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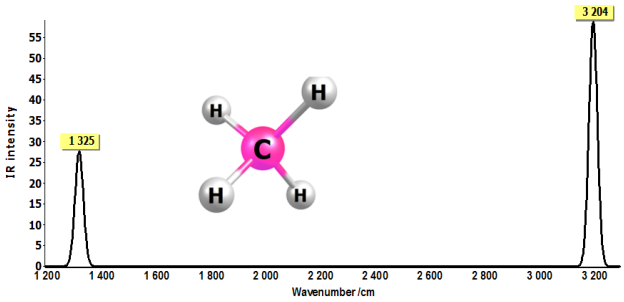

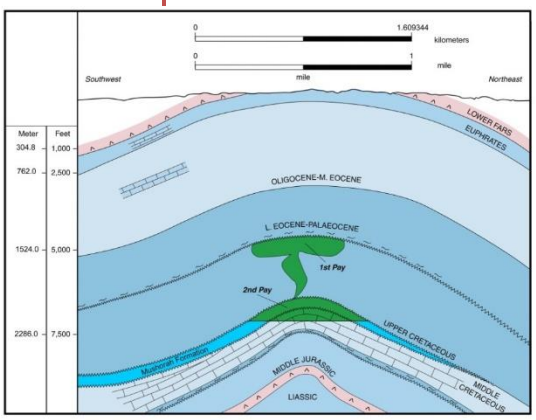

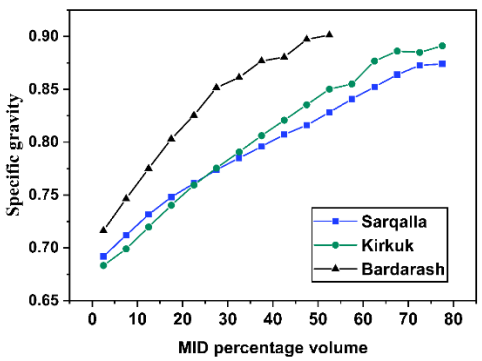

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Circulating prolactin levels in newly diagnosed prostate cancer patients

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Abstract

Objective: the main aim of this study was to assess the role of prolactin in prostate cancer using modern immunoassay methods. Prolactin assays in the past were subject to pitfalls from hook effect and macroprolactinemia; only few previous studies accounted for these pitfalls, therefore and to obtain accurate results, we thought to measure prolactin with modified modern methods. This study also investigated the association of serum prolactin levels with prostate specific antigen and selected steroid and pituitary hormones in prostate cancer patients. **Subjects and methods**: serum was withdrawn from newly pretreated 50 prostate cancer patients and 30 controls. The following parameters were studied using Roche-immunoassay analyzers: prolactin, total (PSA), total testosterone, free testosterone, (SHBG), (DHEA), (E2), progesterone, (FSH), (LH) and albumin; to account for macroprolactinemia, serum samples were pretreated with poly ethylene glycol 6000. SPSS statistical software used to perform students' t test and Pearson's correlation for association studies. P value of < 0.05 was considered significant throughout the study. **Results:** 64% of prostate cancer patients had normal prolactin levels (10.04 ±0.039ng/ml), 22% had raised serum prolactin and had 14% low prolactin levels, the difference in prolactin levels between prostate cancer and control group was not significant. Mean PSA levels were ((234.087 ± 522.695ng/ml) in prostate cancer patients, which was significantly higher in the prostate cancer group when compared to the control group (1.25 ± 1.39ng/ml), $P \leq 0.01$. Prolactin was not associated with PSA levels or with any of the sex and pituitary hormones studied. **Conclusions:** although the majority of prostate cancer patients had normal serum prolactin levels, 22% of the study participants had hyperprolactinemia, patients with higher serum prolactin levels had high Gleason score of 9 or higher and were at stage four, indicating a possible role of higher circulating prolactin at advanced cancer stages.

Introduction

Prostate cancer is one of the most common cancers in males and the second leading cause of cancer death in men. In the past few decades, numerous studies reported the role of endocrine system in cancer pathogenesis. For instance, prolactin may play a role in a variety of cancer types, including breast, colon, and prostate (1). Prolactin has more than 300 various functions in human body, and is not only produced by the anterior pituitary, but it is also synthesized by immune cells, neurons, mammary epithelium, and skin, notably; prostate cells synthesize and secrete prolactin receptors (2). Studies from the 1950s suggested a potential role for

prolactin in the differentiation and growth of the prostate gland (3). Prolactin has similar effects on breast and prostate cancer cells, and in a culture of prostate organ, prolactin promotes cell growth and inhibits apoptosis (4).Interestingly, in prostate cancer prolactin receptor synthesis is expressed highly by prostate gland (1). Despite a well-known study in 2001 (5) concluded that the risk of prostate cancer was not increased by high levels of circulating prolactin, it is not logical to exclude the role of serum prolactin in prostate cancer pathogenesis, as prolactin is generated by human prostate epithelium suggesting a paracrine and autocrine role of prolactin in prostate function distinct from the endocrine prolactin function (1). Moreover, several autoimmune conditions, including systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis, have been linked to hyperprolactinemia, which is more common in women and increases during pregnancy (6). Notably, prolactin assays are subject to many analytical and pre-analytical errors, prolactin assay has two major pitfalls, first, many articles in 2000s reported false hyperprolactinemia levels in macroprolatinoma (tumor size ≥ 1cm) patients, this false low level was explained by high dose hook effect of the assays (7, 8). Secondly, in the past decades, another pitfall of prolactin assay was highlighted, which is interference from macro prolactin (9). Prolactin has three forms; the molecular weight of the monomeric free little prolactin is 23 kDa. The two additional isoforms of prolactin are large (molecular weight between 50-60 kDa) and the big-big prolactin, which has a molecular weight of about 100 kDa and is also referred to as macro prolactin. The big prolactin is composed of a mixture of dimers and trimers of glycolysated free little prolactin with macro prolactin, while the macro prolactin is formed by combination of immunoglobins with glycosylated

prolactin. The free prolactin is the most biologically active form and macro prolactin is the least active isoform (9). To our knowledge only few studies has studied serum prolactin levels in preoperative prostate cancer patients and the results were inconclusive, we also could not find investigations on determining serum prolactin levels accounting for prolactin assay pitfalls. Since epidemiological studies investigating serum prolactin levels in prostate cancer used small sample size, are rare and mostly inconclusive, therefore, we thought revisiting serum prolactin levels in prostate cancer patients. Moreover, a Japanese investigation highlighted that biological and epidemiological variations exist in prostate cancer pathogenesis between western and Asian men (10).

Our study is unique as we 1- we pretreated samples with polyethylene glycol (PEG) 6000 to precipitate big prolactin molecule which is known to interfere with prolactin immunoassays 2- we selected pre-operative newly diagnosed prostate cancer patients. This study's primary goal was to look into patients with newly diagnosed prostate cancer's serum prolactin levels. Additionally, we investigated how serum prolactin levels related to levels of prostate-specific antigen and other sex hormones.

Materials and Methods

Study Design and Population

This study was conducted from September 2021 to January 2022 at the Hiwa cancer hospital. Blood samples were taken from 50 Newly diagnosed prostate cancer patients attending Hiwa cancer hospital. Each participant medical history and demographic information was collected with the aid of specially trained staff at the hospital, the recoded parameters including age, weight, sex, family history, blood pressure and socioeconomic background. Weight of the participants were measured using digital balance with an accuracy of 0.5 kg. At baseline, all patients were asked to fill a questionnaire containing questions about BMI, age, family history of cancer, health-related behaviors, physical activity, occupation, allergy, socioeconomic status, common chronic diseases, smoking, alcohol habits and drug history.

All the patients were informed about the research and consent were obtained from the study participants, the study has approved documentations according to the ethical committee of Sulaimani University and the study was conducted according to the declaration of Helsinki. We excluded patients who might take drugs or conditions affecting our lab results, these included any conditions or drugs affecting lab parameters such as malignancy, liver cirrhosis, acute illness, hypothyroidism or chronic alcoholism, as well as those who were

taking hormones, antiandrogen agents, antifungal agents, or steroidal agents or those who had undergone surgical or medical therapy, obvious neuropathy, psychological disease and urinary tract infection.
Patients also be excluded if they reported a prior history of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer , peptic ulcer or gout, contraindication to the use of aspirin, or current use of aspirin, other platelet-active agents, or vitamin A supplements, Infection or inflammation of the prostate (Prostatitis) or benign prostate hyperplasia , conditions requiring treatment with 5-alpha-reductase inhibitors, abnormal findings on digital rectal examination (DRE) , recent prostate manipulation (i.e., rectal examination within 1 week and lower urinary tract symptoms prostate biopsy, surgery or cystoscopy within 1 month, patients with drug abuse history will also be excluded).

Laboratory Assays

Biochemical Assessment

After a 12-hours fasting, blood was collected from participants and spun within 1 h. Immunoassays from ROCHE-Cobas was used to measure the following analytes: Albumin (3.5-5.5 g/dl) total PSA 0.22 - 6.16 ng/ml, free PSA 0.26 - 4.14 ng/ml, and hormones prolactin (4.04-15.2ng/ml); LH (1.7-8.6 mIu/ml), FSH (1.5-12.4 mIu/ml), estradiol (7.63-42.6 pg/ml) total testosterone (1.93-7.40 ng/ml), SHBG (13-71 nmol/l), DHEA (70-440 µg/dl). Free testosterone was calculated according to Vermuelen et al formula (11) using total testosterone, SBHG and albumin values. Blood test measured by Cobas E 411 (automates the immunoassay reactions utilizing electrochemiluminescence (ECL) (for hormones, total PSA), and cobas c 311 for albumin. Prostate cancers were diagnosed by consultant physicians. Five milliliters of venous blood samples were collected using disposable butterfly needles and had been collected in a serum gel separator tubes, left at room temperature for 5 minutes to clot, then centrifuged for 20 minutes at 4000 rpm to separate the serum. To ensure accurate prolactin measurements, the participants were advised to rest for at least 30 minutes, blood samples were withdrawn between 9-11 am. To exclude macroprolactinemia, the level of prolactin was measured by polyethylene glycol (PEG 6000) precipitation method according to manufacturer's instruction. Samples were pretreated with a 25% aqueous polyethylene glycol 6000 (ratio 1+1), the samples were then centrifuged and the supernatant containing monomeric prolactin was assayed similar to native sample. After running the samples, the dilution effect was considered in the final prolactin measurement.

Statistical Analysis

Data analysis was conducted with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). A p value of ≤ 0.05 was considered significant throughout the study, and all tests were 2-tailed. Independent sample t-test was used to investigate differences in continuous variables between the 2 independent study groups. The association between prolactin, and other study parameters was analysed using Pearson's correlation coefficient. Kolmogorov-Smirnov test was used to assess the normality of data.

Results

Table 1 summarizes the characteristics of the study participants. The study included 50 newly diagnosed prostate cancer patients at various cancer stages and 30 healthy controls. The blood tests were collected before starting cancer treatment. The study also showed that the majority (66%) of the newly diagnosed prostate cancer patients were at stage 4, (16%) were at stage 2 and (12%) at stage 3, while only (6%) were at stage 1. 81.8 % patients with hyperprolactinemia were at stage four and had Gleason score of 9 or more. The table also illustrate general demographic of the patients and the controls. 50% of our cancer patients were in the normal BMI range, 42% were overweight and 8% were obese. In the healthy group, BMI was normal in 26.6%, overweight in 53.33% and obese in 20%. 38% of cancer patients were smokers and 30% of healthy group were non-smokers. 12% of cancer patients had allergy, while 2% of the healthy group had allergies. Notably, 32% of our cancer patients had a family history of cancer and 23% of

control group had a family history of cancer. 22% and 13.3% of cancer and control had type 2 diabetes mellitus respectively. Hypertension was prevalent in 48% and 10% of the healthy group respectively. Both groups didn't take any supplements. Prostate biopsy was used to confirm prostate cancer in all patients and cystoscopy was performed on 44% of the patients. 64% of prostate cancer patients had normal prolactin levels, 22% had elevated prolactin and 14% had hyperprolactinemia. In the control group, 86.7% had normal prolactin levels while 13.3% had elevated serum prolactin levels.

Table 1. General characteristic of the control and prostate cancer patient groups.

	Prostate cancer(n=50)	**Healthy group(n=30)**
Age	71.90 ± 9.76	69.83 ± 7.78
BMI (kg/m2)		
Normal (<25)	50%	26.66%
Over weight (25-30)	42%	53.33%
Obese>30*)*	8%	20%
Smoking status		
Smoker	38%	30%
Non-smoker	68%	70%
Allergy	12%	2%
Family History of cancer	32%	23.3%
Alcohol in take	2%	13.3%
Comorbidities		
Diabetes	22%	13.3%
Hypertension	48%	10%
Physical activity		
Low	32%	2%
medium	58%	13.33%
high	10%	83.33%
Supplement intake	None	None
Prostate Biopsy	100%	-
Cystoscopy	44%	-
Employment	26%	93.33%
Prolactin levels		
Normal	64%	86.7%
Hyperprolactinemia	22%	13.3%
Hypoprolactinemia	14%	None
Total testosterone		
Normal testosterone	88%	93.33%
Elevated testosterone	None	None
Low testosterone	12%	6.66%

88% of the prostate cancer patients had normal testosterone while 93.3% of the control group had normal testosterone. Low testosterone was observed in 12% of the patients and 6.66% in the control group.

PSA levels was significantly higher in prostate cancer patients (234.087 ± 522.695) compared to PSA levels in control subjects (1.25 ± 1.39), $P \leq 0.01$. The results of this study indicated that total testosterone hormone levels, BAT SHBG, LH, DHEAs were significantly higher in prostate cancer patients when compared with the control group While we found that there was no significant difference in FSH, Prolactin, estradiol, free

testosterone, albumin levels between the two groups. One of the most common factors which causes elevated standard deviation is biological variation and our patient cohort were at different stage of cancer, causing large variability in our results.

Table 2. PSA, prolactin levels, estradiol, albumin and androgen profile in prostate cancer patients.

Biomarker	Prostate Cancer patients (N=50)	Controls (N=30)	P value
PSA (ng/mL)	234.087 ± 522.695	1.25 ± 1.39	0.0172*
TS (ng/mL)	4.46 ± 1.58	3.32 ± 1.37	0.0016**
BAT (ng/mL)	1.8 ± 0.87	1.29 ± 0.43	0.0037**
FT (ng/mL)	0.20 ± 0.26	0.131 ± 0.206	N.S
SHBG (nmol/L)	49.26 ± 21.85	37.98 ± 13.48	0.0128*
E2 (pg/ml)	34.75 ± 11.83	40.99 ± 22.62	N.S
Prolactin (ng/ml)	10.498 ± 10.46	10.045 ± 5.83	N.S
Albumin (g/dl)	4.23 ± 0.47	4.22 ± 0.22	N.S
LH (mIu/ml)	14.31 ± 13.84	7.66 ± 0.69	0.0105*
FSH (mIu/ml)	13.99 ± 17.24	20.24 ± 63.80	N.S
DHEAs (ug/dl)	126.77 ± 203.2	232.85 ± 94.48	0.0088**

PSA: prostate-specific antigen, BAT: bioavailable testosterone, SHBG: sex hormone-binding globulin, E2: estradiol, FSH: follicle-stimulating hormone, LH: Luteinizing hormone, (DHEA): dehydroepiandrosterone. *=significant, N. S= none significant, **= very significant

Table 3 illustrates the association between prolactin and hormones (LH, FSH, E2, progesterone, total testosterone, SHBG, free testosterone, total testosterone, DHEA). The results showed that none of the parameters correlated with prolactin in prostate cancer patients.
In addition, we studied the association between prolactin with albumin, CRP, total PSA and free PSA, similarly there was no association between prolactin and any of the parameters studied above.

Table 3. Association of serum prolactin levels with study parameters.

Parameters	Prolactin		
	P	**R**	**R^2**
LH	0.605	0.090	0.0082
FSH	0.929	0.014	0.0002
E2	0.841	0.028	0.0008
Progesterone	0.275	0.313	0.0983
T testosterone	0.335	0.139	0.0194
SHBG	0.910	0.017	0.0003

Free testosterone	0.059	0.269	0.0726
Bioavailable testosterone	0.517	0..093	0.0088
DHEAs	0.754	0.060	0.0037
Albumin	0.218	0.177	0.0314
CRP	0.412	0.220	0.0487
Total PSA	0.614	0.072	0.0053
Free PSA	0.589	0.104	0.0109

*Pearson correlation was used to find the association between prolactin and study parameters.

Discussion

The primary finding of this study is the absence of a statistically significant difference in serum prolactin levels between prostate cancer patients and healthy group, these results were in agreement with a previous study (12). However, 22% of our participants had a serum prolactin concentration greater than 15.2 ng/ml (normal range 4.04-15.2ng/ml), one patient had a prolactin level reaching 72.62 ng/ml, 14% had hyperprolactinemia. These findings show that even though the majority (64%) of prostate cancer patients having normal prolactin levels, there are still a good proportion of the participants with abnormal prolactin levels. This study also showed among 11 patients with raised prolactin levels, 8 of these patients were at stage four of cancer and had a Gleason score of 9 or higher, this might be an indication that prolactin levels change more drastically at advanced cancer stages. These finding are in agreement with few previous research in which they found hyperprolactinemia in about 20% of metastatic prostate cancer patients and 30% of metastatic breast cancer patients (13). Of note, one study has indicated that advanced prostate cancer treatment requires prolactin suppression (14), and another study concluded poor prognosis of prostate cancer patients with persistent hyperprolactinemia (15).

Interestingly, prolactin receptors activate signal transducer and activator of transcription 5 (Stat5) (16). Stat5 is activator of transcription factor critical for prostate cancer cells to survive, prostate cells that were both androgen-dependent and independent died as a result of its inhibition (17). High Gleason score was associated strongly with high Stat5 expression in prostate cancer patients, Stat5 also predicted early disease recurrence (18). Many researchers concluded that despite normal prolactin levels in majority of prostate cancer patients, researchers cannot rule out the role of prolactin in prostate cancer development (5), primarily because the human prostate produces prolactin epithelium and the human prostate expresses prolactin receptors. Another hypothesis suggested that there is a paracrine and autocrine prolactin's role in prostate cancer (5). Moreover an analysis of gene expression revealed 6.7 fold more prolactin expression in rare and more aggressive ducat adenocarcinomas (19); and prolactin concentration in prostatic fluids in prostate cancer patients were higher than controls (1).

To rule out experimental variations and errors, we repeated each measurement twice, also we accounted for factors known to influence serum prolactin levels, these include spontaneous pulsations in hormone secretion, meals, pain, nausea, anxiety, medications, diseases and macroprolactin (20). It is unlikely that our results are affected by medications, since we excluded any patient taking medications affecting serum prolactin levels,

also we excluded patients having thyroid issues or depression related disorders. To avoid variations caused by circadian rhythm, we took blood samples between 9-11 am in the morning and patients were fasting.

Macro prolactin is known to interfere with prolactin immunoassays and cause false raised serum prolactin levels (9). Macro prolactin is a large molecule (150kDa) and consist of monomeric bioactive prolactin (23kDa) and non-reactive immunoglobulin G. To our knowledge, this is one of few studies which involved pre-operative patients and pretreated serum samples with PEG 6000, PEG is used to precipitate macro prolactin this eliminate false positive rise of prolactin.

In this study, 12% of the prostate cancer patients and 6.6% of the control group had low testosterone levels (table1). This is not surprising, since in ageing men, hypogonadism is not rare, our study participants were age matched therefore, the declining testosterone levels might be physiological due to older age.
Many factors might cause low testosterone levels in healthy subjects, these include obesity, stress, metabolic syndrome, depression and malnutrition (21). Also, obese men have lower LH levels indicating a possible disturbance of the hypothalamus pituitary axis. Furthermore, the endocrine system is regulated by circadian rhythm thus samples taken at different timing might contributed to the low testosterone levels. However, low testosterone levels are should be consistent with clinical symptoms and doctors need to exclude secondary causes of low testosterone caused by pituitary tumors (21), our study control were selected and they were healthy, therefore, the declining testosterone levels in this group are most likely due to analytical errors, stress or resultant from natural age.

We also looked in to the relationship of prolactin with total PSA, our results were similar to a previous study (22), in which they also showed no association between prolactin and PSA levels. This is not surprising, since PSA in prostate cancer rises rapidly in most prostate cancer patients, while prolactin levels remained fairly constant (normal) in most of our patients.
The correlation between prostate cancer and androgens levels is also not fully understood, many studies linked aggressive cancers, shorter life expectancy and advanced histological stages with low testosterone levels (23)(24). Although many groups concluded low testosterone levels were associated with poor prognostic factors, but some studies didn't report the same conclusion. A study reported no direct association between hormones (prolactin, growth hormone, testosterone and gonadotrophin) with tumor stage (25), hence pre-treatment serum testosterone's function in prostate cancer development is still under debate. Of note, a study speculated that inhibition of both prolactin and androgen is important to obtain maximum treatment for advanced cancer patients (26). In the current study and unlike some previous studies (23)(27), lower testosterone level was not associated with higher Gleason score and none of our patients had low estradiol levels. Our results were in agreement with few other studies which showed no association between baseline pathological Gleason score and pretreatment serum testosterone level (10)(28). In this research, the percentage of total low testosterone levels was only 12 % (6 out of 50) and 7 % had low free testosterone levels.
Finally, in prostate cancer patients, prolactin didn't correlate with hormones (LH, FSH, E2, progesterone, total testosterone, SHBG, free testosterone, total testosterone, and DHEA).

This is might be explained by the fact the prostate cancer patients were all in small age range (51-88years), these hormones levels change gradually each decade, therefore, since our participants had a small age range, and prolactin also was normal in majority of our study groups, this could be the cause of the lack of association between prolactin and sex hormones in prostate cancer patients. In addition, these discrepancies in testosterone involvement in prostate cancer might be related to different analytical methods, ethnic and racial differences. In particular, it is noteworthy that testosterone measurements also have2 limitations, for example radioimmune assays and chemiluminescence have many limitations, and mass spectrometry is recommended to assess testosterone (29).

Serum prolactin was not associated with prostate cancer risk (30). However, many clinical and *in vitro* studies reported that autocrine prolactin molecules produced by local prostatic cells induce the over activation of prolactin receptor pathway and activate the progression of prostate cancer stages (30).

Many studies have highlighted that the mechanisms of action of serum prolactin in blood is different from prolactin molecule on cellular and molecular levels. There is significant evidence of the existence of prolactin's paracrine and autocrine actions (1). In humans, receptors for prolactin are expressed in the prostate, and this expression of these cellular receptors are particularly elevated in prostate cancer and carcinoma *in situ* (1).

Conclusions

In summary our results showed that at advanced cancer stages, prolactin levels were raised, however, normal prolactin levels were also found in most of our cases, indicating a further investigation focusing on prolactin levels at advanced cancer stage is necessary to reach firm conclusions about the role of prolactin in the pathogenesis of prostate cancer.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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