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## CLINICAL ARTICLE

## Clinical correlates of leiomyoma estrogen and progesterone receptors among Nigerian women

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## ABSTRACT

**Objective:** To compare the expression of estrogen receptor  $\alpha$  (ER $\alpha$ ) and progesterone receptor (PR) in myometrium and leiomyomata tissue, and to correlate their expression with symptoms of uterine leiomyomata. **Methods:** In a cross-sectional study, intraoperative biopsy samples of leiomyomata and adjacent myometrial specimens were obtained from premenopausal women with uterine leiomyomata treated at a center in Nigeria between September 2013 and August 2014. Immunohistochemistry for ER $\alpha$  and PR expression was performed on the samples. The immunoscores of both receptors were correlated with the size and symptoms of the leiomyomata. **Results:** Among 60 pairs of samples, leiomyomata had a higher mean expression of ER $\alpha$  (H-score  $193.42 \pm 64.55$  vs  $153.29 \pm 69.13$ ;  $P=0.01$ ) and PR ( $214.86 \pm 66.56$  vs  $171.53 \pm 63.53$ ;  $P<0.001$ ) than did myometrial tissues. The tumor diameter correlated negatively with the immunoscores of both receptors irrespective of age, parity, and body mass index, but this was only significant for PR ( $\rho=-0.44$ ;  $P<0.001$ ). Down-regulation of PR on leiomyomata was predicted to occur at a diameter of 11 cm. Menorrhagia, dysmenorrhea, and infertility occurred independently of steroid-receptor expression. **Conclusion:** Leiomyomata seem to depend on steroid hormones, but only during early tumor development. This could have implications for the selection of patients for medical management, especially with steroid-receptor modulators.

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## 1. Introduction

Uterine leiomyomata are a major public health concern, being the most common tumors of the female reproductive tract, with an estimated lifetime risk of 80% among black women [1,2]. Whereas the high prevalence of other tumors, such as cervical and breast cancers, has translated into a corresponding volume of research efforts to investigate the pathophysiology of these tumors at the molecular level, there have not been similar efforts for leiomyomata, perhaps because of the rarity of malignant transformation of this neoplasm. However, despite their benign neoplastic character, leiomyomata are independently responsible for considerable ill health, and their surgical treatment often superimposes additional morbidity [3].

Estrogen and, more recently, progesterone have been linked to the development of leiomyomata [4], with the stimulation of steroid receptors on the tumors effecting intracellular growth cascades. Variations in the tissue expression of steroid receptors could therefore be central to the pathogenesis of these tumors [5,6]. The exploration

of this theory is further encouraged by Spellacy et al.'s report [7] that the circulating levels of estrogen and progesterone are normal in women with uterine leiomyomata. The purpose of the present study was therefore to evaluate the expression of steroid receptors on myometrium and leiomyoma tissue pairs, to correlate steroid-receptor expression with the clinical manifestation of the tumor, and to possibly make suggestions that could improve the efficacy of medical treatment of uterine leiomyomata.

## 2. Materials and methods

The present cross-sectional analytical study was conducted at Obafemi Awolowo University, Ile-Ife, Nigeria, between September 1, 2013, and August 31, 2014. Premenopausal women with symptomatic uterine leiomyomata for whom surgical management was planned were recruited before surgery. Women who had been taking hormonal treatment within 6 months preceding the surgery and postmenopausal women were excluded. Approval was obtained from the Research Board of the University Teaching Hospital. All participants provided informed consent.

Relevant data relating to the sociodemographic characteristics, leiomyoma symptoms, and the phase of the menstrual cycle during

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which the specimens were obtained were recorded. During surgery, biopsy samples of 2 cm × 2 cm in size were obtained from the periphery of the largest leiomyoma nodule and from an adjacent area of redundant, normal-looking myometrium. The diameter of the largest nodule from each patient was measured to the nearest 0.1 cm using an inelastic tape measure, and the nodule's location was recorded. Additionally, the total number of nodules enucleated from each patient was recorded.

The specimens were fixed in 10% buffered formalin and embedded in paraffin wax. Thereafter, 3- $\mu$ m sections were stained with hematoxylin and eosin for histological confirmation. Other 3- $\mu$ m sections were then mounted on positively charged slides for immunohistochemistry. Estrogen receptor  $\alpha$  (ER $\alpha$ ) mouse monoclonal antibodies (Novocastra clone 6F11, Leica Biosystems, Newcastle, UK; dilution 1:60) and progesterone receptor (PR) mouse monoclonal antibodies (Novocastra clone 16, Leica Biosystems, Newcastle, UK; dilution 1:100) were used as primary antibodies. The sections were dewaxed in xylene and rehydrated with decreasing concentrations of ethanol. Endogenous peroxidase activity was blocked using 0.5% hydrogen peroxide for 10 minutes. The antigen retrieval solution (0.01 M citrate solution; pH 6) was heated to 100°C using a pressure cooker for 10 minutes, and the sections were then completely immersed and left at full pressure for 1 minute, followed by rapid cooling. The Leica TP1020 (Leica Microsystems, Wetzlar, Germany) automatic tissue processor was used for the rest of the reaction. The sections were rinsed in Tris buffer solution after other protein-binding sites had been blocked by casein solution. The sections were incubated with the primary antibodies (ER $\alpha$  or PR as appropriate) for 1 hour at 60°C and washed in Tris buffer solution for 5 minutes. The sections were then exposed to an avidin–biotin complex solution for 30 minutes and rinsed in Tris buffer solution for 10 minutes. Thereafter, 3,3'-diaminobenzidine solution was added to the sections for 10 minutes and the sections were then washed in water and rinsed in Tris buffer solution. Finally, hematoxylin was added as the counterstain [8,9]. Positive controls were prepared from known weakly ER $\alpha$ - and PR-positive archival specimens of invasive breast carcinomas. Negative controls were prepared by deliberately omitting the primary antibodies during the preparation of the same breast carcinoma specimens.

Each specimen was scored semiquantitatively using the H-score system [10], with the H-score being a product of the intensity of nuclear staining (range 0–3, with 0 indicating no staining and 3 indicating strong staining) and the percentage of tumor cells stained at each intensity across cellular tissue segments on each slide as follows: H-Score = (percentage of cells stained at intensity category 0) × 0 + (percentage of cells stained at intensity category 1) × 1 + (percentage of cells stained at intensity category 2) × 2 + (percentage of cells stained at intensity category 3) × 3.

This produced a numerical variable that ranged from 0 to 300, with an H-score of 75 or more set as the threshold for positivity. The immunoscores of all the specimens were simultaneously but independently, determined by two observers (I.O.A. and G.O.O.-E.) who were masked to the clinical information related to the specimens, using a multi-head light microscope. The average of the two scores was recorded as the final score. Slides with a difference of 10 or more between the scores by the two observers were revisited and a final score was agreed.

The data were analyzed with SPSS version 19.0 (IBM, Armonk, NY, USA). Normally distributed variables were compared using the independent-samples *t* test, whereas the Mann–Whitney *U* test was used for the comparison of variables that were not normally distributed. The relationship between the leiomyoma size and the receptor expression (the H-score) was established using Spearman correlation. *P* < 0.05 was considered statistically significant.

### 3. Results

A total of 68 women were recruited for the study, of whom 47 (69%) women underwent open myomectomy; the remaining 21 (31%) underwent total abdominal hysterectomy. Thirty-seven (54%) surgeries

were performed in the follicular phase of the menstrual cycle. Eight specimens were excluded because of extensive hyaline degeneration and coexisting adenomyosis, which precluded optimal scoring. Therefore, 60 histologically confirmed myometrium/leiomyoma specimen pairs were analyzed.

The mean age of the 60 women for whom specimens were analyzed was 38.39 ± 8.15 years (range 26–53), with 34 (57%) women being nulliparous. The mean duration of symptoms was 20.37 ± 13.46 months (range 3–72). The most common complaints were abdominal swelling (50 [83%] women), menorrhagia (42 [70%]), dysmenorrhea (40 [67%]), and infertility (20 [33%]).

As determined intraoperatively, 23 (38%), 28 (47%), and 9 (15%) of the largest leiomyoma nodules were subserous, intramural, and submucous, respectively. The diameters of the largest leiomyoma nodules ranged from 2 cm to 36 cm, with a mean diameter of 14.8 ± 6.6 cm. Thirty-nine (65%) of the largest leiomyoma nodules were at least 10 cm in diameter, and a median of six nodules (range 1–36) from each woman were enucleated.

For both ER $\alpha$  and PR, the mean H-scores in the leiomyoma specimens were significantly higher than the mean scores for the receptors in the myometrial specimens (Table 1). There was a negative correlation between ER $\alpha$  expression and nodule size, although this was not statistically significant ( $\rho = -0.25$ ; *P* = 0.12). The negative correlation was, however, significant for PR expression ( $\rho = -0.44$ ; *P* < 0.001).

Table 2 compares the mean H-scores for ER $\alpha$  and PR in myometrial and leiomyoma tissues on the basis of the clinical complaints of the women. There was no significant difference in the expression profiles of both receptors between women with dysmenorrhea, infertility, or menorrhagia and those without these symptoms.

Thirty-seven (62%) leiomyoma specimens had a higher H-score for ER $\alpha$  than the myometrial tissue of origin (differential upregulation). The PR expression was similarly upregulated in 42 (70%) leiomyoma specimens. Leiomyoma specimens with lower ER $\alpha$  and PR expression than the myometrial tissue of origin (downregulation) were significantly larger than nodules with upregulated steroid receptors (Table 3). The differential upregulation or downregulation of ER $\alpha$  and PR on leiomyoma nodules was independent of age, parity, and body mass index (Table 4).

A receiver operating characteristics curve was used to assess the extent to which the tumor diameter might be predictive of the upregulation or downregulation of the tumor's ER $\alpha$  and PR. The model predicted that a leiomyoma nodule with a size of 11 cm or more will have a downregulated PR status compared with its adjoining myometrial tissue, with an area under the curve of 0.78, a sensitivity of 76%, and a specificity of 70%. However, the discriminating power for the expression of ER $\alpha$  was poor, with an area under the curve of 0.62.

### 4. Discussion

The findings of the present study confirmed increased expression of both ER $\alpha$  and PR in uterine leiomyomata tissue compared with myometrial tissues among Nigerian women. The expression of both receptors on the leiomyoma nodules, however, declined progressively with increasing nodule size, indicating a reduced dependence of large uterine leiomyomata on steroid receptors.

The mean age of the participants was comparable to the mean age of 38.3 years reported 15 years earlier for the same center [11], but much lower than the mean age of 48.2 years that was reported for white women in the USA [12]. Furthermore, the mean size of the largest nodules of 14.8 ± 6.6 cm in the present study is more than double the mean size of 5.7 cm reported in a similar study in the USA [13]. These observations lend credence to previous reports [14] that leiomyomata are bigger and tend to occur earlier in black women than in white women.

Conventionally, leiomyomata have been considered estrogen-dependent tumors [15]. The significantly higher expression of ER $\alpha$  on leiomyomata specimens than in myometrial tissues, especially in the context of a normal serum estradiol level in women with leiomyomata [7],

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