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## EVALUATION OF SOME HEAVY METALS IN PROSTATE CANCER PATIENTS IN ENUGU

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### ABSTRACT

The evaluation of some heavy metals, in prostate cancer patients and controls in Enugu was carried out. Venous blood samples were collected from 72 participants who gave informed consent for this study at Enugu, Nigeria; 36 prostate cancer patients and 36 healthy subjects as controls. Blood heavy elements were determined using Atomic Absorption Spectrometer. Data from this study were subjected to statistical analysis. Mean, standard deviation, student's t-test and correlations, were determined using statistical package for the social sciences (SPSS). Result shows that, blood Aluminum, Arsenic, Chromium and Lead, were significantly higher ( $0.03 \pm 0.02 \mu\text{g/dl}$ ;  $0.01 \pm 0.01 \mu\text{g/dl}$ ,  $0.02 \pm 0.01 \mu\text{g/dl}$ ;  $0.02 \pm 0.01 \mu\text{g/dl}$ ,  $0.10 \pm 0.04 \mu\text{g/dl}$ ;  $0.03 \pm 0.00 \mu\text{g/dl}$ ,  $13.77 \pm 4.16 \mu\text{g/dl}$ ;  $11.11 \pm 1.37 \mu\text{g/dl}$ , respectively) ( $p < 0.05$  in each case) in prostate cancer patients, compared with controls. The blood Al, As, Cd, Cr and Pd levels were significantly higher ( $p = 0.000$ ,  $p = 0.014$ ,  $p = 0.012$ ,  $p = 0.032$  and  $p = 0.003$  respectively) in prostate cancer patients 0-3 years duration compared with prostate cancer patients 4-6 years' duration.

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The blood Al, Cd, Cr, Pb and Hg levels were significantly correlated ( $p=0.024$ ;  $r=0.377$ ,  $p=0.081$ ;  $r=-0.294$ ,  $p=0.081$ ;  $r=-0.294$ ,  $p=0.002$ ;  $r=-0.503$  and  $p=0.033$ ;  $r=-0.357$  respectively) with free-PSA in prostate cancer patients. From the result of study, it seems that heavy metals are raised in prostate cancer patients, and thus may play some role in the pathogenesis of the disease.

*Keywords: heavy metals, prostate cancer, aluminum, arsenic, chromium and lead*

## MATERIALS AND METHODS

### STUDY AREA

The study was carried out at Christ Our Foundation Specialist Medical Center 23B Hillview Independent Layout Enugu in south eastern region of Nigeria.

### ADVOCACY, MOBILIZATION AND PRE-SURVEY CONTACT

With a letter of introduction from the department, we met the Chief Medical Director(C.M.D) of the Hospital. On request a proposal was submitted to the ethical committee of the Hospital and approval secured. Several meetings were held with the Nurses in charge of oncology unit, days were noted and chosen for the collection of sample. Inform consent was orally gotten from the participants.

### STUDY POPULATION

One hundred and eight(108) subjects were recruited into the study, comprising thirty six(36) controls ,thirty six(36) BPH and thirty six(36) prostate cancer patients in the study population.

**SAMPLE SIZE;** The sample size was determined using the method of Aroye 2004 with the formula  $n=(z^2pq)/d^2$ .

## SELECTION CRITERIA

### 1;INCLUSION

- (i)Men with BPH and men with prostate cancer of 40-70 years,
- ( ii) psa levels > 4.0ng/ml

### 2;EXCLUSION

- (i)Men below 40 years of age.

## STUDY PARAMETERS

The parameters studied in this work include; PSA (total and free),Some heavy metals(Aluminum, Arsenic, Cadmium, Chromium, Lead and Mercury).

## SAMPLE COLLECTION

Six (6)ml of venous blood was collected aseptically from each subject by venipuncture from the anticubital vein. The blood was dispensed into lithium heparin container for heavy metals analysis.

## LABORATORY PROCEDURES

Some of the reagents used were commercially purchased and the manufacturers standard operational procedure (S.O.P) strictly adhered to, while some were self -prepared using chemicals of analytical grade.

## **(A)Determination of Serum Total PSA and free PSA**

### **Immunoenzymometric assay (type 3) (Junker et al.,1997)**

The essential reagents required include high affinity and specificity (enzyme and immobilized), with different and distinct epitope recognition in excess, and native antigen.

### **ASSAY PROCEDURE (total and free PSA).**

After formatting the microplates' wells for each serum, reference calibrator and control, 25µl and 50µl (for total and free PSA )of serum, calibrator and control samples were pipetted into respective wells.100µl of respective enzyme reagent was added, swirled gently for 20-30seconds.The mixture was incubated 30 and 60 minutes for total and free PSA respectively, washed with wash buffer and 100µl of respective substrate solution added and also incubated at room temperature for 15minutes without shaking. Stop solution was added at the end of fifteen (15) minutes of incubation. The absorbance was read using microplate reader at 450nm.

### **Digestion of Samples for heavy elements (metals) (Adrian, 1973)**

**Reagent:**Concentrated Nitric acid(for digestion of blood sample).

### **Procedure**

Fresh whole blood collected in special lead-free tube containing lithium heparin was used for blood heavy elements assay. One (1ml) of whole blood was pipetted into different test tubes, and 1ml of nitric acid was added to each test tube with the sample. The mixture was boiled at 100<sup>0</sup>C for 30 minutes, and then 9mls of distilled water was added to each tube the content was mixed and filtered using filter paper to get clear solution. Then the mixture was transferred for analysis.

**(B)Method of Determination of some blood Heavy Metals**

Atomic absorption spectrophotometry (AAS) as previously described by Alpha, (1995) was the method employed in the assay of blood Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury(Hg), Chromium(Cr) and Aluminum(Al) using Varian AA240 atomic spectrometry.

Atomic spectrometry is designed to determine the amount (concentrated) of an object element in a sample, utilizing the phenomenon that the atoms in the ground state absorb the light of characteristic wavelength passing through an atomic vapor layer of the element.

Normally the apparatus consists of a light source, a sample - atomizer, spectroscope, and a photometer, including a reading system. Some are equipped with a background compensation system. A hallow cathode lamp and a discharge lamb are mainly used for the light source. The sample atomizer is composed of a burner and a gas-flow regulator and also the spectroscope, a grating for double-de-ionized water.

The sample solution was taken for atomic absorption spectrophotometric analysis of blood heavy element using Varian AA240 Atomic Absorption spectrophotometer (APHA, 1995), as previously described.

The specific light source lamp to the lamp housing was fixed, and the instrument was switched on. The source lamp was lighted and the wave length dial of the spectroscope was adjusted to the wave length of the analytical line specified and set at an appropriate current value and slit-width. Using the supporting gas and combustibile gas specified, the mixture of gases was ignited, and the gas flow rate and pressure was adjusted, then the zero adjustment was made after neutralizing the solvent into the flame. Using wave lengths of 228.8nm for cadmium, 217.3nm for lead,

253.7nm for mercury,193.7nm for Arsenic, 576nm for Aluminum, and 357.9nm for Chromium respectively and the instrument was calibrated before use.

A one-point calibration for quick direct concentration analysis was employed. Standard for each of the metals analyzed was employed all through. For each metal analysis, the standard was used to standardize the instrument and the reading was taken. Then the digested sample in duplicates was analyzed by the instrument, and the average absorbance reading noted. Repeated analysis of standard solution confirmed the method's precision. The blood levels of heavy and trace elements were expressed in microgram per deciliter ( $\mu\text{g}/\text{dl}$ ). The detection limit of the instrument was 1  $\mu\text{g}/\text{dl}$ .

### **(C)Atomic Absorption Spectrophotometry (AAS)**

#### **Preparation of Reference Solution**

A series of standard metal solution in the optimum concentration range was prepared. The reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5ml concentrated nitric acid/liter. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

## Calculation

The concentration for blood heavy metals were calculated using the formula.,

$$\frac{\text{Reading of sample}}{\text{Reading of standard}} \times \text{concentration of standards} = R.$$

$R \times \text{Final volume after digestion of sample} = \text{concentration of sample (ug/dl)}$ . Conversion factor

$$\text{ng/dl} \div 10 = \mu\text{g/dl}$$

$$\mu\text{g/L} \times 100 = \mu\text{g/dl}$$

## Statistical Analysis

All data generated from this study were subjected to statistical analysis using statistical package for the social sciences (SPSS) version 20. Values were expressed as Mean  $\pm$  SD, at 95% confidence limit. Results are presented in table.

## RESULTS

The blood Al, As, Cr and Pd levels were significantly higher ( $p=0.000$ ,  $p=0.018$ ,  $p=0.000$  and  $p=0.001$  respectively) in prostate cancer patients compared with controls. The blood Cd level was not significantly higher ( $p=0.706$ ) in prostate cancer patients compared with controls. The blood Hg level was not significantly lower ( $p=0.190$ ) in prostate cancer patients compared with controls.

**Table on study of Blood heavy elements in prostate cancer patients versus controls**

<b>Variables mean±SD</b>	<b>Prostate Cancer patients n =36</b>	<b>Controls n =36</b>	<b>t-value</b>	<b>p-value</b>
<b>Aluminum</b>	0.03±0.02	0.01±0.01	4.245	0.000
Lower 95% C.I	0.02	0.00		
Upper 95% C.I	0.03	0.01		
<b>Arsenic</b>	0.02±0.01	0.02±0.01	2.485	0.018
Lower 95% C.I	0.02	0.01		
Upper 95% C.I	0.02	0.02		
<b>Cadmium</b>	0.10± 0.04	0.09± 0.02	0.380	0.706
Lower 95% C.I	0.08	0.09		
Upper 95% C.I	0.12	0.10		
<b>Chromium</b>	0.10±0.04	0.03±0.00	7.270	0.000
Lower 95% C.I	0.08	0.02		
Upper 95% C.I	0.12	0.04		
<b>Lead</b>	13.77± 4.16	11.11±1.37	3.568	0.001
Lower 95% C.I	12.36	10.64		
Upper 95% C.I	15.18	11.57		
<b>Mercury</b>	0.12±0.01	0.12±0.01	-1.337	0.190
Lower 95% C.I	0.11	0.12		
Upper 95% C.I	0.12	0.12		



## DISCUSSION

The blood Al, As, Cr and Pd levels were significantly higher in prostate cancer patients compared with controls. The blood Cd level was not significantly higher in prostate cancer patients compared with controls. The blood Hg level was not significantly lower ( $p=0.190$ ) in prostate cancer patients compared with controls. The prostate cancer may increase the levels of these heavy metals (Ofor *et al.*, 2016; Ozims *et al.*, 2018; Obeagu *et al.*, 2017)

## CONCLUSION

The blood Al, As, Cr and Pd levels were significantly higher in prostate cancer patients compared with controls. The blood Cd level was not significantly higher in prostate cancer patients compared with controls.

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