Sub-Optimal Plasma Zinc Levels Predict High-Risk PSA Status among Native Nigerian Men with Prostate Cancer

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Abstract

Background:

Sub-optimal plasma zinc level is hypothesized to initiate prostate carcinogenesis which may indicate a link with PSA levels. Hence, this study was an attempt to verify this link among Nigerian men with prostate cancer (PCa).

Methods:

This was a prospective cross-sectional study of 220 histologically-verified PCa patients, conducted in a Nigerian third-level health facility. Clinical, demographic and laboratory variables were obtained and analyzed using SPSS version 21. Plasma zinc and total serum PSA were determined using standard methods.

Results:

High-risk PSA status was observed among the majority (n = 187; 85.0%) of the study cohorts. Most (n = 136; 72.7%) of the cohorts with high-risk PSA status exhibited sub-optimal plasma zinc levels. Inverse relationship existed between plasma zinc and serum PSA among the entire study cohorts (B: -0.401; SE: 2.776; p< 0.001) and among those with high-risk PSA levels (B: -0.309; SE: 2.784; p < 0.001) in unadjusted model. However, the inverse relationship was amplified among the subgroups with high-risk PSA levels (B: -0.322; SE: 0.327; p< 0.001) following adjustment for confounders. Sub-optimal plasma zinc level had greater likelihood of predicting high-risk PSA status in univariate (OR: 2.833; 95% CI: 1.332 – 6.027; p = 0.007) and multivariate (OR: 1.680; 95% CI: 1.157 – 3.937; p = 0.006) logistic regression models than PCa stage, PCa grade, family history of PCa, age, prostate volume, and BMI.

Conclusion:

The study suggests the association of sub-optimal plasma zinc levels with high-risk PSA status among PCa patients. Optimization of zinc status may, therefore, play a role to mitigate high-risk PCa disease. However, further studies are suggested to evaluate the conclusion of this study.

Keywords: *Nigeria, prostate cancer, plasma zinc status, PSA*

INTRODUCTION

Currently, the global community is been challenged by the rising trend as regards the incidence and mortality of prostate cancer (PCa) disease (Taitt, 2018). The disease is gradually approaching epidemic status all around the globe especially among men of the Negroid race (Kelly et al., 2017). A prevalence rate of 15.7% of the disease has been reported in Nigeria (Ukoli et al., 2003). There are suggestions from various quarters that the globalized changing dietary and environmental influences could be augmenting the genetic factors associated with prostate carcinogenesis (Taitt, 2018 & Damber, 2000). There is also some hypothesis regarding the role of the environmental factors in the pathogenesis of the disease. One of such environmental hypothesis is the role of the micronutrients, such as zinc status in males, which has been implicated as a possible factor in prostate carcinogenesis (Giovannucci, 1999).

Zinc is an essential micronutrient for all forms of life, an essential cofactor for more than three hundred human biologic enzymes and plays a vital function in the human reproductive physiology (Kombe, Tsuji, Hashimoto, Itsumura, 2015 & Roohani, Hurrel, Kelishadi, Schulin, 2013). The mineral accumulates in the prostate gland more than ten times the amount found in any other human tissue (Costello & Franklin, 2016). The accumulation of zinc in the prostate gland is made possible by the ability of the gland to extract a high amount of zinc from the extracellular fluid using specialized transport proteins which are unique proteins in the gland (Costello & Franklin, 2016). Therefore, the intra-prostatic status of zinc is virtually dependent on the extracellular zinc status and plasma zinc level is a reliable marker of body zinc status as it responds to dietary and supplemental zinc intake (Kolenko, Teper, Kutikov, Uzzor, 2013 & Costello, Feng, Milon, Tan, Franklin, 2004).

There are lots of experimental and epidemiologic evidence suggestive of the antineoplastic tendencies of zinc in the prostate gland (Costello & Franklin, 2016). The high concentrations of zinc in the gland have been reported to promote healthy growth of the glandular epithelium of prostate gland especially around the most likely cancer-forming zones of the gland (Costello & Franklin, 2016, Kolenko et al., 2013, Costello et al., 2004). Besides, epidemiologic evidence is also consistent that adequate zinc status inhibits PCa initiation and progression through various mechanisms (Costello & Franklin, 2016). Dozens of reports have noted that zinc concentration is significantly reduced in both plasma and PCa tissues of patients with the disease relative to normal controls (Wakwe, Odum, Amadi, 2019, Cortesi et al., 2008, & Onyema-Iloh et al., 2015).

While these studies had suggested that the reduction of zinc concentrations in both plasma and PCa tissues is possibly the initiating event in prostate carcinogenesis, a few have mirrored their study towards the relationship between zinc concentrations and the prostate-specific antigen (PSA) (Abhishek et al., 2017). Wakwe et al. had recently reported the impact of zinc status to predict PCa grade and stage among Nigerian men with histologically-diagnosed PCa disease (Wakwe et al. 2019). Since suboptimal zinc status is hypothesized to enhance prostate carcinogenesis, this may indicate a link between zinc and PSA status. Additionally, the relationship between zinc and PSA in PCa patients is ill-defined in the literature and is essential in understanding the anti-neoplastic roles of zinc in prostate gland physiology (Costello & Franklin, 2016).

Hence, the primary purpose of this study is an attempt to evaluate the relationship between plasma zinc and serum PSA among the histologically-verified PCa patients of Nigerian origin.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted between March 2016 and March 2019 in the Departments of Chemical Pathology, Histopathology, and Urology of the University of Port Harcourt Teaching Hospital (UPTH), South-South Nigeria.

Ethical approval was obtained from the UPTH Research Ethics Committee following the review of all the study protocols and procedures. Informed consent was also obtained from each participant before enrollment. The study procedures and protocols were in tandem with the tenets of the World Medical Association's Declaration of Helsinki of 1964 and as amended in 2013.

The sample size was derived using the formula for sample size determination for evaluating and characterizing proportions in a population greater than ten thousand (Naing, Winn, Rush, 2006). Using PCa prevalence rate of 15.7%, an approximate sample size of 220 was obtained with an anticipated 10% dropout rate inclusive as recently reported (Wakwe et al. 2019).

The study populations were 220 incident, treatment-naïve, and histologically-verified PCa cases with Gleason score grade greater than or equal to six as recommended by the International Society of Urological Pathology as recently described (Wakwe et al. 2019). The PCa cases were subsequently recruited from the Urology Clinic of the hospital using a simple random sampling technique following the application of the eligibility criteria and acquisition of the relevant written and signed informed consent. These patients had been diagnosed accordingly through thorough medical history review, evaluation of clinical features (digital rectal examination), investigations {total serum PSA test, transrectal ultrasound scan of the prostate (TRUS)} and histologic confirmation of PCa on biopsy tissues harvested following trans-perineal prostate biopsy protocol.

The PCa patients were excluded if they met the following conditions: (1) non-consenting patients, (2) those who had undergone previous prostatectomy for benign prostate enlargement, (3) those with other cancers, (4) those who are diabetic, (5) those with chronic renal diseases, (6) those on any drugs (statins, nonsteroidal anti-inflammatory drugs, thiazide, calcium supplement, aspirin, 5α-reductase inhibitors, and exogenous testosterone) documented to influence serum PSA levels in the literature.

Venous blood (fasting) was acquired from each participant between 8 to 10 am daily and the specimen processed accordingly for laboratory analysis of serum total PSA and plasma zinc. The serum total PSA was determined using the Enzyme-linked Immunosorbent Assay method. While the plasma zinc was determined via the flame Atomic Absorption Spectrophotometry (AAS) method using the methodology outlined by Smith and associates (Smith, Butrimovitze, Purdy, 1979). Necessary precautions were taken to prevent environmental zinc contamination of blood samples. Assay precision of laboratory procedures was monitored with the use of three levels of commercial control sera. The system suitability parameters including linearity, accuracy, robustness, and precision were evaluated during AAS methodology for zinc assay and confirmed to be within limits with each parameter having a relative standard deviation of ≤.5%.

Data obtained from each participant were age (years), body mass index (BMI), prostate volume (calculated with the ellipsoidal formula (Aarnink, De la Rossette, Debruyne, Wijkstra, 1996) using the measured dimensions of the prostate following TRUS), family history of prostate cancer, Gleason score grades of PCa tissues, total serum PSA in µg/l, and plasma zinc in µmol/l. PCa staging was done clinically using the American Joint Committee on Cancer (AJCC) guidelines.

Age was arbitrarily stratified into two groups: ≤ 65 years or > 65 years. Plasma PSA level was stratified as mild-risk (PSA < 10 µg/l), intermediate-risk (10 - 20 µg/l) or high-risk (> 20µg/l) based on the D'Amico risk stratification (D'Amico, 1998). Plasma zinc status was stratified based on the recommendations of the International Zinc Nutrition Consultative Group (IZiNCG) as suboptimal (≤ 11.30 µmol/l) or optimal (> 11.30 µmol/l) (Hotz & Brown, 2004). The clinical staging was arbitrarily dichotomized as intra-prostatic PCa disease (≤ cT2) or extra-prostatic PCa disease (≥ cT3) as previously described (Wakwe et al., 2019).

Statistical analysis was executed using the statistical package for social sciences (SPSS) version 21. The continuous data were initially tested for Gaussian distribution using the Shapiro-Wilk test. The non-parametric distributed data were subsequently logarithmically transformed prior to statistical analysis. The continuous variables were summarized with mean (± standard deviations) and their ranges. The categorical variables were presented in numbers and percentages and compared with the Chi-square test or Fisher's exact test as appropriate. Linear regression and binary logistic regression models were utilized to examine the relationships between study variables. An alpha value < 0.05 was chosen as being statistically significant.

RESULTS

This case-only prospective, descriptive and cross-sectional study was conducted in the University of Port Harcourt Teaching Hospital between March 2016 and March 2019. The study population consisted of 220 incident, treatment-naïve, and histologically-verified PCa patients.

Table 1 summarizes the mean ± standard deviation (range) of the entire (n = 220) study population's age, plasma zinc, total serum PSA, Gleason scores, prostate volume, and BMI.

Most (n = 147; p < 0.001) of the study cohorts were elderly (Table 2) and the majority (45.5%) of them responded to having a positive family history of PCa (Table 2).. The majority (n = 187; 85%) of the cohorts also had total serum PSA concentration within the high-risk category using the D'Amico criteria (p < 0.001). However, none of the cohorts had a total serum PSA level in the mild-risk category (Table 2). In terms of disease stage, the majority of the study cohorts had intra-prostatic PCa disease (75.5%) compared to those with extra-prostatic disease (24.5%).

As depicted in table 3, of the 187 (85%) of study cohorts with high-risk serum PSA status, 72.7% (n = 136) of them exhibited plasma zinc levels in the sub-optimal category while 27.3% (n = 51) were in the optimal zinc category (p = 0.008).

In table 4, inverse relationship existed between plasma zinc and serum PSA among the entire study cohorts (B: -0.401; SE: 2.776; p< 0.001) and among those with high-risk PSA status (B: -0.309; SE: 2.784); p < 0.001) in an unadjusted linear regression analysis (Model 1).

However, following the adjustment for age, Gleason grades, prostate volume, BMI and family history of PCa disease in Model 2, the inverse relationship was mildly attenuated but remained statistically significant among the entire PCa patients (B: -0.310; SE: 2.776; p< 0.001), however, this was amplified among the subgroups with high-risk PSA status (B: -0.322; SE: 0.327; p< 0.001) following the adjustment of confounders in model 2 (Table 4).

As illustrated in Table 5, sub-optimal zinc levels had greater likelihood of predicting high-risk serum PSA status in both univariate (OR: 2.833; 95% CI: 1.332 – 6.027; p = 0.007) and multivariate logistic regression models (OR: 1.680; 95% CI: 1.157 – 3.937; p = 0.006) than PCa grade, PCa stage, family history of PCa, age, prostate volume, and BMI.

Table 1: Descriptive characteristics of the continuous variables

Study variables	Mean ± SD	Range
Age (years)	70.22 ± 7.13	56 – 82
Plasma zinc (µmol/l)	10.05 ± 3.10	6.50 – 21.40
Total serum PSA (µg/l)	38.87 ± 5.49	19.50 – 105
Gleason score	7.82 ± 1.46	6 – 10
Prostate volume (cm^3)	36.48 ± 2.69	29.80 – 47.40
BMI (kg/m^2)	27.69 ± 3.47	19.70 -36.70

PSA: Prostate-specific antigen; µmol/l: micromole per liter;
µg/l: microgram per liter; SD: Standard deviation;
BMI: Body mass index.

Table 2: Distributions of the categorical variables

Strata of variables	n (%)	p value
Age stratum (years)		
≤ 65	73 (33.2)	< 0.001*
>65	147 (66.8)	
PSA risk stratum (µg/l)		
Mild-risk	0 (0.0)	
Intermediate-risk	33 (15.0)	< 0.001*
High-risk	187 (85.0)	
Family history of PCa		
No	77 (35.0)	
Yes	100 (45.5)	< 0.001*
NR	43 (19.5)	
Clinical stage stratum		
≤ cT2	166 (75.5)	< 0.001*
≥ cT3	54 (24.5)	

*Statistically significant; PSA: Prostate-specific antigen;
µmol/l: micromole per liter; µg/l: microgram per liter; SD:
Standard deviation; BMI: Body mass index;
NR: No response given

Table 3: Distribution of PSA risk groups based on plasma zinc status

	PSA Risk Stratum (µg/l)			
Plasma Zinc Stratum	Mild-risk n (%)	Intermediate-risk n (%)	High-risk n (%)	p value
Suboptimal (≤ 11.30 µmol/l)	0 (0)	16 (48.5)	136 (72.7)	< 0.008*†
Optimal (> 11.30 µmol/l)	0 (0)	17 (51.5)	51 (27.3)	
Total	0 (0)	33 (15.0)	187 (85.0)	

*Statistically significant; PSA: PSA: Prostate-specific antigen; µmol/l: micromole per liter; µg/l: microgram per liter; †Fisher's exact test.

Table 4: Linear regression analysis between plasma zinc and total serum PSA levels

	Model 1		Model 2	
Study Groups	Beta (SE)	p value	Beta (SE)	p value
Entire cohorts (n = 220)	-0.401 (2.776)	<0.001*	-0.310 (0.293)	<0.001*
PSA risk stratum				
Mild-risk (< 10 µg/l)	-	-	-	-
Intermediate-risk (10 – 20 µg/l)	- 0.094 (0.698)	0.603	-0.087 (0.385)	0.117
High-risk (> 20 µg/l)	-0.309 (2.784)	<0.001*	-0.322 (0.327)	<0.001*

*statistically significant; Beta: Standardized linear logistic regression coefficient;
SE: Standard Error; µg/l: microgram per liter;
Model 1: Unadjusted
Model 2: Adjusted for age, Gleason grades, prostate volume, BMI, family history of PCa disease, and PCa stage.

Table 5: Logistic regression analysis of predictors of high-risk PSA levels

	Univariate			Multivariate		
Variables	OR	95% CI	p value	OR	95%CI	p value
Zinc status						
Optimal (reference)	1.000	–	-	1.000	-	
Sub-optimal	2.833	1.332 – 6.027	0.007*	1.680	1.157 – 3.937	0.006*
Clinical stage						
≤ cT2 (reference)	1.000	-	-	1.000	-	-
≥ cT3	2.646	0.886 – 7.905	0.081	0.806	0.164 – 3.963	0.710
Gleason grade	1.455	1.026 – 2.063	0.036*	1.335	0.754 – 2.363	0.321
Age (years)	1.089	1.035 – 1.145	< 0.001*	1.017	0.949 – 1.090	0.633
Family history of PCa						
No (reference)	1.000	-	-	1.000	-	-
Yes	0.901	0.401 – 2.020	0.801	1.195	0.556 – 5.695	0.321
Prostate volume (cm^3)	0.810	0.704 – 0.932	0.003*	0.928	0.756 – 1.139	0.474
BMI (kg/m^2)	0.424	0.324 – 0.556	< 0.001*	0.432	0.325 – 0.573	< 0.001*

*Statistically significant; OR: Odd Ratio; CI: Confidence Interval;
BMI: Body mass index

DISCUSSION

Convincing evidence exists to suggest that zinc plays pivotal roles in the prevention of prostate carcinogenesis (Costello & Franklin, 2016, Kolenko et al., 2013, Costello et al. 2004). Numerous reports have also proven that patients with PCa have significantly reduced plasma or prostate tissue zinc concentrations compared to those with benign prostate enlargement and the normal controls (Costello & Franklin, 2016). The reduction of the micronutrient in PCa disease had been adjudged by numerous authors to be the cardinal initiating events in prostate carcinogenesis (Kolenko et al., 2013 & Costello et al. 2004). Several investigators had continued to examine the relationship between zinc micronutrient and various biomarkers of PCa disease to better understand the influence of the micronutrient on the disease (Costello & Franklin, 2016, Kolenko et al., 2013, Costello et al. 2004). The association of PCa disease with low zinc status may indicate a link between the mineral and PSA status. This present study was an attempt to examine this relationship among histologically-verified PCa patients.

In this present study, the most significant finding was the association and prediction of high-risk PSA status by suboptimal plasma zinc levels among our study cohorts. This finding is in accord with previous local and foreign reports (Adaramoye, Akinloye, Olatunji, 2010 & Ishii et al., 2004). Adaramoye and colleagues had in their study demonstrated a significant reduction of plasma zinc status in PCa patients with increasing PSA levels (Adaramoye et al., 2010). In that study, Adaramoye and colleagues found that lower zinc levels in PCa was associated with high-risk PSA status (Adaramoye et al., 2010). The depletion of zinc from the prostate that occurs in sub-optimal zinc levels seem to exhaust all the anti-neoplastic effects of zinc as suggested in various reports (Costello & Franklin, 2016, Kolenko et al., 2013, Costello et al. 2004). These authors had posited that suboptimal plasma zinc levels with inadequate intra-prostatic levels of zinc initiate prostate carcinogenesis with resultant high-risk PSA levels through various mechanisms (Kolenko et al., 2013, Ishii et al., 2004).

Ishii and colleagues had recently demonstrated the effectiveness of zinc ions to inhibit several proteases which play roles in PCa cell progression and rising PSA levels (Ishii et al., 2004).

We had also documented an inverse relationship between plasma zinc levels and total PSA status among the entire study cohorts in both the crude and adjusted linear regression models. However, the inverse relationship was more pronounced among the PCa subgroup with the high-risk PSA status which was amplified in the adjusted linear regression analysis.

Similar findings had been noted in few other studies (Goel & Sankhwar, 2009, Darago et al., 2011). Goel and colleagues had documented a similar relationship between plasma zinc and PSA in their study (Goel & Sankhwar, 2009). Darago and associates had observed an inverse relationship between plasma zinc and PSA and concluded that the lowered zinc to total PSA ratio was associated with increasing severity of PCa disease (Darago et al., 2011). In a more recent study, Abhishek and colleagues also reported a weak inverse association (r: -0.10) between plasma zinc and PSA, however, the association was not statistically significant (p = 0.292) (Abhishek et al., 2017). Adaramoye and colleagues had also reported that the inverse relationship between plasma zinc and PSA values in PCa patients signifies a high-risk PCa disease (Adaramoye et al., 2010).

Several investigators had reported that high-risk PSA status is suggestive of PCa progression (Adaramoye et al., 2010, Ishii et al., 2004). Several factors including age, BMI, prostate volume, Gleason grade have also been suggested as factors of PCa progression with resultant high-risk PSA levels (Giovannucci, Liu, Platz, Stampfer, Willett, 2007).

However, we had adjusted these variables in this study and found sub-optimal zinc levels as the major determinant of high-risk PSA status in both crude and adjusted logistic regression models. This finding is in accord with a recent report which documented a positive relationship between low plasma zinc status and PCa grade and stage among native Nigerian men (Wakwe et al., 2019).

Additionally, the depletion of intra-prostatic zinc status has also been advocated as a determinant of PCa progression (Damber, 2000, Giovannucci, 1999). The mechanisms of zinc-induced inhibition of PCa progression, and therefore rising PSA levels, has extensively been investigated (To et al., 2018, Huang, Kirschke, Zhang, 2006). Zinc induces mitochondrial apoptosis by inhibiting nuclear factor kappa beta, thereby reducing cell growth and proliferative potentials of PCa tissues. Zinc reportedly down-regulates androgen receptors thereby inhibiting the proliferative influence of androgens on the prostate tissues, reducing PSA levels in the process (Huang et al., 2006).

The strength of the study is embedded in its prospective design and the enrollment of only the incident, treatment-naïve and histologically-verified PCa cohorts. However, the study was also challenged by some limitations. First, it was solely a hospital-based study carried out in a single setting whose conclusion may lack generalization to the entire population within the study region. Secondly, the histologic Gleason scores were reported by different histopathologist which is subject to inter and intra-individual variation. However, there was consensus on the histologic diagnosis of PCa disease among the histopathologist on each of the biopsy tissues.

CONCLUSION

Most of the study cohorts exhibited high-risk PSA status which was associated with sub-optimal plasma zinc levels. This observation gives credence to the fact that sub-optimal plasma zinc levels define high-risk PSA status which is indicative of high-risk PCa disease. Since high-risk PSA is characteristic of PCa progression, this suggests the implication of sub-optimal plasma zinc levels with PCa progression. Therefore, sub-optimal plasma zinc levels could serve as a marker of PCa disease progression or a target of therapeutic guidance and intervention in the management of PCa disease. However, further studies are recommended to evaluate these findings.

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REFERENCES

Aarnink R.G., De la Rossette J.J., Debruyne F.M., & Wijkstra H. (1997). Formula-derived prostate volume determination. *European Urology*, 29 (4), 399 – 402.

Abhishek A., Singh V., Sinha R.J., Ansari N.G., Siddige M.K.J., Verma M., & Kumar M. (2017). To study the relationship between cadmium, zinc and mtDNA copy number in North Indian patients suffering from prostate cancer. *African Journal of Urology, 23*, 126 – 32.

Adaramoye O.A., Akinloye O., & Olatunji I.K. (2010). Trace elements and vitamin E status in Nigerian patients with prostate cancer. *African Health Sciences*, 10 (1), 2 – 8.

Cortesi M., Fridman E., Volkov A., Shilstein S.S., Chechick R., Breskin A., Vartsky D., Kleinman N., Kogan G., Moriel E., Gladysh V., Huszar M., Ramon J., & Raviv G. (2008). Clinical assessment of the cancer diagnostic value of prostatic zinc. *The prostate*, 69, 994-1006.

Costello L.C., & Franklin R.B. (2016). A comprehensive review of the role of zinc in normal prostate function and metabolism; and its implication in prostate cancer. *Archive of Biochemistry and Biophysics, 611*, 100 – 12.

Costello L.C., Feng P., Milon B., Tan M., & Franklin R.B. (2004). Role of Zinc in the pathogenesis and treatment of prostate cancer: critical issue to resolve. *Prostate Cancer and Prostatic Diseases*, 7, 111-7.

D'Amico A.V., Whittington R., Malkowicz S.B., Schultz D., Blank K., Broderick GA., Tomaszewski J.E., Renshaw A.A., Kaplan I., Beard C.J., & Wein A. (1998). Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *Journal of American Medical Association, 280* (11), 969 – 74.

Damber J.E. (2000). Diet probably plays a role in the development of prostate cancer. *Lakartidningen, 97*(33), 3475 – 80.

Darago A., Sapota A., Matych J., Nasiadek M., Skrzypinska-Gawrysiak M., & Kilanowics A. (2011). The correlations of zinc and insulin-like growth factor (IGF-1), its binding protein (IGFBP-3) and prostate-specific antigen (PSA) in prostate cancer. *Clinical Chemistry and Laboratory Medicine*, 49 (10), 1699 – 705.

Giovannucci E. (1999). Nutritional factors in human cancer. *Advances in Experimental Medicine and Biology, 472*, 29 – 42.

Giovannucci E., Liu E.Y., Platz E.A., Stampfer M.J., & Willett W.C. (2007). Risk factors for prostate cancer incidence and progression in the health professionals' follow-up study. *International Journal of Cancer*, 121 (7), 1571 – 8.

Goel T., & Sankhwar S.N. (2009). Comparative study of zinc levels in benign and malignant lesions of the prostate. *Scandinavian Journal of Urology and Nephrology*, 40 (2), 108 – 12.

Hotz C., Brown K., H. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for control. *Food and Nutrition Bulletin*, 25 (Suppl 2), S99 – S203.

Huang L., Kirschke C.P., & Zhang Y. (2006). Decreased intracellular zinc in human tumorigenic prostate epithelial cells: a possible role in prostate cancer progression. *Cancer Cell International*, 6, 10.

Ishii K., Otsuka T., Iguchi K., Usui S., Yamamoto H., Sugimura H., Yoshikawa K., Hayward S.W., & Hirano K. (2004). Evidence that prostate-specific antigen (PSA)/Zn^{2+} axis may play a role in human prostate cancer invasion. *Cancer Lett*ers, 207 (1), 79 – 87.

Kelly S.P., Rosenberg P.S., Anderson W.F., Andreotti G., Younes N., Cleary S.D., & Cook M.B. (2017). Trends in the incidence of fatal prostate cancer in the United States by race. *European Urology*, 71(1), 195 – 201.

Kolenko V., Teper E., Kutikov A., & Uzzor R. (2013). Zinc and zinc transporters in prostate carcinogenesis. *Natures Review Urology*, 10 (4), 219 – 26.

Kombe T., Tsuji T., Hashimoto A., & Itsumura N. (2015). The physiologic, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiological Review, 95*, 749 – 84.

Naing L., Winn T., & Rush B.N., (2006). Practical issues in calculating sample size for prevalence studies. *Archive of Orofacial Sciences*, 1, 9–14.

Onyema-Iloh B.O., Meludu S.C., Ilooh E., Nnodim J., Onyegbule O., & Mykemba B. (2015). Biochemical changes in some trace elements, antioxidant vitamins and therapeutic importance in prostate cancer patients. *Asian Journal of Medical Science, 6*(1), 95-7.

Roohani N., Hurrel R., Kelishadi R., & Schulin R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences, 18*(2), 144 – 57.

Smith JC., Butrimovitze G.P., & Purdy W.C. (1979). Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clinical Chemistry, 25*, 1487-91.

Taitt H.E. (2018). Global trends and prostate cancer. A review of incidence, detection, and mortality as influenced by race, ethnicity, and geographic location. *American Journal of Men's Health, 12* (6), 1807 - 23.

To P.K., Do M.H., Cho Y.K., Kwon S.Y., Kim M.S., & Jung C. (2018). Zinc inhibits expression of androgen receptor to suppress growth of prostate cancer cells. *International Journal of Molecular Science*, 19, 3062.

Ukoli F., Osime U., Akereyeni F., Okunzuwa O., Kittles R., & Adams – Campbell L. (2003). Prevalence of elevated Serum Prostatic Specific Antigen in Rural Nigeria. *International Journal of Urology, 10*, 315 – 22.

Wakwe V.C., Odum E.P., & Amadi C. (2019). The impact of plasma zinc status on the severity of prostate cancer disease. *Investigative and Clinical Urology, 60*, 162-8.