

Progesterone, Estradiol and their Receptors in Leiomyomata and the Adjacent Normal Myometria of Black Kenyan Women.

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SUMMARY

The contents of progesterone and oestrogen, and their respective receptors in uterine leiomyomata and adjacent normal myometrial tissue in indigenous black women in Kenya were studied. A random selection of twenty women undergoing hysterectomy for uterine fibroids at Kenyatta National Hospital was used for the studies. The myometria contained higher levels of E₂ (181%: P<0.001) and P₄ (240.6%: P<0.001) as compared to the leiomyomata. On the other hand uterine leiomyomata contained significantly higher levels of ER (147.6%: P<0.001) and PR (178.7%: P<0.001) than normal myometria. These findings differ slightly from those reported in black women in developed countries, but support the proposal that manipulation of sex steroids may be useful in the treatment and management of uterine leiomyomata.

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Introduction

Uterine leiomyomata (fibroids) are the most common pelvic tumours in women, first arising during the childbearing period of life. They do not arise during menopause, and are rare after cessation of menstruation [1]. The reproductive implications of fibroids include abnormal uterine bleeding, infertility, ectopic pregnancies, abortions, and pre-mature and difficult labour [2]. Data has accumulated pointing to the involvement of sex steroid hormones (E₂ and P₄) and their respective receptors (ER and PR) in the pathogenesis of fibroids. However, there are conflicting reports on the quantitative levels of ER and PR in the fibroids compared to the normal uterine myometria [3, 4, 5].

Efforts in the present study were directed towards resolving this controversy

and providing information regarding the disease in the black population in Kenya, especially in view of the fact that black women have been reported to have a higher incidence of leiomyomata than other races [6,7,8]. However, the high incidence of the disease reported in the latter population is from work done in black women living in Caucasian populations[6, 7, 8].

The study reported here investigated the levels of progesterone (P₄) and estradiol (E₂), and their respective receptors (PR and ER) in the uterine leiomyomata in indigenous black African women. It is hoped that the findings will provide appropriate data not currently available in our archives. This may enhance the rationale

for the manipulation of sex steroids and their receptors in the management of this disease.

Materials and Methods

Subjects and sample collection

Twenty patients were selected from women undergoing elective hysterectomy for uterine fibroids at the Kenyatta National Hospital, Nairobi, Kenya. Only women of reproductive age (20-45 years) were included in the study.

All the patients studied were premenopausal, black women in the age group 31-42 years. Indications for hysterectomy were either menorrhagia, metrorrhagia and /or dysmenorrhoea. Seven (35%) of the patients suffered from primary infertility while the other thirteen (65%) had secondary infertility. The menstrual status of the patients were determined by medical histories. Fifteen (75%) had menstrual irregularities, three (15%) were in the follicular phase and two (10%) in the luteal phase of their menstrual cycles.

Specimens of uterine leiomyomata and adjacent normal myometria (n=20) were obtained at the moment of hysterectomy. The paired specimens (fibroids and myometria) were immediately placed in ice-block containers and transported to the laboratory where multiple tissue samples were removed for histology while the rest frozen at -20°C until they could be analysed further for hormone and receptor content.

Preparation of cytosol fractions

The method for preparation of cytosols followed the procedures outlined by Bauer and Gorell (1980)[9] for ovine uterus. The frozen tissues were allowed to thaw and 10g of each cut into small pieces. They were then suspended in 40ml TEDG buffer and thoroughly homogenized. The homogenization was accomplished at $0-4^{\circ}\text{C}$ for 18 min with 15 sec bursts using a pre-chilled Sanyo SM3050 tissue blender, followed by 1 min cooling intervals in the freezer. The homogenate was centrifuged in

a Sorvall RC-5B centrifuge at 27,000 Xg for 40 min to yield a supernatant (referred to as cytosol in the remainder of the study). The cytosols were then tipped into sample tubes and stored in a freezer until needed for hormonal and receptor analysis.

Determination of cytosolic protein content

The protein concentration in the cytosol was determined by the routine Lowry et al (1951)[10] method and validated by the Department of Biochemistry, University of Nairobi.

Binding of estrogen and progesterone to their respective receptors

The cytosols (myometria and leiomyomata) were removed from the freezer and thawed. Dilutions of 1:4 (in TEDG buffer) of the fluids were prepared and 0.2ml of each fluid pipetted in triplicates into LP₃ tubes and incubated at 4°C for 48 h with 0.1ml tritiated E₂ (2,4,6,7-³H estradiol) giving about 10,000 counts per minute (cpm). The unbound steroid in the mixture was separated from the steroid bound to the receptors by charcoal adsorption followed by centrifugation at 1000 Xg for 10 min at 4°C . The charcoal pellets were safely disposed of and scintillation cocktail (2,4,5-diphenyloxazol – PPO in toluene) added to the supernatant. This was left to equilibrate over night and then counted in a Beckman LS-7000 liquid scintillation counter for at least 1 min. The same procedure was repeated for progesterone but this time using tritiated P₄ (17*-hydroxy 1,2,6,7, -³H-progesterone).

Determination of the amount of labeled hormone bound to estrogen and progesterone receptors

An indirect method was used to determine the amount of estrogen and progesterone receptors in the leiomyomata and myometria. The amount of labeled hormone bound onto the receptors was first calculated using the amount of the labeled antigen per ml of cytosolic fluid. Thereafter, the amount of labeled hormone bound per mg of cytosolic protein was calculated. The bound

E₂ and P₄ were then expressed as femtomoles per mg protein.

Assays for tissue estrogen and progesterone
The estradiol and progesterone levels in the samples were determined by radioimmunoassay (RIA) using methods outlined in the WHO Matched Reagent Programme Method Manual[11]. Briefly 500ul and 200μl of cytosol were extracted with 10 times volume of diethyl ether for E₂ and P₄ respectively using extraction tubes. The mixture was separated by freezing the aqueous phase and tipping off the ether phase into pre-labeled clean LP₃ tubes and then dried down in a vacuum. In each tube, 2ml of steroid phosphate buffer (containing gelatine and sodium azide at pH 7.4) was added. To 0.5ml of the latter, 100μl of tritiated hormone (10,000 cpm) and 100μl of antibody were also added. The mixture was then incubated overnight and the bound antigen separated from the free hormone by using pre-chilled dextran-coated activated charcoal and centrifuged at 1000Xg for 10min. The bound hormones were tipped off into clean scintillation vials and 4ml toluene/PPO scintillation mixture added and counted in a liquid scintillation counter as earlier described.

Results

Table 1 shows the values of the mean (±) SEM of ER, PR, E₂ and P₄ in the fibroids compared with myometria of the 20 patients. The receptor assay results shows that the uterine leiomyomata contained significantly higher levels of ER (147.6%: p<0.001) and PR (178.7%: p<0.001) compared to the adjacent normal myometria. The total ER in the uterine tissues i.e fibroid and myometria was higher (180.5%) than total PR in the same tissues. On the contrary, the normal myometria contained significantly higher levels of E₂ (181.1%: p<0.001) and P₄ (240.6%: p<0.001) compared to the leiomyomata. The total P₄ was higher (628.4%) than total E₂.

The levels of E₂, P₄, ER and PR in the uterine tissues of patients investigated in the follicular and luteal phases of the menstrual cycles were also compared (Table 2). The results obtained showed that the concentrations of ER and PR were as usual higher in the fibroids compared to the normal myometria in both phases of the menstrual cycles. Also the E₂ and P₄ levels were lower in the fibroids compared with the myometria.

Table 1: Mean±SEM of ER and PR in fmol/mg protein and of E₂ in pmol/mg and P₄ in nm/mg tissue in the myometria and fibroids (n=20).

TISSUE (n= 20)	ER (Mean±SEM fm/mg protein)	PR (Mean±SEM fm/mg protein)	E ₂ (Mean±SEM pm/mg protein)	P ₄ (Mean±SEM nm/mg protein)
Fibroid	28.2±1.6	16.8±0.7	616.9±19.8	3.2±0.34
Myometrium	19.1±0.4	9.4±0.2	1117.6±20.9	7.7±0.25

Table 2: ER, PR, E₂ and P₄ in the follicular and luteal phases of the menstrual cycle.

Follicular Phase (n=3)	ER (Mean±SEM fm/mg protein)	PR (Mean±SEM fm/mg protein)	E₂ (Mean±SEM pm/mg protein)	P₄ (Mean±SEM nm/mg protein)
Fibroid	33.6±2.5	17.8±1.3	650.0±30.5	1.7±0.1
Myometrium	20.0±0.6	13.3±3.4	1169.0±19.0	6.8±0.2
Luteal phase (n=2)	ER	PR	E₂	P₄
Fibroid	22.7±4.3	13.9±1.3	525.2±27.9	4.6±0.1
Myometrium	17.4±0.5	13.8±5.2	1024.2±31.0	8.6±0.2

Discussion

In this study, fibroid and the normal myometrial samples from the same patient (a total of 20 different patients) were used for comparative purposes. The patients recruited were pre-menopausal black females of age group 31-42 years. Difficulties were experienced in obtaining large sample sizes for patients in the proliferative and secretory phases of the menstrual cycle, because most of the patients booked for hysterectomy had menstrual irregularities. The general observation drawn from this study was that the leiomyomata contain higher receptor (ER and PR) concentrations than the adjacent normal myometria. These findings are in agreement with the previous studies [3, 5, 12].

In the present study, though the ER and PR concentrations from the same patient were higher in the leiomyomata than in the myometrium, the ER levels in the myometria and leiomyomata were higher than corresponding levels of PR in the same tissues. But the PR levels in the leiomyomata were raised to the same relative extent as the corresponding ER levels. This was contrary to results obtained by Potgieter et al. [5], who reported higher levels of PR in the myometria and leiomyomata than corresponding ER in similar tissues. Their PR levels in the leiomyomata were not raised to the same extent as the corresponding ER level. Also the results from Fernandez-Moritoli et al [12] showed that PR levels were higher than

ER levels in corresponding myometria and leiomyomata, and that some individuals had much lower PRs than ERs. However, the differences in the levels ER and PR in these three studies could arise from the differences in sample size, methodology (fibroid and myometrium from different or same patient) and from the fact that the phase of menstrual cycles were not controlled in a similar fashion.

The higher levels of ER and PR in the fibroid could explain the occurrence of these tumours during the childbearing period of life and their increase in size during pregnancy [13], since these are physiological states in which circulating levels of sex steroid hormones are relatively high. This also explains the decrease in size of these tumours with GnRH-agonists therapy, whereby circulating levels of estradiol are reduced to menopausal levels [14, 15, 16]. The high levels of receptors in the fibroids determine the capacity of tumors to retain the hormones and could indicate a degree to which the same are sensitive to the hormone action [17]. The lower levels of free hormones (E₂ and P₄) in leiomyomata in this study is explained by this capacity of the tumor cells to bind more of the hormone compared to the adjacent normal myometrium cells.

Regarding interactions between receptors and steroid hormones, it has been stated that a definite biochemical and physiological link exist between ER and PR. It would appear that PR levels are stimulated by estrogens, while the ER and PR levels are

down regulated by progesterone [18, 19, 20]. This could explain the low levels of total ER and total PR during the luteal phase, but fails to explain the high levels of both ER and PR during the follicular phase.

Conclusion

From this study, it is concluded that the relative proportions of steroid hormones and their respective receptors in the individual patients uterine tissue may be important in the pathogenesis of fibroids in the black population in Kenya. This should enable clinicians working in this population to appreciate more the use of chemotherapy in the management of uterine fibroids. However, research should continue in order to identify a non-surgical means of treating this condition. This would be an important public health initiative especially a population where the fibroids occur at a high incidence and surgical complications are common.

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