Hormonal changes in Egyptian patients suffering from prostate cancer and benign prostate hyperplasia

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Abstract: Prostate cancer is the most common malignancy among men. Hormonal factors play an important role in growth of prostate tissue and induction of prostate carcinogenesis. The use of sequential hormonal therapies is a common practice in the systemic therapy of advanced prostate cancer. The aim of this study is to clarify the possibility of use the measuring serum level of androgens (testosterone (T) and adrenal androgens dehydroepiandrostenedione sulphate (DHEA-s) and androstenedione (AD)), gonadotropins (Follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) to improve the accuracy of diagnostic serum prostate specific antigen (PSA), and to examine the effect of these parameters in the progression of prostate cancer from androgen dependent to androgen independent after orchiectomy. Results indicated that serum DHEA-s level showed insignificant decrease in hyperplasia group as compared to androgen independent group (P = 0.185) and a significant decrease in hyperplasia group as compared to androgen dependent group (P = 0.001) while, no significant variation was found between androgen dependent and androgen independent groups (P = 0.341). Combination of PSA and DHEA-s increase the sensitivity of PSA to differentiate between benign hyperplasia and cancer from 93.5% to 96.8%. Serum FSH showed no statistically significant variation between androgen dependent group and hyperplasia group (P = 0.109), while, there was a highly significant increase in androgen independent group as compared to androgen dependent (P = 0.002) and hyperplasia groups (P = 0.001). Serum LH showed statistically significant increase in androgen dependent group (P = 0.001) and androgen independent group (P = 0.001) as compared to hyperplasia group, also, there were a statistically significant variation was found between androgen dependent and androgen independent groups (P = 0.002). Serum T showed statistically significant decrease between androgen dependent group and hyperplasia group (P = 0.021). Also, there was a highly significant decrease in androgen independent group as compared to androgen dependent and hyperplasia groups (P = 0.001). It can be concluded that measuring of DHEA-s together with PSA can be used to increase the differentiation between prostate cancer and BPH and controlling of adrenal androgens and gonadotropin hormones (FSH & LH) would be efficient in delaying the transformation of prostate cancer from androgen dependent to androgen-independent.

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Introduction

Prostate gland is a part of the male reproductive system, it has an endocrine activity (Porter and Ben-Josef, 2001). Prostate cancer is a multi hormonal disease (Porter and Ben-Josef, 2001), it is the fourth most common cancer in men worldwide (Srougi and Dzik, 2000). Projected data for year 2000 indicated that the largest number of new cancer would be prostate cancer and the second leading cause of cancer death in men (Greenlee et al., 2000).

The development of prostate cancer is a multistep process involving androgens and other factors (Risbridger et al., 2001). Prostate cancer initiation and progression is dependant on androgens, principally, T and dihydrotetosterone (DHT) (Ross et al., 1986). Thus, medical or surgical castration constitutes the main line of treatment (Carducci, 2003). Hormonal factors from the hypothalamic–pituitary axis and the male sex hormones, play a significant role in growth of prostate tissue and induction of prostate carcinogensis. The natural history of prostate cancer has long been related to the male hormone testosterone and treatment has focused on depletion of this androgen to slow or prevent growth of prostate cancer tissue (Porter and Ben-Josef, 2001). It has become clear, however, that other hormones rather than androgens, may influence the progression of prostate cancer, with recent interest

focusing on follicle-stimulating hormone (Porter and Ben-Josef, 2001).

Unfortunately, prostate cancer patients treated by castration, after initial improvement, eventually developed (androgen-independent) tumor as evidenced by re-rise of prostate specific antigen and clinical deterioration (Carducci, 2003). After castration, serum testosterone decline to 5% of its original level and this remaining testosterone is derived from the adrenal androgen (Schroder, 2002). Despite these adrenal androgens are weak compared with (T) and (DHT), the development of "androgen-independent" tumor may be related to mutations of androgen receptors that render them responsive to weaker androgenic ligands (Fenton et al., 1997; Algarte-Genin et al., 2004). The persistence of DHEA and AD levels may have clinical significance, as these androgens have been shown to activate both wild-type and mutant androgen receptors in vitro (Culig et al., 1996; Tan et al., 1997). Both steroids have only weak androgenic activities but can be converted into the active androgens (T) and (DHT). Androgen-independent prostate cancer cells express genes corresponding to the enzymes responsible for the conversion of adrenal androgens to testosterone (Stanbrough et al., 2006).

Since the establishment of the androgen dependency of prostate cancer, the treatment of advanced disease has been based on the suppression of testicular androgens (Huggins and Hodges, 1941). Unfortunately, this treatment does not cure the patient as a relapse of prostate tumour growth occurs after an initial period of remission (Trachtenberg, 1987). Androgens of adrenal origin, which are unaffected by standard endocrine therapy (surgical or medical castration), might be the cause of tumour recurrence (Huggins and Scott, 1945). The role of adrenal androgens in the regulation of growth of prostate cancer growth has received limited attention, mainly because of complications encountered with surgical or medical adrenalectomy and because of disappointing clinical results (Mahoney and Harrison, 1945, Murray and Pitt, 1985).

The main androgens secreted by the human adrenal glands are (AD) and dehydroepiandrostenedione (DHEA). They account for 5-10% of circulating androgenic activity in adult males (Coffey and Isaacs, 1981). DHEA-s, the sulfated prohormone of (DHEA), circulates at plasma concentrations higher than any other steroid (Orentreich et al., 1984), and its secretion is not dependent on ACTH or angiotensin II (Hornsby, 1995).

A steroid receptor for DHEA has not been identified, and the hormone is generally considered to exert its effects via conversion to steroid metabolites with estrogenic or androgenic activity (Haning et al., 1991a, b). The persistence of (DHEA) and (AD) levels may have clinical significance, as these androgens have been shown to activate both wild-type and mutant androgen receptors (ARs) *in vitro* (Culig et al., 1996; Tan et al., 1997). Both steroids have only weak androgenic activities but can be converted into the active androgens (T) and (DHT). Androgen-independent prostate cancer cells express genes corresponding to the enzymes responsible for the conversion of adrenal androgens to testosterone (Stanbrough et al., 2006).

Holzbeierlein et al., (2004) found over expression of enzymes involved in the synthesis of cholesterol, the common steroid precursor, from acetyl-CoA and Stanbrough et al., (2006) found over expression of enzymes involved in the synthesis of testosterone and the more potent androgen DHT from cholesterol. Whether the *de novo* synthesis of tumoral androgens from cholesterol or earlier precursors occurs to a substantial and/or clinically relevant degree within prostate tumours requires further evaluation (Mostaghel and Nelson, 2008).

Although orchiectomy induces a rapid fall in plasma testosterone, residual levels of DHT have been reported in prostate tumour tissue of hormonally treated patients (Geller et al., 1984; Belanger et al., 1989). AD and DHEA remaining in the circulation after orchiectomy are likely to be responsible for these observed intra-prostatic DHT levels. It is questioned, however, whether these residual low levels of DHT are, actually stimulatory to the prostate cancer (Van Weerden et al., 1990).

The aim of this study is to study the possibility of use the measuring serum level of androgens (testosterone (T) and adrenal androgens (DHEA-s) and (AD)), gonadotropins (Follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) to improve the accuracy of diagnostic serum prostate specific antigen (PSA), and to examine the effect of these parameters in the progression of prostate cancer from androgen dependent to androgen independent after orchiectomy.

Patients & methods:

Seventy six (76) male urologic patients (age range 53 -80 years, mean 66.25 ± 0.74 years with no significant differences between groups) were involved in this study. Blood sample were collected between 9-12 AM from Ain Shams university hospitals, as well as Electricity hospital and Cleopatra hospital, Cairo, Egypt. The study included three groups: Group 1: Comprised 30 patients having benign prostatic hyperplasia (BPH), Group 2: Comprised 31 patients having localized prostate carcinoma (ADPC) and

Group 3: Comprised 15 patients having androgen-independent prostate carcinoma (AIPC). All the samples by approval from the committee of ethics of National Research Center, Cairo, Egypt.

Testosterone was assayed by ELISA kit purchased from **BioCeck, Inc., USA**. (Cat.No: BC-1115). FSH (EIA-1288), LH (EIA-1289), DHEA-s (EIA-1562) and Androstenedione (EIA-3265) were assayed by ELISA kits purchased from **DRG International, Inc., USA.**

Statistical analysis:

Statistical analysis was performed using the SPSS software package for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. Analysis was carried out using a t-test for comparing two variables. P value considered significant when it was < 0.05. Receiver operating characteristic curve (ROC) was constructed by calculating sensitivities and specificities of studied analyses at different cut-off points.

Results:

Hormonal assay: Various hormones affecting on prostate including (FSH, LH, DHEA-s, T, AD) of different groups are illustrated in figures: 1-5.

Figures (1) shows the mean value ± SE of serum FSH in hyperplasia, androgen dependent and androgen independent groups were (12.76 ± 1.24, 18.59 ± 3.33 and 60.75 ± 11.22 mIU/ml) respectively. Serum FSH showed no statistically significant variation between androgen dependent group and hyperplasia group (P = 0.109), while, there was a highly significant increase in androgen independent group as compared to androgen dependent (P = 0.002) and hyperplasia groups (P = 0.001)

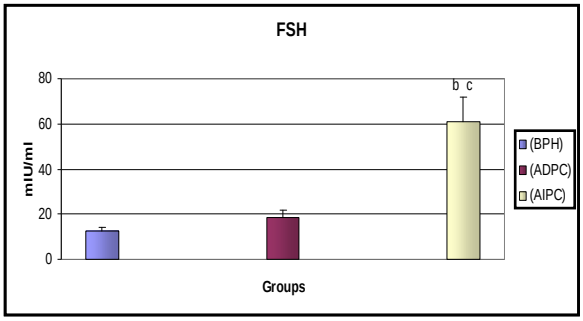

Fig (1): Mean values ± SE of FSH in the different groups

b, significant as compared to hyperplasia group.

c, significant as compared to androgen dependent group

Figures (2) illustrates the mean value ± SE of serum LH in hyperplasia, androgen dependent and androgen independent groups were (8.56 ± 0.62, 18.21 ± 1.2 and 45.27 ± 7.31 mIU/ml) respectively. Serum LH showed statistically significant increase in androgen dependent group(P = 0.001) and androgen independent group (P = 0.001) as compared to hyperplasia group, also, a statistically significant variation was found between androgen dependent and androgen independent groups (P = 0.002).

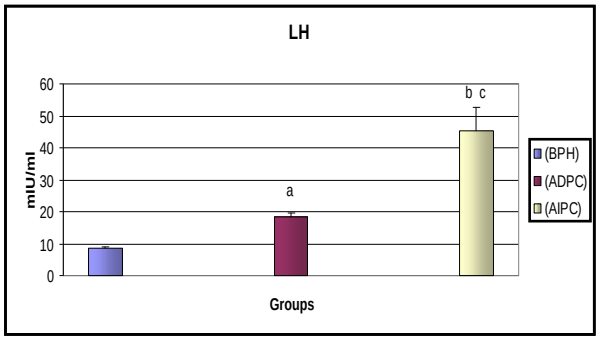

Fig (2): Mean values ± SE of LH in the different groups**.**

a, significant as compared to hyperplasia group.

b, significant as compared to hyperplasia group.

c, significant as compared to androgen dependent group.

Figures (3) reveals the mean value ± SE of serum DHEA-s in hyperplasia, androgen dependent, and androgen independent groups were (0.49 ± 5.25E-2, 0.89 ± 9.59E-2 and 0.71± 0.15 µg/ml) respectively. Serum DHEA-s showed insignificant decrease in hyperplasia group as compared to androgen independent group (P = 0.185) and a significant decrease in hyperplasia group as compared to androgen dependent group (P = 0.001) while, no significant variation was found between androgen dependent and androgen independent groups (P = 0.341).

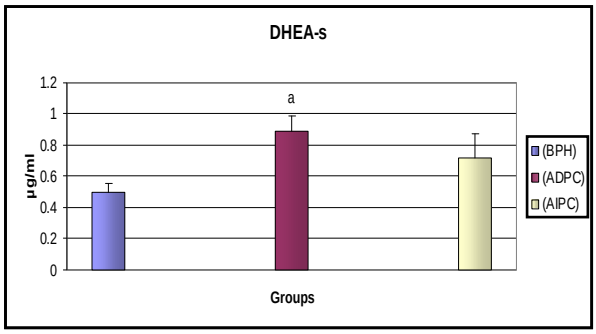

Fig (3): Mean values ± SE of DHEA-s in the different groups.

a, significant as compared to hyperplasia group.

Figures (4) shows the mean value ± SE of serum T in hyperplasia, androgen dependent and androgen independent groups were (4.02 ± 0.24, 2.85 ±

0.43 and 0.37 ± 0.1 ng/ml) respectively. Serum testosterone showed statistically significant decrease between androgen dependent group and hyperplasia group (P = 0.021). Also, there was a highly significant decrease in androgen independent group as compared to androgen dependent and hyperplasia groups (P = 0.001).

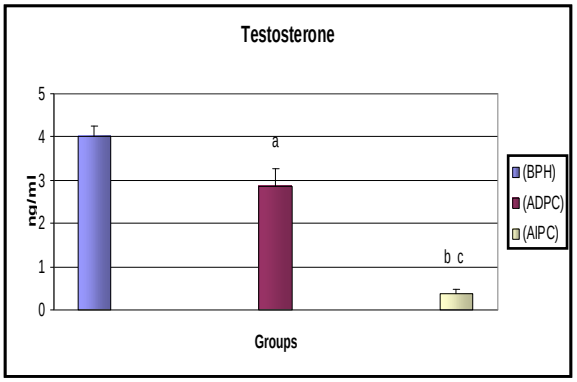

Fig (4): Mean values ± SE of testosterone in the different groups.

a, significant as compared to hyperplasia group.

b, significant as compared to hyperplasia group.

c, significant as compared to androgen dependent group

Figures (5) shows The mean value ± SE of serum (AD) in hyperplasia, androgen dependent and androgen independent groups were (0.74 ± 0.10, 1.20 ± 0.13, 0.86 ± 0.16 ng/ml) respectively. Serum (AD) showed significant increase in androgen dependent group as compared to hyperplasia group (P = 0.008). While, there was no significant variation between androgen dependent and androgen independent groups (P = 0.122) and between androgen independent group and hyperplasia groups (P = 0.538).

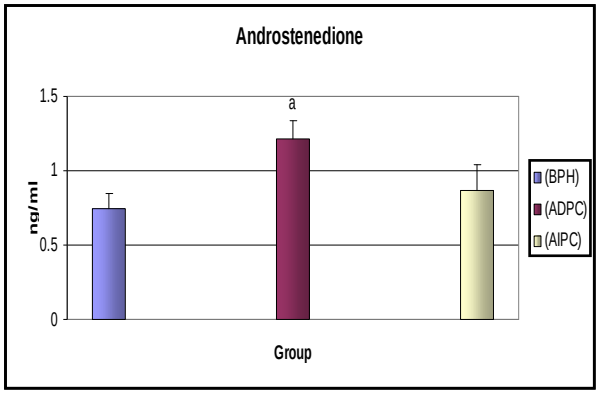

Fig (5): Mean values ± SE of androstenedione in the different groups.

a, significant as compared to hyperplasia.

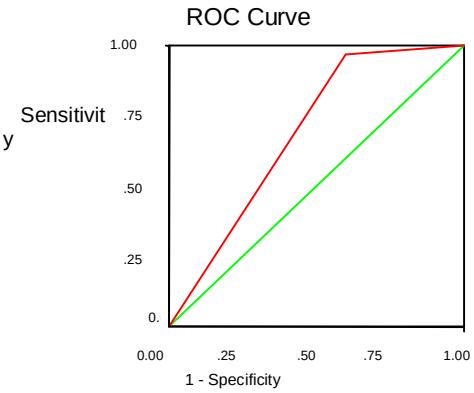

Fig (6): ROC curve for combination PSA and DHEA-s.

Discussion

Prostate cancer is the most common malignancy among U.S. men, a 192,280 new cases was detected in 2009 which represented 25% of all new cancer cases (Jemal et al., 2009). In Egypt the GLOBOCAN 2002 database (compiled by Ferlay et al. for the International Agency for Research on Cancer) estimated the number of new cases per year to be 867 cases (www.emro.who.int/ncd/cancer_globocan.htm). In the period 2002-03 the Egyptian National Cancer Institute at Cairo University reported seeing 238 new cases of prostate cancer out of a total of 9,340 new cancer cases in males (2.6 %). (www.nci.cu.eg)

Chances of developing cancer in men and women have a 35% chance with increasing of age (American Cancer Society 2008). In Egypt aged population represent about 6% of over all population (age 60 years and more) in 2006, it is expected that this percent will be about 9% and 15% in 2015 and 2030 respectively. Between 1986 and 2006 the number of aged populations increased form 2.7 millions in 1986 to 4.4 millions in 2006 (www.idsc.gov.eg/Documents/studies). In spite of the low incidence of prostate cancer in Egypt the increase of the aging population, makes that prostate cancer will become ever more an enormous challenge.

Androgen deprivation is the main treatment of prostate cancer, after initial recovery tumour become not respond to hormonal treatments, in what is referred to as androgen-independent prostate cancer. The mechanisms leading to androgen-independent growth of prostate cancer are complex but are likely to involve

continued androgen receptor (AR) signaling in the face of castrate levels of testosterone. A variety of mechanisms may be responsible for this phenomenon, including activation of the AR by adrenal androgens or exogenous growth factors, amplification of and mutations within the AR itself (Culig et al., 1993; Feldman and Feldman 2001). Although androgen deprivation therapy typically reduces serum testosterone levels by >95%, there is not a proportional reduction in adrenal androgens, such as AD, DHEA-s and DHEA (Labrie et al., 1993).

In our study, serum DHEA-s level in the androgen dependent prostate cancer group was significantly higher as compared to benign hyperplasia group (P = 0.001). In contrast to our data a study by Stahl and colleagues (1992), reported that serum DHEA level was significantly lower in prostate cancer patients than in those without prostate cancer. Comstock et al. (1993) mentioned that, low serum DHEA-s levels were detected in 11% and 12% of the patients in the prostate cancer group and hyperplasia group, respectively, with no significant difference between the groups. Thus they concluded that serum DHEA and DHEA-s levels were not a risk factor for prostate cancer. On the other hand, Eaton et al. (1999) evaluated the changes in the sex hormone values of patients with prostate cancer and normal men in a series of eight prospective epidemiological studies, and they found no difference between the serum DHEA-s levels in the two groups. In another study, no significant difference was found in terms of serum DHEA-s levels between patients with prostate cancer and benign disease (Schatzl et al., 2000).

Spitz (2009) mentioned that, in a case report of a patient with prostate cancer who failed to demonstrate consistent testosterone suppression to castration levels and incomplete suppression of serum PSA, although treated with gonadotropin releasing hormone agonists for 48 months. Serum DHEA, DHEA-s, as well as the androgen metabolite (androsterone glucuronide) was elevated in prostate cancer compared to the other patients. He suggested that those prostate cancer patients who have even marginally elevated adrenal androgens may especially benefit from combined androgen blockade.

In our knowledge, no previous studies have been indicated that the level of gonadotropin hormones (FSH and LH) and level of testosterone are associated with DHEA-s level in prostate cancer patients.

Our results revealed that serum LH had statistically significant increase in androgen dependent group (P = 0.001) and androgen independent group (P = 0.001) as compared to hyperplasia group, also, there was a statistically significant variation was found between androgen dependent and androgen independent groups (P= 0.002). Many authors reported that human adrenal cortex expresses low levels of luteinizing hormone/chorionic gonadotropin receptors (LH/CGR). LH/CGR levels increase in the adrenal cortex after exposure to chronically elevated gonadotropins (e.g. after gonadectomy). In fact, heightened ectopic LH/CGR levels are observed in a subclass of human adrenocortical tumours and gonadotropin responsive adrenocortical hyperplasia and tumours occur in several animal species. These findings suggest that adrenocortical responsiveness to (LH)/ (CG) might be a physiological phenomenon that is amplified in the presence of elevated gonadotropin levels. Experimental and clinical evidence showed that the expression of (LH/CGR) in the adrenal cortex, and that chronically elevated LH/CG levels might stimulate growth and steroidogenesis of the adrenal cortex. Accordingly, several gonad specific genes are expressed including Steroidogenic factor 1 (SF-1) and CYP17 (Bielinska et al., 2003; Bielinska et al., 2005; Bernichtein et al., 2008).

In steroid producing tissues, SF-1 activates the genes encoding the cytochrome P450 steroid hydroxylases (CYPs) and 3β-steroid dehydrogenase (3β-HSD) that together convert cholesterol to steroid hormones (Parker and Schimmer, 1997). SF-1 also targets genes encoding factors involved in cholesterol transport such as steroidogenic acute regulatory protein (StAR) (Sugawara et al., 1996; Caron et al., 1997).

Androgen receptors have been demonstrated in human adrenal glands and the inhibitory effects of testosterone could be androgen receptor mediated (Rossi et al., 1998). Testosterone may inhibit testicular DHEA production either directly or by suppression of LH. However, plasma DHEA and DHEA-s concentrations are determined almost exclusively by the adrenals, with minor contribution from the gonads (about 10%). Thus, testosterone effects are most likely a result of its suppressive effects on adrenal DHEA production (Muniyappa et al., 2006). In our results there was a lower testosterone level in cancer group than those of benign hyperplasia group which may explain the higher DHEA-s in cancer group than benign hyperplasia.

Thus, it can be explained that high serum LH level and low serum testosterone may act together to induce the increase in serum level of adrenal androgen (DHEA-s and androstenedione) in cancer group than that of benign prostate hyperplasia group.

In our results, there was a non significant value in serum level of DHEA-s and androstenedione level between androgen dependent group and androgen independent group which may be contributed to the elimination of the inhibitory effect of testosterone on 3β-HDS which make it competes with CYP17 for the same substrates (pregnenolone and 17-hydroxypregnenolone) (Stalvey, 2002; Swart et al.,

2002), or it may be due to increase of prostate uptake of circulating DHEA-s and androstenedione since intra-prostatic adrenal androgens have been detected at significant levels in patients treated to suppress testosterone (Belanger et al., 1989; Mizokami et al., 2004; Mohler et al., 2004;). Levels of DHEA, DHEA-s and androstenedione were decreased by about 50% in tumour tissue from castrate patients with recurrent prostate cancer, and far exceeded the values of testosterone and DHT in the recurrent tumour tissue (Mostaghel and Nelson, 2008). Since castration-resistant metastatic tumours increased the expression of androgen-metabolizing genes (Holzbeierlein et al., 2004; Stanbrough et al., 2006; Montgomery et al., 2007) these strongly suggests that up-regulated activity of endogenous steroidogenic pathways is driving the outgrowth of 'castration-adapted' tumours, since these androgens can be converted enzymatically within the prostate to the more potent testosterone and DHT (Mostaghel and Nelson, 2008).

From our results it has been hypothesized that adrenal steroids may act a role in prostate cancer and progression to androgen independent cancer after castration.

An important result in this study revealed that , there was a highly significant increase in serum FSH level in androgen independent group as compared to androgen dependent (P = 0.002) and hyperplasia groups (P = 0.001). Increase in serum FSH level following chemical or surgical orchiectomy derives from research on the systemic control of FSH secretion. Testosterone reduces FSH production by decreasing hypothalamic secretion of gonadotropin-releasing hormone (GnRH), which, in turn, inhibits pituitary production and release of LH and FSH (Gleave et al., 1999).

FSH and LH as well as other associated hormones were physiologically regulated by more than just estrogen and androgen levels. Subsequent research identified the prostate as an extra-pituitary source of FSH.

Porter and Ben-Josef (2001) reported that FSH is present in the normal prostate, in BPH, and in prostate cancer. Thus, the old concept of FSH as a pituitary-only hormone, which was previously altered to include its synthesis in the testis and gastro-intestinal tract, now must include the prostate as well. In the development of prostate diseases, therefore, tissue concentrations of FSH rather than plasma concentrations may be more important since *de novo* biosynthesis takes place in the prostate.

Porter and Ben-Josef (2001) have been shown that the prostate contains FSH receptors. Thus, although higher serum FSH levels have been reported in BPH and in carcinoma, its production in the prostate itself might suggest that tissue-specific FSH, especially prostate-specific levels, may be more important. Identification of the FSH receptor in androgen-independent metastatic prostate cancer cell lines and in human malignant prostate tissues, together with evidence that FSH can stimulate proliferation of these cells, suggests that the FSH receptor and its ligand may play a significant role in the growth of androgen independent prostate cancer.

The adrenal precursor, DHEA is converted into testosterone and then DHT by the pathway, either in the plasma or in the prostate itself, as the prostate is able to take up and metabolize adrenal androgens directly (Harper et al., 1974). After castration (medical or surgical), plasma testosterone levels fall to one tenth of normal, but the amount of DHT detectable in prostatic tissue may be 40-50% of normal (Geller et al., 1984). A number of mechanisms have been identified that may contribute to the progression of prostate cancer from androgen sensitive to androgen refractory including the utilization of adrenal androgen by the prostate.

In this study there was statistically significant decrease in serum testosterone level between androgen dependent group and hyperplasia group (P = 0.021). Also, there was a highly significant decrease in androgen independent group as compared to androgen dependent and hyperplasia groups (P = 0.001).

In agreement with our results, Hoffman et al. (2000); Massengill et al. (2003) reported low pre-treatment free and total testosterone levels seem to predict more aggressive disease and metastases. The observation of very low levels of circulating testosterone after castration in prostate cancer has been also reported by Labrie et al. (2009).

Many reports also mentioned that high-grade prostate cancer is associated with low levels of serum testosterone (Miller et al., 1998; Schatzl et al., 2001; Morgentaler, 2007). The reason for this phenomenon is still not clear (Lackner et al., 2008). It has been suggested that a low serum testosterone level could be viewed a potential marker of occult prostate cancer, and it is speculated that testosterone may play a permissive or an inductive role in neoplastic diseases of the prostate, and that DHT may hold a key to understanding how to control and manage some of the neoplastic changes associated with prostatic disease. The presence of DHT and its binding to androgen receptors can directly up-regulate expression of prostate- specific differentiation markers such as PSA (Basaria, 1999; Morales, 2002; Isaacs, 2004; Crawford, 2005).

There is a pressing need for another marker that can be used in conjunction with PSA (Thakur et al., 2004). In our results, at cut-off value of PSA as 4.0 ng/ml and DHEA-s at cut-off value of DHEA-s > 0.8 µg/ml, combination of PSA and DHEA-s increase the

sensitivity of PSA in differentiation between prostate cancer and BPH to 96.8% with specificity 40% and area under the curve equal 0.68. (Fig 6).

From our data it can be concluded that measuring of DHEA-s and PSA can be used as good predictor for the differentiation between prostate cancer and benign prostate hyperplasia.

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