PROFILE OF VITAMIN D LEVELS AMONG AFRICAN MEN PRESENTING FOR PROSTATE CANCER TREATMENT AT KENYATTA NATIONAL HOSPITAL

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H58/76343/2009

MBChB. (UoN)

A DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE OF THE UNIVERSITY OF NAIROBI

DECLARATION

This dissertation is my original work and has not been presented for a degree at any other university.

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ACKNOWLEDGEMENT

All praises to God Almighty, for all the strengths and blessing in completing this dissertation. Special appreciation goes to my supervisors; Dr. Gladwell Kiarie, Professor Mark Joshi and Professor Mungai Ngugi for their constant guidance, patience and support. Their invaluable comments and suggestions throughout the protocol development have contributed to the success of this research.

I would also like to express my appreciation to the patients, of the Kenyatta National Hospital for agreeing to participate in this research. My acknowledgment also goes to my statistician for his exceptional work and the laboratory and IT staff their timely expertise.

Most sincere thanks to all my colleagues, especially Kanyeki, Jackie and Mwololo for their input, guidance, kindness and moral support throughout my study.

Last but not least, my deepest gratitude goes to my beloved parents: Mr. Ezra O. Odhiambo and Mrs. Agnes Nangira Odhiambo and my sisters for their endless love, prayers, encouragement and support. To those who indirectly contributed to this research, your input is much appreciated.

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LIST OF ABBREVIATIONS

1,25-VD…… 1,25 dihydroxycholecalciferol
24-OHase ……. 24 – hydroxylase
25-VD ……..…..25 hydroxycholecaliferol
ADT …...……..……..androgen deprivation therapy
AR…… androgen receptor
BCL ……...…… B-cell lymphoma – 2 gene
BCL-x……. B-cell lymphoma extra large gene
Cdk……...…….. cyclin dependent kinase
CMIA……....chemiluminescene Immunoassay
DKK… ……..……...DickKopf
DU145……..… hormone insensitive non-PSA expressing metastatic prostate cancer brain cell
E2F……..…….……..E2 promotor binding factor
FBS ……..fetal bovine serum
GSK…….. glycogen synthase kinase
HTN……..….hypertension
KNH……..……...……..Kenyatta National Hospital
LNCaP……...……. androgen sensitive prostate cancer metastatic lymph node cell
PCa……...……..prostate cancer
PI……...…….. principle investigator
PSA……..…….. prostate-specific antigen
PTH ……...……..parathyroid hormone
Rb……..……... retinoblastoma gene
RCT……...……..randomized control trial
ROS……..……... reactive oxygen species
T2DM……..…….type 2 diabetes mellitus
TURP……..….transurethral resection of the prostate
UoN……..……...University of Nairobi
UV……..…….. ultraviolet
VDR……..…….. vitamin D receptor
VDRE's……..… vitamin D response elements

ABSTRACT

Background: Few studies have examined vitamin D profile in African men with prostate cancer. Considerable epidemiological, in vitro, in vivo and clinical data support an association between vitamin D deficiency and prostate cancer outcome .The vitamin D status in patients with prostate cancer in Kenya is unknown. This study aimed to determine the profile of vitamin D levels in patients with prostate cancer and to correlate this with patient and disease characteristics.

Methods: This was a hospital-based cross-sectional study that evaluated African men with histologically confirmed prostate cancer who sought ambulatory care at KNH from April 2012 to June 2012. Social and medical histories were obtained by direct interview and the information recorded in questionnaires. Every participant had their anthropometric measurements taken and plasma samples drawn for 25-hydroxyvitamin D (25-VD) concentrations. The relationship between age, body mass index, serum pre-diagnostic PSA and Gleason score on vitamin D status was evaluated using univariate and multivariate analysis.

Results: A total of 162 African men were evaluated. The mean 25-VD was 19.15 ng/ml and 144 (88.9%) men were classified as having vitamin D deficiency (25-VD < 30ng/ml). Of the total population, 29 (17.9%) were severely deficient (25-VD < 10ng/ml), 115 (71%) were moderately deficient (10-29 ng/ml) and 18 (11.1%) were normal (30-100ng/ml). Gleason scores greater than 7 (OR 2.9; 95% CI 1.5-5.5, p = 0.001) and serum PSA ≥ 50ng/ml (OR 2.2; 95% CI 1.7-5.1, p = 0.014) were associated with vitamin D deficiency (25-VD < 20ng/ml) whereas age and BMI were not. Adjusted for age, BMI and serum PSA levels, having Gleason scores higher than 7 was independently associated with vitamin D deficiency (OR 2.5; 95% CI 1.2–4.9, p = 0.01).

Conclusion: Vitamin D deficiency is common in African men with prostate cancer, particularly in those higher Gleason scores. This deficiency may impact negatively on prognosis.

1.0 LITERATURE REVIEW

1.1 INTRODUCTION

Prostate cancer (PCa) remains a major cause of morbidity and mortality in Kenya topping the list among all cancers in men according to the Nairobi Cancer Registry[1]. The GLOBOCAN Project 2008[2] ranks PCa as the second most common cancer in Kenyan men following esophageal cancer. The incidence is much higher in the African-American populations and its risk factors are understudied in the African region including Kenya. Indeed, vitamin D deficiency has been implicated as risk a factor for prostate cancer.[3] In cell culture studies, vitamin D metabolites have a protective action against cancer development[4]. Normal and malignant prostate cells contain vitamin D receptors (VDR),[5] [6] [7]which mediates the antiproliferative action of 1,25 - VD[8]. In addition to its antiproliferative action, 1,25-VD can cause apoptosis[9], induce differentiation[10], inhibit telomerase expression[11], inhibit tumor cell invasiveness[12] and suppress tumor-induced angiogenesis[13].

In 1941, Frank Apperly[14], a pathologist, demonstrated an inverse correlation between levels of ultraviolet radiation in North America and mortality rates from cancers in non-skin sites and proposed that sunlight somehow conferred “relative cancer immunity” to non-skin cancers. After many decades, epidemiologists rediscovered this fundamental insight. Many common cancers, such as cancers of the colon and prostate, display fascinating north – south gradients, with rates that increase systematically with increasing geographic latitude, and show an increased risk among African Americans. African-American men have a higher incidence of prostate cancer than Caucasian men[15]. In addition African-American men have lower serum vitamin D levels as a result of their dark skin pigmentation.

The major source of vitamin D is through sunlight (UV-B)-induced photobiosynthesis in the skin and results from geographical studies suggest an inverse relationship between the level of solar radiation and prostate cancer mortality[16]. Localized disease can be treated relatively successfully by radical prostatectomy[17]. In contrast, treatments for metastatic prostate cancer, including androgen ablation, are initially effective, but the majority of patients develop resistance with consequent development of androgen independent prostate cancer[18]. The rising incidence of prostate cancer and the lack of good, long-term treatments for metastatic disease highlight the

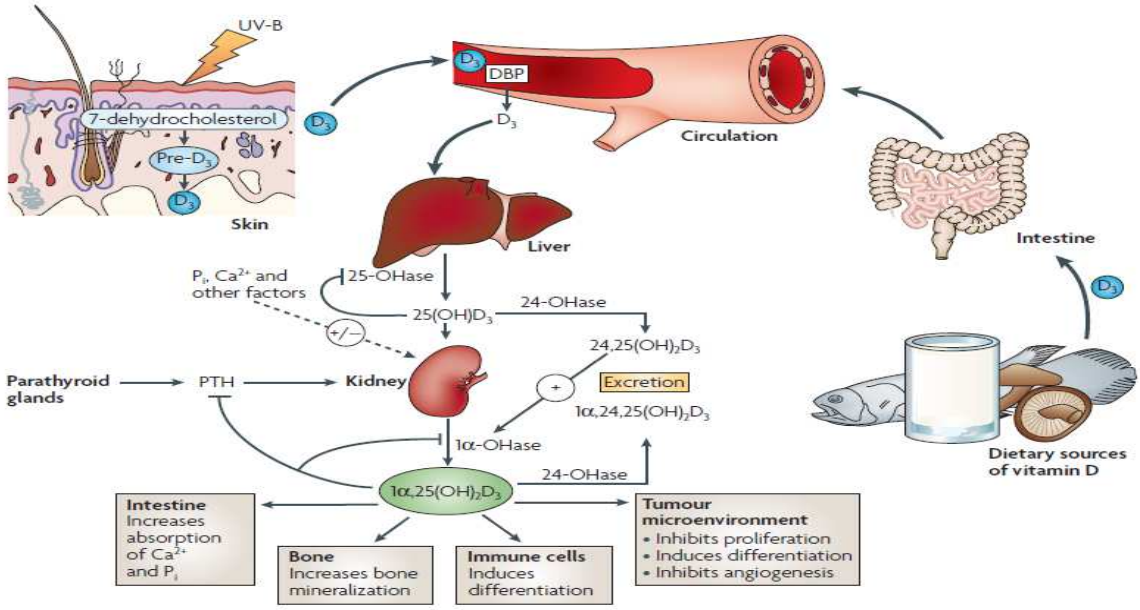
need for new chemopreventive and chemotherapeutic treatments. Vitamin D and its analogues have been proposed as candidates for these treatments.

1.2 VITAMIN D PATHOBIOLOGY

1.2.1 VITAMIN D (photobiology & metabolism)

Vitamin D, or calciferol, is a generic term, and refers to a group of lipid soluble compounds with a four-ringed cholesterol backbone. Vitamin D is best known for its actions in regulating calcium levels and bone remodeling, but recent studies highlight a role for vitamin D in the growth and differentiation of various cell types. It is synthesized in the epidermis by the conversion of its precursor, 7-dehydrocholesterol, into vitamin D3, a reaction catalyzed by the ultraviolet rays of sunlight (non-enzymatic photoisomerization). Subsequent hydroxylation reactions in the liver and kidney produce 1,25-VD. Levels of 1,25-VD are tightly regulated as excess 1,25-VD is inactivated by the enzyme 24-hydroxylase. Vitamin D can also be obtained from natural dietary sources such as fatty fish, fish liver oil, and eggs or from fortified sources such as milk, milk products, and butter. However, for most people, dietary sources contribute a negligible amount of required vitamin D3 compared to that derived from sunlight exposure. The synthesis of vitamin D and its metabolism to 1,25-VD is closely coupled to calcium homeostasis, and is modulated by parathyroid hormone, serum calcium, and phosphorus levels.

Figure 1: Vitamin D Metabolism – Adapted from Nature Reviews: Cancer 2007;7: 688



1.2.2 MECHANISM OF ACTION OF VITAMIN D

Effects of 1,25-VD are mediated through the VDR, a member of the nuclear receptor superfamily that includes receptors for steroids (androgen, progesterone, glucocorticoid and estrogen) as well as for thyroid hormone and retinoids. VDR and other family members are ligand activated transcription factors. 1,25-VD passively diffuses into target cells, binds to VDR, and activates target genes containing one or more vitamin D response elements (VDREs) within their promoters. Target genes include: osteocalcin, osteopontin, calbindin, 24-hydroxylase, and p21[19].VDR also functions as a heterodimer with the receptor for 9-cis retinoic acid, retinoid X receptor (RXR). Target genes of 1,25-VD are transcriptionally activated by heterodimers of VDR and RXR and the transcriptional activities of this complex may in some cases be influenced by 9-cis retinoic acid[20].

Other non-genetic/non-VDR intracellular activites of 1,25-VD include changes in levels of phosphoinositides, increases in intracellular calcium, stimulation of protein kinase C activity, and elevation of cyclic guanosine monophosphate levels.

1.2.3 MECHANISMS OF VITAMIN D IN CANCER PREVENTION.

Numerous mechanisms have been proposed that account for the role of vitamin D in reducing cancer incidence and mortality. Most studies have, however, based their conclusions on epidemiologic data. The mechanisms are (a) up-regulation of adherence and signaling between epithelial cells [21],(b) contact inhibition of proliferation [22], (c) differentiation[23], (d) cell cycle stabilization[24], (e) promotion of apoptosis[25] [26], (f) anti-neoangiogenesis[27], (g) down-regulation of glycogen synthase kinase 3 (GSK-3) which reduces proliferation of colorectal, prostate, and pancreatic cancers in vitro[28], (h) downregulation of the canonical Wnt signaling pathway that is active in colorectal and other cancers[29], (i) increased expression of DKK-1 protein, a tumor suppressor in colon cancer cells having mutations in the Wnt/beta-catenin pathway[30], and (j) down-regulation of DKK-4 transcription; DKK-4 is a target of the Wnt/beta-catenin pathway and is up-regulated in colorectal cancer, increasing cellular autonomy, mobility and invasiveness. The VDR- 1,25-VD complex binds to the promoter of DKK-4, largely preventing its transcription.

Vitamin D metabolites also up-regulate transcription of E-cadherins, the principal epithelial intercellular adherence proteins and induce translocation to the plasma membrane of beta-catenins, proteins whose activity results in anchoring of intercellular junctional proteins to the cytoskeleton, helping maintain the typically cuboidal, polarized shape of most epithelial cells. Vitamin D is not an antioxidant, and therefore it does not prevent ROS species from attacking DNA.

An integrative model has been proposed for cancers of epithelial origin that accommodates the actions of vitamin D and calcium[31]. This model encompasses results of tissue culture research on cell lines and epidemiological findings derived from some of the studies quoted above. The newly proposed model of cancer pathogenesis is termed the Disjunction–Initiation–Natural selection–Overgrowth–Metastasis–Involution–Transition (DINOMIT) model (Fig 2). The model includes the classical concepts of carcinogenesis, such as initiation and promotion, but encompasses the life cycle of malignancies and provides an explanation of the ability of vitamin D and calcium to prevent and potentially arrest the pathogenesis of cancer. Approximately 10% of malignancies are of non-epithelial origin, and this model may not apply to them.

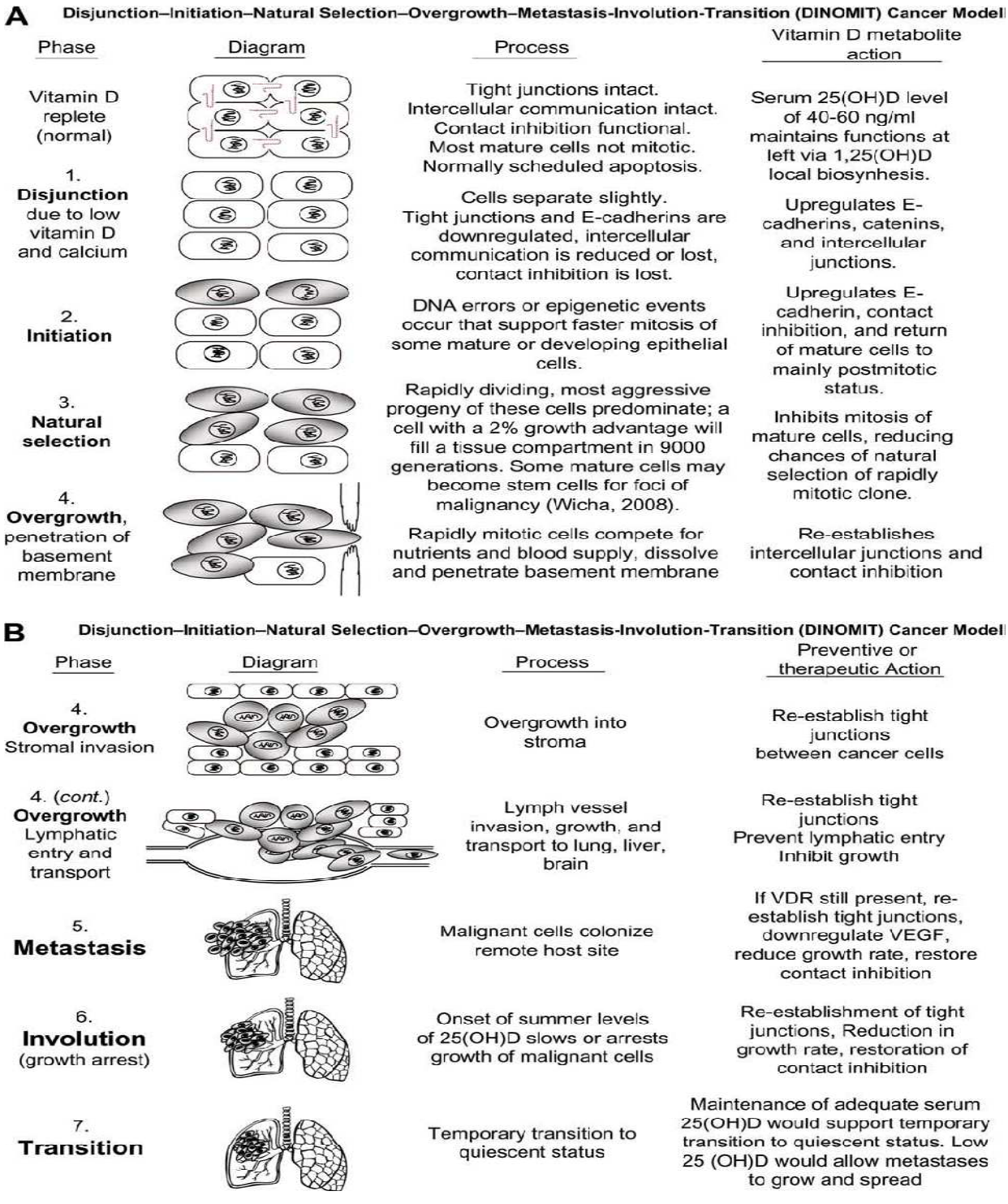


Figure 2: Disjunction–initiation–natural selection–overgrowth–metastasis–involution–transition (DINOMIT) cancer model. Adapted from Elsevier Publications 2009 AEP Vol. 19 No. 7:474

1.2.4 PROSTATE CANCER AND VITAMIN D SPECTRUM

1.2.4.1 SUNLIGHT AND PROSTATE CANCER

The top three well studied risk factors for development of prostate cancer include increasing age, race, and residence in northern latitudes. Schwartz and Hulka showed that African American men have nearly twice the risk of developing prostate cancer as Caucasian men proposing that vitamin D deficiency may be a risk factor for prostate cancer [3]. In addition, they demonstrated that cancer risk factors could be linked to vitamin D deficiency through either reduced sunlight exposure or impaired ability to convert 7-dehydrocholesterol into vitamin. This finding triggered a number of studies revolving around vitamin D and prostate cancer.

African American men have been shown to present with prostate cancer at a younger age and with more advanced disease compared to Caucasian men[32]. Asian men have the lowest risk among these three groups, although the risk for Asian immigrants in the United States increases significantly, suggesting an environmental component in prostate cancer risk[33]. Epidemiological studies have also shown that residence in northern latitudes of the United States increases the risk of developing prostate cancer[34]. This is due to reduced cumulative sunlight hours compared to the tropical region. Furthermore, older men are both less efficient in cutaneous production of vitamin D and may not receive as much sunlight exposure as younger men[35]. The increased melanin content of darker skin absorbs the ultraviolet light necessary for vitamin D3 synthesis[36], suggesting a correlation with race. Finally, residence in northern latitudes is linked to decreased exposure to sunlight and, consequently, to a reduction in vitamin D production.

1.2.4.2 VITAMIN D AND PROSTATE CANCER (GROWTH INHIBITION MECHANISMS)

Vitamin D has been suggested to inhibit prostatic cellular growth through a number of mechanisms, including changes in cell cycle progression and increases in apoptosis as outlined above. Most studies of 1,25-VD action in prostate cancer have utilized human prostate cancer cell lines. The most widely studied cell lines are the LNCaP, PC-3, and DU145 cells.

1.2.4.2.1 CELL CYCLE

LNCaP cells treated with 1,25-VD causes the cells to accumulate in the G0/G1 phases of the cell cycle[37]. During the G1 phase, the decision whether or not to progress through the cell cycle is made, a decision controlled by the retinoblastoma (Rb) gene product. When active, Rb binds and inactivates E2F, a transcription factor critical for progression to the S phase. Cyclin/cyclin-dependent kinase complexes (cyclin/cdk) have been demonstrated to inactivate Rb by phosphorylation and the cyclin/cdk complex is negatively regulated by cdk inhibitor proteins such as p21[38]. This 1,25-VD induced cell cycle accumulation in LNCaP cells involves up-regulation of the p21 cdk inhibitor and a reduction in cdk2 activity [39].

1.2.4.2.2 APOPTOSIS

Previous data has shown that 1,25-VD induces apoptosis in some breast cancer cell lines [40], triggering studies to assess its role in apoptosis in prostate cancer cells. 1,25-VD appears to induce apoptosis in LNCaP cells [9 41 42]. However, the degree of apoptosis reported varies from none (0%)[39] to a small population of the cells (10%)[9] up to 100% of the cell populations [41].

Authors suggest that 1,25-VD induced apoptosis is accompanied by a decrease in the expression of 2 anti-apoptotic proteins, Bcl-2 and Bcl-XL[9]. Bcl-2 over expression substantially reduces LNCaP cell responsiveness to 1,25-VD and blocks the induction of apoptosis by 1,25-VD. Further studies will be required to elucidate specific pathways utilized by 1,25-VD in order to trigger cell death pathways and to determine the relative importance of apoptosis in its ability to inhibit the growth of prostate cancer cells.

1.2.4.3 VITAMIN D AND ANGIOGENESIS AND METASTASIS

1,25-VD has been hypothesized in vivo to reduce angiogenesis in addition to its growth inhibitory effects thereby reducing the ability of tumor cells to metastasize. Angiogenesis is vital for tumor survival and tumor cells must have the ability to stimulate invasion by a blood supply to provide nutrition and oxygen for the rapidly dividing tumor cells. 1,25-VD inhibits endothelial cell growth, sprouting, elongation, and the ability to form networks in vitro due to induction of apoptosis in the sprouting endothelial cells. It also reduces the number of blood vessels in xenograft breast carcinoma tumors in vivo.[43]. In addition, 1,25-VD in combination with 9-cis retinoic acid synergistically inhibits angiogenesis in tumors of various origins [13], suggesting that

combination therapies would be effective in inhibiting tumor growth. Undoubtedly, further studies on 1,25-VD and its ability to inhibit angiogenesis are warranted.

The amount of evidence suggesting 1,25-VD inhibits metastasis is limited. An in vitro study demonstrated that a 1,25-VD analog (1,25-dihydroxy-16-ene-23-yne-cholecalciferol) inhibits the invasion of DU145 cells in an Amgel assay[44] . Furthermore, 1,25-VD and one of its analogs (Ro25–6760) have been shown to reduces the size and number of metastases derived from Dunning prostate tumors in vivo[45].

1,25-VD has also been shown to reduce invasion, adhesion, and migration to laminin, a basement membrane protein, in vitro via down-regulation of two laminin receptors, the α6 and β4 integrins, in the PC-3 and DU145 cells[46].Thus, these few studies suggest that 1,25-VD not only inhibits the growth of prostate cancer cells, but may also reduce the ability of the cells to metastasize.

1.2.4.4 VITAMIN D AS THERAPY FOR PROSTATE CANCER

1,25-VD has proved promising as potential therapy alone and in combination with other agents. A clinical trial tested the potential effects of 1,25-VD in 7 men whose disease had failed either radical prostatectomy or radiation therapy, and who had increasing serum PSA levels[47]. Six of the seven patients showed significant decreases in the rate of increase of serum PSA values, and, interestingly, 1 patient exhibited a drop in serum PSA. Some of the patients had stabilization of their serum PSA values for more than a year. However, all of the subjects also developed hypercalciuria. Thus, whereas 1,25-VD may have some effects on tumor growth in vivo, less calcemic analogs such as EB1089 ought to be tested to try to eliminate the calcemic effects of 1,25-VD treatment.

Cisplatin has been tested in clinical trials as a treatment for prostate cancer and other cancers with little success[48] [49]. Platinum agents (cisplatin or carboplatin) have demonstrated growth inhibitory effects in LNCaP cells and addition of both 1,25-VD and either of these platinum drugs results in greater growth inhibition of the cells than either added alone[50]. Thus, combinations of 1,25-VD and platinum agents may be of benefit as therapy for prostate cancer.

1,25-VD is inactivated by the enzyme 25-hydroxyvitamin D 24-hydroxylase (24-OHase), which is also a transcriptional target of VDR. Therefore, induction of 24-OHase results in rapid 1,25-VD metabolism, which may reduce its ability to inhibit cancer cell growth in vivo. Liarozole, a nonspecific P450 enzyme inhibitor (24-hydroxylase falls into this enzyme class), inhibits the activity of 24-hydroxylase and increases the half-life of 1,25-VD in DU145 cells[51].

These findings perhaps indicate the potential for vitamin D and other forms of differentiation therapy to contribute to our armamentarium for the treatment of prostate cancer. However, further in vivo studies are warranted to determine their utility as combination therapy.

1.3 VITAMIN D DEFICIENCY IN CANCER

Numerous studies have shown a correlation between vitamin D deficiency and prostate cancer risk, progression and aggressiveness. Some studies correlate vitamin deficiency with increases risk while others have linked hypervitaminosis D with increased risk. However, larger studies have failed to demonstrate a statistically significant link between prostate cancer risk and vitamin D deficiency.

1.3.1 PREVALENCE AND CORRELATES OF VITAMIN D DEFICIENCY

In western New York, Trump et al [52] evaluated vitamin D status in recurrent and clinically localized prostate cancer and found a mean 25-VD level of 25.9ng/ml in those with recurrent disease (n=120), 27.5ng/ml in men with clinically localized prostate cancer (n=50) and 24.5 ng/ml in controls (n=100). The frequency of vitamin D deficiency (<20ng/ml) and insufficiency (20-31ng/ml) was 40% and 32% in men with recurrent prostate cancer; 28% had vitamin D levels that were normal (32-100ng/ml). Among men with localized prostate cancer, 18% were deficient, 50% were insufficient and 32% were normal. Among controls, 31% were deficient, 40% were insufficient and 29% were normal. Metastatic disease (P=0.005) and season of blood sampling (winter/spring; P=0.01) were associated with vitamin D deficiency in patients with prostate cancer, while age, race, performance status and body mass index were not. Their team concluded that vitamin D deficiency and insufficiency were common among men with prostate cancer and apparently normal controls as well.

Choo et al [53] in Toronto, Canada examined serum 25-VD levels in a cohort of patients with nonmetastatic prostate cancer. Vitamin D insufficiency was defined as serum 25-VD of less than 30ng/ml. Serum 25-VD levels measured prospectively at baseline and, then, yearly during a 5-year follow-up were analyzed. A total of 106 patients were available for analysis. The median age was 66.3 years. At baseline, the mean 25-VD was 29 g/ml. Sixty-four patients (60.4%) met the definition of vitamin D insufficiency with serum 25-VD (<30ng/ml). Forty (37.7%), 20 (18.9%), and 2 patients (1.9%) had serum 25-VD less than 25, less than 20 and less than 10ng/ml, respectively. On a logistic regression model, season was the only significant variable associated with vitamin D insufficiency. Of a total of 477 serum 25-VD measurements from the baseline and yearly follow-up, 187 (39.2%) met the definition of vitamin D insufficiency. They concluded that vitamin D insufficiency was prevalent among patients with non-metastatic prostate cancer.

Tangpricha et al [54] conducted a small study to determine the prevalence of vitamin D deficiency in an outpatient cancer care clinic at Boston University Medical Center. They included a control group of healthy adults without cancer aged 40 years and above. Vitamin D deficiency was defined as a 25-VD level of 20ng/ml or less. Baseline characteristics were well matched. Of the 56 patients with cancer, 27 (48%) had vitamin D deficiency. In comparison, only 6 (12%) of the 50 healthy control subjects had vitamin D deficiency ($P < 0.05$). The mean 25-VD values were 21.3 ± 10 ng/ml for the patients with cancer versus 33.9 ± 10ng/ml for the healthy control subjects ($P<0.05$).

1.3.2 VITAMIN D DEFICIENCY AND PREDISPOSITION TO CANCER

Baseline vitamin D insufficiency had been linked to subsequent risk of developing PCa. In a large study in Finland, Ahonen et al [5] evaluated pre-diagnostic serum vitamin D levels in 19000 middle-aged men. After a 13-year follow up period, they found that prostate cancer risk, analyzed by quartiles of the 25-VD levels, was inversely related to 25-VD levels. Men with 25-VD concentration below the median had an adjusted relative risk (OR) of 1.7 compared to men with 25-VD level above the median. The prostate cancer risk was highest among younger men (< 52 years) at entry and low serum 25-VD (OR 3.1 nonadjusted and 3.5 adjusted). Among those younger men (< 52 years), low 25-VD entailed a higher risk of non-localized cancers (OR 6.3). The mean age at diagnosis of the patients with 25-VD concentration above the median was 1.8

years higher than that of patients with vitamin D below the median (63.1 vs 61.3 years). Their team concluded that low levels of 25-VD associated with an increased risk for subsequent earlier exposure and more aggressive development of prostate cancer, especially before the andropause.

In contrast, Barnett et al [55] followed 1,433 elderly males and found that in comparison with the lowest quartile of 25-VD, the hazard ratio for the highest quartile of 25-VD was 1.22 (CI 0.50-1.72, p = 0.25), no trend across quartiles (p=0.94) or association with Gleason score was observed. Adjustment for covariates did not alter the results. They found no association between serum 25-VD vitamin D levels and subsequent risk of prostate cancer in older men.

Giovannucci et al [56] studied 25-VD levels and subsequent overall cancer risk in a subset of 1095 men in the Health Professionals Follow-Up Study. They utilized a linear regression model incorporating six personal characteristics (dietary and supplemental vitamin D, race, adiposity, geographic residence, and leisure-time physical activity) as predictors of the plasma levels of 25-VD. They then used this statistical model to compute predicted 25-VD levels for all 47800 men in the cohort and examined whether the 25-VD index was related to subsequent cancer risk. They reported that an increment of 10ng/ml in predicted serum 25-VD was associated with a 17% reduction in total cancer incidence (RR = 0.83 [0.73 to 0.94]) and a 29% reduction in total cancer mortality (RR = 0.71 [0.60 to 0.83]), with even stronger effects for digestive tract cancers.

When we look at the other side of the coin, high vitamin D levels have also been linked to an increased risk of developing prostate cancer. In a large, multicentre, longitudinal, nested case control study in the Nordic countries, Pentti & Ahonen et al found that both low and high levels of serum vitamin D are associated with higher prostate cancer risk.[57] They studied serum 25-VD levels of 622 prostate cancer cases and 1,451 matched controls and found that both low (< 7.8ng/ml) and high (>32ng/ml) 25-VD serum concentrations are associated with higher prostate cancer risk. The normal average serum concentration of 25-VD (16–24 ng/ml) comprised the lowest risk of prostate cancer. In the full study group, a U-shaped prostate cancer risk was observed, with an increasing trend of risk (ORs - 1.3 and 1.5) when the vitamin D level decreased from the reference level of 16 – 23ng/ml. Again, when the vitamin D level increased from the reference level, risk increased (ORs - 1.2 and 1.7). This U-shaped risk of prostate

cancer might be due to similar 1,25-VD availability within the prostate: low vitamin D serum concentration apparently leads to a low tissue concentration and to weakened mitotic control of target cells, whereas a high vitamin D level might lead to vitamin D resistance through increased inactivation by enhanced expression of 24-hydroxylase.

Since there are plans for prostate cancer prevention with vitamin D supplementation alone or combined, these findings might be an important contribution to the strategy because very high serum vitamin D levels may not be the appropriate goal. Very high 25-VD serum levels can be reached without any significant side effects for short periods. Recommendations for vitamin D supplements have been put forward, however, synthetic vitamin D derivatives are favorable for chemoprevention because of their low calcemic effects. This study suggests that moderately high levels of vitamin D for long periods may have adverse effects on prostate cancer risk. Therefore, other carefully planned studies on vitamin D and prostate cancer risk are needed to conclusively elucidate this issue.

When all is put together the statistical significance of these associations declines. In Germany, Yin et al [58] conducted a meta-analysis of longitudinal studies looking at serum vitamin D and prostate cancer risk. They found that overall, eleven original articles were included, ten of which reported on the association between serum vitamin D levels and prostate cancer incidence and one article reported on the association with prostate cancer mortality. Meta-analysis these studies on PCa incidence resulted in a summary OR = 1.03 [0.96-1.11]) associated with an increase of 25-VD by 10ng/ml (P=0.362). No indication for heterogeneity and publication bias was found. It was concluded that according to available evidence from longitudinal studies, serum 25-VD is not associated with PC incidence.

1.3.3 VITAMIN D DEFICIENCY AND PROSTATE CANCER MORTALITY

Vitamin D status has also been linked to prostate cancer mortality. In Oslo (Norway) Tretli et al [59] looked at pre-treatment serum 25-VD levels in 160 men 37 of whom had received prior hormonal therapy assessing associations between these levels and prostate cancer mortality. The serum level of 25-VD was classified as low (<20 ng/ml), medium (20-32ng/ml) or high (>32 ng/ml). A Cox proportional hazard regression model was used to assess the association between

serum 25-VD and cancer mortality. During follow-up, 61 deaths occurred, of whom 52 died of prostate cancer. The median time of follow-up was 44.0 months (range, 1.2-154.6). Serum 25-VD of 20-32ng/ml (RR=0.33[0.14-0.77]) and > 32ng/ml RR 0.16 [0.05-0.43]) were significantly related to better prognosis, compared with the low levels (<20ng/ml). Analysis restricted to patients receiving hormone therapy gave a stronger association. They concluded that the serum level of 25-VD may be involved in disease progression and is a potential marker of prognosis in patients with prostate cancer.

1.3.4 VITAMIN D DEFICIENCY AND ADVANCED PROSTATE CANCER

With regard to disease aggression, a study by Li and colleagues [60] of the Physicians' Health Study cohort found that physicians whose 25-VD and 1,25-VD levels were both below the median, 25-VD of 28 ng/ml and had twice the incidence of aggressive prostate cancer (OR = 2.1 [1.2–3.4], p = 0.05) as compared to men whose levels were above the median. In addition, Corder et al [61] reported that the risk for developing palpable prostate tumors of higher Gleason score increased men with low serum levels of 25-VD, although other studies[62] [63] have found no correlation.

Just recently, Gilbert et al studied the associations of circulating total 25-VD with PSA-detected prostate cancer in a case-control study nested within the Prostate Testing for Cancer and Treatment (ProtecT) trial[64]. Pre-determined categories of total 25-VD were defined as: high: ≥30ng/ml; adequate: 20-<30ng/ml; insufficient: 12-<20ng/ml; deficient: <12ng/ml. They included 1,447 prostate cancer cases (153 advanced, 469 high-grade) and 1,449 healthy controls. His team found that men deficient in vitamin D had a two-fold increased risk of advanced versus localized cancer (OR for deficient vs. adequate total 25-VD = 2.33(1.26-4.28) and high-grade versus low-grade cancer (OR for deficient vs. adequate total 25-VD=1.78(1.15-2.77). However they found no evidence of a linear association between total 25-VD and prostate cancer (p=0.44) or of an increased risk of prostate cancer with high and low vitamin D levels.

They, therefore, concluded that lower 25-VD concentrations were associated with more aggressive cancers (advanced versus localized cancers and high- versus low- Gleason grade), but there was no evidence of an association with overall prostate cancer risk.

1.3.5 EFFECT OF TREATMENT ON VITAMIN D LEVELS

Prostatectomy has not been shown to affect serum vitamin D status as well as androgen deprivation therapy[65]. Use of cytotoxic chemotherapy has been associated with lower vitamin D levels in patients with advanced colon cancer[66] . There are no reports that suggest radiation treatment may lower serum vitamin D levels in prostate cancer.

1.3.6 ADEQUATE VITAMIN D AND CANCER PREVENTION

A few studies have looked at the impact of serum levels of 1,25-VD and its metabolites and the effects on risk for several forms of cancer (including aggressive forms of prostate cancer). Most of these data are however, derived from epidemiologic and observational data. However, one RCT by Lappe et al looked at vitamin D and calcium supplementation and cancer risk reduction in a large population of healthy post-menopausal women[67]. Subjects were randomly assigned to receive 1400-1500 mg supplemental calcium alone (Ca-only), supplemental calcium plus 1100 IU vitamin D3 (Ca + D), or placebo. When analyzed by intention to treat, cancer incidence was lower in the Ca + D women than in the placebo control subjects ($P < 0.03$). Their team concluded that improving calcium and vitamin D nutritional status substantially reduces all-cancer risk in postmenopausal women.

A review article by Garland et al, projected that that raising the minimum year-around serum 25-VD level to 40 to 60 ng/ml would prevent approximately 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer each year, and 75% of deaths from these diseases in the United States and Canada[68]. These conclusions were largely derived from observational studies. They further suggested that intakes also are expected to reduce case-fatality rates of patients who have breast, colorectal, or prostate cancer by half. In addition, the concluded that there are no unreasonable risks from intake of 2000 IU per day of vitamin D, or from a population serum 25-VD level of 40 to 60 ng/ml.

2.0 STUDY JUSTIFICATION

The burden of prostate cancer continues to rise. By the year 2030, the annual incidence is predicted to double, particularly in the developing countries. GLOBOCAN project documented 1087 new cases in Kenya on the year 2008 with an 81% death rate. PCa patients in Kenya present on average on decade earlier .A majority of patients have aggressive and advanced forms at time of diagnosis. This age group represents the main breadwinner male population[69]. Ultimately, families suffer greatly as their economic status deteriorates. It is evident that PCa impacts greatly on morbidity, mortality and economy as well.

Vitamin D deficiency has been shown to be associated with increased risk of developing prostate cancer[5] despite presence of conflicting reports. These deficiency states have also been linked to more aggressive disease, faster progression and higher metastatic potential of prostate cancer[60 61].

The prevalence of vitamin D deficiency in African men is unknown. Furthermore, this data in men with prostate cancer in tropical Africa is unknown. Filling this knowledge gap would serve as benchmark statistics of the magnitude of this problem. This will be a great step forward for further studies looking at vitamin D and prostate cancer in Africa.

3.0 RESEARCH QUESTION

What are the levels of vitamin D in patients with prostate cancer in KNH and how does it correlate with patient and disease characteristics?

4.0 OBJECTIVES

4.1 BROAD OBJECTIVE

- To determine the profile of vitamin D levels among patients with histologically confirmed prostate cancer in KNH and to correlate it with patient and disease characteristics.

4.2 SPECIFIC OBJECTIVES

4.2.1 PRIMARY OBJECTIVE

1. To determine the levels of serum 25-hydroxycholecalciferol in patients with histologically confirmed prostate cancer.
2. To document the Gleason scores and pre-diagnostic serum PSA in the study population.
3. To describe the age, medical history and BMI in the study population.

4.2.2 SECONDARY OBJECTIVE

- To determine the association between serum 25-hydroxycholecalciferol deficiency and age, BMI, Gleason score and serum pre-diagnostic PSA.

5.0 METHODOLOGY

5.1 STUDY SITE

This study was carried out at the Kenyatta National Hospital Cancer Treatment Centre (Radiotherapy Unit) and Urology outpatient clinics.

5.2 STUDY POPULATION

The target population was patients seeking ambulatory care at KNH Cancer Treatment Centre & Urology outpatient clinic with documented histology confirming prostate cancer adenocarcinoma.

5.3 STUDY DESIGN

This study was a hospital based cross-sectional survey

5.4 SAMPLE SIZE

The sample size was calculated using the following method:

$$N = \frac{z^2 \times p(1-p)}{d^2}$$

N=minimum sample size required

z=confidence interval at 95% (standard value of 1.96)

p=estimated prevalence of those with normal vitamin D levels Trump et al [52] study = 28%

(Deficient = 40%, insufficient 32%, **normal = 28%**)

d=margin of error (0.07)

$$N = \frac{(1.96)^2 \times 0.28(1-0.28)}{(0.07)^2}$$

The minimum sample size for this was **159** patients with prostate cancer

5.5 SAMPLING METHOD

Consecutive sampling was undertaken to recruit patients in the aforementioned study sites. This was done between 15-20 patients per week until the desired sample size was achieved.

5.6 INCLUSION AND EXCLUSION CRITERIA

5.6.1 INCLUSION CRITERIA

1. Histologically confirmed prostate cancer.
2. Age over 40 years
3. Written informed consent.

5. 6.2 EXCLUSION CRITERIA

1. Current vitamin D3 supplementation.
2. Incomplete/inconclusive histology report.
3. PCa diagnosis more than 3-months old.

5.7 CASE DEFINITION

Prostate cancer was defined by a histopathology report of a prostate gland biopsy specimen confirming presence of malignancy complete with Gleason scores diagnosed in the preceding three months.

5.8 SCREENING AND RECRUITEMENT

The principal investigator (PI) with the help of research assistants reviewed files of patients attending the urology clinics and cancer treatment centre. This was done everyday just before the beginning of their scheduled routine clinic visit at the respective sites. The files of patients' who met the selection criteria were identified and color-coded for easy identification. Once all color-coded files for that day were identified, subjects were called into the interviewing room after their routine visit and given all the relevant information about the study. Those who gave written informed consent (appendix I) were recruited.

5.9 PROCEDURES

5.9.1 CLINICAL METHODS

The sociodemographic data was obtained from the patient followed by medical history and anthropometric measurements which were used to compute body mass index. Gleason scores and pre-diagnostic serum PSA levels were sought from patient records. This information was subsequently entered into the study proforma (appendix II) for later analysis.

5.9.2 LABORATORY METHODS

2-3 mls of blood was collected from the antecubital fossa in each study participant and immediately put in a plain vacutainer (red top) then subsequently delivered to the Lancet Kenya laboratories at the end of the days' collection for estimation of serum 25-VD concentration. Since all samples were analysed on the day of collection, storage at cool temperatures was not a requirement. Serum vitamin D concentrations were determined by the LIAISON® 25-OH Vitamin D assay technique, an automated chemiluminescent immunoassay (CMIA) method that is rapid, accurate and precise. This method is well validated according to the National Committee for Clinical Laboratory Standards (NCCLS) protocols[70]. In addition, it is comparable to the gold standard liquid chromatography isotope dilution tandem mass spectrometry (LC-IDMS/MS)[71] and correlated well with radioimmunoassay techniques[72]. Serum 25-VD estimation is the optimal test to determine body stores as its metabolite 1-25-VD is rapidly utilized in target organs.

5.9.3 QUALITY ASSURANCE

Standard operating procedures of Lancet Kenya Laboratories (appendix III) for specimen collection, preparation and storage were followed to minimize pre-analytical errors. To ensure quality was maintained, the laboratory tests were carried out in Lancet Kenya laboratories. Machines used were properly calibrated using standard calibration methods and materials and tests assayed against controls. Lancet Kenya laboratory carries out internal and external quality control.

5.10 STUDY VARIABLES

5.10.1 DEPENDENT VARIABLES

- Serum 25-hydroxycholecalciferol levels

5.10.1.1 DEFINITION OF VITAMIN D DEFICIENCY

Vitamin D profile was determined by serum measurement of 25-VD levels as this is the most sensitive and standard modality to assess the levels. We utilized reference ranges provided by Lancet Kenya Laboratories. These levels are in accordance with the universally agreed upon reference ranges. Subjects' vitamin D status was classified as follows:

Table 1: Vitamin D normal laboratory reference ranges

Vitamin D status	25-VD (ng/ml)
Severe deficiency	<10
Moderate deficiency	10-<30
Normal	30-100
Toxicity	>100

For univariate and multivariate analysis, vitamin D levels were stratified as follows:

Table 2: Serum vitamin D correlation status

Vitamin D status	25-VD (ng/ml)
Deficient	< 20
Sufficient	≥ 20

Serum 25-VD levels above 20ng/ml are generally required to realize its anti-tumor properties. Numerous trials have demonstrated cancer risk reduction above this level [5, 67, 68].

5.10.2 INDEPENDENT VARIABLES

- Age
- BMI
- Disease characteristics
    - Histopathological grade (Gleason grading system – Appendix IV)
        - GX – Gleason score cannot be assessed
        - Gleason score $\leq$ 7
        - Gleason score > 7

      Gleason scores of $\geq$ 7 is of prognostic significance. Tumors with these scores tend to be more aggressive [60 61 64].
    - Pre-diagnostic serum PSA levels (ng/ml)
        - < 50
        - $\geq$ 50

      Serum PSA of $\geq$ 50 is also of prognostic significance especially in patients with advanced disease[73].

5.11 DATA MANAGEMENT AND ANALYSIS

Each study proforma was assigned with a unique study serial number to prevent duplication of data collection. All data collected was entered into a password protected computer database using Microsoft access computer software. Statistical analysis was done using statistical package for social scientists (SPSS) version 20 after cleaning and verification with the input of a statistician who had been involved since the beginning of this proposal development. The socio-demographic characteristics, medical history, Gleason scores, serum PSA and vitamin D levels were summarized into means, standard deviations, medians, proportions and frequencies for continuous data and proportions, frequencies and percentages for categorical data. Data summaries were presented using tables, pie charts and graphs.

For comparison purposes, vitamin D deficiency was considered as serum 25-VD concentration of less than 20ng/ml. This was the dependent variable and was tested for association with age, BMI, Gleason score and serum pre-diagnostic PSA using bivariate analysis. All independent variables were categorized prior to secondary explorations. A patient's likelihood of having serum 25-VD deficiency was estimated using odds ratios. Variables were included as potential confounders in multivariate models if they were significantly associated with 25-VD concentrations in unadjusted logistic regression models. Variables that were not significant predictors were subsequently dropped. All the statistical tests were performed at 5% level of significance.

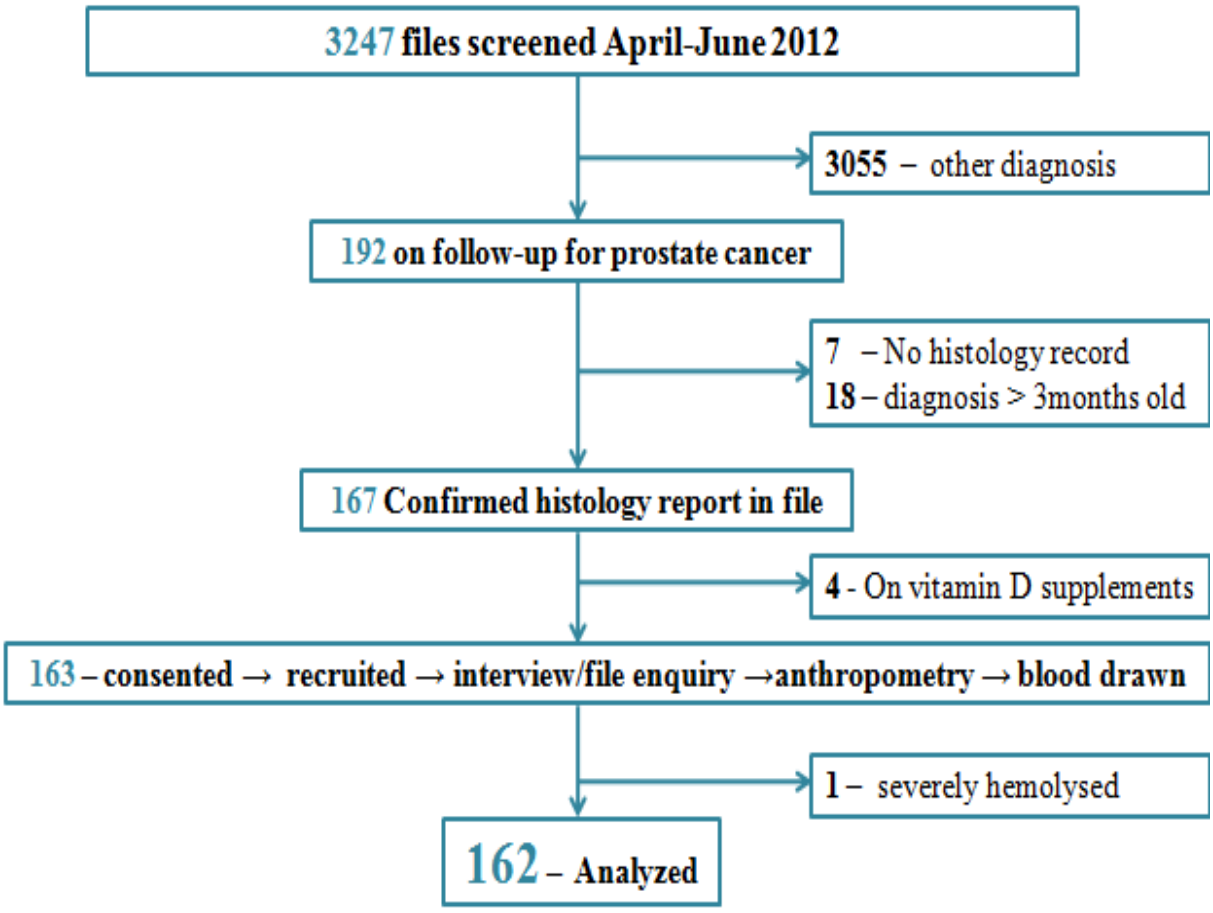
5.13 ETHICAL CONSIDERATIONS

Before commencing, permission to carry out this study was sought from the University of Nairobi's Department of Clinical Medicine and Therapeutics, as well as the KNH/UoN Ethics and Research Committee. Only patients who gave informed consent were recruited into the study. No patient was coerced into participating. There was no discrimination against any patient who declined to participate. All information collected was treated as confidential. Only blood samples intended for study were drawn and thereafter discarded after analysis. Any information that was deemed as important to the management of the patient was communicated to the primary health care provider. The cost of the study was met by the principal investigator.

6.0 RESULTS

Between April and June 2012, the records of one hundred and ninety two patients on follow up for prostate cancer were consecutively sampled at the urology clinic and cancer treatment centre at Kenyatta National Hospital ((Figure 4). Twenty nine patients did not meet the inclusion criteria; eighteen patients had been diagnosed more than three months prior to interview; seven patients did not have any documented prostate cancer histology in the records while four were on oral vitamin D supplements. One hundred and sixty three patients were recruited and subsequently interviewed, anthropometric measurements taken and blood samples drawn for serum vitamin D concentration. Out of those who met the inclusion criteria, one participants' blood sample was severely hemolysed, leaving one hundred and sixty two (162) patients whose data was submitted for analysis.

Figure 3: Recruitment flow chart



6.1 POPULATION CHARACTERISTICS

The study population was relatively elderly with a mean age of 69.27 years. Majority of the patients i.e. seventy-nine (48.8%) were aged between 61 and 70 years. All one hundred and sixty two (100%) patients were married. Sixty-eight (42%) had received post-primary education. One hundred and thirty-eight (85.2%) patients came from Central, Eastern, Nairobi and Coast region (Table 3).

Table 3: Socio-demographic characteristics of the study participants.

Variable	Frequency (%) N = 162	95% CI
Age in yrs (at diagnosis)		
• Mean (SD)	69.27 (8.566)	
• Median (IQR)	68 (64.2-74)	
• Min-Max	47-96	
Age strata		
• ≤ 60	23 (14.2%)	(8.83 – 19.57)
• 61-70	79 (48.8%)	(41.1 – 56.5)
• 71-80	45 (27.8%)	(20.9 – 34.7)
• > 80	15 (9.3%)	(4.83 – 13.77)
Marital status		
• Married	162 (100%)	
Level of education		
• Primary	94 (58)	(50.4 – 65.6)
• Secondary	53 (32.7)	(25.48 – 39.92)
• Tertiary	15 (9.3)	(4.83 – 13.77)
County location		
• Eastern block (*Nairobi, Central, Eastern, N.Eastern & Coast)*	138 (85.2)	(79.73 – 90.67)
• Western block *(Rift Valley, Nyanza, Western)*	24 (14.8)	(9.33 – 20.27)

6.2 CLINICAL CHARACTERISTICS

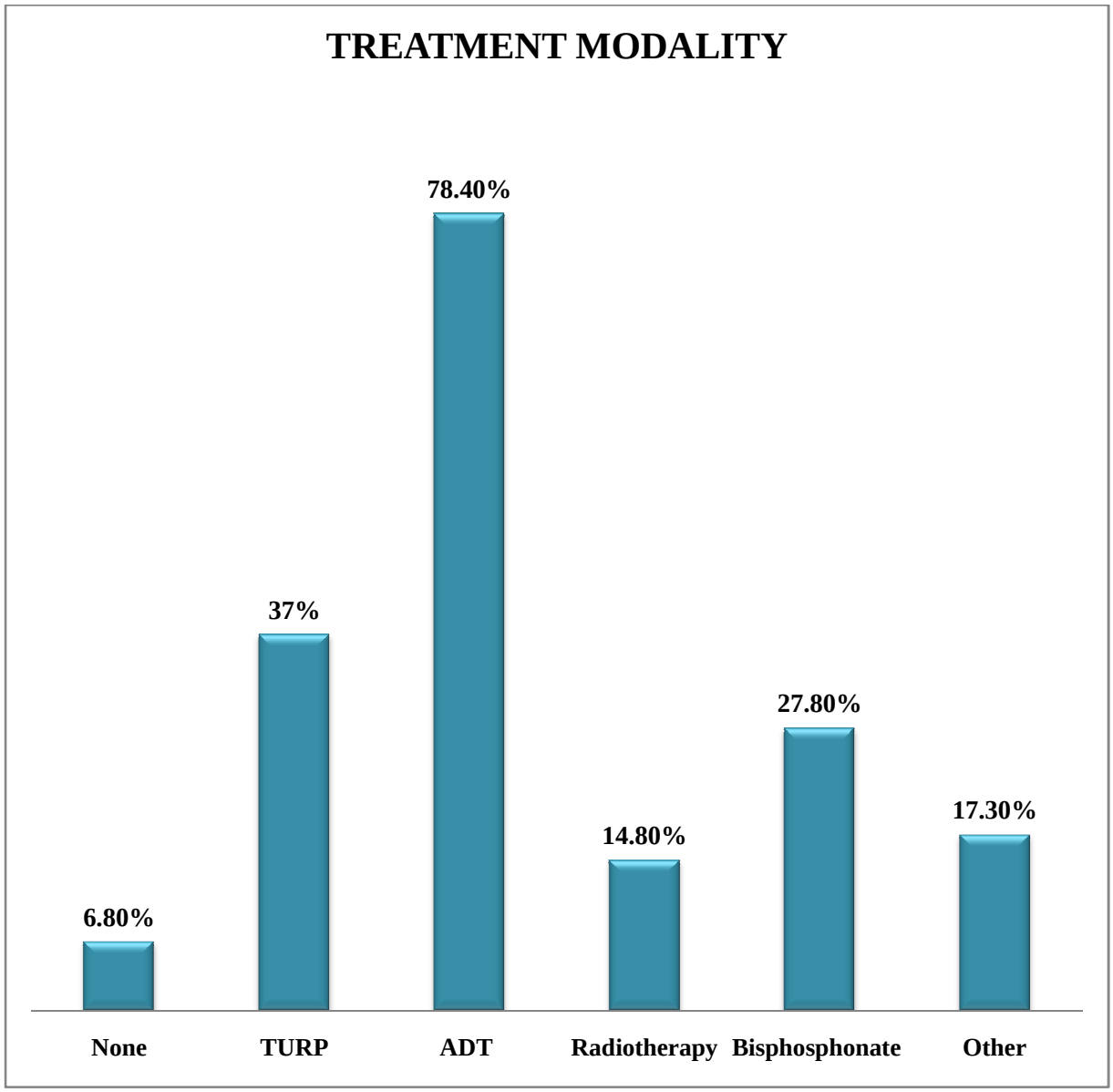
Twenty-six patients (16%) had a 1st degree relative with prostate cancer. Fifty patients (30.9%) had a first degree relative with any other cancer. Twenty-five patients (15.4%) were currently known to have type 2 diabetes whereas forty-three (26.5%) were known to be hypertensive. One hundred and eleven patients (68.5%) were former cigarette smokers while fifteen (9.3%) were current cigarette smokers (Table 4).

Table 4: Study participants' medical history

		Frequency (%) N = 162	95% CI
Family history of Prostate Cancer (1st degree relative)		26 (16)	(10.35 – 21.65)
Family history of any other Cancer (1st degree relative)		50 (30.9)	(23.78 – 38.02)
Known type 2 diabetic		25 (15.4)	(9.84 – 20.96)
Known hypertensive		43 (26.5)	(19.7 – 33.3)
Smoking Status	Current	15 (9.3)	(4.83 – 13.77)
	Former	111 (68.5)	(61.35 – 75.65)
	Never	36 (22.2)	(15.8 – 28.6)

With regard to ongoing and past treatment modalities, participants were on or had received various forms of treatment at the time of interview. Participants may have received more than one treatment modality. One hundred and twenty seven participants (78.4%) were on androgen deprivation therapy i.e. both medical and surgical castration, sixty (37%) had undergone channel TURP for obstructive symptoms, and twenty-four (14.8%) had undergone external beam radiotherapy. Forty-five participants (27.8%) were currently receiving bisphosphonates for metastatic bone disease and twenty-eight (17.3%) were on other modalities of treatment which included systemic chemotherapy with docetaxel, junior aspirin, dexamethasone, herbal therapies and various forms of analgesia. Only eleven participants (6.6%) had not been initiated on any form of treatment at time of interview (Figure 5).

Figure 4: Treatment Modality of Study Subjects

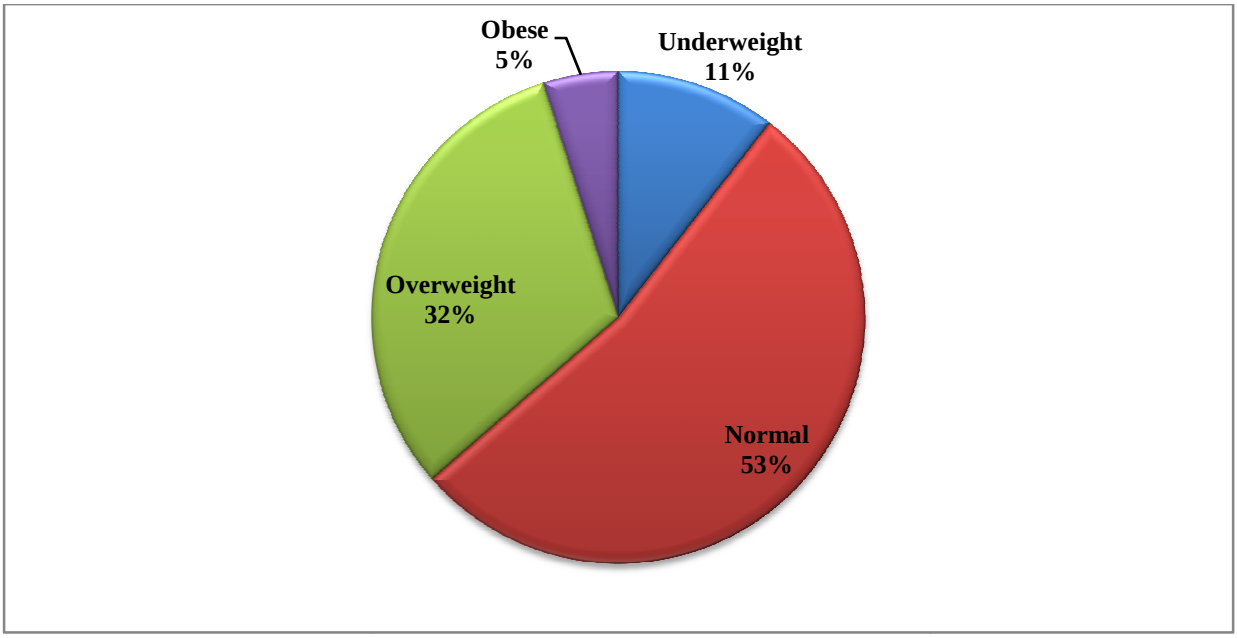


The mean weight, height and BMI were 64.88 kilograms, 1.75 meters and 23.4 kg/m^2 respectively (Table 5). Eighty-six subjects (53.1%) had normal BMI, whereas fifty-one (31.5%) and eight (4.9%) were overweight and obese respectively. Seventeen subjects (10.5%) were underweight (Figure 6).

Table 5: Anthropometric measurements in the study population

Variable	Frequency (%) N = 162	95% CI
Weight in KG		
• Mean (SD)	64.88 (10.96)	
• Median (IQR)	64 (58 – 72.75)	
• Min-Max	40 – 95	
Height in M		
• Mean (SD)	1.75 (1.2)	
• Median (IQR)	1.66 (1.62 – 1.7)	
• Min - Max	1.5 – 1.88	
BMI		
• Mean (SD)	23.4 (3.78)	
• Median (IQR)	23.2	
• Min - Max	14.9 – 32.2	
BMI stratum		
• Underweight	17 (10.5)	(5.78 – 15.22)
• Normal	86 (53.1)	(45.42 – 60.78)
• Overweight	51 (31.5)	(24.35 – 38.65)
• Obese	8 (4.9)	(1.58 – 8.22)

Figure 5: BMI categories



The mean Gleason score was 7.15 with seventy subjects (43.2%) having a Gleason score of greater than 7. The mean and median pre-diagnostic serum PSA was 280.2 and 69.3 ng/ml respectively. One hundred and twenty-six subjects (77.8%) had a serum PSA of more than 20. Fifty subjects (30.9%) had documented bone metastasis (Table 6)

Table 6: Study participants' disease characteristics

Variable	Frequency (%) N = 162	95% CI
Gleason score		
• Mean (SD)	7.15 (1.27)	
• Median (IQR)	7.00 (6 – 8)	
• Min – Max	4 – 10	
Gleason score strata		
• ≤ 7	92 (56.8)	(49.17 – 64.43)
• >7	70 (43.2)	(35.57 – 50.83)
Serum PSA (pre-diagnostic)		
• Mean (SD)	280.2 (910.4)	
• Median (IQR)	63.9 (26.25 – 133.29)	
• Min – Max	0.07 – 8700	
Serum PSA strata		
• <10	12 (7.4)	(3.37 – 11.43)
• 10-20	24 (14.8)	(9.33 – 20.27)
• 21-100	73 (45.1)	(37.44 – 52.76)
• >100	53 (32.7)	(25.48 – 39.92)
Documented bone metastases		
• Yes	50 (30.9)	(23.78 – 38.02)
• No	152 (69.1)	(61.98 – 76.22)

6.3 SERUM 25-HYDROXYCHOLECALCIFEROL PROFILE

We then assayed the blood samples to determine the serum 25-VD concentration in our study subjects. For descriptive and comparison purposes, the interpretation of the serum 25-VD concentration was based on internationally accepted reference ranges as outlined in the methodology section. For purposes of correlation vitamin D deficiency cut point was set at 20ng/ml. Literature has demonstrated that at levels above this, one begins to appreciate the anti-cancer properties of vitamin D [5, 67].

Serum 25-VD concentrations were normally distributed (Figure7) ranging from 4.0 to 46.4. The mean (SD) serum 25-VD concentration in this study population was 19.15ng/ml with a median of 19.35 (8.28) ng/ml and an inter-quartile range of 13.2 to 24.8ng/ml (Table 7). Serum 25-VD cut-off was set at 30ng/ml. One hundred and forty-four subjects (88.9%) had serum 25-VD deficiency. One hundred and fifteen (71%) and twenty-nine (17.9) subjects had moderate and severe deficiency respectively (Figure 8). Eighty five subjects (52.5%) had serum 25-VD levels of below 20ng/ml.

Figure 6: Serum 25-VD profile distribution – Histogram

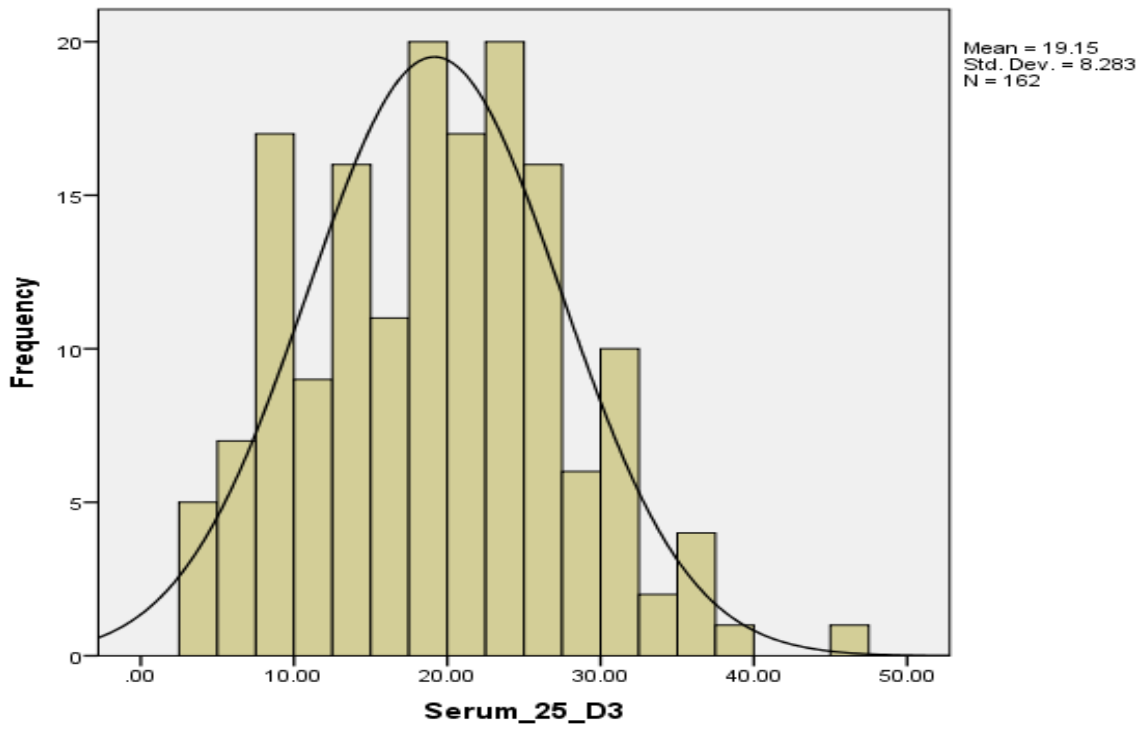
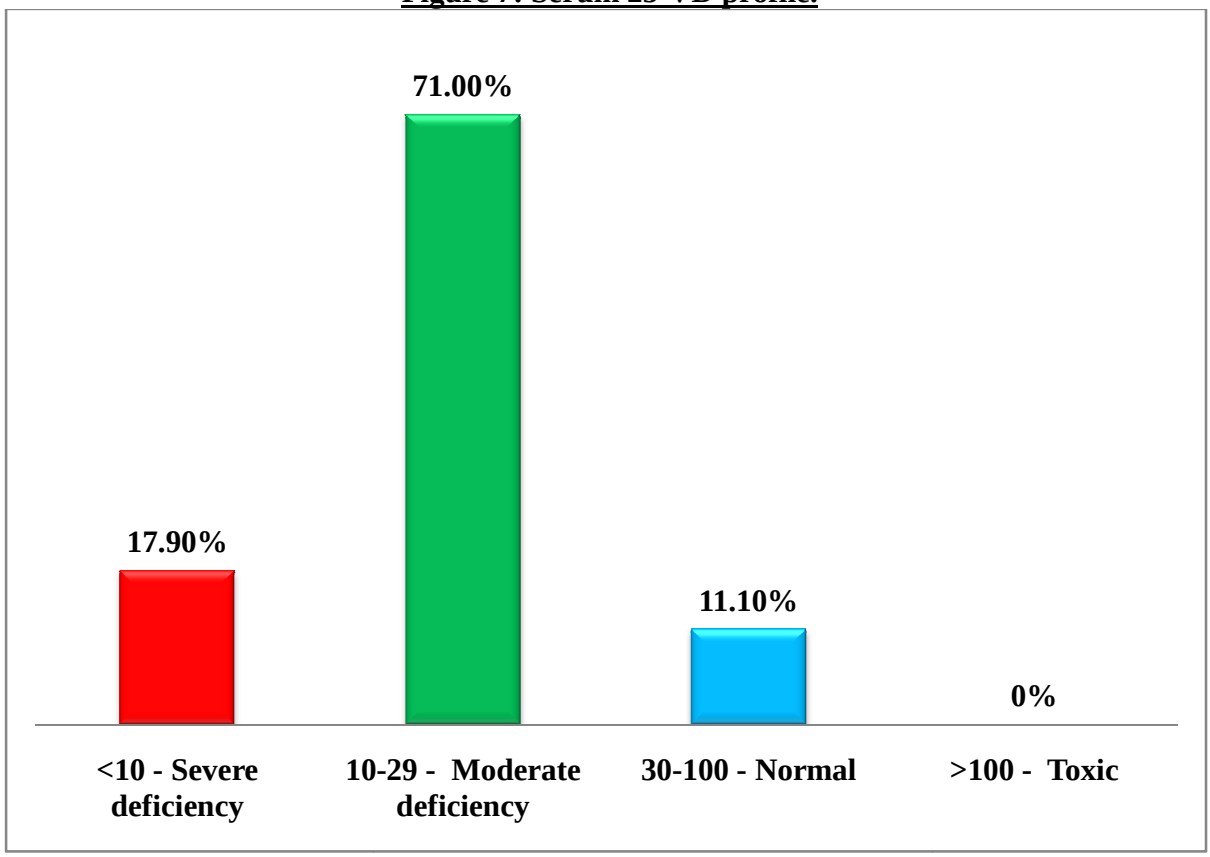


Table 7: Serum 25-VD profile

Variable	F (%) N = 162	95% CI
Serum 25-VD		
• Mean (SD)	19.15 (8.28)	
• Median (IQR)	19.35 (13.2 – 24.8)	
• Min – Max	4 – 46.8	
Serum 25-VD stratum 1		
• < 10 **(Severe deficiency)**	29 (17.9)	(12 – 23.8)
• 10-<30 **(Moderate deficiency)**	115 (71.0)	(64.01 – 77.99)
• 30-100 **(Normal)**	18 (11.1)	(6.26 – 15.94)
• >100 **(Toxic)**	0 (0)	
Serum 25-VD stratum 2		
• < 20	85 (52.5)	(44.8 – 60.2)
• ≥ 20	77 (43.2)	(35.5 – 50.8)

Figure 7: Serum 25-VD profile.



6.4 ASSOCIATIONS BETWEEN VARIABLES

Univariate analysis (Table 8) was then performed to explore associations between serum 25-VD levels and various patient and disease characteristics of interest. These characteristics included patients' age at diagnosis, BMI, Gleason scores and pre-diagnostic serum PSA levels.

Participants ages 80 and above were 2.1 times more likely to have a serum 25-VD deficiency (<20ng/ml) than those aged 60 years and below and though clinically important, this did not reach statistical significance (p = 0.297). For BMI category, those who were categorized as being obese as compared to those who were underweight were less likely to have serum 25-VD deficiency although statistical significance was not reached (OR – 0.3; 95% CI 0.05 – 1.8, p-value = 0.209) (Table 8).

We further explored the association between serum 25-VD levels and disease characteristics i.e. Gleason scores and pre-diagnostic serum PSA levels. In unadjusted univariate analysis (Table 8), participants with a Gleason score of greater than 7 were nearly three times more likely to have serum 25-VD deficiency compared to those with a score of equal to or less than 7 and this was statistically significant (OR – 2.9; 95% CI 1.5–5.5, p-value = 0.001).

For serum PSA category, those with a level of equal to or more than 50ng/ml were twice as likely to have serum 25-VD deficiency and this was also statistically significant (OR - 2.2; 95% CI 1.5–5.1, p-value = 0.014).

In a multivariate model, (Table 9) the association between pre-diagnostic serum PSA and vitamin D deficiency was attenuated leaving Gleason score as the only independent predictor. Men with a Gleason score of greater than 7 were two and a half times more likely to have serum 25-VD deficiency and this was statistically significant. (OR – 2.46; 95% CI 1.2–4.9, p-value = 0.01)

Table 8: Association between Serum 25-VD and Age, BMI, Gleason scores and pre-diagnostic serum PSA

Variable		Vitamin D strata					
		< 20		≥ 20			
		N	%	n	%	OR (95%CI)	P value
Age group	≤60 years	13	56.5%	10	43.5%	**1.0**	
	61-70 years	35	44.3%	44	55.7%	**0.6** (0.2-1.5)	**0.303**
	71-80 years	26	57.8%	19	42.2%	**1.05** (0.4-2.9)	**0.921**
	> 80 years	11	73.3%	4	26.7%	**2.1** (0.5-8.6)	**0.297**
BMI	Under-weight	11	64.7%	6	35.3%	**1.0**	
	Normal	44	51.2%	42	48.8%	**0.5** (0.2-1.7)	**0.310**
	Over-weight	27	52.9%	24	47.1%	**0.6** (0.2-1.9)	**0.399**
	Obese	3	37.5%	5	62.5%	**0.3** (0.05-1.8)	**0.209**
Gleason score	≤7	38	41.3%	54	58.7%	**1.0**	
	>7	47	67.1%	23	32.9%	**2.9** (1.5-5.5)	**0.001**
PSA	<50	29	41.4%	41	58.6%	**1.0**	
	>/=50	56	60.9%	36	39.1%	**2.2** (1.7-5.1)	**0.014**

Table 9: Vitamin D - Multivariate model

Variable	Coefficient	S.E. Of the Coefficient	p value	Or (95% C.I.)
Gleason score	0.901	0.350	0.010	2.463 (1.2 – 4.9)
PSA	0.491	0.347	0.157	1.634 (0.8 - 3.2)

7.0 DISCUSSION

Prostate cancer remains a significant cause of morbidity and mortality in Kenya with majority of patients presenting with advanced disease. Its risk factors are indeed, under-studied in our population. Given that low vitamin D levels have been associated with an increased risk of prostate cancer [5], we sought out to determine the profile of serum vitamin D levels among ambulatory PCa patients at Kenyatta National Hospital. Furthermore, these levels are likely to be lower with advancing age[35] and in those with more aggressive disease [60, 61, 64], hence we explored the associations between serum vitamin D deficiency and age, BMI, Gleason Scores and pre-diagnostic serum PSA.

The study sample was elderly, with a significant burden of advanced disease evidenced by the proportions with poor Gleason Scores, high serum PSA and receiving androgen deprivation therapy which is generally the palliative treatment modality of choice for advanced prostate cancer. The baseline characteristics of the study population were comparable a local study done at KNH by Wasike et al [69] who found a comparable mean age and similar proportions of those with advanced disease.

Serum 25-VD levels of between 30-100ng/ml are considered the normal reference laboratory values which are standardized and validated. Major vitamin D deficiency, which causes rickets in children and osteomalacia in adults, is typically associated with 25–VD levels less than 10ng/ml[74]. Recent investigations have suggested that the threshold for vitamin D deficiency should be the 25-VD level below which PTH secretion begins to rise. The authors of these studies have proposed that the cutoff value for vitamin deficiency may be as high as 32ng/ml [75] [76] [77].

Using a cutoff of 30ng/ml, our study sample demonstrated an alarmingly high prevalence of vitamin D deficiency (88.9%). The mean serum vitamin D level was also very low at 19.15ng/ml (30-100ng/ml). Both Gleason Score and pre-diagnostic serum PSA were found to be significantly associated with vitamin D deficiency on univariate analysis. However, at multivariate analysis, Gleason score was the only independent predictor of vitamin D deficiency. Our study was not powered to show any effect of age and BMI on serum 25-VD levels.

A high prevalence of vitamin D deficiency (36-100%) has been found in other series that evaluated presumably normal populations [78] [79] [80] [81]. Most of these studies were carried out in North America in a predominantly Caucasian population however, one particular study by Jacobs et al[78] in Arizona sampled subjects living in high sun exposure areas and found a high prevalence (77.7%) of vitamin D deficiency (<30ng/ml). The authors of the aforementioned studies carried out in normal populations have proposed that vitamin D deficiency is indeed common and displays significant variations between races and sun exposure.

Trump et al [52] who studied men with PCa and found 72% and 62% prevalence of vitamin D deficiency (<32ng/ml) in those with recurrent (n=120) and localized (n=50) prostate cancer respectively. Their mean serum 25-VD levels were 25.9ng/ml and 27.5ng/ml in those with recurrent and localized prostate cancer respectively. This was higher compared to our study sample whose mean 25-VD levels were (19.15ng/ml) Vitamin D deficiency was more frequent in blacks (p=0.04) and in those with recurrent disease p=(0.006). Choo et al[53] in Canada, studied 106 men with non-metastatic prostate cancer and found a lower prevalence (66%) of vitamin D deficiency (<30ng/nl). This is not surprising as vitamin D deficiency is more prevalent in patients with advanced prostate cancer[60,61,64]. In comparison, vitamin D deficiency was more frequent in our study. The finding of lower concentrations of vitamin D observed in our study is consistent with the known effect of skin pigmentation on endogenous synthesis of 25-VD. Other plausible explanations may include the fact that our study population spent most time indoors, either seeking treatment and/or recuperating due to the impact of advanced disease which has been observed in other patients with receiving cancer treatment [66]. It is estimated that in the above conditions, it takes approximately 12 weeks for vitamin D status to decay from normal to low.

Despite the fact that majority of our sample population had advanced disease, poorer Gleason scores predicted vitamin D deficiency implying that advanced disease is linked to lower vitamin D levels[60,61,64]. Trump et al [52] also demonstrated significant differences in vitamin D levels between those with recurrent prostate cancer compared to localized disease (p=0.005). Despite accepting results from different histopathology laboratories, Gleason scores are less likely to vary due to intricacies inculcated in the reporting criteria compared to PSA levels. Gleason

scores are therefore likely to be a more accurate representative of aggressive disease in this study and may explain why it was a stronger predictor than pre-diagnostic serum PSA.

However, our study did not show any consistent effect of age and BMI on serum 25-VD levels as it was not powered to do so. Trump et al [52] and Choo et al [53] found similar findings in their populations of patients with prostate cancer. Previous studies conducted in predominantly white populations with cancer[82] have noted an inverse association between 25-VD concentration and BMI[83]. This association has been hypothesized to be related to the sun avoidance in those with higher BMI or sequestration of 25-VD in adipose tissue[84]. However, other evidence exists to suggest that adiposity may not be a strong predictor of lower serum vitamin D levels in African Americans as compared to whites[85 86 87].

Indeed, this is the first study looking at profile of serum vitamin D levels among prostate cancer patients at Kenyatta National Hospital. In addition, the vitamin D assay technique that was employed is rapid, accurate, precise, well validated[70] and comparable to the gold standard [71 72]. The American association of Clinical Chemistry in July 2012 rated the performance of this method as excellent to world class. Comparator studies utilized a similar method.

Our study had several limitations. First, its major setback is the lack of a control group that can be composed of age-matched men with no prostate cancer. This study is, therefore, unable to demonstrate whether vitamin D deficiency is more prevalent in those with prostate cancer than controls. Secondly, it had a relatively small sample size which was computed to meet the primary objective. Thus, the interpretation of data calls for caution and the exploratory analysis examining potential variables associated with vitamin D deficiency is limited. Thirdly, it did not address all potential confounders of that can affect serum 25-VD levels. For example, this study did not assess patterns of sun exposure, degree of skin pigmentation and the possibility of liver metastasis impairing hepatic synthesis of 25-VD. Lastly, pre-diagnostic serum PSA levels were not standardized as we accepted results from different laboratories. This may be responsible for the wide ranges and may have influenced our secondary explorations.

Nevertheless, our study was able to determine the profile if serum vitamin D, prevalence of vitamin D deficiency and employ exploratory models to determine factors that might be associated with vitamin D deficiency.

8.0 CONCLUSION AND RECOMMENDATIONS

In our sample of African men with prostate cancer, we found very low vitamin D levels and an alarmingly high prevalence of vitamin D deficiency that is higher than that observed in prostate cancer patients in the Northern latitudes. In addition, vitamin D deficiency was more common in those with aggressive disease.

We recommend further studies to explore:

a. Vitamin D profile – comparative study in patients with and without prostate cancer.
b. Vitamin D profile in earlier stages of prostate cancer.
c. The impact of vitamin D replacement on prostate cancer outcomes in patients with vitamin D deficiency.

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