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The presence of ovarian hormone receptors in the nasal mucosa and their relationship to nasal symptoms*

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SUMMARY

Background: *There is evidence in the literature showing a link between ovarian hormones and changes to nasal physiology.*

Objectives: *The aim of this pilot study was to identify and quantify female hormone receptor positive cells in the nasal mucosa and to establish if there is a correlation with rhinitic symptoms.*

Methods: *Twenty-five adult patients attending a university hospital for routine, elective non-rhinological ENT procedures under general anaesthetic (mainly tonsillectomy) were recruited pre-operatively. Background information about each participant was recorded. Biopsies were taken from the inferior turbinates. These were analysed using immunohistochemistry techniques to assess for the presence of Progesterone, Oestrogen-α (ERα) and Oestrogen-β (ERβ) receptors.*

The mean number of cells positive for the receptors in each biopsy was deduced using a stratified random sampling technique.

Results: *All nasal biopsies were negative for progesterone and ERα receptors. ERβ receptors were present in the mucosal glands in 24 out of the 25 biopsies. Using unpaired t-tests to compare the sexes, smoking status and atopic history no statistical difference was shown between any of these groups ($p > 0.05$). However, the rhinitis quality of life questionnaire score and the mean number of ERβ receptor positive cells per biopsy showed a positive correlation (Pearson correlation of 0.4, $p < 0.05$).*

Conclusions*: The number of oestrogen receptor positive cells appears unaffected by sex, smoking history, hormone status, age or atopy. However, there is a significant positive relationship between the mean number of ERβ positive cells and nasal symptoms. Pharmacological down-regulation of ERβ positive cells may reduce rhinitic symptoms and is the subject of further research.*

Key words: oestrogen receptors, progesterone receptors, female hormones, oestrogen, progesterone, nasal mucosa

INTRODUCTION

Female hormones have been suspected of affecting nasal physiology for many years. As far back as the late nineteenth century it was observed that the menstrual cycle and pregnancy have an effect upon nasal vascularity and mucus secretion [(1-3)]. Recent studies have looked at the effect of menstruation and pregnancy upon nasal physiology [(4-12)]. The mechanism by which female hormones affect nasal physiology remains to be fully elucidated.

A relationship between the histological appearance of the nasal epithelium and phase of the menstrual cycle [(13)] has been shown with young epithelial cells during the menstrual phase, large flat cornified cells during the follicular stage (when hormone levels peak) and rounded or spindled epithelial cells during the luteal phase. It is not known whether or not this is a direct hormonal effect.

The purpose of this study was to determine the quantity of ovarian hormone receptors in the nasal mucosa and see whether there is any correlation with symptom scores, sex, smoking status and atopy.

Footnote: Accepted for poster presentation at the American Academy of Otolaryngology/Head & Neck Surgery, September 2005, Los Angeles

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METHODS

The local committee granted full ethical approval. Patients attending a university hospital for routine elective, non-rhinological ENT procedures under general anaesthetic such as tonsillectomies were recruited pre-operatively. Background information about each patient was recorded including past medical history, drug history and any history of atopy. Patients were then given a rhinitis quality of life questionnaire. The rhinitis quality of life questionnaire score is a validated interviewer-led questionnaire [14]; the higher the score the more symptomatic the patient. Following this the subjects were biopsied at the time of their surgical procedure and the samples were analysed in a dedicated laboratory for immunohistocytochemistry.

Immunohistochemistry

Nasal biopsies were taken from the inferior turbinates as this has been shown to representative of the lateral wall mucosa in the nose [15]; there were no complications as a result of taking these biopsies. The biopsies were fixed in formalin (10%) and paraffin embedded. To stain for ERβ receptors sections were placed on slides, de-waxed in xylene and rehydrated through graded alcohols to water. Slides were then transferred to citrate buffer and microwaved for 15 minutes to maximise antigen retrieval. After washing in water, endogenous peroxidase activity was blocked with 6% hydrogen peroxide in water. Slides were then washed in water and PBS-T20 (phosphate buffer solution – Tween20). Excess moisture was removed from the slides and they were placed in a humidity chamber and blocked with 1:10 normal swine serum for 30 minutes at room temperature. Avidin solution (Vector Laboratories, Peterborough, Cambs, UK) was added to each section for 15 minutes. This was washed off in PBS and followed by adding Biotin solution (Vector Laboratories) for 15 minutes; this Avidin-Biotin stage blocks endogenous biotin. Slides were then drained and incubated overnight at 4°C with 1:50 primary antibody diluted in blocking solution. Negative and positive controls were also prepared. The next day, slides were washed in PBS-T20 for 30 minutes and then incubated with diluted biotinylated swine anti-rabbit immunoglobulins (Upstate Biotechnology, Lake Placid, NY, USA) for 30 minutes. They were washed again in PBS-T20 and incubated with ABC Elite complex (Vector Laboratories) for 30 minutes and washed again in PBS-T20. Slides were then incubated with DAB (diaminobenzidine) (Vector Laboratories) stain for 5 minutes followed by a wash in water. The DAB stain was enhanced by immersion in copper sulphate in sodium chloride for 5 minutes. Slides were then washed in water and counterstained by immersion in haemotoxylin for 1 minute and washed in water again. Slides were finally dehydrated through graded alcohol and mounted.

Similar techniques were used to stain for progesterone and oestrogen-α receptors.

The slides were then assessed using an Axioplan microscope with 400x magnification (Carl Zeiss, Oberkochen, Germany) and images were then captured using a colour video camera (Sony CCD/RGB). The number of glandular cells positive for the receptors was counted and a mean number obtained for each biopsy using a stratified random sampling technique [16].

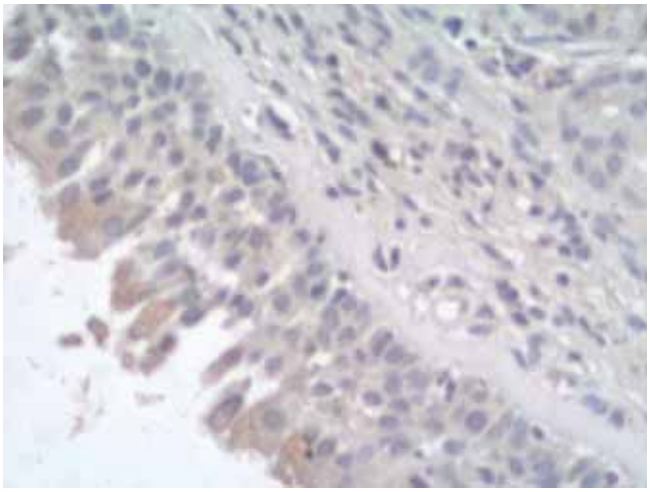

Figure 1. Microscopy of inferior turbinate biopsy with ERβ receptor positive cells in submucosal mucous glands (x400).

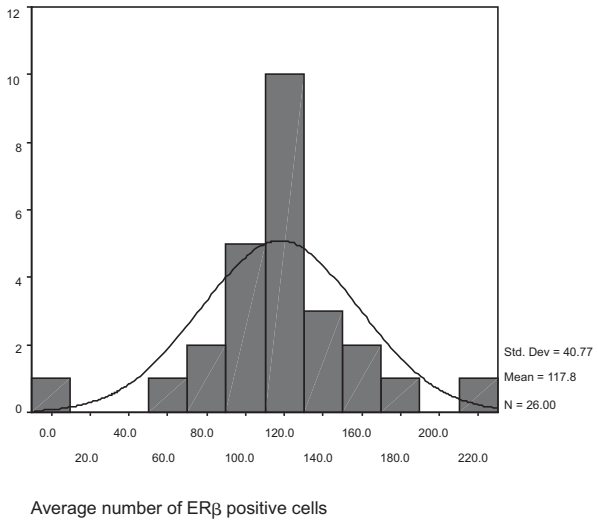

Figure 2. Histogram and normal distribution of ERβ positive cells.

Statistics

The subsequent data was then analysed in conjunction with the patient demographics using SPSS for Windows (SPSS version 14.0, Chicago, Illinois, USA).

RESULTS

Biopsies were taken from a total of 28 patients. Only 25 of the 28 biopsies were analysed as 2 contained no glandular tissue and the file notes were lost for one further patient. From the remaining 25 patients, 16 were male and 9 female ranging from 18 to 68 years with a mean of 41.8 years. In atopic terms, 8 suffered from eczema, asthma and/or hayfever and 17 were non-atopic. None of the nasal biopsies were positive for progesterone or oestrogen-α receptors. ERβ receptors were present

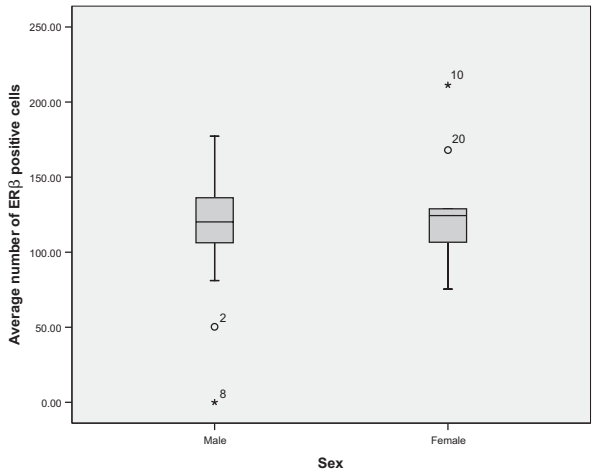

Figure 3. Boxplot comparing mean number of ERβ positive cells by sex.

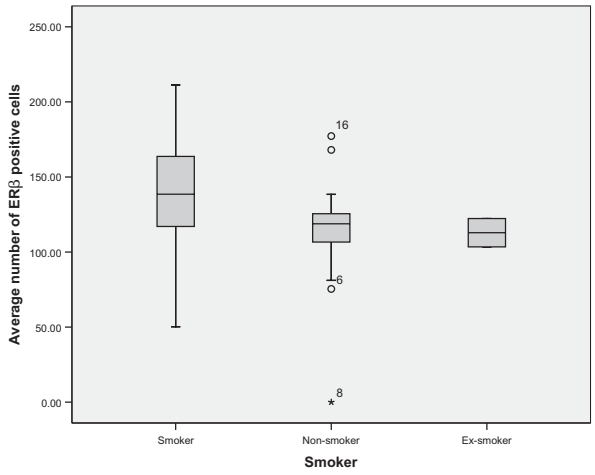

Figure 4. Boxplot comparing mean number of ERβ positive cells between smokers and non-smokers.

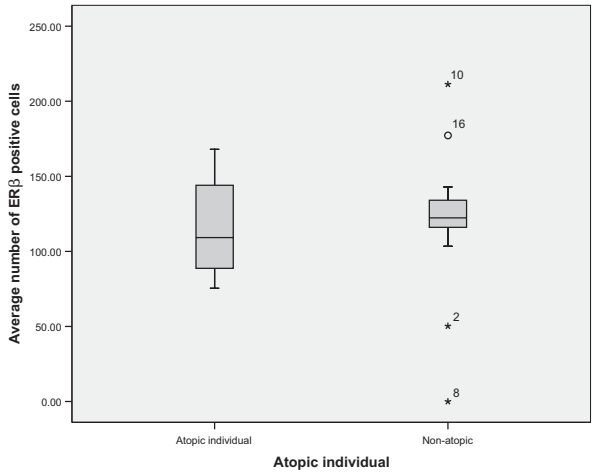

Figure 5. Boxplot comparing mean number of ERβ positive cells between atopic and non-atopic individuals.

in 24 out of 25 of the biopsy specimens. The ERβ receptor positive cells were found in the submucosal mucous glands (Figure 1). No ERβ receptors were found in the connective tissue or blood vessels. The average number of ERβ receptor positive cells found in the nasal biopsies is shown in Figure 2 and demonstrates a normal distribution. Box plots (Figures 3 - 5) illustrate the mean number of receptor positive cells comparing males and females, atopics and non-atopics, and smokers and non-smokers. Using unpaired t-tests to compare the groups there was no statistical difference between any of these groups ($p > 0.05$; Table 1).

There was also no significant relationship between age and ERβ receptor positive cells ($p = 0.531$; Figure 6, Table 2). However the rhinitis quality of life questionnaire scores (Table 3) showed a significant correlation with the mean number of ERβ receptor positive cells per biopsy with a Pearson correlation of 0.4 ($p = 0.043$; Figure 7, Table 2).

DISCUSSION

ERβ receptors have been positively identified and shown to have a potential relationship with rhinitic symptoms. ERα receptors were first discovered in 1958 by Jenson [17] whereas ERβ were discovered much more recently in 1996 [18]. A previous study in 1981 showed that oestrogen receptors are present in the normal nasal mucosa [19]. Following this low levels of oestrogen-receptor-like activity were found in 50% of males and females with chronic rhinitis and weak progesterone receptor activity in a small number of female patients, although immunocytochemical assay failed to demonstrate focal areas of these receptors [20]. All these studies took place prior to the discovery of ERβ. Evidence is also present for the role of hormonal nasal receptors in animal studies. Konno et al. [21] found that systemic administration of oestrogen to guinea pigs caused a significant increase in the density of cholinergic muscarinic receptors in the nasal mucosa whilst progesterone caused a decrease in the density of α1-adrenergic receptors. Another study found that oestradiol injections into guinea pigs caused thickening of the nasal epithelium, spongiosis and oedema of the corium, hyperplasia of glands and increased density of cilia [22]. Common ectodermal derivation of the epithelial cells of the nasal cavity, oral cavity, skin and breast may account for the presence of oestrogen in these tissues [19].

Further work in human studies found that the nasal mucosa of

Table 1. Unpaired t-tests for comparison of subjects by sex, smoking status and atopy.

Categorical variable	p-value
Sex	0.987
Smoking status	0.500
Atopy	0.970

Table 2. Pearson correlation of quantitative variables with ERβ count.

Variable	Correlation coefficient	p-value
Rhinitis score	0.413	0.045
Age	-0.020	0.926

Table 3. Results data.

subject	age	sex	smoking	atopic	QoL	ERβ cells
1	29	F	N	Y	18	111.71
2	53	M	Y	N	1	50.25
3	42	M	N	Y	5	81.1
4	44	M	N	N	14	109.1
5	47	M	EX	N	53	103.45
6	39	F	N	Y	3	75.4
7	41	F	N	Y	41	106.64
8	50	F	N	Y	44	96.3
9	52	F	Y	N	59	211.3
10	23	F	N	N	33	128.9
11	31	F	N	Y	25	124.38
12	51	M	Y	N	11	134.1
13	39	M	N	N	30	125.5
14	41	M	Y	N	1	142.9
15	39	M	N	N	4	177.2
16	18	M	Y	N	6	117
17	19	M	N	N	3	121.5
18	37	F	N	N	35	124.5
19	28	F	N	Y	47	168
20	47	M	N	N	7	118.8
21	60	M	N	N	39	116
22	54	M	N	N	46	138.4
23	68	M	EX	N	12	122.3
24	52	M	Y	Y	46	163.7

QoL = Rhinitis quality of life questionnaire

women became hyperreactive to histamine in connection with ovulation when oestrogen levels reach their peak suggesting a connection between high oestrogen levels and nasal mucosal reactivity [23]. Paulsson et al. examined the nasal biopsies taken from the inferior turbinates of 8 regularly menstruating women and were unable to detect oestrogen or progesterone receptors with immunostaining techniques [5]. Progesterone receptor positive cells have also been demonstrated in fibroblasts with cytoplasmic staining for oestrogen and progesterone receptors in the interstitium of nasal serous glands [24]. Interestingly mucosal abnormalities appear not to have the same presence of hormonal receptors with lower or absent findings in polyps and papillomas [25]. The yield of ERβ receptors in our study is greater than reported in previous studies [26] aided by the greater number of subjects sampled. The positive correlation of number of ERβ receptors and the rhinitis quality of life questionnaire score suggests that the number of ERβ positive cells and the symptoms experienced by a patient are linked. More work needs to be carried out on the relevance of the ERβ receptor in the nasal mucosa; a larger study including healthy volunteers and patients with different nasal pathologies (e.g. allergic rhinitis and chronic rhinosinusitis) is needed to verify our correlation of rhinitic symptoms with the number of ERβ receptor positive cells in the nasal mucosa. In the context of the evidence in the literature as discussed above and the findings from studies in our centre, these results show that further work is required to clarify the missing link between the presence of the ERβ receptors and any effect upon nasal physiology [27].

CONCLUSIONS

Progesterone and ERα receptors were not present in any of the nasal biopsies but ERβ receptors were present in the nasal mucosa of both males and females. The number of ERβ positive cells has a significant correlation with rhinitic symptom scores. Unlocking the secret to the mechanism by which these receptors are activated may enable pharmacological manipulation of this pathway for treatment of rhinitic patients and is the subject of ongoing research in our centre [27].

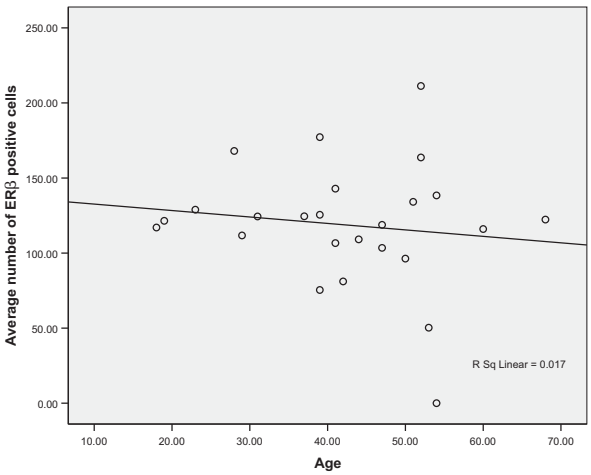

Figure 6. Scatter plot comparing age with average number of ERβ positive cells.

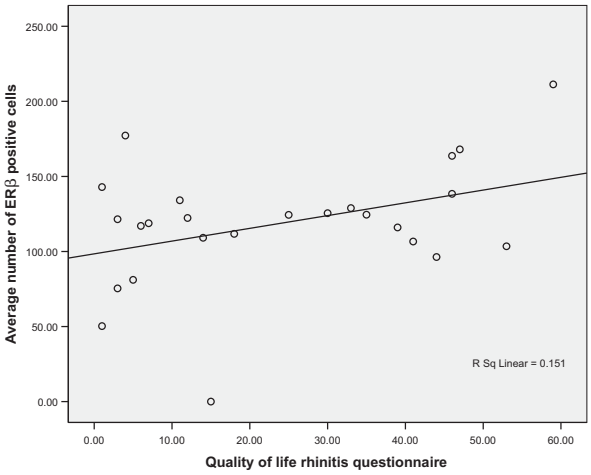

Figure 7. Scatter plot comparing quality of life questionnaire scores with average number of ERβ positive cells.

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