ORIGINAL ARTICLE

Serum Zinc, Total & Free PSA, Free/Total PSA Ratio and Prostate Cancer Association: A Case Control Study

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ABSTRACT

This case case control study was undertaken to see if there is any association of serum Zinc level with histologically diagnosed prostatic cancer patients. Also to re-establish the importance of estimation of serum Prostate specific antigen PSA, (Total, Free and ratio between free and total) in prostate cancer patients. It was evident from the present study that serum Zinc level has got statistically significant difference between control and prostate cancer patients ($p < 0.000$),. Total PSA and ratio of free and total PSA was also significantly different between control and prostatic cancer patients ($p < 0.04$ and 0.006 respectively).However no significant difference was observed in free PSA level between this two groups ($p < 0.282$).

It was concluded from the present study that serum Zinc level could be an adjuvant laboratory test in the diagnosis of prostate cancer and can be started as a routine test in case of suspected prostatic cancer. Also total PSA level and ratio of free and total PSA not free PSA should be done and correlated for diagnosis of prostate cancer.

Key words: Serum Zinc level, PSA (total, Free and ratio), Prostate Cancer

Introduction

Prostate cancer is the most common cancer in men and the second leading cause of death from cancer world wide. Among diagnostic and prognostic markers for prostate carcinoma important ones are total and free PSA estimation, ratio of free and total PSA and recently estimation of Zinc apart from Estrogen receptor (ER) & Progesterone receptor (PR). The three most important procedures for diagnosis of prostatic lesions are per rectal digital examination (PRDE), Ultrasonogram (USG) of Prostate and prostatic biopsy. Many research already done world wide on Zinc, total & free PSA and ratio of free and total PSA and prostate cancer association are conflicting and opposing. Available literature search reveal no work was done on these in Bangladesh. With this background the present study was undertaken to observe if there is any association of serum Zinc level with histologically diagnosed prostatic

carcinoma and the role of total & free PSA and ratio of free and total PSA in diagnosis of prostate cancer association in Bangladeshi patients.

Materials and Methods

We selected 7 patients having histological diagnosis as prostatic carcinoma by light microscopy using routine Hematoxylin and Eosin (H&E stain). These patients attended BIRDEM hospital and other clinics of Dhaka city between the periods of March to November 2010.These patients underwent surgery due to prostate related complaints like acute retention, incomplete voiding, urgency, frequency, dysuria, hematuria and Urinary Tract Infections (UTI).Trans Urethral Resection of Prostate (TURP) surgery was done by the Urologist in these 7 patients and the biopsy was sent for routine H&E diagnosis.. 16 patients of the same age group range but without any clinical complain and evidence of prostatic lesions were selected and 10 ml of blood was taken from both the groups after proper verbal and written consent. Zinc estimation was done by using Zinc Colour Br-PAPAS reagent kit manufactured by Labkit company of Spain.(Packaging code1001350) at Bangladesh Institute of health science (BIHS)at Mirpur,Dhaka. This 5X10ml direct colorometric test was done without depolarization of the sample. At pH8.6 in a buffered media Zinc reacts with the specific complexant 5-Br-PAPS and forms a stable coloured complex. This colour intensity is proportional to the amount of Zinc present in the sample.For quality assurance Hemolysed samples were discarded as it could hamper the result. The working reagent is prepared as per instruction of the manufacturer. All the control and test samples were run by same batch, same time using a RA50 semi autoanalyser in wavelength of 578nm (range 550-5800) using 1cm light path cuvette and room temperature of 30^0C. The abrorbance of the sample and standard were taken against reagent blank. The result was calculated by the following formula.

$$\text{Calculation} = \frac{(A2\text{-}A1)\text{Sample}}{(A2\text{-}A1)\text{Standard}} \times 200(\text{Standard Concentration}) = \text{ugm/dl Zinc in the sample}$$

Control sera run with test sample was Spintrol H for normal (Ref 1002190) and for Pathologoical (Ref 1002210) both supplied with the kit. The normal reference value of Zinc in serum/plasma 68-107 ugm/dl. As per literature supplied it was mentioned that Spinreact reagents did not show any systemic difference when compared with other commercial agents and the gumma correlation coefficient 0.99 and regression equation 1.027X-2.7873.It does not interfere to bilirubin upto15 mg/dl,Hb upto 0.5 gm/dl and

RESULTS & OBSERVATIONS: shown in table I-VI. Table-I

Table I: Showing total PSA, free PSA, Ratio of total and free PSA, serum zinc level in case and control

Variables	Case		Control		*t*	*P*
	No.	Mean ± SD	No.	Mean ± SD		
Age in yrs	7	68.14 ±6.986	16	67.56±7.527	.174	.864
Total PSA in ng/dl	7	71.94± 68.79	16	4.58 ± 7.631	2.584	.041*
Free PSA in ng/dl	6	5.85 ± 9.67	16	1.08 ± 1.261	1.203	.282
Ratio (T & F)	6	15.733 ± 17.70	16	33.744 ± 2.4317	3.079	006**
Serum Zn in½gm/dl	7	142.933 ± 9.95	16	111.11 ± 9.107	7.504	.000**

Table II : Showing age group in case and control

Age in yrs	**Case**		**Control**		χ^2	**p value**
	No	%	No	%		
60- 75	6	86	13	81	.068	.795
76– 80	1	14	3	19		

Table III : Showing total PSA in case and control

TPSA in ng/dl	Case		Control		χ^2	p value
	No	%	No	%		
1 - 25	2	29	16	100	14.603	.000**
>25	5	71	0	0		

Table IV : Showing free PSA level in case and control

FPSA in mg/d	Case		Control		χ^2	p value
	No	%	No	%		
<2	3	50	14	87	3.49	.06
≥2	3	50	2	13		

Table V : Showing ratio of total & free PSA in case control

Ratio of T & F PSA	Case		Control		χ^2	p value
	No	%	No	%		
< 28	5	83	4	25	6.142	.013*
≥ 28	1	17	12	75		

Table VI : Showing serum zinc level case and control

Zn in½gm/dl	Case		Control		χ^2	p value
	No	%	No	%		
Upto 120	0	0	13	81	13.081	.000**
>120	7	100	3	19		

Discussion

Zinc can accelerate cell growth and possibly contribute to cancer[1]. The normal high concentration of zinc in normal prostate gland is significantly reduced in malignant prostatic cancer.[2] Zinc decreases survival of androgen independent prostate cancer cell by modulating the expression of insulin like growth factor (IGF).[3,4] In this case control study we have evaluated and compared the value of serum

Zinc, total PSA, free PSA and ratio between free and total PSA between histologically diagnosed prostate cancer (case) and age matched normal subjects (control). The present study showed that there was statistical significant increase level of serum Zinc found in prostate carcinoma patients than age matched control ($p<0.000$). The serum mean ± SD zinc level was 142.933 ± 9.95 ugm/dl in prostatic carcinoma patients while it was111.11 ± 9.10 ugm/dl in age matched control and the difference was statistically significant($p<0.000$). Previously two other studies showed different type of results.

In one study in India the researchers found strong correlation between plasma Zinc levels and various prostatic diseases. Out of 80 cases studied (20 normal, 50 benign, 10 carcinomatous), Serum Zinc level analysed by atomic absorption spectrophotometry the mean±SD plasma Zinc level in the normal case was 94.5±10.38, for benign prostatic lesion it was 145.4±9.67, 162.4±2.22, 172.7±5.27 (78% rise compared to normal patient) in those with fibromuscular prostate, chronic prostatitis and benign prostatic hyperplasia respectively. Patients with malignancy had a plasma Zinc level of 59.6±3.08(37% fall compared to normal patients). There was a highly statistically significant difference in plasma Zinc level between patients with benign and malignant prostatic disease.[5] The effect of metastasis of carcinoma of the prostate on plasma Zinc levels was not significant($p>0.05$). They concluded that determination of serum zinc levels can be used as a diagnostic and screening tool and may lead to the formulation of methods in which Zinc is used to evaluate prostatic pathology.[6] This research finding corroborate with the present study in relation to benign lesion but contrast with malignant lesion where we found markedly high serum Zinc level. The second study in Germany analysed Zinc in serum of both patients with prostatic carcinoma and men without prostatic cancer. Serum zinc was analysed by flame atomic adsorption spectrometry (FAAS). No significant differences were found between the group with prostatic carcinoma without metastasis and the group used for comparison. The Zinc level in serum of patients with both prostatic carcinoma and metastasis was decreased in comparison to the other groups. A decrease in Zn concentration was also found for men without metastasis after orchiedectomy and hormnone therapy[7]. This is not corroborative with present study. The difference may be due to geographical, racial and methodology of Zinc estimation. Total and Free PSA and ratio of free/total PSA were estimated from serum of the diagnosed prostate cancer cases and the age matched control. Total PSA value and free/total ration showed statistical significant difference between case and control groups ($p<0.04$ and 0.006 respectively). The mean±SD of total PSA was 71.94 ± 68.79ngm/ml for prostate cancer cases and 4.58 ± 7.631 for control. The mean±SD of ratio of free/total PSA was 15.733 ± 17.70 for prostate cancer patients and it was 33.744 ± 2.4317 for control subjects and the difference was statistically significant ($p<0.006$). Our findings were consistent with findings of many authors and contrasting with others. In a study in Japan in 1998 Manabu Kuriyama et al found the determination of free/total PSA ration was considered to be an effective tool discriminating the non prostate cancer cases from those of prostate cancer.[8] They evaluated free/total PSA ratio in 77 cases of prostate cancer and 224 cases of non prostate cancer. They found cut off value of 14 where total PSA was between 5.1-10 ng/ml and 15 where total PSA was between 4.1-10ng/ml. The specificity and sensitivity was 60% & 90% for 14 and 85% & 56.5% for 15. In another study in Japan Yumiko Yokomizoetal in 2009 showed[9] that free/total PSA ration below 11, the cancer rate detection was 55.1% in 12 core biopsy where as this was 48.2% and 17.5% respectively with those of grayzone ie more than 12. In another study in India Chavan Atish et al (2010) found the ratio of free/total PSA was decreased significantly in cancer patients than BPH whereas the total PSA value increased linearly with age and more appropriately used to distinguish BPH and prostatic cancer at an early age.

Regarding total PSA in our study we found mean±SD 71.94 ± 68.79 in prostate cancer patients and total PSA in control was 4.58 ± 7.631 and the difference was statisticsally significant ($p<0.04$). Similar results were found

in some studies abroad. In one study in Japan in 2003 Kazuto Ito et al found usefulness of measuring free/total PSA and PSA in men with initial value of 4.1-10 ngm/ml and subsequent follow up estimation. They found the detection of prostate cancer was increased by 26% in consequitive screening with PSA value of more than 10ngm/ml. The most recent PSA velocity and serum PSA level at last follow up in patients with prostatic cancer were significantly higher than those without prostatic cancer. The cumulative non cancer rate was significantly greater in subjects with %PSA less than cutoff than those with %PSA at the cutoff point or greater in the %PSA cutoffs of 16%. Another study also in Japan by S.Egawa et al in 2002 showed that the probalities of prostate cancer at percent free PSA values of <10.0, >10 15, >15-20, >20-26 and >26 were 58.3, 40.8, 25.3, 14.3 and 7.6% respectively when analysed without regard to findings on palpation when the total PSA value was between 41-10.0ngm/ml.[10,11,12] In our present study we found very high total PSA value in Prostate cancer patients but the free PSA level was not statistically significant as found in this study. Yet in another study in Finland by Finne P et al in 2002 showed that prostate cancer probability depended most strongly on the percentage of free PSA, total PSA, prostatic volume and DRE also contributed to prostate cancer probability, whereas age and family history of prostate cancer did not. More false positive PSA results could be eliminated by using the multivariate risk model rather than the percentage of free PSA ($p<0.001$) or PSA density ($p<0.003$) alone. Wide variation in probability of detecting prostate cancer among screened men with a serum PSA of 4-20ngm/ml was observed.[13]

Prostate specific antigen (PSA) is widely used as a diagnostic marker for prostate cancer because of its high specificity. However elevated serum PSA does not occur only in prostate cancer but also in benign prostatic hyperplasia Prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) are glycoproteins secreted by propstate epithelial cells and have a long clinical history of use as serum biomarkers of prostate cancers.

A low frequency of positive results in patients with prostatic cancer and a high frequency of positive results in those with BPH seem to discourage the use of PSA positive circulating cells in the search for a clinical diagnosis of prostatic cancer.

Thiry percent of patients with localized prostate cancer undergoing radical prostatectomy experience biochemical recurrence with rising serum prostate specific antigen (PSA). More than 50% of these develop distant metastasis. Prostate specific antigen (PSA) has a significant role for the diagnosis of prostate cancer and a significant correlation with bone scan. Immunoradiometric assay has a sensitivity of 79.5% and specificity of 87.5% for prostate cancer. A PSA level above 4ng/ml was found in 79.6% of biopsy positive prostate cancer patients, 20.4% had normal PSA level.[14,15]

Determination of f/tPSA improves differentiation of prosatate carcinoma from BPH. They recommend serum PSA level of 4-20ng/ml, a cut of value of 0.14 to be applied to Iranian patients. A total of 3670 Iranian men older than 40 years were mass checked by PSA based screening.15 They concluded that a f/tPSA thresh hold at <0.18 rather than <0.15 increases the sensitivity for detecting prostate cancer from 85.2% to 94.5% while false positive decreased by 30.8%.

PSA is organ specific but not cancer specific. Several refinements in estimation and interpretation of PSA values have been proposed. These include ratio between PSA values and volume of prostate gland. f/t ratio, the rate of change of PSA with time etc. The f/t ratio is lower in man with cancer ($<10\%$), higher in benign lesions ($>25\%$). PSA is of value for prostatic cancer that are localized and never progress to clinically significant invasive cancers. PSA is of great value in assessing the response to therapy. A high PSA level after prostatectomy or radiotherapy for localized cancer indicates a recurrence or dissemination. IHC is important for localization of PSA antigen which determines whether a metastatic tumor originated from prostate or not.

Conclusions

It is concluded from the present case control study that. high serum Zinc level > 142 ng/ml is indicative of prostate cancer and this test should be started in all diagnostic laboratories as a routine tests and as an adjuvant to normal routine procedure for diagnosis of different prostatic lesions like PRDE,USG of prostate and biopsy. Also it was reestablished that estimation of total PSA (a many fold rise above normal in this study 71.94nmg/ml) not free PSA and the ratio between free and total PSA (cut off value < 15.73%) is more important in the diagnosis of prostatic cancers.

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Limitation

The present study is limited by small number of samples. However this can be used as a baseline data for future research in Bangladesh and other countries in prostate cancer research.

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