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pSerum HLA-G Level as A Prognostic Marker and Its Correlation with Some Important Markers for Malignant and Benign Prostate Hyperplasia

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Abstract. Prostate cancer is the most diagnosed cancer's form in men, and it is the second cause cancer-related mortality after lung cancer, Where it is diagnosed in men who are 50 years and over. Prostate specific antigen (PSA) is often used as a prostate cancer tumor marker. PSA was recently identified in non-prostatic cancer tissues in men and women, casting doubt on its prostatic tissue specificity. PSA is present at low concentrations in the urine of healthy men and at elevated levels in a variety of prostate diseases, including prostatitis and prostate cancer. Thus, a supplementary tumor marker is required for effective cancer diagnosis and follow-up during care. The objective of the study was to evaluate of HLA- G, hK2 and 5-a-reductase levels as a novel and prognostic markers and it's relationships with each of malignant and benign prostate hyperplasia. Samples were collected at the middle Euphrates cancer center Laboratories. The samples were 32 of Prostate cancer, 28 of benign prostate hyperplasia (BPH), and 29 of control group samples. A serum was separated for biochemical tests, where PSA, HLA-G, hK2 and 5- a- reductase was measured by ELISA test. The result showed a significant increase ($p<0.05$) of PSA, HLA-G hK2 and 5- a- reductase levels in both prostate cancer and BPH in comparison with a control group. The correlation study showed presence a negative correlation between the following markers HLA-G and PSA, HLA-G and hK2, HLA-G and 5-a-reductase and PSA and 5-a-reductase concentrations was observed. While our results showed positive correlation between PSA and hK2 and between hK2 and 5-a-reductase concentrations. Conclusion: A significant increase in the levels of all markers in sera of patients, As a consequence, the HLA- G serum level may be used as a novel and simple method for prostate cancer monitoring, detection, and staging.

Keywords: Prostate cancer, BPH, HLA-G, hK2, 5-a-reductase

INTRODUCTION

After lung cancer, with a frequency of 25.3 per 10,000, prostate cancer is the most prevalent form of cancer in middle-aged and elderly men. According to previous research, one man in every six would develop this cancer during his lifetime. It is well established that genetic, hormonal, and environmental factors contribute to the occurrence of this cancer. The occurrence can be seen more often in Northern Europe and the Middle East Africa, with the low incidence in some Asian countries. [1, 2].

Screening , diagnosis , and treatment of prostate cancer in its early stages may extend patients' lives and increase their quality of life and reducing morbidity and mortality [3]. The primary diagnostic methods for prostate cancer are digital rectal examination DRE and prostate specific antigen (PSA) assessment in serum [4]. Prostate specific antigen is a proteolytic enzyme that is secreted by both normal and cancerous prostate cells. Any type of prostate injury, such as hyperplasia, adenocarcinoma, prostatitis, cystoscopy, or senility, has been confirmed to

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increase the serum level of this antigen; however, this antigen is commonly used to diagnose prostate cancer [5-7]. While this specific antigen is expressed more often in patients with prostate cancer, this does not always indicate illness, as elevated PSA levels are often seen in certain persons with benign prostatic hyperplasia and urinary tract infections. PSA testing can be determined by further examinations such as magnetic resonance imaging MRI , computed tomography , ultrasound , and prostate biopsy to confidently detect prostate cancer. However, about 25% of prostate cancers are not detected after the initial biopsy [8].

Previous research has shown that despite of the poor biopsy outcome, the PSA levels rise, but it does not increase in a small proportion of gland lesions.

Therefore, given the limitations and poor diagnostic precision of screening tests, make developing a reliable method of diagnosis is critical [9]. PSA, on the other hand, has drawbacks, including lack of accuracy, which results in prostate cancer over diagnosis [10]. Especially for the so-called gray zone between PSA levels of 4.0 and 10.0 ng/mL, this results in a high rate of negative biopsy. In light of this, additional prostate cancer (PCa) biomarkers could be discovered to improve the accuracy and sensitivity of Pca diagnosis. Numerous promising alternative biomarkers identified as a result of genomic and proteomic technology advancements [11]. In comparison to PSA, human glandular kallikrein 2 (hK2) is a serine protease with a 79 percent amino acid sequence homology to PSA. Previous research indicates that there is a higher expressed of human kallikrein 2 level in Pca than in typical prostate tissues. While these biomarkers continue to have limitations, not present single biomarker to be more useful in diagnosis of prostate cancer than PSA [11]. Additionally to other indicators which considered essential for prostate cancer diagnosis, such as 5-a-reductase, a key enzyme in the growth of the male sexual organs.

HLA -G was recently approved as a tumor marker for lung , breast , eye , ovarian, and gastrointestinal cancers [12]. It is shown that assessing the concentration of HLA-G in serum or plasma improves diagnostic accuracy [13-15]. The aim of this study is to compare this tumor marker level to prostate cancer pathology in patients and assess its value as a new biomarker for detection of prostate cancer.

MATERIALS AND METHODS

Serum specimens were obtained from the subjects with benign and malignant prostatic hyperplasia as well as the healthy men that went to the middle Euphrates cancer center. The average age of the patients was between 50 and 83 years old. The tested samples were 88 samples; the samples of healthy men were 29, 28 samples from BPH, and 32 samples from patients with PCa. The (88) Samples were subjected to a biochemical test. tPSA, HLA-G, hK2 and 5-a-reductase were measured using an immunological method by ELISA reader Huma type. All samples and reagents were brought to the lab at room temperature. All reagents gently combine to avoid foaming. Both phases of the process were performed without interruption once it begins, and biochemical experiments were performed in the Biology Department / Collage of Sciences / University of Kufa laboratories. The used Kits of ELISA were tPSA (toral prostate specific antigen) (2125-300) (Accu-bind company USA in Origin), HLA-G (Human leukocyte antigen-G) (E - EL- H1663) (Elabscience company, china in Origin) and hK2 (Human kallikrein2) (E553Hu) and 5-a-reductase1 (Human Steroid 5-Alpha-Reductase 1) (E2193Hu) (Bioassay technology laboratory company china in Origin). The study of variance table was one–way ANOVA (by Tukey's multiple comparisons test) used for the comparison among subdivided groups in the calculated markers, and the statistical method (Graph Pad prism ver. 5) was used. (Mean ± Stander Error) was used to express the effects. Mega stat (version 10.12) for Excel 2010 was used to quantify correlation to measure the correlation between markers, as well as descriptive statistics and correlation coefficients. (Motulsky, 2003).

RESULTS

The statistical analysis of this study revealed that the levels of PSA, HLA-G, hK2 and 5-a-reductase were statistically significant between all patient's groups ($P < 0.05$). The results showed increase in significant ($p < 0.05$) of PSA level in both prostate cancer (48.60 ± 6.946) and BPH (21.37 ± 3.196) compared with the control group (1.406 ± 0.1412), significant also increase ($p < 0.05$) of PSA level in BPH (21.37 ± 3.196) compared with prostate cancer patients (48.60 ± 6.946) as shown in figure 1. Also, the results of current study revealed that significantly increase of HLA-G level in both prostate cancer (20.89 ± 1.416) and BPH (16.35 ± 1.205) compared with the control group (9.708 ± 1.247), also there was a significant increase ($p<0.05$) in prostate cancer patients compared with benign prostate hyperplasia and as shown in figure 2. As appear in figure 3 our results show increase in significant ($p < 0.05$) of hK2 level in both prostate cancer (409.3 ± 11.60) and BPH (356.0 ± 8.937) compared with

the control group (224.1 ± 9.084), also the results of this study show increase in significant ($p < 0.05$) of hK2 level in BPH (356.0 ± 8.937) compared with prostate cancer patients (409.3 ± 11.60). The present study results showed a significant increase ($p < 0.05$) of 5-a-reductase level in prostate cancer (1.252 ± 0.1432) is compared with the control group (0.6997 ± 0.01455), and also there was significant increase ($p<0.05$) in benign prostate hyperplasia (0.9146 ± 0.05288) compared with prostate cancer patients, While there was a non-significant difference ($p>0.05$) in benign prostate hyperplasia compared with control group as shown in figure 4. About the correlation study, the present study showed presence negative correlation between the following markers HLA-G and PSA, HLA-G and hK2, HLA-G and 5-a-reductase and PSA and 5-a-reductase concentrations was observed as shown in figures 5, 6, 7, and 9. However, the results of this study showed positive correlation between PSA and hK2 and between hK2 and 5-a-reductase concentrations was observed as shown in figures 8 and 10.

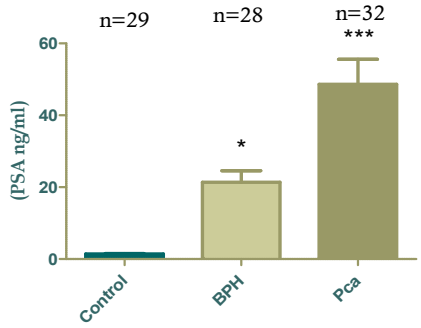

FIGURE 1. The comparison of PSA concentration in the serum between BPH, Prostate cancer with healthy men (control).

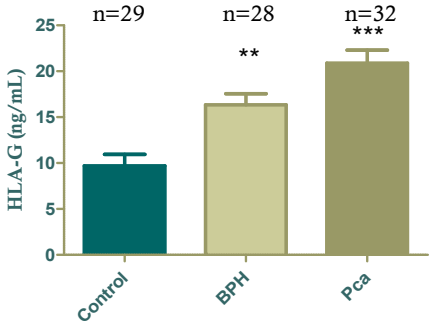

FIGURE 2. The comparison of HLA-G concentration in the serum between BPH, Prostate cancer with healthy men (control).

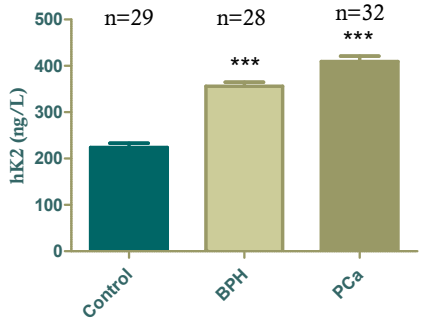

FIGURE 3. The comparison of hK2 concentration in the serum between BPH, Prostate cancer with healthy men (control).

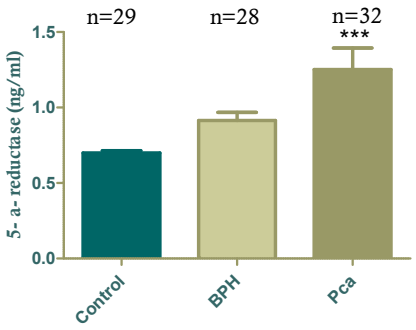

FIGURE 4. The comparison of 5- a- reductase (ng/ml) concentration in the Serum between BPH, Prostate cancer with healthy men (control).

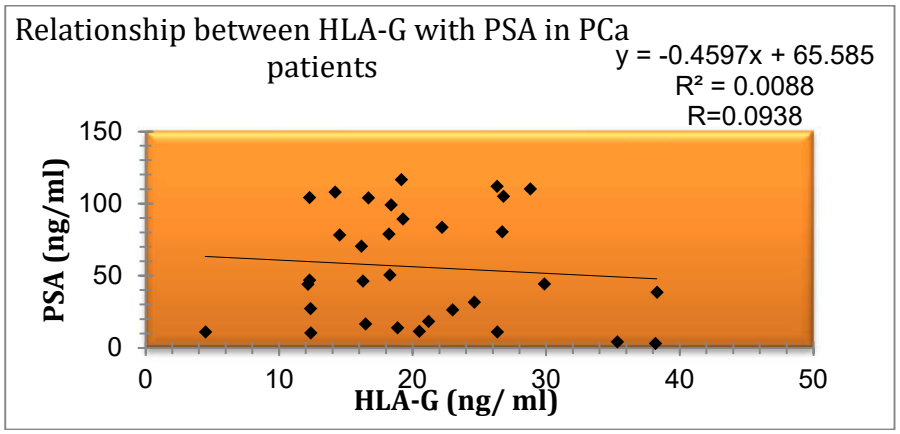

FIGURE 5. HLA-G concentration (ng/ml) correlation with PSA concentrations (ng/ml).

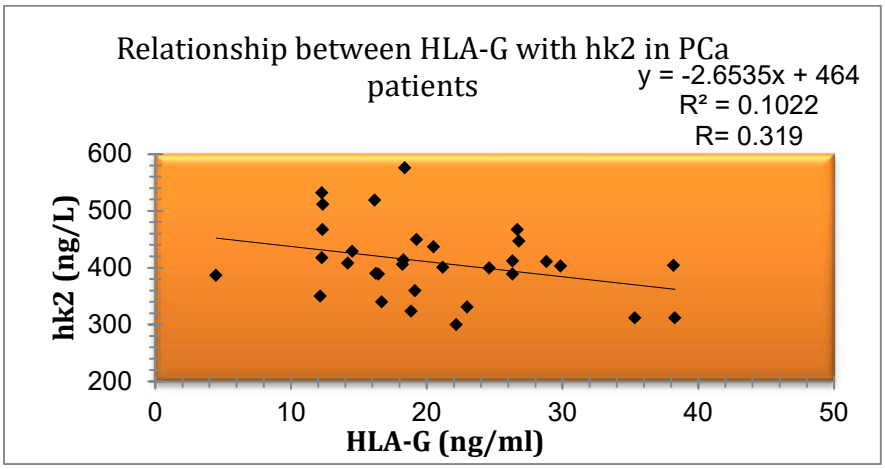

FIGURE 6. HLA-G concentration (ng/ml) correlation with hK2 concentrations (ng/L).

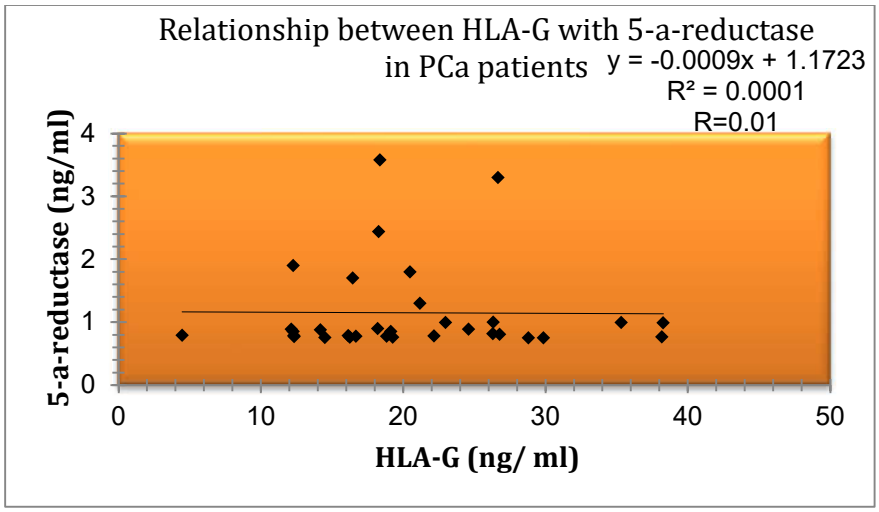

FIGURE 7. HLA-G concentration (ng/ml) correlation with 5-a-reductase concentrations (ng/mL).

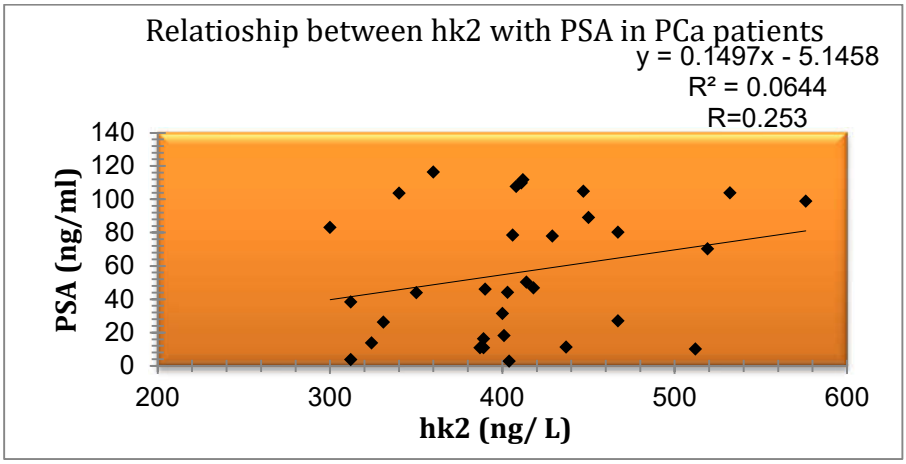

FIGURE 8. PSA concentration (ng/ml) correlation with hK2 concentrations (ng/L).

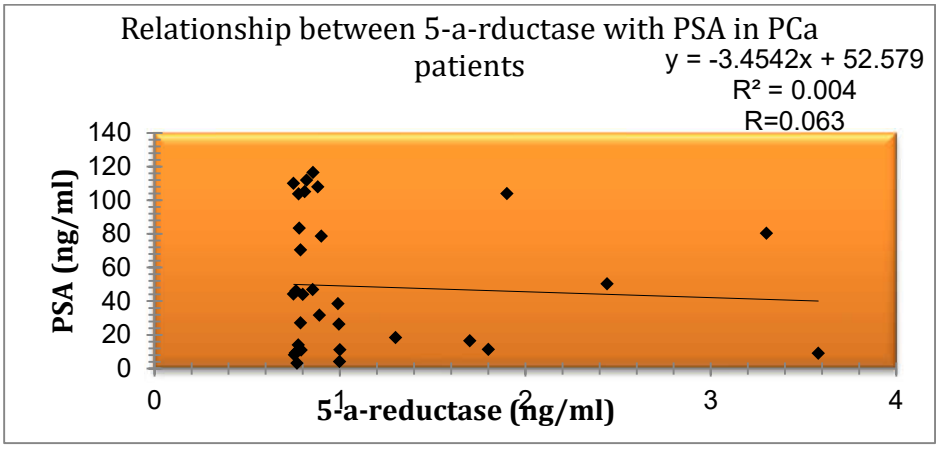

FIGURE 9. PSA concentration (ng/ml) correlation with 5-a-reductase concentrations (ng/ml).

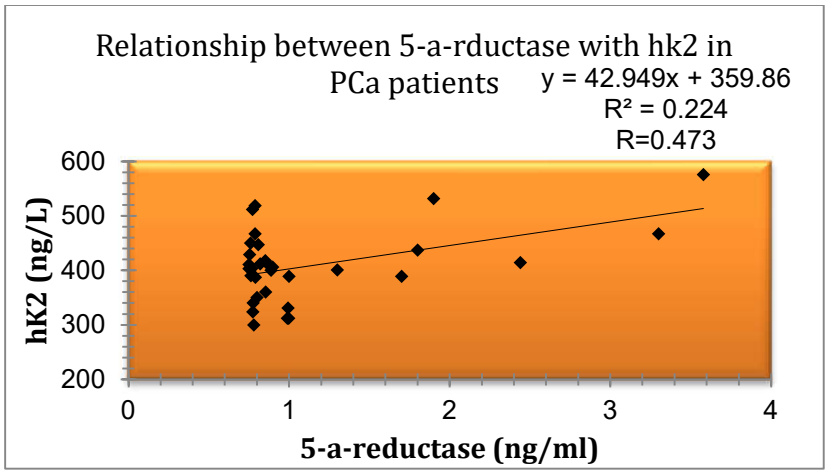

FIGURE 10. hK2 concentration (ng/L) correlation with 5-a-reductase concentrations (ng/ml).

DISCUSSION

This research was aimed to determine the HLA -G serum level as an important tumor marker for prostate cancer screening, diagnosis, and prognosis, as well as to examine the association between HLA-G and several other markers. The results of this study were agreement with Heidari *et al* which said the serum PSA level was high in patients with prostate cancer and benign group in compared with healthy group. Additionally, the mean serum HLA-G level was observed to be elevated in all three classes [16]. The statistical analysis showed a substantial difference in HLA-G serum levels between the three sample groups, with the greatest level belonging to the malignant group and the lowest levels belonging to the healthy group; hence, there is a direct correlation between HLA-G serum levels and cancer stage. Although PSA level determination is routinely used in laboratories for prostate cancer screening, and an increase in the serum level of this marker is interpreted as an indication of increased disease severity, our results suggest that there is no correlation between disease severity, cancer stage, and serum level of PSA. HLA -G is one of the MHC I molecules that functions as an immunosuppressant and a promoter of NK and T cells. [17]. Previous research has established a clinical correlation between elevated HLA-G expression and illnesses such as viral infection , cancer , organ transplantation , pregnancy , autoimmune disease , and inflammation. For the first time, HLA-G expression was detected in trophoblastic cells of the chorion, where it plays a critical role in defending the fetus against the immune system of mother's, fetal development, and the early stages of placenta angiogenesis [18]. Additionally, HLA -G has been identified in individuals undergoing allografts and malignant cell growth in a variety of cancers [19]. Similar to the results of this study, Wang et al. [20] documented a positive association between HLA -G expression and glioma tumor size. Guo et al. [21] concluded in another study after evaluating HLA -E or HLA -G expression in patients with colorectal cancer that there is a direct correlation between HLA -G or HLA -E expression and metastasis rate and mortality. Our results show negative correlation between HLA-G and all other markers in this study, that mean the relationship between HLA-G and these markers are inverse and the higher of HLA-G, the lower of the other markers.

The human leukocyte antigen G (HLA-G) is an immunoregulatory molecule involved in a variety of immunity processes. Since the discovery of HLA-G expression in cancer [22], several pieces of evidence have shown that HLA-G plays a significant role in tumor cells evading immune surveillance and antitumor immune response [23]. In healthy individuals, HLA-G expression is restricted to a few cell types. However, substantial expression exists in some chronic conditions, such as cancer, with different levels of expression depending on the affected tissue [24]. HLA-G can inhibit T and natural killer cell cytotoxicity, induce T cell apoptosis, and induce Th2-type cytokines and unresponsiveness of immune-competent cells to tumor antigens, all of which contribute to cancer progression by encouraging tumor cells to evade immune system-mediated destruction [25]. While little is known about the function of HLA-G in the progression of PCa, there is compelling evidence that these tumors foster immune tolerance early in the course of the disease [26].

Human kallikrein 2 is a serine protease with a sequence similarity to PSA of 79%. Additionally, it is a novel prostate cancer biomarker [27]. hK2 is mainly synthesized in the prostate, secreted as a pro-enzyme in the body, and then released to form active enzymes outside the cell. Blood, sperm, saliva, and other body fluids contain hK2;

additionally, between 80% and 95% of hK2 in the blood is in the free form. Numerous observations have shown the value of serum hK2 in the diagnosis and prognosis of Pca [28]. The results of the present study show significant increase of hK2 level in benign malignant prostate hyperplasia patients and these results agreed with *Mao et al* that show increase in hK2 level in prostate cancer compared with control group [29]. hK2, a subset of the kallikrein protein family, was seen to be a more accurate predictor of prostate cancer development than serum PSA in some studies [30–32]. Serum contains many hK2 variants, including freehK2 and hK2-ACT. The term "free-hK2" refers to both pro-hK2 and mature free-hK2, both of which are increased in prostate cancer [31-34]. A higher Gleason score is correlated with free-hK2. Following radiation treatment, an improvement in free-hK2 may be used to forecast recurrence [35]. This study shows a positive correlation between hK2 and PSA and with 5-a-reductase, which means, the higher the PSA and 5-a-reductase the higher the hK2. While negative correlation between hK2 and HLA-G means, the lower the HLA-G, the higher the hK2.

CONCLUSION

According to the findings of this study, serum HLA -G can be used as a novel and simple method for prostate cancer screening , diagnosis , and staging. Early detection of prostate cancer can help in reducing mortalities and morbidity and improve quality of life for cancer patients. Additionally, hK2 is considered to be more accurate than PSA in detecting prostate cancer.

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