

Evaluation of using Total serum protein and prolactin as a marker in the diagnosis of uterine fibroid

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ashraf.2380@yahoo.com**Abstract:** Aim: To estimate the importance of both total serum protein and prolactin assays as a diagnostic marker for uterine fibroid diagnosis.**Material and Methods:** The present study (A case-control study) was done during the period from May 2016 to November 2016 at El-Hussein University Hospital. 60 women with suffering from uterine fibroid(s) and 60 (healthy normal) were kept as control group and were recruited from the gynecological outpatient clinic were participated in the work. Blood samples were harvested from uterine fibroid subjects pre- and post-surgery. The total protein in the serum was determined by the Biuret method, while prolactin was estimated by ELISA test.**Results:** There were statistically highly significant differences between the studied groups as regards serum prolactin and total protein before surgery, the patients serum from uterine fibroids subjects of pre-surgical operation showed an increase in prolactin concentration (71.8 ± 51.88), while prolactin level post-surgery was averaged (19.69 ± 9.54 ng/ml), in comparison with control group (9.3 ± 5.58 ng/ml). The level of total protein in the serum was comparatively little during pre-surgery in diseased patient group (5.08 ± 2.68 g/dl), while post-surgery the level was nearly as that in control healthy group (6.98 ± 1.15 g/dl vs. 6.86 ± 0.88 g/dl).**Conclusion:** The significant elevation of serum PRL in patients with fibroids and its reduction after surgery points towards the possible use of serum PRL assay in such patients as diagnostic and follow up test. Similarly, total protein in the serum can considered as an assistant confirmative biochemical marker for detection of uterine fibroids.[Ashraf Elshahat Elsayed Ahmed. **Evaluation of using Total serum protein and prolactin as a marker in the diagnosis of uterine fibroid.** *Nat Sci* 2018;16(12):25-30]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). <http://www.sciencepub.net/nature>. 5. doi:10.7537/marsnsj161218.05.**Keywords:** total serum protein, serum prolactin, and uterine fibroid.**1. Introduction**

Leiomyoma (uterine fibroid) the most predominating uterine cancer among women at reproductive age is known as benign tumor of the uterus and arises from the smooth muscle layer and adjacent connective tissues of the uterus and considered the main frequent gynecologic neoplasm (*Geethamala et al., 2016*).

Even though the frequency and ordinary history of the uterine fibroids are not entirely known. Some factors are affecting on the formation and development of uterine fibroid such as age, which predominating in the middle and late reproductive stages and also increased in some races specially in African American women (*Stewart et al., 2017*).

Prolactin (PRL) is a trophic hormone produced mainly by the pituitary gland but also secreted by the extra-pituitary site as (uterus, colon, etc). There are an important role of prolactin hormone for initiating of many types of tumors in reproductive and also in non-reproductive tissues which may be due to local excretion or accumulation. For this reason, PRL level may be used as a monitor for detecting an early development of fibroid tumor or for assessing the efficiency of treatment. Taking into consideration PRL as vigorous contributor in tumorigenesis should motivate and support the expansion of novel treatments aimed at decreasing PRL production or by blocking its receptors (*Somigliana et al., 2007*).

Baban, 2009 found that there is a probable association between the volume of uterine fibroid, and the concentrations of both total protein and PRL, and the probability of using these parameters as a markers for diagnosis of leiomyoma.

2. Patients and Methods**Settings:**

The study conducted at the Obstetrics and Gynecology Department of El-Hussein University Hospital included 120 women. Subjects were recruited from the gynecological outpatient clinic of the hospital and they were randomized by random method into two groups: Group 1 (uterine fibroid group) (study group) included 60 patients with uterine fibroids between 30 and 60 years old. All these patients were prepared to myomectomy or hysterectomy. Group 2 (control group) included 60 healthy normal women between 30 and 60 years old. All were normal with no fibroid and confirmed by U/S.

Selection Criteria:

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 with either hysterectomy or myomectomy.

Laboratory processing:

A sterile blood samples were collected from all patients before operation and 3 weeks after the operation. Sterile blood samples were collected also for protein and prolactin from the control group. The sample had been lifted to be clotted and centrifuged at 800 x 9 for 10 minutes at room temperature. Grossly hemolyzed, lipemic or microbially contaminated specimens might be interfered with the performance of the test and were not to be used. Neither bilirubin nor hemolysis had a significant effect on the procedure. The collected serum samples had been stored at -20°C in a frozen manner. Repeated freezing and thawing of the samples had been avoided.

The circulating level of PRL had been determined by means of ELISA test (enzymatic immunoassay) using prolactin kits (DIMA Ges. F Diagnostika Mbh-Robert- Bosch- Bereitte 23 37079 Goettingen- Germany). Results are expressed in nanograms per milliliter (ng/ml). The considerable average range in the normal females (Menstrual cycle) 2 – 22 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)

Ultrasound study:

Combined abdominal and transvaginal to confirm the diagnosis of uterine leiomyoma (number, size, location, whether submucous, subserous or intramural and the volume of the dominant leiomyoma) and any adnexal masses.

Follow up:

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:

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as the mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Ethical consideration:

Agreement for this study was obtained from the hospital's ethical committee, and an informed oral and written consent were taken from all patients included in the study prior surgery after a very clear explanation of both procedure, adequate providing of information about the study necessities, purpose, and dangers.

3. Results

A total of 120 women recruited to the study were randomly allocated into 2 groups. **Group 1 (uterine fibroid group) (study group):** 60 patients with uterine fibroids between 30 and 60 years old. **Group 2 (control group):** 60 healthy normal women between 30 and 60 years old.

There were statistically highly significant differences between the studied groups as regards serum prolactin and total protein before surgery (p < 0.001) (table 1).

Table (1): Comparison between studied groups before surgery.

	Case group	Control group	St t test	P value
Serum Prolactin before surgery	71.8±51.88	9.3±5.58	6.56	0.001**
<35 ng/ml	16(26.7)	60(100)	FET=31.65	0.001**
≥35 ng/ml	44(73.3)	0(0.0)		
Serum Total Protein before surgery	5.08±2.68	6.86±0.88	3.45	0.001**

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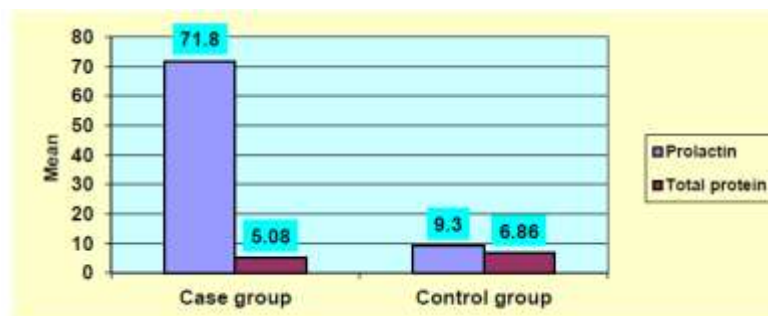


Figure (1): Comparison between studied groups before surgery

There were statistically highly significant differences among case group as regards serum

prolactin and total protein before surgery (p < 0.001) (table 2).

Table (2): Differences among case group before and after surgery.

	Before surgery	After surgery	Paired t-test	P value
Serum Prl / ng/ml	71.8±51.88	16.5±4.68	5.98	0.001**
Serum Total Protein g/dl	5.08±2.68	6.98±1.15	3.48	0.001**

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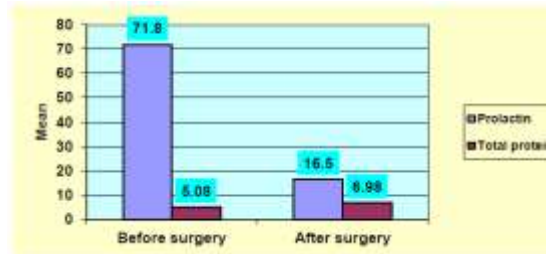


Figure (2): Differences among case group before and after surgery

There was a non-significant difference between serum prolactin and serum total protein ($p > 0.05$) (table 3).

Table (3): Comparison between serum prolactin ≥ 35 ng/ml and serum prolactin < 35 ng/ml concerning serum total protein

Serum Prl / ng/ml	≥ 35 ng/ml	< 35 ng/ml	St t test	P value
Serum Total Protein g/dl	4.97±3.04	5.38±1.36	0.359	0.722

There was statistically a highly significant difference between serum prolactin ≥ 35 ng/ml and serum prolactin < 35 ng/ml concerning number ($p < 0.001$) and a non-significant difference concerning the size of leiomyoma (table 4).

Table (4): Comparison between serum prolactin ≥ 35 ng/ml and serum prolactin < 35 ng/ml concerning number and size of leiomyoma

Serum Prl / ng/ml	≥ 35 ng/ml	< 35 ng/ml	St t test	P value
Number of leiomyoma	7.77±2.05	3.75±2.19	4.68	0.001**
Size of leiomyoma	7.64±2.5	9.25±1.58	1.7	0.101

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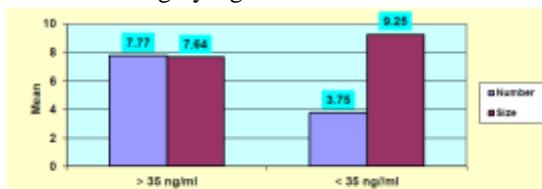


Figure (3): Comparison between serum prolactin ≥ 35 ng/ml and serum prolactin < 35 ng/ml concerning the number and size of leiomyoma

Receiver operator characteristic (ROC) curve was constructed for serum prolactin concentration as a diagnostic marker to confirm the diagnosis of uterine fibroids. Serum prolactin concentration is a good predictor of uterine fibroids.

ROC Curve

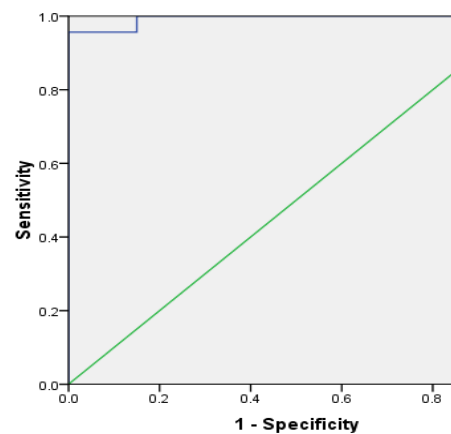


Figure (4): A Receiver-operating characteristic (ROC) curve for the diagnosis of uterine fibroids using serum prolactin concentration.

There was statistically a highly significant difference between the case group and control group regarding prolactin. The cutoff value of serum prolactin level as a diagnostic test of uterine fibroids is 14.35 ng/ml with 100 % sensitivity , 83.3%, specificity, 85.7% positive predictive value and 100% negative predictive value of (table 5).

Table (5): Validity of serum prolactin before surgery in ng/ml

Prolactin	Case group	Control group	X ²	P value
≥ 14.35	60(100)	10(16.7)	42.86	0.001**
< 14.35	0(0.0)	50(83.3)		
AUC	0.989			
Cutoff point	14.35			
Sensitivity	100			
Specificity	83.3			
PPV	85.7			
NPV	100			
Accuracy	91.7			

**=highly sig

Receiver operator characteristic (ROC) curve was constructed for serum total protein concentration as a diagnostic marker to confirm the diagnosis of uterine fibroids.

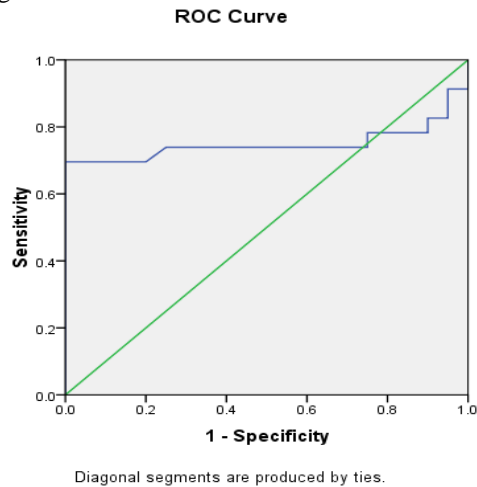


Figure (5): A Receiver-operating characteristic (ROC) curve for the diagnosis of uterine fibroids using total protein concentration.

There was statistically a highly significant difference between the case group and control group regarding total protein. The cutoff value of serum total protein level as a diagnostic test of uterine fibroids is 6.07 gm/dl with 73.3 % sensitivity, 83.3% specificity, 81.5% positive predictive value of and 75.8% negative predictive value (table 6).

Table (6): Validity of serum total protein before surgery in g/dl

Protein	Case group	Control group	X ² test	P value
<6.15	44(73.3)	10(16.7)	19.46	0.001**
≥6.15	16(26.7)	50(83.3)		
AUC	0.804			
Cutoff point	6.07			
Sensitivity	73.3			
Specificity	83.3			
PPV	81.5			
NPV	75.8			
Accuracy	78.3			

**=highly sig.

4. Discussion

Prolactin hormone is a glycoprotein hormone, secreted mainly from anterior pituitary gland, and also from uterine tissues (the endometrium and myometrium) and from uterine fibroids (*Sparic et al., 2016*).

Some investigators, determined the levels of PRL in the serum of patients diagnosed as uterine cervical carcinoma. They recorded abnormal values of PRL concentration in 30.8% of the examined patients. They showed that uterine cervical carcinoma can release a quantities of PRL in the circulation, therefore, PRL secretion could be used as a potential indicator for detecting early occult cancers (*Hsu et al., 1992; Korytnaya et al., 2014*).

Somigliana et al. found that depending on one parameter for detecting of tumor and in the control of tumor seemed to be of limited clinical use. Increasing the number of analyzing a panel of markers is associated with increasing in the accuracy of tumor markers. Nowadays, not be present many panels for cancer detection. The cancer markers include, hormones (indicators of patient health), tumor markers (used nowadays in the diagnosis), auto-antibodies (indicators of immune system activity or stress) and heat shock proteins (indicators of general or systemic stress). Therefore, the present study was aimed to estimate the efficacy of using of serum prolactin and total protein as a possible marker for detection of uterine fibroid affection in women (*Somigliana et al., 2007*).

In our study, there were statistically highly significant differences between the studied groups as regards serum prolactin and total protein before surgery. There were statistically highly significant differences among case group as regards serum prolactin and total protein before surgery. There was statistically a highly significant difference between serum prolactin ≥ 35 ng/ml and serum prolactin < 35 ng/ml concerning the number and a non-significant difference concerning the size of leiomyoma.

Some investigators found proposed that PRL secreted at the late luteal phase and during pregnancy from uterine endometrium, myometrium and considered the second source of PRL secretion, therefore, immunoreactive PRL was detected in vitro culture of myometrium (*Hsu et al., 1992*). Other researchers, showed that PRL concentration are declined after treatment with chemotherapy or hormones of patients suffering from breast cancer. *Cohen et al.*, and they added that no association was found between PRL level and the condition of illness (*Cohen et al., 2000; Saravanan and Anu, 2017*).

Many authors reported the importance of PRL as tumorigenic agent in many types of tumors in the genital system either originating from reproductive and non-reproductive tissues due to local secretion or due to accumulation in those tissues. Due to the role of PRL in inducing tumors in the genital tract, this should excite and stimulate the scientists to develop a recent treatments for the purpose of regressing the tumor volume and inhibiting the release or accumulation of PRL in the uterine tissues or by blocking PRL receptors in the tissues (*Ben-Jonathan et al., 2002; Ding et al., 2017*).

It was found that the level of prolactin in the serum was higher in patients suffering from hyperprolactinemia due to breast cancer in comparison with controls (*Mujagić and Mujagić, 2004; Tworoger and Hankinson, 2008*).

In the study of **Baban (2009)**, about 12.5% of subjects diagnosed as uterine fibroid had normal concentration of serum PRL. The rest of patients (87.5%) all exhibited a higher levels of PRL. There was no mention of how many patients returned to the normal values after the operation. There was no significant correlation between preoperative serum prolactin and the size of the fibroid(s) (**Baban, 2009**).

Many authors observed that total protein and albumin levels in the serum of patients having higher TNF concentration were remarkably decreased than in subjects having low levels of TNF. In addition, uterine leiomyoma individuals showed a decrease in their total serum proteins in comparison with that in healthy control individuals (**Nakashima et al., 1998; Li et al., 2015**).

Receiver operator characteristic (ROC) curve was constructed for serum prolactin and total protein concentrations as diagnostic markers to confirm the diagnosis of uterine fibroids. There was statistically a highly significant difference between the case group and control group regarding prolactin and total protein.

Baban (2009) evaluated the use of total protein and serum prolactin as tumor markers in diagnosing uterine fibroid(s). They concluded that serum total protein and serum prolactin can be applied as assistant or confirmatory biochemical marker for uterine fibroids diagnosis (**Baban, 2009**).

Conclusion

Serum prolactin and serum total protein can be used as an adjuvant biochemical marker to confirm the diagnosis of uterine fibroids. The use of the serum prolactin test for the diagnosis of uterine fibroids is simple, cheap, rapid, and reliable.

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