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EVALUATING ENGRAILED-2 AND CYTOKINES IN URINE WITH SERUM PSA AS POTENTIAL BIOMARKERS IN PATIENTS WITH PROSTATISM AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA
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EVALUATING ENGRAILED-2 AND CYTOKINES IN URINE WITH SERUM PSA AS POTENTIAL BIOMARKERS IN PATIENTS WITH PROSTATISM AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

B. DONALD-BURI, K. PATEL, P. MUSAU, and E. N. FISH

ABSTRACT

***Background*: Prostatism is a clinical syndrome, occurring mostly in older men, usually caused by enlargement of the prostate gland and manifested by irritative and obstructive symptoms. Prostate cancer (CaP) is the most common non-cutaneous and the second leading cause of cancer related death in men. It is a disease in which cells in the prostate gland become abnormal and start to grow uncontrollably, forming tumours. The prostate specific antigen (PSA) test has been shown to be of low specificity and low sensitive and unable to differentiate the various forms of prostatism. Engrailed 2 and pro-inflammatory cytokines may be able to distinguish and stage protatism.**
***Objective*: To evaluate EN-2 and Cytokines in urine with serum PSA as potential biomarkers in patients with prostatism.**
***Design*: Cross sectional study**
***Setting*: Moi Teaching and Referral Hospital, Eldoret, Kenya.**
***Subjects*: Thirty nine (39) respondents were recruited in to the study. Thirteen (13) cases of prostate cancer and twenty six (26) controls were matched by age.**
***Results*: The mean age of cases was 73.1years and controls 71.1 years. Over 92.3% of the cases and 73.1% of controls had PSA values >4.0ng/ml. Both groups had elevated levels of urinary EN-2, which were statistically not significant. IL-6 and TNF-α expressions were higher in cases compared to controls with p-values of 0.0001 and 0.04 respectively. There was a good correlation between urinary IL-6 levels and urinary TNF-α levels (r=0.49)**
***Conclusion*: EN-2 expression in urine was not a predictive marker, however, IL-6 and TNF-α levels were elevated in urine of CaP patients. The potential for using these cytokines as urinary biomarkers to distinguish prostatism should be further explored.**

INTRODUCTION

Prostate cancer (CaP) is the most common non-cutaneous and the second leading cause of cancer related death in men and a disease in which cells in the prostate gland become abnormal and start to grow uncontrollably, forming tumours (1). Approximately 913,000 new cases of CaP were diagnosed worldwide in 2008 (1). It is predicted that the number of cases will almost double (1.7m) by 2030. In Kenya, the estimated numbers of new cases of CaP stands at 1007 per year. African-American males compared to Caucasian males have a greater number of prostatic intra-epithelial neoplasia (PIN) lesions, which are precursors to cancer, and larger tumours, possibly related to the higher levels of testosterone seen in African-American males (2). Among males there is a one in six lifetime probability of being diagnosed with prostate cancer (3). While localised or organ-confined CaP can be cured in a large proportion of patients by surgery or radiotherapy, prostatectomy may be a treatment option in metastatic CaP, although, majority of men with metastatic CaP, refuse the option of prostatectomy. Advanced and

metastatic CaP continues to be associated with a poor prognosis (4). Early diagnosis and treatment of CaP remains a challenge to physicians, as there are a number of key limitations with diagnosis based on prostate specific antigen (PSA), the only marker to diagnose the disease to date. Prostatic carcinoma can be suspected when elevated levels of PSA are found in the blood. However, PSA levels may also be elevated in benign prostatic hyperplasia (BPH) and in prostatitis. Furthermore, an elevated PSA level does not confirm that a patient has prostate cancer. In most laboratories a serum level of 4 ng/mL is used as the cut-off distinguishing between normal and abnormal. However, this simplified approach to serum PSA tests is not appropriate, and has led to the delay in diagnosis of many prostate cancers (3). Studies have shown that 22% of men with a normal DRE and a serum total PSA level between 2.6 and 4.0 ng/ml have CaP, and 81% of them have organ-confined disease (5). Data from the Prostate Cancer Prevention Trial (PCPT) revealed that as many as 15% of men with normal DRE and a serum total PSA less than 4.0 ng/ml have CaP (6). Thus, the PSA test suffers from both low sensitivity and low specificity, causing physicians to rely heavily on the histological examination of prostate biopsy as a differential diagnosis for CaP (3). Prostate biopsy is a special procedure carried out by urologists who are very few in number compared to the population. Most healthcare facilities cannot afford a histopathology laboratory nor hire a pathologist. In addition prostate biopsy is an invasive procedure which is not comfortable to patients. Inflammation, regardless of etiology is thought to incite carcinogenesis by (i) causing cell and genome damage, (ii) promoting cellular replacement and creating a tissue microenvironment rich in cytokines and growth factors that can enhance cell replication, angiogenesis and tissue repair (7). In a recent publication evidence was provided that inflammation may play a significant role in the pathogenesis of prostate cancer via increased activity of inflammatory cytokines, particularly IL-6 (8). Studying pro-inflammatory cytokines in CaP, it was found that there may be a link between high expression of pro-inflammatory cytokines, IL-6 and TNF-α and high serum levels of PSA with the progression of the cancer(9). A recent study demonstrated that EN-2 is expressed in urine and secreted by, CaP cells (11).

MATERIALS AND METHODS

Study design: The study employed a cross sectional design. Male patients of age 50 and above who presented at the urology clinic or admitted at the surgical ward were recruited. Patients who consented to the study were interviewed and questionnaires filled and had their early urine samples collected for EN-2, IL-6 and TNF-α. Blood samples were also collected for PSA test. Their files were also reviewed for abdominal ultrasound and prostate biopsy report to record the differential diagnosis.

Study area: The study was conducted at Moi Teaching and Referral Hospital (MTRH), at the urology clinic and surgical ward. MTRH is situated in Eldoret (the 5th largest town in Kenya) municipality, Uasin Gishu County, North Rift region of Kenya. It is the second teaching and referral hospital in Kenya after Kenyatta National hospital. It lies at 2300 metres above sea level, 310km from Nairobi, Kenya's capital city. Being the only referral hospital in Western Kenya, it has a catchment population of 13 to 15 million, about 40 percent of Kenya's population. It also receives patients from Eastern Uganda and South Sudan.

Sample size and sampling: A sample size of 39, including controls, was arrived at as follows: according to information from the Cancer Registry and average of 14 CaP cases/year had been reported in MTRH. Considering that data collection for this study was going to run for close to a year, the investigator assumed that all cases presenting with CaP within this period were to be recruited into the study. It was presumed that 13 cases may present within the period, and for every case, two controls were allocated, that is, 13+ [2x13] =39. Applying a formula to calculate sample size will require reference to a related study in the same region of which none has been conducted in this region. A large sample size would require a longer duration for the study which was suppose to last for only one year. Data was collected from Jan 2013 to December 2013.

Ethical clearance: Authorisation and clearance to carry out this study was obtained from Moi University (School of Medicine)/Moi Teaching and Referral Hospital (MUSOM/MTRH) Institutional Research and Ethics Committee (IREC). Participation was voluntary and informed consent was sought from all respondents before interview. Confidentiality was strictly observed during and after the interview. There was no monetary compensation for participation in the study; however, the scientific community is going to benefit from the outcome of the study. No risk was reported in the study subjects.

Data management and analysis: Data were obtained from patients files and laboratory data were obtained from the sample analysis. A questionnaire was administered to the participants through an interview. Data were analyzed using SPSS version 20.0 to establish descriptive statistics and derive correlations.

RESULTS

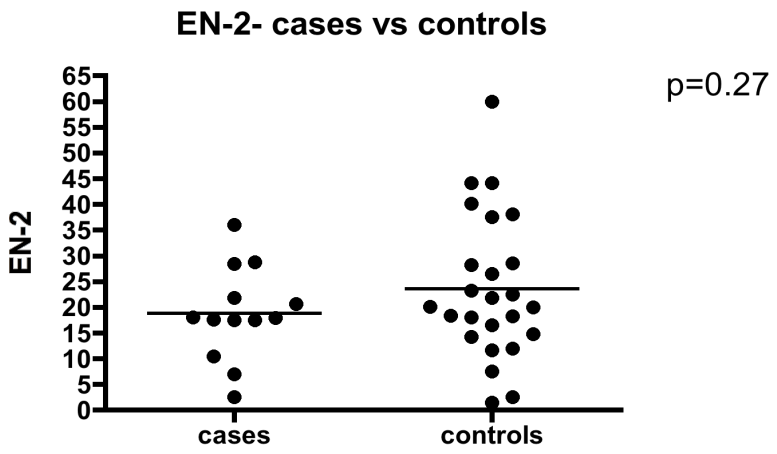
Demographic characteristics: A total of 39 (13 cases and 26 controls) patients, matched by age were included in the study. The mean age for the cases was 73.6 years with an age range of 61 to 94 years, while the mean age for the controls was 71.1 years with an age range 62 to 89 years. Majority of the cases were of the age group 60-69 years, same for the controls (Table 1). Cases and controls were matched by age to rule out age as a disease contributing factor.

Table 1
Results of the different biomarkers in cases and controls with their ages

CASES sample		Serum level	Urine level	Urine level	Urine level	CONTROLS		Serum level	Urine level	Urine level	Urine level
#	age	PSA	EN-2	IL-6	TNF-α	sample #	age	PSA	EN-2	IL-6	TNF-α
1	67	45.15	28.4	399.4	700.8	7	68	100	14.8	54.1	126.5
						11	67	1.42	18.2	12.2	75.3
8	65	44.65	28.8	367.9	150.8	28	64	0.399	7.5	15.2	72.3
						64	64	0.78	23.2	12.3	77.2
15	61	6.4	20.6	398.7	200.3	52	62	100	11.6	200.1	120.2
						35	63	0.604	12	14.1	69.5
16	66	7.82	10.5	306.9	475.7	59	62	22.49	2.5	24.3	71.2
						34	63	22.7	18	12.2	73.2
22	73	100	21.8	65.8	148.5	60	76	1.01	20.1	12.3	77.2
						13	73	15.72	44.1	25.1	148.4
23	79	0.622	17.6	89.5	70.8	48	81	14.96	26.5	15.2	73.2
						29	79	10.25	22.5	25.2	124.3
32	78	100	17.5	5	10.1	26	76	100	18.4	28.4	138.2
						54	77	4.36	16.5	18.2	71.2
40	94	179.43	7	300.5	180.6	37	89	45.95	14.2	17.1	80.3
						57	89	68.89	20	75.1	100.1
43	85	251	17.9	20.3	72.3	21	88	7.625	37.5	12.1	77.1
						58	88	49.39	28.5	12.1	72.6
44	67	100	17.5	20.5	77.1	31	69	5.72	>60	25.7	130.5
						55	68	1	28.2	9.2	74.5
38	65	10.47	18	30.7	10.2	50	68	5.1	38.1	18.1	145.6
						10	70	32.7	21.8	77.2	120.9
3	84	100	36	57.9	125.7	18	80	54.39	44.1	23	146.1
						4	85	2.4	40.1	25.1	130.7
61	73	100	2.56	210.5	122.1	63	73	21.14	1.5	18.1	20.2

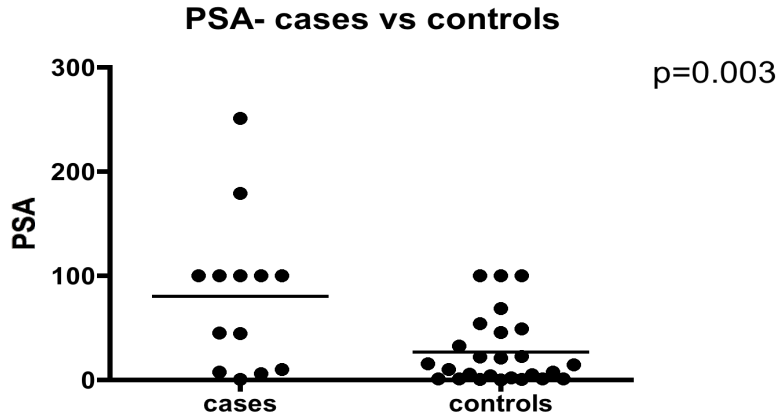
Expression of urinary en-2 levels: Both cases and controls expressed EN-2 in urine with controls showing higher concentration than cases.

Figure 1
Urinary EN-2 concentrations among cases and controls



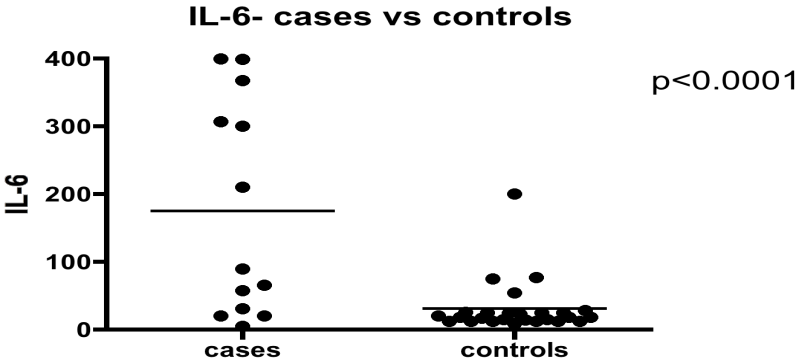
*Expression of serum psa levels:*About three quarters (73.1%) of the controls had PSA values greater than 4.0.

Figure 2
Serum PSA levels in cases and controls



Expression of urinary il-6 levels: The median value for urinary IL-6 levels in CaP was 89.5, while in controls it was 18.2. This difference was statistically significant (p=0.0001) (table 3 figure 4).

Figure 3
Urinary IL-6 levels between cases and controls



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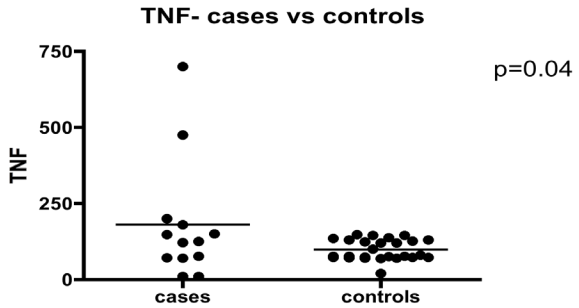
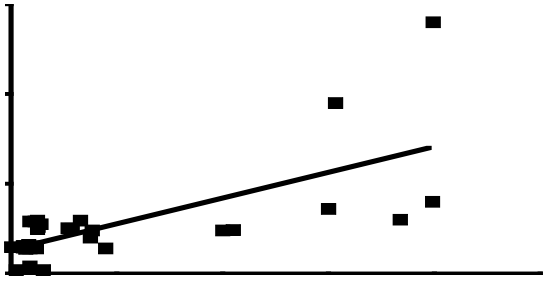
Expression of urinary tnf-α: There was a statistically significant difference in urinary TNF- α levels between case and controls (p=0.04). This biomarker was expressed more in cases (median 125.7) than in controls (median 77.2).

Figure 4
Urinary TNF-α levels between cases and controls



Correlation of urinary il-6 with urinary tnf-α in cases: There was a good correlation between urinary IL-6 levels and urinary TNF-α levels (r=0.49) (fig 6). High IL-6 levels corresponded with high TNF-α level in cases.

Figure 6
Correlating urinary IL-6 levels against TNF-α level in cases



DISCUSSION

Demographic data confirmed that old age is a contributing factor to BPH and prostate cancer. We found out that majority of men are more likely to develop prostate cancer from the age of 60 years. Our findings agreed with those of Hans-Joachim *et al.* (2007), who documented similar observation (10). Although serum PSA concentrations were significantly higher in cases than in controls, p=0.003, 73.1% of the controls had PSA values >4.0ng/ml. This confirms with Catalona *et al* (1997), who showed that 81 % of men with PSA >4 had no CaP (5). This is an indication that the specificity and sensitivity of the PSA test is suboptimal suggesting that PSA could be secreted by both malignant and non-malignant cells, confirming that PSA is prostate-specific and not prostate cancer-specific. There was no statistically significant difference between EN-2 in cases and controls (p-0.27). The presence of EN-2 in urine might not be predictive of CaP prostate or distinguish between CaP and BPH. Data from this study suggest that EN-2 is secreted into urine by both malignant and BPH cells. This contradicts the findings of Richard *et al* (2011) in which urinary EN2 levels were measured by ELISA and had a sensitivity of 66.7% and specificity of 89.3% for cancer detection (11). The study by Richard *et al* had as controls men with no prostetism as opposed to this study in which controls were those with BPH. This could be a possible reason for the difference in findings. Data from this study suggest that EN-2 may not be a reliable diagnostic marker for CaP in Kenya.

Analysis of urinary IL-6 levels in cases and controls revealed it is highly expressed in cases compared to controls. This observation shades more light on the suggestion that inflammation may plays a key role in CaP especially at an early stage. . IL-6 could play a key role in the pathogenesis and progress of CaP. IL-6 may further serve as a diagnostic and prognostic marker suggesting therapeutic targets. TNF-α expression was higher in cases than in controls. A statistically (p=0.04) significant difference was observed. This further confirms that inflammation has a role in prostate cancer development and both markers could serve as diagnostic and prognostic tools. There was a positive correlation (r=0.49) between IL-6 and TNF-α suggesting that both cytokines are expressed in CaP.

In conclusion, this study found that urine may be a sample of choice to differentiate the various forms of prostatism. It also revealed that inflammation may play a major role in the onset and progress of prostate cancer. The study demonstrated that IL-6and TNF-α could be reliable diagnostic and prognostic markers to differentiate prostatism. Changes in inflammation pro-inflammatory cytokines markers may be considered to be of value in discriminating BPH and CaP. The study has also shown that the sensitivity of urinary EN-2 is not significantly higher than that of serum PSA. The EN-2 marker is therefore, not yet useful in application for CaP diagnosis in the study population. It has however, demonstrated that serum PSA is prostate-specific and not prostate cancer-specific. It should therefore be used in association with DRE, pelvic/abdominal ultrasound and/or prostate biopsy.

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