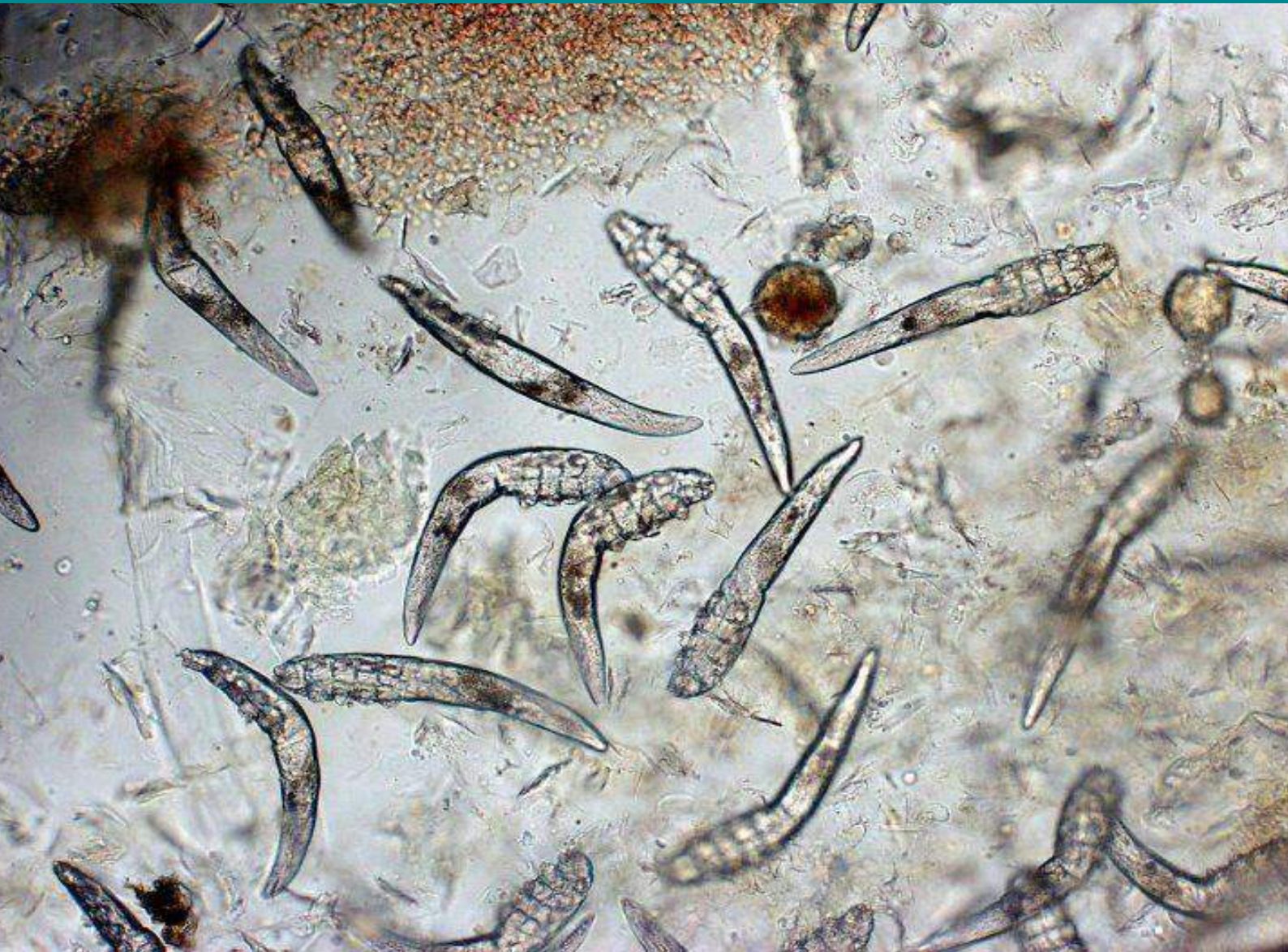# Study of Demodex Mites

A collection of research articles

Introduction

This booklet is a collection of papers addressing the potential role of Demodex mites on dermatological disease which includes original research and review papers of recent decades from all over the world.

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Paper 1

Association of Rosacea with Demodicosis

—— Original Article ——

Association of Rosacea with Demodicosis

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Mohammad-Reza Abbasian MD*, Gita Meshkat-Razavi MD•*

Background: There are controversial reports about the role of *Demodex mites* in pathogenesis of acne rosacea. The aim of this study was to examine the relationship between the presence and number of *Demodex mites* and the pathogenesis of rosacea.

Methods: In this case-control study, the prevalence of *Demodex mites* was studied in facial biopsy of 75 patients with acne rosacea as case group, and in 75 patients with discoid lupus erythematosus and 75 patients with actinic lichen planus as control groups.

Results: The prevalence of *Demodex mites* in patients with acne rosacea (38.6%) was significantly higher than the patients with discoid lupus erythematosus (21.3%) and actinic lichen planus patients (10.6%) ($P < 0.001$).

Conclusion: This study suggests that *Demodex mites* may play a role in pathogenesis of rosacea but it is not clear whether rosacea merely provides a suitable environment for multiplication of mites, or the mites play a role in the pathological changes.

Introduction

Rosacea is a chronic inflammatory disease of skin in young to middle-aged adults, but can occur occasionally in children. Females are more affected than males. Athough the complication of rhinophyma is not common in females who generally experience less severe disease than males.[1]

Although the etiology of rosacea remains a mystery, various factors contribute to this condition.

Its increased prevalence in lighter-skinned races and the histological findings of elastotic degeneration suggest a role for solar irradiation.[2] The occurrence of rosacea-like lesions in carcinoid syndrome and the presence of elevated substance P levels in some patients with rosacea increase the possibility that inflammatory mediators may be involved in the pathogenesis of the disease.[3, 4] Gastrointestinal disturbance (e.g., *Helicobacter pylori* infection), psychogenic stress, hormonal imbalance, sebaceous gland abnormalities, and infections may play roles; however, clinical studies have not approved it.[5 – 12] Histological examination shows dilatation of small dermal blood vessels with thickened walls.[13] Although it can explain the mechanism of flushing, but it does not explain how the papules and pustules in most cases can occur.

It has been proposed that occurrence of papules and pustules are related to the presence of the mite, *Demodex folliculorum* because this is a normal follicular inhabitant. But the etiologic importance of this parasite in the disease process is doubtful because the topical application of sulfur ointment will improve rosacea without affecting the mite populations.[14]

Demodex mites (*D.mites*) are saprophytic mites, which asymptomatically parasitize the human pilosebaceous follicles.[15 – 18] The prevalence of *Demodex* carriers increases with

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age.[15, 19 – 23] A variety of prevalence rates in different age groups have been reported in various studies.[19, 21, 24, 25]

Materials and Methods

We designed a case-control study and examined existing slides at the Pathology Wards of Loghman and Bou-Ali Hospitals in Tehran, Iran. Cases were selected from the patients whose diagnoses had been confirmed by pathologist.

Because *D.mites* are found in normal facial skins,[26] control subjects were selected from patients with discoid lupus erythematosus (DLE) and actinic lichen planus (ALP) whose pathological diagnoses had been confirmed and the role of *D.mites* in their pathogenesis were conclusively ruled out.

The presence of *D.mites, D.folliculorum,* and *D.brevis* was assessed in 75 patients with rosacea, and in 150 age-and sex-matched control subjects. For age matching, we classified the patients in three groups with five-year intervals.

To evaluate *D.mites* colonization, standard skin biopsies were taken from the face in patients and controls, then we coded the slides and a pathologist examined them in four sections (the thickness of the sections was 5 μm). Each sample was counted by light microscopy at standard magnifications (×4, ×10, ×40) and each specimen was examined at least three times.

In this study, we considered no difference between two species of *D.mites* and we examined each slide for presence of mite positivity and total count of mites. Slides without follicle excluded from the study and none of the cases and controls had received treatment at least two months before the skin biopsy.

Data regarding the age at presentation, sex, and previous treatments were obtained from the notes.

Three groups of patients were analyzed:

1- Seventy-five controls who were diagnosed as having DLE (mean age 45 years, range: 20 – 72). Of them 44 (59.7%) were women.

2- Seventy-five controls who were diagnosed as having ALP (mean age 44.7 years, range: 26 – 78). Of them 48 (62.8%) were women.

3- Seventy-five patients who had rosacea (mean age 43 years, range: 21 – 93) and 49 (65.4%) of them were women.

Comparability of control and study groups for sex, age, mite positivity and mite counts was assessed by mean of the Chi-square test and odds ratio.

Results

Pathological findings in skin biopsy of the patients with rosacea were degeneration of collagen fibers due to sun exposure, vascular dilatation, and a nonspecific perivascular and perifollicular lymphocytic infiltration or granulomatous inflammation around hair follicles with no evidence of epidermal changes.

Pathological findings in the DLE patients were hyperkeratosis and well-developed follicular plugging, vacuolar alternation along the dermoepidermal junction and smudged appearance of the dermoepidermal junction, edema of dermis, perivascular infiltration of lymphocytes, and perifolliculitis.

In patients who were diagnosed as having ALP pathological findings were thinned epidermis, liquefaction degeneration of the basement membrane and basal cells, and band-like infiltration of lymphocytes across a thickened papillary dermis obscuring dermoepidermal junction.

Twenty-nine (38.6%) out of the 75 patients with rosacea were infested by *D.mites* compared to 16 patients in DLE (21.3%) group and 8 patients in ALP group (10.6%) (Figure 1).

The prevalence of D. mites (mite positivity) in the group of rosacea patients was significantly higher than controls ($P < 0.001$).

Mite positivity in females with rosacea (20 cases, 40.8%) was higher than males (9 cases, 34.6%). *D.mites* in DLE patients were higher in females (22.7%) than males (19.3%). In ALP
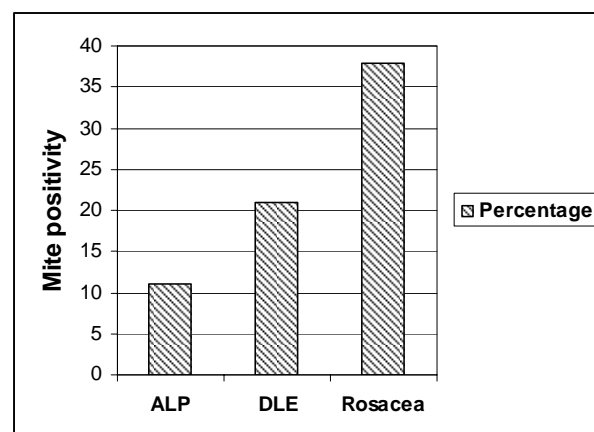

Figure 1. The prevalence of *D.mites* in sections with rosacea compared with DLE and ALP.

patients, mite positivity was 10.7% in males vs. 10.63% in females. None of these differences was significant ($P > 0.01$).

Total mite count was 106 in rosacea patients, 51 in DLE patients, and 15 in patients with ALP.

The mean mite count in patients with rosacea was 1.4 (range: 1 to 13), 0.66 (range: 1 to 8) in DLE patients and 0.2 (range: 1 to 3) in patients with ALP. This difference was statistically significant ($P < 0.01$) (Figure 2).

Odds ratio between rosacea and DLE group was 2.3. Odds ratio between rosacea and ALP groups was 5.2 and between DLE and ALP groups was 2.27.

Discussion

Despite its frequency, the etiology of rosacea is unclear. Rosacea is a chronic disorder of the face, which is more common in females. The development of rosacea is often but not invariably multiphasic.[27]

Several studies have demonstrated that rosacea is mainly a vascular disorder of the skin.[28 – 29] It frequently starts with flushing and redness of the skin, which leads to an increase in the skin blood flow and accumulation of extracellular fluid in the dermis. Edema and elastotic degeneration are because of sun exposure that cause damage to lymphatic vessels. Inflammatory lesions, papules, pustules, and nodules will happen then.

The most severe stage of the disease is rhinophyma, which is due to hypertrophy of nose and proliferation of sebaceous glands, connective tissues, and vessels.

In skin biopsy telangiectasia, edema in upper dermis, dilatation of hair follicles, and perifollicular lymphocytic infiltration are present.
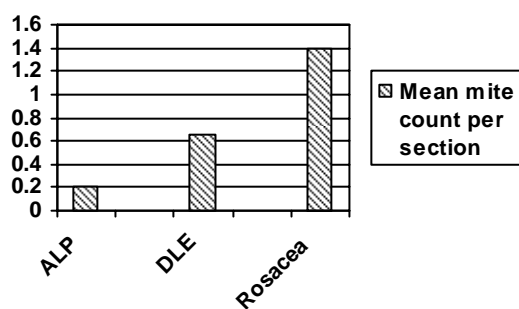
Granulomatous type inflammatory infiltration may be seen.[14] *D.mites* are considered to be involved in the pathogenesis of acne rosacea. They include *D.folliculorum* and *D.brevis*, which are saprophytic mites in human pilosebaceous follicles. For the first time in 1841 Berger and Henle discovered them, but differentiation between them was propounded by Akbulatova.[14 – 37]

D.folliculorum is a transparent and worm-like mite, 0.3 mm long, which occupies the hair follicles, upper the sebaceous glands level. *D.brevis* is smaller than the former and exists solely in depth of sebaceous and meibomian glands.

D.folliculorum is more common than *D.brevis* in human skin. *D.mites* can be found in any age groups except the newborns who are presumably infested soon after birth by direct contact.[24, 30 – 31] The mite population varies with age. It is the lowest in children and adolescents and the highest in the middle age and elderly.[17] No sexual difference in prevalence has been found.[24, 30] *D.mites* have been retrieved from almost every area of human skin but have a predilection for face.

There are different methods for skin sampling to examine *D.mites* such as: adhesive tape, skin scraping, skin impression, hair epilation, comedo extraction, skin surface biopsy, and skin biopsy.[26, 32, 34] Skin surface biopsy and skin biopsy have more commonly been used.

In skin surface biopsy, the mites are intact, alive, mobile, and are easy to detect (Figure 3). It is not a method to study the mite prevalence in the population but to estimate *Demodex* density — or more precisely, *D.folliculorum* density — in each
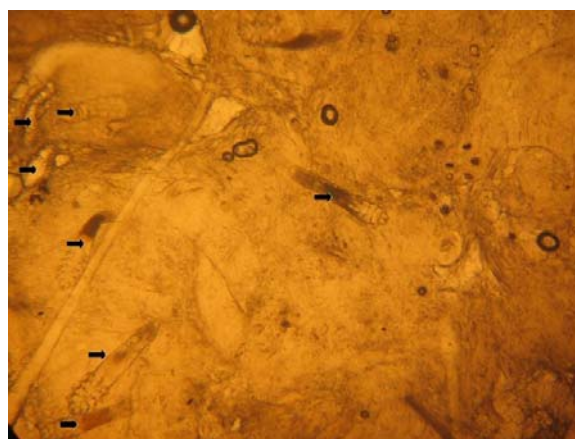
Figure 2. The mean mite count per section in rosacea compared with DLE and ALP

Figure 3. *Demodex mites* in superficial skin biopsy (×40).

subject. The method collects the superficial part of the horny layer and the whole follicle contents, therefore detects the few mites present on the skin surface and the more numerous mites in the pilosebaceous duct.[26] *D.folliculorum* and *D.brevis*, which are principally found in the sebaceous glands and occasionally penetrated in to the dermis, are not detected by this method.[16–35]

It is difficult to find *D.mites* in standard skin biopsy because in histological preparations the mite shrinks rapidly and transforms into a translucent "ghost" sac of chitin.[26]

Our findings showed that the *Demodex* population of the face was increased significantly in patients with rosacea compared to age- and sex-matched control subjects. Whether this increase is opportunistic or contributes to the disease is still to be determined. Because the difference is statistically significant, the possibility of a pathogenic role for *Demodex* must be considered (Figure 4). This finding is in agreement with Roihu and Kariniemi's findings,[33] but is against
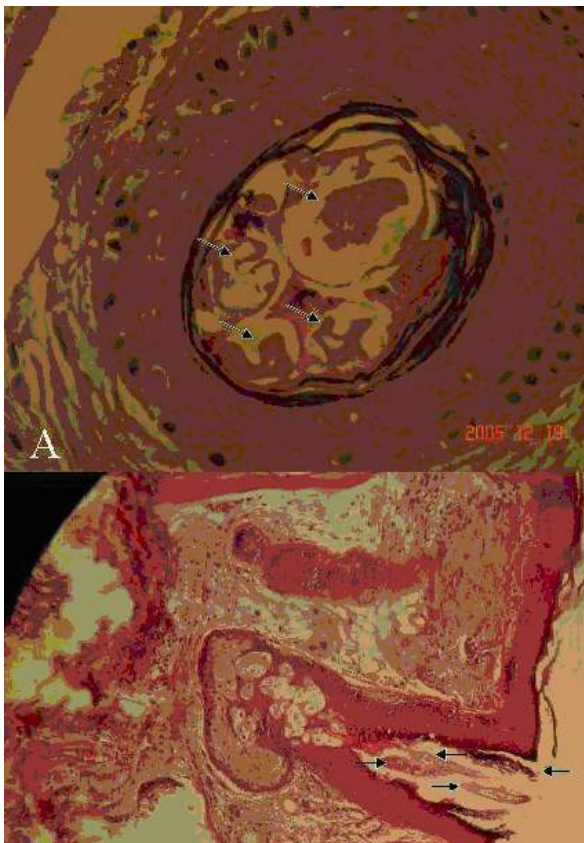
the reports from Marks and Harcout-Webster, and Varotti et al.[36, 27] Most published studies have shown that the prevalence of *Demodex* increases with age.[15, 19–23]

Sengbusch and Hauswrith found a pronounced increase in the prevalence of *D.brevis* with increasing age. Whereas the prevalence of *D.folliculorum* tended to remain more constant.[38] In our study, we did not observe an increase in the mites prevalence in patients older than 40 years.

Mite count in each slide in the rosacea patients (1.4) was significantly higher than the control group (0.66 in DLE group and 0.2 in ALP group), which was against the findings of Roihu and Kariniemi.[33] This discrepancy between our study and Roihu and Karriniemi can be explained by the different methods employed.

Regardless of calculated odds ratios between different groups, we found that possibility of *D.mites* detection in skin biopsy of a patient with rosacea is 2.3 folds higher than a DLE patient and 5.2 folds higher than a patient with ALP.

Considering the results of this study, we can conclude that the prevalence and the number of *D.mites* in rosacea patients are higher than the control subjects. This finding supports the pathogenic role of *D.mites* in rosacea, but whether these mites play a role in initiating rosacea or simply find the lesions of rosacea as a convenient home is still uncertain. However, it is possible that *D.mites* can stimulate an inflammatory reaction that ultimately results in connective tissue damage and telangiectasia. The findings of the present study should be confirmed in a larger patient group.

Figure 4. A) *Demodex mites* in histopathological section of a patient with rosacea (×40). B) Telangiectasia and *Demodex mites* are seen in the histopathological section of a patient with rosacea (×10).

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Paper 2

Potential role of *Demodex* mites and bacteria in the induction of rosacea

Review

Potential role of *Demodex* mites and bacteria in the induction of rosacea

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Rosacea is a common dermatological condition that predominantly affects the central regions of the face. Rosacea affects up to 3 % of the world's population and a number of subtypes are recognized. Rosacea can be treated with a variety of antibiotics (e.g. tetracycline or metronidazole) yet no role for bacteria or microbes in its aetiology has been conclusively established. The density of *Demodex* mites in the skin of rosacea patients is higher than in controls, suggesting a possible role for these mites in the induction of this condition. In addition, *Bacillus oleronius*, known to be sensitive to the antibiotics used to treat rosacea, has been isolated from a *Demodex* mite from a patient with papulopustular rosacea and a potential role for this bacterium in the induction of rosacea has been proposed. *Staphylococcus epidermidis* has been isolated predominantly from the pustules of rosacea patients but not from unaffected skin and may be transported around the face by *Demodex* mites. These findings raise the possibility that rosacea is fundamentally a bacterial disease resulting from the over-proliferation of *Demodex* mites living in skin damaged as a result of adverse weathering, age or the production of sebum with an altered fatty acid content. This review surveys the literature relating to the role of *Demodex* mites and their associated bacteria in the induction and persistence of rosacea and highlights possible therapeutic options.

Rosacea: definition and epidemiology

Rosacea is a common chronic inflammatory dermatosis of the face that affects up to 3 % of the world's population (Buechner, 2005). Skin lesions are usually located in the central regions of the face, involving mostly the cheeks, nose and chin. Occasionally, lesions may be found on sun-exposed areas such as the neckline, the neck and ears; however, the periocular region often remains lesion-free (Powell, 2005). The rash is usually symmetrical and may be described according to associated or underlying symptoms of vascular origin (flushing or permanent erythema, telangiectasias or oedema), as well as the presence of papules and pustules, which can develop secondarily. In some patients, hypertrophy of connective tissue and hyperplasia of the sebaceous glands may occur, resulting in the development of phyma. Rosacea usually affects people between the ages of 30 and 50 and is rare in children. Rosacea affects mostly fair-skinned people with Fitzpatrick skin phototypes I and II (Del Rosso, 2006) and is three times more common in women than in men (Butterwick *et al.*, 2006). In men, the disease has a more severe course and men with rosacea have an increased tendency to develop phyma lesions (Buechner, 2005). The

standard classification system for rosacea identified four basic stages of the disease: erythematotelangiectatic rosacea (ETR) (Fig. 1), papulopustular rosacea (PPR) (Fig. 2), phymatous rosacea, ocular rosacea (Fig. 3) and one variant rosacea, granulomatous rosacea (GR) (Wilkin *et al.*, 2002).

Diagnostic criteria of rosacea include primary features, such as flushing erythema, permanent erythema, papules, pustules and telangiectasias, the presence of which on the convexities of the face justifies the diagnosis of rosacea, and secondary features, such as the feeling of burning or tingling of the skin, oedema, the presence of tarsus, dryness of the skin, ocular symptoms, lesions outside the face and hyperplastic changes, which aid the diagnostic process (Wilkin *et al.*, 2002).

Aetiopathogenesis

The aetiopathogenesis of rosacea remains unexplained, as the pathogenic mechanisms that lead to the development of the skin lesions have not yet been fully elucidated. Possible factors responsible for rosacea may include auto-immune dysregulation, vascular disorders, external factors, degeneration of connective tissue elements, functional

Fig. 1. Erythematotelangiectatic rosacea. Note presence of inflammation on skin and increased vascularization on nose.

disorders of the pilosebaceous unit, nutritional and chemical factors and infectious factors (Crawford *et al.*, 2004, Yamasaki & Gallo, 2009). Over a significant period of time, there have been numerous attempts to connect the etiopathogenesis of rosacea with the presence of some micro-organisms on or within the skin (Lazaridou *et al.*, 2011), including *Demodex* mites and bacteria. It is well established that there is a higher density of *Demodex* mites in the skin of rosacea patients than control patients but the significance of this has been disputed (Vance, 1986; Bonnar *et al.*, 1993; Erbağci & Ozgöztaşi, 1998). This review will explore the current understanding of the role of these organisms in the induction of rosacea.

Demodex mites

There are more than 100 species of *Demodex* mites (class *Arachnida*, subclass *Acarina*) and all are highly specialized, host-specific obligatory commensals of mammals. Various kinds of *Demodex* mites may infest the skin of the host, depending on the preferred area on the skin (Lacey *et al.*, 2009). In many cases, mite infestation is asymptomatic and their role remains unclear (Lacey *et al.*, 2011). The pathogenic role of *Demodex* mites is well-documented in dogs where *Demodex canis* causes demodicosis – a serious, potentially fatal disease connected with numerous skin and ocular symptoms (Gortel, 2006).

Human skin may be inhabited by two species of *Demodex* mites and both have a worm-like shape and are covered by a thin cuticle (Fig. 4). The larger species, *Demodex folliculorum*, is about 0.3–0.4 mm long, has an elongated shape and resides in hair follicles in a cluster consisting of several mites. The smaller species, *Demodex brevis*, is about

0.2–0.3 mm long, has a spindle shape, shorter legs and resides solitarily in the sebaceous or meibomian glands (Raszeja-Kotelba *et al.*, 2004). As *D. brevis* inhabits the deep parts of the skin, it is difficult to extract it without tearing of tissue. Due to the fact that the main food sources for mites in all phases of the development are epidermal cells and sebum components, they reside in skin areas particularly rich in sebaceous glands, such as the face – especially the nose, cheeks, forehead and chin. They may also be found in the external auditory canal, on the chest and in the genital area (Raszeja-Kotelba *et al.*, 2004).

The ultrastructure of *Demodex* mites

The gnathosoma, comprising the mouth and feeding parts, is located in the anterior portion of the *Demodex* body, the rest of the body consists of prosoma and opisthosoma (Fig. 4). The gnathosoma of *D. folliculorum* has sharp, stylet-like chelicerae, more developed than those of *D. brevis*, which are used to cut and take food, and pedipalps, which are used to hold the food. Both species have four pairs of legs in the prosoma (Jing *et al.*, 2005). *Demodex* mites use the chelicerae to cut the epithelial cells of the host skin, secrete lytic enzymes for pre-oral digestion and evacuate liquid cytoplasm components (Desch & Nutting, 1972). In the process of destroying the epithelial cells, the epithelial barrier is often disturbed and the mite penetrates into the dermis stimulating Toll-like receptors (TLR) (Schauber *et al.*, 2007). Proteolytic enzymes (proteases) are among the digestive enzymes secreted by *Demodex* mites. Concrements of serum immunoglobulin IgD and two inhibitors of serum proteases (α-1-antitrypsin and α-1-antichymotrypsin), which might be a specific defensive reaction of the

Fig. 2. Papulopustular rosacea. Characteristics papules and pustules are present on skin of cheek.

host against mites, have been detected on the surface of *Demodex* mites (Tsutsumi, 2004). In atopic dermatitis, proteases produced by house dust mites have been identified as the factor responsible for local skin irritation (Deleuran *et al.*, 1998).

Demodex life cycle

In all phases of their life cycle, *Demodex* mites avoid sunlight. They emerge from the pilosebaceous units at night and migrate across the surface of the skin to find a mating partner, travelling at a speed of about 16 mm h^{-1} (Lacey *et al.*, 2011). The life cycle of *Demodex* mites consists of five phases of development and lasts from 14 to 18 days. The copulation takes place near the entry of the hair follicle. Afterwards, the gravid female moves to the inside of the sebaceous gland, where she deposits eggs, from which the larvae will emerge about 60 h later. Protonymphs and nymphs are the next phases of the *Demodex* life cycle (Lacey *et al.*, 2009; Spickett, 1961).

Due to the fact that *Demodex* mites are obligate parasites of the pilosebaceous units and highly susceptible to desiccation, they are not capable of surviving for long periods outside the host. Routes of transmission are not fully known but it may occur by direct contact as well as through dust. While the skin of new-borns is free of *Demodex folliculorum*, colonization of the skin in humans takes place in childhood or early adulthood. *Demodex*

Fig. 4. *Demodex folliculorum* mite embedded in a hair follicle. The body parts of the mite, including the head–neck segment (a), the body–tail segment (b), the four pairs of short legs attached to the head–neck (c) and the mouth parts (d), are shown. Length, 0.4 mm.

mites are found in representatives of all human races and in all geographical areas (Lacey *et al.*, 2009).

Role of *Demodex* mites in human skin disease

Demodex mites were originally perceived to be commensals, having a symbiotic relationship with the human host. However the opinion about the role of *Demodex* in pathogenesis of many diseases, including rosacea has been changing (Lacey *et al.*, 2009). In some specific conditions in the host system, *Demodex* mites may become potential pathogens. This may happen when the immunological conditions of the host change and new environmental conditions on the skin facilitate the development of *Demodex* mites (Dahl *et al.*, 2004; Whitfeld *et al.*, 2011).

There are certain differences in distribution on the skin between the two species of *Demodex* mites found in the human population. *D. folliculorum* counts are notably higher but *D. brevis* inhabits a larger area of the human body. The proportion of *D. brevis* to *D. folliculorum* also differs among men (1:4, respectively) and women (1:10) (Bohdanowicz & Raszeja-Kotelba, 2001). *D. folliculorum* is more often associated with erythema and epithelial desquamation, whereas *D. brevis* is linked with papulopustular eruption, symmetrical rashes and conditions arising on the background of a pre-existing disease (Akilov *et al.*, 2005).

The extent of *Demodex* colonization in the human population is high (20–80 %), reaching 100 % in elderly people (Elston, 2010). Mite density starts to rise in the sixth decade of life and stays at the same level until the eighth decade of life. Mite density is very low in young adults, even though their levels of sebum production, a potential source of food for mites, are very high (Ozdemir *et al.*, 2005; Aylesworth & Vance, 1982). Patients with papulopustular rosacea produce sebum with an altered fatty acid profile, suggesting that the nature of the sebum, rather than its quantity, may favour the development of *Demodex* mites (Ní Raghallaigh *et al.*, 2012). This finding raises the possibility that non-antibiotic therapies to restore the normal fatty acid composition of sebum may improve skin integrity and inhibit the proliferation of *Demodex* mites.

Due to the fact that *Demodex* mites are commonly found in healthy individuals and the density of mites is generally low, the presence of mites on the skin is not enough to determine pathogenicity. An increase in mite density on facial skin is observed in perioral dermatitis, caused by long-term use of local steroids or other immunomodulating drugs
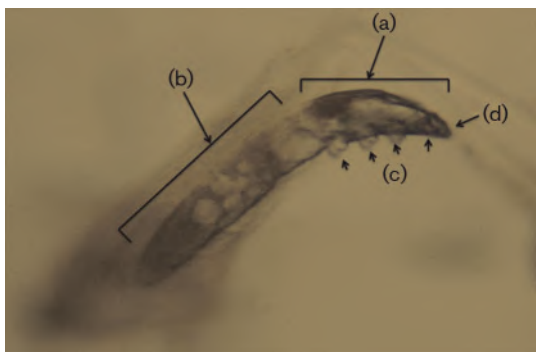
(Fujiwara *et al.*, 2010). Higher numbers of *Demodex* mites have been noted in patients undergoing immunosuppressive therapy, for example children receiving chemotherapy for leukaemia (Ivy *et al.*, 1995), patients with HIV-infection or AIDS (Aquilina *et al.*, 2002; Dominey *et al.*, 1989) and chronic dialysis patients (Karincaoglu *et al.*, 2005).

A positive correlation between high density of *Demodex* mites and the presence of antigens affecting tissue compatibility, HLA Cw2 and Cw4, has been established (Akilov & Mumcuoglu, 2003). Furthermore, increased numbers of mites have been associated with a higher tendency of leukocytes to undergo apoptosis. Such a genetically conditioned decreased immune performance may result in local immuno-suppression and so facilitate survival and replication of *Demodex* mites (Akilov & Mumcuoglu, 2004).

Ayres & Anderson (1932) first suggested a correlation between the presence of *Demodex* mites on the skin and development of various skin lesions (Ayres, 1930). They described a disease entity which they named 'pityriasis folliculorum' and associated its development with the presence of *D. folliculorum* mites. Pityriasis folliculorum is characterized by small, follicular, scaling papules, the feeling of skin dryness and pruritus. Lesions in pityriasis folliculorum are usually unilateral, located mainly on the cheeks, but may also reach the eyelids (Ayres, 1930). Ayres & Ayres (1961) identified a new disease entity, rosacea-like demodicosis, caused by the presence of abundant *D. folliculorum* mites and characterized by erythema, dryness and fine follicular scaling. Later research proved pityriasis folliculorum to be a form of demodicosis, and the most frequent one (54 %), but so discrete and unfamiliar that it was often not diagnosed. Demodicosis is characterized by discrete symptoms of erythema, higher densities of *Demodex* mites per cm^2 (up to 61 mites per cm^2) in comparison to papulopustular rosacea (up to 36 mites per cm^2), and is primarily a disease of the elderly or immunocompromised. A compromised immune system is thought to enable such proliferation of *Demodex* mites in cases of pityriasis folliculorum (Forton *et al.*, 2005).

The mean density of *Demodex* mites on the skin of rosacea patients is 10.8 mites per cm^2 in comparison to 0.7 mites per cm^2 in healthy people. However, when all types of rosacea are taken into account, statistically larger mite densities per cm^2 are found in cases of papulopustular rosacea (Forton & Seys, 1993). Other diseases in which infestation with *Demodex* mites is believed to be the aetiological factor include blepharitis (Czepita *et al.*, 2007) and, in one case, hair loss described in a 6-year-old boy (García-Vargas *et al.*, 2007).

Histopathological examination of skin specimens obtained from control patients revealed the presence of *Demodex* mites in 10 % of all facial skin biopsies and in 12 % of all pilosebaceous units (Aylesworth & Vance, 1982). Skin specimens with histological features of folliculitis revealed that *D. folliculorum* mites were found in 42 % of inflamed

and only 10 % of non-inflamed follicles. Overall, 83 % of all affected follicles demonstrated features of inflammation. However, whether *D. folliculorum* causes folliculitis or simply inhabits inflamed follicles remains unclear (Vollmer, 1996). In a study conducted in patients with papulopustular rosacea, the presence of *D. folliculorum* in follicle secretions was found in 90.2 % of patients and only 11.9 % of control samples. Additionally, histopathological examination of skin obtained from these patients revealed that the presence of *Demodex* mites was connected with severe perifollicular lymphocytary infiltration (Georgala *et al.*, 2001).

It seems that the presence of *Demodex* mites within the skin is more important than their presence on the skin and dermal symptoms occur when mites residing in hair follicles penetrate into the surrounding tissues (Ayres & Ayres, 1961). Most probably, when *Demodex* mites breach the epithelial barrier, their antigens influence the immune system of the host and induce a type IV hypersensitivity reaction. *Demodex* mites may then be attacked by giant cells giving rise to dermal granulomas, which are most often observed in granulomatous acne rosacea. Granulomas are also found in skin biopsies of patients with papulopustular rosacea and even in patients with erythematous rosacea (Hsu *et al.*, 2009).

The causal relationship of *Demodex* mites in skin lesions has been suspected to occur through several mechanisms. They may mechanically block the follicles, leading to distension and causing intra-follicular hyperkeratosis. The presence of mite's chitinous external skeleton may act like a foreign body and contribute to the formation of granulomas. The waste products of *Demodex* mites and/or associated bacteria may activate the elements of innate immune system or stimulate the immune system through the mechanism of delayed hypersensitivity reaction (Bevins & Liu, 2007).

Potential role of *Bacillus oleronius* in rosacea

One hypothesis concerning the role of *Demodex* mites in the induction of rosacea assumes that *Demodex* are vectors for micro-organisms that cause and exacerbate skin lesions (Hsu *et al.*, 2009). The theory has its roots in the fact that clinical improvement was noted in patients with rosacea who were administered tetracycline antibiotics, although these antibiotics neither demonstrate activity against *D. folliculorum* nor reduce their numbers on the skin. It has been suggested that the beneficial activity of antibiotics was due to their anti-inflammatory properties; however, other anti-inflammatory agents, such as steroids or tacrolimus, intensify the symptoms of rosacea or even induce its development (Antille *et al.*, 2004). The fact that only some drugs proved to be effective in the treatment of rosacea suggested that that an unknown bacterium may have a role in the pathogenesis of the disease. Attempts to prove the presence of DNA of Gram-negative intracellular bacterium *Wolbachia pipientis*, which has been detected in various species of mites and nematodes, proved futile in the case of

Demodex mites (Borgo *et al.*, 2009). *Bacillus oleronius* was isolated from a *Demodex* mite, obtained from a patient with papulopustular rosacea (Lacey *et al.*, 2007). The species is an endosporic Gram-negative bacterium (genus *Bacillus*, family *Bacillaceae*) and was first described in 1995 when it was isolated from the hindgut of the termite *Reticulitermes santonensis*, where it most likely plays a symbiotic role (Kuhnigk *et al.*, 1995). The bacterium produces proteins capable of stimulating peripheral blood mononuclear cell proliferation in 16 out of 22 (73 %) patients with papulopustular rosacea compared to only 5 out of 17 (29 %) in control patients. The sera of six other patients with papulopustular rosacea reacted with two antigens isolated from the bacterium: two specific proteins of 62 kDa and 83 kDa, bearing similarity to the heat-shock proteins (Lacey *et al.*, 2007). Another experiment investigated sera from 59 patients with diagnosed rosacea and a statistically significant correlation was demonstrated between positive reactions of the serum from these patients with *B. oleronius* antigens and the presence of *Demodex* mites on their eyelashes and facial skin lesions (Li *et al.*, 2010). Recent work has indicated that a range of *B. oleronius* proteins can activate neutrophils which migrate and produce inflammatory cytokines. It was speculated that the release of *B. oleronius* from dead *Demodex* mites within the pilosebaceous unit could lead to the release of a range of *Bacillus* proteins into the unit, which 'leak' into the surrounding tissue and so attract neutrophils (O'Reilly *et al.*, 2012). If this occurs *in vivo* it would lead to inflammation and tissue degradation in the vicinity of the pilosebaceous unit. Interestingly, inflammation in papulopustular rosacea is often orientated around the pilosebaceous unit, suggesting that the focus of the inflammation is within or adjacent to the unit (Lacey *et al.*, 2007). Exposure of corneal epithelial cells to *Bacillus* proteins results in an aberrant wound healing response, suggesting a possible link between the action of these antigens on the corneal surface and the development of sterile ulcers which are a common feature of ocular rosacea (O'Reilly *et al.*, 2012).

Recent examination of patients with blepharitis has provided further evidence on the pathogenic role of *B. oleronius* (Szkaradkiewicz *et al.*, 2012). The severity of the disease did not correspond with an increased number of *Demodex* mites per lash, with the exception of the five most severe cases, where greater numbers of mites were observed. Statistically significant differences in *B. oleronius* incidence rates were found between patients with severe disease and healthy controls. This might indicate that *Demodex* mites constitute an independent pathogenic factor of blepharitis and the *B. oleronius* bacteria, carried by the mites, most probably play a role as a co-pathogen in the development of more severe forms of blepharitis.

Role of *Staphylococcus epidermidis* in rosacea

Staphylococcus epidermidis has been isolated from the pustules of 9 out of 15 patients with papulopustular rosacea, whereas this bacterium was not detected on unaffected areas of the skin (Whitfeld *et al.*, 2011). *S. epidermidis* was also isolated from the eyelid margins of 4 out of 15 patients with papulopustular rosacea, whereas no pure growth was isolated from the eyelids of age- and sex-matched control subjects. The same study also found that this bacterium was susceptible to antibiotics commonly used to treat rosacea. Facial erythema and increased blood flow in the skin of those with rosacea causes the temperature of the skin to become elevated. Dahl *et al.* (2004) found that *S. epidermidis* secreted more proteins when cultured at 37 °C than at 30 °C and that isolates from rosacea patients' skin were consistently β-haemolytic, whereas isolates from control subjects were non-haemolytic. *Demodex* mites have been shown to transport bacteria around the face (Lacey *et al.*, 2007) so the possibility remains that *S. epidermidis*, along with other bacteria, are moved to areas which favour their proliferation.

Conclusion

Rosacea is a complex disease entity of disputed aetiology. The literature offers numerous arguments supportive of the theory that rosacea is primarily connected with compromised immunity (Forton, 2012). According to this theory, on the skin of healthy, immune-competent individuals, the proliferation of *Demodex* mites is kept under control. In the first stage of rosacea, studied by investigators of the clinical form of pityriasis folliculorum, no inflammation is observed, despite the presence of a large number of *Demodex* mites. This is probably caused by an unidentified, genetic defect of the innate immunity (Akilov & Mumcuoglu, 2003) and/or the localized immunosuppressive influence of the mites (Akilov & Mumcuoglu, 2004). In the later stages of the disease, characterized by developed rosacea, there is an overstimulated reaction of the immune system, which includes elevated levels of serine proteases, kallikrein (KLK5), the presence of abnormal forms of cathelicidins (with lower anti-bacterial potential) (Yamasaki *et al.*, 2007; Schauber & Gallo, 2008) and increased expression of Toll-like 2 receptors (TLR 2), which stimulate the calcium-dependent production of kallikrein (Yamasaki *et al.*, 2011).

Such immunological conditions favour the development of different types of micro-organisms, including *Demodex* mites. Other characteristic features of rosacea patients, such as increased vascularization and elevated temperature, may further promote the growth of the organisms (Whitfeld *et al.*, 2011). Developing *Demodex* mites may be causative agents of rosacea through various mechanisms: they may mechanically block hair follicles, secrete digestive enzymes, destroy the epithelial barrier or trigger reactions of the immune system.

It is believed that *B. oleronius* forms a symbiotic relationship with *Demodex*, as it does in the termite (Kuhnigk *et al.*, 1995). On the skin of humans, this bacterium may occur in the endospore form, which enters the digestive tract of *Demodex* mites when they consume epithelial cells. The dead mites then decompose inside the hair follicles, where they release

significant numbers of bacterial antigens, which have the potential to stimulate a strong immune response (O'Reilly *et al.*, 2012). Thus, the intensification of blepharitis and rosacea, especially the papulopustular variant, may not be induced so much by the presence of the mites alone but by the presence of *Demodex* mites that carry *B. oleronius* in their digestive tract. Empirically confirmed sensitivity of *B. oleronius* to different antibiotics, especially doxycycline (Lacey *et al.*, 2007), might explain the favourable therapeutic effect of the drug in diseases such as rosacea and blepharitis.

The pathogenic role of *Demodex* mites, as well as *B. oleronius* and *S. epidermidis*, in the induction and persistence of rosacea remains an unresolved issue. The lack of an immunological response to *Demodex* mites in healthy skin raises the possibility of localized immunosuppression, facilitating the survival of the mite. Hopefully, the results of further research will bring us closer to understanding the role of microbes in the pathogenesis of rosacea and assist in the development of new and more effective therapies for the treatment of this disfiguring disease.

Acknowledgements

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Paper 3

The hair follicle mites Demodex folliculorum and Demodex brevis: Biology and medical Importance

Review

The Hair Follicle Mites *Demodex folliculorum* and *Demodex brevis:* **Biology and Medical Importance**
A Review

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The hair follicle mites *Demodex folliculorum* and *Demodex brevis* are the most common permanent ectoparasites of man. Man is their sole host. *D. folliculorum* was detected in 1841 by *Henle* and first described in 1842 by *Simon. Akbulatova* [1963] separated a subspecies *D. brevis,* which is understood today as a distinct species of its own [*Desch and Nutting,* 1972]. Worldwide 65 species of Demodex have been described, their geographical distribution coincides with the specific host. Ten of these species which occur in man, horse, cattle, sheep, goat, pig and cat, are known as pathogenic parasites [*Nutting,* 1976a].

Systematics and Biological Data

The two Demodex species found in man, *D. folliculorum* and *D. brevis,* belong to the family Demodicidae of the superfamily Cheyletoidea of the subclass Acari.

The hair follicle mites are spindle-shaped, their body surface is practically hairless and colourless. They are 0.3–0.4 mm long. The four short pairs of legs are located on the anterior third of the body, the posterior portion shows secondary striation. The genital opening is situated dorsally in the anterior portion, an anus is lacking.

Although *D. folliculorum* and *D. brevis* seem to be very similar, the two species may be readily separated on the basis of the following characteristics

[*Nutting, 1976b; Desch und Nutting,* 1972]: (a) All stages of *D. folliculorum* are larger than the corresponding stages of *D. brevis*. (b) The opisthosomal end in *D. folliculorum* is rounded, while in *D. brevis* it is pointed. (c) The length of the opisthosoma in *D. folliculorum* is 7/10 of its body length, in *D. brevis* 1/2 or 1/3. (d) The eggs of *D. folliculorum* are arrow-head shaped (0.1 mm in diameter), the eggs of *D. brevis* are smaller (0.6 mm in diameter) and oval.

The female mite is inseminated in the follicle opening. The gravid mite then makes its way into the hair follicle or a sebaceous gland, where it deposits its eggs. About 12 h elapse between copulation and oviposition. The larva hatches after about 60 h and develops into a protonymph after another 40 h. This stage still lives inside the follicle. After 42 h the protonymph gives rise to a deutonymph, which moves into the follicular opening. There it develops into an adult within 60 h. In this stage the mite lives approximately 5 days. The complete life cycle is therefore about 15 days [*Spickett,* 1961a].

The ratio of female mites to male mites is 1:4.5 for *D. folliculorum,* 1:3.4 for *D. brevis* [*Desch and Nutting,* 1972]. The number of adults generally prevails over the number of juvenile stages. Motility is limited by the mites' very reduced pairs of legs. The mites move only about 8–16 cm/h. All stages show negative phototactic reactions, but are stereotactic. Their tolerance towards heat and dryness is low [*Spickett,* 1961a]. *D. folliculorum* is found practically always posterior down in the hair follicle. It feeds on cells of the follicular epithelium by piercing the cell walls with its stylet-like chelicera and thus harvests the cell contents. *D. brevis* feeds in the same manner on sebaceous gland epithelium [*Desch and Nutting,* 1972].

Epidemiology

The two Demodex species found in man occur worldwide and have been recovered in all investigated racial groups of man [*Nutting,* 1964; *Nutting and Green,* 1976]. The incidence of infestation is distinctly related to the age of the patients examined (table I).

All authors agree that Demodex is most commonly found in the follicles of the nasolabial folds, the nose and the eyelids. Only exceptionally is Demodex recovered from sites other than the facial skin. *Beerman and Stokes* [1934] retrieved Demodex from the skin of the thorax and interscapular region, the mons pubis and the forearms in small numbers. *Breckenridge* [1953]

Table I.

Authors			Incidence of infestation %
Stcherbatchoff	[1903]	100 cadavers	49
Gmeiner	[1908]	100 cadavers	97
Fuss	[1933]	seborrhoeic skin condition	90.9
		dry skin condition	52.4
Norn	[1970a]	follicles of eyelashes and eyebrows in young patients	25
Norn	[1970b]	follicles of the nasolabial folds	72
Orru et al.	[1972]	patients under 20 years	4
		patients of middle age	84
		aged patients	93.2
Kim et al.	[1976]	30 healthy individuals	37

detected Demodices in the follicles of forehead, scalp, ear canal, nipples, mons pubis and gluteal region.

The greatest concentration is found in body sites where sebaceous glands are numerous and sebum production is pronounced. This was confirmed by *Riechers and Kopf* [1969] with a method of dermal-epidermal separation. This would explain why children, whose sebum production is low, are rarely infested with Demodices. In the lower extremities, the number of follicles is limited and the distances between the follicles are great, so that Demodices are hardly ever found there. The same applies to the axilla, where sweat production probably inhibits the development of Demodices [*Norn,* 1972]. The number of mites increases in summer with the increased outside temperature, this may be accounted for by the climatic activation of sebum production [*Akbulatova,* 1963]. The varying rate of infestation in people with a seborrhoeic skin and in people with a dry skin may also be due to the varying sebum production [*Fuss,* 1933].

The transfer of Demodex probably occurs from man to man by body contact. While *D. folliculorum* is found solitary or in groups in the follicular openings, *D. brevis* is only found in the sebaceous gland and mainly as a single specimen [*Desch and Nutting,* 1972]. *Akbulatova* [1963] examined

69 patients and found *D. folliculorum* in 25 patients, *D. brevis* in 16 patients and both species in 28 patients.

Clinical Importance

While in veterinary medicine Demodices are recognized and accepted as strictly species-specific pathogenic parasites in the skin diseases of animal [*Muller and Kirk*, 1978], opinions are still divided as to their medical importance in humans.

Gmeiner [1908] detected no increase in mite numbers in skin diseases. In the older literature, *D. folliculorum* was held responsible for skin alterations such as follicular dyskeratosis with light pigmentation [*Dubreuilh*, 1901], rosacea [*Kaufmann-Wolf*, 1925], zooparasitic pseudotuberculosis [*Bergstadt*, 1925], areata-like alopecias [*Hirst*, 1919] and anular pyoderma-like dermatoses [*Lawrence*, 1916].

Rosacea

In 1930, *Ayres* described a skin disease 'pityriasis folliculorum' in women who used cosmetic preparations rather than soap and water for their daily facial skin care. Antiparasitic treatment and use of soap and water resulted in prompt cure. *Ayres and Anderson* [1932] reported on 17 patients (14 women, 3 men) with a rosacea-like dermatosis, in whom *D. folliculorum* was found in milky vesicles and pustules or in follicular scales. Questioning of the patients revealed that many of them used cosmetic preparations instead of soap and water. Antiparasitic therapy led to improvement of the clinical manifestations with a parallel diminution in the number of mites.

Micropapular lesions on the forehead-scalp-region in 3 bald-headed men were brought into relation with the presence of numerous Demodices by *Miskjian* [1951].

Ayres and Ayres published a report in 1961 on their 30-year experience in 203 patients with 'pityriasis folliculorum' and 'acne rosacea of the Demodex-type'. The latter is described as a rosacea-like dermatosis of external origin, which is to be distinguished from rosacea by its smaller pustules, more superficial granulomas and less oily skin. Demodex was recovered in great numbers from superficial vesicles and pustules. The application of 'Danish ointment' (a sulphur and betanaphthol-containing vaseline) and use of soap and water induced disappearance of the mites and marked improvement or complete cure of the skin disease. In 1962, *Russell* discussed again the possible aetiologic role of *D. folliculorum* in rosacea, and *Spickett* [1962]

stressed the quantitative aspect and related the heaviness of infestation to the frequency of infested follicles or to the number of mites within such follicles.

Hojyo and Dominguez [1976] used cyanoacrylate adhesives for the detection of *D. folliculorum* according to the method of *Marks and Dawber* [1971], the so-called 'skin surface biopsy'. With this method, the whole follicle content is removed from the skin. In 9 out of 10 patients with rosacea they isolated more than 4 mites per follicle, in all of 10 patients with acne and the remaining rosacea patient no mites were found. Acaricide therapy with Danish ointment applied over 12 weeks led to a cure of 10 rosacea patients, but only in 1 acne patient.

These reports, however, are opposed by a few papers with negative results. *Bosse and Nasemann* [1963] found *D. folliculorum* only in 4 out of 12 patients with rosacea. Based on histological examinations before and after local therapy with a 3% sulphur preparation, *Robinson* [1965] excluded a pathogenetic role of *D. folliculorum,* as there was no significant change in the population of mites after therapy. The histological study by *Marks and Harcourt-Webster* [1969] on the aetio-pathogenesis of rosacea led to the opinion that Demodex is of little importance in this disease.

Histological examinations in 10 patients with granulomatous rosacea and lupus miliaris faciei, respectively, carried out by *Grosshans et al.* [1974] revealed remnants of extrafollicular *D. folliculorum* in tuberculoid granulomas in the corium. The authors presume these histological alterations to be a manifestation of a cell-mediated immune reaction to *D. folliculorum* and *D. brevis.*

Seifert [1978] observed a solitary Demodex granuloma in a 9-year-old boy. *Ecker and Winkelmann* [1979] reported on an extensive perioral granulomatosis in a 50-year-old woman. Histology disclosed a lymphohistiocytic infiltrate with foreign body giant cells in the upper dermis and an extrafollicular intact adult *D. folliculorum* in the area of pronounced granulomatous infiltration, a picture consistent with granulomatous rosacea.

Blepharitis

Demodex blepharitis has been known for a long time in the ophthalmologic literature and is well documented. *Raehlmann* first described this disease in 1898. *Morgan and Coston* reported in 1964 on 20 patients with isolated Demodex blepharitis. Burning and itching of the eyelids, redness, scaling, pigmentation about the roots of the cilia, cellular mantles about the basis of the cilia and conjunctival injection were the presenting features. Out of

18 patients with rosacea, 12 had Demodex in their eyelash follicles, while in 54 patients with seborrhoeic dermatitis and seborrhoeic blepharitis and in 30 patients with acne no mites were found in the ciliary follicles.

Ayres and Mihan [1967] also mentioned a Demodex blepharitis in the course of Demodex rosacea, which cleared under 'Danish ointment'.

Post and Juhlin [1963] described Demodex blepharitis as an inflammation of the lid margins with matted, irregularly growing, scanty and loose eyelashes and an accumulation of debris at their base. *Junaid* [1975] reported on 5 patients, 4 of whom were diagnosed as suffering from 'pityriasis folliculorum' and 1 from rosacea. 4 of them also had a Demodex blepharitis. All patients were cured after the application of sulphur preparations within 2–3 weeks.

Based on the examination of 100 biopsies of eyelid skin, *Roth* [1979] denied a pathogenetic role of the mites. He found Demodex in all patients above the age of 70 and in 50% below the age of 50 in the eyelash follicles. Only half of the affected follicles showed perifollicular lymphocytic infiltration.

Perioral Dermatitis

The case reports and photographs in the publications of *Ayres* [1930], *Ayres and Ayres* [1961] and *Ayres and Anderson* [1932] show a remarkable similarity to the clinical picture of perioral dermatitis, which was described in detail by *Frumess and Lewis* in 1957 as light-sensitive seborrhoeid.

Marks and Black [1971] examined 26 biopsies from patients with perioral dermatitis and came to the conclusion that *D. folliculorum* does not play an aetio-pathogenetic role.

Bendl [1976] observed a blepharitis in 4 out of 95 patients with perioral dermatitis. The blepharitis was characterized as Demodex blepharitis, and the coincidence of the two conditions led to a combined therapy with a topical acaricidal preparation (sulfacetamide-sulphur-hydrocortisone lotion) and tetracycline given orally. This combined treatment proved very effective.

Hojyo and Dominguez [1976] detected *D. folliculorum* in 2 out of 6 patients with perioral dermatitis by means of 'skin surface biopsies'. Acaricidal therapy with 'Danish ointment' led to a cure in all 6 patients.

Ohtaki and Irimajiri [1977] treated 2 patients with perioral rosaceiform dermatitis with Lindane (hexachlorocyclohexane). The very irritating papules, which were distributed all over the face with predominance in the perioral area, contained Demodex in great numbers. Lindane produced prompt cure, and Demodices could no longer be found.

Demodex as Vectors of Pathogenetic Organisms

Borrel [1908] mentioned the possibility that *D. folliculorum* might be responsible for the transmission of the leprosy bacillus. This was, however, never proved. The same applies to the transmission of tumour-inducing viruses. *Norn* [1972] detected bacteria on the body surface of the mites by means of electron-microscopy, and *Spickett* [1961b] found bacteria in the gastro-intestinal tract of the mites. *Staphylococcus aureus* was found to a higher degree in cilia infested with mites (69%) than in eyelashes without mites (50%) [*Norn*, 1970a].

Demodicosis in Animals

Demodex canis is an ubiquitous parasite in dogs. The mange-like demodicosis of the dog is the best investigated in the animal kingdom. Clinical symptoms arise in the dog when the number of parasites is increasing [*Bouvier and Gaschen*, 1949; *Muller and Kirk*, 1978]. Young dogs between 3 months and 1 year are preferably infested. Short-haired dogs like dachshunds, beagles, boxers, English bulldogs are more liable to become infested than long-haired dogs. Transmission of the parasite from the bitch to the puppy takes place during the first days after birth.

Two types of demodicosis are observed in dogs, the localized and the generalized form. The localized form occurs most commonly on the face, especially in the periocular area and the commissures of the mouth, less frequently on the forelegs or the back. Most of these cases heal spontaneously, but some may progress into the generalized form. Superinfection with *S. aureus* leads to pyoderma.

The primary lesions are erythemato-squamous, partly elevated patches and acneiform pustules. Pruritus may be so severe in the generalized form as to require euthanasia for the animal. In heavy skin infestations, *D. canis* is also found in internal organs [*Nutting*, 1976a].

Histopathology shows masses of mites and keratin in the dilated hair follicles. The hair is missing. The perifollicular lymphocytic infiltration is moderate in the localized form, very pronounced in the generalized form. The follicular openings are plugged by keratin, debris and dead mites, and intradermal abscesses are present in the generalized pustular form.

The detection of mites *in vitro* is easy to perform. A drop of mineral oil is placed on the skin surface. By firm lateral squeezing, material from the

affected follicles is expressed and can be placed on a microscope slide for inspection. Therapy consists in the application of acaricidal preparations. Antibiotics have to be given in addition for the generalized form.

Feline demodicosis caused by *D. cati* is rare. The eyelids and periocular area are affected. The disease heals spontaneously. Generalized forms have been mentioned in the older literature [*Muller and Kirk*, 1978]. The pathogenetic role of *D. canis* and *D. cati* is not disputed in veterinary medicine, although Demodices, the most frequent permanent ectoparasites of these two domestic animal species, are found quite often in healthy animals.

Conclusions

The hair follicle mites *D. folliculorum* and *D. brevis* are the most common permanent ectoparasites of man, who is their exclusive host. Main habitats are the hair follicles and sebaceous glands of the facial skin. *D. folliculorum* lives in the follicular orifices, *D. brevis* deeper down in the sebaceous glands. The elongated, spindle-shaped form of the mites is adapted to these structures. The rate of infestation in healthy skin is age-dependent and reaches 100% in elderly people.

In veterinary medicine, Demodices are known as the cause of periocular and perioral papulo-pustular dermatoses with a histological picture similar to that of human rosacea. Clinical symptoms appear only after a considerable increase in the number of mites.

The importance of Demodices for human medicine is still the object of an almost 50-year-old discussion. The arguments for an aetiologic role in rosacea and perioral dermatitis, which are possibly identical with or related to the 'pityriasis folliculorum' described by *Ayres and Anderson* in 1932, are based alone on clinical and histopathological observations. Demodices are found in great numbers in the lesions, they disappear under acaricidal therapy, and the skin alterations heal.

The detection of Demodices in the centre of corial granulomas in rosacea [*Grosshans et al.*, 1974] and in granulomas of the facial area [*Ecker and Winkelmann*, 1979; *Seifert*, 1978] and their acceptance as pathogenetic factor is opposed by earlier investigations by *Marks and Harcourt-Webster* [1969] and *Marks and Black* [1971], who attribute no importance to the hair follicle mites in spite of their frequent presence in rosacea and perioral dermatitis.

Spickett [1961a] succeeded in culturing *D. folliculorum* in human sebum

to study their life cycle. The *in vitro* mass culture as a prerequisite for clinical investigations has, to our knowledge, failed so far.

The reliability of the quantitative assessment of mite populations in healthy skin and in lesions of rosacea and perioral dermatitis may be greatly enhanced by using acrylate adhesives, the so-called 'skin surface biopsy', instead of the simple expressing of follicular material [*Hojyo and Dominguez,* 1976].

As with animal demodicosis, the importance of quantitative factors (numbers of affected follicles and numbers of mites per follicle) in the pathogenesis of the two facial dermatoses in man and of internal and external influences (corticosteroids!) must be evaluated. The individual reactivity of the host is yet to be assessed, although on the basis of histopathological investigations, an immunological response to the parasite seems to be implied [*Rufli et al.,* 1981]. Because of the high species specificity of the hair follicle mites, this question can only be answered in the clinical experiment following successful cultivation *in vitro.*

The study of the pathogenetic importance of the Demodex species in rosacea and perioral dermatitis and the integration of the results into modern clinical and epidermiological knowledge is needed, since the aetiology of both skin diseases is still not solved [*Plewig,* 1979].

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Paper 4

Demodex folliculorum on the eyelash follicle of diabetic patients

Demodex folliculorum on the eyelash follicule of diabetic patients

Demodex folliculorum *nos cílios de pacientes diabéticos*

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ABSTRACT

Purpose: To compare the prevalence of *Demodex folliculorum* on the eyelashes of patients with proliferative diabetic retinopathy and healthy voluntaries.

Methods: Type 2 diabetic patients with proliferative retinopathy and age- and gender-matched healthy voluntaries (group control) underwent a slit lamp examination which three eyelashes containing cylindrical dandruff were removed from each lid by fine forceps. The lashes were dyed with fluorescein and the presence of *Demodex folliculorum* was verified by direct visualization under a light microscope. The mites were recognized based on its morphology and peculiar movement. The results were expressed in "positive" when at least one mite on one lash was found and "negative" when no mite was identified. The Chi-square test was used for comparing mites' presence in both groups.

Results: Forty-two patients were included in each group. The age ranged from 50 to 60 years old, with a mean of 56.4 ± 5.2 years. The male:female ratio was 0.6:1. There was no statistically significant difference with regard to age and gender in both groups (p>0.05). *Demodex folliculorum* was significantly more prevalent in diabetic patients (54.8%) than in control patients (38.1%) (p=0.048).

Conclusion: *Demodex folliculorum* was more prevalent in diabetic patients than in healthy voluntaries, independently of gender and age.

Keywords: Blepharitis; Diabetes mellitus; Folliculitis; Eyelid diseases; Mites; Mite infestation

RESUMO

Objetivo: *Comparar a prevalência de* Demodex folliculorum *nos cílios de pacientes com retinopatia diabética proliferativa e voluntários normais.*

Métodos: *Pacientes com diabetes mellitus tipo 2 apresentando retinopatia proliferativa e voluntários normais com mesma distribuição de sexo e idade (grupo controle) foram submetidos a exame em lâmpada de fenda. Três cílios com secreção "em colarete" foram removidos de cada pálpebra com pinça delicada. Os cílios foram corados com fluoresceína e a presença de* Demodex folliculorum *foi verificada por visualização direta através de microscópio de luz. As larvas foram reconhecidas baseadas em sua morfologia e movimentos peculiares. Os resultados foram expressos em "positivo" quando foi encontrada pelo menos uma larva em um cílio e "negativo" quando nenhuma larva foi encontrada. O teste de Chi quadrado foi utilizado para comparar a presença das larvas nos dois grupos.*

Resultados: *Quarenta e dois pacientes foram incluídos em cada grupo. A idade variou de 50 a 60 anos com média de 56,4 ± 5,2 anos. A relação masculino:feminino foi de 0,6:1. Não houve diferença estatisticamente significante com relação ao sexo e idade entre os dois grupos (p>0,05).* Demodex folliculorum *foi significantemente mais prevalente em pacientes com diabetes (54,8%) que no grupo controle (38,1%) (p=0,048).*

Conclusão: Demodex foliculorum *foi mais prevalente em pacientes diabéticos que em voluntários normais, independentemente do sexo e da idade.*

Descritores: *Blefarite; Diabetes mellitus; Foliculte; Doenças palpebrais; Ácaros; Infestações por ácaros*

INTRODUCTION

The *Demodex* sp. is a microscopic elongated mite considered the most common permanent ectoparasite of humans[1]. It has been observed in almost all age, racial and geographical groups[2]. *Demodex* feed on sebum and inhabit skin areas with active sebaceous excretion such as cheeks, forehead and nose[3] and has been implicated in several skin diseases, for instance, acne vulgaris, rosacea, basal cell carcinoma and pityriasis folliculorum[4,5].

In the eyelid, *Demodex folliculorum* can be found in the eyelash follicle and has been suggested as the etiologic agent of blepharitis. Indeed, several studies have demonstrated higher prevalence of *Demodex* on the eyelid of symptomatic patients with blepharitis compared to a control group[6,7]. However, since these mites are frequently found in healthy subjects, their pathogenicity remains controversial[8].

Demodex infestation was also associated with immunodeficiency and various reports have been described this organism in biopsy sample obtained from skin inflammatory conditions in immunosuppressed patients with HIV infection[9] or cancer[10]. In addition, some studies have found higher mite density on the skin surface of potential immunosuppressed subjects, such as hemodialysis[11] and diabetic patients[3].

The aim of the present study was to compare the prevalence of *Demodex folliculorum* on the eyelashes of patients with proliferative diabetic retinopathy and on a normal control group.

METHODS

This study was approved by the institutional research ethics committee and written informed consents were obtained from all participants. This research is in compliance with the tenets of the Declaration of Helsinki.

Type 2 diabetic patients in laser treatment for proliferative retinopathy and age- and gender-matched healthy voluntaries (group control) were invited to participate. Exclusion criteria included pregnancy, diagnosis of diabetes under five years, prior eyelid surgery, known cause of immunosuppression (e.g. HIV infection, hemodialysis), current treatment for blepharitis and concomitant ocular or systemic disease that could interfere with the results of the study.

All subjects underwent a slit lamp examination at a magnification of X25 where three eyelashes containing cylindrical dandruff (Figure 1) were removed from each lid by fine forceps (one eyelash from each third of the eyelid) and placed separately on a glass slide. One drop of fluorescein solution was added and covered with a coverslip. Subsequently, the presence of Demodex was analyzed in the samples under a light microscope at a magnification of X40 and X100 (Figure 2). The examination was always performed by the same ophthalmologist (AJC) immediately after the sampling. The mites were recognized based on its morphology and peculiar movement. The results were expressed in positive (with a least one mite on one lash) and negative (no mite identifiable) and the Chi-square test was used for comparing mites presence in both groups.

RESULTS

Forty-two patients were included in each group. The age ranged from 50 to 60 years old, with a mean of 56.4 ± 5.2 years. The male:female ratio was 0.6:1. There was no statistically significant difference with regard to age and gender in both groups (p>0.05).

Demodex folliculorum was significantly more prevalent in diabetic patients (27.4% of the total population studied) than in control patients (19.0% of the total population studied) as shown in the table 1
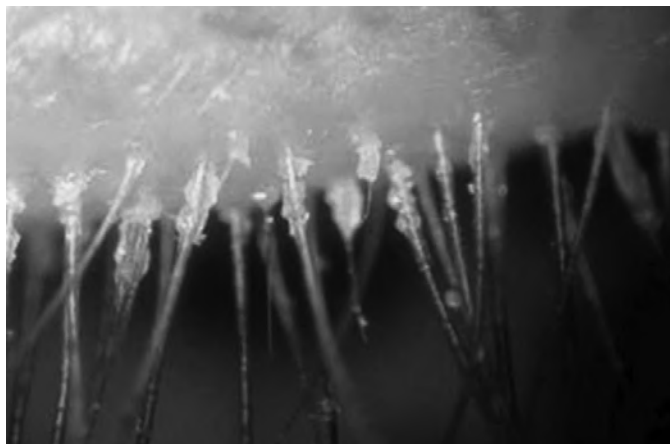

Figure 1. Eyelashes containing cylindrical dandruff under slit lamp examination.
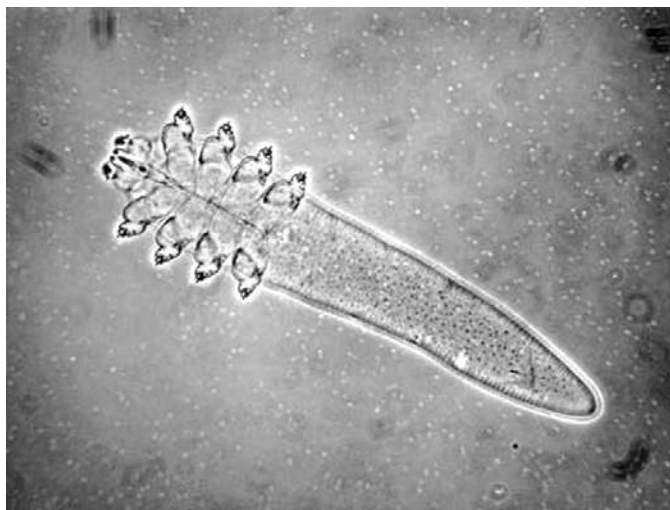

Figure 2. Demodex folliculorum under light microscope (X100 magnification).

(p=0.048). There was a tendency to find Demodex in aged patients. The mean age of positive and negative patients for Demodex were 58.2 ± 1.8 and 54.0 ± 2.8 years, respectively (p=0.09).

In the control group, Demodex was more prevalent in females, but this difference was not statistically significant (p=0.125). In the diabetic group, male gender emerged as protector risk factor (p=0.048).

DISCUSSION

Blepharitis is a commonly progressive chronic illness considered one of the most found ocular disorders in clinical practice. The physiopathology is not entirely known and it represents a therapeutic and diagnostic challenge[12]. Different factors are involved in the pathogenesis of chronic blepharitis, including alteration of the ocular microflora, reaction to exotoxins, allergic response to antigens, changes in the dynamics of the tear film and dysfunction of the meibomian gland[12].

Demodex mites have also been associated with blepharitis and several pathological mechanisms have been suggested. The mites can cause a direct damage in the epithelial cell at the lash follicle[1], induce a reactive hyperplasia and hyperkeratinization[13] or mechanically block of the orifices of meibomian glands[14]. Bacteria were found inside and on the surface of Demodex mites. Some of them, such as staphylococci, produce exotoxins that can directly contribute to unspecific irritative symptoms or induce a host immune reaction[15]. In addition, proteins of the mites and their debris may also elicit a host delay hypersensivity reaction[13].

The data about the prevalence of Demodex in diabetic patients are scarce. Akdeniz et al. found a significantly higher mean mite density and bigger mite mean size on cheeks biopsy of diabetic patients compared with a control group[3]. Clifford et al. analyzed the prevalence of Demodex on eyelashes of 256 subjects and also concluded that mites were more abundant in patients with diabetes[16].

Various reports of Demodex infestation in association with acquired immunodeficiency syndrome and cancer chemotherapy[9,10] and the higher prevalence of Demodex in potential immunosuppressed subpopulations, such as pregnant[17] and hemodialysis patients[11], have suggested that immunological deficiency may facilitate the overgrowth of the mites. Patients with diabetes have an increased risk for infections, but the exact mechanisms of the immunocompromised state are unclear.

Several abnormalities might contribute to the increased susceptibility and severity of infections in diabetic patients, including lower chemotactic activity of neutrophils[18], reduced function of mastocytes[19], poor leukocyte-endothelial cell interactions and decreased quantity of leukocytes in inflammatory lesions[20], low oxidants compounds generation, a reduction in lymph node retention capacity[21] and reduced release of cytokines, such as tumor necrosis factor alpha, interleukins and prostaglandins[22].

In the present study we demonstrated that patients with active proliferative retinopathy showed higher prevalence of Demodex eyelashes infestation. The retinopathy is a severe microvascular diabetic complication that attack specially patients with long-term di-

Table 1. Prevalence of Demodex sp. in diabetic patients and a healthy control group matched by age and gender

	Diabetic patients N (%)*	Control group N (%)*	Total N (%)*
Demodex positve	23 (27.4%)	16 (19.0%)	39 (46.4%)
Demodex negative	19 (22.6%)	26 (31.0%)	45 (53.6%)
Total	42 (50.0%)	42 (50.0%)	84 (100.0%)

*= percentage of the total population of the study, including patients with diabetes and healthy voluntaries. N=84.

sease and poor glycemic control and that are expected to be in greater risk of immunosuppression.

Increased sebum production has been correlated with *Demodex* density[23] and could be other speculative mechanism involved in diabetic patients. An experimental study showed cystic dilatations of hair follicles and altered lipid synthesis in the sebaceous glands of diabetic rats[24]. However this hypothesis is controversial, since others studies have demonstrated that patients and mice with diabetes tend to show a decreased sebaceous gland activity[25,26].

Obviously, for ethic and cosmetic conditions, a generalized epilation of the eyelid is not advised. A simple random epilation may constitute a sampling bias. To improve the chance to detect *Demodex*, the eyelashes with cylindrical dandruff were preferred and fluorescein dye was used to improve the microscopic evaluation as previously described[27].

Demodex infestation has a global distribution without race preference, but it is predominant in females and increases with advancing age[28]. Although the control group was composed by age- and gender-matched voluntaries, there was a tendency to find *Demodex* in aged patients and in the women. The blockage of the meibomian orifices by greasy eye makeup and hormonal alterations are possible factors involved in the higher prevalence in women.

CONCLUSION

Diabetes Mellitus showed to be a risk factor for *Demodex folicullorum* infestation of the eyelid, independently of gender and age. Further clarification of the role of *Demodex* in the physiopathology of blepharitis and the influence of metabolic disturbs are still required.

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Paper 5

Fluorescein Dye Improves Microscopic Evaluation and Counting of *Demodex* in Blepharitis with Cylindrical Dandruff

Fluorescein Dye Improves Microscopic Evaluation and Counting of *Demodex* in Blepharitis With Cylindrical Dandruff

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Purpose: To show whether fluorescein dye helps detect and count *Demodex* embedded in cylindrical dandruff (CD) of epilated eyelashes from patients with blepharitis.

Methods: Two eyelashes with CD were removed from each lid of 10 consecutive patients with blepharitis and subjected to microscopic examination with and without fluorescein solution to detect and count *Demodex* mites.

Results: Of 80 eyelashes examined, 36 (45%) lashes retained their CD after removal. Before addition of the fluorescein solution, the mean total *Demodex* count per patient was 14.9 ± 10 and the mean *Demodex* count per lash was 3.1 ± 2.5 and 0.8 ± 0.7 in epilated eyelashes with and without retained CD, respectively ($P < 0.0001$). After addition of the fluorescein solution, opaque and compact CD instantly expanded to reveal embedded mites in a yellowish and semitransparent background. As a result, the mean total *Demodex* count per patient was significantly increased to 20.2 ± 13.8 ($P = 0.003$), and the mean count per lash was significantly increased to 4.4 ± 2.8 and 1 ± 0.8 in eyelashes with and without retained CD ($P < 0.0001$ and $P = 0.007$), respectively. This new method yielded more mites in 8 of 10 patients and allowed mites to be detected in 3 lashes with retained CD and 1 lash without retained CD that had an initial count of zero.

Conclusions: Addition of fluorescein solution after mounting further increases the proficiency of detecting and counting mites embedded in CD of epilated eyelashes.

Key Words: blepharitis, cylindrical dandruff, *Demodex*, fluorescein

(*Cornea* 2007;26:697–700)

The *Demodex* (class Arachnid and order Acarina) is a microscopic, obligate, elongated mite that is the most common ectoparasite of humans.[1] This ectoparasite has an obvious head–neck part and a body–tail part, of which the former has 4 pairs of stumpy legs. Among a wide range of reported species, only *Demodex folliculorum* and *D. brevis* are found in the human skin. The adult *D. folliculorum* is 0.35–0.4 mm long and is commonly found in small hair follicles. *D. brevis* is 0.15–0.2 mm long and lives deep in the sebaceous glands. Both *Demodex* species often coexist in the same skin area and tend to gather in the face, cheeks, forehead, nose, and external ear tract.[2] In the eye, *D. folliculorum* is found in the lash follicle, whereas *D. brevis* burrows deep into the lash's sebaceous gland and the meibomian gland.[3]

In dermatology, *Demodex* has been implicated in pityriasis folliculorum, papulopustular rosacea, and granulomatous rosacea, and in some cases of isolated inflammatory papules, folliculitis, and hyperpigmentation.[1,4,5] Patients with papulopustular rosacea have been clearly shown to have a higher *Demodex* density than controls.[6–11] Although in ophthalmology *Demodex* has been considered as a cause of blepharitis, whether associated with rosacea or not,[2,3,12–16] the exact pathogenic potential of these mites in eye disorders remains unclear.

To resolve this question, it is important to detect and quantify the extent of this mite infestation in suffering patients. A previously published method relies on microscopic counting of mites in randomly epilated eyelashes mounted with a coverslip after addition of a drop of oil (such as peanut or olive oil).[2] For the following reasons, this method might not detect mites because *Demodex* is predominantly embedded in cylindrical dandruff (CD).[16] First, random epilation may result in a lower count because the chance of detecting *Demodex* is much higher by sampling the lashes with CD than those without. Second, addition of oil may result in undercounting because nonadherent *Demodex* can float away, especially in those lashes without retained CD. Third, if oil is not used, *Demodex* embedded in compact and opaque CD could not be counted unless 100% alcohol is added to stimulate them to migrate out.[16] Unfortunately, the latter maneuver is time consuming, ie, taking up to 20 minutes,[16] and can kill the mite,[17] precluding us from differentiating live from dead mites. Herein, we discovered that these 2 drawbacks could be overcome by adding an aqueous solution containing the fluorescein dye after mounting.

MATERIALS AND METHODS

This prospective study was conducted at the Ocular Surface Center (Miami, FL) in compliance with the tenets of

the Declaration of Helsinki. After we obtained written consent, 10 patients were included in this study. All these patients presented with blepharitis and CD and complained of ocular surface irritation for a prolonged period despite such conventional treatments as baby shampoo lid scrubbing and topical use of steroid, 0.05% cyclosporine and various artificial tears and lubricating ointments. On completion of history taking, eye examination, and external photography, lashes containing CD, defined as scales formed as distinct cuffs collaring the lash root (Fig. 1A), were sampled as described before.[16] In brief, under a slit-lamp biomicroscope at a magnification of ×25, 2 such lashes were removed from each lid by fine forceps and placed separately on each end of a glass slide. Thus, a total of 8 lashes were prepared on 4 slides, each mounted with a coverslip without adding any solution. The counting of *Demodex* by light microscope was performed by the same person (A.K.) within 1 hour after sampling at magnifications of ×100 and ×400. Afterward, the same slide was added with a fluorescein solution, made by wetting a fluorescein strip (FUL-GLO; Akron, Buffalo Grove, IL) with

1 drop of 0.9% NaCl solution to the edge of the coverslip until the lash was immersed. The microscopic counting was repeated by the same person. We also recorded the presence or absence of CD, the species of *Demodex* (*folliculorum* or *brevis*), and the life stage of mites (adult, larva, or egg). Photographs were also taken to compare these 2 methods, ie, with or without fluorescein solution.

All statistical analyses were performed with SPSS 12 (SPSS Inc., Chicago, IL). The unpaired Student t test was used for comparing the *Demodex* count between lashes with and without CD and the paired t test for comparing the *Demodex* count before and after adding the fluorescein solution. $P < 0.05$ was considered to be statistically significant.

RESULTS

Ten patients (6 men and 4 women) with an average age of 63.3 ± 13.4 years (range, 44–82 years) were included in this study. Of the total number of 80 epilated eyelashes, 36 (45%) lashes retained CD, whereas the rest did not. Before

FIGURE 1. Addition of fluorescein solution to enhance visualization of *Demodex* in lashes with retained CD. An example of CD that is found in this eye with blepharitis (A). Microscopic examination of an epilated eyelash without addition of fluorescein solution shows compact and opaque CD and 1 mite free from CD (B, marked by a star). After addition of fluorescein solution, CD instantly expands with liberation of air bubbles (C, arrows) to become more transparent and results in the detection of 3 mites protruding from CD under low magnification (C, marked by stars). However, in the inset marked by a box (C), 6 more mites are easily detected in the yellowish background provided by the fluorescein dye under higher magnification (×400) (D). Before addition of fluorescein solution, mites are not found in another eyelash with compact and opaque CD (E). After addition of fluorescein solution, 3 mites are detected embedded in the CD (F, heads are marked by stars).
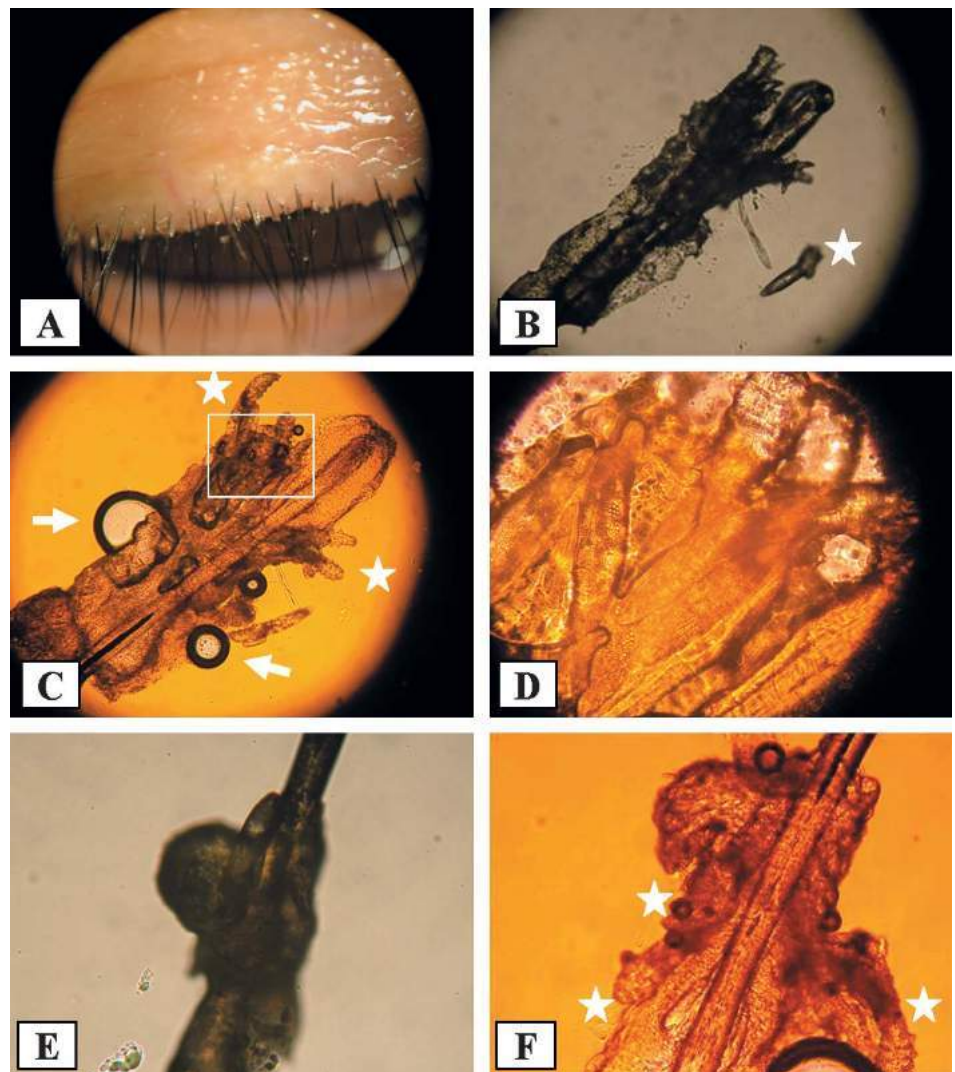
TABLE 1. *Demodex* Count and Species in Lashes With or Without Retained CD Before and After Addition Of Fluorescein Solution

Variable	Demodex Total Count	Demodex sp.		Adult	Larva	Egg
		folliculorum	*brevis*			
Before adding fluorescein						
With retained CD	112	112	0	108	3	1
Without retained CD	37	34	3	36	1	0
Total	149	146	3	144	4	1
After adding fluorescein						
With retained CD	158	158	0	148	7	3
Without retained CD	44	41	3	41	2	1
Total	202	199	3	189	9	4

addition of the fluorescein solution, the mean total *Demodex* count per patient was 14.9 ± 10 and the mean count per lash was 3.1 ± 2.5 and 0.8 ± 0.7 in eyelashes with and without retained CD, respectively. The difference between them was statistically significant ($P < 0.0001$; Table 1).

After addition of the fluorescein solution, the compact and opaque CD was instantly dissolved and expanded (cf. Figs. 1B, C), liberating several air bubbles (Fig. 1C, arrows). As a result, this gave rise to a semitransparent appearance, allowing better visualization of fine structures within and behind the CD and easy detection of mites (Figs. 1C, D, F). Even for lashes without retained CD, this method also helped detect more *Demodex* by eliminating any adherent debris. For example, in the bulb region where CD was not apparent,

addition of the fluorescein solution rendered a more transparent background (Fig. 2). Because of a marked contrast by the yellowish coloration of fluorescein, details of the mite body embedded in CD could be better detected (Fig. 1), and hidden egg and mites could also be revealed (Fig. 2, asterisks).

After addition of the fluorescein solution, the mean total *Demodex* count per patient was 20.2 ± 13.8, which was significantly higher than before adding the fluorescein solution ($P = 0.003$). The mean total count per lash was 4.4 ± 2.8 and 1.0 ± 0.8 in lashes with and without retained CD, respectively (Table 1). These values were significantly higher than those before addition of the fluorescein solution ($P < 0.0001$ and $P = 0.007$, respectively). Eight of 10 patients (80%) showed an average increased count of 6.6 ± 3.3 mites per patient (range, 2–11 mites), and mites were found in 3 lashes with retained CD and 1 lash without retained CD that had an initial count of zero before addition of the fluorescein solution.

After addition of the fluorescein solution, the adult form was found in 93.7% and 93.2% of eyelashes with and without retained CD, respectively. *D. folliculorum* was found in 9 patients, whereas *D. brevis* was found in 1 patient. Intriguingly, we did not find *D. folliculorum* in the patient with *D. brevis*, which was found singly around the lash root or shaft without retained CD.

DISCUSSION

This study showed that addition of the fluorescein solution induced instant dissolution and expansion of CD, which together with a yellowish contrast helped detect *Demodex* embedded in otherwise compact and opaque CD of epilated lashes (Fig. 1). As a result, this unique property

FIGURE 2. Addition of fluorescein solution to enhance visualization of *Demodex* in eyelashes without retained CD. In these 2 epilated eyelashes without retained CD, no mites are detected (A and C, respectively). After addition of fluorescein solution, in 1 lash, the matrix around the eyelash bulb instantly expands with liberation of an air bubble (B, marked by an arrow), allowing us to detect an egg (B, marked by an asterisk). In the other eyelash, although the change of bulb matrix is not as dramatic, 2 mites are readily detected (D, marked by a star at heads).
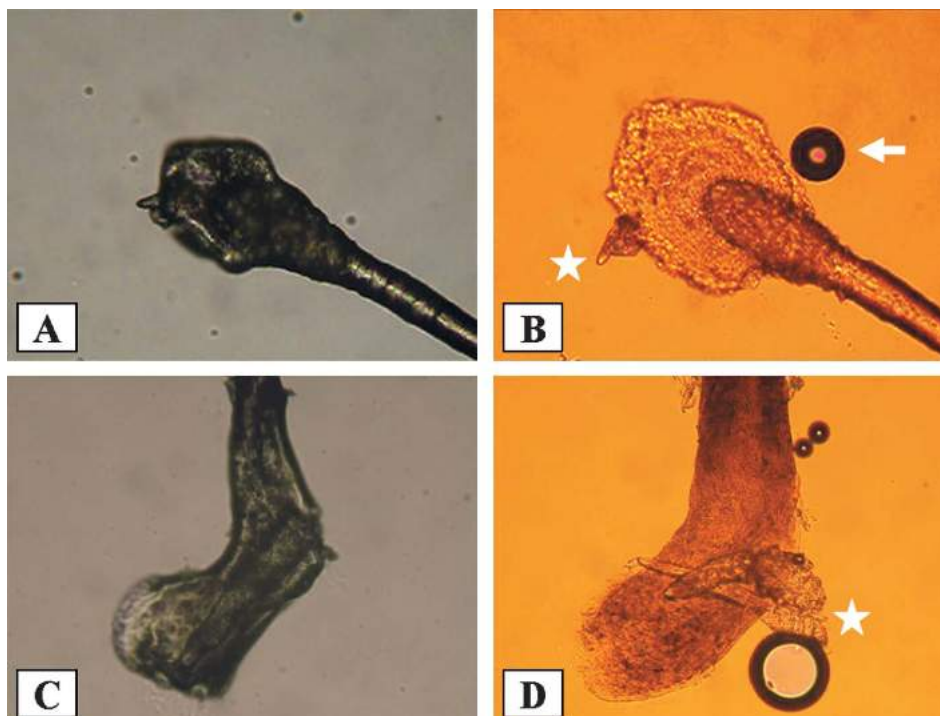
enabled us to obtain a significantly higher count (Table 1). In general, the *Demodex* count per lash in those with retained CD was significantly higher than in those without (Table 1). This finding was consistent with what we have recently reported,[16] further supporting the notion that mites tend to be embedded in CD and that the clinical sign of CD in lashes may be considered pathognomonic for *Demodex* infestation.[16] Furthermore, adult *D. folliculorum* compromised the overwhelming majority of mites detected; *D. brevis* was found in only 1 of these 10 patients. Addition of the fluorescein solution resulted in significantly higher counts per patient and per lash (Table 1). Importantly, in 3 lashes with retained CD and 1 lash without retained CD that before addition of fluorescein solution had not been thought to have mites, *Demodex* was detected after using this method.

The CD is thought to be composed of keratins and lipids.[18] For reasons still not clear to us, the water component of the fluorescein solution was sufficient to lead to rapid swelling of CD, presumably after being absorbed to CD to displace air trapped within. As a result, it liberated air bubbles as shown in Figure 1, for which we used the fluorescein solution. Therefore, adding the saline solution not only results in preservation of the *Demodex* that had a loose contact with the lash at the tip,[16] but also it did help render an otherwise opaque and compact CD with a semitransparent appearance for lashes with retained CD (Fig. 1). This property, coupled with a yellowish background provided by the fluorescein dye, allowed better visualization of the body details of mites embedded in CD (Fig. 1). Taking these findings together, we propose to add the fluorescein solution to improve the proficiency in detecting and counting *Demodex* embedded in CD.

Although our study showed that adding fluorescein solution increased significantly the yield of *Demodex* count in the eyelashes, we did not compare our results to those with addition of saline solution alone or by using an oil drop. When using oil for counting mites, the entire surface of the coverslip must be examined for freely floating mites. However, it is also associated with easier detection of the mites than without any solution by making the cellular debris and mites more transparent. Further studies are required to compare the results of *Demodex* count using fluorescein solution to those with the oil to identify a diagnostic method with the highest yield for mite detection in eyelashes of patients with blepharitis.

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Paper 6

Lichen Planopilaris: Histopathological Study of Vertical Sections of Scalp Biopsies in 44 Patients

Lichen Planopilaris: Histopathological Study of Vertical Sections of Scalp Biopsies in 44 Patients

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Dear Editor,

LPP is one of the main causes of primary cicatricial alopecias. This study was performed for review of histopathological characteristics of LPP, and for the first time the density of hair follicles in vertical scalp biopsies was compared with healthy scalp biopsies. Vertically sectioned scalp 5mm punch biopsies of 44 cases of LPP were examined(H&E and Alcian blue) according to NAHRS criteria (1). Also we reviewed 22 age and sex matched scalp biopsies of autopsies for obtaining criteria for normal follicle number. We found normal values of hair follicles (15.24 ± 3.06), sebaceous glands (9.62 ± 2.29) and arrector pili muscles number (9.05 ± 2.55) in a 5 mm punch biopsy. Based on normal ranges, intensity of reduction in terminal hair was as follows: mild (9-12), moderate (5-8) and marked (1-4 follicles).

Characteristic findings of LPP were: markedly reduced hair density (63.6 %), absence of vellus hair (59.1 %), and follicular lichenoid changes (61.40 %). We found mucinous fibroplasias (50.0 %) and presence of interfollicular mucin (2.3 %). The only significant epidermal change was spongiosis (40.9 %). The most prominent pattern of follicular involvement was lichenoid (58.69%).Other changes included mild to moderate lymphocytic, primarily perifollicular (77.3 %) and perivascular (97.7 %) inflammation, Periinfundibular hypergranolosis (77.3 %), foreign body granuloma (13.6 %),demodex(25.0 %) , max-Josef cleft(38.6 %),epidermal(65.90 %) and follicular civatte bodies(45.45%). Vertical sections are useful in LPP

in which the findings are focally confined to dermo-epidermal junction (DEJ) and superficial dermis (2).

Common findings in LPP are as follows: lichenoid lymphocyte infiltration in follicular DEJ (3-5), wedge shaped hypergranolosis (3, 5), Colloid bodies (5), loss of sebaceous glands and destruction of hair follicle root sheaths (3, 6, 7) and follicular plugging (5). In late lesions, lamellar perifollicular fibrosis is seen around isthmus, and finally the follicles are completely substituted with fibrous tracts (3, 5). Lichenoid infiltrate disappears (5). Clefts may be seen between follicular epithelium and the dermis around it (5). In our study, decrease or lack of terminal hair was seen in 93.1% and vellus hair in 59.1%, arrector pili decrease in 36%, its lack in 9.1%, reduction in sebaceous glands in 36% and its lack in 52%. In Tandon study, these findings were in 100%, 96%, 59%, 19%, 30% and 70% respectively (4).

In LPP, lichenoid changes are seen more than vacuolar degeneration (8). In our study, follicular epithelium changes were lichenoid in 59.13%, spongiotic in 18.18% and vacuolar in 2.27%. In Tandon study, the most common follicular involvement was lichenoid (22%) and spongiotic (15%) (4). On the contrary, in interfollicular epidermis in our samples, vacuolar changes (31.81%) were higher than lichenoid(18.81%) while Tandon has found epidermal involvement to be lichenoid in 7% and vacuolar in 4% (4). Parakeratosis was seen in 13.6%, hyperkeratosis in 68.18% and follicular plugging in 72.7%. In Mehrgan's study follicular plugging has been mentioned as an auxiliary finding in LPP (in 59%) and parakeratosis was not common.[9] In Tendon study, prevalence of parakeratosis, hyperkeratosis and follicular plugging was 15%, 4% and 11%. It may be due to more advanced disease in his study (4).

Epidermal and follicular cytoid bodies were seen in 66% and 45% of our patients. Mehrgan has reported the

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cytoid body prevalence to be 53% (9). In LPP, inflammation is mainly lymphocytic, and in early stages, infindibulum and isthmus of hair are afflicted (10). Inflammation intensity in our patients was mild in 45.5 %, moderate in 38.4 % and severe in 15.9 % and was perifollicular in 77.3 % and perivascular in 97.7 % of cases. In Tandon study, inflammation intensity and location was almost similar to ours.[4] Unlike DLE, mucin is not seen in interfollicular dermis in LPP (3, 10), but there was interfollicular mucin in one of our patients .There may be interfollicular mucin in LPP especially in case of overlap with DLE. Mucinous fibroplasias and perifollicular lamellar fibrosis was seen in 50% and 15.9% of our patients. These changes were seen in 37% and 11% of cases in Tandon study (4). In this study, for the first time a criterion was presented for intensity of alopecia in vertical sections. We recommend a prospective study on cases of LPP with DIF microscopy and mucin stainig.

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Paper 7

Is *Demodex folliculorum* an aetiological factor in seborrhoeic dermatitis?

Is *Demodex folliculorum* an aetiological factor in seborrhoeic dermatitis?

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Summary

Background. Seborrhoeic dermatitis (SD) is a common inflammatory skin disease for which no single cause has been found, although many factors have been implicated. The mite *Demodex folliculorum* (DF) is most commonly seen in the pilosebaceous unit in humans. SD is located in areas that are rich in sebaceous glands, which are also preferred by DF.

Aims. To compare the number of DF parasites in patients with clinical SD and in healthy controls, and to investigate any possible relationship between the number of DF mites and the presence of SD.

Methods. The study comprised 38 patients with SD and 38 healthy controls. Standard random and lesion-specific sampling was performed in the group of patients with SD, whereas standard random sampling only was performed for controls.

Results. *Demodex folliculorum* sampling was positive in 19 patients (50%) and 5 controls (13.1%). Mean DF density was $8.16 \pm 10.1/cm^2$ (range 0–40) and $1.03 \pm 2.17/cm^2$ (1–7) in patient and control groups, respectively. The differences between groups for DF positivity and mean DF density were significant ($P = 0.001$ for each). DF was found in 13 lesional areas in the patient group, but in only 5 areas in the control group ($P = 0.031$).

Conclusions. The number of DF mites was significantly higher in both lesional and nonlesional areas of patients with SD. This suggests that, when other aetiological causes are excluded, DF may have either direct or indirect role in the aetiology of SD.

Introduction

The *Demodex* mite is an asymptomatic, saprophytic ectoparasite that resides in hair follicles and sebaceous glands.[1,2] Only two types of *Demodex* have been identified in humans: *Demodex folliculorum* (DF) and *Demodex brevis* (DB).[1,3] Mites that spend their life cycles in pilosebaceous follicles use sebum and follicular cells as food.[1,4] DF, which is more common than DB, is generally localized to the infundibular area of the hair follicles, whereas DB is localized to sebaceous glands and ducts, which are deeper.[1,5] Both types of follicular mite are often seen on the face (the nasolabial fold, nose, cheeks, forehead, and eyelids) and rarely on the chest and scalp.[1,2,5] DF is the most common ectoparasite in humans.[6] The density of DF on healthy skin is normally $< 5/cm^2$.[7] DF is transmitted to newborns a few days after birth through breastfeeding or close physical contact;[1,8] however, DF density remains low through childhood, owing to low sebum production.[1] Its prevalence increases with age,[2,3] and may reach 100% in elderly adults.[3] It is believed that the increase in the number of DF or its penetration into the dermis causes infestation.[9] The classic clinical forms of DF infestation include pityriasis folliculorum, rosacea-like demodicidosis and demodicidosis gravis.[1,7] In addition, many other clinical forms of DF infestation have been reported in the

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literature, including pustular folliculitis, papulopustular scalp eruption, perioral granulomatous dermatitis, blepharitis, solitary granuloma, papular demodicidosis of the face, follicular spinulosus of the face, seborrhoeic dermatitis (SD)-like lesions, nonspecific facial pruritus with or without erythema, acneiform lesions, and *Demodex* granuloma.[1,5–8,10]

SD is a chronic and superficial inflammatory dermatosis of the skin. It is characterized by erythematous, thin, oily yellow squamae on the scalp, face, chest, back and flexural areas, which are rich in sebaceous glands.[11,12] It affects 1–3% of the population. Although many endogenous and exogenous factors including increased sebum activity, *Pityrosporum ovale* infection, drugs, immunological abnormalities, genetic predisposition, neurological disorders, emotional stress, diet, lifestyle and environmental factors have been implicated, the precise aetiology of SD is not known.[3–17]

SD is most commonly found on the scalp, nasolabial folds, ears, eyebrow and chest, where sebaceous glands abound. DF is also usually seen in follicles of the cheek, nose, forehead, chin, nasolabial fold and eyelid, where sebum is produced in great amounts. In our previous study,[7] we examined the clinical importance of DF in patients with nonspecific facial signs and symptoms, and found that, in addition to the well-known clinical conditions caused by this mite, DF could also cause SD-like erythematous, squamous pityriasiform lesions, suggesting that it may have a role in the aetiology of SD. Thus, this study examined the number and density of DF in lesional and nonlesional areas of patients who presented with SD and compared the results with healthy controls.

Methods

The ethics committee of Inonu University Faculty of Medicine approved the study, and written informed consent was obtained from all patients and controls.

The study comprised patients, either previously or newly diagnosed, presenting with SD to the Dermatology Clinic, İnönü University between February and June 2006. SD was diagnosed clinically. Patients who had pink, yellowish-brown, erythematous patch or plaque lesions covered with thin, oily and yellow squamae localized to the scalp, hairline, eyebrow, eyelashes, glabella, nasolabial fold, ears, external ear canal or breast cleavage were accepted as having classic SD. In total, there were 38 patients [8 women (21.1%), 30 men (78.9%); mean age 36.71 ± 13.20 years, range 16–73]. SD was localized to the scalp in 37 patients (97.3%), nasolabial fold in 34 (89.4%), eyebrow in 24

(63.1%), retroauricular area in 20 (52.6%), chest in 19 (50%) and eyelashes in 7 (18.4%). The number of SD lesional areas was 2 in 4 patients, 3 in 14 patients, 4 in 11 patients and 5 in 9 patients. The most common areas were the scalp and the nasolabial fold. The control group comprised 38 healthy people [11 (28.9%) women, 27 men (71.1%); mean age 55 ± 14.65 years, range 20–67], either medical students or hospital staff, who were matched for age and gender, did not have any disease, and were not receiving any systemic or topical treatment. Exclusion criteria were intertriginous involvement, age < 16 years, pregnancy or lactation, systemic corticosteroid or immunosuppressive treatment, radiotherapy or chemotherapy or topical acaricidal usage during the study period, and use of topical corticosteroids in the previous month.

Demodex folliculorum density was calculated as the number of mites per square centimetre of skin, with $\geq 5/\text{cm}^2$ area considered infestation. DF density was examined in both lesional areas (scalp, eyebrow, eyelash, retroauricular area, nasolabial folds and chest) and standard random areas (forehead, cheek, nose, chin and chest) in the patient group. Only standard random sampling was done in controls. DF was detected using a noninvasive method, standardized skin surface biopsy (SSSB). For SSSB, one side of a microscope slide is coated with a cyanoacrylate adhesive and the adhesive side pressed onto the lesion for 1 min, then peeled off. This procedure lifts off the top of pilosebaceous units, the surface keratin layer and their contents. In hairy areas such as the eyelashes, eyebrow and scalp, three hairs were removed, mounted on a slide and covered with glycerine, and examined for DF under light microscopy ($\times 40$ and $\times 100$ magnification), with a single mite being considered infestation.[9,18] Under microscopy, the mites, which were 0.3–0.4 mm long, had four pairs of short and long legs on the front part of the body.[3,5]

Statistical analysis

Results were compared with the control group. The independent samples *t*-test and Pearson's coefficient analysis were used.

Results

Demodex folliculorum was found in 19 patients (50%) and in 5 controls (13.1%). Mean DF density (evaluating lesional and standard random areas together) was $8.16 \pm 10.10/\text{cm}^2$ (range 0–40) in the patient group and $1.03 \pm 2.17/\text{cm}^2$ (1–7) in the control group.

Table 1 *Demodex follicurum* (DF) counts in patients with seborrhoeic dermatitis and controls.

	DF > 5/cm², n (%)	DF density per cm², mean ± SD (range)
Patients		
Both SD lesions and standard areas	19* (50)	8.16 ± 10.1/cm² (0–40)*
Only SD lesions	13† (34.2)	
Controls	5 (13.1)	1.03 ± 2.17/cm² (1–7)

*P = 0.001; †P = 0.031 (independent samples *t*-test).

Table 2 Frequency of *Demodex follicurum* (DF) (> 5/cm²) in seborrhoeic dermatitis lesional areas.

Location	No. of DF-positive* lesional areas/total no. of lesional areas (%)
Scalp	5/37 (13.5)
Nasolabial fold	12/34 (35.2)
Eyebrow	2/24 (8.3)
Retroauricular	3/20 (15)
Chest	0/19 (0)
Eyelash	2/7 (28.5)

*> 5/cm².

Table 3 Frequency of *Demodex follicurum* (> 5/cm²) in standard random areas of the face and chest in patients with seborrhoeic dermatitis.

Location	No. of patients (%)
Cheek	17 (44.7)
Forehead	15 (39.5)
Nose	9 (23.7)
Chin	7 (18.4)
Chest	0 (0)

The number of DF-positive patients and the mean DF density were significantly higher in the patient group than in the controls in both lesional and nonlesional areas (P = 0.001 for both). When only lesional areas were evaluated in the patient group, DF was positive in 13 (34.2%) patients, and the difference between the patient and control group was again significant (P = 0.031). The number and density of DF in the patient and control groups are presented in Table 1. The number of lesions positive for DF was 5 (13.5%) on the scalp, 12 (31.6%) on the nasolabial folds, 2 (5.3%) on the eyebrow, 2 (5.3%) on the eyelashes, 3 (7.9%) on the retroauricular area and 0 on the chest (Table 2). Using standard random sampling of patients, DF was positive in 17 (44.7%) areas on the cheek, 15 (39.5%) on the forehead, 8 (23.7) on the nose and 7 (18.4) on the chin; no area on the chest was positive (Table 3).

Discussion

SD is a well-known condition with variable severity and unclear aetiology. The variety of proposed causes support the notion that the condition is more complex than an 'oily inflammation of the skin'.[14] SD seen in sebaceous gland-rich areas has been attributed to the increased activity of these glands. Activation of sebaceous glands in puberty explains why SD is common in adolescents and young adults. In addition, the androgen-associated hormonal factors affecting pilosebaceous units explains why the disease is more common in male patients.[14,15] However, SD does not develop in all young adults who have a greasy skin, and the sebum secretion rate of patients with SD can be within the normal range. Therefore, it is believed that rather than being a primary aetiological factor, seborrhoea is a predisposing factor for SD and that SD is not a disease of the sebaceous glands.[15]

The proposal that SD is a superficial fungal disease of the skin developing in sebaceous gland-rich areas has risen from the relationship between Malassezia yeasts and SD.[16,19] *Pityrosporum ovale* is a lipophilic yeast of the Malassezia genus. These yeasts, which are members of the natural flora of the skin, are found in seborrhoeic areas of the body.[13] Owing to their lipase activity, they break down triglycerides into irritant fatty acids that can form desquamation and bring about SD lesions.[20] The number of these yeasts is raised in SD and can be cultured from the lesions.[15] Mirza *et al.*[21] showed that *Pityrosporum* yeasts were higher both in native preparations and in the culture of patients with SD relative to normal individuals and thus, colonization rate increased in SD. Antifungals are effective in the treatment of SD by reducing the number of yeasts, further supporting the involvement of *Pityrosporum ovale* in the aetiology.[13,14,17] Although a correlation between SD severity and yeast density was reported, it was also reported that the number of Malassezia yeasts in patients with SD was not higher than that in controls and that the response to antifungals resulted from the anti-inflammatory effects of the drugs.[15] Furthermore, it was suggested that SD is associated with an abnormal response of the host to the yeasts, but the antibody level was not found to be higher than controls.[14–16] However, it was also suggested that the inflammation was started by reactivation of an immune reaction to antigens produced by *Pityrosporum ovale* or their toxic products and the secretion of some cytokines from the keratinocytes.[14,15]

DF, which is a saprophytic mite of human pilosebaceous units, can be found anywhere on the skin, but primarily on the forehead, cheek, nose, nasolabial fold and eyelid, where sebum production is profuse.[22] It has also been found on the scalp, neck, chest, nipple, penis, mons veneris, hip and buccal mucosa, where ectopic sebaceous glands abound.[3,23] Its presence in healthy individuals suggests the possibility of transmission through contact. Examination of skin biopsies can reveal DF at rates as high as 20–30%. It was established in one study that 10% of 1124 skin biopsies and 12% of 1692 follicles contained follicular mites.[6,22]

The cause of the clinical features in DF infestation is still not known. The hypotheses include immunological deficiency or abnormal immunological reaction of the skin to the parasite.[22] Various explanations have also been put forward for the pathogenic mechanisms: (i) the obstruction of sebaceous canals and follicles by the mite can lead to epithelial hyperplasia, reactive hyperkeratinization and blockage of secretion in addition to increase in bacteria colonization; (ii) there may be a foreign-body reaction to the chitinous skeletons of the mites; or (iii) mites and their discharge products can stimulate humoral and cellular immune reactions and set off inflammation.[1] Georgala *et al.*[24] support the hypothesis that *Demodex* infestation is a type 4 delayed hypersensitivity reaction to an unknown antigen of mite or follicular origin. According to Akilov and Mumcuoglu,[4] as mites cannot penetrate into the basal membrane, they do not encounter the immune system of the skin and therefore the disease develops only in genetically predisposed individuals, hence the reason that the incidence of the disease is higher in patients who have human leucocyte antigen (HLA)-Cw2 and HLA-Cw4 alleles. When planning this study, we did not believe that HLA testing would be feasible without proving the relationship between SD and DF, but our results now suggest that HLA testing may be a useful technique for further study.

Two clinical forms of *Demodex* infestation in humans were first defined in 1930 by Ayres. Pityriasis folliculorum particularly affects women of middle age or older. It is characterized by diffuse, but dull facial erythema, itching and a burning sensation, thin follicular plugs, and squamae that look like sandpaper.[10] Rosacea-like demodicidosis (RLD) clinically resembles rosacea. It is characterized by erythematous and squamous papulopustules on the cheek, perioral area and back of the nose.[22] Lesions are superficial and there is a tendency toward minor papulovesicular and vesiculopustular formation. Additionally, RLD starts abruptly and progresses rapidly. There is no previous flushing, persistent erythema, photosensitivity, sebostatic skin type, tingling or burning sensation, or telangiectasia.[7,10] Demodicidosis gravis, on the other hand, resembles severe granulomatous rosacea. It involves dermal granulomata, central caseation necrosis and mite discharges phagocyosed by foreign body giant cells.[1] A multitude of clinical variants of DF, such as papulopustular scalp eruption, perioral granulomatous dermatitis, blepharitis, solitary granuloma, papular demodicidosis of the face, follicular spinulosus of the face, SD-like lesions, nonspecific facial pruritus with or without erythema, acneiform lesions, *Demodex* granuloma and dermatitis rosaceiformis steroidica have been reported.[1,5–8,10]

In our study, the lesional and nonlesional areas in patients had DF counts and density that were significantly higher than controls. When only lesional areas were evaluated in patients with SD, the number of DF-positive areas was still significantly higher. Thus, it is likely that the explanations for how DF causes SD are similar to those put forward for Malassezia.[9] Reactivation of the immune system by antigens derived from DF or its toxic products can stimulate inflammation, and secretion of cytokines from keratinocytes may induce or aggravate SD. It is possible, however, that SD may be the predisposing factor to DF infestation, instead of the result of such infestation, although there is no support for this possibility in the literature. A possible explanation for the high DF numbers in non-SD areas in patients may be local parasite migration or contact transmission (i.e., by itching).

In conclusion, detection of pathogenic numbers of DF in SD-like pityriasiform lesions of patients presenting with atypical facial signs and symptoms, as described in our previous study, may indicate that DF can have many clinical presentations. The significantly higher numbers of DF in lesional and nonlesional areas of patients with SD compared with controls in the current study supports this idea. Although various theories exist as to the aetiology of SD, its precise aetiology and its relationship with other skin diseases is not yet clear. However, given the results of our study, we believe that DF can play a direct or indirect role in the aetiology of SD in patients in whom other causes cannot be identified. Further studies into the possible role of DF in SD and into the positive results obtained in response to acaricidal treatments in DF-positive patients with SD are needed.
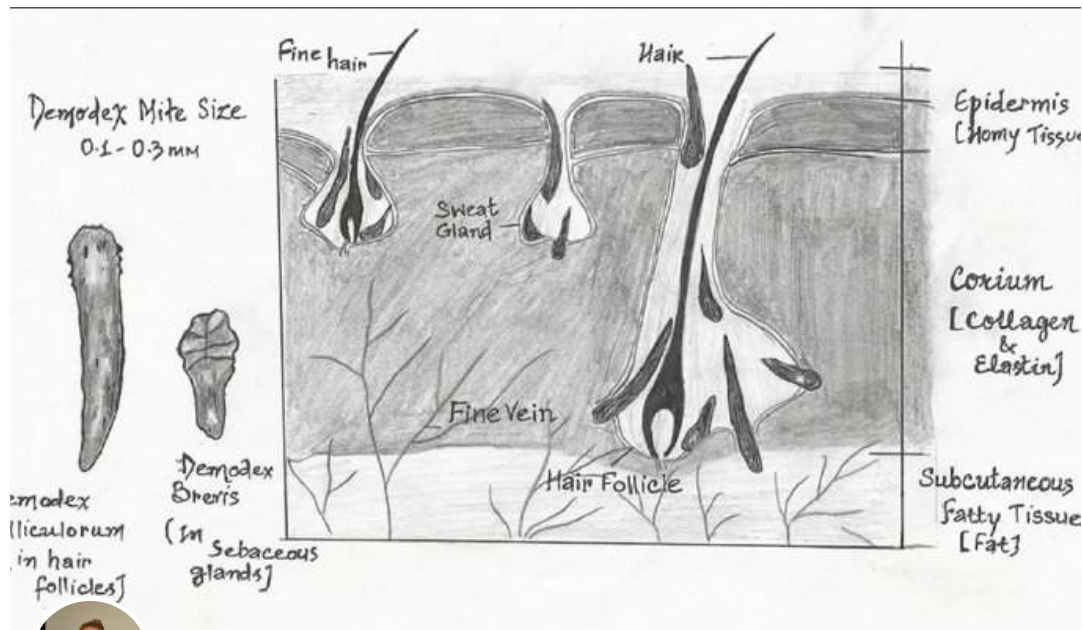
Acknowledgement

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Paper 8

Demodex Mites and the Potential Limitation Facing Patients Post Treatment or Surgery if not Addressed

Fine hair

Hair

Epidermis [Horny Tissue]

Demodex Mite Size 0.1 - 0.3 mm

Sweat Gland

Corium [Collagen & Elastin]

Fine Vein

Hair Follicle

Demodex Brevis (In Sebaceous glands)

Subcutaneous Fatty Tissue [Fat]

emodex lliculorum in hair follicles]

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Following

DEMODEX MITES & THE POTENTIAL LIMITATIONS FACING PATIENTS POST TREATMENT OR SURGERY IF NOT ADDRESSED.

Oct 19, 2015

__What are Demodex (folliculorum) Mites?__ and are they good or bad?

I wish I had stressed this more often over the years with colleagues, peers and mentors alike on the importance of ensuring each patient is free of Demodex mite infestation on the scalp skin, in hair tissue and deep in oil glands pre and post treatment or surgery.

Surgery, Dermatology, Para medical practices, so many other crafts treating the skin, scalp & hair could advance if we are to indentify a patient pre-treatment or surgery of the degree they are host to mites either on the scalp , facial skin or nestled in hair cuticles .

As clinicians, practitioners and staff we should be free of Demodex mites in health

and practice.

Upon a bit of research I am confident you will see the importance of this adventure into understanding scalp and face mites, why I stress in especially treating patients with high counts of <u>Demodex, their follow up treatments and prevention.</u>

I see why Demodex are over looked and misunderstood, for those of us who know what they and those of the other 95% in healing skin, scalp or hair really have no idea what a Demodex mite is or what it is capable of doing to skin and hair along with other potential health risks for that matter.

Because we are all a host to these microscopic cheeky mites that vary in characteristics. They are all part of the same family though, each will find a liking to reside on one part of the body over another and one creature over another with some really amazing forms and shapes. eyes, mouths. rectum and genitals. Pretty amazing.

Research now leads to us to conclude as does heavily supported data in clinical observations these mites certainly contribute to hair loss and scalp disorders by feeding and surviving on the nutrients that our skin and hair needs to function balanced. There appears to be no good reason they exist thus far and can be host to some nanobacteria and nanobes but highly controversal point to ad here.

A hair and scalp clinic doing therapy to regrow or check and halt hair loss for sure this should be one the first stops of a clients service and support cycle .We also do a hair mineral test, which is the latest tool in patient nutritional pathology ensuring the best possible nutritional support along with the best scalp care regime, This I will get into in the next few articles sharing some of the layers to the multi-therapeutic approach we take in treating hair loss and managing regrowth.

I am confident at this stage there is not a healthy balance of mites?, I highly doubt

the existence of one is a good thing. More needs to be done to support me. At this juncture for most to embrace treating patients for skin and scalp mites I doubt will catch on fast but I sense more suitable sources of research from peers will manifest to support my thoughts and expressions as the research is published and shared.

Our observations show Demodex mites play a particular role to poor retention for the newly transplanted grafts in patients so much so that I would highly recommend that as a first step in a patients assessment. Reduction of swelling of mass cells under the scalp is a big step in checking hair loss and recovery, for us retention of the new growth.depends on keeping the scalp perfect ph and alkaline. Free from toxins mites and build up are amazing components in assisted recovery

A good thought for all of us, it is not too late if your patients are post surgery. patients come and gone can now revisit you to get a check up, remove the mites if it warrants by the host having too high a concentration of mites on either scalp, the face and in hair tissue and should most certainly be treated before any real scalp and hair treatment regime or protocol is started or reintroduced back into therapy or treatments.

Mites life Cycle
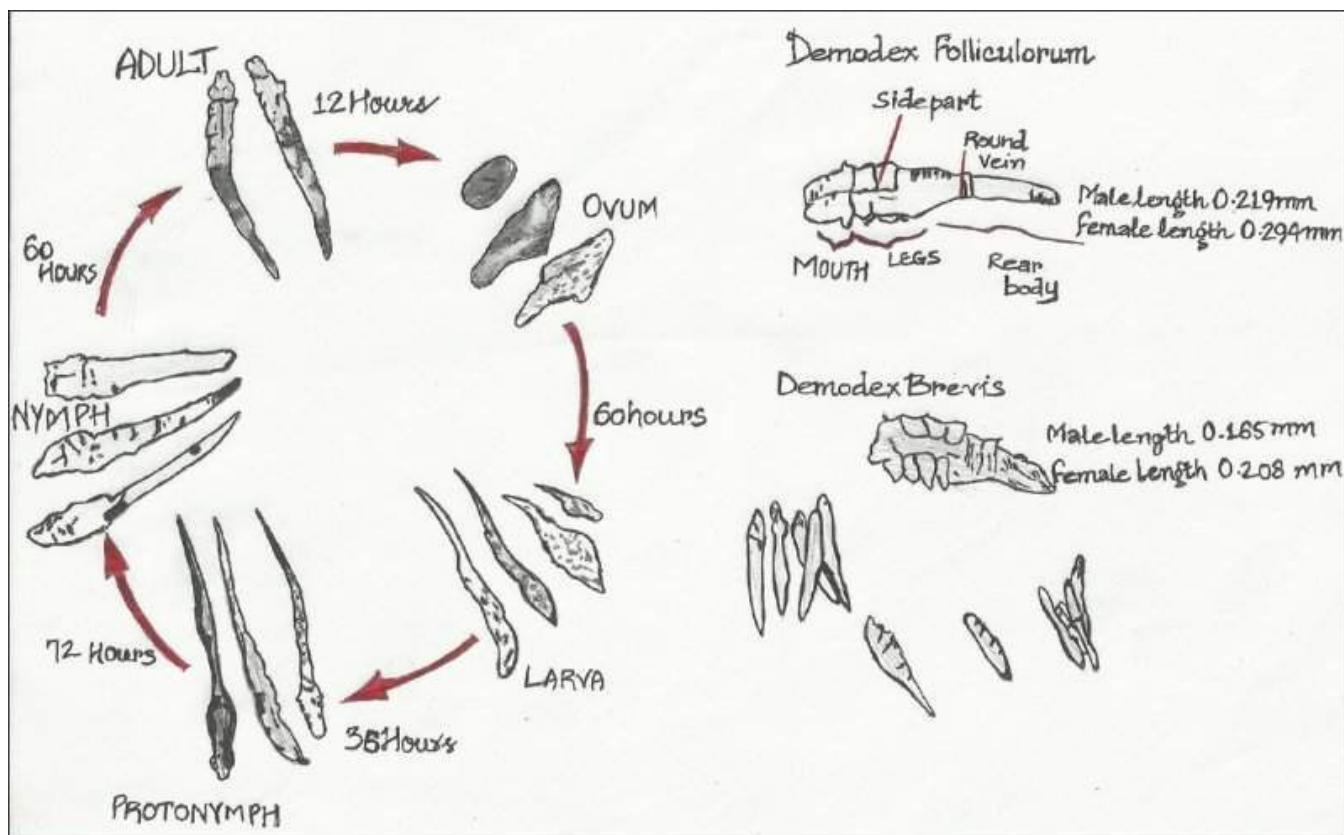
Are invisible to the naked eye, usually measuring between 100 – 300 microns in length. There are numerous different signs of Demodex activity. One of the most obvious signs of the condition is itching, crawling sensation on the face or (and) in the scalp, but most of the time, there is no itching at all, and people are not aware they are infested with Demodex mites.

What damage can Demodex follicle mites do?

Demodex follicle mites live inside the sebaceous glands and hair follicles, sucking nutrients from the hair roots and damaging the cell walls. After mating they burrow into the skin, laying eggs, at times introducing bacteria causing infection to the skin. Throughout the five phases of their life cycle, these mites destroy the skin by excreting wastes and secretions, laying eggs and dying within its layers. After death, their corpses become liquid and decompose inside the skin.

One really amazing find to this journey is having failed treating patients homes

effectively, ie... their intimate belongings and surroundings to minimizing reinfestation. Their good families can also benefit. Early in development of our commercial hair loss control & recovery system launching next year, I missed this step totally. Hence the re-infestation numbers and cases early in treating patients a few years back. Which I can say for certain contributed to some regression and hair shedding on the maintenance program and patients had to return to clinical support treatments to boost the growth and volume back.

As of late I had a chat with our friends at UNGEX, Now we can get it right!...

Sayeed brought something up I had not understood how to do it effectively enough, until yesterday. We can now say Demodex can be managed in the home and that the family is also Demodex free.

If the Demodex mites are not controlled by treating the towels, bedding, furniture then reinfestation of the patient is almost certain. Even in contact with others we know they can jump and reinfest.

So the first step to be taken is with our very own staff, equipment and the clinic.

If practice staff, equipment and the clinic are not examined and treated most likely we are in the cycle to. Skin, nail or hair clinics, salons and even surgical theaters are prone and should be examined and treated. We are all on the best path in assisting recovery this I believe is just one more step in reversing hair loss naturally pre-surgery and taking the work done in surgery just that much further for a patients recovery if you will.

Most clinics, skin or scalp clinic can rid a patient of mites in a few zaps with High Frequency, added serums and tonics but if the home or their office, whether visiting a country or to be simply putting your head on a airplane pillow or head

rest of the bus to the airport means we are probably in contact with a good count of mites.

So how does UNGEX prove they manage that..?

Seyed (M Mallak) Behbahani-*Managing Director at UNGEX Pty. Ltd shared with us he clearly has the solution for a clinic like ours to assist in the patient controlling re-infestation of the Demodex Mites.*

Sayeed's centre has launched the NEWEST BREAKTROUGH TECHNOLOGY & SOLUTION that targets DEMODEX Mites . DEMODEX Treatments are tested and proven to be effective and it is a non-invasive cosmetics procedure. Demodex mites under the Newest Breakthrough Technology is hands down one of the most amazing things to incorporate into our protocol. As a researcher and clinician watching this from baseline to recovery for the patient and for our team from trench to bench is amazing.

A bit of what we discussed at the National Institute for Integrative Medicne is on to how the Demodex can produce the enzyme lipase which is necessary for Demodex to digest the sebum it feeds on. Lipase can adversely affect the quality, condition and appearance of your scalp and hair

If the mites go unobserved, the mite populations can dramatically increase, resulting hair thinning problems. Hair loss, in some cases, pre-mature hair loss can be linked with extended demodex folliculorum activity and much more.

I endorse UNGEX!

The Strand Clinic and Ungex *together not only can -bring hair follicles back but we also bring new health to the home.*

- See more at:http://thestrandclinic.com.au/www/content/default.aspx?
cid=666#sthash.MSDTGUga.dpuf

https://www.ungex.com/aboutus.htm

https://en.wikipedia.org/wiki/Demodex_folliculorum

Paper 9

Human *Demodex* Mite: The Versatile Mite
of Dermatological Importance

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Human Demodex Mite:
The Versatile Mite of Dermatological Importance

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Abstract

Demodex mite is an obligate human ecto-parasite found in or near the pilo-sebaceous units. *Demodex folliculorum* and *Demodex brevis* are two species typically found on humans. *Demodex* infestation usually remains asymptomatic and may have a pathogenic role only when present in high densities and also because of immune imbalance. All cutaneous diseases caused by *Demodex* mites are clubbed under the term demodicosis or demodicidosis, which can be an etiological factor of or resemble a variety of dermatoses. Therefore, a high index of clinical suspicion about the etiological role of *Demodex* in various dermatoses can help in early diagnosis and appropriate, timely, and cost effective management.

Keywords: *Demodex*, *demodicosis*, *demodicidosis*, *ecto-parasite*

Introduction

What was known?
Demodex mite infestation usually remains asymptomatic, but may be an important causative agent for many dermatological conditions.

Demodex, a genus of tiny parasitic mites that live in or near hair follicles of mammals, are among the smallest of arthropods with two species *Demodex folliculorum* and *Demodex brevis* typically found on humans. Infestation with *Demodex* is common; prevalence in healthy adults varying between 23-100%. [1,2] *Demodex* infestation usually remains asymptomatic, although occasionally some skin diseases can be caused by imbalance in the immune mechanism. In this article, we have described the mite and have highlighted its dermatological importance.

General considerations of Demodex
Demodex mite is an obligatory human ecto-parasite, and it is resident in or near the pilo-sebaceous units.[3] About 65 species of *Demodex* are known. Two species *D. folliculorum* and *Demodex brevis*, collectively referred to as *Demodex*, are typically found on humans, occurring in 10% of skin biopsies and 12% of follicles[4,5] [Figure 1]. Identification of these mites dates back to 1841-42 for *D. folliculorum* by Simon and 1963 for *D. brevis* by Akbulatova. [4,6,7]

Figure 1
Demodex mite, an obligatory human ecto-parasite resides in or near the pilo-sebaceous units

Species/genus identification
Demodex is a saprophytic mite that belongs to family Demodicidae, class Arachnida, and order Acarina. [8]

Morphology
Adult *D. folliculorum* mites are 0.3-0.4 mm in length and that of *D. brevis* are slightly shorter of 0.15-0.2 mm length,[2] with females somewhat shorter and rounder than males [Figure 2]. This makes them invisible to the naked eye, but, under the microscope, their structure is clearly visible. It has a semi-transparent, elongated body that consists of two fused segments. Eight short, segmented legs are attached to the first body segment. The eight legs of this mite move at a rate of 8-16 mm/h and this is mainly done during the night as bright light causes the mite to recede into its follicle. The body is covered with scales for anchoring itself in the hair follicle and the mite has pin-like mouth parts for eating skin cells, hormones, and oils (sebum) accumulating in the hair follicles.[2,4,5]

[Figure 2]
Morphology and life cycle of the *Demodex* mite

Sites of involvement
Demodex is an ecto-parasite of pilo-sebaceous follicle and sebaceous gland, typically found on the face including cheeks, nose, chin, forehead, temples, eye lashes, brows, and also on the balding scalp, neck, ears.[4,5] Other seborrheic regions such as naso-labial folds, peri-orbital areas, and less commonly upper and medial region of chest and back are also infested.[2] They may also be found on penis, mons veneris, buttocks, and in the ectopic sebaceous glands in the buccal mucosa.[2]

D. folliculorum is more commonly localised to the face, while *D. brevis* is more commonly found on the neck and chest.[9] Infestation with *D. folliculorum* is more common than with *D. brevis*, but the latter has wider distribution on the body.[4] *D. folliculorum* is usually found in the upper canal of the pilo-sebaceous unit at a density of ≤ 5/sq cm[4] and uses skin cells and sebum for nourishment.[3,10] Several mites, with heads directed toward the fundus, usually occupy a single follicle.[4,11] *D. brevis*, on the other hand, burrows deeper into the sebaceous glands and ducts and feeds on gland cells.[5] Penetration of *Demodex* into the dermis or, more commonly, an increase in the number of mites in the pilo-sebaceous unit of > 5/sq cm,[4] is believed to cause infestation, which triggers inflammation.[10,12] Some authors consider the density of > 5 mites per follicle as a pathogenic criterion.[10,13]

Life cycle
Female *Demodex* are somewhat shorter and rounder than males. Both male and female *Demodex* mites have a genital opening and fertilisation is internal. Mating takes place in the follicle opening and eggs are laid inside the hair follicles or sebaceous glands. The six-legged larvae hatch after 3-4 days, and the larvae develop into adults in about 7 days. It has a 14-day life cycle [6] [Figure 2]. The total lifespan of a *Demodex* mite is several weeks. The dead mites decompose inside the hair follicles or sebaceous glands.

Age/sex consideration of infestation
The number of *Demodex* mites present in the lesion increases with age.[9] The prevalence of infestation with *Demodex* mites is highest in the 20-30 years age group, when the sebum secretion rate is at its highest.[14] Older people are also more likely to carry the mites.[15] Demodicosis is exceptionally seen in children aged <5 years.[16,17] Presumably, *Demodex* passes to newborns through close physical contact after birth; however, due to low sebum production, infants and children lack significant *Demodex* colonisation.[5]

Infestation of both species is more common in males than in females, with males more heavily colonising than females (23% vs 13%) and harbouring more *D. brevis* than females (23% vs 9%).[4]

Mode of transmission

The mites are transferred between hosts through contact of hair, eyebrows, and sebaceous glands on the nose.

Methods of detection on body

Demodex is not easily detected in histological preparations; therefore, skin surface biopsy (SSB) technique with cyanoacrylic adhesion is a commonly used method to measure the density of *Demodex*.[10] It allows the collection of the superficial part of the horny layer and the contents of the pilo-sebaceous follicle;[18] however, it can fail to collect the complete biotope of *D. folliculorum*.[12]

Other sampling methods used in assessing the presence of *Demodex* by microscopy include adhesive bands, skin scrapings, skin impressions, expressed follicular contents, comedone extraction, hair epilation, and punch biopsies.[11,19] The resulting number of mites measured varies greatly depending on the method used.[11] With modern, and more sensitive, assays, the prevalence of *Demodex* in skin samples approaches 100%; therefore, mere presence of *Demodex* does not indicate pathogenesis. Rather, more important in diagnosing *Demodex* pathology is the density of mites or their extra-follicular location.[19]

Predisposing factors

Most people are only carriers of *Demodex* mites and do not develop clinical symptoms. Human demodicosis can be considered as a multi-factorial disease, influenced by external and/or internal factors. [20]
One of the factors for the transition from a clinically unapparent colonisation of mites to dermatoses can be the development of primary or secondary immunodepression.[20,21] Primary immune suppression is most probably based on hereditary defect of T cells, subsequently reinforced by substances that are produced by mites and by bacteria, with intact B cell immunity.[20,22,23] The fact that people and animals with immunodeficiency are prone to infestation with *Demodex* mites has been shown repeatedly.[24,25]

Secondary immune suppression predisposing to demodicosis follows corticosteroid, cytostatic therapy, or due to diseases of an immune-compromised nature such as malignant neoplasia, hepatopathies, lymphosarcoma, and HIV infection.[25,26,27,28] There may, however, be factors other than generalised immunosuppression leading to the development of demodicosis.[29] It has been suggested that infestation may be related to genetic predisposition[30] and also with special types of HLA (Human Leukocyte Antigen), although some HLA types are considered to be resistant to demodicosis.[29]

Immunopathogenesis

Pathogenesis of demodicosis and immune response to mite invasion are poorly understood. [31,32] Many views have been put forth [Figure 3] as follows:

Figure 3
Factors involved in pathogenesis

- Altered immune system, especially in immune-deficient individuals, which eventually causes a skin disorder.
- Hypersensitivity against the mite itself; the evidence being that histopathological examination reveals a dermal infiltrate of lymphocytes, eosinophils, and typical granulomas predominantly composed of CD4+ T helper lymphocytes, often distributed around a *Demodex* body.[33]
- Increased readiness of lymphocytes to undergo apoptosis and increased number of NK cells with Fc receptors is correlated with increased mite density.[34]

- Significant decrease in absolute numbers of lymphocytes and T- cell subsets and significant increase in IgM levels have also been found in patients presenting with *demodex*. *Demodex* proliferation and facial skin lesions.[35]
- Antigenic proteins related to a bacterium isolated from a *D. folliculorum* mite, *Bacillus oleronius*, have the potential to stimulate an inflammatory immune response in patients with papulopustular rosacea by increasing the migration, degranulation, and cytokine production abilities of neutrophils.[36,37]

These findings suggest that colonisation of the skin with *Demodex* could be a reflection of immune response of the host to organism. [34]

Clinical manifestation

Demodex mites are present in healthy individuals and may have a pathogenic role when present in high densities.[13] The infestation may be clinically inapparent, but, under favorable circumstances, these mites may multiply rapidly, leading to the development of different pathogenic conditions.[30,38]

All cutaneous diseases caused by *Demodex* mites are clubbed under the term demodicosis or demodicidosis. It remains unknown if *Demodex* is the underlying cause of these conditions or if *Demodex* mite density increases due to inflammation of affected follicles.[39] It is possible that by blocking the hair follicles, it can cause inflammation or allergic reaction or act as vector for other microorganisms.[40]

These conditions are briefly described below:

Rosacea and Demodex rosacea

Demodex may have a direct role in rosacea or may manifest as rosacea like dermatitis [Figure 4a]. Numerous studies have reported elevated *Demodex* density in patients with rosacea. [4,10,11,41,42]

Figure 4
Clinical photograph showing rosacea (a) and steroid induced rosacea (b)

Human demodicosis may manifest as a dry type of rosacea, termed rosacea-like demodicidosis. [43] Rosacea of demodicosis needs to be differentiated from the common rosacea. *Demodex* type rosacea is characterised by dryness, follicular scaling, superficial vesicles, and pustules, while common rosacea is characterised by oily skin, absent follicular scaling, and being more deeply seated. [44]

Another useful feature is the complete resolution of demodicosis on treatment with scabicide crotamiton or lindane. It has been proposed that failure to wash the face and overuse of oily or creamy preparations supplies the *Demodex* mites with extra lipid nourishment, which promotes reproduction of mites in large numbers, which plugs the pilo-sebaceous ducts and leads to appearance of rosacea-like facial eruption. [45]

Non specific facial dermatitis

Patients presenting with nonspecific facial symptoms such as facial pruritus with or without erythema, a seborrheic dermatitis-like eruption, perioral dermatitis-like lesions and papulopustular, and/or acneiform lesions without telangiectasia, flushing, or comedones have been found to have significantly higher median mite density[39,46] [Figure 5].

Demodex dermatitis may in fact be distinct from rosacea and seborrheic dermatitis, as reported by one group [47] and the presence of facial erythema, dryness, scaling, and roughness with or without papules/pustules may be a result of D. folliculorum proliferation. [48]

Figure 5
Clinical photograph of Demodex induced non specific facial dermatitis

Demodex dermatitis may in fact be distinct from rosacea and seborrheic dermatitis, as reported by one group [47] and the presence of facial erythema, dryness, scaling, and roughness with or without papules/pustules may be a result of D. folliculorum proliferation.[48]

Steroid rosacea

The role of D. folliculorum in the pathogenesis of topical corticosteroid-induced rosacea is controversial.[49,50] It has been reported that the population of Demodex mites is increased in these patients [5,11,51,52] [Figure 4b].

Androgenetic alopecia

Demodex has been implicated in the etiology of AGA. [53] The role of Demodex in AGA has been evaluated to be direct in some studies and indirect in others. The possible mechanisms include the following:

- Induction of inflammation by the presence of an immune-active lipase in Demodex mite.[54] Nowadays, inflammation has been considered to be involved in pathogenesis of AGA.[39,55] It has been proposed that inflammation reaction in AGA is confined to the surrounding area of sebaceous glands and infundibulum, and follicular infiltration with activated T cells results in induced synthesis of collagen by dermal sheath fibroblasts and ultimately replacement of hair follicle with fibrosis takes place.[56,57]
- Altering local hormone metabolism by the inflammatory reaction.[58]
- Sebaceous glands of alopecia-affected hair follicles become larger and more active under the influence of dihydrotestosterone, producing oils at a faster rate and, hence, become a more suitable environment for Demodex. In fact, Demodex infestation is considered to be secondary to AGA and not its cause.
- Exhaustion of the hair bulb and shifting of hair cycle from anagen to telogen through long-term invasion by the parasite.[53]

Madarosis

Infestation of pilo-sebaceous components of the eyelids with D. folliculorum can also result in loss of eyelashes. [59] Demodex mite causes follicular inflammation that produces edema and subsequent easier epilation of eyelashes. It also affects cilia constriction so that lashes become brittle and fall. [60]

Lupus miliaris disseminatus faciei

Several authors suggest that LMDF is a reaction to *D. folliculorum.* … [61] [Figure 6].

Figure 6
A case of clinically and histopathologically proven LMDF

Dissecting folliculitis

The cause of dissecting folliculitis of scalp is not well understood [Figure 7]. It is generally considered to be an inflammatory reaction to components of the hair follicle, particularly microorganisms like bacteria (especially *Propionibacterium acnes*, *Staphylococcus aureus*), yeasts (M Human *Demodex* Mite: The Versatile Mite of Dermatological Importance. [62]

Figure 7
Clinical photograph of dissecting folliculitis leading to cicatricial alopecia

Miscellaneous conditions

Increased number of *Demodex* mites has also been observed in peri-oral dermatitis [Figure 8a], acarica blepharo-conjuctivitis [Figure 8b], grover's disease, eosinophilic folliculitis, papulovesicular facial, papulopustular scalp eruptions, pityriasis folliculorum, pustular folliculitis, *Demodex* abscess, and demodicosis gravis (granulomatous rosacea like demodicosis). [34,39,63]

Figure 8
Clinical photograph of peri-oral dermatitis (a) and blepheritis (b)

Other points of importance

As a vector for transmission

Demodex may act as a vector of transmission of various infections from one area of body to another or between individuals by its potential to ingest and transport various microorganisms that are found in its niche, as demonstrated by potassium hydroxide mount of skin scraping from a mycotic plaque, which showed numerous *Demodex* mites containing spores of *Microsporum canis* inside them. [64]

Prevention/treatment of human demodicosis

Demodex can only live in the human hair follicle and, when kept under control, causes no problems. However, to reduce the chance of the mites proliferating excessively, following preventive measures are important:

- Cleanse the face twice daily with non-soap cleanser
- Avoid oil-based cleansers and greasy makeup
- Exfoliate periodically to remove dead skin cells

After clinical manifestations, the mites may be temporarily eradicated with topical insecticides, especially crotamiton cream, permethrin cream, and also with topical or systemic metronidazole. In severe cases, such as those with HIV infection, oral ivermectin may be recommended. [3,48,66] Go to:

Conclusion

Human demodicosis is caused by the clinical manifestation of otherwise asymptomatic infestation of humans by two species of *Demodex* mite, i.e., *D. folliculorum* and *D. brevis*. The etiological role of this versatile mite should be kept in mind as human demodicosis can present as a variety of clinical manifestations mimicking many other dermatoses. This can help in early diagnosis and proper treatment, thereby saving time and at the same time being cost effective.

What is new?

Demodex mite should be considered as an aetiological factor for a number of dermatoses for their early diagnosis and appropriate treatment. Go to:

Footnotes
Source of Support: Nil
Conflict of Interest: Nil. Go to:

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Paper 10

Ubiquity and Diversity of Human-Associated *Demodex* Mites

RESEARCH ARTICLE

NORTH CAROLINA STATE UNIVERSITY & FORDHAM UNIVERSITY, USA

Ubiquity and Diversity of Human-Associated Demodex Mites

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Abstract

Demodex mites are a group of hair follicle and sebaceous gland-dwelling species. The species of these mites found on humans are arguably the animals with which we have the most intimate interactions. Yet, their prevalence and diversity have been poorly explored. Here we use a new molecular method to assess the occurrence of *Demodex* mites on humans. In addition, we use the 18S rRNA gene (18S rDNA) to assess the genetic diversity and evolutionary history of *Demodex* lineages.

Within our samples, 100% of people over 18 years of age appear to host at least one *Demodex* species, suggesting that *Demodex* mites may be universal associates of adult humans. A phylogenetic analysis of 18S rDNA reveals intraspecific structure within one of the two named human-associated *Demodex* species, *D. brevis*. The *D. brevis* clade is geographically structured, suggesting that new lineages are likely to be discovered as humans from additional geographic regions are sampled.

Introduction

Many organisms live on us and in us… Among the more enigmatic of the multicellular species that live on humans, as well as on other mammals, are mites of the genus *Demodex* (reviewed in [7]), which are common on human faces and other parts of the body [8], [9]. While these mites are well known to dermatologists, ophthalmologists, and veterinarians and have been the subject of study for 172 years (reviewed in [10]), their ubiquity, diversity and evolution are poorly understood. For example, *Demodex* have not been sampled from the vast majority of mammal species, including those that seem very likely to host *Demodex* mites, such as chimpanzees and gorillas. Nor have most human populations been sampled for these mites.

Two species of *Demodex, D. brevis* (Akbulatova 1963) and *D. folliculorum* (Simon 1842), have been described from the human body. In general, *Demodex* live mostly within hair follicles. Biopsies of skin cross-sections reveal *D. folliculorum* to inhabit the area of the follicle above the sebaceous gland, where they appear to ingest cell contents [11]. *D. brevis*, on the other hand, primarily inhabits the sebaceous glands associated with vellus hairs [11], typically at densities of just one to a few mites per gland. With approximately 5 million hair follicles spread across the body [12] and more than 7 billion humans on Earth, the total habitat area available to these mites is immense.

Methods used to collect *Demodex* mites from humans include biopsy, the cellophane tape method (placing tape on the face to stick to the mites), scraping areas where mites are likely to reside, and plucking eyelash and eyebrow hairs. Based on the visual observation of mites collected from healthy individuals by these methods, it appears that approximately 3–55% of humans harbor *Demodex*…

However, because these mites may occur in patches around the body, as in dogs [17], and all existing collection methods sample just small patches of skin (and even incompletely sample those patches), it is difficult to know to what extent the absence of mites in a sample equates to the absence of mites on the body. Intriguingly, in post-mortem studies, mites appear to be present on all adult cadavers (reviewed in [10]). The ubiquity of mites on cadavers might indicate they are universally present on living, adult humans but missed by current sampling methods. Alternately, conditions in which cadavers are found might facilitate colonization by mites and, in doing so, artificially inflate estimates of their incidence.

Even less well understood than the proportion of people (or for that matter, other mammals) that host *Demodex* mites is the diversity of those mites. While two species of human-associated mites have been formally named, they were named based on morphological characters alone [18], [19]. Given that *Demodex* mites inhabit restrictive, specialized environments (hair follicles), some aspects of their morphology, including their small size (~100–200 μM) and general elongate appearance, could reflect convergent evolution among distinct lineages or species groups which would only be discerned by examination of non-morphological data, e.g. by DNA sequence-based differences.

A recent study of human *Demodex* species found genetic differences in the mitochondrial CO1 gene between mite populations that inhabit the eyelashes versus mite populations that inhabit the skin [20]. In addition, studies of another human-associated parasite, lice (*Pediculus humanus*), have found strong genetic structure between geographic lineages [4], [5], [21]. Geographic structure among human-associated *Demodex* lineages is expected, given that these

mites are more intimately associated with the body than lice and seemingly less mobile, yet the minimal data that exist have not yet recovered such variation [22]. Conversely, if *Demodex* lack strong geographic structure, it suggests the movement of mites among humans must occur very frequently (perhaps even with social greeting rituals) and across large geographic distances.

Only recently have molecular studies begun to consider *Demodex* mites. Existing phylogenies and estimates of molecular divergence include very limited sampling of *Demodex* species, are based on few genetic markers, and include only minimal geographic representation.

The DNA sequences that have been obtained from human-associated *Demodex* species come almost exclusively from China (*D. folliculorum* and *D. brevis*) and Spain (*D. folliculorum)* [20], [22]. Studies based on the 16S rRNA gene (16S rDNA) find little variation within *D. folliculorum* and show no geographic structure between samples from China and Spain [22]. However, no molecular data have been considered from *D. brevis* outside of China, and low genetic variation observed for human-associated *Demodex* in previous phylogenies [22] may reflect insufficient sampling rather than the actual genetic diversity of *Demodex* mites.

Here we test a new molecular approach to detect the presence of mites on human bodies and assess the proportion of individuals in one population colonized by mites. We then use phylogenetic reconstruction based on the nuclear 18S rRNA gene (18S rDNA) to better understand the diversity of these mites.

Figures
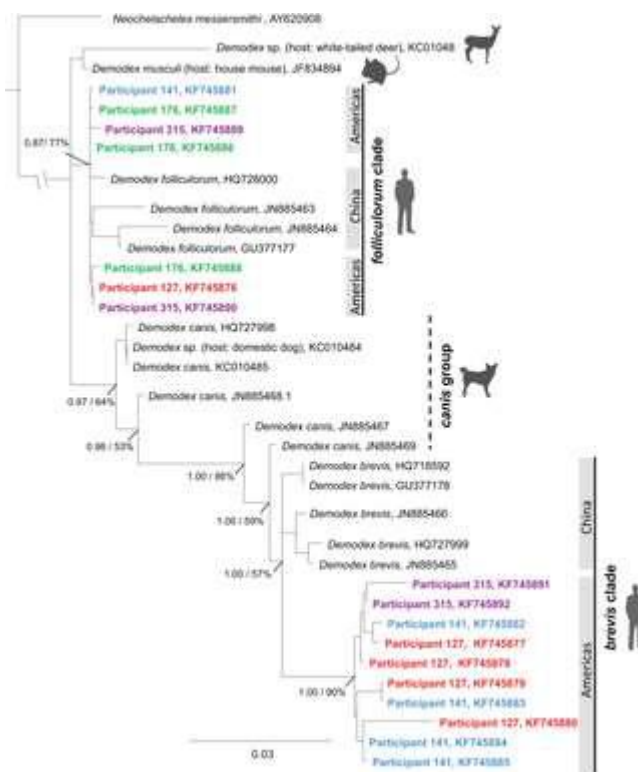
For further images, please refer to the source of the article.

Materials and Methods

Ethics Statement

Participants were sampled by project staff at outreach events. Prior to sampling, each participant was verbally informed about the goals of the project and the sampling protocol. All participants were provided and signed a written Informed Consent form. All human *Demodex* sampling procedures and the participant Informed Consent form were approved by North Carolina State University's Institutional Review Board for the Protection of Human Subjects in Research (IRB), Approval No. 2966.

(a) Sample collection

All sample collections were performed in Raleigh, NC at either the North Carolina Museum of Natural Sciences or North Carolina State University. Each participant was gently scraped with a metal laboratory spatula along the creases of the nose and over the surrounding cheek area.

The facial habitats were chosen based on their high levels of sebum production and ease of pore expression. In addition, Bonnar *et al.* (1993) found the greatest abundance of mites in the cheek area among rosacea patients [23].

Mineral oil was typically applied to the sampled area to facilitate mite removal. After collection, the sebum was moved to a drop of mineral oil on a cover slip fragment where it was inspected to note the presence or absence of visually identifiable mites within the sample. Regardless of the presence or absence of observed mites the entire cover slip fragment with the sebum and mineral oil was transferred to a 1.5 ml microcentrifuge tube and maintained in −20°C for subsequent DNA extraction.

(b) DNA Extraction and PCR

DNA was extracted from the sebum of individual participants, regardless of the presence or absence of an observed mite, using a Qiagen DNeasy Blood & Tissue kit. We followed the manufacturer's supplementary insect protocol, without the initial grinding step. The samples were incubated overnight at 56°C with 180 μl of ATL buffer and 20 μl proteinase K. The final elution step was performed with 150 μl of elution buffer warmed to 56°C.

We used either OneTaq (NEB) or TaKaRa Ex Taq (Clontech), which possess proofreading functions, for all PCR reactions to reduce polymerase induced sequence errors. We designed the primers by aligning all available *Demodex* 16S rDNA or 18S rDNA sequences across the same genes from several other mites and from humans.

In an attempt to design primers that were likely to be unbiased with regards to *Demodex* and have a low affinity for the hosts' DNA, we selected priming sites near the 5′ and 3′ ends of most available *Demodex* sequences that were highly conserved among these mites, yet that were unlikely to amplify these genes from humans.

For this analysis, a set of 19 individuals over 18 years of age and a second set of ten individuals 18 years of age were used. Several 16S rDNA PCR reactions were also sequenced to verify the specificity of the primers. However, data from this gene was not sequenced for most individuals, because this sequence was rather short (~325 bp) and did not contain many phylogenetically informative sites (i.e., two phylogenetically informative sites exist among our 16S rDNA sequences and the *D. folliculorum* sequences available on GenBank).

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Figure 1. PCR based screen for presence of *Demodex* 16S rDNA in samples with no visually identifiable mites. Lanes labeled 1–29 represent samples from single individual participants. Lanes labeled M represent 100 bp molecular weight size markers. (a) PCR products indicate the presence of *Demodex* DNA in 100% of the screened samples from individuals over the age of 18. (b) PCR products indicate the presence of *Demodex* DNA in 70% of the screened samples from individuals 18 years of age.
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The 18S rDNA PCR products were sequenced from four individuals and used for phylogenetic analyses. We chose 18S rDNA for these analyses as this PCR works well with very little incident of non-specific bands (see Figure 1A). Furthermore, the transfer of mtDNA between closely related species has been frequently observed [24]–[26]. By using the nuclear 18S rDNA, we hope to decrease the likelihood of introgression obscuring population or species variation. All sequences were submitted to GenBank (Table 1).

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Table 1. *Demodex* mite species identification based on 18S rDNA gene sequence.
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(c) Sequencing and Phylogenetic Analysis

Because our faces have the potential to harbor many thousands of individual *Demodex* mites, we expect remnants of these mites to be present in our pores and on the surface of our faces, making the clean isolation of *Demodex* DNA from a single mite difficult. Thus, we presume that each of our scrapings is likely to harbor DNA from multiple mites. To obtain sequences from single copies of 18S rDNA from individual mites, we cloned the 18S rDNA PCR products using TOPO TA Cloning Kits (Invitrogen).

We picked and sequenced a minimum of five colonies from each person sampled in this study to get a sense of the diversity within an individual host. The resulting sequences were aligned with *Demodex* sequences available on GenBank using MAFFT v7 [27], with the E-INS-i algorithm, and checked by eye for best alignment. All GenBank sequences are named according to the species names given in GenBank; however, due to the current state

of *Demodex* systematics some sequences are likely improperly designated (particularly dog-hosted species), leading to paraphyly of some taxa.

The 18S rDNA sequence from a mite species, *Neochelacheles messersmithi*, in the same superfamily as *Demodex*, Cheyletoidea, was included as an outgroup for phylogenetic analysis.

To obtain estimates of genetic divergence between 18S rDNA sequences of all taxa included for phylogenetic analysis, Kimura 2-parameter distances (K2P) [28] and total number of nucleotide differences were calculated using MEGA v5 [29]. Genetic distances were calculated for all pairwise sequence comparisons as well as intra- and interspecific means.

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI). Under both methods, gaps in the alignment were treated as missing data. jModelTest 2[30] was used to determine the best-fitting model for the 18S rDNA data set.

Using the corrected Akaike information criterion [31], the TIM2+ I + G model (with two rates of transitions and two rates of transversions) was selected as the best-fitting model for these data [32]. ML analysis was conducted using GARLI 2.0 for Windows [33]. Ten independent search replicates were run under the TIM2+ I + G model, with each replicate run for 100,000 generations. Bootstrap support values for nodes on the ML topology were computed with GARLI by running 1000 bootstrap replicates.

The Bayesian analysis was conducted with Mr. Bayes 3.2 [34]. Two independent runs were performed for 50 million generations, each with four chains (three heated and one cold), uninformative priors, and trees sampled at intervals of 1000 generations. Stationarity was determined by examining standard deviation of split frequencies between the two runs for convergence and examination of average potential scale reduction factor (PSRF). Of the 50,000 trees sampled in each run, the first 10,000 trees were discarded as burn-in and the remaining trees were used to construct a 50% majority rule consensus tree.

Because the standard deviation of split frequencies was observed to drop and remain below 0.01 by 1,500,000 generations (i.e., 1500 sampled trees), our burn-in value of 10,000 was chosen to ensure that trees were sampled well after runs had reached convergence. The harmonic mean of likelihoods was estimated for post burn-in trees using the *sump* command in Mr. Bayes. We assigned putative species sources for new sequences based solely on phylogenetic distance of previously reported species.

Results

Based on the observation of *visually identifiable (microscope testing)* mite specimens within our samples, the prevalence of mites in adults was 14% (n = 253), in line with previous studies [8], [13]–[16]. However, we were able to extract *Demodex* 16S rDNA from 100% of adults over the age of 18 (Figure 1A; Mean age: 37±10.4 years, n = 19). Molecular evidence suggests *Demodex* prevalence is much higher than recognized through visual observation alone. Our results are in line with postmortem studies that find *Demodex* mites present on all adult cadavers (reviewed in [10]).

Based on the observation of intact specimens in samples of young adults 18 years of age, mites were found on only 5.88% (n = 51). Of the ten 18 year olds we examined further for *Demodex* 16S rDNA, we amplified 16S rDNA PCR products from only seven samples. Thus while 100% of adults in our sample hosted *Demodex* mite 16S rDNA, the prevalence and/or detectability in younger individuals appears lower (70%).

For phylogenetic analyses, we amplified, cloned, and sequenced *Demodex* 18S rDNA from four individual humans from whom we identified 17 unique *Demodex* 18S rDNA sequences (Table 1). These sequences reflect the presence of multiple mites within a given sample, even if we assume the presence of sequencing error and potential variation among 18S rDNA copies within the genome.

We combined these sequences with previously published *Demodex* 18S rDNA sequences, representing at least 5 species from 4 mammalian hosts (human: *D. brevis* and *D. folliculorum*, dog: *D. canis*, mouse: *D. musculi*, and white-tailed deer: *D.* sp.) and an additional mite outgroup, *Neochelacheles messersmithi*, from the same superfamily as *Demodex*, Chelyetoidea. Our alignment comprised 1664 bp for 35 sequences (see Material S1 for alignment). The ML analysis yielded a tree with the best score of –ln = 4887.29 (see Material S2 for ML tree file). The Bayesian analysis yielded a 50% consensus tree with harmonic mean of likelihood = −4976.76 (see Material S3 for Bayesian tree file).

The average standard deviation of split frequencies of sampled trees = 0.00119, and the PSRF of sampled trees = 1.000. Phylogenetic analyses conducted with ML and BI yielded largely congruent topologies; minor incongruencies were restricted to placement of sequences with extremely short internodal branch lengths within the *D. folliculorum* clade and as such do not influence our interpretation. The ML topology is shown in Figure 2, with Bayesian posterior probabilities and ML bootstrap support values depicted adjacent to the major nodes of interest.

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As evident in our phylogenetic results, we found substantial genetic diversity among (up to 0.065 K2P distance, up to 20 nucleotide substitutions (nts)) and within *Demodex* species (up to 0.032 K2P, up to 10 nts) (Table S1). Several of our sequences fit within a relatively well-supported *D. folliculorum* clade within which we find low genetic diversity (0.002 K2P, up to 2 nts) even though the individuals sampled included humans from North and South America and sequences from GenBank for individuals from China. Greater diversity is present within the *D. brevis* clade (up to 6.5 K2P, up to 10 nts). Multiple lineages of *D. brevis* appear to be present even on individual humans (within participant diversity: 0.006–0.007 K2P, 2–2.16 nts).

However, the greatest diversity was among geographically distinct human populations (up to 0.032 K2P distance between American and Chinese sequences, 10 nts). Existing sequences of *D. brevis* sampled from humans in China resolve as a monophyletic clade sister to a New World clade composed of samples acquired for this study.

Discussion

Here we tested 29 people for the presence of *Demodex* mites and found that mites were much more common than expected in comparison to methods that rely solely on the visual confirmation of whole mite specimens taken from living humans. When we sampled individuals using traditional approaches, our results were similar to those of the many previous morphologically based studies [8], [13]–[16]; 14% of individuals over the age of 18 had visually observed mites. But when we identified the presence of mites based on the amplification of *Demodex* DNA, we found that every adult over 18 years of age and 70% of 18 year olds had detectable *Demodex* 16S rDNA in the collected sebum of facial samples...

Little is known about the transmission of mites among humans. Recent studies find that many symbiotic microbes are passed directly from mother to offspring during breast-feeding [35] or during birth (especially if birth is vaginal) [36], [37], and dogs acquire their *Demodex* mites as nursing pups [38]. In light of this, the same means of mite transmission seems possible in humans, supported by the fact that in one study, *Demodex* mites were found in 77% of nipple tissue from mastectomies [39].

Yet that we found mites on all adults but only 70% of 18 year olds, suggests that perhaps mite colonization does not strictly occur vertically, from parent to child. These results are in line with earlier morphological (largely post-mortem) studies in which mites were found to be more prevalent on adults than on children (reviewed in [10]). Mites could be more ubiquitous on children than noted in post-mortem studies or herein but at levels or in locations that make the mites difficult to detect even with the use of molecular approaches...

Overall, we found the genetic variation of 18S rDNA within the genus *Demodex* comparable (up to 0.065 K2P) to the level of variation found among other genera within Acari (0.00–0.056 K2P; Ticks: Ixodidae) [41] (Table S1). This diversity suggests *Demodex* is a relatively old genus and even that the divergence between the two named human-associated species, *D. brevis* and *D. folliculorum*, might be relatively ancient. Within *Demodex*, *D. folliculorum* and *D. brevis* exhibit contrasting levels of intraspecific genetic diversity. *D. folliculorum*, which can be found living superficially within pores, show very little variation in the 18S rDNA sequence data we generated (mean of 0.002 K2P, up to 2 nts).

In comparison to *D. folliculorum*, *D. brevis* exhibited higher genetic diversity, not only between mites from the Americas and those from China (up to 0.032 K2P, up to 10 nts) but also among mites collected from the same individual human (0.005–0.009 K2P, 1.6–4.0 nts). Sequences of 18S rDNA from different *D. brevis* samples taken from the same face (of participant 141, Figure 2) exhibited more genetic variation (0.006 K2P, 4 nts) than those of *D. folliculorum* taken from Chinese and North and South Americans (mean 0.002 K2P). The diversity of *D. brevis* 18S rDNA found on individual humans suggests that not only do all adult humans have *Demodex* mites but that colonization is likely to occur more than once.

The Chinese *D. brevis* samples in GenBank and our newly generated samples from the Americas each form monophyletic clades with a relatively deep divergence between them (mean 0.021 K2P, 6.5 nts). The distance between the two *D. brevis* clades suggests strong geographic isolation among populations of *D. brevis*.

Based on sequence divergence, these two populations are as different as are many congeneric species and subspecies. The 18S rDNA variation found between these two geographic populations is similar, for example, to that found between subspecies of parasitic lice, the head louse and body louse (*Pediculus humanus capitis* and *Pediculus humanus humanus*) [5]. *D. brevis* can be found more deeply embedded in sebaceous glands below the skin surface, in comparison to *D. folliculorum* that lives more superficially in the hair follicles.

These contrasting habitat preferences may lead to more frequent transmission of *D. folliculorum* than of *D. brevis*, thus resulting in greater reproductive isolation and geographic structure in populations. However, given our limited geographic sampling, we expect the *Demodex* topology to change as samples from other regions are integrated.

The evolutionary history of the two human-associated *Demodex* species is, at best, poorly understood. *D. folliculorum* was described by Simon in 1842, and as late as 1933, all human *Demodex* were regarded as one, albeit variable, species [42], [43]. It was only in 1963 that *D. brevis* was distinguished from *D. folliculorum* and described as a separate, but closely related, species [18].

Yet de Rojas *et al*. (2012) have demonstrated that interpreting variation in the morphology of the two human-associated *Demodex* mite species is problematic, even when interpreted in light of molecular (16S rDNA) sequence data [20]. The closest relatives for both human-associated species, *D. folliculorum* and *D. brevis,* remain unknown and are likely to remain unknown until these mites are much better sampled from other primates and mammalian hosts in general. Of the described *Demodex* species, only 13 have been sampled for molecular data and included in phylogenetic analyses.

In addition, given that there are over 5000 species of mammals and as of yet, some mammals (such as humans, dogs, and cats) appear to host more than one *Demodex* species, any existing phylogeny represents a minute fraction of the possible species diversity of the genus. *Demodex* are generally considered to be species specific, which would suggest there might be as many as 10,000 *Demodex* species on living mammals if there are two host specific mites per mammal species.

Obviously, this estimate depends both on the ubiquity of *Demodex* mites among mammal species and on their true host specificity, both of which are poorly known. Our phylogeny indicates that the two human-associated mite lineages do not share a recent common ancestor and likely have separate evolutionary histories of transmission to humans. The 18S rDNA sequence does not resolve the sister group to *D. folliculorum*, but places a paraphyletic group of dog-associated mites as the closest relative to *D. brevis*.

The dog mite sequences included here were all acquired from GenBank and are primarily labeled *D. canis*. Yet, there are 3 morphologically distinct *Demodex* species that have been described from dogs (*D. canis, D. injai*, and *D. cornei*) and the molecular delimitation of these dog-associated species is not clear [44]. It seems likely that the sequences labeled *D. canis* included here may actually represent multiple dog-hosted *Demodex* species. Phylogenetic estimates based on 16S rDNA also find that dog-hosted *Demodex* mites share a recent common ancestor with a human-associated species, though in this case *D. folliculorum* and *D. brevis* are both more closely related to goat-associated mites, *D. caprae* [45].

The known habitat of *D. canis* is deep within the pores and is most similar to that of *D. brevis.* It is tempting to posit that *D. brevis* may have colonized humans from wolves during their domestication but any such assertion would be premature. Until other primate species are sampled, the mystery of whether humans acquired *Demodex* mites from our ape/hominid ancestors or through other means such as our interactions with domesticated mammal species will remain.

Author Contributions

The Above article has been condensed. Please refer to the below link for the main source:

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Paper 11

رابطه ی مایت های دمودکس با بروز آکنه روزاسه

رابطه مایت‌های دمودکس با بروز آکنه روزاسه

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چکیده

مقدمه و هـدف: آکنه روزاسه یک بیماری التهابی مزمن و عود کننده پوستی است. با توجه به شیوع آکنه روزاسه و اهمیت شناخت اتیولوژی بیماری و تناقضاتی که در مورد نقش مایت‌های دمودکس در بروز بیماری آکنه روزاسه، این تحقیق روی مراجعین به بیمارستان‌های بوعلی و لقمان طی سال‌های ۷۹ـ۱۳۷۰انجام گرفت.

مواد و روش‌ها: این تحقیق به روش مورد – شاهدی انجام گرفت. گروه مورد براساس گزارش آسیب‌شناسی در بیماران مبتلا به آکنه روزاسه و دو گروه شاهد، یکی بیماران مبتلا به دیسکوئید لوپوس اریتماتوز (DLE) ودیگری بیماران مبتلا بـه لیکن پلان اکتینیک (ALP) بودند. گروه‌ها به لحاظ سن و جنس مشابه‌سازی شده ولام‌هـای تهیـه شـده را بـا تکنیـک نمونه‌برداری پوستی از نظر وجود یا عدم وجود مایت‌های دمودکس و نیز دانسیته آنها بررسی شد. یافته‌های بدست آمده در فرمی ثبت و نقش آنها در بروز آکنه روزاسه تعیین و Odds Ratio آن نیز تعیین گردید.

یافته‌ها: این تحقیق روی ۲۲۵نفر شامل ۷۵نفر در گروه مورد (آکنه روزاسه)، ۷۵نفر در گروه شـاهد DLE و ۷۵نفـر در گروه شاهد ALP انجام گرفت. سن و جنس افراد در این سه گروه به لحاظ آماری اختلافی نداشتند. در گـروه‌هـای شـاهد ۱۶درصد و در گروه مورد ۳۸ درصد مایت‌های دمودکس مشاهده گردید (P<۰/۰۵). وجود این پارازیت شانس بـروز آکنـه روزاسه را ۳/۳ برابر افزایش می‌دهد. تعداد متوسط مایت‌های دمودکس در گروهDLE برابر۰/۶۶ و در گروه ALP برابر ۰/۲ و در گروه مورد ۱/۴ بود (P<۰/۰۵).

نتیجه‌گیری: شیوع و دانسیته مایت‌های دمودکس شانس بروز آکنه روزاسه را افزایش می‌دهد و تحقیق برای تـاثیر درمـان این مایت در بیماری آکنه روزاسه پیشنهاد می‌گردد.

واژه‌های کلیدی: آکنه روزاسه، مایت‌های دمودکس

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مقدمه

آکنه روزاسه یک بیماری التهابی مزمن و عودکننده پوستی است که بیشتر در زنان میانسال ایجاد می‌شود. هرچند این بیماری ناشناخته است اما عوامل اتیولوژیک مختلفی شامل دژنراسیون الاستیک ناشی از آفتاب ، اختلال در عملکرد عروق ، عفونت با هلیکوباکترپیلوری و بیماری‌های دیگر ، Gasterointestinal کلونیزاسیون پوست توسط مایت‌های دمودکس ، عوامل هورمونی ، استرس‌های روحی و افزایش مدیاتورهای هومورال وازواکتیو مثل مادهP ممکن است در اتیولوژی این بیماری دخیل باشند که از این میان نقش مایت‌های دمودکس در آکنه روزاسه بحث‌برانگیز بوده است (۴-۱). مایت‌های دمودکس شامل demodex-fllicularum و demodex brevis مایت‌های ساپروفیتی هستندکه در پوست انسان در واحدهای پیلوسباسه زندگی می‌کنند (۵). در مورد نقش مایت‌های دمودکس در پاتوژنز آکنه روزاسه همچنان اختلاف نظر وجود دارد (۳). از آنجا که مایت‌های دمودکس به صورت ساپروفیت در پوست بسیاری از افراد سالم یافت می‌شود (۶) و همچنین با توجه به این که در بعضی از مطالعات خلاف آن را گزارش نموده‌اند (۷و۸) ، پیشنهاد شده است که مایت‌های دمودکس ممکن است نقشی در واکنش‌های التهابی موجود در آکنه روزاسه بازی کند (۳-۱). لذا به منظور تعیین رابطه مایت‌های دمودکس با بروز آکنه روزاسه این تحقیق روی مراجعین به بیمارستان‌های بوعلی و لقمان در سال‌های ۷۹-۱۳۷۰ انجام گرفت.

مواد و روش‌ها

این تحقیق به صورت مورد - شاهدی با استفاده از لام‌های بافت‌شناسی روی ۲۲۵ بیمار در بیمارستان لقمان و بوعلی در سال ۱۳۸۱ انجام شد. نمونه‌برداری پوستی از ناحیه صورت ۷۵ مورد آکنه روزاسه (گروه مورد) و ۱۵۰ مورد شاهد شامل ۷۵

مورد دیسکوئید لوپوس اریتماتوز[1] و ۷۵ مورد لیکن‌پلان اکتینیک[2] صورت گرفت.

گروه مورد از بیمارانی که تایید تشخیص آکنه روزاسه توسط آسیب‌شناس برای آنها مطرح شده بود ، انتخاب شدند. با توجه به این که مایت‌های دمودکس در افراد سالم هم ممکن است یافت شود ، گروه شاهد از دو گروه از بیماران که تشخیص آسیب‌شناسی داشته و محل نمونه‌برداری هم از ناحیه صورت بود و نیز ثابت شده بود که مایت‌های دمودکس در ایجاد این دو بیماری دخیل نیستند انتخاب گردیدند. این دو گروه شامل بیماران مبتلا به دیسکوئید لوپوس اریتماتوز و لیکن پلان اکتینیک بودند. برای گروه شاهد طبق موارد بالا، افرادی که پاتولوژی لیکن پلان اکتینیک و دیسکوئید لوپوس اریتماتوز را داشتند ، با توجه به تطابق سن و جنس انتخاب شدند. برای تطابق سنی از گروه‌های سنی به فاصله هر ۵سال انتخاب گردید.

پس از مشخص کردن لام‌های آسیب‌شناسی برای گروه‌های مورد و شاهد ، لام‌ها کدگذاری شد. سپس توسط درماتولوژیست تعداد۴ برش[3] از هر لام که هر برش ۵میکرومتر ضخامت داشت ، مورد بررسی قرار گرفت. در این مطالعه وجود یا عدم وجود مایت[4] و تعداد کل مایت‌ها در هر لام تعیین گردید. در این مطالعه هیچ اختلافی بین d.brevis و d.follicularum قائل نشدیم و در مجموع هر دو نوع مایت مورد بررسی قرار گرفت. آن دسته از لام‌هایی که فاقد فولیکول بودند از مطالعه خارج شدند.

داده‌های فوق و نیز خصوصیات بیماران در یک فرم اطلاعاتی ثبت گردید. داده‌های فرم اطلاعاتی طبقه‌بندی و

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[1] DLE
[2] ALP
[3] section
[4] mite positivity

نقش مایت‌های دمودکس با بروز بیماری آکنه روزاسه تعیین و مورد قضاوت آماری قرار گرفت و Odds Ratio مایت‌های دمودکس در دو گروه شاهد و نیز برای هر یک از گروه‌های شاهد تعیین گردید.

یافته‌ها

در نمونه‌برداری‌های پوستی از بیماران مبتلا به آکنه روزاسه یافته‌های آسیب‌شناسی شامل دژنراسیون کلاژن (ناشی از آفتاب) وتلانژکتـازی در درم ، انفیلتـرای لمفوسـیتیک در اطـراف فولیکـول‌هـای مـو و اطـراف عـروق یـا التهـاب گرانولوماتوز در اطراف فولیکول مو بـدون تغییرات واضح اپیدرمی بود.

ازمیان بیماران مبتلا بـه آکنه روزاسه ۲۶نفر مـرد (۳۴/۷ درصد) و ۴۹نفر زن (۶۵/۳درصد) بودند. سن متوسط بیمـاران ۴۳سال و در محدوده سنی ۲۱ تا ۹۳ سال بودند.

در نمونه‌برداری‌های پوستی از بیماران مبتلا بـه دیسکوئید لوپـوس اریتمـاتوز ، هیپرکراتـوز ، فولیکـولار پلاگینیـگ ، واکوئلیزاسـیون لایــه سـلولی بـازال ، ادم درم ، انفیلتـرای لمفوسیتیک دور عروق و دور ضمائم دیـده شـد. از میان ۷۵ بیمار مبتلا بـه DLE ۳۱ نفر مـرد (۴۱/۳درصـد) و ۴۴نفر زن (۵۹/۷درصـد) بودنـد. سـن متوسـط بیمـاران۴۵ و در محـدوده سنی ۲۰تا۷۲ سال بودند.

در نمونه‌بـرداری‌هـای پوسـتی از بیماران مبتلا بـه ALP ، نـازک شـدن اپیـدرم ، دژنراسیون هیـدروپیک لایـه بـازال و انفیلترای band-like در درم دیده شد. از میان۷۵ بیمار مبتلا به ALP،

۲۸ نفر مرد (۳۷/۳درصد) و۴۷نفر زن (۶۲/۷درصد) بودند. سن متوسط بیماران۴۴/۷سال و در محدوده سنی ۲۶تا۷۸سال بودند . این اختلاف سنی و جنسی در گروه مورد و دو گروه شاهد بـه لحاظ آماری معنی‌دار نبود.

جدول یـک نشـان مـی‌دهـد کـه مایـت‌هـای دمودکس در گروه‌های شاهد به طور متوسط ۱۶درصد و در گروه مـورد ۳۸/۷درصد وجود داشته است و این اختلاف به لحاظ آماری معنی‌دار است (P<۰/۰۵) و وجود مایت‌های دمودکس شانس بروز آکنه روزاسه را ۳/۳برابر افزایش می‌دهد.

جدول ۱ : توزیع مبتلایان به آکنه روزاسه و گروه‌های شاهد برحسب داشتن مایت‌های دمودکس ، بیمارستان‌های لقمان و بوعلی در طی سال‌های ۱۳۷۹–۱۳۷۰

گروه	گروه مورد		گروه‌های شاهد			
وجود مایت دمودکس	آکنه روزاسه		لیکن پلان اکتینیک		دیسکوئید لوپوس اریتماتوز	
	تعداد	درصد	تعداد	درصد	تعداد	درصد
دارد	۴۶	۳/۶۱	۶۷	۳/۸۹	۵۹	۸/۷۸
ندارد	۲۹	۷/۳۸	۸	۷/۱۰	۱۶	۳/۲۱
جمع	۷۵		۷۵		۷۵	

تعداد متوسط مایت‌هـای دمودکس در گـروه شـاهدDLE برابر ۰/۶۶ و در گروه شاهد ALP برابر ۰/۲ و در گـروه مـورد (آکنه روزاسه) برابر ۱/۴ بود (P<۰/۰۵).

بحث

این تحقیق نشان داد کـه وجود مایت‌های دمـودکس شـانس بروز آکنه روزاسه را افزایش می‌دهد.

در مطالعه ریهو و کرینمی نیز نتیجه مشابهی به دست آمـد (۵) و در مطالعه آنها نیز شیوع مایت دمودکس در گروه آکنـه روزاسه نسبت به گروه شاهد بیشتر بود. بنابراین بـا نتیجـه ایـن مطالعـه مطابقـت دارد. هرچنـد کـه نتایـج ایـن مطالعـه بانتایـج حاصله در مطالعات هارکوس وبستر و وروتی مغـایرت داشـت (۸و۷). دانسـیته مایـت دمـودکس در هـر لام در گـروه آکنـه روزاسه به طور معناداری نسبت بـه گروه‌های شاهد بـالاتر بـود کـه بـا نتایـج حاصـله در مطالعـه اُزگوسـتاتی و فورتـون مطابقـت داشـت (۶و۹). در حـالی کـه بانتایـج مطالعه ریهو مغایرت داشت

skin biopsy در مطالعات بیشتر استفاده شده است. در روش skin surface biopsy مایت‌ها سالم و زنده بوده و حرکت می‌کنند و به آسانی آنها را می‌توان یافت. به علاوه تمام محتویات فولیکول مو را که در آنجا d.follicularum یافت می‌شوند، می‌توان با این تکنیک به دست آورد. اما با این تکنیک d.brevis را نمی‌توان نشان داد (۹و۱۱). در نمونه‌برداری از پوست مشکل می‌توان مایت‌های دمودکس را پیدا کرد زیرا یک ماده ائوزینوفیلیک متراکم و هموژن به صورت شبحی از کیسه (Ghost sac) مایت را احاطه می‌کند (۱۱).

با توجه به نتایج حاصله از این مطالعه مشخص شد که شیوع و دانسیته مایت دمودکس در گروه آکنه روزاسه نسبت به گروه شاهد بالاتر می‌باشد که این مسأله بر نقش مایت‌های دمودکس در پاتوژنز آکنه روزاسه تأکید می‌کند. با این حال مطالعات بیشتری برای اثبات نقش مایت‌های دمودکس در پاتوژنز آکنه روزاسه لازم است. شیوع و دانسیته مایت‌های دمودکس شانس بروز آکنه روزاسه را افزایش می‌دهند. تحقیق برای تاثیر درمان این مایت در بیماری آکنه روزاسه را پیشنهاد می‌نماید.

تشکر و قدردانی

(۵). این احتمال وجود دارد که شاید اختلاف نتایج در مطالعات مختلف به علت استفاده از تکنیک‌های مختلف جهت بررسی مایت‌های دمودکس بوده باشد.

مایت‌های دمودکس در پاتوژنز آکنه روزاسه دخیل دانسته شده‌اند. مایت‌های دمودکس شامل d. follicularum و d.brevis می‌باشند که مایت‌های ساپروفیتی هستند که در پوست انسان در واحدهای پیلوسباسه زندگی می‌کنند. آنها برای اولین بار توسط برگر و هنل در سال ۱۸۴۱ شناخته شدند و افتراق این دو از همدیگر توسط آکبولاتورا درسال ۱۹۶۳ صورت گرفت (۱۰).

d.follicularum یک مایت شفاف و کرمی شکل است که در انفاندیبولوم فولیکول مو بالای سطح غدد سباسه وجود دارد. d.brevis نسبت به d.follicularum کوتاه‌تر بوده و به صورت منفرد در عمق غدد سباسه و میبومین زندگی می‌کنند. d.follicularum به تعداد بسیار بیشتری نسبت به d.brevis در پوست صورت وجود دارد (۱۰).

تکنیک ما در این تحقیق برای تشخیص مایت‌های دمودکس نمونه‌برداری پوستی بود.

روش‌های مختلفی برای بررسی این پارازیت‌ها استفاده شده است که شامل : skin surface biopsy ، adhesive، skin biopsy ، hair epilation، skin impresion ، skin scraping و comedo extraction band می‌باشند (۵و۱۱) که از این میان از دو روش skin surface biopsy و

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Paper 12

گزارش یک مورد دموديکوزیس در
سر یک دختر ۶ ساله

گزارش یک مورد دموديکوزيس سر در یک دختر ۶ ساله

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۳- استادیار گروه داخلی دانشگاه علوم پزشکی بابل

سابقه و هدف: جنس دمودکس دارای گونه های متعددی است که می تواند در انسان و حیوانات ایجاد بیماری کند. دوگونه از جنس دمودکس، در انسان بیماریزا می باشد. دمودکس فولیکولاروم (Demodex folliculorum) که به هیره فولیکول مو معروف بوده و دومی دمودکس بریویس (Demodex brevis) که در انسان بیماریزا محسوب می گردند. این بیماری در خانمها بیشتر از آقایان دیده می شود. بیماری ایجاد شده می تواند علائمی مشابه درماتیت، جوش های شبیه آکنه، زردزخم واگیر دار و یا ورم پلک چشم(بلفاریت) ایجاد کنند. در این مقاله یک بیمار که به دلیل پوسته ریزی فراوان در سر مراجعه نموده و پس از آزمایش وجود دمودکس در آن ثابت گردیدرا گزارش می نمائیم.

گزارش مورد: بیمار دختر ۶ ساله، ساکن شهرستان بابل بود که به دنبال خارش خفیف و هیپرکراتوزیس (شوره فراوان) در ناحیه پس سر به بخش انگل شناسی و قارچ شناسی دانشکده پزشکی بابل مراجعه نمود که پس از بررسی های لازم و تهیه نمونه از ناحیه سر بیمار دمودکس به مقدار فراوان در نمونه های پوسته سر مشاهده گردید.

نتیجه گیری: نتیجه این مطالعه نشان داد که در کودکان نیز پوسته ریزی پوست سر ممکن است به علت عفونت دمودکسی باشد بنابراین پیشنهاد می شود در بیماریهای اکسفولیاتیو عفونت دمودکسی نیز در تشخیص افتراقی مد نظر باشد.

واژه های کلیدی: دمودکس فولیکولاروم، دمودکس بریویس، هیپرکراتوزیس، دموديکوزيس.

مقدمه

جنس دمودکس گونه های متعددی از هیره های غیر شایع می باشد که بعضی از آنها باعث بروز بیماری گال در حیوانات می شوند. دو گونه از جنس دمودکس در انسان بیماریزا محسوب می گردد. دمودکس فولیکولاروم (Demodex folliculorum) که به هیره فولیکول مو در انسان شهرت دارد و دومی دمودکس بریویس (Demodex brevis) که به هیره غدد سباسه (غدد چربی) معروف می باشد. این هیره ها در فولیکول های مو و غدد چربی بخصوص پلک ها، بینی، گونه ها و قسمت های مجاور بینی و اطراف گوش یافت شده و از ترشحات زیر جلدی به خصوص سبوم تغذیه می کنند. این هیره بسیار کوچک بوده و اندازه آن ۰/۳—۰/۴ میلیمتر سیگاری شکل می باشد(تصویر ۱) دمودکس فولیکولاروم به خاطر شکل خاص آن با هیچ یک از بندپایان دیگری که انسان را آلوده می کند اشتباه نمی شود(۱). هرچند که در بررسی بعضی از افراد ابتلاء زنان را ۲/۵ برابر مردان ذکر کرده اند. این بیماری در

سنین کمتر از ۱۰ سال نادر بوده و بیشتر در افراد بالای ۴۰ سال دیده می شود. هیره ماده در پایه موها در داخل فولیکولها تخم گذاری می کند و از تخم یک لارو ۶ پا خارج می گردد که پس از پوست اندازی به نمف و در پایان به هیره بالغ تبدیل می گردد. کلیه این مراحل حدود ۲ هفته طول می کشد(۲و۱).

تصویر۱. تعداد متعدد انگل در یک میدان میکروسکوپی با درشت نمائی× ۴۰

دمودکس ها می توانند باعث ایجاد درماتیت، جوش هایی شبیه آکنه، زرد زخم واگیردار و یا ورم پلک شوند ولی معمولاً به نظر نمی رسد که اثرات سوئی داشته باشند(۳). برای تشخیص بیماری می توان با استفاده از یک اسکالپل کند استریل شده نواحی مشکوک را خراشیده و تراشه ها را با استفاده از یک قطره گلسیرین یا یک محلول شفاف کننده مانند هیدروکسید پتاسیم در زیر میکروسکوپ بررسی نمود(۴). به طور معمول داروهایی که برای درمان بیماری مورد استفاده قرار می گیرند شامل: سلنیوم سولفید ۰/۵٪، بالزام پرو ۵٪، پماد گوگرد ۱۰٪، مترونیدازول و دیگر داروها می باشد، که معمولاً بمدت ۲ هفته مورد استفاده و کاربرد موضعی قرار می گیرند (۶و۵و۳).

گزارش مورد

دختر ۶ ساله، ساکن شهرستان بابل در مرداد ماه ۱۳۸۴ به دنبال خارش در ناحیه پس سر برای تشخیص عفونت قارچی به بخش انگل شناسی و قارچ شناسی دانشکده پزشکی بابل معرفی

شد. که پس از بررسی لازم و تهیه نمونه از ناحیه سر بیمار هیره دمودکس در زیر میکروسکوپ به میزان بسیار زیاد مشاهده گردید. ضایعات مشاهده شده در ناحیه Lateral -occipital چپ تا ناحیه پس سری بصورت لکه بزرگ و چندین ضایعه کوچک اقماری متوسط و کوچک باضایعات هیپرکراتوتیک، کروت، شوره سفیدفراوان با خارش خفیف بدون ریزش مو همراه بود(تصویر ۲).

تصویر ۲. تصویر ناحیه پس سری جانبی بیمار با شوره های فراوان در ناحیه مبتلا

بیماری وی از ۴ ماه قبل شروع شد و زمانی که مادرش مشغول شستشوی سر او بود متوجه شوره های فراوان در ناحیه سر شده که با مراجعه به پزشک تحت درمان با داروهای ضد قارچی قرار گرفت که موثر واقع نگردید و سپس با تشخیص بالینی آلرژی و درماتیت تماسی مورد درمان قرار گرفت. به علت عدم بهبودی از طرف پزشک برای تشخیص عفونت قارچی به آزمایشگاه معرفی گردید و بعد از نمونه برداری در آزمایشگاه وجود هیره دمودکس به تعداد بسیار زیاد (۵-۳ عدد و در هر میدان میکروسکوپی) و دسته های ۴ تا ۵ تایی و بعضی مواقع منفرد در زیر میکروسکوپ مشاهده گردید.

بحث و نتیجه گیری

هیره دمودکس ازجمله هیره های غیر شایع در آلودگی های انسانی محسوب می گردد این جنس دارای گونه های بسیار متفاوت می باشد که در حیوانات بخصوص عوارض زیادی را ایجاد می کند.

این انگل را یکی از عوامل مهم در ایجاد درماتیت روزاسه می دانند در یک مطالعه نشان داده شد که در ۸۶٪ بیماران مبتلا به درماتیت روزاسه دمودکس فولیکولاروم از این بیماران جدا گردید(۷و۵و۲). این هیره درحیوانات مختلف به ویژه در سگ ضایعات شدیدتری را ایجاد می کندکه این موضوع اهمیت ارتباط افراد را با حیوانات دست آموز در انتقال این آلودگی به انسان بیش از پیش نمایان می سازد(۹و۸).

گزارش حاضر در مورد دختر ۶ ساله است که تنها در سر دارای ضایعات هیپرکراتوز اکسفولیاتیو بوده و هیچگونه ضایعه مشخص دیگری در این فرد در معاینه مشهود نبود. با بررسی های بعمل آمده بیمار هیچگونه بیماری زمینه ای نداشته و از سلامت کامل نیز برخوردار بود. دمودکوزیس قادر به ابتلا در هر دو جنس می باشد هر چند که بعضی از مطالعات میزان ابتلا زنان را ۲/۵ برابر مردان ذکر کردند(۱۰و۴). شکایت اصلی بیمار مذکور پوسته های فراوان، خارش مختصر و خفیف در سر و کاهش محسوس در موی سر بوده که پس از بررسی پوسته های سر هیره های دمودکس وضوح و به تعداد زیاد در زیر میکرسکپ مشاهده گردید.

وجود دمودکس فولیکولاروم در بیماریهائی نظیر آکنه روزاسه آ در ایران نیز همانند دیگر بررسیهای محققان اثبات شده است. مروج و دهقان طی مطالعه ای در سال ۱۳۸۳ نشان دادند که میزان مایت

دمودکس در افراد مبتلا به آکنه روزاسه آ در مقایسه با گروه شاهد بطور معنی داری افزایش داشت(۱۲و۱۱). در مطالعه دیگری که توسط Wesolowska در سال ۲۰۰۵ میلادی صورت گرفت نشان داده شد که نقش دمودکس فولیکولارم و دمودکس برویس در ایجاد بیماری در صورت و سر افراد مسن بسیار شدید ترو بیشتر بود(۱۳). Pena و همکارانش در یک گزارش موردی از یک زن ۳۸ ساله که مشکوک به درماتیت روزاسه آ بود تعداد بسیار زیادی دمودکس فولیکولاروم جدا کرده و آنرا عامل ایجاد بیماری در این فرد ذکر کردند(۱۵و۱۴).

با توجه باینکه احتمال انتقال این جرب از طریق تماس مستقیم و استفاده از لوازم مشترک نظیر کلاه – شانه و روسری یا مقنعه های مشترک وجود دارد لازم است ضمن پیگیری، احتمال ابتلا سایر افراد خانواده و نیز درمان آنها مورد توجه قرار گیرد. بنابراین لازم است که دمودیکوزیس را به عنوان یکی از موارد تشخیص افتراقی درماتیت ها مد نظر قرار داد.

تقدیر و تشکر

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Paper 13

دمودکس فولیکولاروم و روزاسه

■ دمودکس فولیکولوروم و روزاسه

دکتر مهدي پیله ور ، دکتر عباس زمانیان ، دکتر علیرضا منصف ، مهندس خسرو ماني کاشاني.   ص 1

▼ چکیده

نقش دمودکس فولیکولوروم در تعدادي از مطالعات باليني در روزاسه گزارش شده است. از آنجائیکه دمودکس فولیکورروم پارازیت اجباري فولیکول مو مي باشد ممکن است افزایش تعداد آن بیماریزا باشد. در این مطالعه نقش احتمالي دمودکس فولیکولوروم و اهمیت تعداد آن در روزاسه مورد بررسي قرار گرفت.

یک مطالعه توصیفي مقایسه اي گذشته نگر روي 80 مورد بیوپسي از پوست صورت در طي پنجسال (1377 ـ 1372) در بخش پوست بیمارستان سینا همدان که تشخیص روزاسه و غیر روزاسه داشتند انجام گردید. این بیماران شامل 39 مورد روزاسه و 41 مورد غیر مبتلا به روزاسه بوده که از نظر سن و جنس مشابه بیماران روزاسه بودند و محل بیوپسي در دو گروه مبتلا و غیر مبتلا ناحیه صورت بود. از هر نمونه چهار برش مختلف تهیه و میانگین دمودکس در هر دو گروه تعیین گردید. نتایج حاصله با نرم افزار EPI6 مورد تجزیه و تحلیل قرار گرفت و با آزمونهاي آماري 2 و t تست گردیدند.

میانگین دمودکس پوست صورت در مبتلا یان به روزاسه 1/ 103 و براي افراد غیر روزاسه اي 171/0 بود که این اختلاف از نظر آماري معني دار بود ($P < 0.05$) . در مردان و زنان مبتلا به روزاسه و درماتوزهاي غیر روزاسه از نظر میانگین دمودکس اختلاف معني دار وجود نداشت ($P > 0.05$). میانگین تعداد دمودکس در افراد مبتلا به روزاسه بالاي چهل سال در مقایسه با افراد زیر چهل سال اختلاف معني دار داشت($P < 0.05$) . در این مطالعه میانگین دمودکس در افراد روزاسه اي از میانگین گروه کنترل بیشتر بود. ولي جهت تعیین نقش دقیق دمودکس در روزاسه احتیاج به مطالعه آینده نگر با گروه کنترل در پوست نرمال مي باشد.