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A Study to Evaluate the Role of Dihydrotestosterone in the Pathogenesis of Benign Hyperplasia of Prostate and Prostatic Carcinoma in Patients Attending a Tertiary Care Hospital in Eastern India

Authors
Atrayee Roy Choudhury[1], Sharmistha Chatterjee[2], Mousumi Mukhopadhyay[3], Biswajit Majumder[4]
[1]Consultant Biochemist, Apollo Clinic, Newtown, Kolkata
[2]Assistant Professor, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, Kamarhati, Kolkata.
[3]Professor and Head, Department of Biochemistry, Institute of Post-graduate Medical Education and Research, Kolkata
[4]Associate Professor, Department of Cardiology, ICVS, R.G. Kar Medical College, Kolkata
Corresponding Author
Sharmistha Chatterjee
Assistant Professor, Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, Kamarhati, Kolkata
Ph No. 9830477927, Email: *sharmisthacmajumder@yahoo.co.in*

Abstract
Benign hyperplasia of prostate and prostatic carcinoma are two important diseases of aging male with very high incidence rate. Androgens have been implicated in the pathogenesis of both BHP and Ca prostate. Dihydrotestosterone (the 5-α reduced derivative of Testosterone) is the major metabolite of testosterone in prostate. So DHT is thought to play an important role in the development and progression of both the diseases. The present study was aimed at assessing the inter-relationship between the tissue level of 5α-dihydrotestosterone and pathogenesis of benign hyperplasia of prostate and prostatic carcinoma. The tissue level of DHT has been estimated in transition zone and peripheral zone of prostate in BHP and Ca prostate patients the comparative analysis of the above parameters suggests that, 5α-DHT is implicated in the pathogenesis of BHP and Ca prostate in a zone-wise manner. The findings of the present study also reveal that, prostatic tissue level of 5α-DHT may be explored as a possible marker for the diagnosis of benign hyperplasia of prostate and prostatic carcinoma.
Keywords: *Benign hyperplasia of prostate, prostatic carcinoma, Dihydrotestosterone, transition zone, peripheral zone of prostate.*

Introduction

Aging is the progressive, universal decline in functional reserve that varies widely in different individuals and in different organs within a particular individual. Aging is not a disease; however, the risk of developing disease is increased, often dramatically, as a function of age. One important organ, showing such changes with

aging and consequently contributing to significant amount of morbidity and mortality, is the prostate gland. Benign as well as malignant changes in the prostate increase with age. Prostate cancer is the most common cancer in men and the second most common cause of cancer-related death in the United States. From 2006-2010, the median age at diagnosis for cancer of the prostate was 66 years and the median age at death for cancer of the prostate was 80 years [1,2].

The development of benign prostatic hyperplasia an almost universal phenomenon in ageing men[3]. It commences in the fifth decade as a secondary growth spurt, which begins characteristically in the periurethral region (transition zone) as a localized proliferation involving fibronodular and glandular elements. There is considerable evidence that the second spurt, like the growth of the prostrate during puberty, requires a functioning testis and it has been hypothesized that some form of endocrine imbalance in the ageing male might trigger this process[4,5]. On the other hand, prostatic carcinoma, occurring typically in men above the age of 50, predominates in the peripheral zone of prostate . As with BHP, androgens are hypothesised to play a role in the pathogenesis of prostatic carcinoma also [6,7, 8, 9, 10].

In the past, serum hormone levels have been studied in both these conditions [11,12,13] to validate the primary role of androgens in the origin or promotion of these diseases yielding various results [14,15,16]. In the present study, however, the tissue level of the major metabolite of testosterone in prostate, namely, DHT (the 5-α reduced derivative of Testosterone) [17], was measured in a zone wise manner (transition and peripheral zone) in both BHP and carcinoma prostate patients and their co-relation with the serum level of testosterone was studied in the respective patients

Materials and Methods

This observational Cross Sectional study was carried out among the patients attending the outdoor patients department in the Department of Urology, I.P.G.M.E. & R., S.S.K.M. Hospital, Kolkata. Only clinically diagnosed and histologically confirmed patients of BHP and Prostatic Carcinoma were taken as cases in the present study. Patients of Benign Hyperplasia of prostate and Carcinoma of the prostate in the age group of 55 to 85 years were included as cases in the study. Early cases of carcinoma prostate were selected before the initiation of anti-cancer therapy. Patients of BHP and Ca Prostate not proven histologically or already operated or receiving anti-cancer therapy were excluded from the study. The cases were divided into two groups, one-those suffering from BHP and another those with prostatic carcinoma.

After clinical examination, the patients were asked to answer a detailed questionnaire and informed consent was taken from them. Radiological reports (chest X-Ray and ultrasonography of abdomen) are collected from all the patients. Fasting morning blood samples were collected from all the patients in fluoride, clot vials and EDTA vials with proper aseptic precautions and transported with care to prevent haemolysis. A 2 hours post prandial blood sample was also collected in the same way in fluoride vial. After separation of the serum by centrifugation, the serum levels of Testosterone were measured by ELISA. The serum levels of FBS, PPBS, urea, creatinine, total protein, albumin, globulin and SGPT were estimated using auto-analyser. All these estimations were done in the Biochemistry department in I.P.G.M.E.R.

Samples from the peripheral zone of prostate were collected by true cut biopsy, and samples from the transition zone were collected following transurethral resection of prostate (TURP). These Surgical prostate specimens obtained by either transurethral resection (TURP) or ultrasound guided true cut biopsy of prostate were first cleaned of excess blood, charred remnants, connective and adipose tissue, weighed and stored in parafilm at -20°C until extracted. 10 mg sample of the minced tissue was mixed with 500 µl of 50 mMol phosphate buffer (pH 7.4) and

homogenised on ice with mortar and pestle till a homogeneous solution was obtained. 500 µl of homogenate was then mixed with 500 µl of 1 N NaOH and kept in 60˚C for 1 hour in order to dissolve the tissue [18]. After centrifugation of the solution at 2000g for 10 minutes, the resulting supernatant was collected in a 1.5ml eppendrof and further centrifuged at 5000g for 15 minutes. This supernatant was then collected in a 1.5ml eppendorf and stored at -20°C in a chiller tray till estimation of DHT. The tissue preparation was thawed to 23°C in an incubator before using it for ELISA.

The DHT in the prostatic tissue was estimated by solid phase enzyme-linked immunosorbent assay (ELISA) by Demeditec Diagnostics GmbH, Lise-Meitner-Straβe2, D-24145 Kiel (Germany) diagnostic kits using a TECAN ELISA reader.

The numerical variables were analysed and the mean, median and the Interquartile Range were calculated. Association between tissue levels of DHT and serum Testosterone levels were explored through correlation analysis. Pearson's Correlation Coefficient was calculated as both the variables were normally distributed. The tissue level of DHT is compared between groups by the Student's Unpaired T Test. 95% Confidence Interval (CI) of the difference was calculated. The analysis was two tailed and $p<0.05$ was considered as statistically significant.

Results and Analysis

Numerical variables have been summarized as Mean, Standard Error of Mean and Standard Deviation. The Median and Interquartile Range have also been calculated. Association between tissue levels of DHT and serum Testosterone levels have been explored through correlation analysis. Pearson's Correlation Coefficient is calculated as both variables are normally distributed. Similarly, correlation analysis has also been employed to explore association between tissue levels of DHT and serum PSA levels. Here also Pearson's Correlation Coefficient is calculated as both variables are normally distributed. Tissue level of DHT is compared between groups by Student's Unpaired T Test. 95% Confidence Interval (CI) of difference has been calculated. Analysis is two tailed and $p<0.05$ is considered as statistically significant.

Table 1: Comparison of 5α DHT level in prostate tissue between transition zone (TZ)and peripheral zone (PZ)of prostate in BHP patients:

	TZ BHP (ng/mg wet tissue)	PZ BHP (ng/mg wet tissue)
Minimum	4.780	0.5600
25% Percentile	4.990	0.573
Median	5.395	0.650
75% Percentile	6.035	0.718
Maximum	6.840	0.790
Mean	5.533	0.651
Std. Deviation	0.645	0.083
Std. Error of Mean	0.132	0.029
Upper 95% CI	5.806	0.721
Lower 95% CI	5.261	0.582
P value (two tailed)	P<0.0001	
Difference between means	4.882 ± 0.2310	
95% confidence interval	4.247 to 5.517	

As shown in this table, the prostatic tissue level of DHT is much higher in transition zone (5.533 ± 0.645 pg/mg of wet tissue) compared to peripheral zone (0.6513 ± 0.083 pg/mg of wet tissue). Tissue level of DHT is calculated to be approximately 8.5 folds higher in transition zone than peripheral zone of prostate in benign hyperplasia of prostate ($P<0.0001$).

Table 2: Comparison of 5α DHT level in prostate tissue between transition zone and peripheral zone of prostate in Ca prostate patients

	TZ Ca (pg/mg wet tissue)	PZ Ca (pg/mg wet tissue)
Minimum	1.830	7.160
25% Percentile	1.955	8.565
Median	2.160	8.900
75% Percentile	2.310	9.208
Maximum	2.380	9.980
Mean	2.127	8.869
Std. Deviation	0.191	0.634
Std. Error of Mean	0.053	0.142
Upper 95% CI	2.242	9.166
Lower 95% CI	2.011	8.572
	P<0.0001	
P value summary		
Difference between means	-6.742 ± 0.1819	
95% confidence interval	-7.133 to -6.371	

As shown in this table, the prostatic tissue level of DHT is much higher in peripheral zone (8.869 ± 0.191 pg/mg of wet tissue) compared to transition zone (2.127 ± 0.634 pg/mg of wet tissue). Therefore, tissue level of DHT is approximately 4.17 folds higher in peripheral zone than transition zone of prostate in case of prostatic carcinoma (P<0.0001).

Table 3: Comparison of the prostatic tissue levels of 5α DHT in transition zone (TZ) of prostate in patients of BHP and Ca prostate

	TZ BHP (pg/mg wet tissue)	TZ Ca prostate (pg/mg wet tissue)
Minimum	4.780	1.830
25% Percentile	4.990	1.955
Median	5.395	2.160
75% Percentile	6.035	2.310
Maximum	6.840	2.380
Mean	5.533	2.127
Std. Deviation	0.645	0.191
Std. Error of Mean	0.132	0.053
Upper 95% CI	5.806	2.242
Lower 95% CI	5.261	2.011
Difference between means	3.406 ± 0.184	
95% confidence interval	3.033 to 3.780	
P value (two tailed)	P<0.0001	

The mean value of DHT in transition zone of prostate in case of BHP is 5.533 ± 0.645 pg/mg of wet tissue whereas in case of Ca prostate it is 2.127 ± 0.191 pg/mg of wet tissue. Therefore, the tissue level of DHT in transition zone of prostate in BHP is approximately 2.6 folds higher in comparison to Ca prostate. (P<0.0001)

Table 4: Comparison of 5α DHT level in prostatic tissue of the peripheral zone between BHP and Ca prostate patients:

	PZ BHP (pg/mg wet tissue)	PZ Caprostate (pg/mg wet tissue)
Minimum	0.560	7.160
25% Percentile	0.573	8.565
Median	0.650	8.900
75% Percentile	0.718	9.208
Maximum	0.7900	9.980
Mean	0.6513	8.869
Std. Deviation	0.083	0.634
Std. Error of Mean	0.029	0.142
Upper 95% CI	0.721	9.166
Lower 95% CI	0.582	8.572

Difference between means	-8.218 ± 0.2275
95% confidence interval	-8.868 to -7.750
P value (two tailed)	P<0.0001

The value of DHT in peripheral zone of prostate in case of BHP is 0.6513 ± 0.083 pg/mg of wet tissue whereas in case of Ca prostate it is 8.869 ± 0.634 pg/mg of wet tissue. Therefore, prostatic tissue level of DHT in peripheral zone of prostate in Ca prostate is approximately 13.62 folds higher in comparison to BHP (P<0.0001).

Table 5: Comparison of serum Testosterone level between benign hyperplasia of prostate and prostate carcinoma patients:

	BHP (ng/mL)	Ca Prostate (ng/mL)
Minimum	10.80	20.10
25% Percentile	11.68	22.20
Median	12.80	23.40
75% Percentile	14.03	26.45
Maximum	15.60	28.70
Mean	12.89	24.03
Std. Deviation	1.319	2.645
Std. Error of mean	0.2331	0.4605
Upper 95% CI	13.37	24.97
Lower 95% CI	12.42	23.09

P value (two tailed)	P<0.0001
Difference between means	-11.13 ± 0.5210
95% confidence interval	-12.18 to -10.09

Thus, serum Testosterone level is approximately 1.86 folds higher in case of Ca prostate (24.03 ± 2.645 ng/mL) in comparison with benign hyperplasia of prostate (12.89 ± 1.319 ng/mL), (P<0.0001).

The results of the study are briefly summarized here. In case of BHP, the level of 5DHT in the transition zone is about 8.5 times greater than in the peripheral zone. But, in case of Ca Prostate, the level of 5DHT in the peripheral zone is 4.17 times greater than in the transition zone. Further, the serum level of Testosterone is found to be higher in prostatic carcinoma (24.03 ± 2.645 ng/mL) compared to BHP (12.89 ± 1.319 ng/mL). Correlation analysis between prostatic tissue level of DHT and serum Testosterone level reveals that,

prostatic tissue level of DHT correlates with serum Testosterone level in case of Ca prostate patients when data from peripheral zone of prostate was taken into account (p=0.005) but not when transition zone was considered. In contrary, tissue level of DHT has no such correlation with serum Testosterone level in case of BHP (data from both transition and peripheral zone were considered separately).

Discussion

As BHP and Ca Prostate involve anatomically different zones of prostate, so the Prostatic tissue level of DHT, which is the active metabolite of Testosterone in Prostate tissue, in a zone wise manner, might be helpful for a better insight into the pathogenesis of these diseases [19,20,21].

As BHP primarily involves Transition zone of Prostate and Ca prostate primarily involves peripheral zone of prostate, so, in this study, prostatic tissue level of DHT has been measured in both transition and peripheral zones of Prostate gland in both diseases

In the present study, the findings show 8.5 folds increase in tissue level of DHT in Transition zone of prostate compared to Peripheral zone of prostate in BHP patients. Again, there is 4.17 folds increase in tissue level of DHT in Peripheral zone of prostate compared to Transition zone in prostate in Ca Prostate patients. The tissue level of DHT in Transition zone of Prostate is 2.6 folds higher in BHP compared to Ca Prostate. Whereas, the tissue level of DHT in Peripheral zone of Prostate is 13.62 folds higher in Ca Prostate compared to BHP.

These values indicate that, androgens, to be more specific, Testosterone and its active metabolite 5α-DHT may be implicated in the development of BHP and prostatic carcinoma.

In this study, only early diagnosed cases of Ca Prostate have been included, before the initialize-ation of anti-cancer therapy, because, anti-cancer therapy can influence the tissue level of DHT.

In the present study, the findings show 8.5 folds increase in tissue level of DHT in Transition zone of prostate compared to Peripheral zone of prostate in BHP patients. Again, there is 4.17 folds increase in tissue level of DHT in Peripheral zone of prostate compared to Transition zone in prostate in Ca Prostate patients. The tissue level of DHT in Transition zone of Prostate is 2.6 folds higher in BHP compared to Ca Prostate. Whereas, the tissue level of DHT in Peripheral zone of Prostate is 13.62 folds higher in Ca Prostate compared to BHP.

These values indicate that, androgens, to be more specific, Testosterone and its active metabolite 5α-DHT may be implicated in the development of BHP and prostatic carcinoma.

In this study, correlation analysis has been done between tissue level of 5α DHT and serum level of Testosterone in case of BHP and Ca 105 prostate. As described earlier, Testosterone is converted to its active metabolite 5α dihydrotestosterone by the enzyme 5α reductase. It is found in the present study that, the increased prostatic tissue level of DHT does not correlate with the serum testosterone level in case of BHP (taking into account values from both zones of prostate separately). This finding suggests that there may be some deviation from the normal physiological process of conversion of Testosterone to 5α DHT in prostate tissue in case of BHP. This absence of correlation may also be attributed to the probability that the rate of dihydrotestosterone formation exceeds the rate of its destruction and turnover in hyperplastic prostate tissue[22]. It is possible that, dihydrotestosterone formation is selectively increased in periurethral tissue rather than in the gland as a whole[22], and the increase in DHT level in transition zone compared to the peripheral zone of prostate in case of BHP lends support to this possibility. Finally, some unexplored pathways other than the direct reduction of testosterone within the gland might account for the accumulation of dihydrotestosterone in the hyperplastic prostate gland, specifically in the transition zone of prostate [22].

Thus, other than throwing some insight into the pathogenesis of BHP and Ca Prostate, this study elucidates the role of tissue level of 110 5α-dihydrotestosterone as a diagnostic and probable prognostic marker in Benign Hyperplasia of Prostate and Prostatic Carcinoma. This may be useful in establishing the diagnosis of these diseases as biopsy results are often non-confirmatory and inconclusive. Furthermore, the required amount of prostatic tissue is very little and is easy to achieve following TURP and true cut biopsy of prostate. Thus tissue level of 5α-DHT may be explored as a potential diagnostic tool for these very common diseases of aging male.

Moreover, prostatic tissue level of 5α-DHT in a zone wise manner may be further explored as a potential marker in order to predict future development of prostate carcinoma in apparently benign prostate tissue in patients undergoing TURP or true cut biopsy of prostate. So, patients with apparently benign prostatic tissue but having a high tissue level of DHT can be recommended to undergo frequent follow up so as to avert any untoward incident in future. Future prospective studies can be carried out in this regard.

Summary and Conclusion

Benign hyperplasia of prostate and prostatic carcinoma are two important diseases of aging male with very high incidence rate. Androgens have been implicated in the pathogenesis of both BHP and Ca prostate. Testosterone is the major circulating androgen in men. Dihydrotestosterone (the 5-α reduced derivative of Testosterone) is the major metabolite of testosterone in prostate. So DHT is thought to play an important role in the development and progression of both the diseases. The present study was aimed at assessing the inter-relationship between the tissue level of 5α-dihydrotestosterone and pathogenesis of benign hyperplasia of prostate and prostatic carcinoma.

Correlation analysis between prostatic tissue level of DHT and serum Testosterone level reveals that, tissue level of DHT does not correlate with serum Testosterone level in case of BHP (in case of data from both zones calculated separately) and in case of prostate carcinoma patients when data from transition zone of prostate is used. But tissue level of DHT correlates with serum Testosterone level in case of Ca prostate patients when data from peripheral zone of prostate is taken into account ($p=0.005$).

So, the comparative analysis of the above parameters suggests that, 5α-DHT is implicated in the pathogenesis of BHP and Ca prostate in a zone-wise manner. The findings of the present study also reveal that, prostatic tissue level of 5α-DHT may be explored as a possible marker for the diagnosis of benign hyperplasia of prostate and prostatic carcinoma.

References

1. Perls T, Terry D: Understanding the determinants of exceptional longevity. Ann Intern Med. 2003; 139:445
2. Tinetti ME et al: Potential pitfalls of disease-specific guidelines for patients with multiple conditions. N Engl J Med. 2004; 351:2870.
3. Barry MJ, Roehrborn CG. Benign prostatic hyperplasia. BMJ. 2001; 323:1042.
4. Ofner P, Harris RS, Wool IG, Loraine JA, and Thimann KV. Effects and metabolism of hormones. Academic Press, Inc., New York. 1968; 26: 237.
5. Geller J. Benign prostatic hyperplasia: pathogenesis and medical therapy. Source: Department of Medical Education, Mercy Hospital and Medical Center, San Diego, CA 92103-2180. J Am Geriatr Soc. December 1991; 39(12):1208-16.
6. Peter H. Gann, Charles H. Hennekens, Jing Ma, Christopher Longcope, Meir J. Stampfer. Prospective Study of Sex Hormone Levels and Risk of Prostate Cancer. Journal of the National Cancer Institute. August 21, 1996; Vol. 88,No. 16.
7. Isom-Batz G, Bianco FJ Jr, Kattan MW, et al. Testosterone as a predictor of

pathological stage in clinically localized prostate cancer. J Urol. 2005; 173:1935-1937.

8. Kelly Chiles, MD, Stanton Honig, MD. Relationships of Testosterone and Prostate Cancer: A 2011 Perspective. May 01, 2011.
9. Pierorazio PM, Ferrucci L, Kettermann A, et al. Serum testosterone is associated with aggressive prostate cancer in older men: results from the Baltimore Longitudinal Study of Aging. BJU Int. 2010; 105:824-829.
10. Nomura AM, Kolonel LN. Prostate cancer: a current perspective. Epidemiol Rev. 1991; 13:200-27.
11. Farnsworth, W. E., and J. R. Brown. E. P. Vollmer, editor. Testosterone metabolism in the prostate. *In* Biology of the Prostate and related tissues. National Cancer Institute Monograph. 1963; 12: 323.
12. Farnsworth, W. E., and J. R. Brown. Metabolism of Testosterone by the human prostate. J. Amer. Med. Ass. 1963; 183: 436.
13. Shimazaki, J., H. Kurihara, Y. Ito, and K. Shida. Testosterone metabolism in prostate; formation of androstan-17beta-ol-3-one and androst-4-ene, 17-dione, and inhibitory effect of natural and synthetic estrogens. Gunma J. Med. Sci. 1965;14: 313.
14. Chamberlain, J., N. Jagarinec, and P. Ofner. Catabolism of [4-36C] testosterone by subcellular fractions of human prostate. Biochem. J. 1966; 99: 610.
15. Gloyna, R. E., and J. D. Wilson. A comparative study of the conversion of testosterone to 17beta-hydroxy-5alpha-androstan-3-one (dihydro-testosterone) by prostate and epididymis. J. Clin. Endocrinol. Meatb. 1969; 29: 970.
16. Siiteri Pentti K and Wilson Jean D. Dihydrotestosterone in prostatic hypertrophy: I. The formation and content of dihydrotestosterone in the hypertrophic prostate of man. J. Clin Invest. September 1970; 49 (9): 1737-1745.
17. Tsutomu Nishiyama, Toshihiko Ikarashi, Yutaka Hashimoto, Kazuya Suzuki and Kota Takahashi. Association Between the Dihydrotestosterone Level in thProstate and Prostate Cancer Aggressiveness Using the Gleason Score. The Journal of Urology.Vol. 176, 1387-1391, October 2006. DOI:10.1016/j.juro.2006.06.066.
18. Qing Ji, Lilly Chang, Frank Z. Stanczyk, Murad Ookhtens,1 Andy Sherrod, and Andrew Stolz. Impaired dihydrotesto-sterone catabolism in human prostate cancer: a critical role of AKR1C2 as a pre-receptor regulator of androgen receptor signalling. Pub Med. Cancer Res. 2007; 67: 1361–9.
19. R. Bruce Montgomery, 1 Elahe A. Mostaghel, Robert Vessella, David L. Hess, Thomas F. Kalhorn, Celestia S. Higano, Lawrence D. True, and Peter S. Nelson. Maintenance of Intratumoral Androgens in Metastatic Prostate Cancer: A Mechanism for Castration-Resistant Tumor Growth. Pub Med Central, Cancer research. 2008 June 1; 68(11): 4447–4454. doi: 10.1158/0008-5472.
20. Mohler JL, Gregory CW, Ford OH, 3rd, et al. The androgen axis in recurrent prostate cancer. Pub Med. Clinical Cancer Research.2004;10:440–8.
21. Mark A. Titus, Michael J. Schell, Fred B. Lih, Kenneth B. Tomer and James L. Mohler. Testosterone and Dihydrotesto-sterone Tissue Levels in Recurrent Prostate Cancer. Clinical Cancer Research. July 1, 2005; 11: 4653. doi: 10.1158/1078-0432.
22. Thorpe A, Neal D: Benign prostatic hyperplasia. Lancet. 2003; 366:1359.