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Full Length Research Paper

An assessment of the effect of exposure to gasoline vapours on the functionality of the prostate gland using serum total and prostatic acid phosphatase

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In this study, changes in the activities of serum total and prostatic acid phosphatase (TACP and PACP, respectively), alkaline phosphatase (ALP), gamma( )-glutamyltransferase (GGT) and testosterone level were assessed in male rats exposed wholly to 17.8±2.6cm^3 hr^{-1}kg^{-1}m^{-3} day^{-1} of gasoline vapours (8 hours daily, 6 days/week) for 20 weeks in exposure chambers. The results showed that exposure to gasoline vapours caused a significant increase ($P < 0.05$) in serum TACP, PACP, ALP, GGT and testosterone levels, by 74.12 ± 4.71, 74.00 ± 2.80, 53.00 ± 3.6, 87.17 ± 2.5 and 83.42 ± 5.2 percents, respectively, in male rats. Increase in serum acid and alkaline phosphatases, - glutamyltransferase and testosterone levels, observed in this study to be associated with exposure of male rats to gasoline vapours, is known to be implicated in various prostatic clinical conditions, including prostatic cancer.

Key words : Gasoline vapours, acid phosphatase, -glutamyltransferase, testosterone.

INTRODUCTION

It has been observed and reported that exposure to gasoline vapours caused such clinical conditions related to those that are caused by smoking, including haematotoxicity, hepatotoxicity, nephrotoxicity, and alterations in some biochemical activities associated with lipid metabolism in rats (Uboh *et al*.,2005; Uboh *et al*.,2007a,b,c; Uboh *et al*.,2008) . Also, our earlier studies showed that gasoline vapours increased serum follicle stimulating hormones (FSH), luteinizing hormones (LH) and testosterone in male rats ; and decreased serum FSH, LH, oestadiol and progesterone in female rats(Uboh *et al*.,2007c). On the basis of the various clinical conditions observed to be associated with gasoline vapours, it was suspected that exposure to gasoline vapours may also affect the activity / concentration of total and prostatic acid phosphatase, hence the activity of the prostate gland in male rats.

Acid phosphatase activity / concentration has been reported to be elevated in the sera of males with metastatic prostatic cancer (Fang *et al.*,2008; Saito *et al.*,2006; Taira *et al.*,2007). This indicates that increased serum acid phosphatase levels is of great clinical importance in the diagnosis of prostatic cancer. The acid phosphatases are a group of enzymes capable of hydrolyzing esters of orthophosphoric acid in an acid medium. Acid phosphatase activity is widely distributed in human tissues and acid phosphatases represent a heterogeneous group of enzymes containing many isoenzymes, each specific for one type of tissue. The human prostate is particularly rich in this enzyme and serum enzyme levels have been used as a tumor marker of prostate cancer (Merick *et al.*,2005). Hence, the enzyme is used as a one of the markers for prostatic cancer. Also, higher levels of – glutamyl transferase (GGT) are found in the prostate glands, suggesting that high serum GGT level, in the presence of other incriminating factors, may be related to prostatic cancer (Strasak *et al.*, 2008).

In recent years, studies on prostatic disorders, such as benign prostatic hyperplasia (BPH), have focused more attention on the metabolic syndrome and its major endocrine aberration-hyperinsulinaemia (Hammarsten and Hogstedt,2001; 2002; Nanadeesha *et al.*,2006; Ozden *et al.*,2007). According to Laaksonen *et al*.(2003; 2004), a reduced testosterone level has been linked to the metabolic syndrome. However, the prostate gland tissue is known to be a testosterone-dependent organ

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and, consequently, when the testosterone level decreases as a result from an increasingly pronounced metabolic syndrome, the growth-stimulating effect on the prostate gland by other aberrations might possibly be reduced. Ross *et al.*(1992) however, demonstrated that young African-American men had serum testosterone levels that were approximately 15% higher than their white counterparts and have suggested that this difference is enough to explain the increased risk for prostate cancer in African- American men. The hormonal hypothesis seems to be one of the most important hypotheses in prostatic cancer etiology, and efforts are continuing to improve the understanding of androgen action in prostatic cancer (Imamoto *et al*.,2009). Although evidence from epidemiological studies of an association between circulating levels of androgens and prostatic cancer risk has been inconsistent, the traditional view that higher testosterone represents a risk factor for prostatic cancer appears to have little evidentiary support (Morgentaler,2007). However, epidemiological and biological differences in prostatic cancer are generally believed to exist between Western and Asian men. Investigating the association between serum testosterone levels and prostatic cancer in a Japanese population thus seems important (Immamoto *et al*.,2008).

A number of studies have found that cigarette smoking may be a risk factor for the development of prostate cancer (Hsing *et al*.,1990). This report demonstrated a relative risk of 1.8 and 2.1 for cigarette smoking and chewing tobacco, respectively. A case–control study of 382 men by Fincham et al.(1990), however, did not find a link between smoking and prostate cancer. Smoking is known to cause serious health hazards, some of which may be similar to those caused by various environmental pollutants. Gasoline vapours contribute various ubiquitous pollutants, which may pose threat to health, into the environments. Human health risks from intermetent, low- dose exposure to gasoline vapour is not quite consistent. To identify the potential health risk of chronic exposure to unleaded gasoline (UG), American Petroleum Institute sponsored a cancer bioassay, in which B6C3F1 mice and F-344 rats were exposed to UG vapour for 6 hrs/day, 5days/week for 2 years. The results indicated that the carcinogenic effects detected were the induction of male rat kidney tumours and female mouse liver tumours. The kidney tumours were believed to result from the interaction of the metabolites of certain isoparaffinic components of UG with a male rat-specific renal protein, 2µ- globulin (Chun *et al*.,1992; Hard *et al*.,1993). The accumulation of this protein in proximal tubule cells may lead to cytolethality, regenerative cell proliferation and ultimately, renal cancer (Borghoff *et al*.,1992). Other reports also indicate that UG vapours stimulate the growth of diethyl nitrosamine-induced hepatic preneoplastic lesions in mice, and induce an enzyme activity associated with cytochrome P_{450} 2B (Standeven and Goldsworthy,1994; Staneven *et al*.,1994). This study assessed the effect of exposure to gasoline vapours on the functionality of the prostate gland using serum total and prostatic acid phosphatase, alkaline phosphatase, gamma( ) - glutamyl transferase and testosterone levels in male rats.

MATERIALS AND METHODS

Animals and animal handling

Fourteen male albino Wistar rats weighing 100-150g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar, Nigeria and used for this study. The animals were allowed one week of acclimatization to laboratory conditions and handling, after which they were distributed, according to weight into two groups (Control and Test groups) of seven rats each. The animals were housed individually in cages with plastic bottom and wire mesh top (North Kent Co. Ltd) and fed with normal rat chow (Guinea Feeds Product) purchased from the High Quality Livestock Feeds stores, Calabar, Nigeria. They were supplied with tap water *ad libitum* throughout the experimental period. The animals in the control group were maintained in the gasoline vapours-free section of the animal room adequately ventilated under standard conditions (ambient temperature, 28 ± 2^{o}C, and relative humidity, 46%, with a light/dark cycle of 12/12h). While animals in the test group were kept in the exposure chambers (vapours cupboards) previously saturated with premium motor spirit (PMS) blend of gasoline vapours during the exposure periods. The liquid gasoline (PMS blend) was obtained from the Mobil Refueling station, Marian Road, Calabar, Nigeria.

All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

Exposure to gasoline vapours

The animals in the test group were wholly exposed to 17.8$cm^3h^{-1}m^{-3}kg^{-1}$(target concentration) of vapourized Premium Motor Spirit (PMS) blend of unleaded gasoline (UG) vapours for 8 hr/day, 6days/week, for 20 weeks in a glass exposure chambers(1.5m × 0.9m × 2.1m). Exposure conditions were chosen to reproduce those used in our previous studies (Uboh *et al*.,2007a; Uboh *et al*.,2007b; Uboh *et al* .,2008). The exposures were routinely conducted from 9.00am to 5.00pm on week days, including holidays to mimic workplace exposure. The chamber design, exposure generation system, and monitoring system were the same as those previously described (Uboh *et al*.,2007a; Uboh *et al*.,2007b; Uboh *et al*.,2008), with chamber concentrations of the UG determined daily. The average daily chamber concentrations of UG during exposure periods were 17.8±2.6cm^3 $h^{-1}m^{-3}kg^{-1}$ (about 85 .4 percent of target concentration) . At the end of each day's exposure period, the animals were transferred to gasoline vapours- free section of the experimental animal house and maintained under the same standard conditions as the animals in control groups until the next day. During the exposure period, the initial and final volumes of liquid gasoline were respectively recorded before and after daily exposure. The daily differences in volume were used to estimate the relative concentrations of vapours used in this exposure method.

Collection and handling of blood serum for Analyses

Twenty-four hours after last exposure, the animals were anaesthetized with chloroform vapour and dissected. Whole blood from each animal was collected by cardiac puncture into well-labeled non-heparinized sample tubes and allowed to clot for 3 hrs

Table 1. Effect of gasoline vapours on some serum prostatic cancer markers in rats

Group	TACP (U/L)	PACP (U/L)	ALP (U/L)	GGT (U/L)	Testosterone(ng/ml)
Control	10.88±1.02	3.46±0.74	203.15±12.86	46.30±3.44	3.74±1.20
Test	18.95±2.18*	6.02±1.82*	310.79±14.33*	84.81±9.95*	6.86±2.16*

Data are presented as mean ± SEM, n = 7, *P < 0.05 compared with control.

TACP = Total acid phosphatase, PACP = Prostatic acid phosphatase, ALP = Alkaline phosphatase, GGT = – glutamyl transferase.

in iced water. The serum was separated from the clots after centrifuging at 10,000 rpm for 5 min, using bench top centrifuge(MSE Minor, England), into well-labeled plain sample bottles, and used for assays.

Biochemical assays

Biochemical analyses carried out included measurement of the concentration of gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total and prostatic acid phosphatase (TACP and PACP, respectively) and testosterone in the serum. The measurements of the activities / concentrations of serum ALP, PACP and TACP were done by spectrophotometric determination of their absorbances, using analytical grade laboratory reagent kits obtained from Biosystems Laboratories (S. A. Costa Brava, Barcelonia, Spain) . While reagent kits from Randox Laboratories (United Kingdom) were used to assess the concentration of serum GGT. All absorbance readings were taken with DREL3000 HACH model spectrophotometer. Also, the serum was assayed for testosterone using enzyme immunoassay methods. The immunoassay reagent kits were obtained from Diagnostic Automation Inc., 23961 Craftman Road, Suite E / E, Calabasas, CA 91302. Microplate reader (Dialab Instruments Ltd.) was used in taking the absorbance readings. Calculations of the concentration of the hormone was done according to the method described in the kits manual.

Statistical analysis

Students' t-test was used to evaluate the significance of the difference between the mean value of the measured parameters in the test and control groups. A significant difference was accepted at P< 0.05.

RESULTS

This study assessed the effect of exposure to gasoline vapours on serum total and prostatic acid phosphatase (TACP and PACP, respectively), alkaline phosphatase (ALP), gamma( ) - glutamyl transferase (GGT) and testosterone levels in male rats. The results of this study are summarized in Table 1. From the results obtained in this present study, it was observed that exposure to gasoline vapours caused a 74.12 ± 4.71, 74.00 ± 2.80, 53.00 ± 3.6, 87.17 ± 2.5 and 83.42 ± 5.2 percent increase in serum TACP, PACP, ALP, GGT and testosterone levels, respectively, in male rats. These results gave a clear indication that exposure to gasoline vapours produced in a significant increase (P < 0.05) in serum TACP, PACP, ALP,GGT and testosterone levels in male rats (Table 1).

From these results, it is evidenced that exposure to gasoline vapours may results in clinical conditions associated with increased serum acid and alkaline phosphatase, gamma glutamyl transferase and testosterone, such as prostatic disorders**.**

DISCUSSION

The epidemiologic and screening studies performed in the past years have raised several important questions about the causative factors and pathogenesis of this disease, but a definitive cause for various prostate disorders, such as prostate cancer, has not been well established. It has been apparent for several years that the age-related incidence rates as well as death rates from clinical prostate disorders vary dramatically from country to country. It is now generally accepted that the development of a fully malignant cancer cell requires multiple malignant genetic events, including those that initiate cell transformation as well as those that promote or encourage the transformation process (Pienta *et al*.,1989). Generally, prostate cancer for instance, as most other cancers, develops as the result of an interplay of genetic and epigenetic events, both of which may be affected by environmental risk factors (Carter and Coffey,1990; Carter *et al*.,1990a; Carter *et al*.,1990b; Pienta and Esper,1993).

The results of this study showed that exposure to gasoline vapours caused increase in serum total and prostatic acid phosphatase, alkaline phosphatase, gamma glutamyl transferase, and testosterone male rats. These results gave an indication that exposure to gasoline vapours may adversely affect the functionality of the prostate glands. The elevation of serum level of these bio-indices have been reported to be associated with prostatic cancer (Fang *et al*.,2008; Ross *et al*.,1992; Saito *et al*.,2006; Taira *et al*.,2007). This indicates that these enzymes and the androgen can be used as biomarkers for the assessment of prostatic cancer. Particularly, prostatic acid phosphatase is reported to have emerged as the world's first clinically useful tumor marker (Taira *et al*.,2007). The observations made from the result of this

present study therefore gave an indication that exposure to gasoline vapours may be considered to be one of the predisposing environmental risk factors for prostatic disorders. Although the specific mechanism(s) through which exposure to gasoline vapours causes increase in serum total and prostatic acid phosphatase, alkaline phosphatase, gamma glutamyl transferase, and testosterone male rats is not understood, several intriguing reports, especially the report by Ross *et al*.(1992), suggest that the endogenous level of androgenic hormones, either testosterone, or dihydrotestosterone, may play a pivotal role in the cause of prostate cancer. Generally, the androgens are believed to be important in the aetiology of prostate cancer. And several studies have identified relationships between pretreatment serum levels of testosterone and both clinical stage of prostate cancer and patient survival, suggesting that pretreatment serum testosterone level has potential as a prognostic factor for prostatic cancer (Chen *et al*.,2002; Freedland *et al*.,2005).The involvement of these hormones would explain, at least in part, why discerning risk factors for prostate disorders have been so difficult because androgen levels in an individual would result from the interplay of several different genes, including those for testosterone, 5-α-reductase, sex hormone binding globulin, and estrogen, as well as the environmental influence on these gene products, such as zinc intake, smoking, vitamin A, and dietary fat.

The results of this study suggested a probable increase risk for the development of prostatic disorders to be associated with exposure to gasoline vapours in rats model. The observations made in this study therefore increase the list of potential risk factors for prostatic disorders. However further study, using PSA (Prostatic specific Antigen) and histopathologic examination of the prostate tissue, to confirm and identify the specific probable risk of prostatic disorders observed to be associated with exposure to gasoline vapours in rats model is in progress. Occupation and smoking have been to be among the risk factors for prostatic cancer (Pienta and Esper, 1993). However, several studies examining the risk for prostate cancer and occupation and physical activity have had mixed results (Elghany *et al*., 1990). Checkoway *et al*.(1987), in a case–control study, found that 75% of 40 patients with prostate cancer had a history of farming compared with 37.5% of control men with benign prostatic hyperplasia. Also, other industries or occupations that have been associated with a higher incidence of prostate cancer include mechanics, newspaper workers, plumbers, and men in rubber manufacturing industries, but these reports have not been confirmed conclusively[7]. Industries that have received the closest scrutiny as increasing the risk for prostate cancer have been those in which workers are exposed to cadmium. Several studies, with varying results, have been done to determine whether cadmium exposure places an individual at greater risk for prostate cancer (Elghany *et al*.,1990). Most of these studies tend to support the hypothesis that cadmium exposure weakly increases the risk for prostate cancer. And it has been suggested that cadmium increases the risk for prostate cancer by interacting with zinc (Elghany *et al*.,1990). Also, a number of studies have found that cigarette smoking may be a risk factor for the development of prostate cancer (Giovannucci *et al*.,1999) . Moreso, Hsing *et al*.(1990) earlier reported the relative risks of 1.8 and 2.1 for cigarette smoking and chewing tobacco, respectively. The results of this present study identified a significant risk of prostate dysfunction to be associated with exposure to gasoline vapours in rat model. Hence , it may be concluded that exposure to gasoline vapours is likely to be one of the potential risk factors for prostatic disorders.

POTENTIAL CONFLICTS OF INTEREST: None

REFERENCES

Borghoff SF, Youtsey NL, Sweenberg JA (1992). A comparison of European high test gasoline and PS-6 unleaded gasoline in their nephropathy and renal cell proliferation. *Toxilet*. 63:21-33.

Carter HB, Coffey DS (1990). The prostate: an increasing medical problem. *The Prostate*. 16:39-48.

Carter HB, Piantadosi S, Isaacs JT (1990a). Clinical evidence for and implications of the multistep development of prostate cancer. *J. Urol*. 143:742-746.

Carter BS, Carter HB, Isaacs JT (1990). Epidemiologic evidence regarding predisposing factors to prostate cancer. *The Prostate*. 16:187- 197.

Checkoway H, DiFerdinando G, Hulka BS, Mickey DD (1987). Medical, life-style, and occupational risk factors for prostate cancer. *The Prostate*. 10:79-88.

Chen SS, Chen KK, Lin AT, Chang YH, Wu HH, Chang LS (2002). The correlation between pretreatment serum hormone levels and treatment outcome for patients with prostatic cancer and bony metastasis. *BJU Int*. 89: 710.

Chun JS, Burleigh-Flayer HD, Kintig WJ (1992). Methyl tertiary butyl ether: vapour inhalation oncogenicity study in F344 rats. *Bushy Run Research Center (BRRC)'s Report*; 91NOO13B. BRRC, Export, P.A.

Elghany NA, Schumacher MC, Slattery ML, West DW, Lee JS (1990). Occupation, cadmium exposure,and prostate cancer. *Epidemiol.* 1:107-115.

Fang LC, Dattoli M, Taira A, True L, Sorace R, Wallner K (2008). Prostatic acid phosphatase adversely affects cause-specific survival in patients with intermediate to high-risk prostate cancer treated with brachytherapy. *Urol.*.71(1):146-150.

Fincham SM, Hill GB, Hanson J, Wijayasinghe C (1990). Epidemiology of prostatic cancer: a case–control study. *The Prostate*. 17:189-206.

Freedland SJ, Isaacs WB, Platz EA, Terris MK, Aronson WJ, Amling CL (2005). Prostate size and risk of high-grade, advanced prostate cancer and biochemical progression after radical prostatectomy: a search database study. *J. Clin. Oncol*. 23: 7546–7554.

Giovannucci E, Rimm EB, Ascherio A, Colditz GA, Spiegelman D, Stampfer MJ, Willett WC (1999). Smoking and Risk of Total and Fatal Prostate Cancer in United States Health Professionals1. *Cancer Epidemiol. Biomarkers Prev*. 8(4): 277 - 282.

Hammarsten J, Högstedt B (2001). Hyperinsulinaemia as a risk factor for developing benign prostatic hyperplasia. *Eur. Urol*. 39: 151–158.

Hammarsten J, Högstedt B (2002). Calculated fast-growing benign prostatic hyperplasia—a risk factor for developing clinical prostate cancer. *Scand. J. Urol. Nephrol*. 36: 330–338.

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR (1993). Hazard evaluation of chemicals that cause

accumulation of alpha2? - globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. *Environ. Health Perspect*. 99: 313 -349.

Hsing AW, McLaughlin JK, Schuman LM, Bjelke E, Gridley G, Wacholder S (1990). Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. *Cancer Res*. 50:6836-6840.

Imammoto T, Suzuki H, Yano M, Kawamura K, Kamiya N, Araki K (2009). Does presence of prostate cancer affect serum testosterone levels in clinically localized prostate cancer patients? *Prostate Cancer Prostatic Dis.* 12: 78–82. doi:10.1038/pcan.2008.35.

Imammoto T, Suzuki H, Yano M, et al.(2008). The role of testosterone in the pathogenesis of prostate cancer. Int. J. Urol. 15(6) : 472 – 480.

Laaksonen DE, Niskanen L, Punnonen K, Nyyssönen K, Tuomainen TP, Valkonen VP (2003). Sex hormones, inflammation and the metabolic syndrome: a population-based study. *Eur. J. Endocrinol*. 149: 601–608.

Laaksonen DE, Niskanen L, Punnonen K, Nyyssönen K, Tuomainen TP, Valkonen VP (2004). Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care*; 27: 1036–1041.

Morgentaler A (2007). Testosterone deficiency and prostate cancer: emerging recognition of an important and troubling relationship. *Eur. Urol*. 52: 623–625.

Ozden C, Ozdal OL, Urgancioglu G, Koyuncu H, Gokkaya S, Memis A (2007). The correlation between metabolic syndrome and prostatic growth in patients with benign prostatic hyperplasia. *Eur. Urol*. 51: 199–206.

Pienta KJ, Esper PS (1993). Risk factors for prostate cancer. *Ann. Int. Med*. 118(10): 793 – 803.

Pienta KJ, Partin AW, Coffey DS (1989). Cancer as a disease of DNA organization and dynamic cell structure. *Cancer Res*. 49:2525-2530.

Ross RK, Bernstein L, Lobo RA, Shimizu H, Stanczyk FZ, Pike M, et al. (1992). 5-α-reductase activity and risk of prostate cancer among Japanese and U.S. white and black males. *Lancet*. 339:887-889.

Saito T, Kitamura Y, Komatsubara S (2006). Prognosis of prostate cancer with elevated prostatic acid phosphatase. *Acta Urologica Japonica*; 52(3):177-180.

Standeven AM, Blazer T, Goldsworthy TL (1994). Investigation of antiestrogenic properties of unleaded gasoline in female mice. *Toxicol. Appl. Pharmacol*. 127:233 - 240.

Standeven AM, Goldsworthy TL (1994). Identification of hepatic mitogenic and cytochrome p-450 - inducing fractions of unleaded gasoline in B6C3F1 mice. *J. Toxicol Environ. Health*. 43: 213 - 224.

Strasak AM, Rapp K, Brant LJ, Hilbe W, Gregory M, Oberaigner W et al. (2008). Association of -glutamyltransferase and risk of cancer incidence in men: A prospective study. *Cancer Res.* 68(10): 3970 - 3977. doi: 10.1158/0008-5472.CAN-07-6686

Taira A, Merrick G, Wallner K, Dattoli M (2007). Reviving the acid phosphatase test for prostate cancer. *Oncol. (Williston Park)*.21(8):1003-1010.

Uboh FE, Akpanabiatu MI, Ebong PE, Eyong EU (2005). Evaluation of the toxicological implications of inhalation exposure to kerosene and petrol fumes in rats. *Acta Biologica Szegiend*. 49 (3-4): 19-22.

Uboh FE, Akpanabiatu MI, Ebong PE, Umoh IB (2007a). Gender differences in the haematotoxicity and weight changes associated with exposure to gasoline vapours in wistar albino rats. *Acta Toxicologica*; 15(2): 125-131.

Uboh FE, Akpanabiatu MI, Atangwho IJ, Ebong PE, Umoh IB (2007b). Effect of gasoline vapours on serum lipid profile and oxidative stress in hepatocyte of male and female rats. *Acta Toxicologica*; 15(1): 13-18.

Uboh FE, Akpanabiatu MI, Ekaidem IS, Ebong PE, Umoh IB (2007c). Effect of inhalation exposure to gasoline fumes on sex hormones profile in Wistar albino rats. *Acta Endocrinol (Buc)*. 3(1):23-30. URL:http://acta-endo.ro/

Uboh FE, Akpanabiatu MI, Alozie Y (2008). Comparative effect of gasoline vapours on renal functions in male and female albino wistar rats. *J. Pharmacol. Toxicol*. 3(6): 478-484.