Chapter 6 Association of environmental pollutants like lead and cadmium with incidence of benign prostatic hyperplasia in patients of western India.

6.1 Introduction
6.2 Experimental design
6.3 Results
6.4 Discussion
6.5 References

6.1 Introduction

It is clearly demonstrated in earlier chapter that low levels of lead and cadmium exposure cause disruption in HPG axis by interacting at cellular and molecular level. Also, it has been shown that antioxidant enzymes are inhibited by both heavy metals leading to increased reactive oxygen species, which are known disruptors for various disease manifestations. Male infertility is one of the manifestations which correlated with heavy metal exposure in many studies. Male accessory glands like prostate do play an important role in the male reproductive function. Very few reports are available on association of environmental pollutants and prostatic disorder such as prostate cancer (CaP) and Benign prostatic hyperplasia (BPH) (Ogunlewe and Osegbe, 1989; Feustel, 1986).

The incidence of prostatic disorders has been studied and documented in various parts of the globe. CaP is the most common type of cancer in men and is the second leading cause of death. BPH is characterized by the nonmalignant overgrowth of prostatic tissue surrounding the urethra, ultimately constricting the urethral opening and giving rise to associated lower urinary tract symptoms (LUTS) such as urgency, frequency, nocturia, incomplete bladder emptying, and weak urine stream (AUA, 2003; Wei et al., 2005). Not all men with histologic BPH develop LUTS that requires intervention. Several processes, such as prostatic infarction, acute or chronic inflammation as a result of ongoing prostatitis, or in some cases, incidental adenocarcinomas of the gland, may foster the development of symptomatic BPH (Oesterling, 1996). As BPH nodules that originate in the transition zone grow, the remainder of the gland becomes compressed; if sufficient elasticity is present in the rest of the gland, little or no urethral compression will result. Conversely, if the remainder of the gland cannot expand freely, urethral constriction and obstruction of the urinary tract may occur (Oesterling, 1996). There is also a dynamic component to BPH, which can show substantive variation between individuals, relating to the neuronal control over prostatic smooth muscle tone by alpha$_1$A-adrenergic receptors

(Chapple, 1996). The interindividual differences in the static and dynamic components of BPH will determine which men are affected by LUTS

Apart from LUTS, there are various biochemical markers which are used for accessing the functionality of prostate gland. Amongst them, **(1)** Prostate specific antigen (PSA) is well established as a biochemical marker of choice in BPH & prognosis (Small & Roach, 2002). It is present in higher-than-normal levels (0 and 4 ng/ml) in the blood of men with BPH & prostate cancer. **(2)** Prostatic Acid Phosphatase (PAP) is primarily produced by the prostate gland and considered as marker enzyme for prostatic disorder (Pappas AA, Gadsden RH). PAP levels elevate due to altered membrane permeability or necrosis of malignant cells resulting in enzyme leakage in the blood stream. Elevated PAP values found in 84-100% of patients with CaP (Bahnson RR, Catalona WJ, 1987). However, PAP is not sensitive enough to be used as a screening test and is not suitable for detecting CaP in early stages of disease. **(3)** Measurement of the maximum urinary outflow rate (Qmax) is used for assessing the patients complaining for the lower urinary tract symptoms (LUTS). Qmax has 2 major implications that there might be obstruction at the level of urethra due to enlargement of the prostatic gland or bladder outflow obstruction (BOO). For clinical categorization cut of values have been identified where by men with Qmax < 15 (ml/sec) have approximatly 70% chance of having obstruction at the level of urethra or BOO, where as men having Qmax > 15 have 65% chance of having urethral obstruction/BOO. (Poulsen et al., 1994)

Certain risk factor for manifestation of BPH involves advanced age, African-American race, first-degree family history of the disease, environmental influences, cigarette smoking and heavy metal exposure.

Besides age and race, a positive family history is recognized as one of the strongest epidemiologic risk factors for prostate cancer (Gronberg *et al 2003).* The probable reason for the prostate carcinogenesis in patients with positive family history is single nucleotide polymorphism (SNP) occurring in the prostate cancer susceptibility genes. Some researchers investigated the association between benign prostatic hyperplasia and common single-nucleotide

polymorphisms (SNPs) in the AR (Xq11-12) (Kenji et al., 1999) and PSA (19q13) genes (Gunes et al., 2007). All four genes are involved in the synthesis of estradiol, testosterone and dihydrotestosterone (DHT), which can influence prostate growth and function. Environmental influences play a role in the development of prostate disorders because migration studies of Japanese immigrants to the United States have found that the risk of prostate disorders increased 5-fold with the change of environment (Angwafo, 1998).

One potential modifiable risk factor is cigarette smoking. There are several hypothetical mechanisms through which cigarette smoking may enhance BPH risk. Reports have suggested that, cigarette smoking may alter circulating levels of steroid hormones. In particular, cigarette smoking has been associated with higher levels of bioavailable testosterone and lower levels of bioavailable estradiol in men. Studies found significant positive correlations between cigarettes smoked/day and serum total androstenedione as well as total and free testosterone in men (Kym et al., 2001). This is significant because testosterone and its more potent metabolite DHT are necessary not only for normal prostate development and growth but also appear to enhance cell proliferation in the prostate (Marker et al., 2003), which potentially could be associated with malignant transformation. Effectively, cigarette smoking may establish a hormonal milieu that is favorable for the development or progression of BPH. In addition, cigarettes contain significant levels of cadmium, which has been linked to prostate hyperplasia due to its estrogen mimicking effect. Either or both of these mechanisms could support an association between cigarette smoking and BPH.

Cadmium is a known human carcinogen and is linked to BPH & prostate cancer in epidemiologic and laboratory animal studies demonstrated by ATSDR, 1999. Food and cigarette smoke are the largest sources of lead and cadmium exposure in the general population. Smokers have a daily lead and cadmium intake that may be twice that of non-smokers. Lead and cadmium are sulfhydryl reactive metals and they have three major properties, which mechanistically explain how they elicit a majority of their toxic effects. First they are transition

metals that promote hydrogen peroxide, hydroxyl radical and lipid peroxidation. The pro-oxidant properties of the metals are exaculated by their inhibitory effect on antioxidant processes. They have high affinities for glutathione (GSH), and deplete the cellular GSH (Quig, 1998) leading to pathogenesis.

From the literature, it is clear that cadmium's estrogen mimicking capability, causing onset of tumor formation in the prostate gland. The mechanism of which is still under investigation. In view of this the present study was carried out to understand the association of environmental pollutants (cadmium and lead) with the incidence of BPH in patients of western India. The importance of our study is reflected by the fact that, this demographic study has been carried out directly in the target tissue, i.e. the prostate from patients undergoing TURP (Trans Urethral Resection of Prostate). The objective of the present study is to ascertain the correlation of BPH and its severity to smoking and hereditary factors and Qmax, Cd levels and antioxidant status.

6.2 Experimental design

About 120 prostate samples from BPH patients (~1g tissue weight) were collected from the Sujay hospital, Baroda in ice-cold conditions (4°C) in normal saline and brought immediately to lab for further processing. Tissue samples of BPH patient's was collected in hospital through advance technology i.e. TRUP and HOLEP.

Around 0.5 g of prostate sample was used for metal estimation by atomic absorption spectrophotometer (AAS). Rest of tissue was weighed and homogenized in chilled (4°C) isolation medium (0.25 M sucrose, 10 mM Tris-HCl buffer pH 7.4, 1 mM EDTA and 250 µg BSA/ml). The isolation of mitochondria and post-mitochondrial fractions was carried out according to the procedures described in chapter 2. The mitochondrial and post-mitochondrial fractions obtained were subjected to biochemical estimations/analysis of reactive oxygen species related parameter. ROS parameters include GSH (Beutler E and Gelbart T, 1970), LPO (Ohkawa and Ohishi, 1979), SOD (Marklund S and Marklund G,

1974), Catalase (Hugo EA 1987) and GSHPx (Hafeman et al., 1974). The activity of ACP (Bowers and McComb, 1975), was estimated in post-mitochondrial fraction. Protein estimation has been carried out to express enzyme activity in terms of specific activity (Lowary et al., 1951). The serum PSA was measured using immunoassays (The Monobind USA Kits) two to three days prior to surgery, as it is clinical marker for prostatic disorders.

Prostate sample was also used for histological observations by standard histological techniques. Histological observation was made for every sample of BPH patients under the light microscope.

Questionnaire was prepared for patient history record. Patients were asked several questions about their dietary habits, additive habits and environmental pollutant exposure status at work place or at place of residence and if the patient had any genetic lineage of BPH.

The total numbers of samples were analyzed on the basis of correlation/trends in relation with various variables using SPSS software programme.

PATIENT HISTORY Questionnaire

PROSTATE DISORDERS – QUESTIONNAIRE

Department of Biochemistry
Faculty of Science
The M.S. University of Baroda
Vadodara 390002

DATE: ___ ___ ___ SERIAL CODE: □□□□□

INVIGILATOR: _____

PERSONAL DETAILS:

Name: _____

Age:_____ years.

Education: _____

Address: _____

_____

Phone: _____

Native: _____

Diet: Vegetarian / Non-vegetarian / Omnitarian

Additive Habits: Tobacco / Smoking / Alcohol / Others; _____

Occupation: _____

Q- Are you exposed to a pollutant(s) at your work place or at your place of residence? Yes / No

If Yes, Nature of pollutant: Land / Water / Air / Others; _____

HISTORY OF ANY MAJOR ILLNESS:

Q- Are you suffering from any lifestyle disorder(s)? Yes / No

If Yes, Nature of disorders: Diabetes / Obesity / Cholesterol disorder / Cardiac disorder / Other; _____

Symptoms associated: _____

Duration of disorder: _____

Q- What made you suspect that you have a prostatic disorder?

_____

Q- Any one in your family ever detected with a prostatic disorder? Yes / No

If Yes, First / Second / Third degree relative. Relation: _____

PROSTATIC DISORDER(S):

Diagnosis (tick):

[ ] PSA Screening (If Yes, Level: _____ ng/ml blood / serum)

[ ] Digital Rectal Examination (DRE)

[ ] Trans Rectal Ultrasound (TRUS)

[ ] Prostate Needle Biopsy

[ ] Others; _____

Suspected Prostatic Disorder (tick):

[ ] Prostatitis, (If Yes, State: Acute / Chronic)

[ ] Benign Prostatic Hyperplasia (If Yes, State: Mild / Moderate / Severe)

[ ] Prostatic Cancer (If Yes, State: Benign / Metatstatic

[ ] Prostate Intra-epithelial Neoplasia (PIN) Grade: I / II / III

[ ] Gleason Grading Stage: 1 / 2 / 3 / 4 / 5, Gleason Score: __+__=_____)

Any other associated symptoms? Yes / No

If Yes, Symptoms: _____

Treatment (tick):

[ ] Antiandrogenic drugs (If Yes, which: _____)

[ ] Any other drug (If yes, nature: _____, which: _____)

[ ] Transurethral Resection of the Prostate (TURP)

[ ] Laser removal of the diseased portion

[ ] Complete removal of the whole prostate gland

Post treatment recovery: Excellent / Good / Poor / No response

Post treatment side effects / symptoms (if any): _____

General Comments: _____

_____

6.3 Results

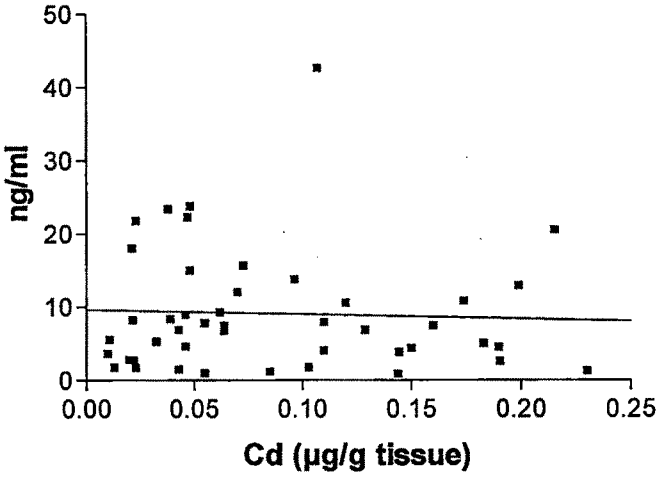
The total numbers of tissue samples obtain from the hospital were 120, from this 116 samples were of BPH and 4 were of prostate cancer. The results represent the correlation(s)/trend(s) of 116 numbers of patients suffering from benign prostate hyperplasia. The known biochemical marker PSA, PAP and clinical marker Qmax were correlated with both lead and cadmium content. Strong correlation was observed with increasing Cd content to that of Qmax (Figure 3) and PAP (Figure 2) value without any relation with Pb content (Figure 5 & 6). PSA level did not show any significant correlation with cadmium (Figure 1) and lead (Figure 4) content.

Efforts were also made to correlate severity of BPH from histopathological analysis with Pb and Cd content. Again Cd showed positive relation with severity. Few representative slides are shown in figure 7.

Record of patient history was also maintained so as to obtain information regarding the dietary habits, additive habits (cigarette smoking, tobacco chewing, alcoholism), and environmental pollutant exposure and genetic lineage of BPH. On the basis of the record, patients was divided in to two groups, those having additive habits of smoking or not and having any first or second-degree genetic lineage of BPH or not.

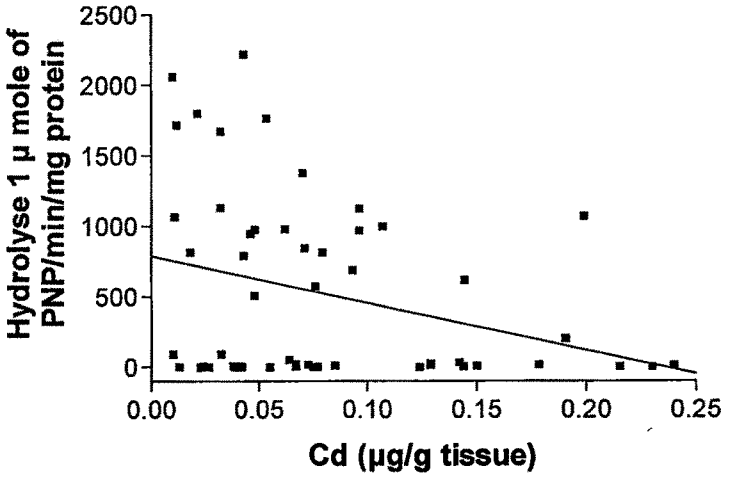
Parameter such as metal content (Cd and Pb) and Qmax was analyzed in smokers and non smokers. Amongst the metals, cadmium was significantly more in smokers compared to non-smokers (Figure 8) while Pb did not showed any significant change (Figure 9). Mean Qmax was found to be significantly lower in smokers than non smokers (Figure 10). Qmax of genetically predisposed patients was significantly lower compared to genetically nondisposed BPH patients (Figure 12). Also the incidence of BPH in genetically predisposed patients was found to be higher at an early age (Figure 13). Patients when divided in to three age groups (55-64, 65-74 and 75-84) and compared with Qmax (Figure 11) it was found to be decreased significantly.

Figure 1. **Correlation of Cd content with PSA level in human serum of BPH patients.**



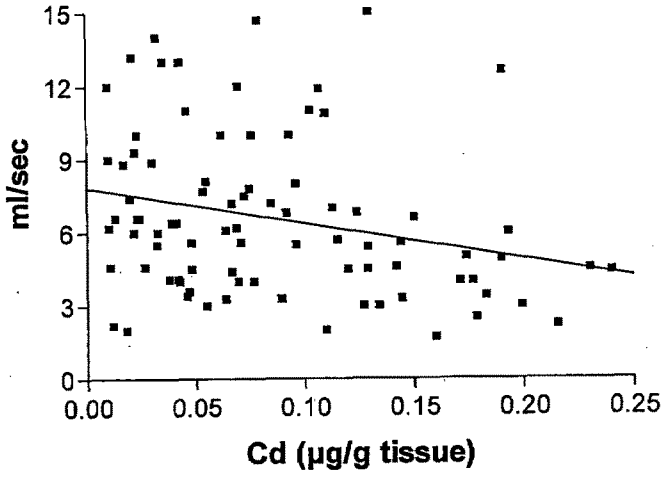
r^2=0.002; ns (N=45)

Figure 2. **Correlation of Cd content with PAP in human prostate of BPH patients.**



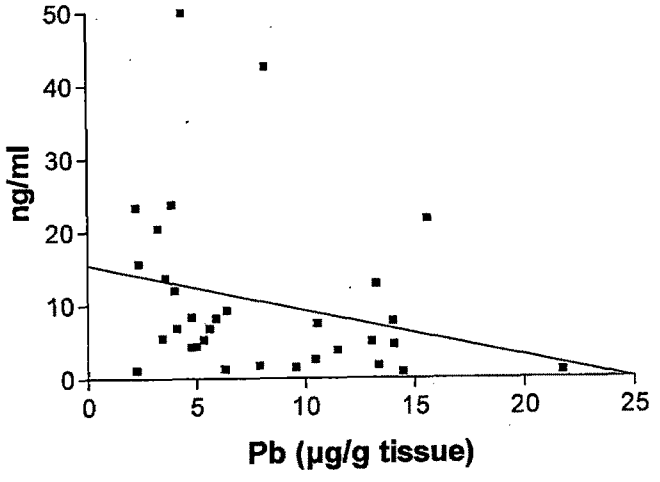
r^2=0.095; $p<0.05$ (N=54)

Figure 3. Correlation of Cd content with Qmax level of BPH patients.



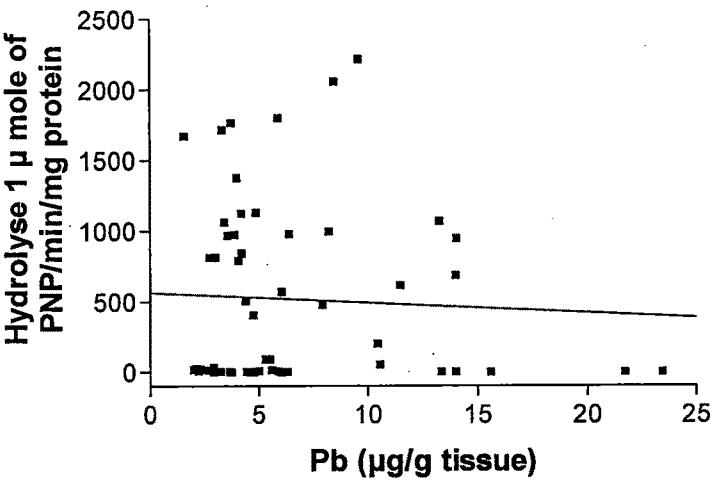
r^2=0.07; P<0.05 (N=85)

Figure 4. Correlation of Pb content with PSA level in human prostate of BPH patients.



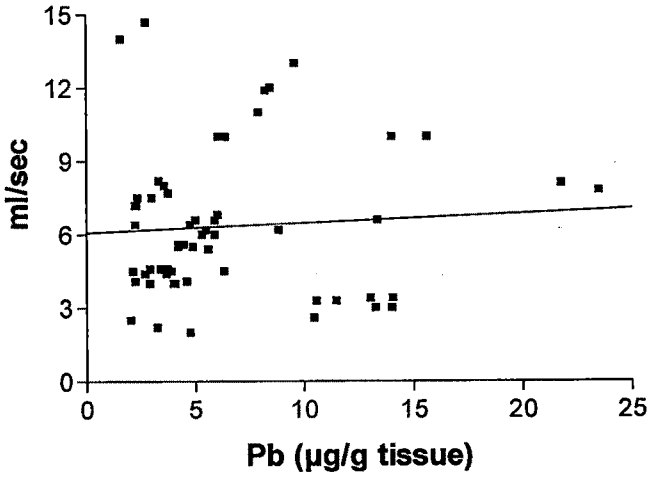
r^2= 0.07; ns (N=34)

Figure 5. Correlation of Pb content with PAP activity in human prostate of BPH patients.



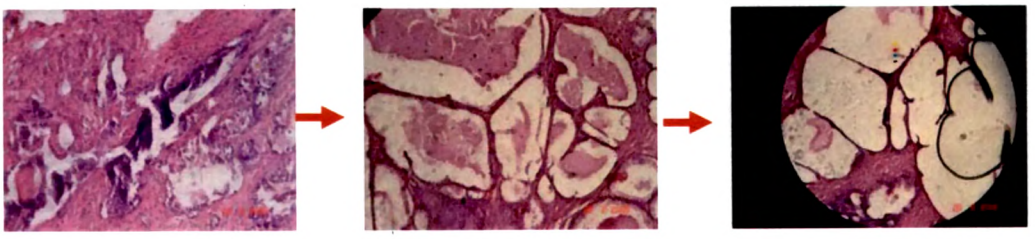
r^2= 0.003; ns (N=54)

Figure 6. Correlation of Pb content with Qmax level in human prostate of BPH patients.



r^2= 0.004; ns (N=58)

Figure 7. Correlation of Cd with histopathological observation of BPH patients.

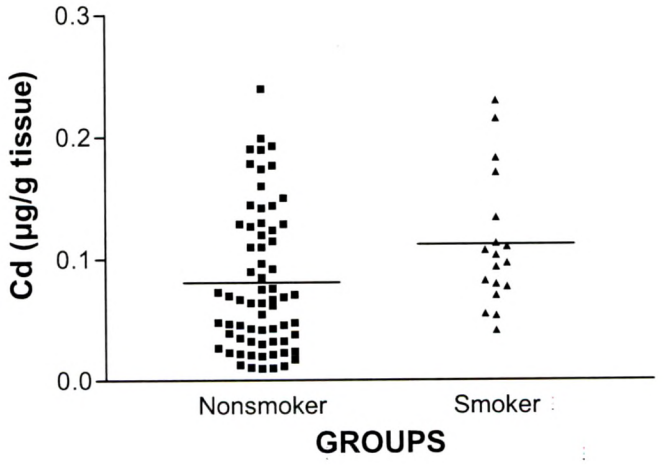


Cd=0.024 ug/g tissue

Cd=0.077 ug/g tissue

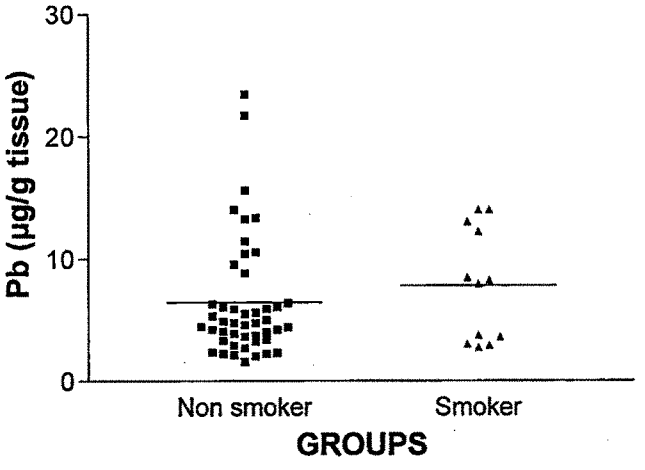
Cd=0.133 ug/g tissue

Figure 8. Cd levels in nonsmokers and smokers: An effect of smoke in BPH patients.



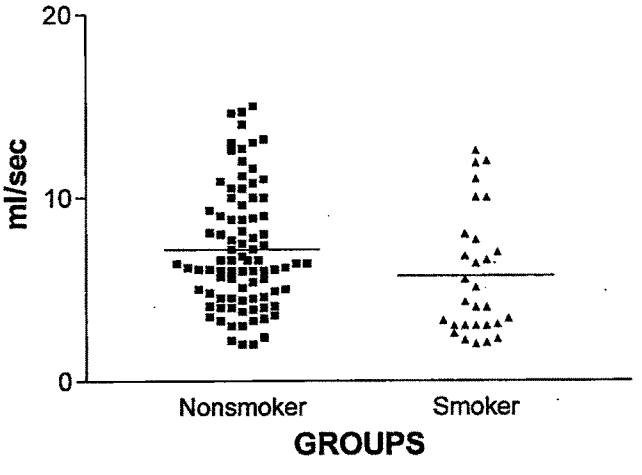
Horizontal bars indicate mean. Nonsmoker = 67, Smoker = 18; $P<0.05$

Figure 9. Pb levels in nonsmokers and smokers: An effect of smoke in BPH patients.



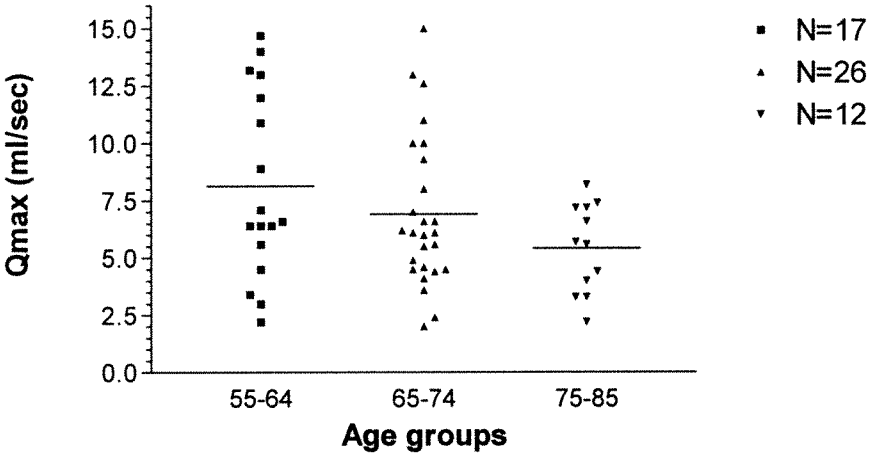
Horizontal bars indicate mean. Nonsmoker = 46, Smoker = 12; ns

Figure 10. Qmax levels in nonsmokers and smokers: An effect of smoke in BPH patients.



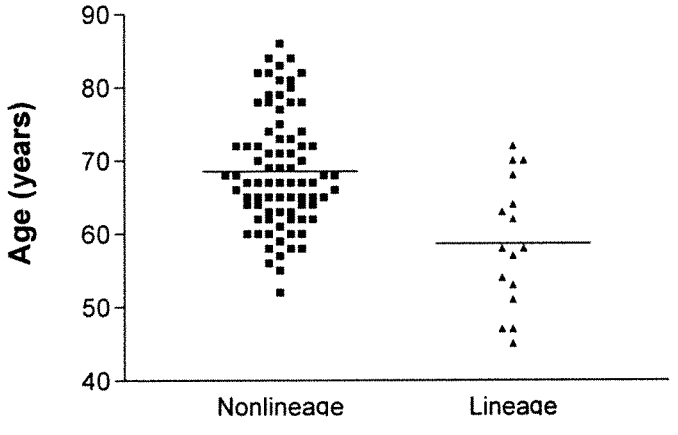
Horizontal bars indicate mean. Nonsmoker = 87, Smoker = 29; $P<0.05$

Figure 11. Age dependent effect on Qmax level in BPH patients.



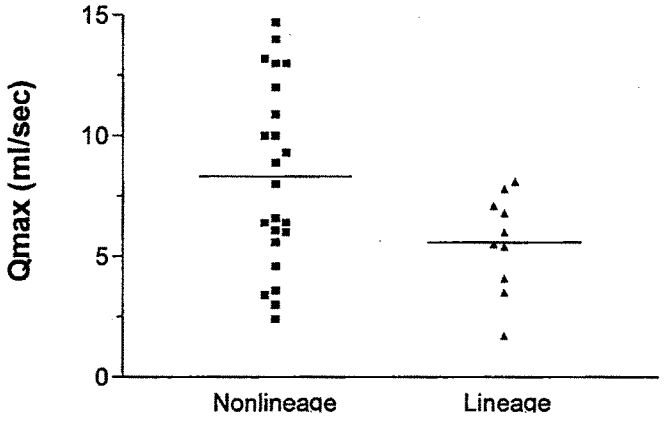
Horizontal bars indicate mean. 55-64 vs 75-85 ($P<0.05$)

Figure 12. Age of genetically non lineage and lineage BPH patients.



Horizontal bars indicate mean. Nonlineage = 87, Lineage = 29; $P<0.001$

Figure 13. Qmax of genetically non lineage and lineage BPH patients between 45 to 64 age.

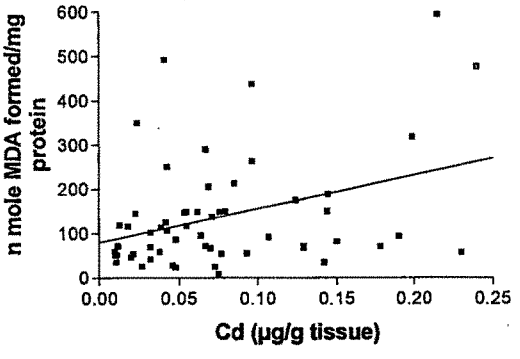


Horizontal bars indicate mean. Non lineage = 23, Lineage = 10; $P<0.05$

Oxidative stress parameters such as lipid peroxidation, glutathione level and antioxidant enzyme activity (SOD, catalase and GPx) from mitochondrial and post mitochondrial fraction are correlated with the levels of Cd and Pb as representation of environmental pollutants in BPH patients. Both the mitochondrial (Figure 14(A)) and post mitochondrial (Figure 14(B)) LPO levels were found to be significantly increased with increase in Cd levels (positive correlation). SOD activity demonstrated decreasing trend with respect to increased Cd levels in both sub cellular fractions (negative correlation; Figure 15(A) & 15(B)). Catalase activity does not exhibit any changes with Cd accumulation (Figure 18). In both mitochondrial (Figure 16(A)) and post mitochondrial (Figure 16(B)) fractions, GPx activity was significantly increased and positively correlated with increasing Cd content. Non enzymatic antioxidant such as GSH was although showing negative correlation with Cd (Figure 17(A) & 17(B)) and Pb (Figure 22(A) & 22(B)) concentration, not demonstrating statistical significance. Similarly, statistical correlation of all antioxidant enzyme activity was carried out with the Pb content in BPH patient. Significant positive trends were obtained in MDA levels with Pb accumulation in both the fraction used (Figure 19(B) & 19(B)). SOD (Figure 20(B) & 20(B)) and GPx (Figure 21(B) & 21(B)) enzyme activity exhibited negative correlation, while Catalse activity

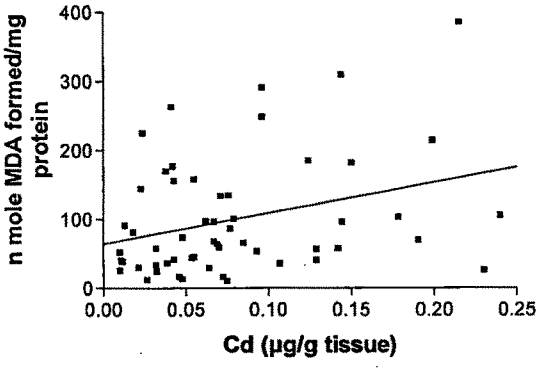
Figure 14. Correlation of Cd content with (A) mitochondrial and (B) post mitochondrial LPO level in human prostate of BPH patients.

(A)



r^2= 0.128; P<0.01 (N=58)

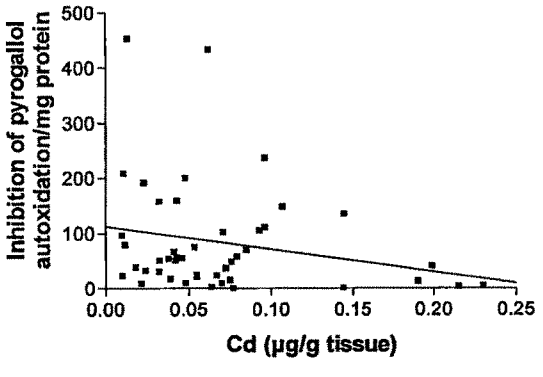
(B)



r^2= 0.097; P<0.05 (N=56)

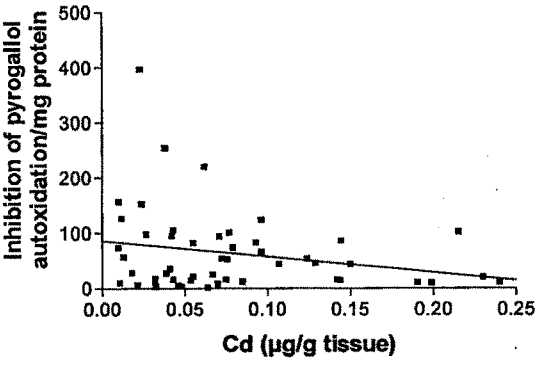
Figure 15. Correlation of Cd content with (A) mitochondrial and (B) post mitochondrial SOD activity in human prostate of BPH patients

(A)



r^2= 0.051; ns (N=47)

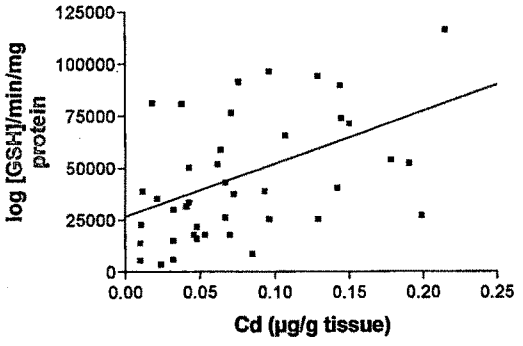
(B)



r^2= 0.054; ns (N=50)

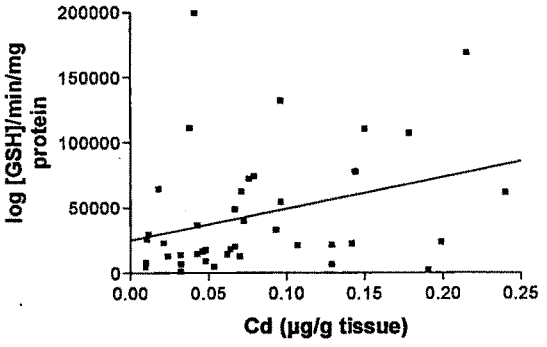
Figure 16. Correlation of Cd content with (A) mitochondrial and (B) post mitochondrial GPx activity in human prostate of BPH patients.

(A)



r^2= 0.177; P<0.01 (N=42)

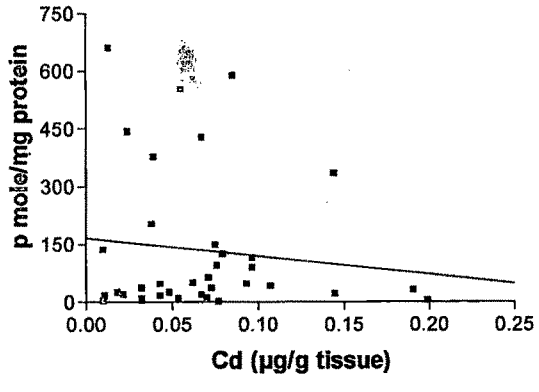
(B)



r^2= 0.1; P<0.05 (N=42)

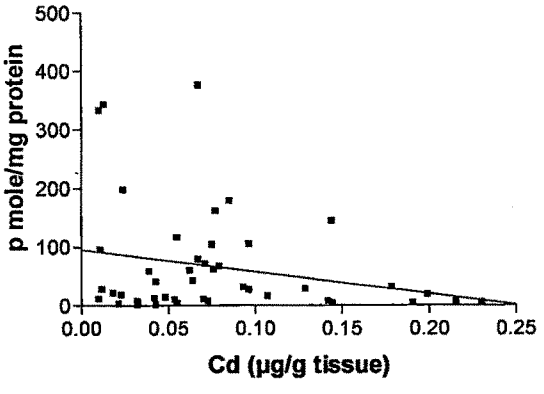
Figure 17. Correlation of Cd content with (A) mitochondrial and (B) post mitochondrial reduced glutathione level in human prostate of BPH patients.

(A)



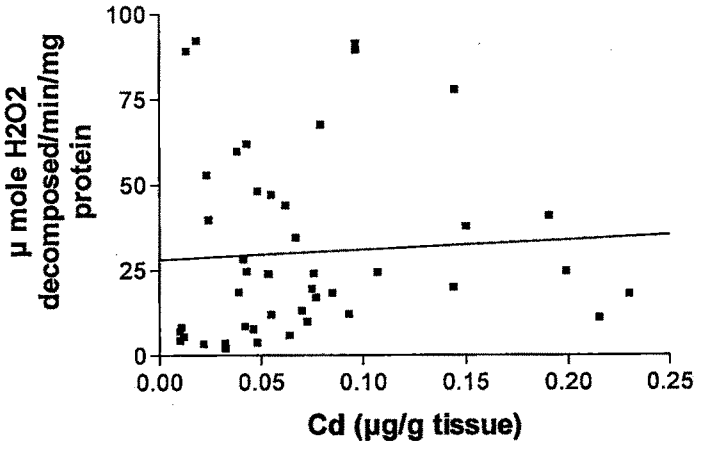
r^2= 0.014; ns (N=37)

(B)



r^2= 0.055; ns (N=45)

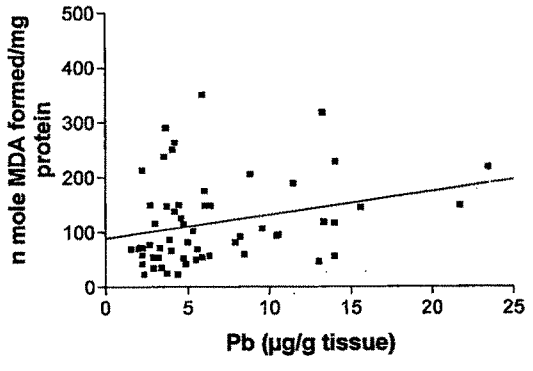
Figure 18. Correlation of Cd content with post-mitochondrial catalase activity in human prostate of BPH patients.



r^2= 0.003; ns (N=45)

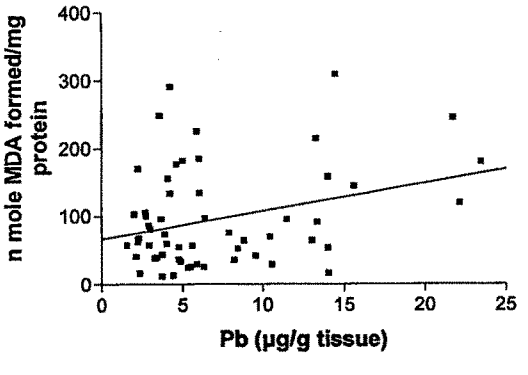
Figure 19. Correlation of Pb content with (A) mitochondrial and (B) post mitochondrial LPO level in human prostate of BPH patients.

(A)



r^2=0.07; p<0.05 (N=58)

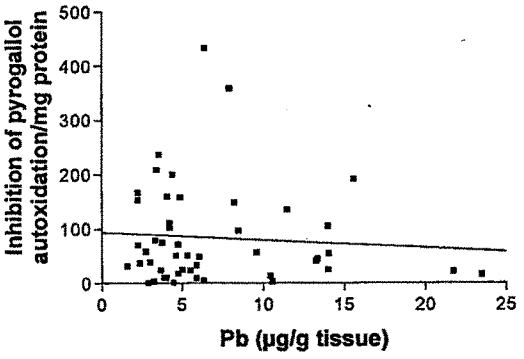
(B)



r^2=0.091; p<0.05 (N=59)

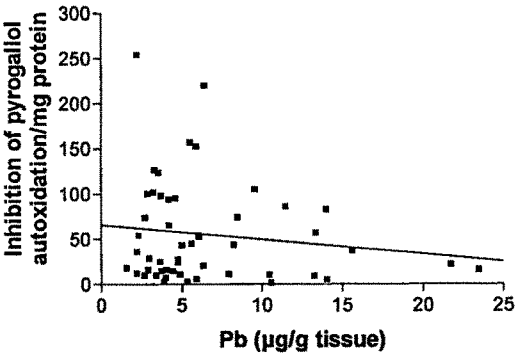
Figure 20. Correlation of Pb content with (A) mitochondrial and (B) post mitochondrial SOD activity in human prostate of BPH patients.

(A)



r^2=0.006; ns (N=48)

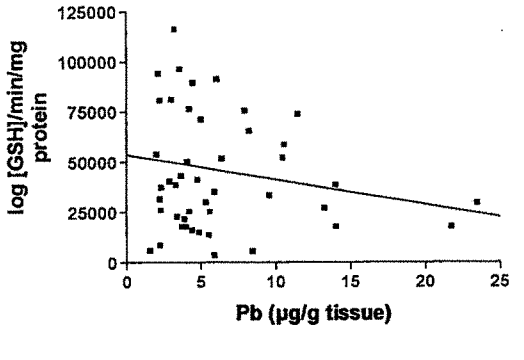
(B)



r^2=0.02; ns (N=52)

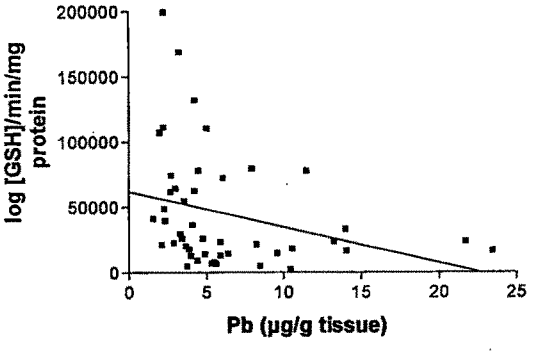
Figure 21. Correlation of Pb content with (A) mitochondrial and (B) post mitochondrial GPx activity in human prostate of BPH patients.

(A)



r^2=0.034; ns (N=46)

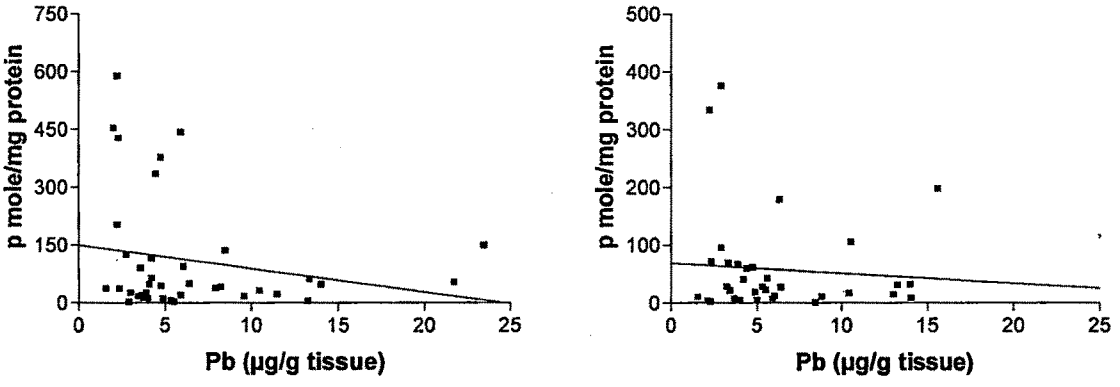
(B)



r^2= 0.08867; P<0.05 (N=46)

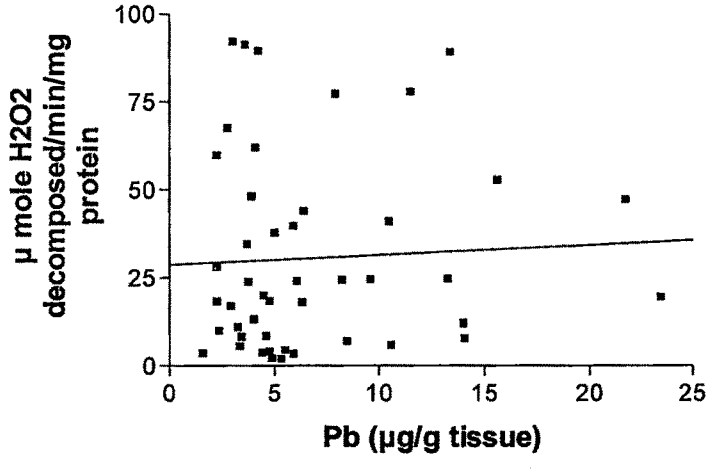
Figure 22. Correlation of Pb content with (A) mitochondrial and (B) post mitochondrial reduced glutathione level in human prostate of BPH patients.

(A) (B)



r^2= 0.04124; ns (N=39) r^2= 0.006; ns (N=35)

Figure 23. Correlation of Pb content with catalase activity in human prostate of BPH patients.



r^2=0.002; ns (N=47)

(Figure 23) has no correlation with Pb concentration. However, except LPO all other correlation with Pb is insignificant.

6.4 Discussion

To ascertain association of environmental pollutants with reproductive disorders, study was carried was carried out with patients undergoing TURP for BPH. The patients were divided according to positive family history. Positive family history has been recognized as one of the strongest epidemiologic risk factors for prostate cancer (Gronberg et al 2003). The plausible explanation for early incidence and higher severity of BPH in genetically predisposed people are probably SNPs (single nucleotide polymorphisms) in genes of various enzymes and even in certain receptors like androgen receptors, estrogen receptors (α and β isoforms; Kenji et al., 1999).

Since BPH is considered to be an age related disorder we divided the patients into 3 age groups. Qmax was used as a measure of severity for different age groups and found to have inverse relation with Qmax. Furthermore, with increase in age there is increase in the 5α-reductase activity in prostate tissue (Berry et al., 1984). Which will again imbalance hormonal milieu and cause BPH.

Patients were also divided into two groups namely, smokers and non smokers from their history. Cadmium content was higher in prostate samples of smokers as compared to nonsmokers. Qmax of the smokers is significantly lower than the non smoker patients suggesting the higher levels of severity of BPH in smokers. There are several potential mechanisms whereby cigarette smoking may increase risk of prostate cancer and hyperplasia. One is the ability of cigarette smoking to increase bioavailable testosterone and decrease bioavailable estradiol, which may alter the hormonal milieu favoring higher androgenic exposure to the prostate (Field et al 1994). Another possible mechanism for an association between smoking and prostatic hyperplasia is exposure to carcinogenic substances found in cigarettes. For example, cadmium and lead are inorganic toxicant also found in cigarettes (Kalcher et al 1993, Saldivar et al 1991, yeargen et al 1992). In 1993, the International Agency for Research on Cancer

designated cadmium as a human carcinogen. Although not directly mutagenic in the prostate, cadmium has been shown to indirectly induce prostate carcinogenesis through interaction with the androgen receptor (Ye et al., 2003). Studies also reported that cadmium has the property of activating the androgen receptor response, in human prostate cancer cell lines. Furthermore, when applied in combination with androgen, cadmium enhances androgen-mediated transcriptional activity in the prostate (Ye et al., 2003). Chronic cadmium exposure in rats has been shown to induce prostate tumors in the presence of normal testicular function. Therefore, chronic smoking in men with otherwise normal testicular function and androgen levels may effectively increase their androgen exposure through the interaction of cadmium with the androgen receptor, thus increasing the risk of prostate cancer and prostatic hyperplasia over a period of time.

There are many other factors which play a role in causing the incidence of BPH and increasing its severity. It is well known that toxic metals act as a catalyst in generation of reactive oxygen species (Monteiro et al., 1991; El-Maraghy et al., 2001). In present study, a linkage between an increase in cellular reactive oxygen radicals causing lipid peroxidation due to metal accumulation and the pathogenesis of BPH has been well established. Patients with higher accumulation of cadmium content also, demonstrated more imbalance in antioxidant enzymes. Mechanism of such alteration has been discussed in earlier chapter (chapter 5). When the antioxidant control mechanisms are exhausted or overrun, the cellular redox potential shifts toward an oxidative stress, in turn, increasing the potential for damage to cellular components and severity of BPH.

Thus, cadmium as pollutant along with ageing and positive family history demonstrated association with severity of BPH.

6.5 References

Angwafo FF. Migration and prostate cancer, an international perspective. J Natl Med Assoc 1998, 90, S720-S723.

AUA Practice Guidelines Committee. AUA guideline on management of benign prostatic hyperplasia. Ch-1, Diagnosis and treatment recommendations. J Urol 2003, 170, 530-547.

Bahnson RR, Catalona WJ. Adverse implications of acid phosphatase levels in the upper range of normal. J Urol 1987, 137, 427-430.

Berry SW, Coffey DS, Walsh PC, et al. The development of human benign prostate hyperplasia with age. J Urol. 1984, 132, 474-479.

Beutler E, Gelbart J. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med 1985, 105, 581-584.

Bowers GN Jr., McComb RB. Measurement of total alkaline phosphatase activity in human serum. Clin Chem 1975, 21, 1988-1995.

Chapple CR. Selective a1-adrenoreceptor antagonists in benign prostatic hyperplasia, rationale and clinical experience. Eur Urol. 1996, 29, 129-144.

Chen Y, Ye S, Huang Y, Zheng W. Stage-dependent expression of androgen binding protein mRNA in sertoli cell of rat testis Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2000, 22, 287-289. Chinese.

El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA. Effect of cadmium and aluminum intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol 2001, 15, 207-214.

Feustel A, Wennrich R. Zinc and cadmium plasma and erythrocyte levels in prostatic carcinoma, BPH, urological malignancies, and inflammations. Prostate. 1986, 8, 75-79.

Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB, The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. J Clin Endocrinol Metab 1994, 79, 1310-1316.

Gronberg H. Prostate cancer epidemiology. Lancet. 2003, 361, 859-864.

Gunes S, Bagci H, Sarikaya S, Bilen CY, Kara N. Prostate-specific antigen and 17-hydroxylase polymorphic genotypes in patients with prostate cancer and benign prostatic hyperplasia. DNA Cell Biol, 2007, 26, 873-878.

Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr. 1974, 104, 580-587.

Hickey K, Do KA, Green A. Smoking and Prostate Cancer review. 2001, 23, 115-125.

Hugo EA. Catalase. In Methods of Biochemical Analysis. Bergmeyer HU, Bergmeyer J, Grabt M editors. 3rd volume, New York VCH Publishers, 1987, 277-282.

Kalcher K, Kern W, Pietsch R. Cadmium and lead in the smoke of a filter cigarette Sci Total Environ. 1993, 128, 21–35.

Kenji M, Terai A, Oka H, Segawa T, Ogura K, Yoshida O, Ogawa O. Androgen receptor CAG repeat length polymorphism in benign prostatic hyperplasia (BPH): Correlation with adenoma growth. The Prostate, 1999, 41, 253 - 257.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951, 193, 265-275.

Marker PC, Donjacour AA, Dahiya R, Cunha GR Hormonal, cellular, and molecular control of prostatic development. Dev Biol 2003, 253, 165-174.

Marklund S, Marklund G. Involment of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974, 47, 469-474.

Monteiro HP, Bechara EJ, Abdalla DS. Free radicals involvement in neurological porphyrias and lead poisoning. Mol Cell Biochem. 1991, 24, 73–83.

Oesterling JE. Benign prostatic hyperplasia, a review of its histogenesis and natural history. Prostate Suppl 1996, 6, 67-73.

Ogunlewe JO, Osegbe DN. Zinc and cadmium concentrations in indigenous blacks with normal, hypertrophic, and malignant prostate. Cancer. 1989, 63, 1388-1392.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979, 95, 351-357.

Pappas AA, Gadsden RH Sr. Prostatic acid phosphatase, clinical utility in detection, assessment, and monitoring carcinoma of the prostate. Ann Clin Lab Sci 1984, 14, 285-291.

Poulsen A, Schou J, Puggaard L, Torp-pederson L. Prostatic enlargement, symptomology pressure/flow evaluation: interrelation in patients with symptomagic BPH. Scand J Urol Nephrol Suppl, 1994, 157, 67-73.

Quig D, Cysteine metabolism and metal toxicity. Altern Med Rev. 1998, 3, 262-270.

Rizzo S, Attard G, Hudson DL. Prostate stem cells S. Rizzo et al. Prostate epithelial stem cells. Cell Prolif. 2005, 38, 363–374.

Saldivar L, Luna M, Reyes E, Soto R, Fortoul TI. Cadmium determination in Mexican-produced tobacco. Environ Res. 1991, 55, 91–96.

Small EJ, Roach M 3rd Prostate-specific antigen in prostate cancer, a case study in the development of a tumor marker to monitor recurrence and assess response. Seminars in Oncology 2002, 29, 264–273.

Waalkes MP. Cadmium carcinogenesis in review. J Inorg Biochem. 2000, 79, 241–244.

Wei JT, Calhoun E, Jacobsen SJ. Urologic Diseases in America project, benign prostatic hyperplasia. J Urol 2005, 173, 1256-1261.

Yeargan R, Maiti IB, Nielsen MT, Hunt AG, Wagner GJ. Tissue partitioning of cadmium in transgenic tobacco seedlings and field grown plants expressing the mouse metallothionein I gene Transgenic Res 1992, 1, 261–267.