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Assessment of Prostatitis and Histological Investigations in Okigwe Imo State and Umuahia, Abia State

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Abstract: The Assessment of Prostatitis Antigens and Histological findings was carried out in Okigwe L.G.A of Imo State and Umuahia North L.G.A in Abia State, Nigeria. A total of 1000 patients were examined in the two locations, 800 for Umuahia and 200 for Okigwe. Blood and tissue biopsy specimens were collected aseptically using standards Microbiological Laboratory Techniques. The patients were examined for Prostate Specific Antigen (PSA) both quantitative and qualitative tests and tissues processing tests. Out of 200 patients tested for PSA in Okigwe, 119 (59.5%) and 129 (64.5%) were the results obtained for quantitative and qualitative tests respectively. PSA test in Umuahia was 416 (52.0%) and 433 (54.1%) for quantitative and qualitative tests respectively. In this research, the patients PSA levels that was above 4.0ng/ml were sent for PSA for tissue histological processing test into benign and malignant classifications. A total of 535(53.5%) tissues were processed in the two locations, 24(4.5%) were malignant while 511(95.5%) were benign. It was observed that out 119 positive PSA patients in Okigwe, 6(5.0%) were malignant while 113(95.0%) were benign. In Umuahia, out of 416(50.0%) tissues processed, 18(4.3%) tissues were malignant while 398(95.7%) were benign prostatic hyperplasia (BPH). These patients were from the age group of 50 years and above with much number from the age group of 80 years and above. Earlier detection of PSA could prevent prostate biopsy that occurs due to increase level of PSA. Again, early detection and proper treatment can reduce the high level of PSA that aggravate prostate cancer.

Keywords: Prostatitis, Biospy, Benign and Malignant.

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Introduction

Prostatitis is a very common and multifaceted disease. It affects men of above 50 years of age and it is estimated that about 50% of all man will experience various forms of prostatitis at some time during their lives. It is the most common urological disorder in men under the age of 50 and the third most common urological disorder, after benign prostatic hyperplasmia and prostate cancer in men older than 50 (Collins *et al*., 2003). Common symptoms include pain (genitourinary, pelvic or rectal), voiding difficulty and sexual dysfunction. Men older than 50 years and above have a longer time to develop prostatitis disease than their younger counterparts (Ogwuegbu *et al*., 2018).

Scientists believe that prostate cancer begins with prostatic intraepithelial neoplasia (PIN) which means the microscopic changes in the size and shape of prostate cells. It is important to identify men with significant risk factors for prostate cancer. Risk factors include age, race/ethnicity, family history, and classification of PIN. Age is one of the strongest risk factors, with a rapid increase in risk after age 50 years. The risk is almost doubled if a man has a brother or father with prostate cancer, suggesting that there is a genetic component (Christine, 2012). If time is not critical and appropriate equipment is available, quantitative method of PSA screenings is preferable to detect the exert level of prostatitis (Nickel, 2003; Ogwuegbu *et al*., 2018). Age specific ranges to improve cancer and the continuous use of 4.0 ng/ml as standard cut-off point to consider biopsy. According to Cancer Research UK (2015) more than 34,000 new cases are diagnosed every year, making it the most common cancer in men in UK. It has been reported that there is significant increases in incidence of prostate cancer in many countries, including the UK (Sharp, 2000).

Materials and Methods

Study Areas

The study population came from two cities in South Eastern Nigeria; Okigwe L.G.A of Imo State and Umuahia North of Abia State, Nigeria. They are patients referred and patient attending Healing Cross Hospital Umuahia and God Heals Hospital Okigwe for urological cases. (The tests were analyzed at Healing Cross Diagnostic Center Umuahia).

Ethical Clearance

The clearance to obtain specimens and work with the people in the various hospitals was given by the Heads Medical Directors in charge of the locations after submitting the clearance letter from the Abia State University, Uturu ethical clearance committee to the various hospitals.

Study Population

A total of 1000 people were sampled in the two locations:- 800 were screened from Umuahia while 200 were screened from Okigwe. (okigwe is a semi-Urban city). Their ages range from 21- 80 and above with 7 class intervals and they were placed in age bracket of 10 intervals (eg, 21-30, 31-40 etc).

Specimen Collection For Prostate Specific Antigen (PSA) Test

1. About 3ml of Venous Blood of the target individual was withdrawn and put into a clean serum gel test tube (plan) and allowed to stand for 10-15minutes to clot.
2. The blood specimen was capped and labelled.
3. This serum was collected with pipette and stored at 20^0C. until required for use according to the manufacturer's of the equipment instruction (Cheesbrough, 2006).

Examination of Blood for PSA Using Acon Cassette Materials

Test cassettes, Droppers pipette, Buffer
Serum specimen, Timer.

Procedure

The PSA cassettes were removed from their sealed paunch
The PSA cassettes were placed on a cleaned and leveled surface.
With the dropper pipette, about 1ml of serum (approximately 40ul) was transferred onto the specimen area of the cassette.

One drop of buffer was transferred to the sample well on the PSA cassette to allowed the serum to migrate membrane and generate colour line.
The test was interpreted within 5 minutes. (A single line on the Acon cassette shows negative while double lines shows positive) (Cheng-Ching *et al*., 2015).

Examination of PSA Using Microtiter Plate Reader

All reagents were brought to room temperature and mixed by gently inverting the containers severally before use. The washing buffer was prepared by mixing 1 volume of wash buffer Concentrate into 60 ml of distilled water. The desired number of coated wells for the test were secured in the holder 50ul of standards, specimen and control was dispensed into the appropriate wells. 100ul of Zero Buffer was dispensed into each well. They were thoroughly mixed for 30 seconds. The mixed samples were incubated at room (18-25^{0}C) for 60 minutes in an incubator. The incubated mixture was removed by emptying the plate contents into a suitable waste container for disposal. The emptied microtiter wells were rinsed 5 times with distilled water. The wells were strike sharply onto absorbent paper to remove all the residual water droplets used. 100ul of Enzyme Conjugate Reagent was dispensed into each well and gently mixed for 10 seconds. The wells were incubated at room temperature (18-25^{0}C) for 60 minutes. The incubated mixture was emptied into a suitable waste container. The well was rinsed and emptied 5 times with distilled water with washing buffer. The wells was stroked sharply onto absorbent paper to removed residual water droplets used. 100ul of TMB Reagent was dispensed into the wells and gently mixed for 10 seconds. It was incubated at room temperature for 20 minutes. The reaction of the mixture was stopped by adding 100ul of Stop Solution in each well and gently mixed for 30 seconds to make sure that the blue color changes to yellow color completely. It was read using microtiter plate reader at 450 density within 15 minutes (Cheng-Ching *et al*., 2015).

Tissue Collection through Biopsy

- Trans rectal ultrasound and prostatic biopsy were carried out on the patient whose prostate specific antigen value was up to 10ng/ml.
- An ultrasound probe was gently inserted 3-4 inches into the rectum.
- The probe emitted sound wave that converted video images corresponding to different prostate zones.
- In one second about 10-12 tissue specimen were taken using the biopsy gun needle that was fired through the rectal wall.

Tissues Processing For Histological Examination

- ✓ The biopsied specimens were fixed in 10% formal saline.
- ✓ Due to the presence of calcium that clumped on the tissue, some of the tissues were decalcified with a chelating agent (EDTA).
- ✓ The tissue was dehydrated with ascending grades of alcohol; 30%, 50%, 70%, 90%, absolute alcohol 1, 2 & 3.
- ✓ The tissues were de-alcoholized with a clearing agent (Xylene).
- ✓ It was embedded in a molten paraffin wax and allowed to solidify.
- ✓ The tissue was sectioned using microtome.
- ✓ The sectioned tissue was dewaxed using water bath at 40^0 C -50^{0}C.
- ✓ The tissues were gently dried on a hot plate at 50%.
- ✓ The tissues were re-hydrate in two changes of alcohol; 95% alcohol for 2 minutes, 70 % alcohol for 2 minutes and briefly washed in water.
- ✓ A preservative and an adhesive solution of thymol was used in fixing the tissues on a clean grease free slide to prevent lifting during staining (Kiernan, 2016).

Results

The effect of diagnostic method on the prevalence of prostatitis in the study areas was shown in (table 1) using quantitative and qualitative methods. In Okigwe, the diagnosis difference with qualitative method was 10(5.0%) and in Umuahia 17(2.1). showing the total percentage diagnosis difference of 24(2.4%). The prevalence of the PSA level in prostatitis patients according to age in Okigwe and Umuahia using qualitative method (using Acon strip) was shown in (fig 1). Out of 200 patient tested in Okigwe, 129 (64.5%) were positive with 80 years and above had the highest positive result of 18 (90.0%) while 21-30 years had the lowest with 1 (20.0%).

In Umuahia, out of 800 patient tested with PSA strip 433 (54.35) patient were positive. Patients in 80 years and above had the highest PSA value of 40 (80.0%) while 21-30 years of age had the lowest positive result of 3(15.0%). Table 2: shows the Malignant and Benign Prostatic Hyperplasia results according to age distribution in Okigwe and Umuahia. In Okigwe, 6(5.0) specimen were malignant with 4(3.3) from the age of 80 years and above while 2(1.6) occurred from the age of 71–80. In Umuahia, 18(4.3) specimen were malignant with the highest occurrence from the age of 80 years and above with 9(2.3) and the least from 51-60 years with 1(0.2). Plate (1) shows the malignant prostate tissue slide, which shows the presence of fused/confluent gland, fibrotic stroma and hemorraige. Plate (2) shows The Benign prostate tissue slide that is made up of papillaries, fibromuscular stroma, epithelia and myo-epithelia cells. Plate (3) is the Ultrasound Image of an Enlarged Prostate Gland before Biopsy.

Table 1. Impact of diagnostic method on the prevalence of prostatitis in the study areas

	Okigwe	Umuahia				Total		
Diagnostic Methods	**NS**	**NI**	**%**	**NS**	**NI**	**%**	**NS**	**NI**	**%**
Qualitative	200	129	64.5 %	800	433	54.1 %	10000	562	56.2 %
Quantitative	200	119	59.5 %	800	416	52.0 %	10000	535	53.5 %
Difference	-	10	5.0 %	-	17	2.1 %	-	24	2.7 %

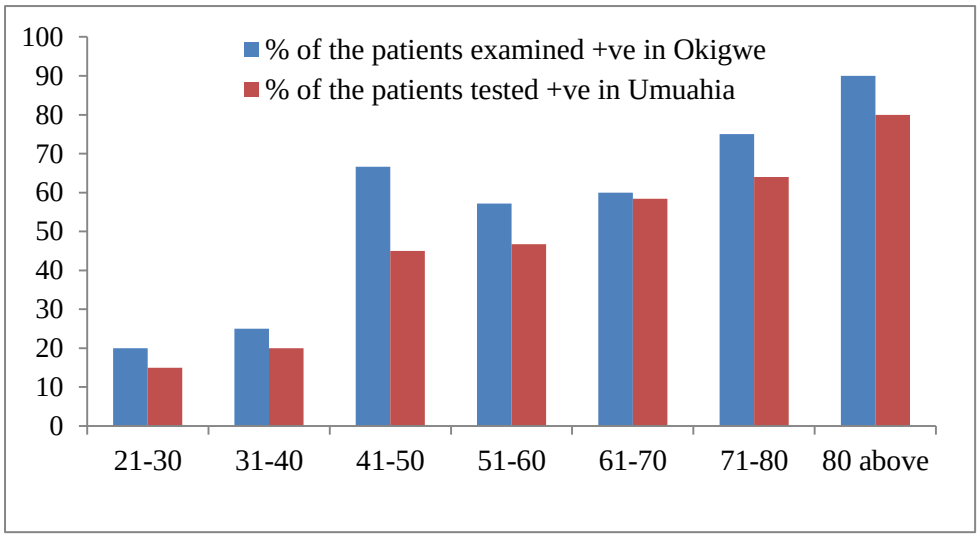


Figure 1. Prevalence of the PSA level in Prostatitis patients according to age in Okigwe and Umuahia using qualitative methods

Table 2. Malignant and Benign Prostatic Hyperplasia results according to age distribution in Okigwe and Umuahia

Age	No. of PSA positive patients in Okigwe	No. of malignant patients in Okigwe	No of benign patients in Okigwe	No. of PSA Positive patients in Umuahia	No. of malignant patients in Umuahia	No of benign patients in Umuahia
21-30	0 (0.0)	0 (0.0)	0 (0 .0)	1 (15.0)	0 (0.0)	1(0.2)
31-40	1(25.0)	0 (0.0)	1(2.7)	7 (20.0)	0 (0.0)	7(1.7)
41-50	8 (66.6)	0 (0.0)	8(10.7)	40 (45.0)	0 (0.0)	40((10.0)
51-60	20 (57.2)	0(0.0)	20(17.8)	68(46.7)	1(0.2)	67(16.8)
61-70	28 (60.0)	0(0.0)	28(26.8)	100 (58.4)	2(0.4)	98(24.6)
71-80	45 (75.0)	2(1.6)	43(38.4)	158 (64.0)	6(1.4)	152(38.1)
80 >	17 (90.0)	4(3.6)	13(12.5)	42(80.0)	9(2.1)	33(8.2)
Total	119 (59.5)	6(5.0)	113(95.0)	416 (50.0)	18(4.3)	398(95.7)

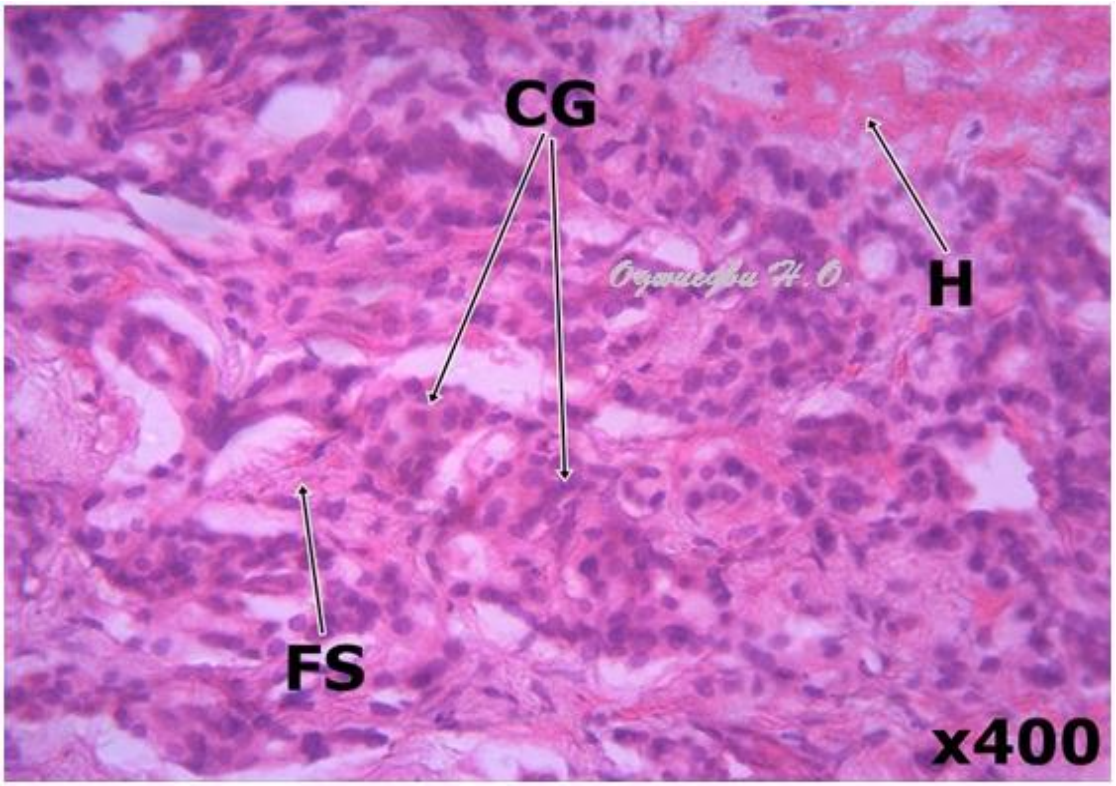


Plate 1. The malignant prostate tissue

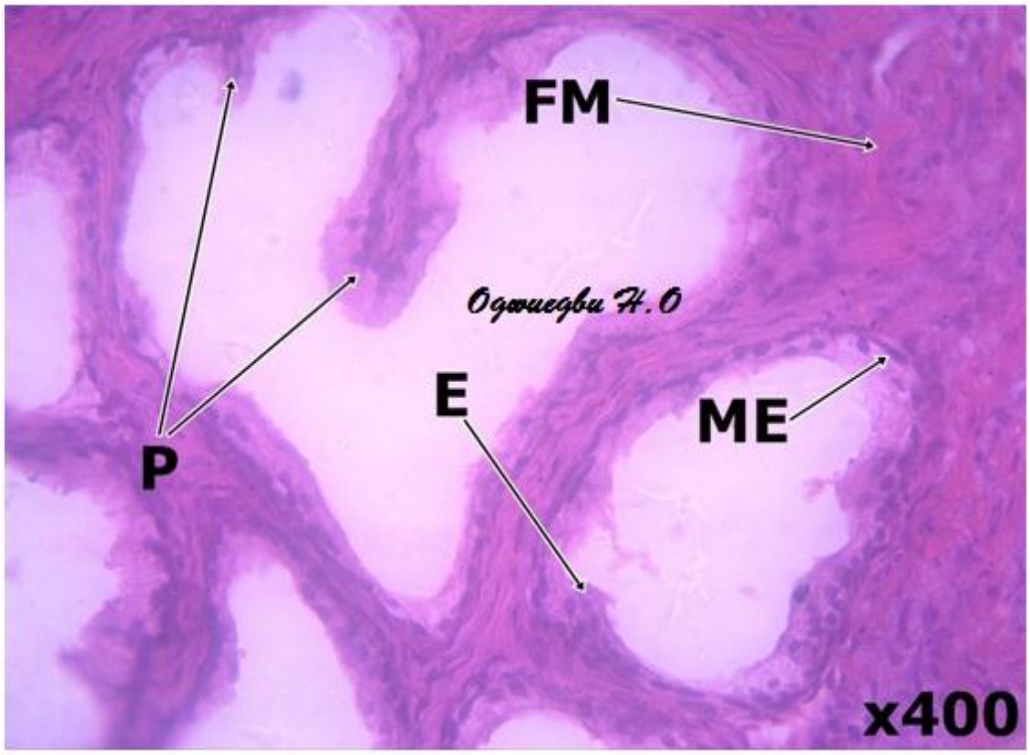
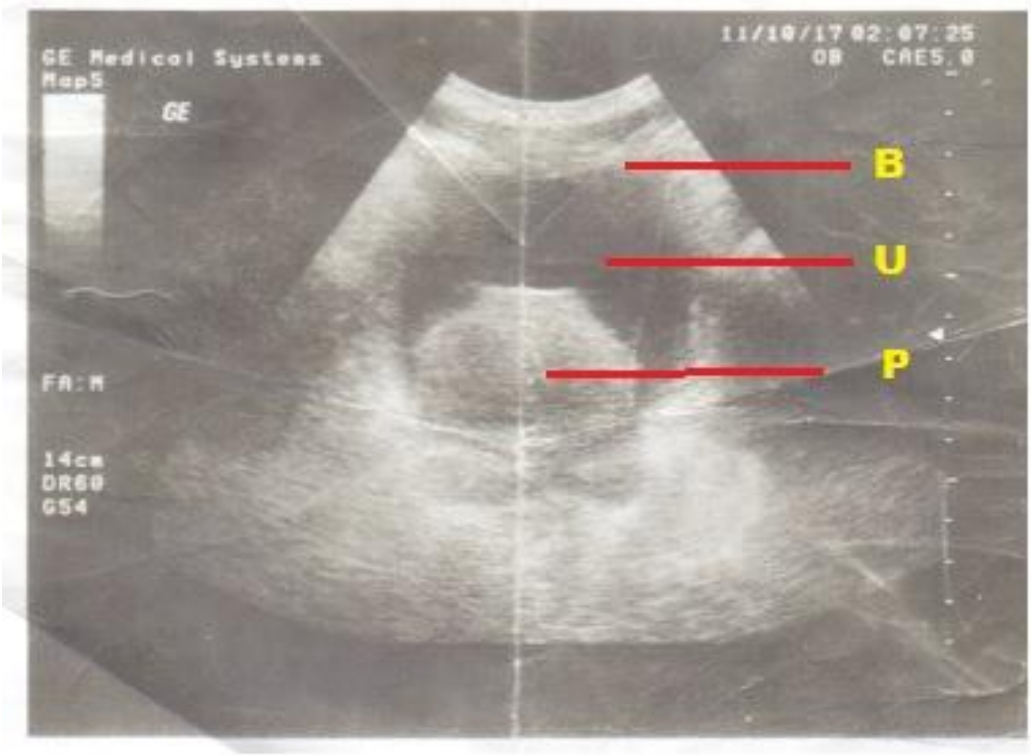


Plate 2. The Benign prostate tissue



B – Bladder, U –Urine, P– Enlarged Prostate
Plate 3: The Ultrasound Image of an Enlarged Prostate Gland before Biopsy

Discussion

In this research, the prevalence of prostatitis was examined in Umuahia and Okigwe both in Eastern Nigeria using prostate specific Antigen (PSA) screening tests. The study lasted for 12 months period. A total of one thousand (1000) patients were screened (two hundred from Okigwe and eight hundred from Umuahia) using both qualitative and quantitative tests. A total prevalence of 562(56.2%) was recorded for qualitative and 535(53.5%) for a quantitative. In Okigwe, the prevalence was 129(64.5%) for qualitative and 119(59.5%) for quantitative while in Umuahia it was 433(54.1%) for qualitative and 416(52.0%) for quantitative. The diagnostic methods of quantitative and qualitative diagnosis of prostatitis in the studied areas showed variations with diagnostic difference of 10(5.0%) in Okigwe and 24(2.7%) in Umuahia indicating significance difference between the methods (P=0.05). This observation corresponds with those of Cheng-Ching *et al.,* (2015), Ogwuegbu *et al.,* (2018) and Nickel (2003) who believed that PSA rapid test and clinical chemistry test are mutually exclusive methods and could defer.

In this research, the patients PSA levels that was above 4.0 ng/ml were sent for PSA biopsy for histological processing into benign and malignant classifications. A total of 535(53.5%) tissues were processed in the two locations, 24(4.5%) were malignant while 511(95.5%) were benign. It was observed that out 119 positive PSA patients in Okigwe, 6(5.0%) were malignant while 113(95.0%) were benign. In Umuahia, out of 416(50.0%) tissues processed, 18(4.3%) tissues were malignant while 398(95.7%) were benign. These patients were from the age group of 60 years and above with much number from the age group of 80 years and above. The positive CA patients were from the age of 60 years and above , this finding tallies with Scher et al., (2015) who said that within subject's risk factors for prostate cancer, family history and a highly significant association was observed as compared to risk seen in all ages. Age is an established risk factor for prostate cancer.

In the two locations; the benign and malignant slides were viewed and explained by a pathologist. The benign prostatic hyperplasia (BPH) slide is made up of papillaries, fibromuscular stroma, epithelia and myo-epithelia cells. The epithelia cells present in benign showed that they do not lined by the usual bi- epithelia layer and they made up of luminal epithelia and myo-epithelia cells. The benign usually display papillaries architectural design which is made up of vascular core lined by bi-cellular layer. While in malignant slide, it is made up of fused/confluent gland, fibrotic stroma and hemorraige. The hemorriage could caused due to biopsy or from the blood vessels. The confluent and individual glands seen in the malignant slide influence the stroma by giving it a fibrotic response which leads to excessive piloferation in the prostatic malignant slide. This work tallies with Collins *et al.,* (2003) who said that Prostatitis is a very common and multifaceted disease that it affect men of older ages and it is estimated that about 50% of all man will experience systems of prostatitis at some time during their lives.

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