Analysis of Estrogen and Progesterone Receptors in Lesional and Normal Skin of Patients with Acne Rosacea¶

ROZASELİ HASTALARDA LEZYONEL VE NORMAL DERİDE ÖSTROJEN VE PROGESTERON RESEPTÖRLERİNİN ARAŞTIRILMASI

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Summary

Background: There is a plenty of clinical evidence suggesting that rosacea may be a hormonally mediated disorder. Hypothetically, an overexpression of progesterone receptors, in conjunction with a reduced expression of estrogen receptors within the lesional skin might play a role in the development of rosacea.

Objective: The aim of the present study was to evaluate the expression of estrogen and progesterone receptors in lesional and uninvolved skin of patients with rosacea.

Methods: For this purpose 20 lesional cutaneous biopsies and 5 non-lesional cutaneous biopsies from patients with rosacea were studied by immunohistochemical method for the expression of estrogen and progesterone receptors.

Results: Immunohistochemical examination showed that 2 (10%) of the 20 lesional biopsy specimens expressed progesterone receptors. None of the lesional biopsy samples expressed estrogen receptors.

Conclusion: Although these findings fail to provide presumptive evidence for a role of estrogen and progesterone receptors in rosacea, there remains the possibility that unopposed androgenic stimulation might be involved in the etiopathogenesis of rosacea.

Key Words: Rosacea, Estrogen, Progesterone, Receptors

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Özet

Giriş: Rozase etyolojisinde hormonal faktörlerin rol oynayabileceğine ilişkin çok sayıda kanıt bulunmaktadır. Lezyonel deride östrojen reseptörlerinin azalması ve progesteron reseptörlerinin artışı hipotetik olarak rozase oluşumunda önemli olabilir.

Amaç: Bu çalışmada rozaseli hastaların lezyonel ve normal deri dokularında östrojen ve progesteron reseptörlerinin ekspresyonunun saptanması amaçlanmıştır.

Hastalar ve Yöntem: Bu amaçla rozaseli 20 hastanın lezyonel deri örneklerinde ve gönüllü 5 hastanın non-lezyonel deri örneklerinde immünhistokimyasal metod ile östrojen ve progesteron reseptörleri çalışılmıştır.

Bulgular: Lezyonel deri biyopsilerinden 2'sinde (10%) progesteron reseptör varlığı saptanmış, ancak hiç bir örnekte östrojen reseptörleri gösterilememiştir.

Sonuç: Bu bulgular rozase etyolojisinde östrojen ve progesteron reseptörlerinin önemi hipotezini desteklememekle birlikte bloklanamayan/ karşı konulamayan androjenik uyarı hipotezinin araştırılması gerektiğini düşündürmektedir.

Anahtar Kelimeler: Rozase, Östrojen, Progesteron, Reseptör

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Acne rosacea is a common chronic progressive and recurrent disorder involving the skin and eye and that begins insidiously (1-4). Symptoms include facial flushing, erythema, telangiectasia, inflammatory lesions (papules and pustules), occasionally lymphedema and hypertrophy of sebaceous glands, connective tissue and vascular tissue of the nose (1-9). The most common sites of involvement are the cheeks, nose, chin, forehead and V of neck (3-5).

The etiopathogenesis of the disease is not clearly understood. Current evidence regarding the pathogenesis favors a multifactorial disorder (2,6). Hormonal influences have been proposed to contribute to the origin of the disease based on clinical and electron microscopic observations (9).

The present study was designed to evaluate the expression of estrogen and progesterone receptors in lesional and uninvolved skin of patients with rosacea.

Materials and Methods

Selection of Patient and Control Group

This study was designed as a prospective study including 32 consecutive patients with rosacea diagnosed at the Dermatology Department of Kırıkkale University Faculty of Medicine between May 2000 and May 2001. Rosacea was diagnosed by typical clinical features and histology. Patients were enrolled into the study irrespective of age and sex. Nine patients, receiving oral and/ or topical therapy for rosacea and 3 patients who refused biopsy of lesional skin were excluded from the study.

A 4 mm punch biopsy specimen was obtained from exposed facial skin lesions of each patient. In addition non-lesional unexposed skin of postauricular area was biopsied in 5 patients among the 20 patients who gave informed consent for biopsy of normal skin. Biopsy samples were fixed in 10% formalin and embedded in paraffin.

Immunohistochemical Analysis

Five micron-thick sections were obtained by microtome and transferred into adhesive slides. The sections were kept in the autoclave at 37˚C for 16 hours and at 60˚C for 20 minutes. Then they were deparaffinized and dehydrated by immersion into xylene twice for ten minutes and into alcohol twice for two minutes. Then, the specimens were incubated in 3% H_2O_2 for five minutes to inhibit activation of endogenous peroxidases. All preparations were transferred into high pH "Target Retrieval Solution" (EDTA, pH: 9.9) and kept in the microwave oven (750 watt) twice for five minutes. By using Shandon Sequenza^{Tm} manual staining device for standardization, classical avidin-biotin-peroxidase method and DAB chromogen were applied for immunohistochemical analysis of estrogen (monoclonal, prediluted; DAKO; Denmark) and progesterone (monoclonal, prediluted; DAKO; Denmark) receptors. A mammary carcinoma and an endometrial biopsy specimen served as internal positive control samples for estrogen/ progesterone receptors. A negative control consisting of nonimmune serum was used in all cases for both immunohistochemical markers.

Mayer's hematoxylin was used as counterstain and slides were examined by light microscopy. The results of immunostaining were analyzed semiquantitatively. The amount of staining was evaluated according to the percentage of positively stained cells around the hair follicles, sebaceous glands and basal layer of epidermis and was recorded as follows: (-) no expression; (+) weak expression; (++) mild expression; (+++) strong expression. An additional estimation was performed, considering the intensity of immunostaining, which was recorded as weak (+), mild (++) or strong (+++).

Results

The patient group comprised 3 males and 17 females. The age range was 18-70 years (mean: 43.15; median: 43.00). The duration of the disease varied from 1 to 25 years (mean 5.75 years; median: 4.00 years). Eight (40%) of 20 patients had erythematotelangiectatic type of rosacea and 11 patients (55%) had papulopustular type of rosacea. One patient (5%) had granulomatous type of rosacea.

Immunohistochemical examination showed that 2 (10%) of the 20 lesional biopsy specimens expressed progesterone receptors. None of the lesional and control biopsy samples expressed estrogen receptors. Internal positive controls (mammary carcinoma and endometrial biopsy sample) consistently demonstrated strong expression of estrogen and progesterone receptors. The amount of immunostaining for estrogen and progesterone receptors in rosacea and control specimens is shown in Table 1.

In 2 lesional biopsy specimens showing (+) progesterone receptor immunostaining, the amount and intensity of immunostaining were mild and the expression was localized to the sebaceous glands. All 5 control specimens lacked progesterone receptor expression.

Table 1. The amount of immunostaining for estrogen and progesterone receptors in rosacea and control specimens

	Estrogen receptor		Progesterone receptor	
	Patient	Control	Patient	Control
(-)	20 (100%)	5	18 (90%)	5
(+)	0	0	2 (10%)	0
(++)	0	0	0	0
(+++)	0	0	0	0

Discussion

There is plenty of clinical evidence suggesting that rosacea may be a hormonally mediated disorder. A significant number of patients with rosacea are perimenopausal women (10,11). Menopause is associated with a decline in ovarian estrogen production and a rise in FSH and LH levels with subsequent vasomotor symptoms and skin changes (12). The vasomotor instability during menopause provokes flushing and rosacea is frequently triggered and exacerbated during menopause (1,2,9-11,13,14). Endocrine disorders have been found in females with rosacea (5) and the disease is associated with historical and clinical abnormalities of hormonal origin including menstrual abnormalities, acne vulgaris, polycystic ovary syndrome and hirsutismus (4,11). Furthermore, the premenstrual exacerbations of rosacea have been strongly linked to progesterone (15) and supported by a report of rosacea associated with the use of synthetic progesterone-releasing (levonorgestrel) intrauterine contraceptive device, upon removal of which the disease completely resolved (16). Finally, there are reports on the efficacy of oral ovulation inhibitors (estrogens) and cyproterone acetate in female patients with acne rosacea and such treatment modalities led to a decrease in flushing as well as to complete recovery of papulopustular lesions (11,17). These observations raise the possibility that rosacea may not only develop as a consequence of genetic predisposition and provocative environmental factors (6,9), but may also be contributed by endocrine influences. Although serum sex steroid levels have been consistently found normal in rosacea (9,13,18), the role of local cutaneous endocrine milieu can not be discarded. Therefore we hypothesized that demonstration of a reduction in the estrogen receptors along with an enhanced expression of the progesterone receptors within the lesional skin (as compared with the normal skin) could provide supportive evidence for a role of sex steroids in the pathogenesis of rosacea.

Estrogens are C18 steroids secreted from the granulosa cells of the ovary (12,19). An enzyme called aromatase in the endoplasmic reticulum catalyses the synthesis of estrogens from C19 steroids (androgens). The primary sites of aromatase expression in females are the ovarian granulosa cells in premenopausal female and adipose tissue and skin fibroblasts in postmenopausal female (19). In the postmenopausal female and in male, aromatization of androgens in peripheral tissues is the primary mechanism for estrogen formation (19,20). Estrogen action in most tissues is mediated through the estrogen receptor, a member of a large superfamily of nuclear receptors (19). The present study showed that the expression of estrogen and progesterone receptors was decreased or did not exist at al in lesional and normal skin of patients with rosacea. Despite the sensitivity of the immunohistochemical method and the use of monoclonal antibodies in our study, the basis of negative results may potentially be attributed to the expression of estrogen and progesterone levels beyond the detectability level of immunohistochemistry. This hypothesis is supported by the consistent strong expression of estrogen and progesterone receptors by endometrial biopsy sample and mammary carcinoma specimen used as positive internal controls in this study. In the medical literature, there is only one study by Schmidt et al (9) investigating the receptor status in rosacea. The authors examined estrogen and androgen receptor levels in the skin of 11 male and 14 female patients with rosacea by using the saturation analysis technique. They found no significant increase in receptor levels or distribution between lesional and normal skin of patients with rosacea. Data from

their study suggested that rosacea formation is not governed by endocrine alterations. The results of our study is consistent with that of Schmidt et al. Although our results can not rule out the possibility of an endocrine influence at microvascular level, rosacea is probably not dominated by the overproduction of estrogen and progesterone and overexpression of their receptors in lesional skin. However, we believe that unopposed androgenic stimulation might contribute to the pathogenesis of the disorder. In the menopausal ovary the hormonal production of both estrogen and androgens are decreased. However, estrogens decline further creating a relative increase in the androgen to estrogen ratio. This relative increase in androgens may cause an increase in the number of hyperandrogenic symptoms in menopause (12). The effect of progesterone on the development of rosacea in menopausal women is probably unimportant, since the levels of this hormone significantly decrease during menopause, owing to the lack of ovulation. The available data and our experience on the use of topical and oral estrogen preperations in the treatment of postmenopausal rosacea implicate that opposing the androgenic stimulation by estrogen therapy results in improvement of flushing and papulopustular eruption of rosacea. Therefore, further studies investigating the hormonal mechanisms, particularly focusing on the activities of the enzymes 5-α-reductase and aromatase; and the expression of androgen receptors in lesional and non-lesional skin are awaited to clarify the pathogenesis of rosacea.

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