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Original research article

ASSESSMENT OF SERUM FERRITIN ALONG WITH SERUM PSA IN DIFFERENTIAL DIAGNOSIS OF BENIGN PROSTATIC HYPERTROPHY AND PROSTATE CANCER

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Abstract

Background: Ferritin plays a key role in the development of prostate cancer (PCa). Our earlier studies showed that the knockdown of ferritin heavy chain (FTH) suppressed the migration and invasion of the prostate cancer cell line (PC3). However, the mechanisms behind FTH in the cell migration regulation of PCa have not been thoroughly investigated.

Aims and Objectives: An assessment of serum ferritin along with serum PSA in differential diagnosis of Benign Prostatic Hypertrophy & Prostate Cancer.

Materials and Methods: The case control study was conducted at Nil Ratan Sircar Medical College and Hospital, in the Department of Biochemistry in the time period of May-June 2022, with the prior permission of the authority. The study included 30 patients with PCa, 52 patients with BPH and 60 controls visiting NRS Medical College. All the patients with PCa and BPH were confirmed histopathologically. Subjects with history of other cancer, liver cirrhosis or viral hepatitis, and prior treatment for carcinoma prostate or BPH were excluded from the study. Written informed consent was obtained in the preferred language (Bengali/English/Hindi) from all participants. Blood samples were collected in clotted vial and serum was separated by centrifugation. Serum ferritin & serum Free PSA were measured using chemiluminescence immunoassay analyzer (Advia Centure XP).

Results and Observations: It is evident that the mean free PSA and serum ferritin levels are significantly elevated in BPH and Prostate Cancer subjects compared to the controls. We found statistically significant differences between control versus prostate cancer ($p < 0.001$) and between BPH and prostate cancer ($p < 0.001$). We did not find statistically significant differences between control and BPH subjects (p 0.28). We

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found statistically significant differences between control versus prostate cancer ($p<0.001$) and between BPH and prostate cancer ($p<0.001$). We did not find statistically significant differences between control and BPH subjects (p 0.27). We observed statistically significant positive correlation between free PSA and ferritin levels in prostate cancer ($p<0.001$) and we did not find statistically significant correlation between free PSA and ferritin levels in BPH subjects.

Conclusion: The amount of serum free PSA and serum ferritin in PCa patients, BPH patients, and normal controls was measured. The levels of ferritin were verified to be significantly higher in the blood of PCa patients than in that of BPH patients and controls. In many research it was found that serum ferritin play a significant role in the proliferation, apoptosis, and migration of prostate cancer. From all these analysis we concluded that ferritin can be used to distinguish PCa patients from BPH patients.

Keywords: Prostate specific antigen (PSA), Prostate cancer (PCa), ferritin, benign prostatic hyperplasia (BPH), differential diagnosis

Introduction

Serum ferritin, the storage form of iron, is elevated in many diseases and has emerged as a novel biomarker for many kinds of cancers. With a growing number of studies indicating that ferritin plays a vital role in oncogenesis, researchers have attempted to use ferritin as a biomarker for disease detection, therapy monitoring, and drug resistance surveillance. According to Wang *et al.*, serum ferritin-binding PSA detection can improve the diagnostic accuracy of PCa. In PCa patients, serum ferritin is significantly correlated with total PSA (tPSA), free PSA (fPSA) and fPSA-tPSA ratio, withslight correlations found in the corresponding BPH controls However, the role of ferritin in the progress of PCa is still unclear. Studying the mechanism of ferritin in the progression of PCa is of great significance for early diagnosis, treatment, and prognosis.

The S100 protein family, which comprises 21 members, has a high degree of structural similarity. Nonetheless, the members are functionally non-interchangeable. This protein family regulates cellular responses through the function of intracellular Ca2+ sensors and extracellular factors. An increasing number of studies have shown that dysregulation of S100 protein expression is a common event in many human cancers. Elevated levels of S100P were found to be correlated with progression to metastatic disease. The disorder of S100P is involved in multiple types of tumorigenesis. Meanwhile, S100A4 has been implicated in invasion and metastasis, and upregulation of its expression can be seen in a variety of tumor cells, including in prostate, pancreatic, breast, ovarian, renal, and brain tumors Also, S100A2 was dysregulated in prostate and breast cancer and found to have tumor-suppressing effects Moreover, other proteins, like S100A6 and S100A10 are dysregulated in multiple kinds of cancers. Prostatic carcinoma (PCa) is the second most common type of carcinoma in males worldwide. Also, it is the sixth leading cause of cancer death among men. The global burden of PCa is expected to reach about 1.7 million new cases, and 499 000 associated deaths by 2030 [1]. As per the population-based cancer registries (PBCRs) of India, prostatic carcinoma is the second most common type carcinoma among Indian males in cities like Delhi, Kolkata, Pune and Thiruvananthapuram. Similarly, in other cities of India like Bangaluru and Mumbai it is the third most common cancer type in male.

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Overall, PCa has been among the top ten leading sites of cancers in India according to a study published in 2014 [2].

In the diagnostic work-up of prostatic diseases, Prostate specific antigen (PSA) has been used as an important diagnostic as well as prognostic marker. However, the inability to differentiate other diseases of the organ from PCa poses a significant challenge, for example, a moderately increased PSA (4-10 ng/ml) overlaps both Benign prostatic hyperplasia (BPH) and also PCa. Furthermore, in some patients with PCa may have "normal" PSA levels (≤ 4 ng/ml) [3]. Thus, PSA in isolation, probably, may not be the most suitable biomarker for prostate cancer, given its general lack of specificity and sensitivity [4]. To increase diagnostic accuracy and reduce the number of unnecessary screening procedures including biopsies [5, 6], a new or complementary noninvasive biomarker is the need of time.

Serum ferritin, the storage form of iron, is seen elevated in many diseases like leukemias, lymphomas, hyperthyroidism, etc. A few recent reports have shown that serum ferritin expression was up-regulated in many malignant conditions like that of breast, liver, lung and urinary tract [7-11].

In this case control study we have evaluated the serum ferritin levels along with serum PSA levels to differentiate patients with PCa and BHP from normal controls.

Materials and Method

The case control study was conducted at Nil Ratan Sircar Medical College and Hospital, in the Department of Biochemistry in the time period of May-June 2022, with the prior permission of the authority. The study included 30 patients with PCa, 52 patients with BPH and 60 controls visiting NRS Medical College. All the patients with PCa and BPH were confirmed histopathologically. Subjects with history of other cancer, liver cirrhosis or viral hepatitis, and prior treatment for carcinoma prostate or BPH were excluded from the study.

Written informed consent was obtained in the preferred language (Bengali/English/Hindi) from all participants. Blood samples were collected in clotted vial and serum was separated by centrifugation. Serum ferritin & serum Free PSA were measured using chemiluminescence immunoassay analyzer (Advia Centure XP).

Results

Table 1: Shows the mean values of Free PSA (ng/mL) and Serum Ferritin (ng/mL) in Control, BPH and Prostate Cancer Subjects

	Control	**BPH**	**Prostate Cancer**
Free PSA	0.73±0.4	1.8±0.8	10.4±8.2
Serum Ferritin	75±48.6	133±80	685±415

It is evident that the mean free PSA and serum ferritin levels are significantly elevated in BPH and Prostate Cancer subjects compared to the controls.

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Table 2: Shows the comparison of ANOVA test results with post hoc analysis for Free PSA levels between the three groups

	P value
Control vs BPH	0.28
Control vs PCa	<0.001
BPH vs PCa	<0.001

This table represents the comparison of ANOVA test results with post hoc analysis for free PSA levels between control, BPH and Prostate Cancer subjects. We found statistically significant differences between control versus prostate cancer (p <0.001) and between BPH and prostate cancer (p <0.001). We did not find statistically significant differences between control and BPH subjects (p 0.28).

Table 3: Shows the comparison of ANOVA test results with post hoc analysis for serum ferritin levels between the three groups

	P value
Control vs BPH	0.27
Control vs PCa	<0.001
BPH vs PCa	<0.001

This table represents the comparison of ANOVA test results with post hoc analysis for serum ferritin levels between control, BPH and Prostate Cancer subjects. We found statistically significant differences between control versus prostate cancer (p<0.001) and between BPH and prostate cancer (p<0.001). We did not find statistically significant differences between control and BPH subjects (p 0.27).

Table 4: Shows Pearson's Correlation Coefficient for PSA and ferritin in Prostate Cancer and BPH subjects

	Correlation coefficient	P value
Prostate cancer	0.85	<0.001
BPH	-0.24	0.09

We observed statistically significant positive correlation between free PSA and ferritin levels in prostate cancer (p<0.001) and we did not find statistically significant correlation between free PSA and ferritin levels in BPH subjects.

Discussion

Prostate cancer (PCa) is the deadliest cancer after lung cancer in men and also has the highest incidence of cancer worldwide [12]. Tissue biopsy is the gold standard for the diagnosis of the prostate [13], while serum prostate-specific antigen (PSA) remains the most widely used biomarker for the management of early PCa [14]. Although PSA has extensive clinical applications, increased PSA levels can be found in benign prostatic

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hyperplasia (BPH) and other diseases [15]. When the patient's total prostate-specific antigen (tPSA) level is in the gray area (4-10 ng/mL), the diagnostic specificity is only 25-40% and may cause unnecessary biopsy in some patients [16]. Therefore, the development of more sensitive and specific tumor markers and oncogenes to improve the diagnostic accuracy and prognosis in PCa is urgently needed. We evaluated the value of free PSA and serum ferritin for discriminating between Prostate Cancer (PCa) and Benign prostatic hyperplasia (BPH). From all the samples that we have collected we first separate each sample based on their free PSA and serum ferrtin level. We categorized them into 3 classes one in Prostatic Cancer group, other 2 is Benign prostatic hyperplasia and Control.

In the table 1 shows the mean and SD of serum Free PSA and serum ferritin levels between the three groups control, BPH, and Carcinoma of Prostate. We found elevated levels of free PSA and serum ferritin in BPH and prostate cancer groups compared to control groups (table 1). Then ANOVA test with post hoc analysis was performed between three groups (control, PCa and BPH) for serum free PSA. We found statistically significant differences in serum free PSA between PCa& BPH and PCa and control (p value<0.001) but no significant different was found between control and BPH group. Similarly, we found Statistically Significant differences serum ferritin between PCa& BPH and PCa and control (p value<0.001) but no significant different was found between control and BPH group (table 1 and 2).

Finally, Correlation test was conducted between serum Free PSA and serum Ferritin among PCa group (table 4). In the result Significant correlation were found between serum PSA and serum ferritin (p value <0.001). Serum PSA and serum ferritin were extremely co-related.

Another Correlation test was conducted between serum Free PSA and serum Ferritin among BPH group (table 4). No significant correlation were found between serum PSA and serum ferritin (p value 0.93). Their value of correlation coefficient were in negative. So that means in the BPH group serum PSA and serum ferritin were not correlated.

Serum ferritin is the storage form of iron, which is elevated in many diseases. Some recent reports have shown that serum ferritin expression was up-regulated in many tumour-associated diseases such as breast cancer, liver cancer, and lung cancer [17-20]. Other reports have also suggested an association of serum ferritin with urinary tract tumours.

In a study, researcher collected urine samples from patients with PCa and BPH, and from healthy volunteers. Using an immunohistochemical approach, they found that ferritin was differentially expressed in tissues from the patients with PCa and BPH. Furthermore, to eliminate the influence of water content on urine protein analysis, they evaluated the differences in the ferritin levels in the urine of PCa and BPH patients using both ferritin and the ferritin-creatinine ratio (FCR) as indicators. Ultimately, they found that urine ferritin shows potential as a useful biomarker for PCa diagnosis.

In another study researchers collected blood samples of 3 groups, patients with PCa, BPH and control (healthy individual). They systematically evaluated the diagnostic and prognostic value of using circulating ferritin as a non-invasive biomarker for prostate cancer in a large-scale case-control sudy. Their results provide compelling evidence to support the correlation between both circulating and tissue ferritin levels and prostate

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cancer risk. Moreover, their findings suggest a clear relationship between serum ferritin levels and the risk, diagnosis and prognosis of prostate cancer. So, ferritin can help to distinguish PCa patients from BPH patients. The major limitation of our study was the small quantity of samples analyzed. Therefore, large-scale studies and more samples are needed for further validation. Determination of the mechanism of action of serum ferritin in prostatic progression will help to further improve the pathogenesis of prostate cancer and provide a new biomarker and an important theoretical basis for the diagnosis of prostate cancer.

Conclusion

The amount of serum free PSA and serum ferritin in PCa patients, BPH patients, and normal controls was measured. The levels of ferritin were verified to be significantly higher in the blood of PCa patients than in that of BPH patients and controls. In many research it was found that serum ferritin play a significant role in the proliferation, apoptosis and migration of prostate cancer. From all these analysis we concluded that ferritin can be used to distinguish PCa patients from BPH patients.

References

1. Ferlay J, Shin HR, Bray F. International Agency for Research on Cancer; Lyon, France: GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No, 2010, 10.
2. Shalu Jain, Sunita Saxena, Anup Kumar. Epidemiology of prostate cancer in India; Delhi: Elsevier, 2014.
3. Thompson IM, Pauler DK, Goodman PJ, *et al*. Prevalence of Prostate Cancer among Men with a Prostate-Specific Antigen Level ≤4.0 ng per Milliliter. N Engl. J Med. 2004;350:2239-2246.
4. De Vincentis G, Follacchio GA, Frantellizzi V, Liberatore M, Monteleone F, Cortesi E. Prostate-Specific Antigen Flare Phenomenon During 223 Ra-Dichloride Treatment for Bone Metastatic Castration-Resistant Prostate Cancer: A Case Report. Clin Genitourin Cancer. 2016;pii:S1558-767330104-5.
5. Thompson IM, Pauler DK, Goodman PI, Tangen CM, Lucia MS, Parnes HL, *et al*. Prevalence of prostate cancer among men with a prostate-specific antigen level < or = 4.0 ng per milliliter. N Engl. J Med. 2004;350:2239-2246.
6. Draisma G, Etzioni R, Tsodikov A, Mariotto A, Wever E, Gulati R, *et al*. Lead Time and Overdiagnosis in Prostate-Specific Antigen Screening: Importance of Methods and Context. J Natl Cancer Inst. 2009;101:374-383.
7. Draisma G, Boer R, Otto SJ, Van der Cruijsen IW, Damhuis RA, Schröder FH, *et al*. Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. J Natl Cancer Inst. 2003;95:868-878.
8. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochim Biophys Acta. 2010;1800:760-769.
9. Jézéquel P, Campion L, Spyratos F, *et al*. Validation of tumor-associated macrophage ferritin light chain as a prognostic biomarker in node-negative breast cancer tumors: a multicentric 2004 national PHRC study. Int J Cancer. 2012;131:426-437.

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10. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. Cancer Causes Control. 2007;18:1047-1053.
11. Cujuc D, Golubovic S, Bojic-Trbojevic Z, Ilic N, Baricevic I, Nedic O. Differential diagnosis of liver diseases using serum biomarkers. JBUON. 2010;15:141-146.
12. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2019;69:7-34.
13. Litwin MS, Tan HJ. The Diagnosis and Treatment of Prostate Cancer: A Review. JAMA. 2017;317:2532-42.
14. Popov SV, Guseinov RG, Skryabin ON, *et al*. Prognostic significance of prostate-specific antigen in defining indications for initial prostate biopsy. Urologiia, 2018, 92-7.
15. Chistiakov DA, Myasoedova VA, Grechko AV, *et al*. New biomarkers for diagnosis and prognosis of localized prostate cancer. Semin Cancer Biol. 2018;52:9-16.
16. Nguyen-Nielsen M, Borre M. Diagnostic and Therapeutic Strategies for Prostate Cancer. Semin Nucl. Med. 2016;46:484-90.
17. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochim Biophys Acta. 2010;1800:760-769.
18. Jézéquel P, Campion L, Spyratos F, *et al*. Validation of tumor-associated macrophage ferritin light chain as a prognostic biomarker in node-negative breast cancer tumors: a multicentric 2004 national PHRC study. Int J Cancer. 2012;131:426-437.
19. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. Cancer Causes Control. 2007;18:1047-1053.
20. Cujuc D, Golubovic S, Bojic-Trbojevic Z, Ilic N, Baricevic I, Nedic O. Differential diagnosis of liver diseases using serum biomarkers. JBUON. 2010;15:141-146.