RESEARCH

Cortisol and Health-related Quality of Life as Prognostic Indicators for Prostate Cancer Risk in West African Black Men in Nigeria, Cameroon and the USA: The CaPTC Cohort Study

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ABSTRACT

Poor understanding of the clinicopathological features of prostate cancer (CaP) in Black men (BM) is one of the major challenges implicated in the management and prevention of the disease. The development of CaP involves an accumulation of multiple oncogenic events with associated increase in prostate specific antigen (PSA) and stress related hormones such as Cortisol. This research aims to examine the role of Cortisol and health-related quality of life (HRQoL) in CaP development. Data was collected as part of a large CaPTC Familial CaP Cohort Study. The HRQoL indicators were measured and salivary Cortisol levels evaluated by enzyme immunoassay. CaP tissue expression patterns of cortisol and Annexin V were also studied by immunohistochemistry. The HRQoL indicators showed a significant difference between participants with and without physical activity (P = 0.025), stress (P = 0.008) and self-care (P = 0.005). There was significant increase in salivary cortisol levels in the CaP patients compared to CaP-free participants (P = 0.003). The salivary cortisol level for the CaP patients ranged from 1.029 µg/dL to 0.037 µg/dL while the range for the CaP-free participants was from 0.139 µg/dL to 0.026 µg/dL. In addition, we found increased expression of the cortisol protein in CaP patients with Gleason score 8 compared to those with lower scores and the CaP tissues showed overexpression of Annexin V protein. Salivary and tissue cortisol levels with an accompanying Annexin V expression may serve as important biomarkers for CaP diagnosis and prognosis in West African Black men.

KEYWORDS: Prostate Cancer, African Black Men, Nigerian Men, Cameroonian Men, Cortisol, Health Related Quality of Life

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INTRODUCTION

Prostate cancer (CaP) is a significant public health challenge that has disproportionately overburdened Black men of African ancestry with increased prevalence, poor prognosis and heavy mortality rates (Odedina *et al.*, 2009 (a); Bray *et al.*, 2013). A number of studies have documented significant CaP burden among Nigerian and Cameroonian Black men in West Africa and the diaspora (Odedina *et al.*, 2009 (b); Kumar *et al.*, 2009; Akinremi *et al.*, 2011; Odedina *et al.*, 2011(a); Odedina *et al.*, 2011 (b); Enow Orock *et al.*, 2012; Ferlay *et al.*, 2015; Kaninjing *et al.*, 2017; Odedina *et al.*, 2017). In African Americans, the incidence and mortality rates of CaP are disproportionately higher than in Caucasians, and the prognosis worse, due largely to genetic factors (Odedina *et al.*, 2009 (b); American Cancer Society, 2013). Personal factors (such as lack of awareness), provider factors (such as shortage of human resources) and healthcare systems factors (such as limited access to appropriate treatment) contribute to the late CaP presentation with metastatic disease as the first presenting features at diagnosis in Black men of African ancestry (Farré and Kibera, 2018). It is pertinent to note that available diagnostic and prognostic tools for CaP which includes prostate specific antigen (PSA) lack specificity and sensitivity. This makes it difficult to distinguish between aggressive and nonaggressive state of the disease for proper stratification, further evaluation and therapeutic intervention (Farran *et al.*, 2018). To explore the distinct biological and environmental etiology of CaP, the Prostate Cancer Transatlantic Consortium (CaPTC - https://epi.grants.cancer.gov/captc/), a US National Institutes of Health (NIH)/ National Cancer Institute (NCI) – Epidemiology and Genomics Research Program (EGRP) approved consortium, utilizes multilevel, collaborative, transdisciplinary, translational, and global team science research approach to study Black men globally. Specifically, CaPTC investigators address the complexity of CaP taking into consideration the ethnic heterogeneity and geographical classifications of Black men with African ancestry. This approach is pertinent to understanding the etiology of African-associated CaP risk and utilizing the diverse West African population will be of immense benefit.

The initial state of CaP and its progression involves an accumulation of multiple oncogenic events resulting in gene amplification, which is associated with increased levels of androgens and prostate-specific antigen (PSA). The PSA has been reported to be higher in Black men compared to Caucasians, however, this has been associated with complications and controversies (Visakorpi et al., 1995; Moul et al.., 1996; US Preventive Services Task Force Recommendation Statement, 2018). The complications with increase in PSA level are aggravated by several abnormal mutations that make CaP cells more aggressive via the stress and cortisol axis mechanisms and an increase in the amino acid content within the cancer microenvironment (Gaddipati *et al.*, 1994; Zhao *et al.*, 2000; Wang *et al.*, 2013; Tee, 2013; Huang et al., 2018). In addition, amino acids via anabolic pathways generate nucleotide and membrane biomolecule precursors for aggressive CaP cells (Andrew *et al.*, 2013). Perhaps, this explains why leucine may serve as a source of fuel for aggressive prostate cancer cells. However, it has not been unequivocally demonstrated that leucine is secreted mostly by Cortisol overexpression.

The Cortisol, an important glucocorticoid hormone, is synthesized in the body by adrenal

cortex via adrenocorticotropin stimulation. Therefore, cortisol fluctuation as a result of stress has been linked to allostatic load-associated diseases including immune suppression (Kirschbaum and Hellhammer, 1999). Only about 5% of Cortisol is unbound and freely circulate to the tissues including salivary gland for biological activity, whereas the remaining 95% is bound to several components of the blood including corticosteroid-binding globulin (Ekins, 1990; Walker *et al.*, 1978). Thus, saliva provides a simple and non-invasive means of assessing unbound cortisol level because blood cortisol levels may be affected by the corticosteroid-binding globulin activity (Walker *et al.*, 1978).

Health-related quality of life (HRQoL) has also been reported to be associated with PSA status in men with CaP (Gidron *et al.*, 2011). Since a deficiency in Cortisol secretion results to quiescent immune system and overexpression of Cortisol contribute to suppression of immune responses and possible tumorigenesis (Munck and Naray-Fejes-Toth, 1995; Coussens and Werb, 2002), our study focused on the expression pattern of Cortisol in CaP tissue cells, taking into considerations the Gleason score by immunohistochemistry. The ability of Cortisol to regulate immunological and inflammatory processes prompted us to explore the expression pattern of Annexin V, an intracellular protein with affinity to phosphatidylserine, which is expressed on the surface of physiologically stressed cells and functions in inhibition of cancer angiogenesis (Blankenberg, 2009).

In line with our long-term goal of addressing the burden of CaP in West African men, the primary objective of this study was to examine the role of Cortisol and HRQoL in the development of CaP among Nigerian and Cameroonian men living in Nigeria, Cameroon and United States. Findings from this study show significant differences in HRQoL, salivary cortisol level and tissue Cortisol expression pattern in the CaP patients compared to CaP-free participants. These findings may have a significant impact on CaP risk and prognosis, and may contribute to the understanding of the genetic, environmental and behavioral etiological factors associated with the disease in Black men.

METHODS

Study Participants and Recruitment

The participants recruited for this pilot study were Nigerian and Cameroonian Black men within the age bracket of 35 to 70 years and residing in Nigeria, the Republic of Cameroon and the United States of America (US) - Participants were recruited as part of the large-scale CaPTC study focused on studying a familial cohort of West African men in multiple countries. The CaPTC study (which is ongoing) is using the heterogeneity of the study participants, including geographical locations, to explore the genetic, environmental and behavioral etiological factors associated with CaP. The study inclusion criteria were: (1) West African men regardless of the history of CaP diagnosis; (2) men between the age of 35 and 70 years; and (3) men who consented to complete the study survey. Participants were recruited from multiple settings using a flyer, including at clinics and diverse community settings such as social organizations, churches, mosques, health events and also business facilities.

Study Variables and Measures

The study variables were: (1) health-related quality of life indicators including exercise, stress, ability to self-care and perceived emotional health status,-;

(2) personal history of CaP,-; and (3) demographic information, including age, religion, ethnicity, education, income, employment, country of residence, and previous history of cancer. These variables were assessed using the standardized Global Prostate Cancer Measure for Black men that was developed by CaPTC and the African Caribbean Cancer Consortium (AC3).

Data Collection

Ethical approval was obtained at each study site prior to data collection. The ethics approval include implementing informed consent process for each participant. Following informed consent approval by participants, the key study personnel in charge of data collection administered the survey and collected saliva drool using a saliva kit. The salivary samples were collected from 8am to 12 noon. Participants were provided a monetary incentive or a T-shirt for the time taken to participate in the study.

Statistical Considerations and Data Analyses

The study data entry and management was conducted using the Research Electronic Data Capture (REDCap) software. Subsequently, data were exported into the PC-SAS analytical software for data analysis. Frequency analysis of the variables was conducted to confirm responses are appropriately entered and errors corrected. The internal consistency of the study scales was calculated to establish the reliability of the scales. The study variables were evaluated using descriptive statistics of means procedure for continuous variables and frequency analyses for categorical variables. Microsoft Excel (Microsoft Office Professional Plus 2013; Microsoft Corp., Redmond, WA, USA) was used to produce the charts. Subsequently t-test statistical analysis was used to determine the differences in cortisol levels and expression using the SPSS software, version 20.0 (IBM Corp., Armonk, NY, USA). A *P* -value of <0.05 was considered statistically significant.

Salivary Cortisol Analysis by Enzyme-linked Immunosorbent Assay

The salivary cortisol was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Salimetric Inc, College Park, PA) as per manufacturer's procedure. All ELISA experiments were performed in duplicate analysis.

Tissue Cortisol Analysis by Immunohistochemistry

Immunohistochemistry (IHC) was performed on 5-µm CaP formalin fixed paraffin embedded (FFPE) tissue and non-malignant prostatic FFPE tissues for cortisol and Annexin V protein expression using Anti-Cortisol (ABIN3208586) and Anti-Annexin V (BIN4964891) antibodies (antibodies-online Aachen, Germany). Briefly, the slides containing tissue sections were baked for 1 hour, then deparaffinized with 100% xylene at room temperature for 1 minute, and hydrated in a graded alcohol stages consisting of 30-second dips each in 100% and 95% ethyl alcohol diluted in water (total volume is 5 mL) at room temperature, and finally hydrated in water. Sections were incubated in 3% hydrogen peroxidase in water at room temperature for 10 minutes to block endogenous peroxidase activity. The slides were then washed, blocked and incubated at room temperature for 30 minutes. The slides were incubated with the Anti-cortisol antibody and in 5 µg/ml dilutions and with HRP-linked secondary antibody in blocking buffer. The same protocol was used for Anti-Annexin V (BIN4964891) antibody staining using 2.5 µg/ml. The nuclei of the cells were counterstained with haematoxylin (blue). The expression level was categorized as low and high based on a combined score of intensity

and distribution. The expression was categorised base on intensity of the stain (0 = absent; 1 = weak; 2 = moderate; 3 = strong) and distribution (per cent of tumour positive).

RESULTS

A total of 500 Black men of West African descent participated in the study. Of the 500 Black men, 85% are resident in Nigeria, 8% in the USA and 7% in the Republic of Cameroon. Demographic characteristics of the participants are displayed in Table 1. The results revealed that 8% (40) of the participants had previous diagnosis of prostate cancer whereas 1% (4) had previously been diagnosed with other malignancies such as colorectal, liver, nasopharyngeal cancers and sarcoma. About 82% (401) of the participants were non-smokers and 85% (427) were employed.

Table 1. Demographic Characteristic for the CaPTC Cohort Study.

Characteristics	Frequency	Percent
Demographic characteristics, n = 500		
Country of resident		
Nigeria	428	85.60
Cameroon	34	6.80
USA	38	7.60
Age range of participants recruited		
35-44	216	43.20
45-54	160	32.00
55-64	82	16.40
65 and above	42	8.40
Personal history of cancer		
Prostate cancer	40	8.00
Colorectal cancer	2	0.40
Liver cancer	1	0.20
Nasopharyngeal	1	0.20
Sarcoma	1	0.20
Do not know	455	91.00
Marital status		
Married or living as married	460	92.00
Never married or divorced/ widowed	37	7.40
Non-disclosed	3	0.60
Smoking status		
At present	20	4.00
In the past	72	14.40
Never	329	68.80
Non-disclosed	79	15.80
Education		
8 to 11th grade	87	17.4
High school	108	21.6
University	294	58.8
Non-Disclosed	11	2.20
Employment Status		
Employed	427	85.40
Not employed	63	12.60
Non-Disclosed	10	2.00
Household income		
Less than $25,000	419	83.80
$25,000 to $49,999	10	2.00
$50,000 to $74,999	9	1.80
$75,000 and above	15	3.00
Non-disclosed	47	9.40

Participant's health-related quality of life

Five relevant factors associated with HRQoL indicators were examined in this study: exercise, stress, ability to self-care, health status perception and the need for specialized equipment towards personal daily life support. Results show significant differences (P = 0.0353) in participants physical activity levels within the last month, with 57% of participants reporting up to 30 days active exercise, 19% reporting 0 to 9 days of no exercise, 3% reporting 10 to 19 days of no exercise, and 4% reporting 20 to 30 days of no exercise within the last month (see Figure 1). There was also significance difference (P = 0.0080) across stress levels of participants within the last month. Sixty-four per cent (64%) of participants reported absence of emotional stress for 30 days within the last month, 13% reported presence of stress for 0

to 9 days, 3% reported presence of stress for 10 to 19 days and 3% reported presence of stress for 20 to 30 days (Figure 1).

There were significant differences (*P* = 0.0051) in participants' report on self-care within the last month. Sixty-three (63%) indicated that they had not been able to carry out self-care for 30 days, 16% indicated that they had not been able to carry out self-care for 0 to 9 days, 2% had not been able to carry out self-care for 10 to 19 days and 3% had not been able to carry out self-care 20 to 30 days.

In general, most of the participants reported good healthcare status. Eighty-four per cent of the participants indicated that they were in good health while 14% reported poor or fair health within the last month. Furthermore, 94% of the participants reported that they had no need for specialized equipment to carryout daily personal activities while 4% reported that they needed special equipment for daily life activities within the last month.

Table 2 compares HRQoL indicators between participants with history of CaP and CaP-free participants. The HRQoL indicators show no significant difference in exercise (*P* = 0.1441), stress (P = 0.1789), ability to self-care (*P* = 0.1434), health status perception (*P* = 0.3066), and need for specialized equipment (*P* = 0.3784) between patients with history of CaP and CaP-free participants.

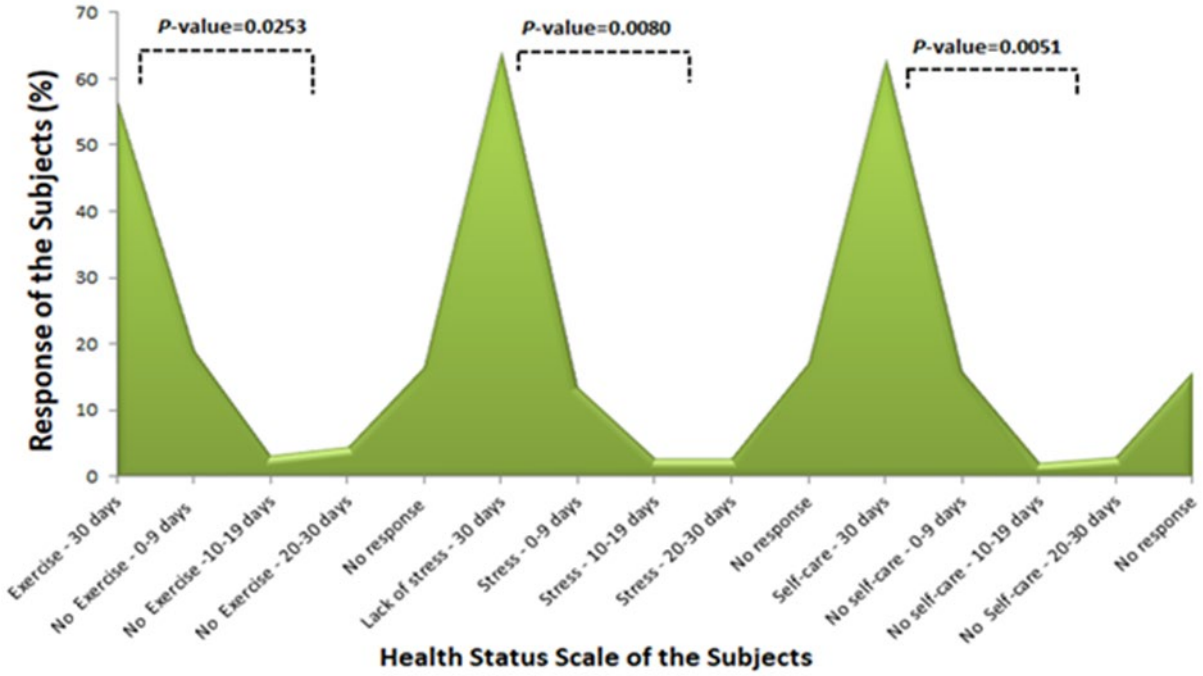


Figure 1. Summary of Health Related Quality of Life of participants recruited.

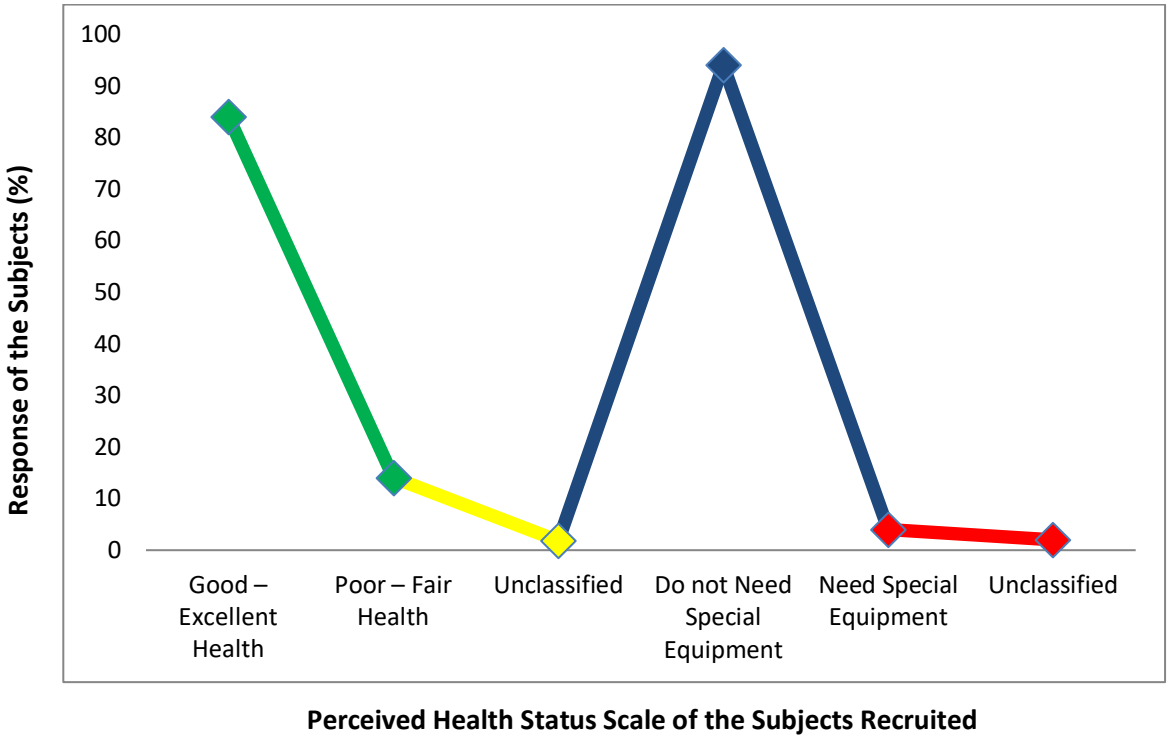


Figure 2. Response of perceived health status of the participants.

Table 2. Comparison of health-related quality of life for participants with history of prostate cancer and prostate cancer-free participants.

Variable s	Participants with CaP History N=40 (%)	Participant without CaP History N=460 (%)	P-value
Exercise			
30 days Active Exercise	19 (47.5)	289 (64.3)	0.1441
No Exercise 0-9 days	13 (32.5)	101 (21.9)	
No Exercise 10-19	2 (5.0)	14 (3.0)	
No Exercise 20-30 days	3 (7.5)	13 (2.8)	
No response	3 (7.5)	43 (7.9)	
Stress			
No stress 30 days	22 (55.0)	316 (70.1)	0.1789
Stress 0-9 days	8 (20.0)	61 (13.3)	
Stress 10-19 days	2 (5.0)	14 (3.1)	
Stress 20-30 days	3 (7.5)	13 (2.8)	
No response	5 (12.5)	56 (10.7)	
Ability to self-care			
Self-care 30 days	25 (62.5)	290 (63.0)	0.1434
No self-care 0-9	6 (15.0)	74 (16.1)	
No self-care 10-19	3 (7.5)	7 (1.5)	
No self-care 20-30	2 (5.0)	13 (2.8)	
No response	4 (10.0)	76 (16.5)	

Health status perception			
Good to excellent	20 (50)	390 (84.8)	0.3066
Poor to fair	17 (42.5)	63 (13.7)	
No response	3 (7.5)	7 (1.5)10	
Need for specialized equipment			
Do not need	24 (60)	436 (94.8)	0.3784
Need	13 (32.5)	11 (2.4)	
No response	3 (7.5)	13 (2.8)	

Salivary cortisol levels of CaP patients and CaP-free participants

Results from the salivary cortisol analysis by ELISA show significant increase in expression (P =0.003) of salivary Cortisol levels (0.18±0.03) in the prostate cancer patients (N=10) compared to the values (0.08±0.01) from the CaP-free participant (N=40) (Figure 3A). The maximum value of the salivary Cortisol level among the CaP patients was 1.029 and the minimum value was 0.037. The maximum and minimum values of the salivary Cortisol in CaP-free participants were 0.139 and 0.026 respectively (Figure 3B).

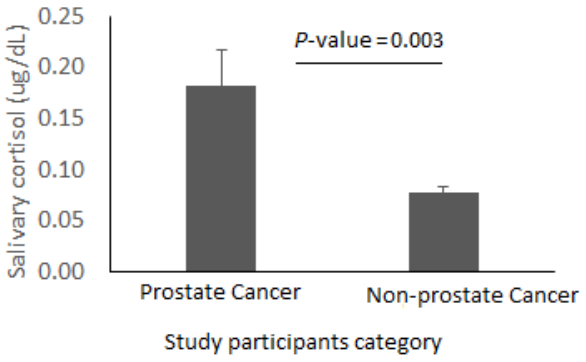


Figure 3A. Salivary Cortisol levels of few of the participants recruited.

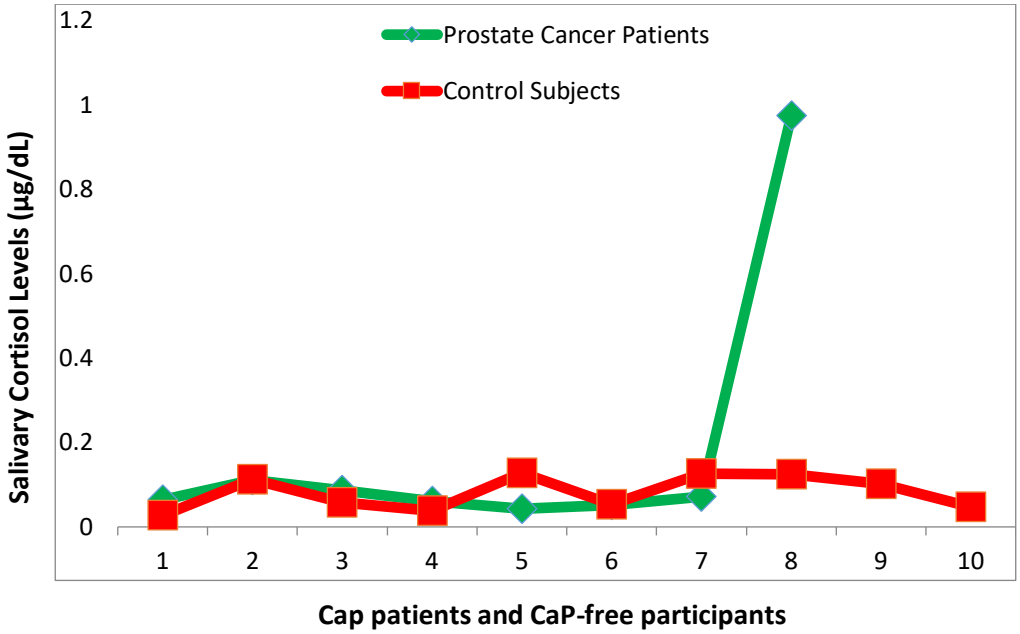
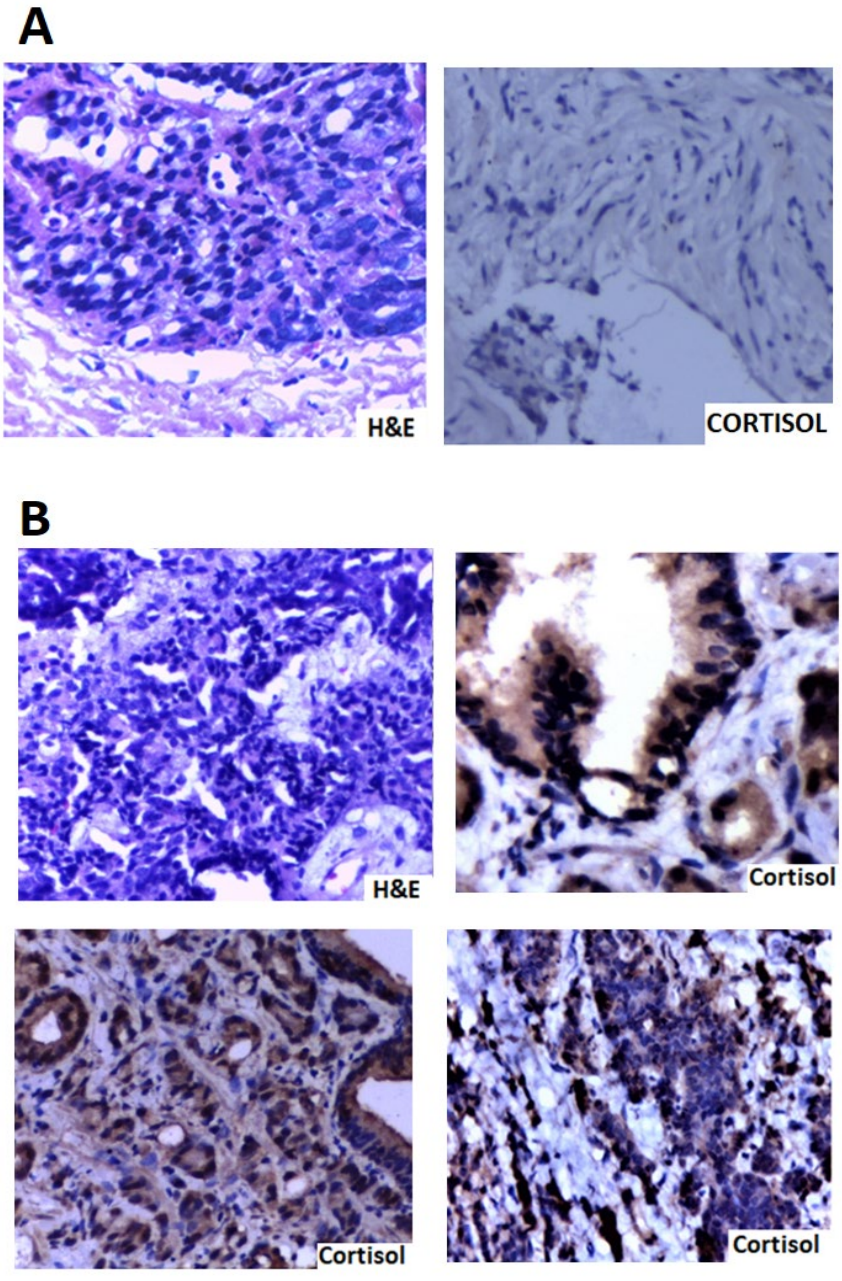


Figure 3B. Salivary Cortisol levels from a portion of CaP patients and CaP-free participant recruited. One of the CaP participants in position 8 above showed abnormal increase in cortisol even after repeating the analysis for accuracy.

Tissue Cortisol and Annexin V expression in CaP patients and CaP-free participants

Result show overexpression of Cortisol protein in the CaP cases with Gleason score of 8 compared to those with lower Gleason scores. The CaP-free tissues show negative expression of the Cortisol protein (Figure A). Figure 4B contains H&E stained sections of CaP showing CaP cells and 3 different sections stained for IHC with the Anti-Cortisol antibody signifying strong expression of the cortisol protein. The sections are CaP cases with Gleason score of 8 . Also shown in the IHC result is moderate expression of Cortisol protein in the CaP cells with lower Gleason score of 6 and below (Figure 4C). All but one of the CaP cases stained with Anti-Annexin V antibody show increased expression of Annexin V protein (Figure 4D).



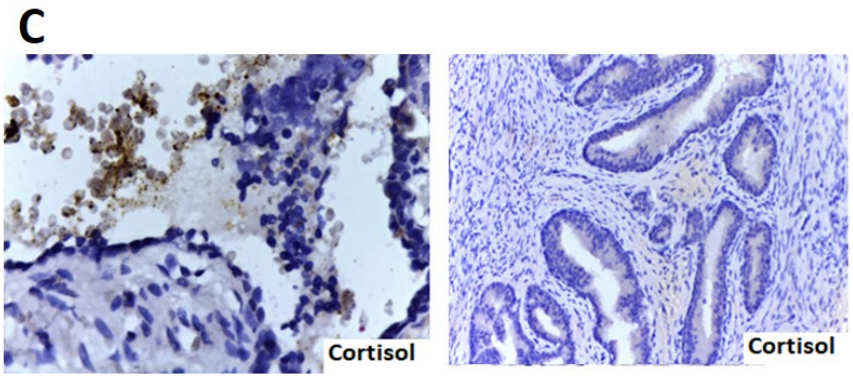


Figure 4A. Left: Benign or non-malignant prostate tissue stained with Haematoxylin and Eosin (H&E), and Right: Anti-Cortisol antibody stain for showing no cortisol expression in the prostate. Figure 4B: Cortisol expression pattern in CaP cells with Gleason score 8. The top left photograph is an H&E stained section of CaP tissue showing malignant cells. The other photographs show sections of CaP cells with Gleason score 8 stained by immunocytochemistry with Anti-Cortisol antibody; there is strong expression of Cortisol. Fig 4C: Expression pattern of Cortisol in CaP cells with Gleason Score 6 and below. The IHC result show moderate expression of the Cortisol in the cells.

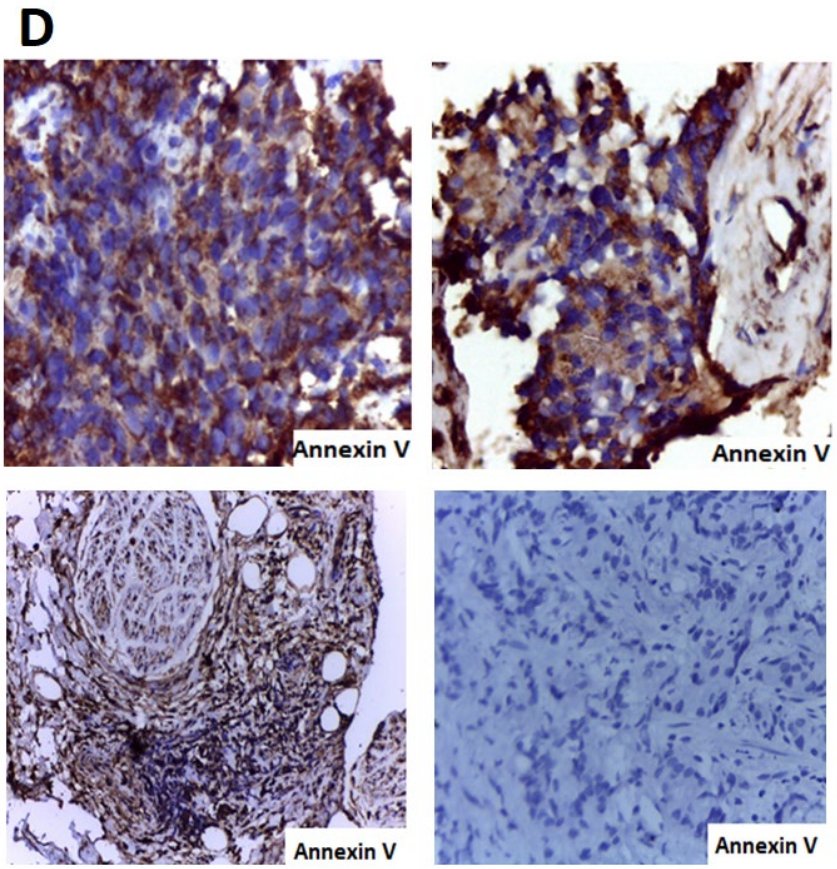


Figure 4D. Expression pattern of Annexin V protein in CaP cells. The stain shows all but one of the CaP sections stained with Anti-Annexin V antibody to have increase expression of the protein.

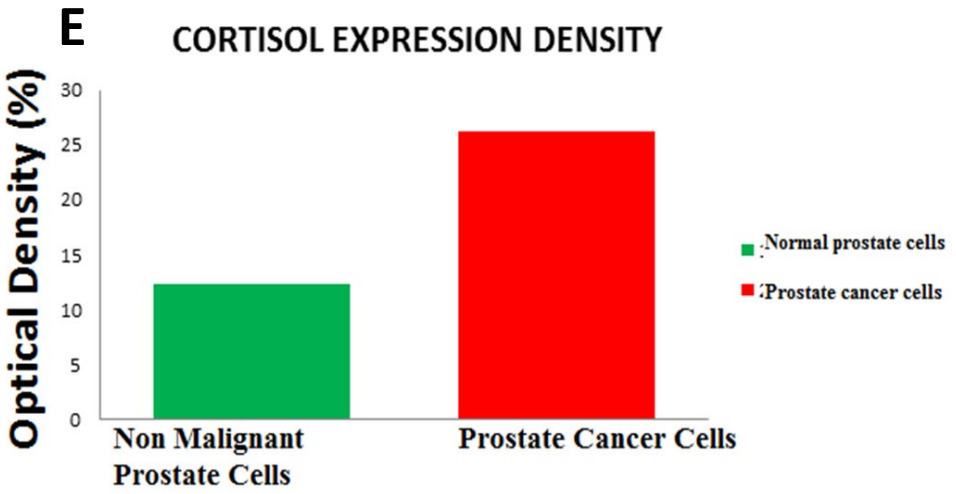


Figure 4E. Show H score depicting pattern of Cortisol expression in normal prostatic lesion and CaP cells. The score show 2-fold increase in Cortisol expression in the CaP sections.

DISCUSSION

The results from this CaPTC pilot study of 500 West African Balck men within the age bracket of 35 and 70 years show variation in HRQoL indicators such as exercise, stress, and ability to self-care, health status perception and the need for specialized equipment in carrying out daily life activities. The most interesting finding was, indicators associated to HRQoL themes appeared to have the existence of about 3% of the respondents reporting their inability to carry out daily exercise, self-care and hence consistent stress for 20 to 30 days within period of one month. The percentage of the HRQoL indicators increased slightly to about 19% for the participants when increasing the number of days for physical activity by 10 days. Poor health-related quality of life parameters which interfere with physical, functional, social and emotional states of an individual have been associated to worsen disease prognosis especially in African American men with CaP (Blankenberg 2009; DeSantis *et al.*, 2012). Previous studies have showed that Western lifestyle, reduced physical activity and high fat diet intake are related to CaP risk (Freedland and Aronson, 2009; Fradet et al., 2009). Stress and psychosocial factors are also associated with the onset and prognosis of malignant tumours. A recent meta-analysis of 165 studies reports that psychosocial factors are predictive of cancer prognosis, independent of initial tumor stage and other confounders (Chida *et al.*, 2008). Of the 500 study participants, 40 were patients diagnosed with CaP, thus contributing to the percentage of the participants with poor health-related quality of life in this research.

Results that compare HRQoL indicators between participants with history of CaP and CaP-free participants show no significant differences between the two groups. The lack of differences in the HRQoL indicators between the groups may be associated to the small number of CaP participants (N=40) as compared to CaP-free participants (N=460) recruited in this study. However, since the study recruitment is on-going, further effort will lay emphasis on recruiting sufficient CaP patients.

The salivary Cortisol level analysis of 40 participants without CaP and 10 patients with CaP

showed a significant increase in the mean average Cortisol level from 0.08±0.01 to 0.18±0.03 respectively. However, one of the patients with CaP showed a four-fold increase in salivary Cortisol level compared to the highest level in the non-CaP participants even after repeating the analysis to rule out possible errors. Previous studies have shown that Cortisol levels are associated with circadian disruption and poor health status which in turn contribute to immune dysfunction and CaP risk (Touitou 1983; Coussens and Werb, 2002; DeSantis *et al.*, 2012; Tai *et al.*, 2016). Findings from this study further showed about 14% of the respondents to have poor health status, while 4% needed specialized equipment to be able to perform regular daily activities, probably for reasons, not unrelated to stress.

Additionally, the expression patterns of Cortisol protein at tissue level were assessed by immunohistochemistry to further explore the role of the protien in CaP pathogenesis. The results obtained show an increase in the expression of the Cortisol in CaP cells, which was directly associated with increase in Gleason score and hence the disease aggressiveness. The luminal cells of the glandular epithelium in CaP secrete various hormones including androgens (Feldman and Feldman, 2001) and perhaps Cortisol for a role in cellular proliferation and apoptosis. Previous report shows that stress increases cytokines level especially interleukin 6 on the hypothalamic-pituitary-adrenal axis (Zhou et al., 1993) which may result to fluctuation in the body Cortisol level. The T877A androgen receptor mutation in the CaP microenvironment increases the binding affinity of the androgen receptor to Cortisol and its metabolite, cortisone, creating a cortisol responsive CaP cells leading to androgen independent growth and higher tumour grade (Zhao et al., 2000). Interestingly, the abnormal mutations of the androgen receptor molecule results in the ability of cortisol to bind to the androgen molecule thereby functioning as a "pseudo-androgen", promoting the increase in secretion of PSA and making CaP cells more aggressive (Gaddipati *et al.*, 1994; Zhao *et al.*, 2000). These factors explain the potential source and role of cortisol in CaP. CaP cells with metastatic features including metastatic castration-resistant phenotypes have been reported to be highly dependent on amino acid uptake through the L-amino acid transporters (LATs) for cellular growth and proliferation, as well as malignant transformation (Wang *et al.*, 2013). The LATs are part of ATF4 genes that function in the exchange of branch chain amino acids for intracellular amino acids (Tee, 2013). Amino acids via anabolic pathways have been reported to generate nucleotide and membrane biomolecule precursors for aggressive CaP cells (Tee, 2013). Perhaps this explains why leucine may serve as a source of fuel for aggressive prostate cancer cells. The study also shows increased tissue expression of annexin V protein in the CaP cells. Annexin V have been reported to regulate immunological and inflammatory processes and commonly expressed on the surface of stressed cells for inhibition of malignant tumour angiogenesis (DeSantis et al., 2012).

Previous findings have shown that abnormal increase in stress contributes to increase in Cortisol, inflammation and immune dysfunction via activation of the hypothalamic-pituitary-adrenal axis (Blankenberg, 2009). These underlining mechanisms have been proposed to play significant role in prostate cancer pathology and poor prognosis (Touitou *et al.*, 1983; Gidron *et al.*, 2011; Coussens and Werb 2002; DeSantis *et al.*,

2012; Tai et al., 2016). The limitation of our pilot study includes the small sample size of participants with CaP compared to CaP-free participants. Additional research parameters which include but not limited to inflammatory markers are necessary to increase our understanding of the role of Cortisol in the development and prognosis of CaP in Black African men. Further in-depth studies that take into consideration all the necessary parameters are required.

CONCLUSION

In conclusion, this study to a large extent has demonstrated associations of salivary Cortisol level, tissue cortisol expression activity and HRQoL of life in West African Black men in Nigeria, Cameroon and the USA with or without CaP. The study show that salivary and tissue Cortisol levels with an accompanying Annexin V expression may serve as important biomarkers for prostate cancer diagnosis and prognosis in West African Black men. The findings further suggest the possibility that tissue and salivary Cortisol in combination with indicators of HRQoL may mediate prostate cancer risk in West African Balck men.

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Conflict of interest

The authors declare that no competing or conflict of interests exist. The funders had no role in study design, writing of the manuscript, or decision to publish.

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