PROLACTIN PROFILES AND THEIR MOLECULAR WEIGHTS IN SERUM AND TISSUES OF PATIENTS WITH UTERINE LEIOMYOMA IN IRAQ

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Abstract

Background: Uterine leiomyomas are benign tumors arising from the myometrial compartment of the uterus. They are the most common gynecologic neoplasm, occurring with a remarkable frequency in more than 70% of women at their reproductive age. The female reproductive tract is known to be an extrapituitary source of the hormone prolactin. Prolactin (PRL) is a polypeptide hormone of growth hormone/cytokine family. In serum, PRL occurs in various molecular forms with different molecular weights, including the physiologically active monomeric form called the little PRL, the big PRL, and the big big PRL, that is also called macroprolactin.

Objectives: To study the PRL profiles with its molecular weights in patient's serum, leiomyomas, and myometrium compared with the PRL profiles of a normal myometrium.

Subjects and methods: Circulating prolactin of patient group (n=57) as well as their tissues prolactin [(leiomyomas and myometrium)] and [normal myometrium] of the control group (n=45) was assayed using the Prolactin Kit (Biomérieux). Prolactin profile was detected using the polyethylene glycol 8000 precipitating method to separate the big big prolactin from the monomeric and the big prolactin isoforms. Disk gel electrophoresis technique was used to confirm the prolactin isoforms and to calculate their molecular weights.

Results: A highly significant difference was found between the leiomyoma prolactin and patient's myometrium prolactin as well as between the leiomyoma prolactin and the normal myometrium prolactin ($P<0.0001$), while no significance was found between patient's myometrium and the normal myometrium prolactin. Also a high significance ($P<0.0001$) was found between the patients serum prolactin and their leiomyoma prolactin, leiomyoma size, while a significant value ($P<0.05$) was found between leiomyoma prolactin and their sizes. In this study the prolactin isoforms in patient's serum, leiomyoma and myometrium as well as in the normal myometrium of control group were monomeric and big prolactin with different molecular weights. Only one sample of patient's serum had big big prolactin isoform.

Conclusion: Serum prolactin level was increased in patient with uterine leiomyoma with a significant positive correlation with the leiomyoma size and its prolactin produced from it. Hyperprolactinemia may be also caused by macroprolactin in patients with uterine leiomyoma because the big forms of PRL have a decreased bioactivity, they do not cause clinical symptoms of hyperprolactinemia.

Key words: Prolactin, uterine leiomyoma, Prolactin profile.

Introduction

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. Leiomyomas are classified by their location in the uterus and may be as small as a pea or as large as a grapefruit. [1,2]

Prolactin is initially identified as a pituitary gland hormone; several studies have demonstrated that prolactin is also produced by uterine tissues, including the endometrium, myometrium, and uterine leiomyomas.[3] The significance of prolactin production in leiomyomas is not yet well defined; however, interest in this hormone has been stimulated by

the finding that prolactin acts as a mitogen for vascular smooth muscle. Because leiomyomas are mitotically active during the luteal phase, the inhibition of leiomyoma prolactin production by progesterone tends to cast some doubt upon the role of this hormone in fibroid growth.[4] However, in a recent study, treatment of leiomyoma and myometrial cell cultures with a prolactin-neutralizing antibody inhibited cell proliferation, leading the authors to conclude that prolactin may be an autocrine or paracrine growth factor for both leiomyoma and myometrial cells.[5] At this date, it would seem that the prolactin story is unfinished, evolving, and worthy of further study.

Human prolactin (PRL) is synthesized as a pre-hormone with a molecular weight of 26,000 kDa. When the pre-prolactin is cleaved, the resulting polypeptide has a molecular weight of 23,000 kDa, and this monomeric form accounts for the majority of total PRL. Prolactin (PRL) in human serum has been classified into three main species on the basis of molecular mass: monomeric PRL, big PRL and big, big PRL (bb-PRL), called 'macroprolactin', with molecular masses of 23 kDa, 50–60 kDa and 150–170 kDa respectively.[6] Although the nature of bb-PRL is heterogeneous, the most common form of macroprolactin is a complex of PRL and immunoglobulin G.[7,8] Macroprolactin is recognized, in various degrees, by immunoassays for PRL and has a slower clearance from serum than PRL, causing diagnostic confusion in evaluating hyperprolactinemic conditions. The incidence of macroprolactinemia ranges from 15% up to 26% of all hyperprolactinemic sera and represents the main cause of inter assay variability for PRL dosage.[9,10]

Recognizing the presence of macro-prolactin may help define the etiology in patients with idiopathic hyperprolactinemia, and in some cases recognition of macroprolactinemia might eliminate the need for extensive diagnostic tests or pituitary imaging. This is especially important because 10% of healthy subjects have radiographic evidence of a pituitary adenoma.[11]

PEG precipitation is a relatively simple and inexpensive technique but is not specific or quantitative. A percentage recovery of greater than 65% confirms the presence of monomeric PRL whereas a percentage recovery of 40% or less is very sensitive for detecting significant amounts of macroprolactin. Recovery between 40% and 65% indicates a sample may contain macroprolactin and oligomeric PRL, in addition to the monomeric form. In these cases, separation method such as gel filtration chromatography or electrophoresis would be necessary to confirm the presence of macroprolactinemia.[12,13]

The presence of macro-prolactinemia in a patient with no clinical suspicion of hyperprolactinemia could obviate the need for a pituitary magnetic resonance imaging or other testing.[9]

Smith *et al*. 2002; also noted that some patients with macroprolactinemia have elevated levels of monomeric PRL and suggest that the diagnosis of macroprolactin be used only when a PRL level falls to a level seen in sera from normoprolactinemic subjects treated with PEG. Although this would help ascertain whether an excess of monomeric PRL is present along with macroprolactin, it would require establishment of new reference ranges for all PRL assays[10]

Methodology

Samples in this study were collected during the period from May 2004 to June 2006 at Obstetrics and Gynaecology departments of three hospitals in Baghdad city (Al-Khadimyia teaching hospital, Al-Noor, and Al-Saadoon Hospital).It included a sample of cases group and a comparison group. The cases group included female patients at their reproductive age who were diagnosed previously by their physicians as patients with uterine fibroids after proper physical and gynecological examination which confirmed by ultrasound findings, they were prepared for laparatomy either for total abdominal hysterectomy or myomectomy. All patients were with normal pituitary image, none of them was on any drugs known to increase serum prolactin level in the last six months. None of them was known to complain of diabetes mellitus, pituitary, and thyroid, renal or psychiatric disease.

Ten milliliters of venous blood were aspirated from leiomyoma patients just before operation, left to clot, and then centrifuged. Part of it was used for measuring the serum PRL level at the same day of operation by using the Prolactin Kit (Biomérieux); [measurement range of the VIDAS PRL kit is 0.5-200 ng/ml]. The range of expected values for the normal menstruating women is (5-35 ng/ml), Prolactin level was considered normal up to 35 ng/ml. Patients after operation with abnormal serum PRL level (>35 ng/ml) were sent to the Magnetic Resonance Imaging (MRI) or Computerized Tomography (CT) to examine their pituitary .

Uterine fibroids introduced in this study were identified grossly at surgery and confirmed by histological examination to be fibromatous leiomyomatous tissue without malignancy. They were immediately immersed in ice-cold saline solution after recording their dimensions, types, and localized their position in the uterus. Leiomyomas were dissected free from surrounding myometrium. When two or more leiomyomas were present in the same uterus, samples from several of them were pooled and diced.[14]

A host myometrium sample also was taken from each leiomyoma patient. Also these tissue samples were immersed immediately in cold saline as described for leiomyoma samples.

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. No blood sample was taken from those pregnant women because their serum PRL level is already higher 10 folds than the normal level.

Tissue samples were kept at –20°C before processing up to 2 weeks. Then they were weighed, and sliced with scalpel in petri dish standing on a dry ice. Slices were thawed and minced with scissors, then homogenized with (0.02) M Tris buffer pH 7.4 with a ratio of 1:3 (w:v) tissue to buffer solution using a mechanical homogenizer.[15]

The homogenate then was filtered through ten layers of nylon gauze, and centrifuged in cooled centrifuge at 4°C in order to precipitate the remaining intact cells and the intact nuclei at 4000 xg, which express the multiple of the gravitational force for 30 minutes.[16] Supernatant was then used to estimate the PRL level in it by the same (Biomérieux) kit.

Macroprolactin screening method

All serum and tissue supernatant samples were process as following:

Because the high concentration of polyethylene glycol (PEG) precipitate macroprolactin from serum, the precipitation method was carried out by adding 200 µl of serum or tissue homogenate supernatant to 200 µl of 250 g/L PEG 8000 solution (in distilled water, kept at 4°C), mixed for 1 minute with a vortex mixer. The mixture was centrifuged for 5 minutes at room temperature at 9500 xg and determined again the amount of PRL in the supernatant using the same miniVIDAS. The PRL recovery was calculated according to the following formula (Fabio *et al.*2001).[17]

$$\frac{\text{PRL level after PEG precipitation}}{\text{PRL level before PEG precipitation}} \times 100$$

Recoveries ≥65% are classified as predominantly monomeric hyperprolactinemia, and recoveries of ≤ 40% as predominantly high molecular weight forms (macroprolactin). Values between 40-65% were classified as indeterminate and they were all submitted to gel electrophoresis.

Determination of prolactin molecular weight

A method of disk-electrophoresis in sodium dodesyl sulphate (SDS) was used to determine the molecular weight of prolactin in both serum and tissue homogenate supernatant.[18] A protein standard kit (Promega; Low and high-range protein molecular weight markers ranging from 14 up to 150 KDa molecular weight) were used and applied for gel electrophoresis at the same time of sample processing.

In Table (1), LM patients shows a high mean value of PRL level in their serum which is equal to (147.6 ± 102.6) ng/ml compared

with normal serum PRL level range in healthy menstrual women (5-35 ng/ml).

The mean PRL level of LM was greater than the PRL of myometrium in same patient group (16.9 ± 12.0) (6.8 ± 3.2) ng/ml respectively, while mean of PRL in the myometrium of control group was found (5.6 ± 2.5) ng/ml.

Analyses were done using SPSS computer program version 10.0, Statistical analysis included descriptive statistics (mean, standard deviation SD, frequencies and percentage), and Student's t-test was used for comparing means of two variables. P value equals or less than 0.05 was considered significant.

Results

Fifty-seven women patients with leiomyoma were included in this study. Their mean age±SD was 37.9±9.2 years and age range was (28-47) years. Twenty patients (35.1%) underwent hysterectomy while 37 patients (64.9%) had myomectomy.

Eighty-eight leiomyoma samples with different uterine sites were harvested from those 57 women.

The comparison group, on the other hand, included 45 pregnant women who had cesarean section for maternal or fetal causes. The mean age±SD of this group was 30± 5.1 years and the age range (17-43) years. None of them had leiomyoma which was confirmed by ultrasonography.

1- Uterine leiomyoma patients may have LM(s) accumulate in one uterine site, while others may show LM(s) in many different sites (intramural, submucousal, subserosal, broadligament and cervical). The size of these LM(s) was ranging from 0.11 cm^3 to 138.3 cm^3.
2- Also it shows the percentage of each PRL isoform found in serum, LM and myometrium of same patient group comparing with PRL isoform found in the myometrium of control group.

Table (1)
Mean and standard deviation of prolactin and prolactin isoforms in both patient and control groups.

	Patient			***Control****
	Serum	***Uterine▲ leiomyoma***	***myometrium***	***myometrium***
Prolactin Level (ng/ml)	147.6 ± 102.6	16.9 ± 12.0	6.8 ± 3.2	5.6 ± 2.5
Prolactin isoforms:				
Monomeric PRL	(32)56.14%	(60)68.18%	(47)82.46%	(35)77.78%
Big PRL	(24)42.11%	(28)31.82%	(10)17.54%	(10)22.22%
Big big PRL	(1) 1.75%	----------	----------	---------
Total	**(57)100.0%**	**(88)100.0%**	**(57)100.0%**	**(45)100.0%**

(*) No serum sample was taken from this group because the prolactin level is already in a pregnant woman is higher 10 folds than the normal.
Note: Mean serum prolactin for healthy menstrual women was found 18.6 ± 2.3 ng/ml.
(▲): Eighty-eight fibroids were found in 57 LM patients because those patients may have more than one uterine fibroid in the same site.

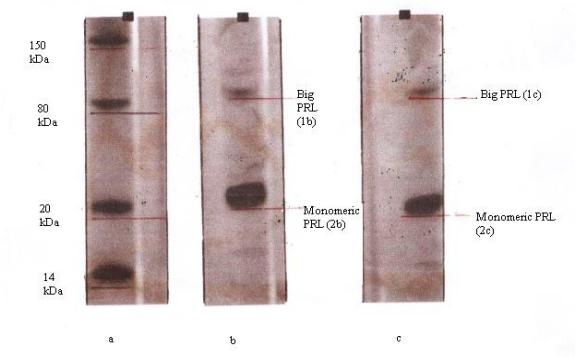
3- Monomeric PRL was found in both patient and control groups. Serum of 32 patients out of 57 (56.14%) were with monomeric PRL, while (68.18%) and (82.46%) represents the percentage of monomeric PRL in their LM and myometrium respectively. Thirty-five (77.78%) out of 45 control myomerium had monomeric PRL isoform.

4- Big PRL isoform represents the second PRL isoform found predominantly in both groups. 24 (42.11%) out of 75 LM patients had big PRL isoform in their serum. Tissues (LM and myometrium) of same patient group as well as myometrium of control group had also big PRL isoform with percentages (31.82%) (17.54%) and (22.22%) respectively.

5- Only one patient serum sample out of 57 had big big PRL isoform. And no big big PRL was found in tissues of patient and control groups.

Prolactin molecular weight

To evaluate the molecular weight (MWt.) of each PRL isoform in both patient and control group, samples were underwent disk gel electrophoresis technique and then all separated result images were introduced in the PhotoCaptMWt. Program to calculate the PRL MWt.



Protein standard and serum sample pre- and post- polyethylene glycol treatment. Two bands exhibit representing big and monomeric PRL isoforms.
***(a)*: Protein standard Bands represented (150, 80, 20, 14) kDa respectively in descending manner.**
***(b):* Double bands (1b and 2b) represented big and monomeric PRL isoforms in pre-polyethylene glycol serum sample.**
***(c):* Double bands (1c and 2c) represented big and monomeric PRL isoforms in post-polyethylene glycol serum sample.**

All calculated MWt. results were compared with the recovery PRL percentage [dividing the post-PEG PRL level by the pre-PEG PRL level] as shown in Table (2).

Table (2)
Comparison between the recovery PRL percentage and the calculated molecular weight in both patient group and control group, were R%=recovery PRL percentage, MWt. = molecular weigh.

Prolactin profiles	Patient						Control	
	Serum		*Leiomyoma*		*Myometrium*		*Myometrium*	
	R%	MWt	R%	MWt	R%	MWt	R%	MWt
Monomeric	77.6±8.2	24.9±9.7 16.5±4.5	80.5±8.3	16.5±5.0	89.2±5.2	16.7±6.9	87.0±2.7	16.7±5.7
Big	53.6±7.7	71.6±15.7	59.5±4.4	70.5±11.2	62.9±15.4	78.5±14.9	54.9±8.5	74.4±13.2
Bigbig	19.05	201.5	----------		----------		----------	

R%= Recovery prolactin percentage, MWt= molecular weight in kDa (kilo Dalton).

In Table (2), serum, LM and myometrium of same patient group had monomeric PRL isoform. Two types of monomeric PRL isoforms were found in patient's serum, [24.9±9.7 and 16.5 ± 4.5] kDa. While the LM and myometrium PRL of same patient group had monomeric isoform with Mwt. 16.5 ± 5.0 and 16.7 ± 6.9 kDa. Control myometrium was also found with 16.7 ± 5.7 kDa molecular weight.

The big PRL isoform was found with 71.6±15.7, 70.5 ±11.2 and 78.5±14.9 kDa for serum, LM and myometrium respectively of same patient group. Big PRL isoform was also

found in control myometrium with Mwt. 74.4±13.2 kDa.

The only big big PRL isoform found in this study was in one patient's serum with Mwt. 201.5 kDa. All these calculated Mwt(s) in serum and tissues of both study groups were matched with their expected isoforms from the recovery PRL percentage.

Discussion

Mitchell *et al.* 1989 have reported that 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 mean results of this study as shown in Table (1) in which the amount of PRL in LM was greater than myometrium PRL of the same patient and greater than the control myometrium PRL, while no significant differences was found between patient and control myometrium PRL.[19]

Daly *et al.* 1984 said that leiomyoma has the ability to synthesize prolactin which 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.[14]

In this study the most predominant PRL isoform found in both LM patient and control groups was the monomeric PRL. Ben-Jonathan *et al.* 1996 have mentioned that explants of normal myometrium as well as proliferative leiomyomas (fibroids) secreted immunoreactive PRL (monomeric isoform) into culture medium.Bigbig PRL isoform was not found in patient tissues or control myometrium. But it was found only in one patient serum.[20] This isoform did not appear lonely, it was found with both monomeric and big PRL isoforms in the same patient serum. This bigbig case can be considered as the first one found among LM patients. No cases or reports were found during the study search that had bigbig PRL isoform in LM patients.

In the 1980s, macroprolactin-aemia was first identified as a new type of hyperprolactinemia. It was found to occur in 8±2.5% of patients with hyperprolactinaemia. This entity was defined by the bigbig PRL isoform being the only or the predominant form and was claimed to be poorly symptomatic and idiopathic. Although the nature of these large forms is still under debate a tumoral origin has been suggested.[13,21] Suliman *et al.*, 2003, have reported that hyperprolactinemia is characterized by the presence of excess monomeric prolactin in serum. This finding agreed with serum results among the patient group whom have abnormal serum PRL level >35 ng/ml in this study. They also said that macroprolactinemia can be defined by the presence of excess serum macroprolactin together with non pathologic monomeric PRL concentrations. In addition to that, the macro PRL isoform is only found when there is large amount of PRL aggregation and conjugated with IgG. There is no reason can be given till now by researchers as an explanation about this inactive form, only that appearance of this isoform is due to the renal delay clearance which is due to its large molecule.[22]

Corbacho *et al.*, 2002 have reported that PRL does not circulate as a single molecular species but as a family of related proteins. Circulating PRL in humans appears to consist of 5 isoforms: the classical 23 kDa molecule, a glycosylated PRL of 25 kDa, a 16 kDa fragment of PRL, dimmers of 50-60 kDa (big PRL), and aggregates of >100 kDa (bigbig PRL). They mentioned that the actions of different members of the PRL family on angiogenesis provide one of the clearest examples directly relating PRL functional diversity to its structural heterogenecity. So, full length PRL was considered to be inactive on blood vessel growth until showed its potential as a proangiogenic factor. Conversely, the enzymatically cleaved 16 KDa N-terminal fragment of PRL has a well defined anti-angiogenic effect.[23] Gel filtration technique was not used because it is difficult, expensive not recommended to be used nowadays.[24] It is also relatively insensitive requiring high levels of PRL, and since big and bigbig PRL forms represent only (6.1-42%) of

the total PRL immunoreactivity and this agreed with what Blacker *et al.*, 1994 reported.[25]

The monomeric PRL molecular weight varying between 16 up to 25 kDa for both serum and tissues. This help explanation that PRL secreted from the LM is from the same source of smooth cell myometrium Table (2). The abnormal level of serum PRL in patient group, which is predominantly consisting of the monomeric with mean MWt. of PRL 16.5±4.5 kDa, is due to the ectopic PRL production of the LM, although, we can see, the monomeric MWt. in serum PRL found 24.9 kDa which is greater than that found in the tissues. Also it has big PRL isoform with mean MWt. of 71.6 ± 15.7 kDa.

The 16 kDa prolactin is a PRL fragment retains PRL-like effects; it is mitogenic in the pigeon Crop-sac and in the Nb2 lymphoma cell bioassays. It has mammary mitogenic activity in the rat in vivo and it is both mitogenic and lactogenic in rat mammary cells in culture.[26] 16 kDa PRL could reach the circulation from different sources, including the pituitary gland and extra pituitary tissues.[23]

The predominant PRL isoforms found in the control group were both monomeric and big PRL with mean MWt. of (16.7±5.7, 74.4±13.2) kDa respectively. And because this group consist of only pregnant women haven't any uterine fibroid, this result agreed with Corbacho *et al.* 2002 when reported that the concentration of 16 kDa PRL was elevated in pregnant women close to the day of delivery. [23]

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الخلاصة

الخلفية: الأورام العضلية الرحمية الملساء leiomyomas ، أو ما يشار إليها عادة باسم "الأورام الليفية", هي عبارة عن أورام حميدة تنشأ من حجيرات الرحم العضلية,تعرف بإمكانية تمييزها بشكل جيد لإمتلاكها إنقسام خيطي واطيء نسبيا" مع احتفاظها بالشكل الاملس للعضلة.تعد هذه الأورام الاكثر شيوعا" عند النساء في سني التناسل و تحدث بنسبة تصل إلى أكثر من 70%. من المعروف أن الجهاز التناسلي عند النساء يعتبر كمصدر آخر للبرولاكتين خارج الغدة النخامية extrapituitary . البرولاكتين هو بولي ببتيد(كثير الهضمية), وزنه الجزيئي 23 كيلو دالتون, ينتمي إلى عائلة هرمونات النمو/السايتوكايين, و يفرز بشكل رئيسي من قبل lactotrophs الواقعة في الجزء الأمامي للغدة النخامية فضلا" عن إفرازه بتراكيز منخفضة من قبل أنسجة أخرى. في المصل, يتواجد البرولاكتين بأشكال جزيئية مختلفة, من ضمنها الشكل الأحادي الفعال وظيفيا" و المسمى أيضا" بالبرولاكتين الصغير Little PRL (L-PRL) والذي وزنه الجزيئي 23 كيلو دالتون.والبرولاكتين الكبير Big PRL (B-PRL) والذي وزنه الجزيئي 50–60 كيلود التون والكبير الكبير Big Big PRL (BB-PRL) الذي وزنه الجزيئي 150–170 كيلو دالتون والمسمى أيضاً بـ (macroprolactin).

الهدف : لدراسة نمط البرولاكتين مع أوزانه الجزيئية في كل من المصل ,الأورام العضلية الرحمية والحجيرات العضلية الرحمية لمرضى هذه الأورام

المواضيع والطرق : تم تحليل برولاكتين الدم والأنسجة (الأورام العضلية الرحمية والحجيرات العضلية) لمجموعة المرضى وعددهم (57) وكذلك برولاكتين الحجيرات العضلية الطبيعية لمجموعة السيطرة وعددهم خمسة واربعين

بأستخدام عدة البرولاكتين من شركة (Biomerieux) أما نمط البرولاكتين فتم الكشف عنه بأستخدام طريقة

بالعقدالرحمية كون الانماط الكبيرة من البرولاكتين تقل فيها الفعالية الحياتية وبالتالي لا يتسببون في اية عوارض واضحة.

الترسيب بالبولي أثيلين كلايكول (polyethylene glycol 8000) لفصل البرولاكتين الكبير الكبير عن شكلي البرولاكتين الأحادي والكبير. وأستخدمت تقنية الترحيل الكهربي الهلامي القرصي لتعزيز أشكال البرولاكتين ولحساب أوزانهم الجزيئية .

النتائج: لقد وجدت قيمة عالية الدلالة بين برولاكتين الأورام العضلية الرحمية وبرولاكتين الحجيرات العضلية للمرضى وكذلك بين برولاكتين الأورام العضلية الرحمية وبرولاكتين حجيرات العضلة الطبيعية ($P<0.0001$) في حين لم توجد أي قيمة ذات دلالة بين برولاكتين الحجيرات العضلية للمرضى مقارنةً لمجموعة السيطرة الطبيعية. كما وجدت قيمة ذات دلالة عالية ($P<0.0001$) في مصل المرضى وبرولاكتين أورامهم العضلية وحجم الأورام , وبين نسبة برولاكتين المصل / برولاكتين الورم مع الحجم , في حين وجدت قيمة ذات دلالة ($P<0.05$) بين برولاكتين الأورام العضلية وحجمها.

يتضح أن شكل البرولاكتين السائد في هذه الدراسة هو البرولاكتين الأحادي monomeric, والذي وجد في كل من مصل المرضى وأنسجتهم (ألأورام والحجيرات العضلية) بوزنين جزيئيين (16.5 ± 4.5و 24.9±9.7) كيلو دالتون, (16.7±6.9و 16.5±5.0) كيلو دالتون على التوالي . أما متوسط الوزن الجزيئي للبرولاكتين الأحادي في مجموعة السيطرة الطبيعية فكان (16.7±5.7) كيلو دالتون. وكان الشكل السائد الثاني للبرولاكتين هو البرولاكتين الكبير , حيث وجد بوزن جزيئي مقداره (71.6±15.7) كيلو دالتون في مصل المرضى في حين كان (70.5±11.2) و (78.5±14.9) كيلو دالتون في الأورام والحجيرات العضلية على التوالي . هذا وقد بلغ الوزن الجزيئي لبرولاكتين الحجيرات العضلية الطبيعية (74.4±13.2) كيلو دالتون . لقد وجد نموذج واحد فقط للبرولاكتين من نوع كبير كبير Big Big في مصل احدى المريضات وبوزن جزيئي مقداره (201.5) كيلو دالتون .

الاستنتاج: ان مستوى البرولاكتين في المصل يزداد مع زيادة برولاكتين الورم العضلي وحجمه عند المرضى . نمط البرولاكتين الكبيرالكبير ممكن ان يكون هو السبب في ارتفاع مستوى البرولاكتين في الدم عند المرضى المصابين