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Serum Fatty Acid Synthase Level in Patients with Prostate Cancer and Benign Prostatic Hyperplasia

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Abstract

Background: Human Fatty Acid Synthase is highly expressed in many human cancers. Previous studies have shown that this enzyme is expressed at very high levels in prostate cancer and that the growth of prostate cancer cell line can be inhibited by pharmacological inhibitors that target this enzyme. Additionally, some studies have reported that this enzyme is overexpressed not only in tissue, but also in serum of patients with various cancers. The aim of this study was to,evaluate serum levels of this enzyme in patients with prostate cancer and in patients with benign prostatic hyperplasia as well as to investigate whether it can be used as a biomarker for detection of prostate cancer and benign prostatic hyperplasia. **Methods:** By using an FASN ELISA kit, we measured serum levels of Human Fatty Acid Synthase in 35 patients with prostate cancer and 35 patients with benign prostatic hyperplasia. We also measured serum FASN levels of 35 healthy volunteers enlisted as normal controls. **Results:** Serum FASN levels in prostate cancer patients were significantly higher than in healthy control subjects, but FASN levels in patients with benign prostatic hyperplasia were not significantly higher than in healthy control subjects. **Conclusions:** Serum FASN levels are expressed at significantly high levels in human prostate cancer. Serum FASN levels were not expressed at significantly high levels in human benign prostatic hyperplasia. FASN serum levels may be additional biomarker for prostate cancer detection.

Keywords: *Fatty acid synthase, prostate cancer, benign prostatic hyperplasia, ELISA.*

Introduction

Prostate cancer (PC) and benign prostatic hyperplasia (BPH) are common prostate tumors. Prostate cancer is the most widely recognized non-cutaneous malignancy tumor in men [1]. Benign prostatic hyperplasia is a non-cancerous increase in size of prostate gland. It is the most common benign tumor found in men [2].

Although transrectal ultrasound-guided prostate biopsy is gold standard in the diagnosis of prostate cancer, it can be hurtful with possible side effects of biopsies including pain, serious infections and bleeding [3].

Additionally, serum prostate-specific antigen (PSA) levels do not have a direct correlation with increasing grade and stage of prostate cancer [4].Thus, a novel biomarkers that have a stronger association with prostate cancer and have direct correlation with increasing grade of prostate cancer and have less side effects are needed.

A marker that is considered in this study is human fatty acid synthase (FASN), a metabolic enzyme that catalyzes the biosynthesis of longchain fatty acids [5]. FASN was first identified as oncogenic antigen 519 in patients with a poor prognosis for breast cancer [6]. In normal human tissue de novo fatty acid synthesis is suppressed and the low levels of lipogenic enzymes expression are maintained. Normal cells preferentially depend on dietary lipids to satisfy their metabolic needs. In contrast, increased lipogenesis is a major hallmark for tumor progression with cancer cells change to dependence on de novo fatty acid synthesis to support rapid cell growth [7]. The main enzyme responsible for the synthesis of fatty acids in the cell is fatty acid synthase (FASN) [8]. FASN has been found to be common sense overexpressed in about every type of cancer and is associated with their progression and development [9]. This enzyme is overexpressed not only in tissues, but also increased enzyme concentration in serum of patients with various cancers such as colorectal

cancer, gastric carcinoma and esophageal neoplasia [10- 12]. Serum FASN levels are elevating in patients with prostate cancer compared with healthy controls [13]. In addition, the levels of serum fatty acid synthase are associated with stage of disease in patients with colorectal cancer [14].

The aim of this study was to evaluate serum levels of this enzyme in patients with prostate cancer and in patients with benign prostatic hyperplasia as well as to investigate whether it can be used as a biomarker for detection of prostate cancer and benign prostatic hyperplasia.

Materials and Method

Study subjects

The present study included 35 patients with prostate cancer (15 patients underwent trans urethral resection or prostate biopsy surgery and 20 patients from oncology hospital. In addition, 35 patients with benign prostatic hyperplasia who were diagnosed and had blood samples collected between September 2018 and April 2019. Patients were divided into three groups; control, BPH and PC. Fasting serum samples were collected from patients preoperative or prior to treatment in addition to serum samples from 35 healthy individuals (controls).

FASN ELISA

A total of 100µl serum was analyzed using a commercially available ELISA kit, FASN ELISA (cusabio biotech), according to manufacturer's recommendations. Using a pipette, a volume of 100µl of standard and sample was added per well. Then covered with the adhesive strip provided, incubated for 2 hours at 37°C, then the liquid of each well was removed by inverting plate and rapidly flicking the liquid away from the plate. Using a pipette, a volume of 100µl of Biotin-antibody (1x) was added to each well and covered with a new adhesive strip. Incubated for 1 hour at 37°C, then each well was aspirated and washed, and the process was repeated two times for a total of three washes. It was washed by filling each well with wash buffer (200µl) using an auto-washer and it was left to stand for 2 minutes. After the last wash, any remaining wash Buffer was removed by decanting. The plate was inverted and blot it against clean paper towels. Then by pipette (100µl) of HRP-avidin (1x) was added to each well. The micro titer plate was covered with a new adhesive strip and incubated for 1 hour at 37°C. The aspiration/wash process was repeated for five times as in step 6, then (90µl) of TMB substrate was added to each well and incubated for 15-30 minutes at 37°C in the dark. The a volume of 50µl of stop solution was added to each well using a pipette, the plate was gently tapped to ensure thorough mixing. The optical density was determined of each well within 5 minutes, using a micro plate reader set to 450nm.

Statistical Analysis

Statistical analysis was carried out using Microsoft excel 2013 and SPSS version 20. The numerical data expressed as mean ±SD. Furthermore, comparisons between mean serum concentrations of FASN in control and study (PC and BPH) groups were performed. All *P* values were two sided and statistical significance was set at $P \leq 0.05$. Receiver Operating Characteristics (ROC) curve was calculated to estimate the sensitivity and specificity of the used FASN as biomarker and discriminatory ability.

Results

In this study, ELIZA method was used for estimation of the concentration of FASN in sera of patients with prostate cancer, benign prostatic hyperplasia and healthy controls (Figure 1). FASN concentration in sera of patients with prostate cancer ranged from (0.87ng/ml) to (24.19ng/ml) with a mean±SD of 5.8±4.9ng/ml, and showed a significant elevation in comparison with control group (P=0.001; Table 1). On the other hand, the mean±SD FASN concentration in sera of benign prostatic hyperplasia patients was (3.3±1.8) and showed no significant elevation in comparison with control group (P-= 0.17; Table 2).

Table (1) Comparison between serum FASN concentrations in PC and control groups

Mean±SD		**P value**
PC group	Control group	
5.8±4.9	2.7±2	0.001

Table (2) Comparison between serum FASN concentrations in BPH and control group s

Mean±SD		P value
BPH group	Control group	
3.3 ±1.8	2.7±2	0.17

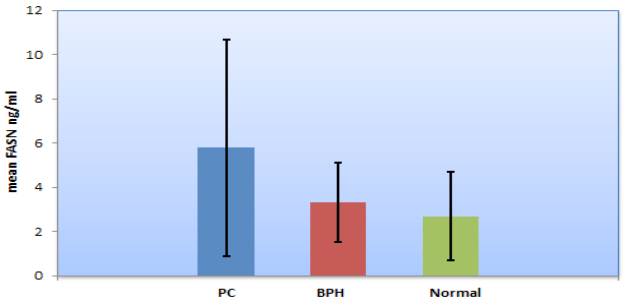

Figure 1 Concentration FASN in PC, BPH and control groups.

The Receiver Operator Characteristic (ROC) curve showed a significant discriminatory ability of increased serum FASN levels for PC (Figure 2).

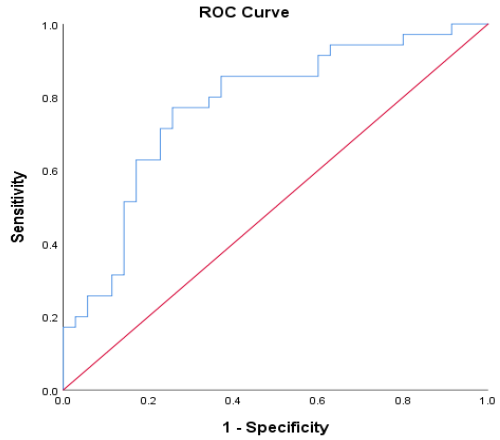

Figure 2 ROC curve

The cut-off value of the serum FASN concentration in patients with prostate cancer was (3.31ng/ml) with sensitivity of 71%, specificity of 23% and an area under curve of 0.77; Table 3).

Table 3 Sensitivity and Specificity of FASN ROC curve in patients with prostate cancer

Cut-off value	Specificity	Sensitivity	Area under curve
3.31ng/ml	23%	71%	0.77

Discussion

A novel biomarker that has stronger association with prostate cancer and has direct correlation with increasing grade of prostate cancer is needed. Identification of serum protein markers of prostate cancer and benign prostatic hyperplasia together with other markers already known could help provide such a non-invasive diagnostic and prognostic screening tool. FASN is a major component of the de novo fatty acid synthesis pathway that catalyzes the oxidation NADPH-dependent formation of large free fatty acids from two carbon donors. A strong association was found between increased FASN levels in malignant tissue and the presence of other unfavorable prognostic indicators in primary prostate cancer, as determined by immunohistochemistry methods [15]. Patients with prostate cancer rich in FASN display significantly poorer clinical prognosis than those with prostate tumors containing lesser amounts of FASN enzyme.

In this study, FASN concentrations were determined in sera of patients. The findings indicated that the concentrations of FASN in sera of patients with prostate cancer were higher than in sera of healthy subjects. We have detected high FASN concentration in 71% of participant patients with prostate cancers depending on FASN cut-off value and the mean±SD was (5.8±4.9ng/ml).

This finding was in agreement with study of [13], however, t the mean and standard deviation of serum FASN in that study was (0.79±0.76 units/I) which was different from the mean and standard deviation in our study, because the (ng/ml) unit was used in our study to measure sera FASN concentration.

This was the first study that serum concentration of FASN in patients with benign prostatic hyperplasia was determined. The results shown that no significant different between BPH and healthy subjects groups (P= 0.17; Table 2). This approved that FASN has a role in development of prostate cancer and indicating that serum FASN concentration could aid as a diagnostic marker for prostate cancer, because most men with an elevated PSA level turns out not to have prostate cancer; just about 25% of men who have a prostate biopsy due to an elevated PSA level actually are found to have prostate cancer when a biopsy is done [16] and novel biomarkers that have stronger association with prostate cancer are needed.

A receiver operatory characteristic (ROC) curve is a graphical plot used to show the diagnostic ability of binary classifiers. It was first used in signal detection theory, but now it is used in many other areas as medicine. ROC curve is instituted by plotting the sensitivity against the (1- Specificity).

Sensitivity is the ratio of observations that were correctly predicted to be positive out of all positive observation. It equals:

True positive/ (True positive + False negative).

In contrast, specificity is the ratio of observations that are correctly predicted to be negative out of all negative observations. (1 – Specificity) equals:

False positive /(True negative + False positive)

In this study discriminatory ability of increased serum FASN levels for PC was tested by ROC curve (Figure 2). The area under curve was 77% and this indicated how well serum FASN can distinguish between patients with prostate cancer and healthy subjects.

The sensitivity was 71%. This meant that the positive detection rate was 71%. This is higher than positive detection rate in study of [13] which was 53%. The latter study had only 29 samples with prostate cancer and may be this the cause that made their results vulnerable to bias, additionally the incubation time and temperature of the first incubation ELISA method in that study were overnight at 4°C and this is different from current study where the incubation time was 2 hour at 37°C.

Conclusion

This study suggested that serum FASN levels were expressed at significantly high levels in human prostate cancer. Thus, FASN serum level may be an additional biomarker for prostate cancer detection with a sensitivity and specificity of 71% and 23%, respectively. The study concluded that there was no significant different between serum FASN of patients with BPH and healthy subjects. In addition, serum FASN concentration is not useful biomarker for diagnosis of PBH. This approved that FASN has a role in development of prostate cancer. Subsequent studies should investigate how serum FASN levels relate to clinical responses to prostate cancer therapies. Subsequent studies to investigate relationship between transcription factors of FASN and Gleason score. Determine the cut-off value of serum FASN concentration for each class of Gleason score in patients with prostate cancer. Also, studying correlation between serum concentration of FASN with stage of prostate cancer is highly recommended.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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