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Evaluation of Serum Sarcosine, Total and Free Testosterone Levels in Patients with Prostate Disorders in Sokoto, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author NKN is the principal investigator, design the work and proof read. Authors POA, IAM and LSB are co-investigators, involved in the design and proof reading. Author MKD is involved in the statistical analysis and production of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Although radiation and surgery are generally regarded as effective for treatment of prostate cancer (PCa) in majority of men, diagnosis and prognosis remains poor in patients with progressive disease. Disease –specific metabolites represent the effective end points with considerable ability to identify men at increased risk of disease progression. In the current study, serum levels of Sarcosine, free and total testosterone (fTesto and tTesto) were assayed to evaluate the tumorigenic properties of PCa in our locality. In this study, 150 prostate cancer, 200 benign prostatic hyperplasia (BPH) Patients diagnosed and 200 volunteer matched controls were evaluated. Serum sarcosine were 64.94±0.81 nmol/dl, 118.70±1.80 nmol/dl and 134.13±2.21 nmol/dl in PCa, BPH patients and

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controls respectively. Serum tTesto and fTesto levels were 5.09±0.15 ng/ml, 5.12±0.11 ng/ml and 13.42±0.26 pg/ml, 5.72±0.20 ng/ml, 13.93±0.24 ng/ml and 11.73±0.47 pg/ml in PCa, BPH patients and controls respectively. Values differ significantly ($p<0.05$) between PCa, BPH patients and controls in all the analytes. Attempt was also made to define the reference ranges of these analytes in various age groups of the controls. We recommend the inclusion of Serum levels of Sarcosine, tTesto and fTesto into multiplex biomarker panel for PCa and BPH detection in our localities.

Keywords: Prostate cancer; sarcosine; free and total testosterone.

1. INTRODUCTION

The identification of prostate disorders remains problematic; no single, simple procedure exists for a reliable diagnosis. Prostate specific antigen (PSA), while non-invasive and easily measurable in serum, is not specific owing to false-positives from Prostate cancer (PCa), benign prostatic hyperplasia (BPH), inflammatory conditions and prostatic trauma. Additional diagnostic information must therefore be obtained by transrectal ultrasonography (TRUS) and digital rectal examination (DRE) to assess prostate size and morphology. Final confirmation of a malignancy requires histopathological analysis of several biopsies [1]. These present challenging obstacles in developing successful therapeutic modalities and screening tools [2]. Malignant prostate cells progress through a series of genetic and epigenetic changes leading to aberrant proliferation, angiogenesis metastasis to secondary sites and androgen independence [3]. These pro-oncogenic pathways and key signaling molecules and bye-products, are currently being examined at molecular and cellular levels for identification of novel markers that will indicate specific tumour properties in individual patients [4].

Sarcosine, also known as N-methylglycine with chemical formula CH_3NHCH_2COOH, was firstly isolated and named by a German Chemist Justus von Liebig in 1847. It is a non-proteinogenic amino acid that occurs at an intermediate product in the synthesis and degradation of amino acid glycine [5]. Biosynthesis of sarcosine has been shown to be certainly affected by cancerogenesis [6], greatly increased during progression of a prostate disorders and other metastasis process [7] and can be determined in the urine, tissue and plasma samples [6].

Androgens on the other way, are known to be essential for normal prostate growth and support [8] but the influence of testosterone in the development of prostate disorders has been a controversial issue. There is a long-standing belief that the higher serum testosterone concentrations contribute to the development of PCa. Other several studies have indicated that low serum testosterone concentrations are associated with an increase risk of PCa [9]. In a new approach to this diagnostic dilemma, it has been argued that screening for changes in metabolite concentrations could be used to identify a panel of biological markers that may improve the diagnostic performance of benign, localised and metastatic prostate disorders [1].

It is known that the serum levels of total and free testosterone are altered in patients with prostate cancer and could be considered as a marker for more aggressive disease [10,11]. Not much work has been done in Nigeria in this subject area as to document the findings even though it is utilized in clinical practice. In this study, serum sarcosine, considered as a novel marker [12,13] in the investigation of prostrate disorder was evaluated and relationship of the findings were compared with those of serum total and free testosterone in the studied subjects. The current PSA-based test for the diagnosis of prostate cancer lacks sensitivity and specificity, resulting in missed diagnosis and unnecessary biopsies. In the current study, we aimed to evaluate the interplay of serum sarcosine, total and free testosterone levels in patients with prostate disorders in Sokoto, North-West Nigeria. Examination of biomarker combination panels provides promise for early and precise prostate cancer diagnosis, and potential for the development of tumourigenic pathways defining individual tumours.

The hypothesis of the study is to find out if there is any significant difference in the levels of the total and free testosterone in BPH and prostate cancer.Also, is there any significant difference between subjects with prostate disorders (BPH and PCa) and apparently healthy subjects.

2. MATERIALS AND METHODS

This study was done in Sokoto, capital of Sokoto State Nigeria with a population of approximately 2.5 million people. Sokoto State is in the dry Sahel, surrounded by sandy savannah and isolated hills. It is located between longitudes 49'E Ecd 6°54'E and latitude 12°N and 13°58'N.

The study is an original research work conducted in the Urology Unit, Usmanu Danfodiyo University Teaching Hospital Sokoto. There is no evidence of any previous works carried out in Sokoto and its environs.

The variables studied were sarcosine, total and free testosterone lvels. They were analyzed in BPH and PCa subjects and compared with the apparently healthy subjects who served as controls.

One hundred and fifty (150) Pca and two hundred (200) BPH Patients aged 30-90 years, from the Urology Unit, Usmanu Danfodiyo University Teaching Hospital Sokoto Nigeria, who had undergone Transrectal Ultra Sonography (TRUS), Digital Rectal Examination (DRE), and/or histologically confirmed and diagnosed to have either prostate cancer or BPH were recruited in the study. Two hundred (200) Control subjects, who were apparently healthy volunteers from among the staff in the hospital and other volunteers were also recruited in the study. Informed consent from all the participant and institutional ethical approval was obtained. Serum Sarcosine was measured by the Colourimetric method (BioVision Research Products, 980 Linda Vista Avenue, Mountain View, CA 94043 USA. Serum total and free Testosterone were estimated using Enzyme Linked Immunoassay (ELISA) [10].

The parameters were analyzed considering age ranges and serum prostate specific antigen levels with the specified ranges. The BPH and PCa subjects were confirmed in the Urology Unit using TRUS and PSA tests. Sample size was determined using the formula of Naing et al. 2008.

The data were analyzed for relationships between the variables using the appropriate statistics (ANOVA, t-test and ROC (Receivers Operating Characteristics curve).

3. RESULTS

Results of the current study are shown on Tables 1 and 2 and Figs. 1-4, are in line with the hypothesis. The mean values of serum sarcosine for PCa patients, BPH patients and the control are shown in Table 1. There is a significant different between the patients groups and controls in serum sarcosine, total and free testosterone (P <0.01), while the difference between BPH and PCa patients total and free testosterone were similar (p>0.01).

Defined reference ranges using the control subjects (Apparently healthy men) for serum sarcosine, total and free testosterones age-wise are shown in Table 2.

Table 1. Mean values of serum sarcosine and other biochemical analytes (mean±SEM) in patients and controls

Subjects	N	Sarcosine (nmol/dl)	Total testosterone (ng/ml)	Free testosterone (pg/ml)
BPH	200	118.70±1.80	5.12±0.11	13.93±0.24
PCa	150	134.13±2.21	5.09±0.15	13.42±0.26
Controls	200	64.94±0.81	5.72±0.20	11.73±0.47
F		91.905 (72.782)	1.511	3.884
p-value		<0.01	>0.01	<0.01
BPH vs PCa		<0.05	>0.05	>0.05
BPH vs Control		<0.05	<0.05	<0.05
PCa vs Control		<0.05	<0.05	>0.05

***n** = sample size; **SEM** = standard error of mean,*
***BPH** = Benign prostatic hypertrophy. PCa = Prostate cancer*
P – value =significant
p< 0.05 Significant (There is significance difference between groups)
p> 0.05 Not significance (There is no significance difference between groups)

Table 2. Defined reference ranges using the control subjects (Apparently healthy men) for serum sarcosine and testosterone

	tTesto (ng/ml)		FTesto (ng/ml)		Sarcosine (ng/dl)	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
CONTROLS (40-60YRS) (n =200)	5.72	3.74-7.70	11.73	7.13-16.33	64.94	53-77.41
CONTROLS (30-40YRS) (n =106)	5.88	3.76-8.00	12.18	7.25-16.71	66.02	54.57-77.55
CONTROLS (41-50YRS) (n =55)	5.64	3.76-7.42	11.79	7.59-16.19	62.93	52.64-73.22
CONTROLS (51-60YRS) (n =27)	5.71	3.62-7.80	9.08	3.29-14.87	67.93	56.71-79.15
CONTROLS (61-70YRS) (n =11)	4.40	3.68-5.12	8.83	5.82-11.84	55.55	44.84-77.26

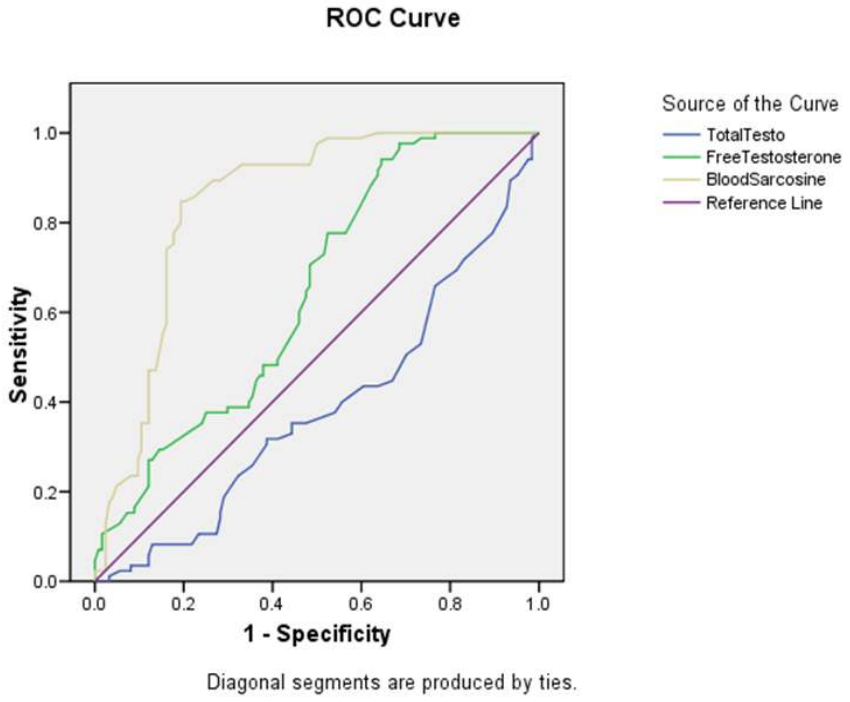

Fig. 1. ROC curve for total testosterone, free testosterone and serum sarcosine for the BPH group in PSA range 0-10 ng/ml

Testo*= Testosterone*

BPH*= Benign Prostatic Hypertrophy*

4. DISCUSSION

Metabolites play essential role in understanding the biological reactions and thereby the changes in their levels contribute to the development of new diagnostic and therapeutic interventions [11]. Elevated levels of sarcosine free and total testosterone may correlate well with progression of prostate disorders, making them interesting in the field of less invasive biomarkers.

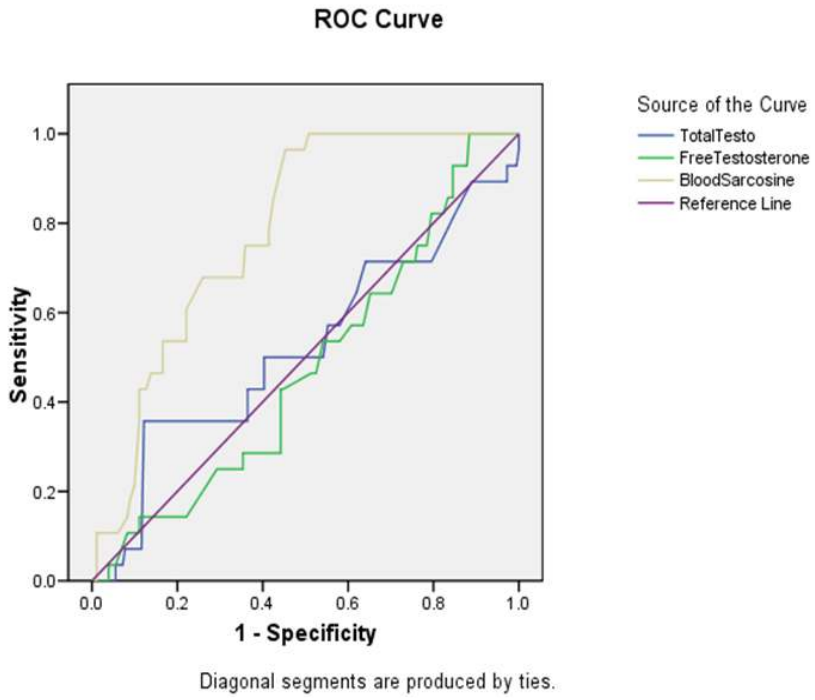

Fig. 2. ROC curve for total testosterone, free testosterone and serum sarcosine for the cancer group in PSA range 0-10 ng/ml

Testo= *Testosterone*
PCa= *Prostate Cancer*

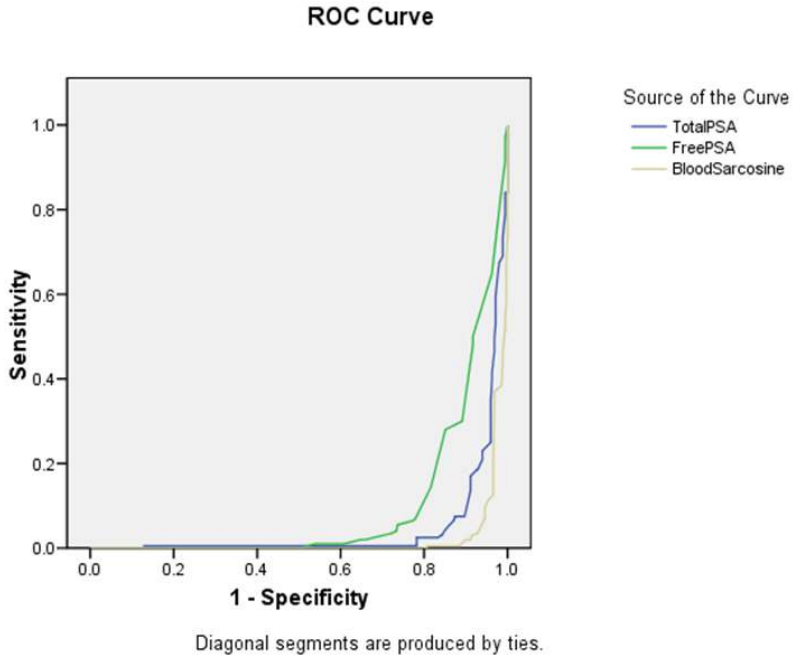

Fig. 3. ROC curve for total PSA, free PSA and serum sarcosine for the control group

PSA = *Prostate Specific Antigen*

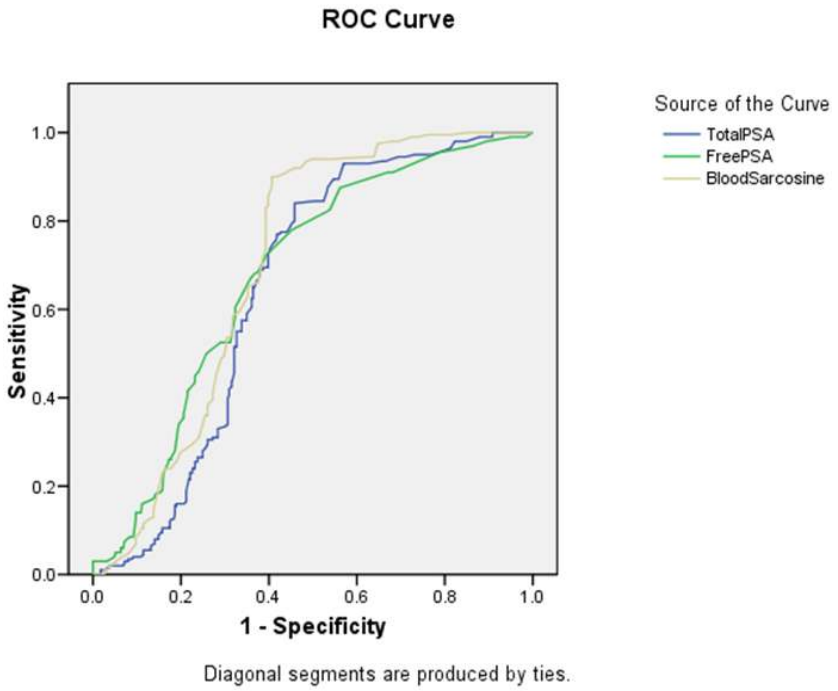

Fig. 4. ROC curve for total PSA, free PSA and serum sarcosine for the BPH group
PSA = Prostate Specific Antigen

Our results showed decreased serum concentration of sarcosine in control subjects remain the same, even if these control subjects are staggered in very narrow age bracket, indicative of its stability in non disease state. Additionally, we report here a significant difference in serum sarcosine level between PCa and BPH patients, which also are statistically different with the values observed in controls. These affirmed the proposal that sarcosine (N-methylglycine) pathway may have a tumour-promoting role in patients with PCa [14,15], reported that a metabolite tumor profile need to be highly dependent on its origin, and exhibits unique patterns and differentiation status. Serum sarcosine may, therefore, have an important role in selecting men with low grade/low risk PCa BPH who should undergo active surveillance programmes [12]. Similar to our findings were reported elsewhere as sarcosine discriminated better than prostate specific antigen (PSA) between PCa patients and patients with no evidence of malignancy (NEM) within the PSA grey zone of 2–10 ng/ml [13]. Because of the well-known problems of low specificity and the low positive predictive value of PSA in early PCa detection, this promising finding not only drew the intense interest of the urologic community, but was also highlighted and discussed in leading scientific journals [16-18]. More work in this area will herald the usefulness of this biomarker.

For many years it was believed that higher total testosterone contributed to prostate cancer and caused rapid cancer growth, and numerous studies with multiple designs and contradictory conclusions have investigated the relationship between total testosterone and prostate cancer development [19]. Age-related changes in androgens are also widely accepted as the main factors involved in the pathogenesis of BPH [20]. Previous studies have shown a positive correlation between BPH and changes in free testosterone (FT). In the current study, we found significantly higher tTesto and significantly lower f Testo levels when compared with both PCa and BPH patients. However, both tTesto and fTesto levels were similar in both PCa and BPH patients. The relationship between total testosterone and prostate disorders has been an area of interest among physicians for decades. Conflicting results have been reported on the relationship between total testosterone and subsequent prostate cancer. Much of this controversy appears to be based on conflicting study designs, definitions and methodologies. Nevertheless, this study adds to the increasing

evidence that it is important to measure testosterone levels in men with prostate disorders to aid the diagnostic performance.

5. CONCLUSION

In conclusion, the findings from the study on subjects with prostrate disorders (PCa and BPH) revealed elevated levels of sarcosine, and tTesto and decrease level of fTesto. The stable concentration of serum sarcosine at varying age bracket may point to its diagnostic value. Elevated level of t Testo and decreased level of f Testo when compared with control showed a unique pattern that can be validated. Prostate specific antigen (PSA), though considered as Gold standard, are found in patients with normal prostate function, benign prostate hyperplasia (BPH) and prostate cancer [21] Taken together, these studies substantiate the role of sarcosine, total and free Testo in prostate disorders progression and highlights the potential of using these metabolites to develop a multi-plex based markers for prostate diseases. The association between testosterone and prostate cancer risk and progression remains controversial. However, sarcosine may be combined with PSA and testosterones as tools in identifying men with insignificant PCa and thus highlights the potential of using these metabolites to develop a panel of markers for screening and monitoring for prostate disorders. Serum testosterone levels should be considered as additional tool in identifying men with clinically insignificant prostates for active surveillance. Thus serum sarcosine and testosterone levels should be considered as possible routine tool in identifying men with clinically insignificant prostate disorder for active surveillance. Thus serum sarcosine and testosterone levels

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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