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Determination of sarcosine in urine as a predictor of prostate cancer using Enzyme linked immunosorbent method

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Abstract

Prostate cancer is a type of malignancy that is defined by abnormal development of cells in the prostate tissue. Prostate cancer needs early intervention since its incidence and prevalence is high across the world leading to high morbidity and mortality. Prostatic specific antigen test which is the commonly used screening test in Kenya and across the world is nonspecific, expensive and inaccessible to many people in rural setting who are in need. The definitive histological test is invasive and requires specialized facilities and personnel. This study sought to investigate sarcosine in urine as a predictor of prostate cancer to supplement prostatic specific antigen test in the diagnosis of prostate carcinoma. Cross sectional study design was employed in this study for all suspected prostate cancer identified according to clinical assessment during the study period. Midstream urine samples of about 30mls was collected in plastic tubes, centrifuged and supernatant collected and analyzed using ELISA method for sarcosine. Raw data obtained was tabulated in excel and transferred to statistical package for social science. Differences in means and standard deviation from various age groups was analyzed using one-way Anova and Independent t test. The Bonferroni was used as post Hoc to test the means that were significant from others. Significance level was set at 95%. The concentration of sarcosine (4.30±0.11nmol/ml) in prostate cancer participants was significantly higher than the concentration (0.47±0.06nmol/ml) of control participants using ELISA ($p<0.001$;). Hence Sarcosine in urine needs to be analyzed for the testing of prostate tumor since it is raised in confirmed prostate carcinoma participants as compared to negative control units. The age groups of the prostate tumor participants had no significant variation in sarcosine concentration using ELISA method ($p=0.57$). Similarly, the age groups of the control individuals were not significantly different in sarcosine concentration ($p=0.17$). Future studies need to dwell in incorporating sarcosine metabolite in urine.

Keywords: Sarcosine, Urine, Diagnosis, Prostate cancer, ELISA (enzyme linked immunosorbent assay).

INTRODUCTION

Prostate cancer refers to a type of malignancy that occurs on glandular cells of the prostate gland [1]. There are different types of prostate cancer the most common being adenocarcinoma occurring in 99% of all prostate cancers [2, 12] In 2020,7.3% newly diagnosed prostate cancer cases and 3.8% prostate cancer related deaths were reported worldwide [4]. In Africa, cancer of the prostate is a burden recording a high incidence and mortality, with 9.4% new cases and 7.6% deaths [2, 12].

In East African countries, prostate cancer has been reported in health facilities but no studies have been done on native populations. Incidence reports are expected to rise to over 85% by 2030 [11]. Mitigation measures that include accessible and reliable screening methods are needed to reduce the mortality cases associated with prostate cancer. Kenya records incidence rates of about 8.1% and mortality cases of about which translates to 6.6% of patients suffering from prostate cancer succumbing to the disease [4]. Garissa County lacks the capacity for mass screening due to lack of accessibility to testing since screening is only done at Garissa County Referral Hospital. Lack of diagnosis in most of the facilities is due to limited resources and this is worsened by lack of neither specific policies nor effective strategies for controlling the disease. This therefore signified the need for the development of ELISA screening procedure for sarcosine in urine which is non-invasive and less expensive in order to reach the nomadic population who are the majority in Garissa County. Screening and early diagnosis of prostate malignancy is the most effective intervention tool for prostate cancer. This study aimed at determining correlation between prostate gland abnormality and its secretory products and whether the relationship could be used to provide alternative to prostate cancer diagnosis. Variability of sarcosine in urine among different age

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groups of prostate and non-prostate cancer participants was determined to show their levels and distribution.

MATERIALS AND METHODS

Study site

The study site was Garissa County referral hospital. It is located approximately one kilometer from Garissa town. It is the only level five facility in the county that offers specialized testing of prostate cancer. Most of health facilities are inaccessible due to poor road networks and therefore leading to health burden of diseases and conditions to the local population who are the majority in Garissa and northeastern province at large. A cross-sectional analytical study design involving the number of participants as determined by Fischer *et al* 2006 was used. [21]. Sarcosine levels of individuals with confirmed prostate cancer was compared with those not confirmed for prostate cancer according to histological finding [22].

Sample collection and processing

Midstream urine samples were collected from confirmed and those not confirmed of prostate cancer in the oncology clinic-Garissa County Referral Hospital. All the samples were centrifuged at 1000rv/min for 5 minutes. Supernatant was collected in fresh labelled plastic tubes and kept at -80 degrees centigrade till analysis of sarcosine.

Sarcosine analysis

Analysis of sarcosine employed a sandwich quantitative Elisa assay principle. The plate was precoated with human sarcosine antibody. Sarcosine antigen in the sample was transferred to the wells and attached to the antibodies present in the plate. Antisarcosine antibody was transferred to the mixture and reacted with sarcosine present in the reaction mixture. Horse dish peroxidase enzyme was added. On addition of Substrate, the solution changed color and the reaction stopped by addition of a stop solution and optical density read calorimetrically at 450nm.

Sample and Reagent preparation

Samples and reagents were placed on the working bench free on contamination to allow them to thaw at room temperature. The samples were centrifuged at 3000r/min for 20mins with centrifuge set at 25 degrees centigrade. Supernatant was transferred to clean Eppendorf tubes labelled S1-S31 and Sc1 to Sc 31.

Standards and blank control preparations

Clean Eppendorf tubes were clearly labelled std5, std 4, std3, std2, std1 in duplicates. Blank control tubes were labelled blank 1 and blank 11 in duplicates.

Standards were diluted in the following manner:120ul of standard (16nmol/ml) was diluted with 120ul of diluent and transferred to tubes labelled standard5 generating 8nmol solution.

2-fold serial dilutions (1:2) with standard diluent was done in duplicates generating 4nmol/ml, 2nmol/ml, 1nmol/ml and 0.5nmol/ml.

Preparation of wash buffer

20ml of the concentrate was diluted into 480ml of distilled water in a clean falcon tube to yield 500ml.

Procedure

96 well plate diagrams were first drawn to give the layout of analytes i.e., Standards, samples, sample controls and blanks in duplicates. 50ul of standard solutions were transferred using multichannel pippete to wells No.5 to No.1 in duplicates. 40ul of samples were transferred to sample wells in duplicates. 10ul of antisarcosine antibody was added to samples. 50ul horse dish peroxidase was added to sample wells, standards and mixed well.

Blanks were prepared by transferring 40ul of distilled water and 10ul of antisarcosine antibody.

All the solutions were mixed sealed and incubated at 37 degrees centigrade for 60mins.The plate was then washed every minute up to five times in 300ul wash buffer.

The plate was bloated with paper towels after each wash taking care not to suck air bubbles which obscures the binding sites. 50 ul of substrate A was added to all wells and 50 ul of solution subsequently added.

The plate was covered with new sealer and incubated at 37 degrees centigrade for 10 mins in the dark. 50 ul of stop solution was transferred to all wells changing the blue color to yellow. Microreader plate at 450nm was used to read the optical density of each well within 10mins.The standard curve was then generated and used to determine concentration of samples.

Statistical data analysis

Raw data was transferred to an excel sheet. Analysis was done in statistical package for social science (SPSS) software version 26. Descriptive statistics were expressed as standard error of the mean, range, mean variance, standard deviation minimum value and maximum value for quantitative data, as well as frequency and percentage in the characterization of participants in terms of age groups. An inferential statistic independent t-test was used to compare concentrations of sarcosine among prostate cancer patients and controls. The influence of age on the dependent variable was investigated by aggregation of participants in 10 years age range. Concentrations of sarcosine among the age groups of participants was compared using one way analysis of variance. Bonferroni post hoc test was used in case of mean differences using Anova.

Significance was set at 95% and data presented in tables, pie charts and graphs.

RESULTS

Participants demographic information

Sixty-two male participants attending prostate cancer screening in Garissa County Hospital were enrolled.

Age group: The participants mean age was 66.80±12.76. The participants were assigned age groups of <60, 60-69, 70-79 and ≥80 years old. The age group of 60-69 had the highest percentage of participants of 32.3%, followed by less than 60 (30.6%), greater than 80 (19.4%) and 70-79 (17.7%)

Prostate cancer status of the participants

Among the participants, 31 were prostate cancer positive, whereas 31 were prostate cancer negative (controls).

Table 1: Characteristics of participants

	Study participants	
Variable	**Frequency**	**Percent**
Age groups		
<60	19	30.6
60-69	20	32.3
70-79	11	17.7
≥80	12	19.4
Prostate status		
Prostate positive	31	50
Prostate negative	31	50
Total	62	100

Variability of Sarcosine for prostate cancer diagnosis

The prostate cancer participants had a standard error of the mean, variance, range, standard deviation, mean, minimum value and maximum value of sarcosine concentration of 0.11, 0.36, 1.81,0.60,4.03, 3.30 and 5.11nmol/ml, respectively. In contrast, the sarcosine concentration of control participants had a mean of 0.47 with a standard deviation of 0.35, standard error of the mean of 0.06, variance of 0.12, range of 1.00 and minimum and maximum values of 0.04 and 1.04nmol/ml (Table 4.4). The concentration of sarcosine (4.30±0.11nmol/ml) in prostate cancer participants was significantly higher than the concentration (0.47±0.06nmol/ml) of control participants using ELISA (p<0.001;).

Table 2: Concentrations of sarcosine among prostate cancer and normal control participants using ELISA

Std. = standard

	Sarcosine concentration (nmol/ml)	
Descriptive statistics	**Prostate cancer**	**Control**
Mean	4.30	0.47
Std. error of mean	0.11	0.06
Std. deviation	0.60	0.35
Variance	0.36	0.12
Range	1.81	1.00
Minimum	3.30	0.04
Maximum	5.11	1.04

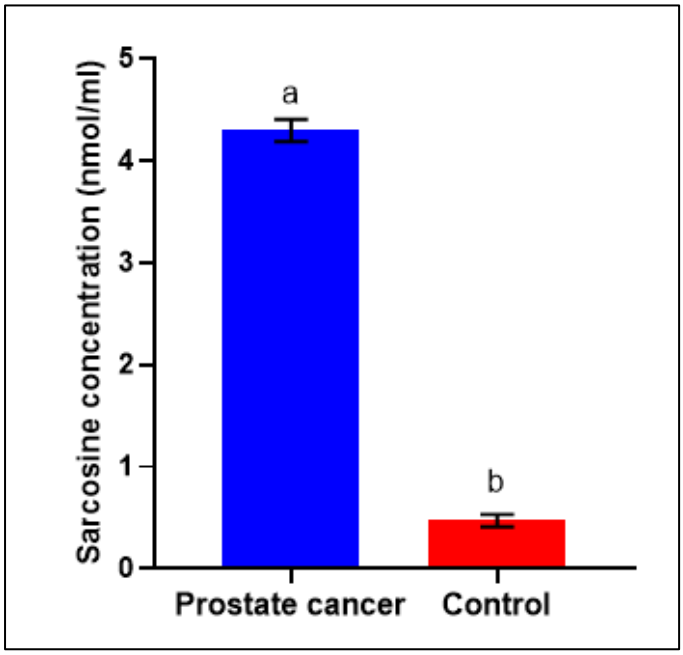

Figure 1: Sarcosine concentration in prostate cancer and control participants using ELISA. Bars with a different lowercase letter are significantly different using an independent t-test (p<0.05)

There was no significant variation between the age groups of the prostate cancer patients and the sarcosine concentration using ELISA (p=0.57; Table 2.9.1). The age groups of <60, 60-69, 70-79 and ≥80 had sarcosine concentrations of 4.03±0.39, 4.51±0.13, 4.30±0.28 and 4.23±0.19nmol/ml, respectively (Figure 2.9.1, Table 2.9.1). Similarly, the age groups of the control individuals had no significant difference in sarcosine concentrations (p=0.17; Table 4.5). The age groups of <60, 60-69, 70-79 and ≥80 had sarcosine concentrations of 0.57±0.09, 0.34±0.0.09, 0.32±0.16 and 0.92±0.00nmol/ml, respectively (Figure 2.9.1; Table 2.9.1).

Table 3: Sarcosine concentration by age groups of prostate cancer patients and controls using ELISA

	Sarcosine concentration (nmol/ml)	
Age groups	**Prostate cancer**	**Control**
<60	4.03±0.39	0.57±0.09
60-69	4.51±0.13	0.34±0.09
70-79	4.30±0.28	0.32±0.16
≥80	4.23±0.19	0.92±0.00
p value	0.57	0.17

Mean±standard error of the mean had no significant difference in the ANOVA test (p>0.05).

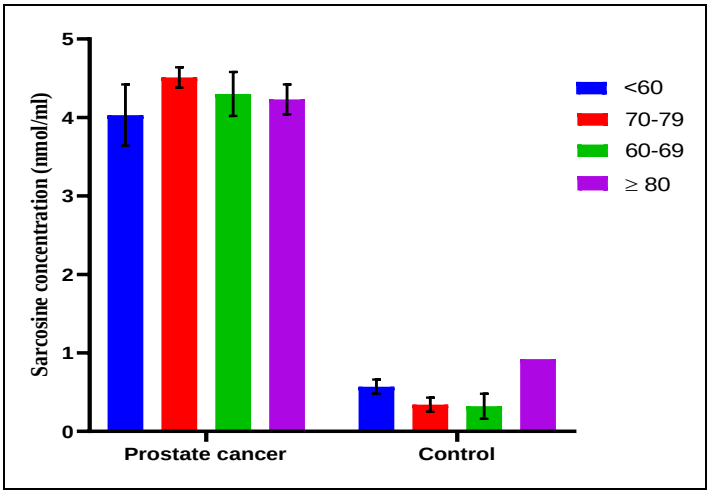

Figure 2: Sarcosine concentration by age groups of prostate cancer patients and controls using ELISA

DISCUSSION

The mean age of participants for prostate cancer study was 66.81 years. The mean age was slightly lower than another study done in Europe by maxwell *et al*., 2020 whose, mean age of study participants was 71.5 years.

The above trend in the current study can be attributed to the long distances covered to reach the testing facility the only public facility in the county of Garissa hence a low turn up with people of old age ie.70-79 years and >80 years. Limited resources are also another challenge since most residents of Garissa County are nomads and depend entirely on cattle and other animals for their livelihood. As a result of perennial droughts, most have been rendered poorest since many animals have died.

Variability of Sarcosine in ELISA diagnosis

The mean concentration of sarcosine in prostate cancer participants were significantly higher at 4.30 nmol/ml than those of control participants (0.47nmol/ml) in ELISA diagnosis P<0.001

The study compares with another study done by European urology Journal where sarcosine concentration levels in urine were higher when compared with control participants [23]. Their study failed to show sarcosine concentration in urine a biomarker of prostate cancer since it was comparing sarcosine with creatinine.

Jiang *et al*., 2010 innovated a new method for the quantification of sarcosine metabolite using GC-MS (Gas chromatography and Mass spectrophotometry [17]. The study noted average sarcosine concentration were higher in prostate cancer patients compared to the controls. There was no significant variation between the age groups of prostate cancer patients and sarcosine concentration in ELISA diagnosis p=0.57. The age groups of <60,60-69,70-79 and ≥80 had sarcosine concentrations of 4.30±0.39, 4.51±0.13, 4.30±0.28, 4.23±0.19 respectively.

The above study differs with a study done by Makni *et al*.,2021 using electrochemical sensors but their results showed that there no significant difference in sarcosine levels between age groups [13]. The current study confirms the need for adoption of ELISA method for analysis of sarcosine in urine of prostate carcinoma patients to supplement prostatic antigen test in the diagnosis of malignancy of the prostate tissue.

The means and standard error of means in control participants were also not significantly different(P>0.05). Gkotsos *et al*.,2017 noted in their findings that, there was no correlation between sarcosine concentrations and age [5].

CONCLUSIONS

ELISA method for the detection of sarcosine in urine in a promising procedure to supplement prostatic specific antigen testing of malignancy of the prostate. Urine collection is noninvasive therefore mass screening for prostate cancer can receive a good response from the population.

Ethics approval and consent to participate

Ethical approval was sought from Kenyatta university ethical review board and Garissa County referral hospital administration. The approval and documentation to carry out the research was sourced from the national commission for science, technology and innovation . Informed consent forms were also availed to all the participants of the study.

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Conflicts of interest

None declared.

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