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Research Article

# Prognostic Risk for Prostate Cancer According to Secretor Status

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**Abstract:**
**Background:** Prostrate cancer is a leading cause of cancer mortality in Nigerian males. The Prostate Specific Antigen (PSA) is known to be a specific marker for the diagnosis of prostate cancer. **Objective:** To investigate the association between secretor status and prostate specific antigen levels among apparently healthy males. **Materials and Methods:** This was a cross sectional study of 120 serially recruited apparently healthy males aged 40 years and above. Blood group of the subjects were determined by the Tile Hemagglutination test, the secretor status by the Tube Hemagglutination test while the PSA level was determined by Sandwich Enzyme-Linked Immunosorbent Assay. Data was analyzed using the Statistical Package for Social Sciences Version 23. $P < 0.05$ was considered significant. **Result**: 41.7% of the subjects who are non-secretors have PSA values of 4ng/ml and above compared to 10.2% for secretors. **Conclusion**: This finding suggests that individuals who are non-secretors may be at risk for developing prostate cancer compared to secretors.

**Keywords:** Secretor status, prostate specific antigen, prostate cancer

## Introduction

There is currently a disproportionate high prostate cancer-related mortality among Nigerian males compared to the Caucasian population [1]. Prostate Specific Antigen (PSA); a serine protease enzyme secreted almost exclusively by the prostate gland has remained the standard biomarker for diagnosing prostate cancer [2,4]. Secretors are individuals who produce A, B and H substances in their body fluids (saliva, sweat, tears, semen, serum, bile, colostrum) while non-secretor are individuals who do not produce these antigens in their body fluids. While secretors have the genotype SeSe/Sese, non-secretors have the genotype sese [4]. Recent studies have demonstrated associations among individuals secretor status, risk and prognosis in various diseases [5,6]. However, to date, few studies have investigated the association of the secretor

status with prostate cancer among individuals in the Nigerian population. The aim of this study was to investigate the association of the secretor status with risk of developing prostate cancer among apparently healthy individuals in Enugu, Nigeria.

## Materials and Methods

### Study Setting

The study was carried out in the Enugu State University of Science and Technology (ESUT) Teaching Hospital, Enugu, Nigeria. The ESUT Teaching Hospital is the major tertiary health facility for the state and is located at the center of the capital city also known as Enugu for easy accessibility to residents. Enugu State is made up of three senatorial zones namely Enugu West and North. The senatorial zones are divided into seventeen Local Government Areas comprising 450 communities. The state derived its name from the name of its capital and largest city, Enugu. It has an area of 7,161km$^2$ with a population of 3,267,837, lies between longitudes 6$^o$ 30'E and latitudes 5$^o$ 15'N and 7$^o$ 15'E. It is boarded by Abia State and Imo State to the South, Ebonyi to the East, Benue and Kogi States to the North and Anambra to the West. It comprises mainly the Igbo speaking tribe of South Eastern Nigeria, about 50% of which lives in the rural areas [7].

### Study Design

This was a cross sectional study involving 120 apparently healthy males aged 40 years and above recruited by convenient sampling within Enugu State University Teaching Hospital between April and December, 2023.

### Sample Size

The required minimum sample size was determined using the Lesley Kish's formula for estimating minimum sample size in health studies [8].

$$n = \frac{Z^2Pq}{d^2}$$

Where

n = minimum sample size

Z = two-sided percentage of point of the normal distribution corresponding to the

Required significant level (0.05) = 1.96.

P = prevalence of prostate cancer in a previous study = 25% (9) = 0.025

q = complimentary probability of P = 1-P

d = tolerable alpha error or level of precision = 5% = 0.05

n = 1.96 x 0.025 x (1-0.025/0.052)

= 3.8416 x 0.025 x 0.932/0.0025 = 38

**Inclusion Criteria**

Males aged 40 years and above were included in the study.

Males who had normal digital rectal examination (DRF) were included.

Only subjects that gave informed consent after an explanation of the research procedure and aim were included.

**Exclusion Criteria**

Males below the age of 40 years were excluded.

Males with abnormal digital rectal examination and/or diagnosed with conditions such as prostatitis and benign prostrate hyperplasia were excluded.

Subjects who did not give informed consent after an explanation of the research procedure and aim were excluded.

**Ethical Consideration**

The ethical clearance for the study was obtained from the Ethics Committee of the Enugu State University of Science and Technology Teaching Hospital Enugu with reference number NP/C-MAC/RA/035/101.4/38. Informed consent was obtained from all participants before being recruited for the study.

**Sample Collection**

Five milliliters (5ml) of venous blood were collected from subjects following standard venipuncture technique. About 2ml was dispensed into EDTA bottles for the determination of patients' blood group. The remaining 3ml of blood was centrifuged at 5000 revolution per minute for 5 minutes and the serum separated and stored at about -20$^{o}$C for the determination of prostate specific antigen levels.

**Determination of Blood Group**

The subjects blood group were determined using the slide haemagglutination technique with commercially prepared antisera (anti-A, anti-B, anti-AB and anti-D). 5% washed red blood cells were prepared using normal saline. Drop of washed red cell was mixed with equal drop of antisera in a grease-free white tile. The mixture was rocked for 10 minutes and observed for agglutination to detect the different blood groups of the subjects.

**Determination of Prostate Specific Antigen**

The prostate specific antigen level was determined by the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The desired number of coated wells in the holder was secured, 50µl of standard, specimen and controls were dispensed into the appropriate wells. 50µl of zero buffer was added into each of the well which was thoroughly mixed for 30 seconds and then incubated for 60 minutes at room temperature. The incubated mixture was removed by emptying the plate contents into a waste container and the microtitre wells were rinsed and emptied for five (5) times

with wash buffer and the wells were stroked unto blotting paper to remove all residual water droplets followed by the addition of 100µl of enzyme conjugate reagent dispensed into each well and was gently mixed for 10 seconds, and then incubated at room temperature for 10 minutes and the plate contents were emptied after incubations and the microtitre wells rinsed and emptied 5 times with distilled water and stroked sharply onto absorbent paper to remove residual water droplets. 100 TMB solution was dispensed into each well and was mixed for 10 seconds; this was incubated at room temperature for 20 minutes and the reaction was stopped by adding 100µl of stopping solution to each well which was gently mixed for 30 seconds to allow color development (blue to yellow) and the color read with a microplate reader with optical density set at 450mm.

### Determination of Secretor Status

After proper rinsing of the mouth of each participant with distilled water, a clean rubber band was given to the participants to chew to increase salivation. After discarding the first few drops, about 3mls of saliva was collected into a sterile plain container for the determination of secretor status. The saliva was transferred into a test tube and placed in a boiling water bath for 10 minutes to denature salivary enzymes. It was then cooled and centrifuged at 10,000 RPM for 5 minutes; the supernatant was harvested, equal volume of it was placed into three labelled tubes A, B and H. Equal volume of diluted Anti-A, Anti-B and Anti-H were added to the appropriate tubes; respectively. Control were included to ascertain the antisera. Each tube was mixed and incubated at room temperature for about 10 minutes. A drop of standard red cells A, B and H was added into the corresponding tubes A, B and H. Each content of the tubes was mixed and incubated further for 10 minutes at room temperature. Reaction or agglutination were observed. Control tubes was observed for agglutination to confirm potency of antisera. Absence of agglutination or reaction in tube A, B and or H indicates the presence of corresponding A, B and/or H substance in the urine. Agglutination in any test sample tube indicated absence of A, B and/or H substance in the urine.

### Data Analysis

Data was analysed using IBM statistical package for social sciences (SPSS) for windows version 23, Armok, NY, USA. This was presented with descriptive statistics as frequencies and percentages.

## Result

A total of 120 apparently healthy adult males participated in the study. The distribution of ABO blood group among the subjects are: blood group A 23 subjects, B 20 subjects, AB 10 subjects and O was 67 subjects. Out of the 23 subjects with blood group, 21 are secretors while 2 are non-secretors. For the 20 subjects with blood group B, 18 are secretors while 2 are non-secretors. Out of 10 subjects that were blood group AB, 9 were secretors while 1 was a non-secretor. Among the 67 blood group O subjects in the study, 60 are secretors while 7 are non-secretors (Table 1). The distribution of the secretor status among the subjects based on the RhD group (Table 2) revealed that out

of the 113 Rhesus positive subjects, 104 are secretors while 9 are non-secretors. Also, out of the 7 Rhesus negative subjects, 6 are prostate specific antigen (Table 3) revealed that subjects in the higher range had higher mean prostate specific antigen levels compared to those in lower secretor status (Table 4) revealed that 10.2% of secretors had serum prostate specific antigen values above 4ng/ml compared to 41.7% for the subjects who are non-secretors.

**Table 1.** Distribution of secretor status among the subjects based on ABO blood group

| Blood group | Secretor (%) | Non secretor (%) | Total (%) |
|---|---|---|---|
| A | 21(19.4) | 2(16.7) | 23(19.2) |
| B | 18(16.7) | 2(16.7) | 20(16.7) |
| AB | 9(8.3) | 1(8.3) | 10(8.3) |
| O | 60(55.5) | 7(58.3) | 67(55.8 |
| TOTAL | 108 | 13 | 120 |

**Table 2.** Distribution of secretor status among the subjects based on RhD blood group

| Blood group | Secretor (%) | Non secretor (%) | Total (%) |
|---|---|---|---|
| Rh (+) | 104(86.6) | 9(7.5) | 113(94.7) |
| Rh (-) | 6(5) | 1(0.83) | 7(5.8) |
| TOTAL | 110 | 10 | 120 |

**Table 3.** Age based concentrations of prostate specific antigen level of the subjects

| Age Group | Number | Mean ± SD |
|---|---|---|
| 40 – 49 | 44 | 6.31 ± 11.4 |
| 50 – 59 | 36 | 9.18 ± 15.20 |
| 60 – 69 | 22 | 10.23 ± 5.30 |
| 70 and above | 18 | 11.66 ± 8.79 |

**Table 4.** Prognostic risk for prostate cancer among the subjects based on secretor status

| PSA Level (ng/ml) | Secretor (%) | Non secretor (%) | Total (%) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| < 1 | 42(38.9) | 3(25) | 45(37.5) |
| 1.00 – 1.90 | 30927.8) | 2(16.7) | 32(26.7) |
| 2.00 – 2.90 | 25(23.1) | 2(16.7) | 27(22.5) |
| ≥ | 11(10.2) | 5(41.7) | 16(13.3) |
| TOTAL | 108 | 13 | 120 |

## Discussion

Prostate specific antigen is normally present in blood at low levels with increase in its levels suggesting the risk of cancer. In Nigeria, reference range of less than 4ng/ml are usually considered normal result, values above 4ng/ml are considered high while values between 4 and 10ng/ml are considered risk for prostate cancer [10]. In the present study, 41.7% of the subjects who are non-secretors have prostate specific antigen values of 4ng/ml and above compared to only 10.2% for the secretors suggesting that non-secretor are at high risk for developing prostate cancer compared to secretors. Studies on the relationship between secretor status and cancer has shown different results [11]. However, our present findings is in tandem with the claim for a significant association of non- secretion of ABH substances with various diseases [5,6]. The major differences between secretors and non-secretors is in their pattern of expression; the FUT 1enzyme (non-secretor) gene is expressed predominantly in erythroid tissue giving rise to FUT 1 enzyme whose products reside on erythrocytes whereas the FUT 2 (secretor) gene is expressed predominantly in in the body fluids and tissues giving rise to FUT 2 enzyme (secretor enzyme) a product that reside on body fluid. Prostrate caner is a multi-factorial disease, thus, the increased risk for prostate cancer among the non-secretors could be as a result of influence of environment factors such as exposure to engine exhaust, fuel, dust, heavy metals, high intake of animal fats like milk, intake of red meat on the FUT 2 gene expression. Thus, studies involving the relationship between FUT 2 gene expression and activities of prostate specific antigen (as it plays vital role in cell-cell adhesion, a primary characteristics of any cancer cell) are needed to support the present finding. Also, studies involving a larger sample size and multi centers are recommended to support the present finding.

## Conclusion

It can however be concluded based on the prostate specific antigen levels among the subjects that individuals who are non-secretors are at risk for developing prostate cancer compared to secretors.

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