0	0–4	0	1	0.66 ± 0.19	NS
Controls	70	22 (31.4)	–	0	0–7	0	1	0.66 ± 0.15	–

PD, patients with perioral dermatitis; SPD, treated with topical steroids; NSPD, not treated with topical steroids; Q1, first quartile; Q3, third quartile; SEM, standard error of the mean. The p values for each group refer to comparison with the control group.

(mostly mometasone and betamethasone) diluted in various creams. The topical steroids were prescribed mostly by general practitioners, only a few patients obtained them from relatives or friends without their doctor's knowledge. The remaining 29 patients had received no topical steroids prior to their visit to the outpatient dermatology clinic (NSPD group).

For all patients, the nature and duration of previous treatment were noted. Patients treated with systemic antibiotics before the dermatological examination were not included in the study. None of the patients had received topical antimicrobial therapy in the 4 weeks prior to the examination. None of the patients or controls suffered from an immune deficiency disorder, and none had been treated with immunosuppressive medication.

Methods

In each subject, a skin surface biopsy was performed on the chin; an area of 1 cm^2 was examined. For the biopsy, a circle 11.5 mm in diameter was drawn on a glass slide (a surface area of approx. 1 cm^2) and a drop of cyanoacrylate glue was placed at the centre of the circle. The adhesive-bearing surface of the slide was applied to the chin and left in place for about 1 minute. After removal, two or three drops of immersion oil were applied to the specimen, which was then covered with a cover slide. The specimens were examined by light microscopy at ×4 and ×100 magnifications. Each specimen was searched twice within 2 h of sampling. The mite density for each person was recorded. The clinical examination, skin surface biopsy and microscopy were always done by the same investigator.

Statistical analysis

The χ^2 test was used to compare the prevalence of mites in patients and control subjects; Fisher's exact test was applied when appropriate. Because of the non-normal distribution of mite counts, the groups studied were compared with the Kruskal-Wallis test. Pair-wise comparisons were then made with the Mann-Whitney U-test, using a Bonferroni correction for the p values. Linear regression analysis was used to evaluate the association between treatment duration and mite density in the SPD group. Patients' ages were analysed with Student's t-test. The chosen level of significance was $p=0.05$.

Table II. *Comparison of mite density in two groups of patients with perioral dermatitis (SPD – treated with topical steroids, NSPD – not treated with topical steroids) and in control subjects (C)*

Groups compared	n	Mean rank differences	p value
C and SPD	123	36.32	0.000
NSPD and SPD	82	24.03	0.000
C and NSPD	99	2.56	0.629

RESULTS

D. folliculorum was found in 62.2% of all patients with PD and in 31.4% of controls. The difference in mite prevalence between patients and the control group was significant ($\chi^2=13.12$; $p<0.001$). The mite was detected in 75.5% of patients in the SPD group and in 37.9% of patients in the NSPD group (Table I). The difference in mite prevalence between the two patient groups was significant ($\chi^2=9.69$; $p<0.002$), but the difference between the NSPD group and the control group was insignificant ($\chi^2=0.15$; $p=0.69$).

The data on mite density for each group studied are shown in Table I. The mite densities were significantly different in the three groups (control, NSPD and SPD) (Kruskal-Wallis test: $p<0.001$). The mite densities were similar in the first two groups, but the SPD group had substantially higher values. The Mann-Whitney test confirmed this observation, the p values being significant even when the Bonferroni correction was applied (Table II). Furthermore, a mite density >5/cm^2 was significantly more common in the SPD group than in the NSPD group (Fisher's exact test: $p<0.001$) (Table III).

Neither the patient group nor the control group showed a significant increase in mite density with age (linear regression: $p=0.86$).

Table III. Demodex folliculorum *density above and below the threshold of 5/cm^2, obtained in two groups of patients with perioral dermatitis (SPD – treated with topical steroids, NSPD – not treated with topical steroids) and in control subjects*

Mite density	SPD (n=53)	NSPD (n=29)	Controls (n=70)	Total (n=152)
0	13	18	48	79
1–5/cm^2	22	11	21	54
>5/cm^2	18	0	1	19

The p value for the comparison of density between the SPD and the NSPD groups is <0.01.

The correlation between mite density and duration of treatment with topical steroids in the SPD group is shown in Fig. 1. Data from 52 patients were evaluated; in one patient the duration of treatment was unknown. The results of linear regression are significant ($p<0.001$) and the coefficient is positive (b=0.322; SE=0.08) implying that mite density increases with length of treatment. However, the correlation is not very strong (r=0.48), which means that while length of treatment affects mite density, the variation in mite density explained with the length of treatment is rather low ($R^2=0.23$).

DISCUSSION

The first description of PD, referred to as 'light sensitive seborrhoeid' (18), dates back to 1957, but its aetiology has not been adequately explained to the present. An exogenous cause has been postulated by some authors (9). As the disease affects mainly young and middle-aged women, who habitually use moisturizing creams, the pathomechanism of excessive hydration of the horny layer, impairment of barrier function and proliferation of the skin microflora has been proposed (19). In several studies, microbial factors were assessed with the use of routine bacteriological and mycological swabs, which failed to yield pathogenic organisms (9, 15).

The role of *D. folliculorum* in PD has been explored with the use of histological (6, 9) and skin scarification techniques (7, 10). Histological studies showed no evidence of increased colonization by the mite (6). However, histology is not considered an appropriate method for studying the pathogenic role of *D. folliculorum*. Mites shrink and transform in histological preparations and are not easily detected (11, 20). As the mite is a normal inhabitant of the human pilosebaceous unit, the mere detection of mites in skin specimens has no clinical relevance. Only quantitative analysis can have pathogenic implications (4, 21). Although skin surface biopsy has also false-negative results (22), it allows for numerical assessment of mites in the superficial part of the stratum corneum and from contents of pilosebaceous ducts in a measured area of skin (17, 23). It is a standardized and non-invasive method in which mites stay alive and are easy to detect microscopically.

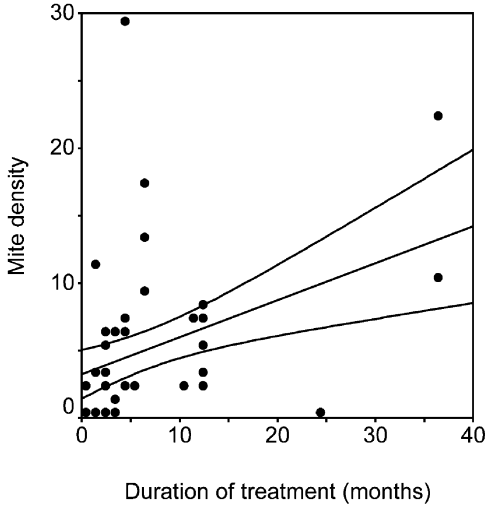

Fig. 1. Linear regression model for the mite density (n/cm^2) depending on duration of treatment with topical steroids in patients with perioral dermatitis. r=0.48, $p<0.001$.

The surface area studied in our patients (1 cm^2) was the same as in some studies in rosacea patients (11). Considering that *D. folliculorum* is thought to be present in most healthy adults (4, 11, 24), the prevalence of 31% found in our control subjects seems to be low. This may be partly explained by the small area sampled, but the main reason is probably the sampling site. In other studies, *D. folliculorum* was rarely retrieved from the chin compared with other face locations (5, 12, 25). In two studies of rosacea patients performed with skin surface biopsy (5, 12), mite prevalence on the chin did not exceed 32% in control subjects. The reported prevalence rates vary also with the techniques used (11, 13). When other sampling methods were employed, even lower prevalence rates were found in healthy individuals (14, 26).

Taken as a group, our patients with PD had significantly higher mite prevalence and mite density values than the control group. However, these differences were entirely due to the subgroup of patients who had been treated with topical steroids. In the NSPD group, neither the prevalence nor the mite density differed significantly from the values for the control group. We also found a positive correlation between mite count and duration of treatment with topical steroids. A long treatment time significantly increased the odds for an excessive *D. folliculorum* value. We were particularly interested in mite counts above the level of 5/cm^2, considered to have a definite pathogenic implication in rosacea (11). Such abnormal high densities were found only in the SPD group and were observed mainly in patients who had used topical steroids for >3 months. In 13 patients in the SPD group, *D. folliculorum* was not detected. Ten of them had used topical steroids for <2 months, and the remaining three only intermittently for <3 months. Therefore, increased density of *D. folliculorum* seems to be the consequence of prolonged treatment with topical steroids. Besides the duration of treatment, the potency of the drugs used and the frequency of their usage are probably also important.

The high mite density found in the SPD group can be attributed to the immunosuppressive action of topical steroids (27). Immunological status is probably the most important factor in the defence of human skin against arthropods (28). If the defence is compromised, either because of systemic or local factors, mites may begin to multiply above the normal limit and become pathogenic.

Demodicidosis is a common problem in systemically immunocompromised patients (29–31). Patients treated with cortisone have been found to have elevated mite counts in facial skin (20, 32). Rufli et al. (7) observed a high mite prevalence in patients with PD previously treated with topical steroids. Particularly high density values for *D. folliculorum* were found in patients with steroid-induced rosacea (10, 12). In the study of Forton & Seys (11) no significant relationship between previous therapy and *D. folliculorum* density was noted in rosacea, but only a small number of their patients had received topical steroids. A few recent reports have suggested that *Demodex* mites may proliferate also in patients treated with the new topical immunosuppressive drugs tacrolimus and pimecrolimus (33, 34).

Several mechanisms of an inflammatory reaction to *D. folliculorum* have been suggested. An immunohistochemical study by Georgala et al. (14) indicated that a cell-mediated immune response of the delayed type of hypersensitivity may occur in patients with rosacea and a positive *D. folliculorum* finding. The authors concluded that this reaction could be triggered by antigens probably originating from the mite. Rufli & Büchner also observed a predominance of helper T cells in *Demodex* granuloma (35). A humoral immune response, mechanical blockage of the follicle, and a possible vector role of *D. folliculorum* for some pathogenic microorganisms have also been incriminated (4, 36, 37). If *D. folliculorum* escapes from a ruptured follicle into the dermal tissue, its chitinous skeleton can act as a foreign body and induce a foreign body granulomatous reaction (3, 28). This has been observed in a patient with a papulopustular rosacea-like eruption involving the perioral region (38). In patients with rosacea and rosacea-related diseases previous treatment with topical steroids has been found to increase the likelihood of epitheloid granulomatous inflammation (10).

Whether or not an increase in mite density may aggravate PD still remains to be determined. PD is a disease of multifactorial aetiology, and the intensity of the inflammatory reaction cannot depend solely on the density of *D. folliculorum*. An ecological balance of microorganisms on the surface of the stratum corneum which constitute normal face flora may also be disturbed. Certain bacteria, especially staphylococci and lipophilic coryneforms, or *Malassezia* spp., may proliferate in the initial phase. Fusiform bacilli from the mouth have also been implicated, but have not been cultured (39). Constant overhydration of the skin due to habitual use of moisturizing creams could provoke microbial changes in predisposed individuals. Proliferation of these microorganisms might also be enhanced by topical steroids (16). To our knowledge, quantitative bacteriological or mycological studies in PD have not been performed, and the influence of topical steroids on the microbial population in this disease has not been adequately explained (16, 39).

The majority of our patients were successfully treated with topical metronidazole and systemic tetracyclines. In mild forms of the disease, topical metronidazole alone was prescribed and was often successful also in patients who had not been treated with topical steroids. Metronidazole was not found to have direct acaricidal activity (40), therefore its efficacy must be related to its anti-inflammatory and antioxidant action (4).

Our observations suggest that increased density of *D. folliculorum* in PD is a secondary phenomenon, associated with previous treatment with topical steroids. In our opinion, it has no major role in the pathogenesis of this disease, but it may be an important co-factor in aggravating the inflammation in patients treated with topical steroids. In any study exploring the pathogenic role of *D. folliculorum*, previous therapy, especially with topical steroids, must be considered in interpreting the results.

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