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Antinuclear antibodies in postinfectious smell loss - a pilot study*

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Abstract

Background: Numerous diseases are mediated by autoimmune mechanisms some of which have been shown to be associated with reduced olfactory function, e.g. systemic lupus erythematosus, Sjögren syndrome, psoriasis vulgaris. Postinfectious smell loss is a frequent cause of olfactory dysfunction. However, its nature is not fully understood, but connections to immune mediated processes might be possible. The primary aim of the present study was therefore to investigate the possible relation of postinfectious smell loss with autoimmune processes.

Methods: Fifty-two patients (35 female, 17 male; mean age 57 years ± 12 years) with postinfectious smell loss and 56 controls (21 female, 35 male; mean age 58 years ± 13 years), with olfactory dysfunction of other causes (sinunasal, posttraumatic or idiopathic) were included in the present study. The patients' olfactory function was measured using the extended test battery of the "Sniffin' Sticks". Furthermore venous blood samples were taken and analyzed for antinuclear autoantibodies.

Results: Patients showed anti-nuclear antibodies (ANA) significantly more frequently compared to controls, although ANAs were typically only present at cut off levels of 1:100. In addition, there was a significant association between the presence of ANAs and odor thresholds.

Conclusions: Results from this study indicate that postinfectious olfactory dysfunction may be associated with autoimmune processes, as they show significantly more ANAs – although not at a clincially significant level - compared to patients with olfactory deficits of other origin.

Key words: antinuclear antibodies, olfaction, autoimmune disease, smell, auto antibodies

Introduction

The sense of smell is unique and important, as a decreased sense of smell may lead to various impairments such as a reduced quality of life [(1)], distorted flavor perception [(2)] and changes in dietary behavior [(3)]. Furthermore, an intact olfactory system is crucial to protect subjects from harm, such as fire, smoke, eating rotten food [(4)]. Causes of reduced olfactory function vary and may be divided in sinunasal (72%) and non sinunasal origin. Of the non sinunasal causes postinfectious smell loss is with 11% the most frequent [(5)].

Neurological disorders and autoimmune processes have been shown to be associated with impairments of olfactory function [(6,7)]. In several autoimmune diseases [(8)] decreased olfactory function has been shown, e.g. systemic lupus erythematosus [(9)], pemphigus vulgaris [(10)], psoriasis vulgaris [(11)], Sjögren syndrome [(12)] and Wegener's disease [(13, 14)]. In addition, reduced olfactory function has been shown in several other diseases, which are discussed to be mediated by autoimmune processes, e.g. multiple sclerosis [(15)].

Animal models such as olfactory bulbectomy in rats were used to highlight the association between immune-mediated processes, depression and olfaction. Bulbectomy led to a reduced olfactory function, induced depression and changed cellular and humoral immunity [(16)]. Other animal models could show

impaired olfactory function and depressive mood induced by the injection of anti-ribosomal antibodies [17, 18]. These antibodies have been shown to be able to bind to and penetrate neuronal cells [19]. Therefore, the hypothesis of the present study was that the presence of autoantibodies (ANAs) influences the olfactory function negatively.

There is a plethora of different autoantibodies, e.g. tissue specific ones or antinuclear antibodies (ANA), which are found in many autoimmune diseases. The interpretation of ANA presence depends on both titer and pattern, furthermore it needs to be taken into account that ANA are also found in healthy individuals with a prevalence in the general population of 13.8% [20] and an even higher perecentage of affected healthy people above the age of 80 years [21].

Therefore the aim of the present study was to investigate the possible relation of patients with postinfectious smell loss and autoimmune processes.

Material and Methods

This prospective pilot study study was performed in the Smell and Taste Clinic at the Department of Otorhinolaryngology-Head and Neck Surgery, "Technische Universität" Dresden after it had been approved by the local ethics committee (EK 189062013). It has been conducted according to the Declaration of Helsinki. Patients were given detailed information about the study and written informed consent was obtained.

Patients

Fifty-two patients with postinfectious smell loss and 56 controls with olfactory dysfunction of other origin were included in the present study. Postinfectious smell loss was diagnosed from medical history and after exclusion of sinunasal causes of the smell loss. Prior to inclusion in this study, all patients underwent a thorough ENT examination, including nasal endoscopy. Exclusion criteria were as follows: age under 18, pregnancy or breast feeding period, intake of anticoagulants, the existence or suspected coagulation disorder and the presence of autoimmune diseases such as systemic lupus erythematodes, sjoegren syndrome.

Study protocol

Patients' olfactory function was measured once. To avoid chemosensory desensitization, participants of the study were asked to not smoke, eat, or drink anything other than water for 1 hour prior to all measurements. Furthermore, a blood sample of 10ml was taken from all participants.

Assessment of orthonasal olfactory function

Olfactory function was analyzed by means of the validated, standardized "Sniffin' Sticks" extended test battery [22, 23] (Burghart GmbH, Wedel, Germany).

This test combines the phenyethlalcohol (PEA) odor threshold (THR), odor discrimination (DIS) and 16-item odor identification subtest (ID). It is based on pen-like odor-dispensing devices [22]. For odor testing procedure the cap of the pen was removed and the felt-tip was presented for approximately 3 sec, 2 cm in front of the subjects' nostrils. Measurements started with the threshold subtest, where 16 different PEA concentrations were used. The participants were presented a triplet of pens, of which only one contained the odor (PEA-rose like smell), while the other two pens were without odor, containing the solvent, propylene glycol, alone. The presentation of the triplets started with the lowest concentration and was finished – in case of anosmic subjects - with the highest one. Testing procedure was performed in a staircase manner: after identifying the odor containing pen twice in a presented triplet, a reversal of the staircase was started until the patient could no longer identify the odor containing pen. The threshold score was built from the mean of the last four out of seven staircase reversals.

For the DIS subtest, also 16 triplets were used, with two pens containing the same odor and the third a different one. The participants' task was to identify the different odor.

Lastly, the odor ID test was performed which consists of 16 pens. Here, participants were asked to match the odor and the corresponding verbal descriptor presented in a list of 4 desciptors each.

Gustatory screening test

Gustatory function was screened with the "taste sprays", which contain suprathreshold tastants sprayed onto the tongue. They had to be identified as sweet, sour, salty or bitter [24]. This test provided information whether the patient was able to recognize and differentiate different taste qualities [25].

Blood samples

Blood samples were collected from all subjects. Serum was immediately separated and stored at -30°C until the blood analysis was performed. Commercially available indirect immofluorescence tests (Euroimmun, Lübeck, Germany) were used to detect total antinuclear antibodies (ANA) [26], on human-epithelial type 2 cells (HEp-2-cells). Titers <1:100 were considered negative. In addition the following tissues and cells were tested for immunlobulin AGM: $thyroid_{monkey}$, $parathyrdoid_{monkey}$, $adrenal\ gland_{monkey}$, $ovarium_{monkey}$, $placenta_{monkey}$, $testis_{monkey}$, $pituitary\ gland_{monkey}$, $hypothalamus_{monkey}$, $pancreas_{monkey}$, $liver_{monkey}$, $kidney_{monkey}$, $lung_{monkey}$, $esophagus_{monkey}$, $urinary\ bladder_{rat}$, $stomach_{monkey}$, gut_{monkey}, $parotid\ gland_{monkey}$, $spermatozoa_{human}$, crithidia lucillae, $granulocytes_{human}$, $thrombocytes_{human}$, $lymphocytes_{human}$, $neurons_{monkey}$, $cerebellum_{monkey}$, $cerebrum_{monkey}$, $spinal\ cord_{monkey}$, $skeletal\ muscle_{monkey}$, $cardiac\ muscle_{monkey}$, HEp-2-cells, $kidney_{rat}$, $stomach_{rat}$, $liver_{rat}$

Table 1. Comparison of olfactory scores between patients and controls.

	Patients	Controls	p value
Threshold part	3.5 ± 2.6	3.0 ± 3.0	0.43
Discrimination part	9.5 ± 2.8	7.1 ± 3.1	<0.001
Identification part	8.8 ± 3.5	6.3 ± 3.3	0.002
TDI score	21.9 ± 7.6	15.8 ± 6.6	<0.001

Table 2. Distribution of auto antibodies in patients versus controls.

	Patients		Controls		P value
	<1:100	≥1:100	<1:100	≥1:100	
ANA against HEp2cells	23	29	46	10	<0.01
Anti-Myelin	51	1	56	0	0.48
pANCA	51	1	56	0	0.48
IF DNA-ANCA	51	1	56	0	0.48
Anti-vascular endothelial IgG	50	2	56	0	0.23
Thyroglobulin IgG	49	3	55	1	0.35
Ovary	51	1	56	0	1.00
Spermatozoa	51	1	54	2	1.00
Stomach	50	2	54	2	1.00
Neutrophils	50	2	54	2	0.68
Skeletal muscle	48	4	49	7	0.53
Kidney	51	1	56	0	0.48

Table 3. Comparison of olfactory scores between patients with ANAs and controls without ANAs.

	Patients with ANAs	Patients without ANAs	p value
Odor threshold	1.2 ± 0.5	3.5 ± 3.2	0.001
Odor discrimination	5.3 ± 3.2	7.6 ± 2.9	0.056
Odor identification	5.3 ± 3.5	6.6 ± 3.2	0.31
TDI score	12.0 ± 7.1	16.7 ± 6.3	0.040

Statistical analysis

All statistical analyses were performed using the program SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Significance level was set at $p<0.05$. The Fisher exact test and the Chi-squared tests were used for non-parametric statistics and t-tests for parametric statistics were used wherever appropriate.

Results

Patient characteristics

108 participants were included between May 2014 and November 2016 in the Smell and Taste clinic at the Department of ORL of the TU Dresden. 52 of all participants (35 female, 17 male; mean age 57 years ± 12 years) were patients with postinfectious smell loss (further referred to as "patients"), and 56 subjects (21 female, 35 male; mean age 58 years ±13 years) were controls suffering from a smell disorder of other origin (sinunasal, post-traumatic or idiopathic; further addressed as "controls"). Mean duration of the disease was 20.9 ± 53.5 months in patients and 54.3 ± 70.1 months in controls.

Analysis of olfactory function

There were significant differences in olfactory function (DIS, ID, TDI) between patients and controls. Patients scored significantly better compared to controls in DIS, ID subpart, resulting in a significantly higher TDI score (Table 1).

Analysis of anti-nuclear antibodies

There was a significant difference for the presence of antinuclear antibodies between patients and controls. Patients showed significantly more frequently antinuclear antibodies than controls with an odds ratio of 3.65 (23 patients with ANAs vs 29 without, and 10 controls with ANAs vs 46 without). This was true for ANA (Table 2). There was no significant relation between the presence of ANAs and the sex ($p=0.11$), presence of phantosmia/parosmia ($p>0.055$). Olfactory function was associated with the presence of ANAs (THR $p=0.001$; TDI $p=0.04$) (Table 3). However, this was not the case for the discrimination and identification part of the Sniffin Sticks test (DIS $p=0.06$; ID $p=0.31$).

Discussion

The major result from this study was that, when compared to controls, patients with postinfectious olfactory loss exhibited significantly more frequently antinuclear antibodies (ANAs) although it has to be kept in mind that the effect was only found for a reatively low level of 1:100 which is clinically not significant. In immune dysregulation autoantibodies are present, e.g. ANAs. ANAs are also be found in healthy subjects, especially in individuals >80 years with a frequency of 31% [21]. In the present study 50% of patients with postinfectious olfactory loss showed ANAs. These results suggest that postinfectious smell loss might be related to immune mediated processes as reduced olfactory function has been already shown in several autoimmune meditaed diseases such as multiple sclerosis [27], pemphigus vulgaris [10], psoriasis vulgaris [11] and Sjögren syndrome [12]. Interestingly, autoimmune diseases are linked with the major histocompatibility complex genes (MHC) [28]. In their vicinity several human olfactory receptor-genes (MHC-linked olfactory receptor genes) are found [29]. In line with this, the presence of ANAs (THR $p=0.001$; TDI $p=0.04$) in this study was shown to be associated with reduced values in the Sniffin' Sticks THR and TDI scores in patients. This was not true for the discrimination and identification part of the Sniffin Stick's test (DIS $p=0.06$; ID $p=0.31$) though, possibly indicating that autoimmune processes

mostly affect the periphery of the olfactory system.
One limitation of the present study is the inhomogenous sex distribution among the two groups (patients vs controls), as women have been shown to exhibit higher ANA levels compared to men [30, 31]. A further limitation is that we have not compared patients with a group of people without any olfactory disorder. Further studies should also question patents about the subjective severity of olfactory loss – apart form the psychophysical assessment of olfactory function with olfactory tests – and use longer follow-up periods.
Although it has to be maintained that the titer of the autoantibodies was at a subclinical level, still the presence of autoantibodies might provide a meaning. Future studies should address the possible predictive value of these changes in relation to the prognosis of patients with olfactory loss.

Conclusion

In conclusion, this study suggests that autoimmune processes may be associated with postinfectious smell loss, as these patients show significantly more ANAs compared to patients with olfactory deficits of other origin.

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Authorship contribution

UWD was part of the study design, analysed data, wrote the manuscript; AMZ designed the study, collected data, helped writing the manuscript; TH designed the study, analysed data, wrote the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interests regarding the content of this manuscript.

Ethics approval and consent to participate

Approved by the local ethics committee (EK 189062013). Patients were given detailed information about the study and written informed consent was obtained.

Consent for publication

Not applicable.

Availability of data and materials

Data will be made available upon request.

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