

Clinical significance of oestrogen and progesterone receptors in the growth and symptomatology of uterine fibroids

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ABSTRACT

Background: Uterine fibroids are responsible for significant morbidity in a large proportion of the female population of the reproductive age worldwide. Hence, there is a need to determine the levels of oestrogen and progesterone receptors in relation to the growth and symptomatology in a purely African population noted for high incidence of uterine fibroids.

Objectives: To determine the levels of oestrogen and progesterone receptors in normal myometrium and uterine fibroids and ascertain whether there are any significant clinical associations.

Materials and Methods: This was a prospective study conducted at the Obstetrics and Gynaecology Department in collaboration with the Morbid Anatomy Department, both at the University of Benin Teaching Hospital (UBTH). Tissue specimens obtained from uterine fibroids and normal myometrium during surgeries performed on patients with a pre-operative diagnosis of uterine fibroids were histologically examined. The concentrations of oestrogen and progesterone receptors were histochemically determined for the selective tissue slides. The results and the socio-demographic characteristics of the patients were used to generate a database for analysis.

Results: A total of 262 cases of uterine fibroids were analysed. Those presenting with lower abdominal mass had more oestrogen receptors in uterine fibroids (57.0%, $P = 0.014$), whereas more progesterone receptors were found in those presenting with menorrhagia ($P = 0.001$). A comparison of oestrogen and progesterone receptors in uterine fibroids and normal myometrium showed significantly higher levels of oestrogen and progesterone receptors in fibroids than in normal myometrium ($P = 0.000$).

Conclusion: The concentrations of oestrogen and progesterone receptors in uterine fibroids were significantly higher than those in normal myometrium. The steroid dependence of the growth and symptomatology of uterine fibroids may be related to the steroid receptor level. Identification and quantification of the concentrations of oestrogen and progesterone receptors will be useful in the prognostication and the development of newer treatment modalities for uterine fibroids. Further research in this area is clearly warranted.

Key words: Myometrial tissues; oestrogen receptors; progesterone receptors; uterine fibroids; University of Benin Teaching Hospital.

Introduction

Uterine fibroids, also known as uterine leiomyoma, is a benign tumour of the uterus composed of smooth muscles

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and varying amounts of connective tissue.^[1] It is the most common tumour of the uterus among women of reproductive age.^[1,2] Uterine fibroids are seen in approximately 20–25% of women of reproductive age.^[3] Autopsy studies have shown higher incidences of uterine fibroids of various sizes and shapes, though many were asymptomatic.^[1] They frequently cause serious gynaecological problems such as pelvic pain, menorrhagia, dysmenorrhoea, reduced fertility and recurrent pregnancy loss.^[4,5] In addition, uterine fibroids is the most common indication for hysterectomy all over the world, especially in Nigeria.^[6,7]

The pathophysiology of uterine fibroids has not been explored satisfactorily despite the high prevalence rate and tremendous influence on women during their reproductive years.^[1-3,8,9] The relationships between cytogenetic changes of individual fibroids and hormonal mechanisms are not very clear.^[3,8,9] There are reasons to believe that somatic mutations induce changes in the myometrial cell clones resulting in a progressive loss of growth regulation.^[3,8,9] Further development of the tumour is steroid hormone dependent and the main hormones implicated are oestradiol and progestogens, with the population of receptors to these hormones increased in uterine fibroids.^[3,9] This is supported by the observation that generally fibroids do not occur before the menarche and they shrink markedly after the menopause.^[1,2,5,8,10] Although the increased sensitivity to oestradiol is important for the growth of uterine leiomyomas, high circulating oestradiol levels are not a necessary requirement.^[2]

The physiological effects of oestrogen are mediated by oestrogen receptors (ERs), among them, ER is more highly expressed in uterine leiomyomas than in normal myometrium suggesting a possible link between uterine leiomyomas and ER-expression level.^[8,9] In past, oestrogen was considered to play an important role in the growth of uterine fibroids. Later studies have shown that there is an increased concentration of progesterone receptors (PR) in leiomyoma tissue than the normal myometrium.^[3,8,9] Accumulating evidence indicates that the effects of steroid hormones on the growth of fibroids are mediated by specific genes coding for growth factors and their receptors, either directly or via steroid-responsive transcription factors, which consist of protein products of cell cycle-related genes.^[1] The steroid hormone/receptor complex in itself may function as a transcription factor, binding to elements that are located in the promoter region of steroid-responsive genes.^[9] Most fibroids grow slowly and the mechanisms responsible for their neoplastic potential are not completely known.

Recently, increasing interest has been directed towards pharmacological treatment of fibroids, although these are

for symptomatic relief and not for cure.^[9] Two randomised controlled trials have shown that progestins, when used as add-back therapy in combination with gonadotrophin releasing hormones (GnRH) agonists, attenuate or reverse the inhibitory effects of GnRH agonists on uterine fibroids.^[10,11]

Worldwide, uterine fibroids are responsible for significant morbidity in a large proportion of the female population of reproductive age. Studies from developed countries have established high levels of ER and PR expression in uterine fibroids than in normal myometrium of women.^[3,9,10]

This study was undertaken to compare the concentrations of ER and PR in fibroids and normal myometrium obtained during surgery and to determine the clinical significance.

Materials and Methods

This was a prospective study conducted at the Department of Obstetrics and Gynaecology and the Immunohistochemistry unit of the Department of Morbid Anatomy, University of Benin Teaching Hospital, Benin-City, Nigeria. The sample size was determined using the formula by Taylor based on the prevalence of about 25%. The calculated sample size was 288 patients.^[3,5,12]

Tissue specimens obtained from surgeries performed on patients with a pre-operative diagnosis of uterine fibroids from 1st January 2009 to 31st December 2009 were histologically examined. Tissue specimens from both the uterine fibroids and normal myometrial tissues from the same patients were taken for analysis. A semi-qualitative analysis of ER and PR, where the number of receptors that took up stain were quantified, was done.

The tissues were fixed immediately in 10% normal saline for 24 hours, following which good representative sections were obtained and placed in tissue pre-labeled cassettes for processing. The tissue processing involved the use of automatic tissue processor (LEICA TP 1020) and entailed dehydration in 70% alcohol.

The tissues were then transferred into toluene bath 1 for 2 hours and toluene bath 2 for 2 hours. Thereafter, wax impregnation of the tissues was carried out in thermostatically controlled molten wax for 2 hours. The tissues were then taken through the process of blocking out, trimming and sectioning. The sections were made at 3-micrometer gauge and then floated on water, in a water bath, while the sections were picked with pre-labelled APEC-coated slides. The sections were well drained and heated at 60°C for about an hour on a hot plate. The samples

were then subjected to immunohistochemistry procedure using Novolink.

The results were recorded on data sheets and analysis was done with personal computer using the Statistical Package for Social Sciences version 20.0 (SPSS IBM Corp, Armonk, NY) and GraphPad InStat 3 software, (GraphPad Software Inc., San Diego, CA) with *P* value of <0.05 level of significance at 95% confidence interval.

Results

A total of 288 tissue specimens were collected from fibroids and paired with the same number from normal myometrium. Of the 288 tissue specimens, 262 were found to be adequate for analysis and therefore formed the basis of this study.

The mean ± SD age of the patients was 35.98 ± 5.93 years. The mean parity ± SD was 1.12 ± 1.87. Most of the patients presented within the age range of 35–39 years (29.40%) and >40 years (29.80%). Uterine fibroids were seen more among nulliparous patients (61.80%), and the incidence reduces with increase in parity [Table 1]. Most of the patients (172; 65.6%) presented with lower abdominal mass, whereas 143 (54.6%) presented with menorrhagia. Thus, menorrhagia and lower abdominal mass were the common presenting symptoms. Fifty-seven patients (21.76%) had more than one clinical feature at presentation.

When clinical features were compared with the quantity of ERs as suggested by the degree of slides staining, it was observed that most were mildly stained in patients with menorrhagia, lower abdominal pains and pressure symptoms. None of the slides were deeply stained and only small fractions were moderately stained for ERs [Table 2].

When the clinical features were compared for quantity of PRs, all the 3 clinical features considered had varying degrees of specimen staining for PRs. Menorrhagia was associated with more progesterone receptors in the uterine fibroids, with a *P* value of 0.001 which was statistically significant [Table 2].

When the tissue specimens stained for ERs and PRs were compared between the histological samples, it was observed that 172 (65.6%) slides from uterine fibroids took some stain (mildly or moderately stained) for oestrogen compared to 65 (24.8%) for the normal myometrial tissues [Table 3].

For progesterone receptors, 220 (84%) of the slides from uterine fibroids had various degrees of staining compared to only 129 (49.20%) from normal myometrium. While

Table 1: Socio-demographic data and the presenting symptoms

Variables	Numbers	Percentages
Age (years)		
≤19	-	0
20-24	4	1.50
25-29	38	14.50
30-34	65	24.80
35-39	77	29.40
≥40g	78	29.80
Total	262	100.00
Parity		
0	162	61.80
1-4	78	29.80
≥5	22	8.40
Total	262	100.00
Presenting symptoms		
Menorrhagia	143	54.60
Lower abdominal mass	172	65.60
Pressure symptoms (Urinary frequency)	40	15.30
Total	**	**

**Multiple responses were obtained as presenting symptoms, as most patients had more than one symptom at presentations

Table 2: Clinical symptoms compared with the concentrations of progesterone and estrogen receptors in uterine fibroids

	Receptor concentration				<i>P</i>
	(-, +, ++, +++)				
Progesterone receptors					
Menorrhagia	24 (16.8%)	53 (37.1%)	35 (24.5%)	31 (21.7%)	0.010
Lower abdominal mass	26 (15.1%)	47 (27.3%)	67 (39.8%)	32 (18.6%)	0.074
Pressure symptoms	8 (20.8%)	11 (27.5%)	12 (30.8%)	9 (22.5%)	0.603
Oestrogen receptors					
Menorrhagia	47 (32.9%)	88 (61.5%)	8 (5.6%)	0 (0.0%)	0.840
Lower abdominal mass	59 (34.3%)	98 (57.0%)	15 (8.78%)	0 (0.00%)	0.014
Pressure symptoms	17 (42.5%)	23 (57.5%)	0 (0.00%)	0 (0.00%)	0.160

-- Not stained; + - Mildly stained; ++ - Moderately stained; +++ - Deeply stained

Table 3: Comparison of progesterone and oestrogen receptors concentrations in normal myometrium and uterine fibroids

Receptor concentration	Normal myometrium		Uterine fibroids		<i>P</i>
	no	%	no	%	
Progesterone receptors					0.000
-	133	50.8	42	16.0	
+	91	34.7	84	32.1	
++	38	14.5	91	34.7	
+++	0	0.0	45	17.2	
Total	262	100	262	100	
Oestrogen receptors					0.000
-	197	75.2	90	34.4	
+	65	24.8	157	59.9	
++	0	0.0	15	5.7	
+++	0	0.0	0	0.0	
Total	262	100	262	100	

-- Not stained; + - Mildly stained; ++ - Moderately stained; +++ - Deeply stained

only 42 (16.0%) of uterine fibroid slides did not show any form of staining, 138 (50.80%) from normal myometrium

did not [Table 3]. A comparison of the two groups showed statistical significant difference ($P = 0.000$).

Discussion

This study has further confirmed that uterine fibroids are common within the ages 25 and 40 years – the reproductive age of most women, as has been shown in other studies.^[1,3] The results showed that 180 (68.70%) of the 262 patients studied were within the ages of 25 and 39 years. This confirms the common occurrence of uterine fibroids. Approximately two-thirds (61.80%) of our patients were nullipara, with 56.5% having between 1–4 miscarriages in consonance with the previous reports, associating uterine fibroids with nulliparity, reduced fertility and recurrent pregnancy losses.^[4,5,11,13] Although most were asymptomatic, those presenting with symptoms had lower abdominal mass in 65.6% of cases, which was closely followed by menorrhagia 54.6%.

Findings from this study indicate a statistically significant association ($P = 0.014$) between the presence of ERs in uterine fibroids and the specific symptomatology of lower abdominal mass. Although increase in abdominal mass was seen in older patients in this study, it was discovered that increase in the size of the lower abdominal mass had no relationship with the degree of staining for ERs.

Several anti-oestrogen compounds have recently become available and useful for the medical treatment of symptomatic uterine fibroids.^[7-9,14] This includes the use of selective ER modulators as well as the use of pure anti-oestrogens.^[7-9,14,15] Drugs such as Luprorelin acetate depot, a gonadotrophin-releasing hormone (GnRH) agonist, which has been found to cause hypo-oestrogenism followed by the regression of uterine leiomyomas are also useful in the management of uterine leiomyomas.^[8-10]

Patients presenting with menorrhagia had a high concentration of PRs in their uterine fibroids (P value of 0.001), which was highly statistically significant. This justifies the use of gonadotrophin-releasing hormone agonist in the management of patients with uterine fibroids presenting with irregular menstrual blood loss (menorrhagia) with consequent anaemia. They cause amenorrhoea with restoration of normal haemoglobin levels and significant reduction in the sizes of the uterine fibroids.^[9,10] Anti-progesterone compounds seem promising, of value is the use of Mifepristone 25 mg per day for 6 months.^[16,17] Treatment with mifepristone also induces regression of uterine leiomyomas.^[16] This has been clearly demonstrated in this study with uterine fibroids having high levels of PRs.

Approximately a quarter (24.8%) of normal myometrial tissue slides were mildly stained for oestrogen receptors whereas about two third (59.9%) of uterine fibroids tissues were mildly and moderately stained (5.7%) with a cumulative percentage of 65.6%. This observation is consistent with earlier studies, majority of which have demonstrated that ERs and PRs are greater in fibroid than normal myometrium.^[3,8,9,17]

The finding of greater staining for progesterone receptors in uterine fibroids (84.0%) as against that in normal myometrium (49.2%) in this study is at variance with other studies. Pollow *et al.* found fibroid steroid hormone receptor concentrations to be lower for PRs as against normal myometrium, however, the ER concentrations were more in the uterine fibroids compared to normal myometrium.^[18] However, other studies lend credence to the findings in this study that both ER PR levels are higher in fibroid than normal myometrium.^[3,8-10,17]

Otubu *et al.* found a significantly higher concentration of 17β -oestradiol in fibroids than normal myometrium, especially in the proliferative phase, whereas no difference in the concentration of progesterone was found.^[19] The authors speculated that the higher levels of oestradiol in the fibroids could be related to lower levels of the enzyme 17β -hydroxysteroid dehydrogenase, which accelerates the conversion of oestradiol to oestrone. Our study did not take into cognisance the phase of the cycle when the specimens were taken.

When the quantity of ERs in uterine fibroids were compared to that in normal myometrium, there was a statistically significantly higher concentration of receptors in fibroids than in normal myometrium ($P = 0.000$). On comparing the quantity of PRs in uterine fibroids and normal myometrium, it was significantly higher in the former than in the latter ($P = 0.000$). This agrees with the first report in literature by Sadan *et al.*, which found that ERs and PRs in leiomyoma were significantly higher than in normal myometrium ($P = 0.0002$).^[20] This study has demonstrated that the concentrations of ERs and PRs are greater in uterine fibroids than that in the normal myometrium and also that there is a relationship between ERs and PRs with the pattern of clinical presentation of uterine fibroids.

Uterine fibroids have a substantial impact on women's reproductive health, they cause significant morbidity, including prolonged or heavy menstrual bleeding, pressure symptoms, pain and in rare cases, reproductive dysfunction particularly in our environment. Thus, both the economic cost and adverse effect on quality of life are substantial. Surgery remains the mainstay of fibroid treatment, and

various minimally invasive procedures have been developed in addition to hysterectomy and abdominal myomectomy, which have made little impact where we practice because of late presentation, ignorance and poverty.^[5] Formation of new fibroid masses after these conservative therapies remains a significant challenge. Although medications that manipulate concentrations of steroid hormones are effective, their side-effects limit long-term use. A better approach would be the manipulation of the steroid-hormone milieu with specific hormone antagonists. There has been little evidence-based evaluation of therapy. Research into the basic biology of these neoplasms will continue to add new treatment options for the future as the role of growth factors and genetic mutations in these tumours are better understood.

The absence of tissue stain for ERs and PRs in some tissue samples from uterine fibroids sites requires further studies. Not considering the phase of the menstrual cycle at the time of specimen collection for this study is considered a limitation.

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Conflicts of interest

There are no conflicts of interest.

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