Research Article

Studies on the Occurrence of Prostate Cancer in Gujrat, Punjab, Pakistan and its Association with Testosterone Level

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Abstract | The purpose of the present study is to detect the association between the levels of testosterone with prostate cancer patients by the evaluation of a tumor marker Prostate-specific antigen (PSA). Prevalence was recorded in Gujrat, Punjab, Pakistan. Blood samples of normal and prostate cancer males were taken from Gujrat, Punjab, Pakistan. Information including age, marital status, smoking, Prostate-specific antigen (PSA), testosterone, and any previous record was obtained with the patient's consent. Exclusion and inclusion criteria were defined. The samples of control and patients were analyzed through ELISA protocol to check the testosterone and Prostate-specific antigen (PSA). Testosterone significantly affects the prostate-specific antigen (PSA) with ($P<0.05$). In the cases, the mean testosterone, age, PSA was 38.45±36.66, 66.28±6.386 and 9.655±6.656, respectively while in the control group mean testosterone, age and PSA were 194.1±84.08, 59.35±6.794 and 1.295±0.809 respectively. The present study concludes testosterone levels as negatively affected by prostate specific antigen (PSA). The prevalence of Prostate cancer recorded in Gujrat, Punjab, Pakistan was 2%. Our study may prove feasible for the detection of prostate cancer in aged males. Testosterone replacement therapy may prove to be effective in retrieving the complications induced by prostate cancer.

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Introduction

Prostate cancer that is the second prevalent cancer worldwide and major cause of mortality in men (Lee *et al*., 2023). This type of cancer frequently develops in the periphery of a prostate gland; putting pressure on the urethra (Guess, 2001). The prostate gland is a tube-like alveolar gland present between the bladder neck and pelvic (Miller and Torkko, 2001).

The prostate is an exocrine gland, which is a part of the semen gland that assists in semen ejaculation (Oyelekan *et al*., 2020). A clinical marker is used to detect prostate cancer in which neural serine protease encodes for 15 genes (Diamandis, 2000).

This prostate specific antigen did not exist in one place and it is released into the glandular lumen from the cells of epithelium (Balk *et al*., 2003). Certain binding proteins of the prostate release IGF-I has a direct association between IGF and PCa (Kingshott *et al*., 2021).

Androgen induce the production of prostate-specific antigen and age-related reproductive decline leads to balanced redox state into oxidation conditions (Leisegang *et al*., 2017).

Prostate cancer has common symptoms like obstruction of the urinary tract, enlarged prostate gland, nocturia (Hamilton and Sharp, 2004). Hypertension and waist circumference are the major factors that cause this infection to spread (Esposito *et al*., 2013).

Prostate cancer has involved certain other health risks related diseases in human beings with cardiovascular diseases (Gupta *et al*., 2018). Prostate cancer could be prevented by a greater intake of vegetables and fruits (Riboli, 2001).

In a specific clinical strategy, androgen replacement therapy is used to treat prostate cancer in its advanced stages (Rove and Crawford, 2014). External beam radiation therapies are the most important treatment in localized prostate cancer (Moon *et al*., 2006). Prevalence was found to be 14.8% at age of 40 years on a global study while 36.8% are in the age of 80 or above (Lee *et al*., 2017).

Prevalence of deficiency of testosterone in men was reported 9.0% in the age of 45-54 years and 16.5% in the age of 55-64 years and 18.3% in 65-74 years age (Moskovic *et al*., 2013). Whereas 100 common loci are participating in prostate cancer with a risk factor of 38.9% (Eeles *et al*., 2013).

Chances of appearance of the prostate disease have increased from 55 to onwards effectively (Bashir, 2015). Study is conducted to check that a decrease in serum-free testosterone is an indicator for the severe disease (Hoffman *et al*., 2000).

This study may help in future hormonal therapies to treat prostate cancer patients.

Materials and Methods

Prostate cancer patients were chosen along all symptoms of this disease. A written consent was gathered from the affected person participating in the scenario after discussing with them. Different parameters of the patients like the name, age, gender, smokers and non-smokers, occupation, marital status, treated or untreated and previous history of disease were recorded. All males were residents of Gujrat city, Punjab, Pakistan. This study was approved by the Institutional Committee of Ethical practice of the University of Gujrat.

Blood sampling

The blood sampling was done at civil hospital Gujrat, Punjab, Pakistan. Sample collection was done from January 2021 to June 2021. The blood samples were collected from prostate cancer patients (n=60) and normal males (n=60) to analyze the level of testosterone during prostate cancer. At a time of collection of blood, patient was in a sitting position. The sampling area was cleaned by alcohol dipped cotton swab.

The patient's erect arm was tourniqueted for a minute before the vein (median cubital vein) from which blood was drawn was found. The syringe was injected at an angle of 15 degrees and tourniquet was removed when the blood started flowing. A blood sample (5ml) was collected in the syringe, and then it was stored in two types of vials: EDTA vials in which 25 ml of the total blood was added, which was used for a testosterone test.

The second vial was Gel and clot-activator vial in which 2.50-3.50ml blood was added to perform fasting blood prostate specific antigen (PSA) test. The vials containing the blood samples were labeled correctly for the details which include patient name, phone number MR (Medical Record) number, date, and time of blood collection.

Then after 30 minutes of the blood collection, centrifugation of the clotted vials at 3000-3500 rpm was performed. On the other hand, the EDTA vials were rotated in a pathological lab while being shaken 5 to 10 times.

Vials centrifugation was used to separate blood serum from the blood cells and proteins. Then these vials were stored at 2-8 °C in the refrigerator and after this, they were transported for laboratory testing in the Pathological lab of civil hospital, Gujrat.

Blood tests

Blood samples were transferred into the laboratory to perform tests such as testosterone and prostate specific antigen (PSA) with fasting morning serum samples. To check the level of testosterone the Micro plate Enzyme Immunoassay technique, ELISA testosterone test kits (Catalogue no KAPD-1559).

Free prostate specific antigen (PSA) ELISA kit (Catalogue no KAPD-0209) was used, in which different reagents of Mark chemical company determined the concentration of testosterone and recorded light units at the end.

Assay for testosterone test

I formatted the micro plate wells of both groups thoroughly and secured the necessary micro titer wells in the holder for each serum reference. With the aid of a pipette, 10 mL of serum reference containing specimen and control was added to the designated wells, along with 50 mL of ready-to-use testosterone enzyme reagents.

Micro plates were gently shaken out of the well's contents to mix the reagents through the process of aspiration or decantation, and if their decantation step was followed, the plate was blotted dry with absorbent after being gently swirled to mix all the reagents for 20–30 seconds. These plates were then incubated at (18–25 °C).

Wash buffer of about 350µL was added then blot, tap or aspirate then manual or automatic plate washer was used. 100µL of working substrate solution was added to each well, reagents were always added in a similar order to lessen the time differences of reactions between all wells. Incubation was done for fifteen minutes all the plates and 100µL of stop solution were absorbed into all wells and for 15-20 seconds mixed gently.

And after this read the absorbance of every well at 450nm by using the reference range of wavelength 620-630nm which was used to reduce the imperfection with the use of a micro titer well reader after completion of the whole reaction (As per described in the Kit's manual).

Assay for PSA test

All coated wells were in desired amount placed in suitable holder. Placed 100ml sample, standards or control inside suitable wells, 100ml sample diluent was dropped into every well, gently stir for 10 seconds and mixing had done very carefully in this step then incubated for 60 minutes at 37°C. Micro titer wells were cleaned and with washing buffer these were emptied five times.

All residual water droplets were removed by throwing the well onto absorbent paper. 200ml of enzyme conjugate reagent was dispersed into each well, they were mixed for 5 seconds with incubation for 60 minutes at 37 °C. Into suitable waste container mixture was removed.

Then 100ml TMB solution was poured into each well and incubated for 20 minutes in the dark at room temperature, reaction was stopped by the addition of 100ml of stop solution into each well then gently mixed to ensure that the blue color was altered into yellow color. Optical density was read at 450nm by using a micro titer plate reader after reaction has been completed.

Data analysis

All the mean standard values were calculated by using SPSS (Statistical Packages for Social Science) version 21.0. And Standard Error Mean (±S.E.M) was also calculated. Variations in control and treatment groups were checked by using un-paired *t*-test. Pearson's find out the association between variables. The P value < 0.05 was found statistically significant.

Results and Discussion

A curve plotted using the absorbance values determined from each reference standard over vertical or Y-axis and the concentration in ng/ml on the horizontal or X-axis of graph paper. PSA concentration was calculated by using a micro plate reader (EMP CR-201-Chem Plate Reader).

Calculations

Testosterone concentration was highly affected in prostate cancer patients in old age males that may contribute to abnormal semen production, muscle weakness. Other parameters like PSA and testosterone were also analyzed in this study.

There was a significant difference in the testosterone of the normal and diseased males. Demographic studies showed a total of 120 cases out of which 60

were normal subjects. All were selected with the same demographic profile. All parameters like testosterone, PSA, age, and smoking were highly variable.

In the cases, the mean testosterone, age, PSA was 38.45±36.66, 66.28±6.386 and 9.655±6.656, respectively while in the control group mean testosterone, age and PSA were 194.1±84.08, 59.35±6.794 and 1.295±0.809, respectively as illustrated in Table 1.

Table 1: *Comparison of means in control and cases.*

	Mean	SD
Control		
Age	59.35	±6.794
PSA	1.295	±0.809
Testosterone	194.1	± 84.08
Cases		
Age	66.28	±6.381
PSA	9.655	±6.656
Testosterone	38.45	± 36.66

The testosterone mean levels of normal were higher than prostate cancer patient i.e. mean±SEM 194.1±10.85 and 38.45±4.733. The PSA levels of normal were comparatively low than prostate cancer patients i.e. mean ±S E M 1.29±0.10 and 9.655±0.859 and the difference of means was 8.36±0.754 as indicated in Table 1.

And the prevalence calculated in the Gujrat area was only 2% according to our sample size. R-squared value calculated was 0.22 as indicated in Tables 2 and 3 with significant p-value 0.00.

Table 2: *Comparison in testosterone levels of cases and control by unpaired t-test T-test and levene test for equality of means and variances.*

t-value	13.149
P-value	0.000
Mean difference	155.714
S.E.M difference	11.842
F-value	23.353
95% CI	179.1
R-Squared	0.22

The level of testosterone in control group was significantly higher as compared to treatment group as illustrated in Figure 1. There was a higher level of Prostate specific antigen in non-smokers as compared to smokers as illustrated in Figure 2.

Table 3: *Comparison between PSA levels of Cases and Control by unpaired t-test T-test and levene test for equality of means and variances.*

t-value	9.657
P-value	0.000
Mean difference	8.3600
S.E.M difference	0.8657
F-value	42.480
95% CI	6.645
R-Squared	0.22

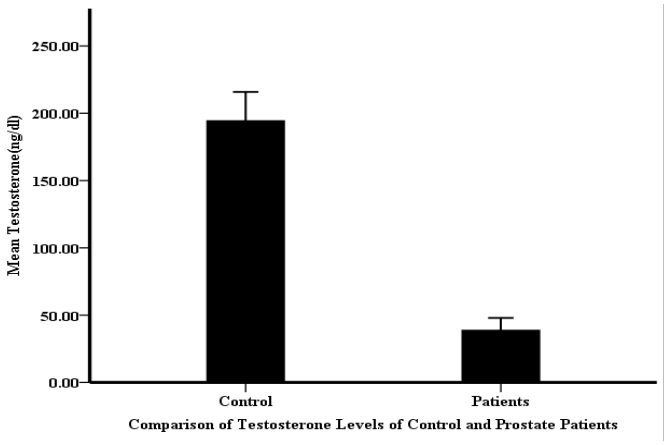

Figure 1: *Testosterone levels of control and patients.*

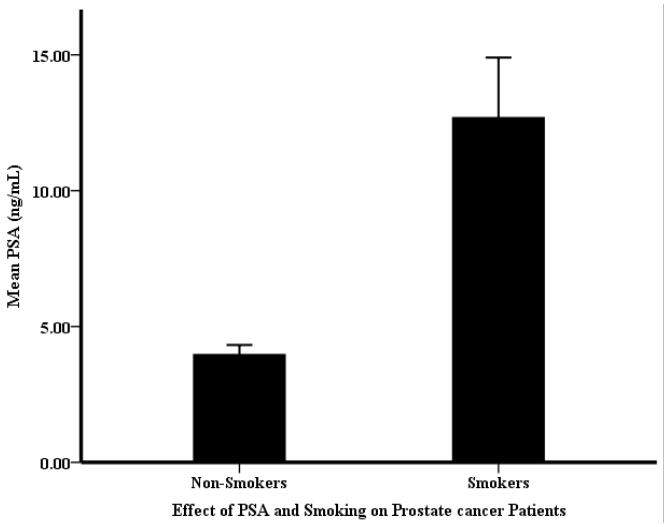

Figure 2: *Effect of smoking on PSA levels.*

The level of prostate specific antigen (PSA) in treatment group was significantly higher as compared to control group as illustrated in Figure 3.

In another study, it has revealed that patients with a decreased testosterone showed higher risk of prostate cancer spreading our results are in accordance with

these research findings (Shin *et al.*, 2010).

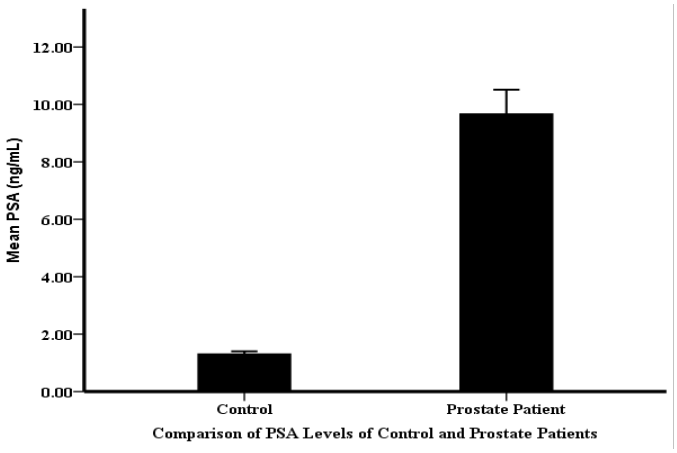

Figure 3: *Antigen levels in control and patients.*

The level of PSA was higher in males of old age group of prostate cancer as compared to normal case as indicated in Figure 3. Furthermore, the results of our study were confirmed by the (Kim *et al.*, 2020) that explained higher level of PSA was seen in prostate patients as ($P<0.05$). Higher level of PSA can result in prostate gland cells tumors and complications during hypogonadisom as ($P<0.05$).

The level of PSA was higher in prostate males of old age group with smoking as compared to normal non-smoker males as indicated in Figure 2. Furthermore, the results of our study were confirmed by the (Tyagi *et al.*, 2010) that explained higher level of PSA was seen in smokers prostate cancer patients as ($P<0.05$).

Our results resemble the other findings that a positive association exists between the older age person and the attack of a tumor. In our study relation of hormone and antigen, PSA was also checked in control and patients with respect to the age (Bostwick *et al.*, 2004).

Our results matched with another experiment in which higher serum PSA level acted as an indicator for severe prostate tumors and level of PSA was increased in patients as compared to the testosterone and positive association was found between these two groups (Catalona *et al.*, 1991).

Conclusions and Recommendations

The impact of testosterone on prostate cancer was evaluated in which decreased level of testosterone has significantly enhanced the risk of prostate cancer and study reported enhanced values of prostate-specific antigen (PSA) as testosterone level decreases from the normal value in prostate cancer patients as compared to control group.

During the age of 60-85 a decreased level of testosterone is present that may affect the prostate gland of males. We analyzed the association of testosterone with prostate cancer in males and its prevalence in Gujrat.

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Novelty Statement

The present study is new in every aspect and would be beneficial for men in improving health.

Author's Contribution

S.R. and S.S. conceived of the presented idea. S.S. and K.N. developed the theory and performed the Experimentation. R.A.M., K. S. and S.I. verified the analytical methods. S.R. encouraged K.N. and M.I. to investigate the Occurrence of Prostate Cancer in Gujrat, Punjab, Pakistan and its Association with Testosterone Level and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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