

ENDOMETRIAL HORMONE-RECEPTOR ASSAY: A NEW TOOL IN THE INVESTIGATION ON MASTODYNIA

Preliminary report

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SUMMARY

As breast dysfunctional pathology does not appear constantly correlated to significant variations of steroid plasma levels, we purposed to verify whether the control of tissutal mechanism of hormone vehiculation can provide more reliable informations, in order to identify early endocrine disorders and to check more properly the effectiveness of the treatment from both a qualitative and a quantitative standpoint.

In this view we assayed the receptors for estradiol and progesterone in the endometrium of women with mastodynia and healthy controls, to verify whether endometrial tissue receptivity may be useful in the control of breast tissue receptivity; in fact, both the tissues are hormone-dependent and equally exposed to the hormones of the menstrual cycle.

Mastodynia has long been investigated by several Authors (¹⁻¹⁰) and is commonly believed to depend on a condition of hyperestrinism, either absolute or relative (¹⁻⁷), which has been demonstrated through estradiol and progesterone plasma level radioimmunoassay performed using polyclonal antibodies. These considerations suggested a progestin treatment for the symptom. On the other hand, there are researchers (⁸⁻¹⁰) who deny a real involvement of hyperestrinism or progesterone deficiency in the genesis of this picture: they base their opinion on the lack of correlations, in their series, between hormonal plasma levels and symptomatology.

In the attempt to understand why data in literature are discordant and to help in clearing-up the genesis of the clinical picture, we deepened our investigation to the tissutal level.

In fact, steroid hormones are known to need intranuclear translocation by specific proteic carrier in order to express their metabolic action (¹¹); these proteins, the hormone-receptors, have been largely studied over the last 10 years, and quite recently it was demonstrated that no chemico-physical difference does exist for estrogen and for progesterone-receptors in the different target tissues of the same individual (¹²) and that the mechanism of action of the receptors is the same in the different tissues (¹³).

These last findings support the rationale of our research: to evaluate the receptorial capacity of the breast through the assay of endometrial cytoplasmic receptors. The endometrium, in fact, exposed to the same hormonal stimulation as breast tissue, does respond to the variations of circulating hormone levels and, what is most important, can be easily obtained even for repeated examinations.

MATERIAL AND METHODS

For this study we selected 28 patients affected by mastodynia and 58 healthy control

patients without any breast symptomatology.

The selection was always performed by the same physician. We considered affected by mastodynia the woman who presented mono or bilateral breast spontaneous pain lasting at least eight days before menstruation, regularly occurring in at least the six previous cycles.

In patients and controls we performed colposcopy and endometrial cytology in the periovulatory period; endometrial samples were with-

powder was then transferred into pre-chilled Corex-glass (20×34 mm) and then taken to the Cold-Room. All the steps of the process were carried out in the Cold-Room to assure a constant reaction temperature.

The powder was suspended in TEDMo-Buffer pH 7.4 at 4 °C (Tris-Ultra Pure 0.01M-Schwarz/Mann; EDTA 0.0015M-Fisher Sc. Co.; DTT 0.5mM-Calbiochem Behring Co.; Molybdic Acid 5mM-Sigma Ch. Co.) HCl 12N.

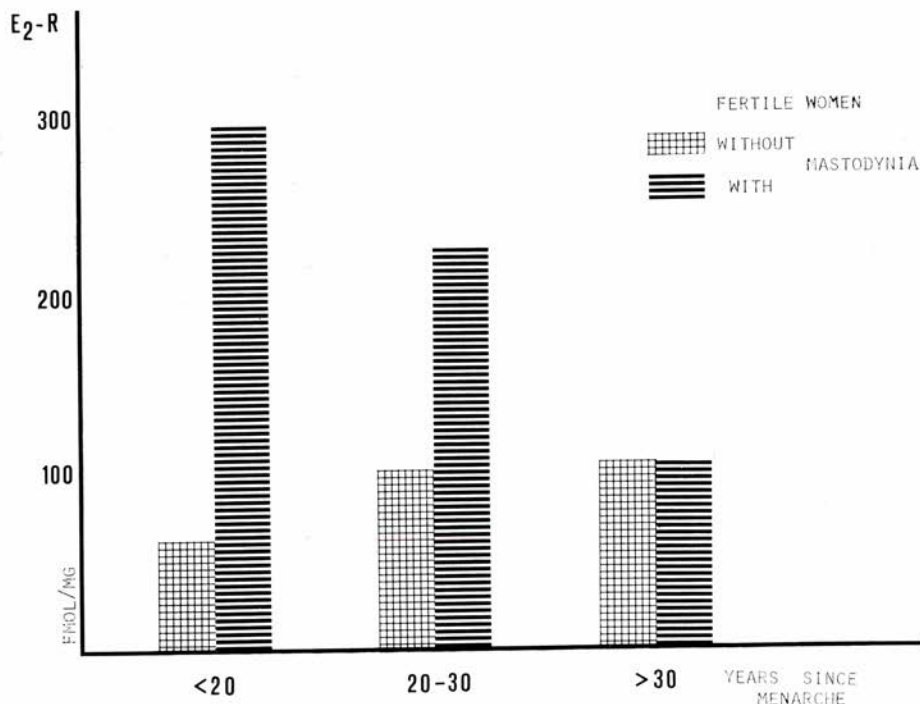


Fig. 1. — Behaviour of cytoplasmic estradiol receptor levels in the endometrium of women with mastodynia and controls, in the different periods of the fertile age.

drawn by the same flexible Ch 8/40 cm Nelaton catheter used for suction cytology: the amount of endometrial tissue was sufficient for receptor assay (dry weight of 0.1-0.3 gms after freezing).

The double cytologic control allowed a better endocrine setting for the sample and a more correct interpretation of receptor assay. Blood and mucus were washed away by cold H₂O_d before storing in liquid Nitrogen.

Receptor assay was carried out by DCC in SSA-Method^(14,15,16); each frozen sample was pulverized in Microdismembrator-Braun and the

The tissue was homogenized with Polytron-Brinkman PT 10 ST by three 10 sec bursts at the very lowest setting; the homogenate was then centrifuged in TGA-65 Kontron at 105,000 g for 1 hour. The cytosol was sucked by cold Pasteur and, after the estimate of nucleic acids, properly diluted. 200 λ of diluted cytosol was then incubated, in triplicate, with the respective competitors; 1 hour later the radiolabelled steroids were added and let to react for 16 hours. (³H-R5020 2.5×10^{-9} M, Cold R5020 2.5×10^{-7} M, DHT 1×10^{-6} M, F 1×10^{-6} M; ³H-E₂ 8×10^{-11}

M, DES 8×10^{-9} M). DCC-1% BSA was added to each tube and, after 15 min. of vigorous shaking in Varvel FVF20A, the tubes were centrifuged at 3000 rpm for 15 min. in ALC965R. 500 λ was read in a Betamatic-Kontron with an efficiency of 57% in Toluol-PPO-POPOP. The proteins were assayed according to the Lowry Method on the LKB-Ultrospec 4050, and the results were expressed in femtomoles/milligrams.

matic women coming as outpatients to the periodic gynecologic check-up, while in the other series reporting data on the endometrial receptivity in fertile cycles (¹⁸⁻²³) all the patients had been sent for diagnostic endometrial biopsy, and only after histology some of them were singled out as normal.

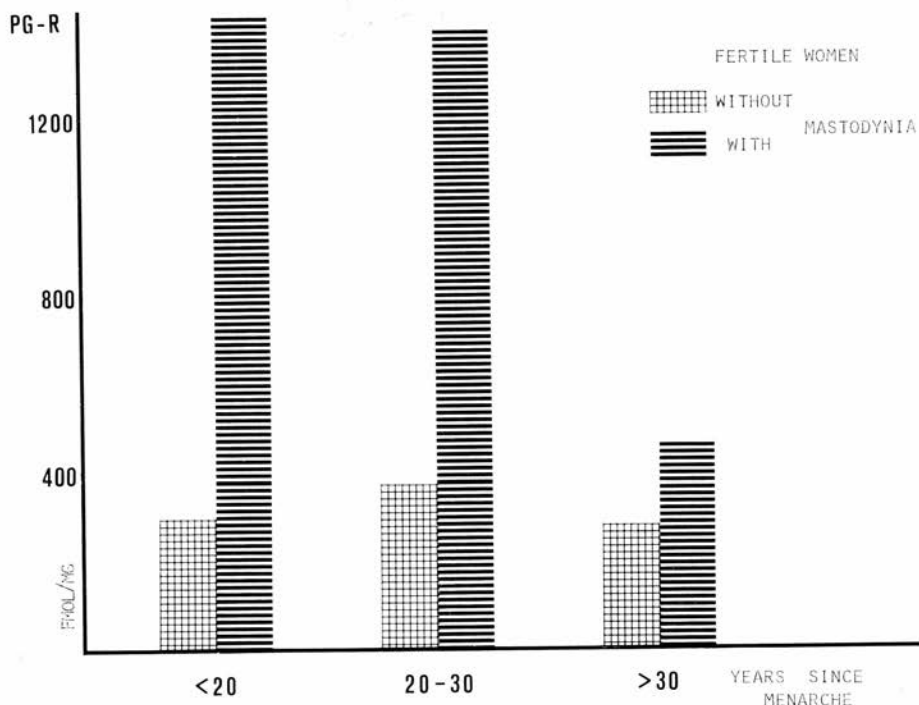


Fig. 2. — Behaviour of cytoplasmic progesterone receptor levels in the endometrium of women with mastodynia and controls, in the different periods of the fertile age.

RESULTS

The results of our study are showed in figs. 1, 2 and 3: the data are subdivided according to hormonal exposition, that is the time elapsed since the menarche; the three figures illustrate respectively the behaviour of E_2R , PgR and PgR/E_2R ratio in women with mastodynia and controls. As we pointed out (¹⁷) our controls are healthy normally menstruating asymptomatic

The analysis of our results suggests considerations immediately clear in figs. 1, 2 and 3: the levels of cytoplasmic receptors for estradiol, which in normal patients increase only after 20 years of exposition to the hormones of the menstrual cycle and seem thereafter stable around constant values (fig. 1), show an opposite trend in patients with mastodynia: in the latter the levels are greatly higher than in the controls for exposition periods less

than 20 years, and then tend to decrease in the following decades and become overlapping to normal patients' only after an exposition time of at least 30 years.

The cytoplasmic concentration of progesterone receptors (fig. 2), which in control patients is almost constant with

gesterone and estradiol receptors (PgRc/E₂Rc) in patients with mastodynia and controls. While in the controls this ratio tends to diminish progressively as exposition time increases, in patients with mastodynia this ratio remains constant around values like those we find in the

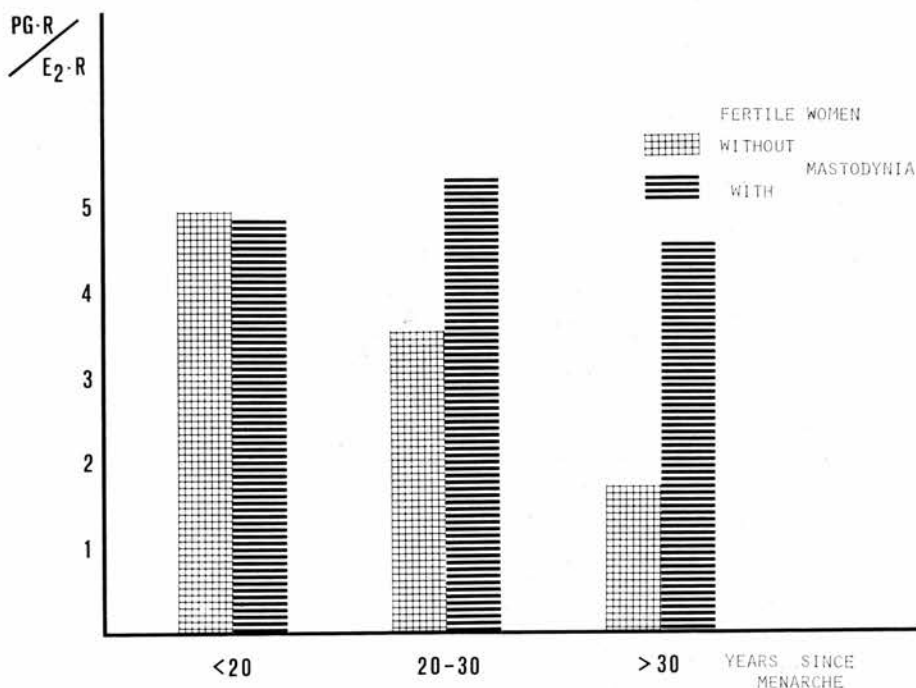


Fig. 3. — Variations of the ratio between cytoplasmic progesterone and estradiol receptors in the endometrium of women with mastodynia and controls, in the different periods of the fertile age.

slightly higher levels for 20-30 years of exposition, is highly increased in women with mastodynia in which we find levels five-fold higher than in the controls for exposition periods up to 30 years since the menarche. Only after 30 years of hormonal exposition these value decrease remaining, however, higher than in the controls.

Anyway, we believe that the most interesting consideration concerns the variation of the ratio between cytoplasmic pro-

controls for periods less than 20 years since the beginning of the fertile age.

DISCUSSION

The study of hormonal receptivity of breast tissue through the assay of steroid cytoplasmic receptors in the endometrium allows a further step towards the comprehension of the mechanisms responsible for dysendocrine breast diseases.

The controversy about the real importance of hyperestrogenism or progesterone

deficit in the pathogenesis of this condition might be overcome or clarified by the investigation of the tissutal receptor picture.

The analysis of our data leads us to believe that in patients with mastodynia a higher activity of estrogens is present, documented by the increased levels of cytoplasmic receptors for estradiol and progesterone known to be directly correlated with estrogen stimulation.

The high cytoplasmic progesterone receptor levels, particularly in the first two periods of hormonal exposition, may lead one to hypothesize a defect in the translocation of the receptor into the nucleus; this, in turn, might be due to a reduced concentration of circulating progesterone and be consequently ascribable to progesterone deficit. The decreased opposition by progesterone would then allow the prevalence of estrogen activity, in agreement with the data presented by some Authors (¹⁻⁷).

On the other hand, the finding of cytoplasmic estradiol receptor levels four-fold higher in patients with mastodynia than in the controls already in the first period of hormone exposition, lets us hypothesize a predetermined back-ground, eventually due to genetic causes. This hypothesis might explain why patients with mastodynia may have normal hormone plasma levels: in them the appearance of the symptom would be attributed to an altered tissutal modulation of the action of hormones present in normal plasma concentrations.

Finally, whether the altered tissutal receptivity for estradiol and progesterone is considered the cause or the consequence of a dysendocrine picture, the assay of hormone receptors in the endometrium of women with breast diseases seems to be a promising qualitative improvement in the comprehension and individuation of those defects of tissue receptivity that may lead to dysplastic and/or neoplastic growth.

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