



## Prostate Specific Antigen (PSA) Screening among Apparently Healthy Men of African Descent in Sokoto, North Western, Nigeria

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors OE, IZ, RAS and IPI designed the study. Author ASM performed the statistical analysis. Author OE wrote the protocol and first draft of the manuscript. Authors AF and DI managed the analyses of the study. Authors EKU and OOI managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** Globally prostate cancer is the sixth leading cause of cancer-related death in men. Prostate Specific Antigen (PSA) is present in small amount in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer and other prostate-related disorders. The aim of this present study was to determine the PSA levels among healthy men of African descent resident in Sokoto, North Western Nigeria.

**Methods:** Testing was carried out using the CTK Biotech PSA kit (CTK Biotech Inc, San Diego, USA). The Onsite PSA Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of prostate specific antigen (PSA) in human serum or plasma at a cut-off level of 4.0 ng/mL.

**Result:** We investigated the PSA levels in 106 consecutively recruited men of African descent with age range and mean age of 40-70 years and  $44.75 \pm 7.91$  years respectively. We observed a PSA of  $> 4\text{ng/mL}$  among 7 (6.6%) of subjects studied. Men  $> 60$  had the highest prevalence of raised PSA level (33.3%) compared to those younger men ( $p= 0.001$ ). We observed a higher prevalence among farmers (11.1%) compared to business men, civil servants and students.

**Conclusion:** In this present study we observed a high prevalence of PSA  $>4\text{ng/mL}$  and a positive and significant correlation between age and raised PSA levels among men in Sokoto, North Western Nigeria. We recommend an annual PSA blood test for men  $\geq 40$  years of age and that those with a PSA value  $> 4.0 \text{ ng/mL}$  should be considered for further evaluation. There is also the need to build capacity among biomedical and medical staff in Nigeria to facilitate effective diagnosis of prostate cancer. There is also the need for increased prostate cancer awareness in the area to facilitate voluntary testing.

*Keywords: Prostate specific antigen (PSA); apparently healthy men; Sokoto; Nigeria.*

## 1. INTRODUCTION

Prostate Cancer has become the number one cancer in men with increasing incidence and morbidity in men of black African ancestry [1]. PSA is present in small quantities in the serum of men with healthy prostates, but is often elevated in patients with prostate cancer or other prostate-associated disorders [2]. Although (PSA) is a controversial laboratory test for the diagnosis of prostate cancer, it however remains a useful parameter for monitoring treatment. Prevalence of PSA levels greater than  $4\text{ug/l}$  among US men is 14% [3]. Elevated serum PSA concentration has been reported as a common finding in patients with prostate cancer, benign prostate hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues, but not in apparently healthy men, men with non-prostate carcinoma, apparently healthy women, or women with cancer. Studies have suggested that serum PSA is one of the most useful tumour markers in oncology. PSA measurements can enhance early prostate cancer detection particularly when combined with digital rectal examination (DRE). It may also serves as an accurate marker for assessing response to the treatment of prostate cancer. Therefore, measurement of serum PSA concentration can be an important tool in monitoring patients with prostate cancer and in determining the potential and actual effectiveness of surgery or other therapies.

Previous report in Nigeria [4] found that 85.1% prostate cancer patients had total PSA above the normal cut off level. The mean total PSA levels observed in prostate cancer subjects was found to be  $92.6\text{ug/l}$  in Ibadan [5] and  $106\text{ug/l}$  in Ile Ife [6] both in South Eastern Nigeria. There is paucity of data on PSA levels in men, the risk of prostate cancer among men in Sokoto state in North Western zone of Nigeria is not known and it is not known whether there are regional differences in prostate cancer incidence or prevalence in different regions of Nigeria. This present study is aimed at investigating the pattern of prostate-specific antigen (PSA) distribution among men in Sokoto, Nigeria.

## **2. MATERIALS AND METHODS**

### **2.1 Methods**

Testing was carried out using the CTK Biotech PSA kit (CTK Biotech Inc, San Diego, USA). The Onsite PSA Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of prostate specific antigen (PSA) in human serum or plasma at a cut-off level of 4.0 ng/mL. It is intended to be used as a screening test and as an aid in the diagnosis of prostate cancer. Any reactive specimen with the On Site PSA Rapid test must be confirmed with alternative testing method(s) and clinical findings.

### **2.2 Summary and Explanation of the Test Procedure**

PSA is a serine protease with a molecular weight of approximately 34,000 daltons containing 7% carbohydrate by weight. PSA is immunologically specific for prostatic tissue, existing in normal, benign hyperplastic, in malignant prostate tissue, in metastatic prostate carcinoma, and in prostate fluid and seminal plasma [7]. The On Site PSA Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of a burgundy colored conjugate pad containing anti-PSA antibody conjugated with colloid gold (PSA antibody conjugates), a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with polyclonal anti PSA antibody, and the C band is pre-coated with goat anti-mouse IgG antibody. When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the test cassette. Elevated PSA if present in the specimen will bind to the PSA antibody conjugates. The immune complex is then captured on the membrane by the pre-coated anti-PSA antibodies, forming a burgundy colored T band, indicating a PSA positive test result. Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immune complex of goat anti- mouse IgG /mouse IgG-gold conjugate regardless of color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

### **2.3 Sample Collection**

Blood specimen was collected by venipuncture into a plain tube without anticoagulant. Blood sample was allowed to clot and serum was separated by centrifugation. Serum sample was carefully withdrawn into a new pre-labelled tube. Samples were tested as soon as possible after collecting. When testing had to be delayed, samples were stored at 2°C - 8°C. Samples stored at 2°C - 8°C was viable for testing for up to 5 days. The manufactures standard operating procedure was followed strictly.

### **2.4 Assay Procedure**

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
3. Label the device with specimen's ID number.

4. Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 2- 3 drops (about 60- 90  $\mu$ L) of specimen into the sample well making sure that there are no air bubbles.
5. Set up the timer and read the results in 10 minutes.
6. Results were negative if only the C band is developed and indicates that no detectable PSA is present in the specimen. If both C and T bands are developed, the test is positive and indicates that the level of PSA in the specimen is higher than 4 ng/mL. If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below.

## **2.5 Limitations of Test**

The On Site PSA Rapid Test is limited to the qualitative detection of PSA at a cut-off level of 4.0 ng/mL in human serum or plasma. The intensity of the test band does not have linear correlation with the level of PSA in the specimen. A negative result for an individual subject indicates the level of PSA is not detectable but may not preclude the possibility of prostate cancer.

## **2.5 Inclusion Criteria**

Inclusion criteria include age  $\geq$  40 years, history of African descent, residence in Sokoto State, no previous history of prostatitis-related symptoms, no history of prostate tissue sampling at least two weeks prior to testing and willingness to provide informed consent prior to testing.

## **2.6 Exclusion Criteria**

The following persons were excluded from this study; persons < 40 years, patients with prostatitis-related symptoms, Caucasians, non-residents of Sokoto State, history of prostate tissue sampling at least two weeks prior to testing and refusal to provide informed consent prior to testing

## **2.7 Study Design**

This study was a prospective case study designed to determine the PSA level among men of African descent residing in Sokoto, North Western Nigeria. The effect of socio-demographic factors were also compared statistically.

## **2.8 Informed Consent and Ethical Clearance**

Written informed consent was obtained from all participants recruited into this study. Ethical clearance was obtained from the Ethical committee in the Faculty of Medical Laboratory Science Hospital Sokoto, Nigeria.

## 2.9 Statistical Analysis

Statistical analyses were conducted using SPSS (version 11; SPSS Inc., Chicago, IL) software. Data were expressed as mean  $\pm$  standard deviation. Mean PSA were calculated and compared across various subgroups using the two-sample independent t-test and Mann–Whitney U-test, respectively. PSA was summarized as grouped data classified into 2 levels;  $< 4$  ng/mL and  $> 4$  ng/mL. An alpha value of  $< 0.05$  denoted a statistically significant difference in all statistical comparisons. Correlation was compared using a version of linear regression analysis.

## 2.10 Study Population and Site

Subjects for this prospective case study included 106 men consecutively recruited men of African descent resident in Sokoto Nigeria. Study was carried out in the service laboratory in the Faculty of Medical Laboratory Science in Usmanu Danfodiyo University in Sokoto, North Western Nigeria. All the participants gave their written, informed consent and were offered pre- and post-test counselling.

## 2.11 Study Area

This present research work was carried out in the cosmopolitan city of Sokoto, in North Western Nigeria. Sokoto State is located in the extreme North Western part of Nigeria near to the confluence of the Sokoto River and the Rima River. With an annual average temperature of  $28.3^{\circ}\text{C}$  ( $82.9^{\circ}\text{F}$ ), Sokoto is, on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under  $40^{\circ}\text{C}$  ( $104.0^{\circ}\text{F}$ ). The warmest months are February to April when daytime temperatures can exceed  $45^{\circ}\text{C}$  ( $113.0^{\circ}\text{F}$ ). The rainy season is from June to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission respectively. Report from the 2007 National Population Commission indicated that the state had a population of 3.6 million [8].

## 3. RESULT

We investigated the PSA levels in 106 consecutively recruited men of African descent with age range and mean age of 40-70 years and  $44.75 \pm 7.91$  years respectively. PSA cut-off of  $> 4$ ng/mL was considered significant. We observed a PSA of  $> 4$ ng/mL among 7 (6.6%) of subjects studied. Table 1 shows the prevalence of high PSA levels ( $> 4$ ng/mL) among subjects studied. Men  $> 60$  years had the highest risk of prostate cancer (33.3%) compared to those younger. We grouped all subjects above 40 years and compared them against those who were  $< 40$  years. We observed that the prevalence of PSA  $> 4$ ng/mL was significantly higher among subjects  $> 40$  years (7.1%) years compared to those  $< 40$  years (5.6%) ( $p= 0.001$ ). Table 2 show the distribution of PSA results  $> 4$ ng/mL among subjects based on age range. We observed a higher prevalence among farmers compared to business men, civil servants and students. Table 3 show the distribution of PSA values  $> 4$ ng/mL based on professional groups.

**Table 1. Age-related distribution of PSA level higher than 4 ng/mL**

Age range (years)	Number screened	Number positive	% Positive	p-value
< 41	36	2	5.6	0.0001
41-50	48	3	6.3	
51-60	19	1	5.3	
> 60	3	1	33.3	

**Table 2. The prevalence of PSA level higher than 4 ng/mL among subjects**

PSA testing result	Number of subjects	% of subjects
Negative	99	9.34%
Positive (4.0 ng/mL)	7	6.6%

**Table 3. Distribution of PSA level higher than 4 ng/mL based on occupational groups**

Occupational groups	Number tested	Number positive	% Positive
Civil Servants	53	3	5.66
Business Men	15	1	6.67
Farmers	17	2	11.77
Traders	14	1	7.14
Students	7	0	0

#### 4. DISCUSSION

In this present study, we observed a prevalence of PSA value > 4ng/mL among 6.6% of men of African descent residing in Sokoto, North Western, Nigeria. Our finding is consistent with findings from previous reports in other parts of Nigeria; Kano 16.5% [9], Zaria 9.2% [10], Benin 7.13% [11] and Maiduguri 6.15% [12]. There are several challenges associated with the effective diagnosis of prostate cancer using PSA testing in Nigeria; poor epidemiological data; PSA checks are not practiced routinely in Nigeria, role of lifestyle and behavioral patterns associated with prostate cancer are known and education about prostate cancer is often sparse [13]. There is remarkable lack of awareness of prostate cancer among the Nigerian urban populace. Previous report however found that 81.5% were willing to be screened for the disease [14]. Prostate cancer research in Nigeria is growing and seems multifaceted [15]. Prostate cancer rates in African-American have been reported to be high, suggesting genetic predisposition [16]. Previous reports in Nigeria indicated an increasing incidence of prostate cancer [17]. Developing countries appear to be undergoing a cancer epidemic similar to that in developed countries. There are several reasons that may be responsible for the low incidence of prostate cancer in the past; under reporting and lack of adequate diagnosis, impact of lifestyle changes (smoking and alcohol use, dietary change changes from core traditional to western foods, consumption of canned foods with chemical additives and preservatives and increased use of processed beverages [18]. Prostate cancer second only to liver cancer is presently the most common cancer among men over 18 years of age, with an increased relative frequency ratio of 13.2% in 1980–1988 to 16.14% in 1989–1996 [19].

In this study we have used PSA testing and cut-off > 4ng/mL as an index for determining the risk for prostate cancer among apparently healthy men in Sokoto Nigeria. There is divided

opinion about the utility and relevance of PSA testing in the diagnosis of prostate cancer. The United States Preventive Services Task Force (USPSTF, 2012) [20] does not recommend PSA screening, noting that the potential benefits of testing does not outweigh the treatment-associated harm and that the test may result in over diagnosis and overtreatment because most prostate cancer is asymptomatic for life and treatments involve risks of complications including impotence (erectile dysfunction) and incontinence. The USPSTF concludes "the potential benefit does not outweigh the expected harms. Also the National Health Service in the United Kingdom does not mandate nor advise for PSA test, but allows patients to decide based on their doctor's advice [21]. Over diagnosis was defined as the detection of prostate cancer through PSA testing that otherwise would not have been diagnosed within the patient's lifetime [22]. In the United States, Food and Drug Administration (FDA) on the other hand approved the PSA test for annual screening of prostate cancer in men of age 50 and older. PSA levels between 4 and 10 ng/mL (nanograms per milliliter) are considered to be suspicious and consideration should be given to confirming the abnormal PSA with a repeat test. If indicated, prostate biopsy is performed to obtain tissue sample for histo-pathological analysis. PSA testing may help 1 in 1000 men avoid death due to prostate cancer. PSA level > 4ng/mL is not equal to prostate cancer. Histological diagnosis is the golden standard. However general opinion seems to favour the use of PSA testing in the possible diagnosis and the determination of predisposition to prostate cancer but with a caveat that the medical community use the test as wisely and possibly in conjunction with histological analysis [23].

In this study among men of African descent, we observed that prevalence of PSA value >4ng/mL was significantly higher in men above 40 years compared to those < 40 years. Age and race seems a determining factor in the potential use of PSA measurement in prostate cancer diagnosis [24]. Our observed prevalence is however higher than a 1.7% prevalence of PSA of  $\geq 4$  ng/mL obtained from a previous hospital- based report in Nigeria [5]. A population based study in a rural Nigerian community obtained a prevalence of PSA > 4ng/mL among 15.7% of men > 50 years [25]. A previous report among Chinese men confirms that PSA level correlates with age [26]. It seems that ethnic differences exist in the age-related distribution of serum PSA in men [27]. Our finding although lower is also consistent with reports from other population and PSA-based screenings from other parts of the world; US (10–15%) [28-29], Netherlands (9.6%) [30], Singapore (13.1%) [31], Germany (17.2%) [32], Sweden (17–17.2%) [33], South African (15.2%) [34]. Our prevalence was also higher than a 3.4% prevalence of PSA > 2ng/mL observed among Japanese men [35]. Previous report suggest that the distribution and cut-off value of the serum PSA level in Korean men differ from those in other races and that the median and 95th percentile serum PSA levels of Korean men younger than 50 years were higher, but those for men 50 years old or older were lower than in other races [36]. The possible reasons for the low prevalence observed in our study compared to those observed in other developed countries could be due to under reporting and lack of adequate diagnosis. An elevated level of prostate-specific antigen (PSA) is correlated with the presence of prostate cancer. There is advocacy that for black men  $\geq 40$  years and men at high risk with a family history of the disease, a strategy consisting of an annual PSA blood test and digital rectal examination seems to be prudent. Use of age- and race-specific reference ranges for PSA seems the most appropriate approach in high-risk population as ours. Specifically among black men 40-49 years of age, those with a PSA value > 2.0 ng/mL should consider further evaluation [37]. There is advocacy for race-specific PSA reference ranges for the early detection of prostate cancer. This has been based on previous report that seems to indicate a higher age-specific serum prostate-specific antigen (PSA) values in African-American (AA) men without prostate cancer compared to white men [38]. Recent report

however does indicate that the minor differences in PSA reference ranges between AA and white men may not be of sufficient magnitude to recommend the use of race-specific PSA reference ranges for screening [39]. Our finding is also consistent with a previous report which reviewed of the medical records of 826 consecutive men who underwent one or more prostate biopsies at the Veterans Affairs Medical Center in Shreveport, USA. Data from a total of 752 consecutive men who were either white or African-American and whose indication for biopsy included a serum PSA of greater than 4.0 ng/mL and/or an abnormal digital rectal examination were analyzed to determine if serum PSA levels were associated with the patient's age, race, or prostate volume in men without prostate cancer. Of the 498 men without prostate cancer, 367 (74%) men were white and 131 (26%) were black. There were no racial differences in age or calculated prostate volume. Serum PSA levels and calculated PSA density were significantly (both  $p < .0001$ ) higher in African-American men than in white men. For African-American and white men, serum PSA values of greater than 4 ng/mL were associated with prostate cancer with sensitivities of 89.5% and 81.9%, respectively, and specificities of 38.2% and 52.3%, respectively [40]. Systematic review of Pub Med, EMBASE and Cochrane to examine the published literature reporting the cost-effectiveness of PSA-based screening seem to all concluded that PSA-based screening is readily available, does not require expensive equipment and is essential not invasive but a cost effective option in the diagnosis of prostate cancer particularly among younger men ( $\leq 60$  years of age) and at higher PSA levels ( $\geq 3$  ng/ml) [41].

We observed a higher prevalence of PSA value  $>4$ ng/mL among farmers (11.77%) compared to business men 6.67% and civil servants (5.66%). It is not known whether professions associated with extensive physical activity play a role in raised PSA values  $>4$ ng/mL. Physical activities (cycling) has been reported in a previous report to cause an average 9.5% increase in PSA, in healthy male cyclists  $\geq 50$  years old, when measured within 5 minutes post cycling. Based on this finding, the authors suggest a 24-48 hour period of abstinence from cycling and ejaculation before a PSA test, to avoid spurious results [42]. Also another previous report seems to suggest that extensive physical activity should be avoided before blood sampling for diagnostic purposes and, in case of an increase, the PSA concentration should be controlled after an exercise test [43].

## **5. CONCLUSION**

In this present study we observed a prevalence of PSA  $>4$ ng/mL of 6.6% and a positive and significant correlation between age ( $\geq 40$  years) and raised PSA levels among men in Sokoto, North Western Nigeria. We recommend an annual PSA blood test examination for men  $\geq 40$  years of age and that those with a PSA value  $> 4.0$  ng/mL should consider further evaluation. There is also the need to build capacity among Medical Laboratory Scientist and Medical staff in Nigeria to facilitate effective diagnosis of prostate cancer. There is also the need for increased prostate cancer awareness in the area to facilitate voluntary testing.

## **CONSENT**

All authors declare that 'written informed consent was obtained from the patient for the publication of this case report.



## **ETHICAL APPROVAL**

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## **ACKNOWLEDGEMENT**

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## **LIMITATIONS**

This study had several limitations; Samples size for this preliminary study is small. We hope to undertake a larger and multicentre study in future. Previous reports indicates that Prostate cancer incidence goes up with age. The number of older subjects included in this study is small. We hope to optimize the number in subsequent study.

## **COMPETING INTERESTS**

The authors declare that to the best of their knowledge, there are no conflicts of interest and no competing financial interests in relation to this work

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