Serum protein and prolactin as diagnostic markers

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ABSTRACT

الأهداف: تَقييم استعمال مصل البرولاكتين و البروتين الكلي في مصل الدم كعلامة في تَشخيص الورم الليفي الرحمي.

الطريقة: أجريت هذه الدراسة خلال الفترةَ من مارس 2004 إلى اكتوبر 2005 – مستشفى الكرخ – بغداد. اشتملت هذه الدراسة 32 مريضة مصابة بالورم الليفي الرحمي و30 إمرأة طبيعية صحية. أخذ نموذج الدم من مجموعة المرضَى قبل وبعد الجراحة. وتم قياس البروتين الكليِّ في مصل دم المرضى بطريقة بايوريت والبرولاكتين بتقنية المني فايدس على التوالي.

النتائج: اظهر مستوى البرولاكتين في مصل مرضى الأورام الليفية الرحمية قبل عملية رفع الورم ارتفاع ملحوظ مقارنة مع مستوى البرولاكتين في نفس المرضى بعد إجراء الجراحة ومجموعة التحكم, 169.64±133.11ng/ml 19.69±9.54ng/ml و18.93±5.16ng/ml على التوالي). وقد لوحظ أن البرولاكتين في مصل المرضى يزداد بزيادة عدد العقد الليفية وبشكل لا يعتمد لا على موقع الورِم ولا على حجمه. وان مستوى البروتين الكلي في مصل المرضَى كان منخفض نسبياً قبل جراحة . 5.56±9.66g/dl عما هو بعد الجراحة 6.83±0.9g/dl وكذلك مقارنة مع مجموعة السيطرة 7.18±0.75g/dl.

خاتمة: يمكن أَن يستعمل كلا من مصلي البرولاكتين والبروتين الكلي كعلامة كيماوية حيوية في تَأكيد تشخيصِ الأورام الليفية الرحمية.

Objectives: To evaluate the use of serum prolactin and total protein as a tumor marker in diagnosing uterine fibroid(s).

Methods: A case control study was carried out from March 2004 to October 2005 at Al-Kharch Hospital in Baghdad, Iraq. Thirty-two patients with uterine fibroid(s) and 30 healthy normal women were involved in the study. Blood was collected from uterine fibroid patients before and after surgery. The serum total protein was measured by the Biuret method, and prolactin by the mini VIDAS ELFA technique (enzyme linked fluorescent assay).

Results: The serum of patients with uterine fibroids before surgery showed an elevated prolactin level (169.64±133.11 ng/ml), compared with their prolactin after surgery (19.69±9.54 ng/ml), and with the control group (18.93 ± 5.16 ng/ml). This also increased with increasing fibroid number independently of the site, or the size of the fibroid. Serum total protein was relatively low in the patient group before surgery (5.56±9.66 g/dl), and returned to a healthy reference level after they underwent surgery (6.83 ± 0.9 g/dl), similar to the control group level (7.18±0.75 g/dl).

Conclusion: Serum prolactin and serum total protein can be used as an adjuvant biochemical marker to confirm the diagnosis of uterine fibroids.

Saudi Med J 2009; Vol. 30 (11): 1411-1415

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Received 27th July 2009. Accepted 6th October 2009.

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Uterine fibroid (leiomyoma) is the most common benign uterine tumors in women of reproductive age. Many studies have suggested that uterine leiomyomas are monoclonal tumors derived from a single neoplastic myometrial cell, and neoplastic transformation of myometrium to fibroids occurs in less than 1% of fibroids and remains to be elucidated.[1] The size of the uterine fibroid may be as small as a pea, or as large as a grapefruit.[2] Although the incidence and natural history of uterine fibroids is not fully understood,[3] uterine fibroid formation and development varies with age, increasing in the late reproductive period, and with ethnic origin, with African American women being disproportionately affected.[3] They are usually found during routine pelvic exams. The increase of serum prolactin (PRL) levels among reproductive aged women

is especially high in those women presenting with reproductive or menstrual dysfunction.[4] It was found to affect approximately one-third of infertile women.[5] 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 myomectomy is usually carried out, when the possibility of having children after the surgery is planned.[2] Prolactin was initially identified as a pituitary gland hormone, but several studies have demonstrated that PRL is not only produced by the pituitary gland, but it is also produced by uterine tissues, including the endometrium, myometrium, and uterine fibroids.[6,7] Hsu et al[8] assayed the sera of 743 patients with uterine cervical carcinoma for levels of circulating PRL. They observed markedly abnormal serum PRL levels in 30.8% (229/743) of the patients. These studies show ectopic production of PRL by uterine cervical carcinoma, and PRL production may be a potential marker for detecting early occult tumors, or for gauging the effectiveness of therapy for human cervical carcinoma. In recent years, magnetic resonance imaging (MRI) has gained popularity. However, it does not add clinically relevant information in most cases. Ultrasonography appears to be as efficient as MRI in fibroid detection, and essentially as good for assessing their size and location, if the uteri have less than 5 lesions.[9] Conversely, when the number of lesions is higher, the MRI exceeds the ultrasound's technical limitation in precise fibroid mapping and characterization.[10] Somigliana et al[11] reported that reliance upon one tumor marker in the management of cancer appears to be of limited clinical utility. Many studies revealed that the clinical accuracy of tumor markers can be increased by analyzing a panel of markers. Currently, no such panel for cancer exists. Monitoring assays that may increase the clinical accuracy of patients in panels include: tumor markers (currently used in diagnostics), hormones (indicators of patient health), heat shock proteins (indicators of general or systemic stress), and auto-antibodies (indicators of immune system activity or stress). Gómez de Terreros et al[12] demonstrated that the concentration of tumor markers in bronchoalveolar lavage (BAL [fundamental technique in the diagnosis of different respiratory diseases including lung cancer]) is expressed in relation to total protein. The aim of this study was to investigate the possible relationship between circulating levels of PRL and total protein with the size of uterine fibroid, and the possibility of these parameters to be used as a diagnostic marker.

Methods. This study was carried out from March 2004 to October 2005 at the Obstetrics and Gynecology Department of Al-Kharch Hospital in Al-Kharch, Baghdad, Iraq. Patients with diabetes mellitus, pituitary and thyroid, renal or psychiatric disease, or using any drug known to increase the level of PRL in their sera, at least for the last 6 months were all excluded from this study. Only uterine fibroid(s) patients with normal pituitary image were included in this study. They were all selected for surgical treatment either with hysterectomy or myomectomy. Written consent was taken from all the patients prior to operation. Ethical approval and patient permission was obtained prior surgery for the local ethics committee and to conduct the study. Thirty-two patients were included in this study with a mean age ± standard deviation (SD) of 42.16 ± 6.51 years, and age range of 31-53 years. Fibroid(s) were harvested from different sites of the uterus including intramural, submucosal, subserosal, broad ligament, and cervical. They were identified grossly at surgery, and confirmed by histological examination. Ten out of 32 (31.25%) complained of infertility. Healthy normal women (n=30) were taken as a control group. Their mean age ± SD was 38.2 ± 6.9 years, and age range 28-49 years. They were all normal without fibroids, which was confirmed by ultrasonography. Blood samples were drawn under sterile conditions from patients prior to operation, left to clot, and centrifuged at 800 x g for 10 minutes at room temperature. The circulating levels of PRL were determined by means of mini-VIDAS using a Prolactin Kit (Biomérieux Inc., Durham, North Carolina, USA). The measurement range of the VIDAS PRL kit is 0.5-200 ng/ml. The range of normal values for menstruating women is 5-35 ng/ml. Patients with serum PRL level >35 ng/ml were considered as hyperprolactinemic. The total serum protein (g/dl) was determined at 645 nm using the RANDOX Total Protein Kit with Biuret method (Randox Laboratories Ltd., Krumlin, United Kingdom) The patients were followed up after their surgical treatment, and serum PRL and total protein were re-estimated again after 3 weeks after surgery to compare their levels with those estimated before. The same kits and instruments were used to measure all samples.

Statistical analysis was performed using the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, IL., USA). Descriptive statistics such as mean, standard error (SE), correlation, linear regression and one-way ANOVA were used to evaluate the significance (p-value) between study variables before and after surgery. A p-value of <0.05 was considered statistically significant.

Results. The mean standard error (mean ± SE) of the patient's serum PRL before surgery (n=32) was 169.64 ± 133.11 ng/ml, which was higher than after

surgery (19.69 ± 9.54 ng/ml), and higher than in healthy normal women (18.6 ± 2.3 ng/ml) (n=30). There was a decrease in the patient's total serum protein (5.56 ± 9.66) before surgery, compared with total serum protein in the controls (18.93 ± 5.16 ng/ml), and after surgery (19.69 ± 9.54 ng/ml). The mean ± SE size of fibroid(s) harvested from different uterine patient's sites was 28.52 ± 27.64 cm^3. Only 4 out of 32 (12.5%) uterine fibroid patients had normal serum PRL levels, although their fibroid sizes ranged from 1-100 cm^3. The remaining 28 patients (87.5%) all had high levels of PRL in their serum, and so were considered as non-pituitary hyperprolactinemic. One patient may have more than one fibroid in the same uterine site. Thus, 73 fibroids were harvested from all patients' study. Their fibroid sizes ranged from 0.09-280 cm^3 (**Table 1**). As shown in **Table 2**, the patient's serum PRL gradually increased with the number of uterine fibroids. The lowest mean ± SE level of serum PRL was found in patients with one fibroid, while the highest mean ± SE was found in patients with 8 fibroids. **Table 3** illustrates the significance between serum PRL and total serum proteins before and after surgery. A positive correlation with positive linear regression equations was found between serum PRL and total serum protein before and after surgery as shown in **Figures 1 & 2**. To ensure these correlations before and after surgery, a positive linear regression, and correlations also were found between serum prolactin and its ratios with serum total protein before and after surgery (**Figures 3 & 4**).

Table 1 - Serum prolactin level among patients group with their number of fibroids.

Serum prolactin, ng/ml	n	(%)	Range of fibroids size (cm^3)
≤35	4	(12.5)	1 - 100
>35	28	(87.5)	0.09 - 280
Total	**32**	**(100)**	

Table 2 - Range of serum prolactin (PRL) level in patient group (n=32) according to their fibroid(s) number.

Number of fibroids	n	(%)	Serum PRL standard error
1	15	46.88	141.78 ± 14.39
2	3	9.37	160.48 ± 13.2
3	5	15.62	189.8 ± 34.53
4	4	12.5	512.34 ± 38.85
5	2	6.25	554.40 ± 41.75
6	1	3.13	596.98 ± 0.00
8	2	6.25	623.08 ± 96.85

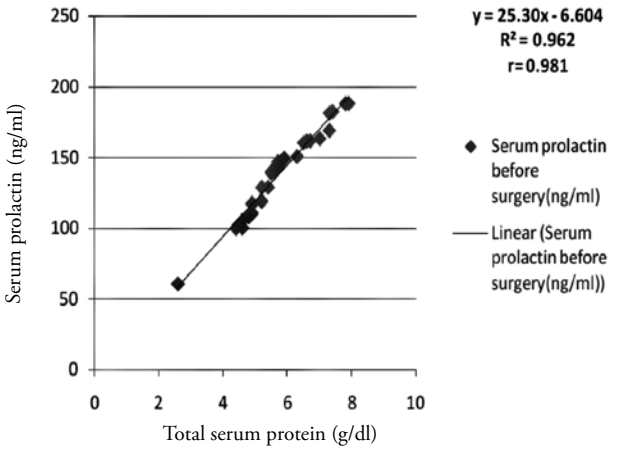

Figure 1 - Linear regression between serum prolactin and total serum protein before surgery.

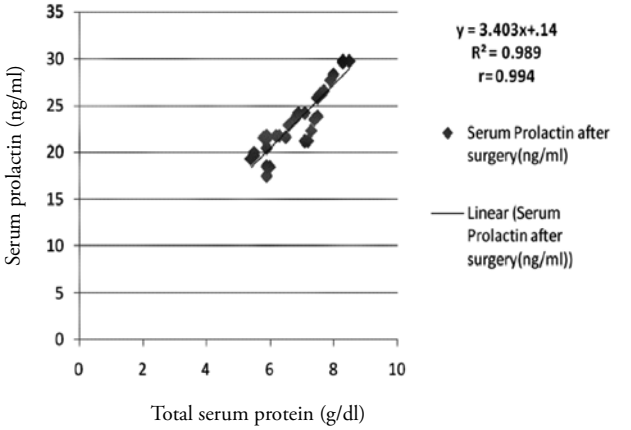

Figure 2 - Linear regression between serum prolactin and total serum protein after surgery.

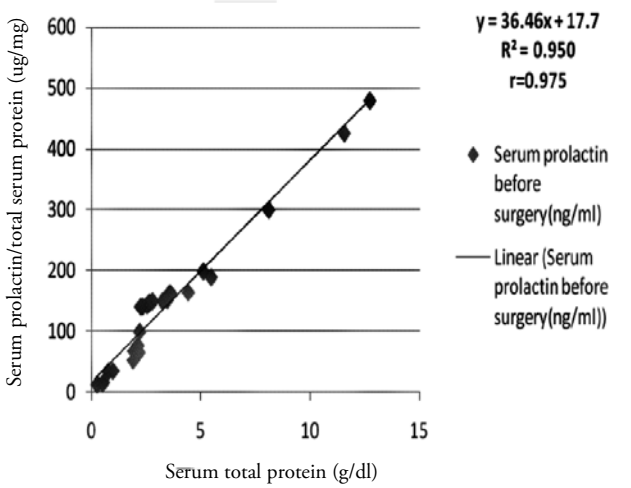

Figure 3 - Linear regression between serum prolactin and its ratios with serum total protein before surgery.

Table 3 - One-way analysis of variance for prolactin and total protein in patient serum before and after surgery.

Proteins	Mean ± SE	95%CI	*P*-value
Before surgery			
Serum PRL, ng/ml	169.64 ± 133.11	123.82 - 183.34	0.0001
Total serum protein, g/dl	5.56 ± 9 .66	5.38 - 5.77	0.0001
After surgery			
Serum PRL, ng/ml	19.69 ± 9.54	17.50 - 21.76	0.0001
Total serum protein, g/dl	6.83 ± 0.9	6.68 - 7.06	0.0001

CI - confidence interval

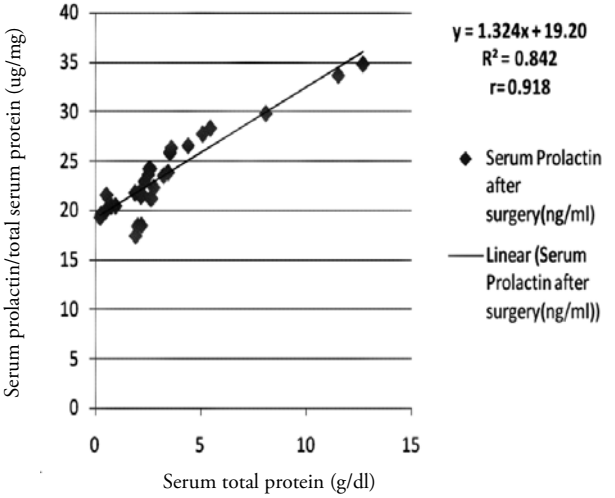

Figure 4 - Linear regression between serum prolactin and its ratios with serum total protein after surgery.

Discussion. First, it should be mentioned that the only limitation faced in this study was following up patients after surgery. The follow up is dependent basically upon patient cooperation, and so only such cooperative patients were included in this study. Indinnimeo et al[13] reported that serum concentrations of PRL, which is a trophic hormone produced by the pituitary gland, have been shown to be raised in certain group of patients with cancer. It was detected in 0-20% of the colon cancer by immunohistochemistry and in the plasma in 6-53% of the patients. Hsu et al[8] concluded that surgical removal of cervical carcinoma resulted in normalization of serum PRL concentrations. This explains the increase of PRL concentration level in the patients, and the decline in their levels to a normal level after surgery. In addition to the decidualized endometrium of the late luteal phase and of pregnancy, the human myometrium has been proposed as a second source of uterine PRL, since immunoreactive PRL was found in supernatants from myometrial explant cultures.[14]

Prolactin can function as a circulating hormone and as a cytokine. The explanation of this function is based on PRL production and its distinct regulation in extrapituitary sites, its binding to membrane receptors of the cytokine receptor super family, and activation of signaling pathways that promote cell growth and survival. Many studies showed increasing evidence of PRL and say that PRL plays a role in several types of cancer in the reproductive and non-reproductive tissues via local production, or accumulation. Considering PRL as an active participant in tumorigenesis should inspire and encourage the development of novel therapies aimed at reducing tumor growth by suppressing PRL production, or by blocking its receptors.[15]

Nakashima et al[16] concluded that serum total protein and albumin levels in patients with elevated serum TNF levels were significantly lower (p<0.05) than the corresponding values in patients with undetectable serum TNF levels. Uterine leiomyoma patients in this study showed a decrease in their total serum proteins compared with the same variable of normal healthy women (controls). Molina et al[17] on other hand, analyzed S-100 serum concentrations in healthy controls and patients with benign and malignant diseases. They concluded that S-100 is a useful marker for melanoma, and abnormal levels of this tumor marker may be found in benign and malignant diseases.[17]

Patients with elevated serum PRL levels were considered as non-pituitary hyperprolactinemic as shown in **Table 1**. The increase in patient's serum PRL before surgery does not depend on the size of uterine fibroid(s), but it depends on the number fibroids found in their uterus (**Table 2**). This elevated level of serum PRL was significantly decreased (p<0.0001) after surgery (**Table 3**). Mujagic and Mujagic[18] found also that the circulating levels of PRL before treatment in breast cancer patients with hyperprolactinemia were significantly higher (U=125.5, p<0.01) in comparison with controls. Cohen et al[19] concluded that PRL levels are decreased following hormonal or chemotherapy in patients with breast cancer, and there is no correlation between PRL serum level and the state of disease. Also, a slight increase was detected after surgery in uterine patients serum total protein and a highly significant value (p<0.0001) was found as shown in **Table 3**. Yonemura

et al[20] reported in their study that supplementation with alfacalcidol can increase protein intake and serum albumin concentration in hemodialyzed patients with hypoalbuminemia, probably through the suppressed tumor necrosis factor activity. Dalmasso[21] reported that changes in protein quantities or specific modifications can be discovered by comparing the complement of proteins expressed in a diseased state with proteins expressed in a normal state. The relevance of proteomics to disease biomarker discovery lies in the fact that proteins constitute the final form of gene expression, and that the function, or dysfunction of a protein and the pathways that it participates in are often dependent on post-translational modifications that are not reflected in changes in mRNA expression.[21]

In conclusion, a highly significant correlation was found between serum PRL and its ratio with serum total protein. This result will ensure and support the relation between the patient's serum PRL, total serum protein with their fibroid number, enabling to suggest these variables as a simple biochemical marker supporting other clinical findings. Although Suat et al[22] examined serum total protein and other biochemical parameters in type 2 diabetic mellitus (DM) patients, they also applied their findings as a ratio with total protein and they found that total sialic acid (TSA), lipid-associated sialic acid (LSA), TSA/total protein, and LSA/TP have interactive connections with DM. These parameters can be used as a diagnostic index for patients with DM.[22] For further studies, it is recommended to study patient's types of total protein before and after surgery in their serum and types of protein in their fibroid tissues to establish a comparison between these types and confirming their use as diagnostic markers.

References

1. Tai CT, Lin WC, Chang WC, Chiu TH, Chen GT. Classical cadherin and catenin expression in normal myometrial tissues and uterine leiomyomas. ***Mol Reprod Dev*** 2003: 64: 172-178.
2. Walters CA, Daly DC, Chapitis J, Kuslis ST, Prior JC, Kusmik WF, et al. Human myometrium: a new potential source of prolactin. ***Am J Obstet Gynecol*** 1983; 147: 639-644.
3. McWilliams RB. Reproductive Endocrinology and Gynecology. updated 2005 [cited 2009 July 4] Available from URL: http://www.houstonfertilityspecialist.com/articles/art_fibroids.html
4. Corenblum B. Disorders of prolactin secretion. Copeland LJ, Jarrell JF, McGregor JA. Text book of Gynecology. Philadelphia (PA): WB Saunders Co.; 1993. p. 447-467.
5. Rebar RW, editor. Advances in the Management of Hyperprolactinemia. American Society for Reproduction Medicine 53rd Annual Meeting; 1997 18-22 October, Cincinnati, USA. Columbus(Ohaio): Ohaio Academy of Family Physicians.; 2008. p. 1536.
6. Myers ER, Barber MD, Gustilo-Ashby T, Couchman G, Matchar DB, McCrory DC. Management of uterine leiomyomata: what do we really know? ***Obstet Gynecol*** 2002; 100: 8-17.
7. Payson M, Leppert P, Segars J. Epidemiology of myomas. ***Obstet Gynecol Clin North Am*** 2006; 33: 1-11.
8. Hsu CT, Yu MH, Lee CY, Jong HL, Yeh MY. Ectopic production of prolactin in uterine cervical carcinoma. ***Gynecol Oncol*** 1992; 44: 166-171.
9. Flake GP, Andersen J, Dixon D. Etiology and pathogenesis of uterine leiomyomas: a review. ***Environ Health Perspect*** 2003; 111: 1037-1054.
10. Dueholm M, Lundorf E, Hansen ES, Ledertoug S, Olesen F. Accuracy of magnetic resonance imaging and transvaginal ultrasonography in the diagnosis, mapping, and measurement of uterine myomas. ***Am J Obstet Gynecol*** 2002; 186: 409-415.
11. Somigliana E, Vercellini P, Daguati R, Pasin R, De Giorgi O, Crosignani PG. Fibroids and female reproduction: a critical analysis of the evidence. ***Hum Reprod Update*** 2007; 13: 465-476.
12. Gómez de Terreros Caro FJ, Alvarez-Sala R, Prados MC, Callol L, Gómez de Terreros Sánchez FJ, Villamor J. How to express tumor markers in bronchoalveolar lavage. ***Int J Biol Markers*** 2004; 19: 67-71.
13. Indinnimeo M, Cicchini C, Memeo L, Stazi A, Ghini C, Ricci F, et al. Plasma and tissue prolactin detection in colon carcinoma. ***Oncol Rep*** 2001; 8: 1351-1353.
14. Gellersen B, Bonhoff A, Hunt N, Bohnet HG. Decidual-type prolactin expression by the human myometrium. ***Endocrinology*** 1991; 129: 158-168.
15. Ben-Jonathan N, Liby K, McFarland M, Zinger M. Prolactin as an autocrine/paracrine growth factor in human cancer. ***Trends Endocrinol Metab*** 2002; 13: 245-250.
16. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M. Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. ***Clin Cancer Res*** 1998; 4: 1743-1748.
17. Molina R, Navarro J, Filella X, Castel T, Ballesta AM. S-100 protein serum levels in patients with benign and malignant diseases: false-positive results related to liver and renal function. ***Tumour Biol*** 2002; 23: 39-44.
18. Mujagic Z, Mujagic H. The relationship of circulating prolactin levels to the size of primary tumor in breast cancer patients. ***Turkish Journal of Biochemistry*** 2004; 29; 277-281.
19. Cohen AD, Cohen Y, Maislos M, Buskila D. Prolactin serum level in patients with breast cancer. ***Isr Med Assoc J*** 2000; 2: 287-289.
20. Yonemura K, Sugiura T, Yamashita F, Matsushima H, Hishida A. Supplementation with Alfacalcidol increases protein intake and serum albumin concentration in patients undergoing hemodialysis with hpoalbumineamia. ***Blood Purif*** 2004; 22: 210-215.
21. Dalmasso EA. Proteomics for Marker Discovery: Protein Chip Technology, 2-D Gel Electrophoresis, and Mass Spectrometry. ***Journal of Clinical Ligand Assay*** 2002; 25: 117.
22. Ekin S, Meral I, Gunduz H, Mert N. Comparative study of total protein, and total and lipid-associated serum sialic acid levels in patients with type 2 diabetes mellitus. ***J Clin Lab Anal*** 2003; 17: 124-126.