

# Prolactin receptors in uterine leiomyomas

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## ABSTRACT

**الأهداف:** للتعرف على موقع مستقبل البرولاكتين عند مرضى الأورام العضلية الرحمية النساء وحجيرات الرحم العضلية من نفس المريض ومقارنتها مع الحجيرات الرحمية العضلية عند النساء الغير مصابات بالورم.

**الطريقة:** إجريت هذه الدراسة بكلية الطب – جامعة النهرين – بغداد – العراق، خلال الفترة مابين 2004م وحتى 2006م. تم جمع نماذج الأورام من قسم النساء والولادة من أربعة مستشفيات وهم: مستشفى الكاظمية التعليمي، مستشفى النور، مستشفى الكرخ، ومستشفى السعدون. أخذت مقاطع من أورام صغيرة وكبيرة مع أنسجة الحجيرات العضلية من (53) مريضة، و(40) مقطع من الحجيرات العضلية الطبيعية من نساء ليس لديهن أورام للمقارنة. تم صبغ جميع المقاطع لإظهار مواقع مستقبلات البرولاكتين فيها PRLR على غشاء هذه الأورام.

**النتائج:** أظهرت جميع النماذج نتائج ايجابية، وتباينت مواقع وأعداد المستقبلات للبرولاكتين من مقطع آخر ومن مريضة لاخرى ومقارنة مع الغير مرضى.

**خاتمة:** إن الزيادة في عدد مستقبلات البرولاكتين هي متوقعة والتي بالتالي تشير إلى أن نسيج الحجيرات العضلية المأخوذة من نفس المريضة تكون غير طبيعية.

**Objective:** To identify the location of prolactin receptors in patients with uterine leiomyomas and their host myometrium as well as normal myometrium.

**Methods:** A case control study was conducted at the College of Medicine Al-Nahrain University, Baghdad, Iraq during the period from 2004-2006. The samples were collected at Obstetrics and Gynecological Departments of 4 hospitals in Baghdad City (Al-Khadimiya Teaching Hospital, Al-Noor, Al-Kharch, and Al-Saadoon Hospital). Sections from large and small tumors (n=53) with their host myometriums and from normal myometriums (n=40) were stained immunohistochemically for prolactin receptors.

**Results:** Prolactin receptors were positively seen in all cases examined including patient and comparison tissues, in the form of dark brown staining. Staining

was heterogenous and varied in intensity from one case to another and sometimes from one area to another in the same section.

**Conclusion:** The increase in prolactin receptors in leiomyoma is expected given that the underlying host myometrium is abnormal.

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Uterine fibroids are tumors made of connective tissue and smooth muscle. They grow slowly within the wall of the uterus or attach to the uterine wall. Most fibroids are non cancerous, although in some rare cases they may become cancerous. This occurs in less than 1% of fibroids. A uterine fibroid may be as small as a pea or as large as a grapefruit. Other terms used for a uterine fibroid are leiomyoma or myoma of the uterus.<sup>1</sup> Fibroids are usually found during routine pelvic exams. If the patient has severe menstrual symptoms or other pelvic problems, an ultrasound scan maybe recommended determining the cause of the problems. For fibroids that require treatment, the gynecologist may suggest a hysterectomy or myomectomy, and the latest one is usually carried out when the possibility of having children after the surgery is planned.<sup>1</sup> Prolactin (PRL) was initially identified as a pituitary gland hormone, but several studies have demonstrated that prolactin is also produced by uterine tissues, including the endometrium, myometrium, and uterine leiomyomas.<sup>2</sup> The prolactin receptor (PRLR) belongs to the class 1-cytokine receptor family and it was first identified during the seventies. Class 1-cytokine receptors are single-pass transmembrane proteins that contain an extracellular, a transmembrane, and an intracellular domain.<sup>3</sup> The extracellular region of the PRLR is 210 amino acids and

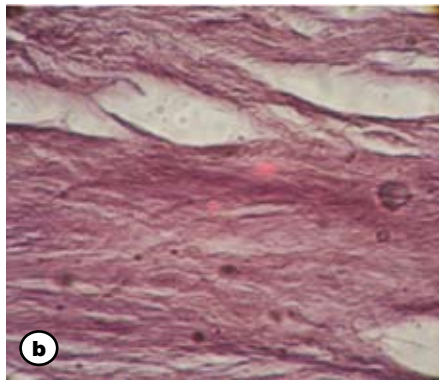
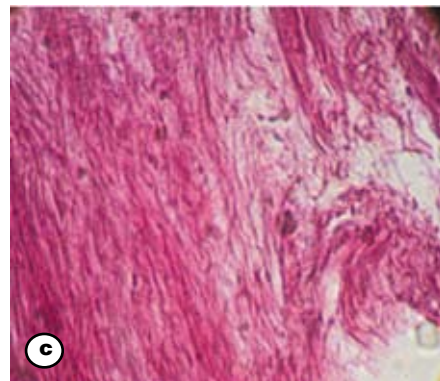
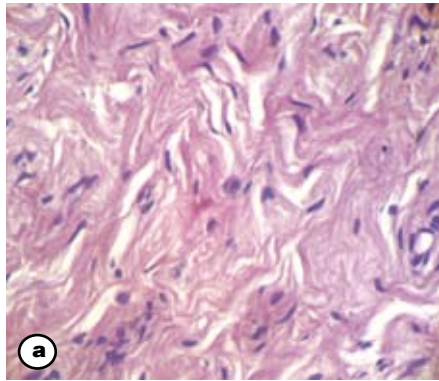
contains 2 subdomains, D1 and D2.<sup>4</sup> These extracellular subdomains are structured into 2 types 3 fibronectin-like domains that consist of 7 antiparallel  $\beta$ -strands divided into 2  $\beta$ -sheets that are connected by a 5 amino acids linker.<sup>5</sup> When ligands bind to the extracellular domain, the PRLRs will homodimerise, resulting in activation of intracellular signaling systems.<sup>4</sup> The aim of the study is to identify the location of prolactin receptors in patients with uterine leiomyomas, and their host myometrium as well as normal myometrium

**Methods.** This study was carried out during the period from 2004-2006 at the Obstetrics and Gynecology Departments of 4 hospitals in Baghdad City (Al-Khadimiya teaching hospital, Al-Noor, Al-Kharch, and Al-Saadoon Hospital). Patients with uterine fibroids who were selected for surgical treatment (myomectomy or hysterectomy) were included in this study, those with uterine adenomyosis, endocrine disease, or malignancy were excluded from the study. Written consent was taken from all the patients prior to operation. Ethical approval was not needed in this study as its dealing with histopathological specimens. Sixty-two women patients were collected from the 4 hospitals, 9 of them (14.5%) were excluded (one of those 9 had breast cancer and underwent a chemotherapy treatment; another had thyroidectomy, 6 with adenomyosis, and one with micropituitary adenoma). A total of 53 women patients with leiomyoma were included in this study. Their mean age $\pm$ SD was 39.9 $\pm$ 5.5 years, and age range was 31-49 years. Eighty-six leiomyoma samples with different uterine sites including intramural, subserosal, submucosal, broad ligament, and cervical with size ranges from 0.09-136.5 cm<sup>3</sup> were harvested from those 53 women. The comparison group, however, included 40 pregnant women who had cesarean section for maternal or fetal causes. The mean age $\pm$ SD of this group was 38.4 $\pm$ 4.9 years and the age range 30-48 years. None of them had leiomyoma, which was confirmed by ultrasonography. Uterine fibroids included in this study were identified grossly at surgery and confirmed by histological examination to be fibromatous leiomyomatous tissue. The prolactin receptor was detected in tissue sections by using an immunohistochemical staining method with monoclonal antibody (BIOCARE detection kit). The immunohistochemical staining of leiomyoma sections revealed brown spots, which indicate the presence of prolactin receptor on the membrane of leiomyoma. A myometrium sample from the same leiomyoma patient also was taken. The comparison group was pregnant women who underwent cesarean section during the same period, ultrasonography was used to confirm the absence of leiomyoma among those women. A myometrium sample was taken from each woman in the comparison group and compared with myometrium

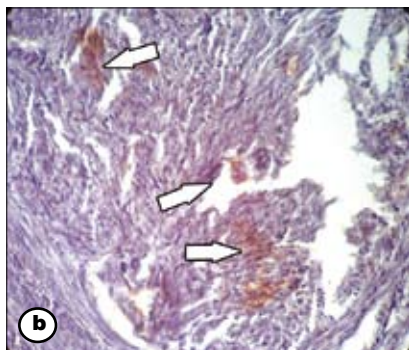
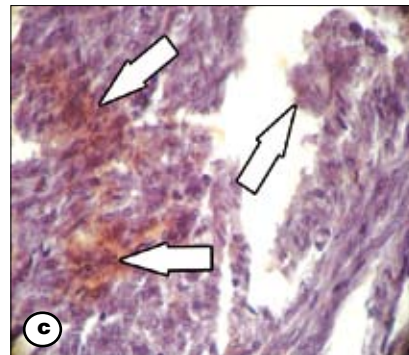
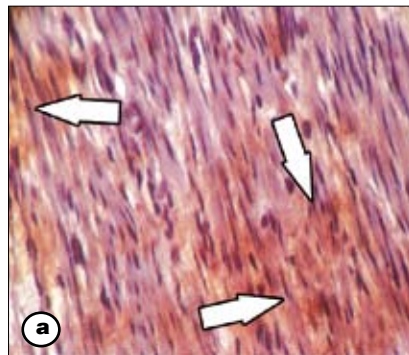
of leiomyoma patients. Routinely processed, formalin fixed paraffin embedded tissues is suitable for use with the monoclonal antibody to prolactin receptor (quartet Cat.# 161803701) when used with BIOCARE detection kit and AB500 Chromogen System (Cat.# DB801R). The recommended tissue fixative is 10% neutral buffered formalin (Sheehan and Hrapchak 1980, Fluka, Germany). Each section should be cut at approximately 5 $\mu$ m thickness and placed on positively charged glass slid. Staining of formalin/paraffin tissues requires digestion with pepsin 1mg/ml Tris-HCL. Cat.# 401603799 for 15 minutes at room temperature. After application of streptavidin-HRP (streptavidin horseradish-peroxidase), rinse tissue sections in PBS wash buffer.

**Results.** The hematoxylin and eosin stained sections of uterine tissue, were used to confirm the presence of uterine leiomyomas from which they were harvested. Figure 1 shows the stained uterine leiomyomas sections of patients with their host myometrium and normal myometrium. Prolactin receptors were positively seen in all cases examined, including patient and control tissues, in the form of dark brown staining spots (Figure 2). They were formed as a result of reacting the monoclonal antibody of prolactin receptor with the extracellular portion of the receptor. Staining was heterogenous and varied in intensity from one case to another and sometimes from one area to another in the same section. As the intensity of brown spots increased, the prolactin receptors increased, and this occurred in uterine leiomyomas.

**Discussion.** In this study, it was found that the mean age of patients with leiomyoma was 39.9  $\pm$  5.5 years, which is considered as late reproductive age or premenopausal age. This result was in agreement with Cramer and Patel,<sup>6</sup> who mentioned that uterine leiomyoma is the most common gynecologic neoplasm, occurring with a remarkable frequency in more than 70% of reproductive age women. Also, Cheryl et al<sup>7</sup> reported that risk increases with age during the premenopausal years, but tumors typically regress and/or become asymptomatic with menopause. Mitchell et al<sup>8</sup> reported that leuomorphi (LM) PRL secretion is significantly greater than myometrial PRL secretion for the same patient, and they found that LM PRL secretion increased with time whereas myometrial PRL secretion did not. This finding agrees with the spotting results of this study as shown in Figure 2, in which the amount of PRL in LM was greater than myometrium PRL of the same patient and greater than the comparison myometrium PRL, and as the prolactin level increased, its receptor will increase also. Daly et al<sup>9</sup> said that leiomyoma has the ability to synthesize prolactin, which



**Figure 1** - Hematoxylin and eosin stained section for a) uterine leiomyoma, b) patient's myometrium, c) normal myometrium.



**Figure 2** - Brown spots indicated the location of prolactin receptors at tissue membrane using monoclonal antibody: a) uterine leiomyomas section, b) patient myometrium section, c) comparison myometrium section.

increases the evidence that cells of mesenchymal origin that arise near the paramesonephric ducts have a latent ability to express the genome for prolactin synthesis, and the appearance of prolactin synthesis in leiomyoma in-vivo suggests that this potential genome expression is activated either in smooth muscle cells or stromal cells during the transformation of normal cells to leiomyoma cells. Bhatavdekar et al<sup>10</sup> also studied the ectopic production of PRL by colorectal adenocarcinoma, and by assaying the circulating PRL and carcino embryonic antigen using immunoradiometric assay and radioimmunoassay kits, respectively in preoperative blood and tumor-draining venous blood samples of colorectal carcinoma patients. They concluded that these multiple approaches confirmed that PRL is produced by colorectal carcinoma cells and by looking at its prognostic value and correlation with disease activity; it may provide a new insight into treatment for patients with colorectal carcinoma.<sup>10</sup>

Brown spots show heterogeneity in their numbers and accumulation. The difference was clearly obvious between accumulation and number of brown spots in the leiomyomas sections, including their different sites, and the myometrium sections from the same patient. The leiomyomas sections as shown in Figure 2 had more distributed brown spots than myometrium of same patient. And the latent shows less accumulated brown spots with less distribution. While myometrium sections of comparison group (non-fibroid pregnant women) exhibits more accumulation and numbers of brown spots than those in patient myometrium. The distributed spots may vary also from one area to another in the same tissue section especially in the myometrium sections. Gill et al<sup>11</sup> studied the expression of PRLRs in normal, benign, and malignant breast tissue by an immunohistological method and they concluded that because prolactin plays an important role in the proliferation and differentiation of normal breast epithelium, they suggested that the higher expression of PRLR noted in the cells of most benign and malignant breast lesions, compared with normal cells, could be an important factor in the pathogenesis of these diseases, rather than a reflection of the high proliferate activity of the abnormal cells. They also studied the normal breast PRLR, they had seen positively in all cases examined, in the form of dark brown staining of the luminal borders of the epithelial cells lining the ducts and acini. Staining was heterogenous and varied in intensity from one case to another and sometimes from one area to another in the same section. Myoepithelial cells were negative, in addition to fibrous tissue and blood vessels. They mentioned also that there is a significant relation between the estrogen receptor and PRLR in female with invasive breast carcinoma ( $p < 0.05$ ). Richards et al<sup>12</sup> examined the estrogen receptor content of fibromyomata, in relation to the estrogen receptor

content of their host myometria and normal myometria. Estrogen receptor levels in fibromyomata are thought to be influenced by the endometrial cycle, increasing through the proliferative phase into the early secretory phase before falling just prior to the onset of menses. They concluded that the increase in estrogen receptors in fibromyomata is expected given that the underlying host myometrium is abnormal. The phasic differences in receptor content may help explain the higher mitotic indices in tumors from secretory phase uteri. Furthermore, the differential staining patterns of the nuclei may be related to differences in gene regulation as a result of the fibromyomata's heightened sensitivity to estrogen.

In conclusion, the increase in prolactin receptors in leiomyoma is expected given that the underlying host myometrium is abnormal. For further study, it is recommended to count the brown spots which represent the prolactin receptors in abnormal myometrium, and measure its intensity by special technique.

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