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MAIN ARTICLE

Isolated itching of external auditory canal: clinicopathological study with immunohistochemical determination of antimicrobial peptides

B ACAR[1], G GÜLER SIMSEK[2], S OGUZTUZUN[3], M ZAIM[1], R MURAT KARASEN[1]

[1]*Departments of Otorhinolaryngology,* [2]*Pathology, Kecioren Training and Research Hospital, Ankara, and* [3]*Department of Biology, Kırıkkale University, Turkey*

Abstract

Objective: This study aimed to demonstrate the histological and immunohistological features of skin biopsy specimens from patients complaining of isolated itching of the external auditory canal.

Materials and methods: A prospective, case–control study was performed of 24 patients undergoing evaluation for contact dermatitis of the external auditory canal, and 24 controls. Skin biopsies were examined histologically by a single, blinded dermatopathologist, using light microscopy, to determine histopathological characteristics. The immunohistological presence of the antimicrobial peptides human β-defensin-3 and LL-37 cathelicidin was also assessed. Findings for patients and controls were compared.

Results: There was a statistically significant difference in the degree of inflammation, comparing patients and controls ($p < 0.05$). There was no significant difference in the presence of spongiotic changes, comparing patients and controls ($p > 0.05$). Furthermore, the patients' skin biopsies did not show pronounced expression of human β-defensin-3 or LL-37 cathelicidin.

Conclusion: Histological and immunohistological examination of skin biopsies from cases of isolated itching of the external auditory canal did not support a diagnosis of dermatitis.

Key words: External Auditory Canal; Otitis Externa; Pruritus

Introduction

Isolated itching of the external auditory canal is diagnosed in cases with no identifiable aetiology, e.g. diabetes mellitus, hepatic and renal conditions, or lymphoma, leukaemia or other malignancies. A few clinical trials have investigated such primary ear itching, although it is the complaint most frequently encountered by otolaryngologists.[1] Isolated itching of the external auditory canal occurs mostly in middle-aged women.[2] Physical examination of the external auditory canal is generally normal, and there are few treatment modalities available to relieve the itching. Therefore, treatment is unsuccessful in some patients, and pruritus persists or recurs. Some authors believe that patients with pruritic ears probably also suffer from allergic contact dermatitis.[2]

Skin biopsy is one of the most important diagnostic tools in cases of dermatoses such as allergic contact dermatitis. In cases of allergic contact dermatitis, histopathological examination may reveal the presence of background acanthosis, perivascular lymphocytic infiltration, dermal eosinophilia or hyperkeratosis.[3]

Antimicrobial peptides play important roles in the human immune defence system, activating inflammatory cells and regulating adaptive immunity.[4,5] In human skin, the β-defensins and cathelicidins have been the most extensively studied families of antimicrobial peptides. Antimicrobial peptides contribute to the immune defence against skin pathogens by direct antimicrobial activity. Previous studies have demonstrated that cathelicidins are produced by the eccrine apparatus and secreted in human sweat.[6] The LL-37 cathelicidin was first isolated from neutrophils, and is also secreted by keratinocytes in inflammatory skin disorders.[7,8] Human β-defensin-3 is inducibly expressed by human lesional psoriatic scales and cloned keratinocytes.[8]

This study aimed to compare the histopathological appearance and LL-37 and human β-defensin-3 content in skin biopsy specimens from patients with

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isolated itching of the external auditory canal, and from normal controls.

Materials and methods

Patients and design

Before commencement, approval for the study was obtained from the insitution's ethics committee.

The study included 24 female patients complaining of recurrent external auditory canal itching and scheduled to undergo skin biopsy, and 24 female controls with conductive deafness associated with dry perforation, without ear pruritus. The study subjects and controls were aged between 33 and 45 years. In the study group, skin biopsies were taken from the outer part of the external auditory canal. In the control group, skin biopsies were taken during tympanoplasty, from the tympanomeatal flaps. Excisional biopsies were taken, including epidermis and dermis but not subcutaneous tissue.

Subjects were examined for coexisting atopic and contact dermatitis, by an experienced dermatologist in a public medical centre, according to a routine procedure. Skin patch testing was also performed.

Exclusion criteria for the patient group comprised: (1) a diagnosis of contact dermatitis and other dermatoses; (2) systemic disorders such as atopic dermatitis; (3) otoscopic evidence of fungal or bacterial infection, tympanic membrane perforation, or other middle-ear pathology; (4) long-term topical steroid use; (5) self-treatment with topical agents for intermittent ear discharge; and (6) a positive skin patch test for a contact allergen. Exclusion criteria for the control group comprised otorrhoea, ear itching and self-treatment with a topical ear agent for any complaint.

Histopathological examination

Prepared microscopy slides from the previously performed skin biopsies were evaluated in a blinded fashion by a single dermatopathologist, using light microscopy. Biopsy slides were stained with haematoxylin and eosin.

The histological characteristics assessed included: acanthosis, hyperkeratosis, spongiosis, lymphocytic infiltrate, dermal and epidermal acute and chronic inflammatory cells (e.g. eosinophils, neutrophils and lymphocytes), lymphocyte exocytosis, and dyskeratosis. Characteristics were noted to be present or absent.

Immunohistochemical examination

Biopsy tissue was fixed in 10 per cent buffered formalin and embedded in a paraffin block. Sections of 4 mm thickness were cut. One section in each block was stained with haematoxylin and eosin to show tissue morphology.

For immunohistochemical analysis, endogenous peroxidase activity was blocked by incubating the sections in 1 per cent hydrogen peroxide (volume for volume) in methanol for 10 minutes at room temperature. Sections were subsequently washed in distilled water for 5 minutes, and antigen retrieval was performed for 5 minutes using 0.01 M citrate buffer (pH 6.0) in a domestic pressure cooker. The sections were then transferred to 0.05 M Tris-HCl (pH 7.6) containing 0.15 M sodium chloride. After washing in water, the sections were incubated at room temperature for 30 minutes with normal swine serum (for anti human β-defensin-3 and LL-37) diluted 1:20 in 0.05 M Tris-HCl containing 0.15 M sodium chloride, to block non-specific binding. The sections were then covered with the primary antibodies against human β-defensin-3 and LL-37 (Phoenix Pharmaceuticals Inc., Phoenix, Arizona, USA), diluted 1:500 in 0.05 M Tris-HCl containing 0.15 M sodium chloride, and left at 4°C overnight. After washing in 0.05 M Tris-HCl containing 0.15 M sodium chloride for 15 minutes, sections were incubated at room temperature for 1 hour with secondary antibody (swine anti-rabbit immunoglobulin-biotinylated) at a dilution of 1:100. Sections were then treated with avidin-biotin peroxidase complex (Dakopatts, Glostrup, Denmark). Diaminobenzidine was used to visualise peroxidase activity in the tissues. Nuclei were lightly counterstained with haematoxylin. Sections were then dehydrated and mounted.

Both positive and negative controls were included in each run. Positive controls for human β-defensin-3 and LL-37 consisted of sections of tonsillitis tissue. In the negative controls, 0.05 M Tris-HCl containing 0.15 M sodium chloride was used in place of the primary antibody.

The immunohistochemically stained sections were examined under light microscopy by a pathologist blinded to patients' clinical information. The distribution, localisation and characteristics of immunostaining were recorded. The presence of a brown colour in the cytoplasm of epithelial cells was evaluated as positive staining.

In addition, the intensity of the immunoreaction was scored by both pathologists. For each antibody, the immunoreaction intensity was scored using a semi-quantitative scale, as negative, mildly positive, moderately positive or strongly positive. Scoring differences between the observers were resolved by consensus.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences version 15.0 for Windows software program. The independent sample *t*-test was used to compare age between groups. The chi-square and Fisher's exact tests were used for statistical evaluation. A level of $p < 0.05$ was used as the threshold value to indicate statistical significance.

Results

We identified as potential study subjects 24 female patients complaining of ear itching, and 24 controls without ear pruritus, evaluated between 1 January and 1 November 2009. (Isolated itching of the external

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auditory canal is seen more frequently in women; thus, only female subjects were enrolled, in order to homogenise our subject group.)

There were no statistically significant differences between the two groups with respect to age.

Histopathological examination showed no evidence of allergic contact dermatitis (e.g. acanthosis, hyperkeratosis, spongiosis and/or active inflammation) in either group. The patient group showed no statistically significant differences regarding histopathological characteristics (i.e. lymphatic dilation, dermal hyalinisation and vascular ectasia) or immunohistochemical findings (i.e. for human β-defensin-3 and LL-37), compared with the control group ($p > 0.05$). However, a significantly greater degree of inflammation was found in the patient group (37.5 per cent) compared with the control group (12.5 per cent) ($p = 0.048$).

All data are presented in Table I.

Discussion

Isolated itching of the external auditory canal comprises itching without any obvious skin changes, other than those induced by scratching. Some authors have termed this condition 'itchy ear syndrome'.[9] Isolated itching of the external auditory canal must be distinguished from all other forms of pruritus for which an underlying cause can be ascertained. Pruritus may be a major manifestation of diabetes mellitus, hepatic and renal conditions, and lymphoma, leukaemia and other malignancies. Therefore, it must be emphasised that underlying causes must be actively sought and excluded in each case. Primary pruritus of the ear appears to be more common in middle-aged and elderly women, compared with younger women. Compulsive scratching may lead to secondary otitis externa due to bacterial infection.

TABLE I
DATA FOR PATIENTS AND CONTROLS

Parameter	Patients*	Controls†	*p*
Age (mean ± SD; y)	39.3 ± 3.7	39.1 ± 3.2	0.78
Lymphatic dilation (*n* (%))	9 (37.5)	4 (16.7)	0.108
Dermal hyalinisation (*n* (%))	2 (8.3)	6 (25)	0.125
Inflammation (*n* (%))	9 (37.5)	3 (12.5)	0.048
Vascular ectasia (*n* (%))	1 (4.2)	0 (0)	0.317
HBD3 staining (*n* (%))			
– Negative	16 (66.7)	19 (79.2)	0.466
– Mildly +ve	7 (29.2)	2 (8.3)	
– Moderately +ve	2 (8.3)	2 (8.3)	
– Strongly +ve	1 (4.2)	1 (4.2)	
LL37 staining (*n* (%))			
– Negative	18 (75)	18 (75)	0.913
– Mildly +ve	2 (8.3)	1 (4.2)	
– Moderately +ve	2 (8.3)	2 (8.3)	
– Strongly +ve	2 (8.3)	3 (12.5)	

*$n = 24$; †$n = 24$. SD = standard deviation; y = years; HBD3 = human β-defensin-3; +ve = positive

Most otolaryngologists treat external auditory canal itching with ear drops containing a keratolytic substance, 2 per cent salicylic acid in alcohol, and low-potency topical steroid.[10] If pruritus interferes with sleep, antihistamines may be helpful. When no cause for itching has been established, treatment remains largely symptomatic, and this contributes to treatment failure and recurrence. Treatment failure is worrying for both patient and clinician.

In most patients with essential pruritus, the original condition is believed to be allergic contact dermatitis.[1] Allergic contact dermatitis occurs in individuals previously exposed to and immunologically sensitised to a particular chemical (e.g. hair care product constituents such as cocamidopropyl betaine, fragrances, proteins, lanolin, parabens or formaldehyde). Haptens also activate keratinocytes to secrete immunomodulatory cytokines. The clinical result is itching and irritation of the skin.

Dermatopathology textbooks have traditionally classified allergic contact dermatitis under the histological spectrum of 'spongiotic dermatitis'.[11] The histological characteristics of standard allergic contact dermatitis typically include acanthosis, hyperkeratosis, spongiosis, psoriasiform changes, and an active cellular infiltrate rich in eosinophils. None of our subjects' histopathological slides showed spongiotic changes, in either group. Inflammatory changes were present in the patient group, but these were minimal and composed of lymphocytes. Patients with the finding of lymphatic dilation were 37.5 per cent in all cases. In the absence of other histopathological findings, such a finding does not support a diagnosis of dermatitis. Such inflammatory changes may indicate previous chronic infection of the outer meatus, e.g. external otitis media or folliculitis. In no cases did we detect neutrophils or eosinophils, indicators of acute inflammation. The absence of these active agents of acute inflammation excludes allergic contact dermatitides and other acute dermatitides.

The antimicrobial peptides are involved in many immune defence functions, and play a role in some inflammatory skin disorders such as psoriasis and dermatitis.[8]

Human β-defensin-3 is a recently described epithelial antimicrobial peptide which effectively kills a broad spectrum of multi-drug-resistant bacteria. Its messenger RNA has been detected in primary keratinocytes and other human epithelial cells. Expression of human β-defensin-3 is significantly induced by inflammatory stimuli in the stratum corneum.[12]

Another antimicrobial peptide, the cathelicidin LL-37, is also produced by epithelial cells, neutrophils and macrophages.[7,13] Marchini *et al.*[14] identified the presence of LL-37 in inflammatory skin disorders involving hyper- and parakeratosis and acanthosis.

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Therefore, in addition to histopathological examination of external auditory canal skin biopsies, the current study aimed to assess the association between allergic contact dermatitis and immunohistochemical staining for human β-defensin-3 and LL-37. However, we could find no clear evidence for significant involvement of either human β-defensin-3 or LL-37; both patient and control groups had a similar intensity of positive staining for both these antimicrobial peptides.

- **Isolated itching of the external auditory canal involves itching without any obvious skin changes or identifiable aetiology**
- **The condition mostly occurs in middle-aged women**
- **In such cases, histopathological and immunohistochemical examination of external auditory canal skin biopsies did not support a diagnosis of dermatitis**
- **In most patients with this condition, other causes should be considered (e.g. psychological factors)**

Our results provide no clear histopathological evidence to indicate the presence of allergic contact dermatitis in patients with isolated itching of the external auditory canal. Therefore, other aetiologies should be considered in such patients, such as psychological factors (e.g. somatisation disorder).

Conclusion

Histological and immunohistochemical examination of skin biopsies from patients with isolated itching of the external auditory canal did not support a diagnosis of dermatitis. We believe that further studies of such patients are required, particularly in order to determine the incidence of somatisation disorder.

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Address for correspondence:
Dr Baran Acar,
Department of Otorhinolaryngology,
Kecioren Training and Research Hospital,
Pinarbasi mahallesi Sanatoryum caddesi Ardahan sok no 1,
Kecioren 06310,
Ankara, Turkey

Fax: + 90 312 356 90 22
E-mail: drbaranacar@gmail.com

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