UNIVERSITY OF GHANA

THE IN Volvement OF Transient receptor potential melastatin (TRPM) member 6 and 7 channel regulatory proteins and imbalance of calcium and magnesium ions in the aetiology of benign prostatic hyperplasia.

This thesis is submitted to the University of Ghana, Legon in partial fulfilment of the requirement for the award of MSC Medical Laboratory Sciences degree.

In the Department of Medical Laboratory Sciences,

College of Health Sciences

By

Illias Suleman

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DEDICATION

This thesis is dedicated to Allah, my saviour and my help, my parents Mr. Sulemana Ibrahim and Mrs. Abena Ayishatu Ibrahim.
DECLARATION

I, Illias Suleman of the Department of Medical Laboratory Sciences of the University of Ghana, do hereby declare that, with the exception of the cited articles and references, this project work was duly carried out by me and the results obtained herein are a true reflection of the work done under the supervision of Prof. George A. Asare and Dr. Seth Amanquah.

Illias Suleman (10552215) ..............................................
(Student) Signature
Date: ..............................................

Prof. George A. Asare ..............................................
(Supervisor) Signature
Date: ..............................................

Dr. Seth Amanquah ..............................................
(Supervisor) Signature
Date: ..............................................
ACKNOWLEDGEMENT

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>1, 25(OH)₂D₃</td>
<td>1, 25 dihydroxyvitamin D₃</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUA</td>
<td>American Urological Association</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BPH</td>
<td>Benign prostatic hyperplasia</td>
</tr>
<tr>
<td>CCR</td>
<td>Coiled coil region</td>
</tr>
<tr>
<td>DHT</td>
<td>Dihydrotestosterone</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital rectal examination</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>IPSS</td>
<td>International prostate symptoms score</td>
</tr>
<tr>
<td>LUTS</td>
<td>Lower urinary tract symptoms</td>
</tr>
<tr>
<td>MgNuM</td>
<td>Magnesium-nucleotide-regulated current</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PCa</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>QOL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>REA</td>
<td>Repressor of estrogen activity</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3,5,5 Tetramethylbenzidine</td>
</tr>
<tr>
<td>TRP</td>
<td>Transient receptor potential</td>
</tr>
<tr>
<td>TRPM</td>
<td>Transient receptor potential melastatin</td>
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ABSTRACT

Background: Benign prostatic hyperplasia is a well-known and commonly diagnosed urological condition in the aging male population, which affects over 50% of men in their 60s and increased to 90% by the age of 85 years. The condition is normally accompanied by lower urinary tract symptoms that impacts negatively on the quality of life. Upon all the problems that BPH cause in the life of men, the exact aetiology is unknown. Several mechanism have been mentioned that contribute to the pathogenesis of BPH but no mechanism have been proven to solely cause BPH. The role of abnormal Ca$^{2+}$ and Mg$^{2+}$ levels in the development of many human diseases have been documented. These studies associated the development of human diseases as a result of imbalance of Ca$^{2+}$ and Mg$^{2+}$ to either defect in expression of TRPM6 and TRPM7 channels or abnormal levels of ions in water and diet. A study recently has shown the existence of this calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) imbalance in BPH condition however, the exact mechanism that mediates this imbalance is unknown.

Aim: The aim of the study was to investigate the involvement of TRPM6 and TRPM7 channel regulatory proteins and their association with calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) imbalance in the BPH patients.

Method: The study was a quasi-experimental study design. Venous blood sample of 4 ml were obtained from 36 BPH patients by convenient sampling method and age matched control and prostate cancer patients were also added. The blood samples were centrifuged after clotting to get the serum. Calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) concentration in the serum were then assayed using spectrophotometry. ELISA was used to determine the levels of TRPM6 and TRPM7 channel regulatory proteins in the serum. The data obtained was analysed using unpaired student t test, ANNOVA and correlation on Graphpad prisms version 6.05.

Results: The calcium (Ca$^{2+}$) measured in the study was high in control (2.44±0.36) followed by prostate cancer (PCa) samples (2.09±0.73) and then BPH (1.92±0.63). There was a significant difference between BPH and control (P=0.0005) and PCa and control (P=0.0466). Magnesium (Mg$^{2+}$) also followed the same trend with significant difference between BPH both control (P<0.0001) and PCa (P<0.0001). Albumin and globulin level for the samples were also determined with low albumin level in PCa subject (35.75±10.29) and globulin being highest in PCa (36.27±14.18) with BPH globulin level between the groups (19.23±9.15). TRPM6 level measured in the sample was high in control subjects with almost the same level in BPH (0.94±0.5) and PCa (0.93±0.40). Significant was recorded between control and both BPH (P<0.0001) and PCa (P<0.0001). TRPM7 level was very high in BPH (0.82±0.43) followed by control with PCa recording very low level (0.48±0.10). Ca$^{2+}$/Mg$^{2+}$ ratio was also high in BPH (2.59±1.09) whiles PCa result was the least among the groups. Both TRPM6 and TRPM7 showed a positive correlation with serum calcium (Ca$^{2+}$) and corrected calcium (Ca$^{2+}$). There was also a positive correlation between TRPM7 and albumin and globulin. A positive relationship was observed between age and TRPM6 in control group however, inverse relationship was observed in both BPH (r=-0.1730) and PCa (r=-0.4741). A negative relationship was however observed between age and TRPM7 in control group with inverse relationship in BPH (r=0.3249) and negative relationship in PCa (r=-0.4080).

Conclusion: The imbalance of Ca$^{2+}$/Mg$^{2+}$ ratio was observed in BPH in this study. TRPM7 may be involved in the imbalance of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) ratio in BPH subject. It was not clear the role of TRPM6 in calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) imbalance in BPH subject.
CHAPTER ONE

INTRODUCTION

1.1 Background

Benign prostatic hyperplasia (BPH) is a well-known urological condition, which manifests as non-malignant enlargement of the prostate gland resulting from the unregulated progressive proliferation of prostatic stromal and glandular epithelial cells. This histologic condition represents the most common noncancerous form of growth and inevitable disorder that occurs in the periurethral transition zone of prostate surrounding the urethra among aging male population (AUA Practice Guidelines Committee, 2003; Briganti et al., 2009; Lepor, 2005). Histopathologic BPH is a rare condition before the fourth decades of life and the prevalence increases with age. It is an important public health problem, affecting over 50% of men aged 60 years and reaches 90% by the age of 85 years (Allkanjari and Vitalone, 2015). However, the symptoms of BPH can start as early as 30 years and by the age of 50 years, about 50% of men with these symptoms will start exhibiting the histological evidence of BPH (Roehrborn, 2005; Kapoor, 2012).

The clinical manifestations of BPH are collectively called lower urinary tract symptoms (LUTS), which also increases with age. The development of these symptoms in BPH patients is attributed to the obstruction of bladder outlet from the enlarged prostatic tissue and increased smooth muscle tone and resistance. As a result, the prostatic urethra is compressed, which restrict the free flow of urine from bladder (Dias, 2012). This constriction and disruption of urine flow may give rise to associated LUTS such as urinary hesitancy, delay in initiating micturition,
intermittency, involuntary interruption of voiding, weak urinary stream, straining to void, a sensation of incomplete emptying, urinary frequency, nocturia, urgency and incontinence (Lepor, 2004). The development of LUTS in part also depends on the response of detrusor muscle of the bladder to this obstruction.

BPH is often, but not always, the cause of LUTS. Some men with clinically diagnosed BPH showing histologic evidence of the condition do not exhibit LUTS. LUTS are also neither specific to nor exclusive of BPH but more than 50% of men in their 60s to as many as 90% of the octogenarians present with LUTS (Sarma and Wei, 2012). Moreover, the size of the prostate gland does not certainly relate to LUTS. BPH with LUTS may occur in some men without enlargement of the prostate, and enlargement may exist in the absence of LUTS (Briganti et al., 2009; AUA Practice Guidelines Committee, 2003; Simon and Zieve, 2012). The appearance of these bothersome symptoms impact negatively on the quality of life of the BPH patients by interfering with normal daily activities, sleep pattern, sexual relations and comprised feeling of well-being (Allkanjari and Vitalize, 2015; Kirby, 2000).

The two factors known to predetermine the onset of BPH is the presence of testes and advancing age but the exact aetiology or factors that initiate and promote the proliferative process in prostate is unknown. The resemblance between BPH and embryonic morphogenesis of prostate has led to assumption that BPH might result from a “reawaken” in adulthood of the embryonic induction processes (Roehrborn, 2008). Various mechanisms seem to be involved in the pathophysiology of BPH, which include hormonal alterations, metabolic syndrome, age-related tissue modifications as well as inflammation (Briganti et al., 2009). The observed increase in number of cell could be due to epithelial and stromal cell proliferation or impairment in the apoptosis process leading to cellular accumulation.
Calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) ions have been implicated to regulate numerous important physiological functions of the body, which includes muscle contraction, cell signaling, transmitter release, cell proliferation and differentiation, cell cycle, gene transcription, cell death and as a cofactor (Zhang et al., 2014; Berridge et al., 2000). Calcium and magnesium belong to the same family in the periodic table and share the same homeostasis regulatory system but in the cell ionized magnesium antagonizes the action of ionized calcium in many physiological processes, regardless of similar chemical reactivity and charge (Dai et al., 2013; Brown and MacLeod, 2001; Iseri and French, 1984). Calcium and magnesium ions also compete for intracellular binding site (Grabarek, 2011). The concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) in the cell is tightly regulated and this correlate with physiological well-being in human. Abnormal levels of these ions in the cell have been associated with the development of human diseases like prostate cancer, hypertension, type 2 diabetes, cardiovascular diseases, pre-eclampsia and many other chronic conditions (Long and Romani, 2014; Rodriguez et al., 2003). These conditions occur as a result of the disturbance in the Ca\(^{2+}\) and Mg\(^{2+}\) homeostasis, which is mediated by either defects in the ion transport channels or abnormal levels of micronutrients in both water and diet (Hoenderop and Bindels, 2005; Long and Romani, 2014).

Importantly, the maintenance of tight ionic balance between Ca\(^{2+}\) and Mg\(^{2+}\) is needed for proper physiological functions in the body (Callera et al., 2009). This balance is about the state of the body store of calcium relative to magnesium at equilibrium over some extended time period. Studies have shown the protective effect of preserving the correct concentration of Mg\(^{2+}\) (thus, low Ca\(^{2+}\)/Mg\(^{2+}\)) against the development of disorders in many human organs (Yang et al., 2000; Dai et al., 2007; Rubenowitz et al., 2000; Sahmoun and Singh, 2010). However, hypomagnesaemic disorders enhances the physiologic effects and increase intracellular
concentration of calcium (Dai et al., 2011; Dacey, 2001; Iseri and French, 1984; Nielsen et al., 2007), and this has been associated with the initiation or progression of numerous disease conditions. Extracellular increase in Ca$^{2+}$ or decrease in Mg$^{2+}$ further increased influx of Ca$^{2+}$. Impairment of these ionic balance especially high Ca$^{2+}$/Mg$^{2+}$ ratio can alter the normal physiological functions of the cell and may lead to irregularities in many biological activities, such as cell differentiation, proliferation, DNA repair and carcinogenesis (Wolf et al., 2007). Therefore, both influx and efflux of Ca$^{2+}$ and Mg$^{2+}$ needs to be properly regulated for efficient intracellular ion activities and homeostasis.

Ca$^{2+}$ and Mg$^{2+}$ homeostasis or balance is carried out by the parathyroid gland, intestine, bone and kidney, which serve as a negative feedback structure to counter disparity in the ions (Van Der Wijst et al., 2009; Peacock, 2010). These organs are equipped with ion channels, pumps, binding proteins internal stores and buffers which are capable of detecting minor changes in the Ca$^{2+}$ and Mg$^{2+}$ concentration in the cells and also their restoration to the normal level (Sun et al., 2015). Mg$^{2+}$ was recently discovered to share common ion transporters with Ca$^{2+}$, which could possibly account for their persistence competition (Hoenderop and Bindels, 2005). Transient receptor potential cation channel, subfamily melastatin-like, member 6 and 7 are two major ion channel proteins noted for their transport of Ca$^{2+}$ and Mg$^{2+}$ into and out of the cell. They form part of the transient receptor potential (TRP) superfamily of ion channels and in the subfamily TRP melastatin-like group, which are diverse group of voltage-independent cation permeable channels(Montell, 2003; Pedersen et al., 2005).

TRPM6 and TRPM7 are divalent cation selective channels, which are highly permeable to Ca$^{2+}$ and Mg$^{2+}$. TRPM6 and TRPM7 uniquely combined cation channel with enzymatically functional C-terminal serine/threonine protein kinase domain in their structure (Runnels et al., 2001; Yee et
These channels are actively involved in regulation of Ca$^{2+}$ and Mg$^{2+}$ homeostasis necessary for biological functions. Mutation in the gene that encodes for TRP channels can lead to alteration in the channel property and function and these have been attributed to development of many human diseases. Autosomal recessive form of familial hypomagnesaemia with secondary hypocalcemia for instance occurs as a result of mutation in TRPM6 genes (Walder et al., 2002; Fleig and Penner, 2004; Schlingmann and Gudermann, 2005).

1.2 Problem statement

Benign prostatic hyperplasia with bothersome LUTS constitutes a major impediment globally to the health of all aging men. Approximately, 75% of men over the age of 50 years experience symptoms arising from BPH, and 20-30% of men attaining 80 years of age require surgical intervention for management of BPH (AUA Practice Guidelines Committee, 2003; Briganti et al., 2009). The prevalence of BPH in Ghanaian men was determined using International Prostate Symptoms Score (IPSS), digital rectal examination and symptomatic BPH as 19.9%, 62.3% and 13.3% respectively (Chokkalingam et al., 2012). Despite the negative health impact of BPH like acute urinary retention, recurrent urinary tract infection, hematuria, bladder calculi and renal insufficiency on aging men, the aetiology of the condition is still largely unresolved, however diseases are best treated when cause and effect relationships are established. Although mechanisms such as hormonal alteration, metabolic syndrome, diet, growth factors and defect in apoptotic genes have been implicated in the pathophysiology of BPH, there is no single mechanism that has proven to cause the condition. The occurrence of BPH for instance, requires the presence of male androgen but there is no clear correlation between concentration of the circulating male androgen and the prostate size in aging men (Roehrborn, 2008).
Ca^{2+} and Mg^{2+} in their appropriate levels are involved in plethora of cellular and physiological processes of the body (Zhang et al., 2014). The decrease in Mg and intranuclei increase in Ca^{2+} with advancing age has been shown but aging is a major risk factor for the development of BPH (Barbagallo and Dominguez, 2010; Tvedt et al., 1989). The correlation between abnormal levels of Ca^{2+} and Mg^{2+} and development of many human disorders have also been established in several studies (Sun et al., 2015; Simon et al., 2013; Konrad et al., 2004; Agus, 1999). However, some of these studies associated the cause of the disorders to Ca^{2+}/Mg^{2+} imbalance rather than the respective deficiencies among the individual ions. Dia et al. (2007) and Sun et al. (2012) respectively concluded on the association of high Ca^{2+}/Mg^{2+} ratio to the development of colorectal and high-grade prostate cancer. The disturbance in Ca^{2+} and Mg^{2+} balance in the studies was implicated to either mutation in the expression of TRPM6 and TRPM7 genes or abnormal intake of the trace elements in diet and water. More recently, Asare et al. (2017) has also concluded on the existence of this high Ca^{2+}/Mg^{2+} imbalance in BPH patients however, the mechanism that leads to this ionic imbalance in BPH patience is not known to the best of my knowledge. Therefore, this study seeks to find out whether the level of expression of the TRPM6 and TRPM7 regulatory channel proteins could be involved in the imbalance of Ca^{2+}/Mg^{2+} ratio associated to BPH.

1.3 Significance of study

The knowledge on the mechanism under which Ca^{2+} and Mg^{2+} balance is regulated in BPH patients could help in the proper understanding of the cause and it’s progression to bothersome LUTS. The study could also lead to appreciation of the factors that drive the prostate towards increased cell proliferation as men age and this is a potential source of knowledge for the development new therapies in the treatment of a clinical condition. For instance, the study could
lead to the production of new medicine that will target TRPM6 and TRPM7, if they are found to be involved in the modification of the Ca\(^{2+}\) and Mg\(^{2+}\) balance. It could also result in the prescription of mineral supplement of the mineral lacking to BPH patient with poor Ca\(^{2+}/\)Mg\(^{2+}\) balance. This may restore the ion balance and promote normal ionic homeostasis in the cell. The outcome of the studies could promote the measurement of TRPM6 and TRPM7 channel regulatory proteins as part of the routine diagnostic procedure for the cause of Ca\(^{2+}/\)Mg\(^{2+}\) imbalance in BPH condition. The study could also aid in education of men on the association of imbalance of Ca\(^{2+}/\)Mg\(^{2+}\) and BPH development and the possible mechanism that could alter this ionic balance.

1.4 Aim

The aim of the study was to investigate the involvement of TRPM6 and TRPM7 channel regulatory proteins and BPH and their association with calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) imbalance in the BPH patients.

1.5 Objectives

- To determine the level of calcium (Ca\(^{2+}\)), and magnesium (Mg\(^{2+}\)) in the serum samples of controls, BPH and prostate cancer patients.
- To determine the level of TRPM6 and TRPM7 channel regulatory proteins in the serum of controls, BPH and prostate cancer patients.
- To determine the association between levels of calcium (Ca\(^{2+}\)), magnesium (Mg\(^{2+}\)), Ca\(^{2+}/\)Mg\(^{2+}\) ratio and the channel regulatory proteins expression in controls, BPH and prostate cancer patients.
1.6 Hypothesis

There is no association between the TRPM6 and TRPM7 channel regulatory proteins and imbalance of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) in the BPH.
CHAPTER TWO

LITERATURE REVIEW

2.1 The Prostate

2.1.1 The prostate gland development, structure and functions

The prostate gland is a small-branched tubuloaveolar exocrine gland that forms part of the male reproductive system (Prince and Williams-Ashman, 1961). The prostate is the largest male accessory gland that is located low in the pelvic, inferior to the bladder and in front of the rectum (Royce, 2013). The embryonic development of the gland begins roughly at 10 weeks in the human fetus and by week 12, the lobes of the gland become apparent. The prostate gland develops from the epithelial buds of the urogenital sinus epithelium under the influence of the underlying mesenchyme and androgens. The buds proliferate to form branching cords, which begins to grow into a lumen-giving rise to a network of tubules and alveoli (Aaron et al., 2016; Lowsley, 1912).

The prostate is about the size of a pea at birth and remains less identical at prepubertal stage but starts to go through morphologic changes into adult phenotype at the initiation of the puberty. The prostate gland attains it normal size, which is shaped like inverted pyramid or walnut, during early 20s of a man life. The gland remains stable until about mid-40s, where in most men; the prostate begins to grow again through hyperplasia (Simon and Zieve, 2012). The prostate is made of a number small glands surrounded by stroma, which provides support. Prostatic urethra and a pair of ejaculatory ducts traverse the gland, with the former dividing the prostate into left and right lobe. The prostate gland surrounds the first 2 cm of the urethra and weighs about 15 g
to 20 g and averagely measures 3.4 cm in length, 4.4 cm in width and 2.6 cm in thickness (Amis, 1994).

Lowsley introduced the system of dividing the prostate gland into five lobes, which was solely based on the anatomic position in the embryonic and fetal gland. This system divided the prostate into anterior lobe, middle lobe, posterior lobe and a pair of lateral lobes (Lowsley, 1912). This lobar sub-division of the prostate became popular and was accepted for decades even though the lobes are not apparent in adult human prostate. However, McNeal proposed the current zonal nomenclature, which is used to describe the structure of the human prostate. Fibroelastic tissue layer encapsulate the gland and extends inward to form a septa dividing the prostate into different zones. The zones are the peripheral zone, central zone, transition zone and anterior fibromuscular stroma (McNeal, 1984; McNeal, 1981). The prostatic zonal anatomy had significant pathological implications. The peripheral zone consists of the posterior portion, apical and lateral areas of the prostate. This is the largest zone, which encompass over 70% of glandular tissue of the prostate. It encloses most of the central zone and part of the distal portion of the urethra. This zone is a common site for prostatic adenocarcinoma with 70-75% of cancer cases occurring in this area. The central zone is a wedge of glandular tissue that constitutes about 25% of the prostate gland. This area is situated at most part of the base of the prostate and extends posteriorly to surround the ejaculatory duct. The central zone is noted for few but aggressive forms of prostate adenocarcinoma.

The transition zone is a small region in the prostate that forms about 5% of the glandular tissue. This zone surrounds the prostatic urethra. The development of age related benign prostatic hyperplasia occurs in this site and, less commonly, the development of 20-25% of adenocarcinoma. The anterior fibromuscular stroma is non-glandular and composed of only
muscle and fibrous tissue, which forms the entire anterior surface of the prostate gland. The bladder neck is contiguous with this zone and fibromuscular stroma shields the anterior surface of the three glandular regions (McNeal, 1981; McNeal, 1984; Simpson, 1997). The prostate gland function by secreting slightly acidic fluid that forms about 20-30% of the semen volume. The seminal vesicle secretion combined to make the semen slightly basic, which prolongs the sperm lifespan. The prostatic fluid provides nutrients, optimal ionic milieu and pH for sperm in the seminal fluid (Untergasser et al., 2005). The prostate additionally produces prostate specific antigen (PSA), protease and prostatic acid phosphatase, which liquefy the viscous semen to facilitate sperms motility. The prostate also produces an enzyme 5 alpha reductase that function by converting testosterone into a more active dihydrotestosterone.

2.1.2 Prostate pathology

The common complications that normally affect the prostate gland are the prostatitis, prostate cancer and benign prostatic hyperplasia (Ejike and Eze, 2015). Prostatitis, which is the inflammation of the prostate mostly caused by infection, account for about 2% of the prostatic diseases, while BPH the non-malignant enlargement of the prostate and prostate cancer that is any cancer within the gland respectively constitute 80% and 18% of the prostate problems (Bock-Oruma et al., 2013). These prostatic pathologies can coexist but the presence of one does not guarantee the chance or lead to other.

2.2 Benign prostatic hyperplasia (BPH)

BPH is the most common and progressive proliferative urological disorder characterized clinically by noncancerous enlargement of the prostate gland in the aging men population. The worldwide estimation of patients who visit and get diagnosed of either primary or secondary
BPH yearly by physician is around eight (8) million (Yoshimura et al., 2013). This histologic change of the prostate in BPH patients occur as a result of unregulated stromal and glandular epithelial hyperplasia that arise from the periurethral and transition zone of the prostate surrounding the urethra (Briganti et al., 2009; Lepor, 2005; McNeal, 1978). BPH can be classified into microscopic, macroscopic and clinical BPH based on the manifestation or developmental stage of the condition. Microscopic BPH is the histologic evidence of the enlargement in the prostatic stromal and epithelial tissue. The enlargement of the prostate as a result of stromal and glandular epithelial proliferation also represents macroscopic BPH but the clinical manifestations that occur after the enlarged prostate give rise to clinical BPH (Lepor, 2004). Nearly all aging men population develop histologic BPH but only about half of these men will proceed on to develop macroscopic BPH. About 50% of the men with macroscopic BPH also develop the actual clinical BPH (Isaac and Coffey, 1989). Though BPH involves increment of both stromal and glandular epithelial cells as a result of the proliferation, however, it mainly the surge in number of stromal smooth muscle cells that is responsible for increase in the volume of prostate (Alonso-Magdalena et al., 2009). The ratio of stromal to epithelial cells in a normal prostate is estimated to be 2:1 but this can increase to 5:1 under BPH condition (Prabhav and Bairy, 2009; Lepor, 2005).

2.2.1 Prevalence of BPH

Studies have been conducted on the prevalence of BPH in many different human geographical areas and reported different results. This variation in the prevalence report might be due to differences in the study method, geographical differences, sampling bias, variable criteria for defining condition and many others. However, the increase in both age and BPH prevalence was common among these studies. Histologic BPH was estimated to affect one-in-four men in their
50s, one-in-three men in their 60s and one-in two men in their 80s and over (Robert et al., 2009). Similarly, BPH was also reported to affect 42% of men aged 51-60, 70% of those aged 61-70 and 90% of men with 81 years and over (Nickel, 2006). The American Urological Association (AUA), mentioned the prevalence of histologic BPH to be rare in those below 40 years but range from over 50% at 60 years and reaches approximately 90% by the age 85 (AUA Practice Guidelines Committee, 2003; Briganti et al., 2009). However, the symptoms of BPH can start as early as 30 years and by the age of 50 years, about 50% of men with these symptoms will start manifesting the histologic evidence of BPH (McVary, 2006; Kapoor, 2012). A study among Ghanaian men, which was based on different evaluation methods reported prevalence of 19.9%, 62.3% and 13.3% using international prostate symptoms score (IPSS), digital rectal examination and symptomatic BPH respectively (Chokkalingam et al., 2012).

### 2.2.2 BPH symptoms

The enlargement of the prostate has been associated with the development of lower urinary tract symptoms (LUTS), which is a common manifestation in BPH patients. LUTS suggestive of BPH also increases with age and negatively impact on quality of life through interference with daily activities and normal sleep pattern (Wei et al., 2008). The enlarged prostate compresses the urethra at the neck of the bladder and increases the resistance to urine flow. A capsule surrounding the prostate inhibits outward expansion of the gland as it proliferates. It then expands inward leading to compression of the prostatic urethra (Edwards, 2008). This can predisposed a BPH patient to development of bothersome LUTS, which are generally classified into voiding (obstructive) and irritative (storage) symptoms. The obstructive symptoms consist of urinary hesitancy, delay in initiating micturition, intermittency, involuntary interruption of
voiding, weak urinary stream, straining to void, a sensation of incomplete emptying and terminal dribbling. Irritative symptoms also comprise of urinary frequency, nocturia, urgency, incontinence and bladder pain or dysuria (McVary, 2003; Allkanjari and Vitalone, 2015). The development of the bladder outlet obstruction mentioned to caused LUTS is having static component, which involves the enlarged prostate gland impinging on the urethra and dynamic component that is due to increase in smooth muscle tone of the bladder neck (Prabhav and Bairy, 2009). The development of LUTS in part also depends on the response of detrusor muscle of the bladder to this obstruction (Reynard, 2004).

Though studies have mentioned that the enlarged prostate, leads to bladder outlet obstruction and this causes LUTS in BPH patients, however, this assumption has been referred to by other two studies as a gross over simplification because their research reviewed a very poor correlation between the above mentioned conditions and there is no sufficient evidence to support that concept (Jacobsen et al., 2001; Barry et al., 1993). Additionally, BPH is often, but not always the cause of LUTS. Some men with clinically diagnosed BPH showing histologic evidence of the condition do not exhibit LUTS. LUTS are also neither specific to nor exclusive of BPH but more than 50% of men in their 60s to as many as 90% of the octogenarians present with LUTS (Sarma and Wei, 2012). Moreover, the size of the prostate gland does not certainly relate to LUTS. BPH with LUTS may occur in some men without enlargement of the prostate, and enlargement may exist in the absence of LUTS (Briganti et al., 2009; Simon and Zieve, 2012). Persistence of untreated BPH with bothersome symptoms and obstruction of urine flow may lead to the development of serious complication like urinary tract infection, hematuria, acute urinary retention, bladder calculi and renal insufficiency and failure (Curtis, 2006; McVary, 2006).
2.2.3 Diagnosis of BPH

The complete diagnosis of BPH involves physical examination, laboratory investigations, taking detailed medical, urologic and neurologic history of the patient (Sarma and Wei, 2012; AUA Practice Guidelines Committee, 2003). Men with BPH, initially hardly come to the hospital to complain of enlarge prostate, however, they presents a set of abnormal urinary symptoms or complication from hostile bladder outflow obstruction which through the application of the above methods BPH is diagnosed without confusing it with other conditions with similar symptoms.

2.2.3.1 Medical history

A detailed medical history that focus on urinary tract symptoms, bladder dysfunction conditions, family history of prostate diseases, general health issues, frequency of micturation, history on other comorbidities, medication, previous surgical procedure and other information needed to rule out causes of LUTS other than BPH (AUA Practice Guidelines Committee, 2003; Nash, 2012). The medical history should distinctly establish symptoms, it severity and impact on patient quality of life (QOL) in order to exclude other diseases. The International Prostate Symptoms Score, a modification from the America Urological Association (AUA) symptoms index, which is a questionnaire, appears to be a major tool employ in the evaluation and quantification of LUTS severity and it impact on patient QOL. The IPSS consist of seven questions on bothersome LUTS and one question that address the patient perception of his QOL. Based on the symptom score, three categories of symptom severity are described, i.e. mild (0-7), moderate (8-19) and severe (20-35). The use of these symptom categories assist urologist in choosing suitable treatment for a patient. Attention should be paid to the final QOL question,
which give physician insight on how the patient feels about his symptoms and the need for early
treatment (Tanguay et al., 2009; Madersbacher et al., 2004).

2.2.3.2 Physical examination

Digital rectal examination (DRE) and neurological assessment should be performed to physically
examine the prostate for the cause of the LUTS and it should be systematically done basing on
patient medical history and observed abnormalities. The DRE involves palpating and percussion
of the prostate to examine the shape, size, tenderness, nodule, and symmetry to exclude any
evidence of prostate cancer (Kijvikai, 2009; Madersbacher et al., 2004; Tanguay et al., 2009).
This is done by inserting a gloved finger in the rectum to the back of the prostate and this can
provide valuable information on voiding dysfunction. Usually, DRE tends to underestimate the
prostate size, however this can be rectify by using ultrasound or other instrument to confirm the
true size of the prostate. Neurological evaluation that focuses on the assessment of ambulatory
status of the patient, general mental status, lower extremity neuromuscular function, rectal
sphincter tone and functional status of somatic, sensory and motor part of sacral reflex should be
carried to ascertain whether the cause of LUTS is not due neurological abnormalities (AUA

2.2.3.3 Laboratory evaluation of BPH

Laboratory blood and urine investigation are also essential in checking for non-BPH causes of
LUTS in aging men population such as prostate, kidney and bladder cancer, urethral strictures,
urolithiasis, bladder stone, urinary tract infection etc. Urinalysis is performed using dipstick and
microscope (AUA Practice Guidelines Committee, 2003; Rahman et al., 2012). This test is necessary when screening for hematuria and urinary tract infection (UTI). Urine culture, cytology, serum creatinine and glucose may also be tested for depending on patient’s history (Abrams et al., 2013). The measurement of serum prostate specific antigen (PSA) is also encouraged in the diagnosis of BPH, especially in men with 10 years life expectancy and who can be a candidate for prostate cancer management (Madersbacher et al., 2004). Elevated serum PSA in BPH patients indicates a higher risk of symptom progression, deterioration of flow rate and acute urinary retention but very high PSA value may increase the chance of diagnosing prostate cancer. Other optional non-invasive investigations such as urine flow rate, cystoscopy, postvoid residual measurement, pressure-flow studies, transrectal ultrasound are sometime perform based on the situation (McVary, 2003; Tanguay et al., 2009).

2.2.4 Risk factors

Genetic factors, metabolic syndrome, lifestyle, racial differences and diet have been mentioned in studies to influence the growth of prostate or BPH development aside age and androgen which are well documented BPH risk factors (Parsons, 2010; Eaton, 2003)

2.2.4.1 Metabolic syndrome

The clinical constellation of metabolic defects that results from dietary and other lifestyle practices defined metabolic syndrome. The association of metabolic syndrome or its components with BPH development have been observed in studies (De Nunzio et al., 2012). Increased risk of BPH development was noticed in patients with type 2 diabetes mellitus/glucose intolerance, hypertension, obesity/high BMI, hyperinsulinemia and low HDL cholesterol (Corona et al., 2014; Gupta et al., 2006; Hammarsten et al., 1998). High levels of total cholesterol, Low Density
Lipoprotein, triglyceride and reduced levels of high density lipoprotein increases the risk of BPH whiles cholesterol-lowering medication may decrease the risk (Moyad and Lowe, 2008).

### 2.2.4.2 Genetic factors

Genetic component is also reported to play a role in BPH development. The genetic factor does not only influence risk of BPH formation but also severity. Family history of a first degree relative developing BPH under the age of 60 years increases man’s risk of clinical BPH (Nash, 2010). About half of men with BPH who undergoes on surgery under the age of 60 years are having heritable form of the disease and is an autosomal dominant pattern of inheritance (Parsons, 2010; Pearson et al., 2003). The part play by genetic in BPH condition was shown in monozygote twins (Walsh et al., 1994).

### 2.2.4.3 Lifestyle

Factors such as cigarette smoking, diet, alcohol and physical activity have been observed to influence BPH risk. The consumption of diet high in polyunsaturated fatty acid and red meat increases the risk of prostate enlargement (Suzuki et al., 2002). However, fruits and vegetables rich diet intake inversely correlate with BPH (Ranjan et al., 2006; Rohrmann et al., 2007). Similarly, Asian diets which are high in plant based products reduce BPH risk compared to animal based diet of western countries that increases BPH risk. Additionally, moderate and regular alcohol intake, cigarette smoking at certain intensity and increase in physical activity have been associated to the reduction in risk of BPH development, however some studies reported on increased surgical risk in BPH patient with high intake of alcohol and no or increase risk of BPH for smoking (Parsons, 2007, Parsons; 2010, Rahman et al., 2012). Heavy alcohol
consumption and frequent smoking increases the risk of developing severe LUTS (Maharajh et al., 2015).

### 2.2.4.4 Racial differences

The prevalence of histological BPH was shown to be similar in all racial groups, however clinical BPH had greater prevalence in African-American than in Caucasians (Ekman, 2000). The rate of moderate to severe LUTS prevalence and surgery for BPH was lower among the Asian race (McNicholas and Mitchell, 2008). Therefore racial and geographical location of men also impacts on prostate growth. African-Americans risk of developing BPH was also mentioned in a study as either similar or possibly higher than the Caucasians (Roehrborn and Ray, 2006).

### 2.2.5 Aetiology of BPH

Functional testes and age advancement are the two vital factors known to influence the onset of BPH development. The exact aetiology of BPH have not been established, in spite of the acute urinary retention, recurrent urinary tract infection, bladder calculi, renal insufficiency caused by BPH and the intense research effort (Roehrborn, 2008). Three theories have been proposed over the years to explain the causative mechanism of BPH, however, there is no enough concrete evidence to prove any of the theory alone causing BPH. Hormonal theory, stem cell theory and stromal-epithelial interactions theory were proposed to explain the cause of BPH development (Allkanjari and Vitalize, 2015; Bosch, 1991).

#### 2.2.5.1 Hormonal theory

2.2.5.1.1 Androgen hypothesis

There were no evidence of BPH development in men castrated before puberty and androgen
deprivation causes a reduction in prostate volume (Bosch, 1991). This clearly shows the importance of male androgen especially the active metabolite dihydrotestosterone (DHT) in prostate growth and development. Initial studies indicated that tissue concentration of DHT increases as men age and is the major cause of the prostate enlargement seen in aged men population (Kristal et al., 2008; Marks et al., 2008). However, Walsh et al. (1983), reported that the levels of DHT in both BPH and normal prostate tissue are the same and that the previously reported rise of DHT in BPH tissue was due to artifacts related to DHT metabolism (Marcelli and Cunningham, 1999; Walsh et al., 1983). Therefore, only DHT cannot lead to BPH development though it is important for prostate growth.

2.2.5.1.2 Estrogen hypothesis

The imbalance of estrogen and androgen in aging male population has been also associated to the pathogenesis of BPH. Estrogen and androgen play synergistic role in the development of BPH (Bosch, 1991). The age dependent increase in expression of aromatase and estrogen receptors with corresponding decrease in testosterone levels in prostatic tissue has been reported (Royuela et al., 2001; Prezioso et al., 2007). Increasing the levels of internally derived estrogen can induce proliferation and enlargement of the prostate (Chen et al., 2012). However, the inhibition of aromatase production in a clinical trials did not reduce prostate size and also few estrogen receptors are found in BPH tissue (Marcelli and Cunningham, 1999).

2.2.5.2 The Stem cell theory

The theory proposed that stem cells of the prostate develop in response to androgen until they attain their full number and this can determine the size of the prostate under BPH condition (Bosch, 1991; Marcelli and Cunningham, 1999). Alteration in the properties of small percentage
stem cells present in the prostate could lead to development of BPH. The pluripotent stem cells can proliferate and differentiate into mature cells via clonal expansion to increase the number of cells (Bosch, 1991). The cells undergoes programmed cell death at maturity, however, aging reduces cells death through obstruction of maturation process in the terminally differentiated cells (Roehrborn and McConnell, 2007). Prostate size is influence by the balance between cell growth and cell death. Under the stem cell theory, BPH development was associated with abnormal increase in number of stem cells, increase in clonal expansion of the of proliferating and mature cells, decrease rate of apoptosis of mature cells or combination of the three process (Bosch, 1991). Proliferation of cells and down regulation of cell death have been observed in BPH (Claus et al., 1997).

2.2.5.3 The stromal-epithelial interaction theory

2.2.5.3.1 Embryonic reawakening hypothesis

The observation of new glands budding out and branching to form new alveoli in the small transitional zone of the prostate by McNeal gave rise to embryonic reawakening hypothesis of BPH development. The hypothesis associated BPH development to result from reawakening of embryonic induction processes in the adulthood and mentioned re-awaken of stromal-hormonal factor to induce this prostate epithelial proliferation (McNeal, 1990; McNeal, 1978; Ho and Habib, 2011). The theory indicated that stromal induced hyperplastic changes in the glandular epithelium, however, only the prostatic mesenchyme was observed to have inductive potential after culturing human BPH stromal fibroblast and epithelial separately and together (Cunha et al., 1980).
2.2.5.3.2 Growth factor hypothesis

Abnormal levels of growth factors such as basic fibroblast growth factor, keratinocyte growth factor, epidermal growth factor and transforming growth factor β from either the prostatic glandular epithelial or stromal tissue have been proposed to mediate the reawakening of embryonic growth potential. High concentrations of these factors were detected in BPH tissue compared to normal prostate tissue. The growth factors act through either autocrine or paracrine signaling to stimulate the prostatic stromal and epithelial cell hyperplasia (Bosch, 1991; Ho and Habib, 2011; Marcelli and Cunningham, 1999).

Though some of the proposed mechanisms of BPH development by the above theories are to some extent consistent with the pathophysiology of the condition, however, there are discrepancies that hinder the full acceptance of any of the theory to solely lead to BPH development and current evidence is insufficient.

2.3 Trace element

Trace elements form an important part of the human body and using atomic absorption spectrophotometry, concentration of some major trace elements such calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Cadmium (Cd), Nickel (Ni) and Copper (Cu) were evaluated in both malignant and benign prostate samples. The concentration of calcium and magnesium were however found to be higher in malignant prostate compared to the benign among other metals (Yaman et al., 2005). Calcium and magnesium have been shown to be present in different parts of human prostate gland including the secretory vessels, cytoplasm of both hyperplastic and normal prostate and among others (Asare et al., 2017; Tvedt et al., 1989). They are essential for development and performance of different physiological functions that maintains human health. Disturbances in these trace elements appear to play a crucial role in the pathogenesis and rapid
progression of human diseases. The associations between calcium, magnesium and other minerals in the development of diseases have been carried out in the prostate gland, breast and other human organs.

2.3.1 Magnesium (Mg$^{2+}$)

Magnesium is the fourth most common cation in the body and second most prevalent intracellular cation after potassium (Rubin, 2007). Compared to other biological cations, Mg$^{2+}$ possesses the smallest ionic radius and largest hydration radius. Mg$^{2+}$ ion is hexacoordinated and tightly held in its first hydration shell. Distinctively, this underlies Mg$^{2+}$'s special roles in catalysis, biological structures and control of diverse physiological processes (Payandeh et al., 2013). Magnesium (Mg$^{2+}$) in the appropriate concentration is involved in a plethora of physiological functions of the human body including cell cycle regulation, neurological excitability, muscle contraction, cell signaling, cell proliferation, mitochondrial integrity, and also modulating ion transport. It serves as an important cofactor for over 350 enzymatic reactions including metabolic processes like oxidative phosphorylation, protein synthesis, deoxyribonucleic and ribonucleic acid metabolism (Touyz, 2008).

Primarily magnesium (Mg) is an intracellular cation with about 53% of the body total magnesium found between bone cell, 27% in intracellular compartment of muscles, 19% in soft tissues, 0.5% in erythrocyte and 0.3% in serum (Fawcett et al., 1999). Magnesium in the serum exist in three forms; free, bound to protein and complexes with anion. Approximately about 1% of magnesium is found in the extracellular compartment of human body with 55-60% which is physiologically active circulating freely in ionic state and or 30% are protein bound mostly albumin and 5-10% forms complexes with anions such as citrates and phosphates (Külpmann and Gerlach, 1996; Innerarity, 2000). The biological functions undertaken by magnesium are
carried out by the physiologically active free ionic fraction. The body obtains magnesium from foods mostly unprocessed diets and drinking water. The chief sources of magnesium in the body are whole seeds, nuts, green leafy vegetables, unrefined whole grains, legume, fruits, meats and hard water. Its absorption is inhibited by phytate, fibre, alcohol and excess calcium (Coudray et al., 2003).

2.3.2 Calcium (Ca$^{2+}$)

Calcium (Ca$^{2+}$) is ubiquitous and versatile intracellular signal molecules that function as a second messenger in cell signaling pathways. The intracellular concentrations of Ca$^{2+}$ are much lower compared to extracellular fluids that bath the cells. Through active extrusion mechanism and relatively impermeable nature of plasma membrane to Ca$^{2+}$ the cell maintains a large electrochemical gradient across the membrane. However, electrical potential and damage to the membrane tends to promote influx of Ca$^{2+}$ into the cell (Pu et al., 2016). Calcium (Ca$^{2+}$) in the human body perform most of its functions through signaling pathways and the signal can be localized or take the form of release waves that sweep through entire cell and may range in duration from microseconds to hours depending on the kind of activity. Calcium (Ca$^{2+}$) signaling encodes information that combines with other signal transduction cascades to control different and specific cellular processes starting from cell life at fertilization to apoptosis to terminate cell life cycle. These physiological functions includes cell proliferation, differentiation, cell cycle, gene transcription, muscle contraction, cell secretion, motility and migration (Berridge et al., 2000; Brown et al., 2011).

Calcium is a major structural component of the bones and about 99% of the total body calcium is found in bone tissues with only 1% of the calcium deposited in the extracellular compartment of
body (Kopic and Geibel, 2013). Serum levels of the extracellular calcium are only 0.1-0.2% which exist in three fractions. About 45-50% of the total serum calcium is free, ionized and biologically active, 40% is bound to protein mainly albumin and the remaining 10-15% is chelated to anions like bicarbonate, citrate, phosphate, sulphate and lactate (Seaton et al., 1983; Baird, 2011). Calcium in the body is mostly obtained from food and water. Diary food products are the richest source of dietary calcium in the body with plant based foods like legume, green leafy vegetables, tofu, broccoli and kali family supplementing the body needs of calcium however the amount they contribute is small compare to dairy products. High oxalate, phytate and some drugs can also affect the rate of calcium absorption in the body (Greger, 1987).

2.3.3 Calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) transport and homeostasis

The concentrations of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) in the human body are tightly controlled. The levels of these trace elements in the body are regulated by joint interplay between three organs: calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) intestinal absorption from diet and water, their storage and exchange with the bones and changes in renal calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) reabsorption capacity, however parathyroid gland secret hormones that modulate the movement of these ions (Hoenderop and Bindels, 2008). Diet and water serves as the main source of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) that enters the body and they are both (re)absorbed from the intestine and kidney by two distinct pathways that constitute active transcellular and passive paracellular pathways (Beggs and Alexander, 2017; Kerstan and Quamme, 2002). The paracellular pathway allows direct exchange of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) between two compartments which involves transport through the epithelial spaces, however the transcellular pathway transport the trace elements through interior of epithelial cells across at least two plasma membrane barriers.
The absorption of luminal intestinal dietary calcium (Ca\textsuperscript{2+}) can occur through passive concentration gradient-dependent paracellular and active ATP-dependent transcellular pathways. The transcellular transport has been extensively reported to take place in the duodenum where 80% calcium uptake occur in low calcium diet and 10% in high calcium diet however, other studies mentioned jejunum, cecum and proximal part of the colon to also mediate the transport of calcium through this pathway (Khanal and Nemere, 2008). Saturable transcellular pathway is regulated by 1, 25-dihydroxyvitamin D\textsubscript{3} (1, 25(OH)\textsubscript{2}D\textsubscript{3}) and is most efficient when calcium supply in the diet is low. This multistep transport system encompass the movement of luminal Ca\textsuperscript{2+} into the enterocyte, the translocation of Calcium (Ca\textsuperscript{2+}) from point of entry to the basolateral membrane and lastly extrusion of Ca\textsuperscript{2+} from the cell into circulatory system (Wongdee and Charoenphandhu, 2015).

The apical influx of Calcium (Ca\textsuperscript{2+}) in this pathway is mediated through some selected Ca\textsuperscript{2+}, channels which transient receptor potential channels mostly TRPV5 and TRPV6 are the largely predicted members. Studies have however, reported the full function of transcellular pathway in TRPV5 and TRPV6 knockout/down mice and human (Benn et al., 2008; Nakkrasae et al., 2010) therefore is possible that other TRP channels like TRPM7 which are ubiquitously expressed in the body and intestine and TRPM6 also commonly expressed in the brush border membrane and colon could mediate calcium transport in this pathway. Calbindin (Calbindin-D\textsubscript{9k}) a major calcium (Ca\textsuperscript{2+}) binding protein then binds, buffer and shuttle Ca\textsuperscript{2+} from entry point to the basolateral membrane where it is extruded from enterocyte into extracellular fluid through Ca\textsuperscript{2+}-dependent ATPase or in exchange for Na\textsuperscript{+} by Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. Calbindin-D\textsubscript{9k} expression in the cell is stimulated by 1, 25(OH)\textsubscript{2}D\textsubscript{3} (Christakos, 2012a).
In contrast, paracellular pathway account for majority of calcium absorption from the intestine (over 80%) and is the prefer method when dietary calcium concentration is high. Paracellular intestinal calcium transport occurs predominantly in the jejunum and ileum. Paracellular calcium transport is non-saturable and energy-independent pathway that mediate calcium transport down their electrochemical gradient (Pu et al., 2016). The transport of calcium in this pathway is regulated by a tight junction composed of proteins from claudins family. Claudins-2, -12, and -15 are the members implicated in the intestinal calcium absorption. The flux through this tight junction is influence by size and charge selectivity. Calcitiol (1, 25(OH)2D3) has been shown to also regulate paracellular calcium transport by increasing the permeability of the tight junction through reduction of claudin 3, aquaporin 8, and cadherin 17 expression (Christakos, 2012a; Christakos, 2012b).

The filtered calcium (Ca2+) in the kidney is reabsorbed along the nephron to maintain the concentration in the serum. Similarly, the kidney reabsorption of filtered calcium (Ca2+) is also mediated by both active transcellular and passive pracellular transport pathways. Bulk of the filtered calcium (Ca2+) in the nephron, approximately 70% in the proximal convoluted tubule and 20% in the thick ascending limb, is reabsorbed through the passive paracellular pathway (Beggs and Alexander, 2017). Paracellular transport of calcium across the nephron also moves down an electrochemical gradient. The tight junction which regulates calcium permeability is made of claudins-2 in the proximal tubule and claudins-16 and -19 in thick ascending limb. The remaining filtered calcium (Ca2+) is reabsorbed in the distal convoluted tubule and collecting tubule via active transcellular route with only 1-2% excreted in the urine. The active
transcellular transport also involves the same apical entry in to the cell, intracellular binding and shuttling and basolateral extrusion as described for intestinal transport (Bronner, 1998). However, the major difference is only TRPV5 was mentioned to be channel that mediate calcium entry and calbindin-D_{28k} is the binding protein which buffer and shuttle calcium (Ca^{2+}) to the basolateral membrane for extrusion.

Similar to calcium (Ca^{2+}), magnesium (Mg^{2+}) (re)absorption occur in both the intestine and kidney. Active transcellular and passive paracellular are also the transport pathways for dietary intestinal magnesium (Mg^{2+}) uptake. Small intestine and to a lesser extent colon are the major sites for intestinal magnesium (Mg^{2+}) absorption, although some is taken up through large intestine (Lameris et al., 2012). Paracellular transport mechanism which covers most parts of the small intestine is the prefer method when magnesium concentration is high and is responsible for majority of the intestinal magnesium transport. This unsaturable pathway drives magnesium (Mg^{2+}) through a tight junction down a concentration gradient and is energy-independent. Claudins-2 and -12 protein have been proposed to form the tight junction in this pathway but claudins proteins have not been directly discovered (Hou et al., 2009). The absorption of Na^+ followed by water provide the driving force for Mg^{2+} transport. The active transcellular magnesium transport in the intestine take place in ileum, colon and is similar to calcium intestinal transcellular transport except that the channels known to mediate magnesium entry are mainly TRPM6 and TRPM7 and also the binding protein which buffer and shuttle magnesium to basolateral membrane for extrusion is unknown though some studies have mentioned the same calbindins to perform that function in magnesium transport (Quamme, 2008). The extrusion occur through Na^+/Mg^{2+} exchanger and this is most efficient pathway when dietary Mg^{2+}
concentration is low. Calcitiol (1, 25(OH)2D3) has been also shown to stimulate intestinal Mg2+ absorption (De Baaij et al., 2012).

The kidney reabsorption of magnesium (Mg2+) and calcium (Ca2+) are also similar in that they both mediate reabsorption through paracellular and transcellular pathways however, the level of reabsorption in the segments of the nephron were different. Through passive paracellular pathway, approximately and in contrast to calcium, 20% and 70% of the filtered Mg2+ are respectively reabsorbed in the proximal convoluted tubule and thick ascending limb of the nephron which depend on lumen-positive electrochemical gradient, filtered load, net salt and water reabsorption. Claudins-16, and -19 are known to compose of the tight junction that regulate the Mg2+ permeability (De Baaij et al., 2012; Hou et al., 2009). The remaining 10% of filtered Mg2+ is reabsorbed by active transcellular transport in distal convoluted tubule (DCT) and this defines the final urinary Mg2+ excretion which is around 3-5% however, this can reduce to 0.5-1% in the presence of hypomagnesemia condition. TRPM6 channels in DCT mediate apical reabsorption of the filtered Mg2+ which is then shuttle to the basoletaral membrane for extrusion through coupled Na+ gradient owning to the action of basolateral Na+/K+-ATPase (Glaudemans et al., 2010; Nijenhuis et al., 2005).

Calcium (Ca2+) and magnesium (Mg2+), transported to the blood are carried to various organs, tissues and cells which the prostate gland form part, where different functions are executed by these ions. The expression of TRPM6 and TRPM7 that are major Ca2+ and Mg2+ transport channels in the cells of the prostate gland have been shown (Wang et al., 2007). However, most or excess of the calcium (Ca2+) and magnesium (Mg2+) in the blood is stored in the bones which
serve as a buffer to maintain the optimum concentration of these ions in the cells. They can be released when plasma concentration of Calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) is low (Wallach, 1990; Gropper et al., 2005).

The homeostasis of calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) in the cells are maintained by the steady state between influx, efflux and storage mechanisms. Ionized calcium and magnesium in both extracellular and intracellular are physiologically active and need to be regulated. The homeostasis of Ca\(^{2+}\) and Mg\(^{2+}\) in the body are tightly controlled by efficient feedback control system through dynamic action of intestinal absorption, exchange with bones and renal excretion mediated by complex integration of hormones, parathyroid hormone, calcitriol (vitamin D) and calcitonin (Hoenderop and Bindels, 2008). These organs are equipped with channels, pumps, buffers and binding protein that mediate their homeostatic functions. Calcium sensing receptors on kidney tubule and parathyroid gland monitor Ca\(^{2+}\) and Mg\(^{2+}\) concentration in the blood. The secretion of parathyroid hormone is influence especially by low plasma Ca\(^{2+}\) concentration and this action down regulate calcitonin release from thyroid gland. Increase level of parathyroid hormone in the body leads to synthesis of calcitriol from 25-OH vitamin through 1-hydroxylase (Moe, 2008).

The body initially control the levels of calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) via reduce or increase intestinal absorption and also reabsorption or excretion of excess ions by the kidney. As mentioned above, high level of calcitriol (1, 25(OH)\(_2\)D\(_3\)) result in both intestinal and kidney (re)absorption of Ca\(^{2+}\) and Mg\(^{2+}\) through respective stimulation of calbindin-D\(_{9k}\) and calbindin-D\(_{28k}\) synthesis which binds and translocate Ca\(^{2+}\) and Mg\(^{2+}\) to basolateral membrane for extrusion.
Parathyroid hormone and calcitriol can also binds to receptors on osteoblasts and stimulate osteoclasts to release Ca^{2+} and Mg^{2+} (Gropper et al., 2005). High concentrations of Ca^{2+} and Mg^{2+} in the body initiate a conformation changes in the calcium sensing receptors through IP_3 and this signal the parathyroid gland to discontinue the release of parathyroid hormone. Calcitonin synthesis from the parafollicular cells of the thyroid gland can be activated by the rise in blood calcium level. This can act to increase bone reabsorption of calcium by inhibiting vitamin D activation and osteoclasts activity (Brown, 1991).

Parathyroid hormone (PTH) seems to be more associated with calcium (Ca^{2+}) homeostasis, however magnesium (Mg^{2+}) is needed for parathyroid hormone secretion and studies have reported low levels of parathyroid hormone under hypomagnesemia state which was restore to normal by magnesium supplementation. Magnesium (Mg^{2+}) is again needed to for PTH effect on intestine, bone and kidney. On the other hand, high concentrations of Mg^{2+} appear to inhibit parathyroid hormone secretion. Studies have also shown that estrogen deficiency can negatively affect Ca^{2+} balance and increases bone loss and also 17β-estradiol regulate renal expression of TRPM6 which promotes Mg^{2+} reabsorption in the kidney (van Abel et al., 2002; Groenestege et al., 2006).

2.3.4 Calcium (Ca^{2+}) and magnesium (Mg^{2+}) associated abnormalities

The maintenance of normal Ca^{2+} and Mg^{2+} homeostasis in the body correlate with the physiological well-being in human, however disturbances in their near constancy levels which could be either hypo or hyper have been associated to the development of many detrimental conditions in the body. This could result from reduced (re)absorption from intestine and kidney, abnormal parathyroid function, chronic intake of the trace elements, low magnesium affecting
PTH secretion, vitamin D deficiency etc. The dysfunction in the regulation of calcium (Ca\textsuperscript{2+}) have implicated to the development of prostate cancer (Rodriguez et al., 2003), neurodegenerative diseases like Parkinson, Alzheimer, cardiovascular diseases, Pre-eclampsia, kidney stone, osteoporosis, colorectal cancer and other cancer situations that occurs in the body (Sun et al., 2015; Massa et al., 2014; Hofmeyr et al., 2014; Sorensen et al., 2012; Bolland et al., 2010). Abnormal level of Mg\textsuperscript{2+} has been also associated to development of cardiovascular diseases like hypertension, stroke, sudden cardiac death, atherogenesis, cardiac arrhythmia vasospasm, inflammatory condition and atherosclerosis (Altura et al., 2010; Long and Romani, 2014). Neurological and reproductive instabilities also linked to low level of Mg\textsuperscript{2+} are migraine, Parkinson disease, epilepsy, Pre-eclampsia, pre-term birth and neurological degeneration (Farzin and Sajadi, 2012; Vink et al., 2009). Muscle dysfunction, bone wasting, hepatic fibrosis, osteoporosis, diabetes mellitus type 1&2, asthma and many other conditions (Long and Romani, 2014).

2.3.5 Calcium and magnesium balance or Ca\textsuperscript{2+}/ Mg\textsuperscript{2+} in the body

The cellular influx and efflux control of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} should be tightly regulated for effective intracellular ion activities and balance. Ca\textsuperscript{2+} and Mg\textsuperscript{2+} balance or ratio is the state of the body store of calcium (Ca\textsuperscript{2+}) relative to magnesium (Mg\textsuperscript{2+}) at equilibrium over extended time period (Treadwell, 2011). Calcium and magnesium belong to the same family in the periodic table and share the same homeostasis control system. They are regulated together by a negative feedback system and have been recently shown to share common transporters. However, Mg\textsuperscript{2+} antagonizes the action of Ca\textsuperscript{2+} in many physiological processes, regardless of similar chemical reactivity charge (Dai et al., 2013; Hardwick et al., 1991; Iseri and French, 1984). Studies have shown Ca\textsuperscript{2+} and Mg\textsuperscript{2+} to be in a constant competition for both kidney and intestinal (re)absorption,
membrane binding sites and as messengers to regulate process like proliferation. Additionally, increase in level of body Ca\(^{2+}\) over Mg\(^{2+}\) inhibit the absorption of both ions and also Mg\(^{2+}\) deficiency enhances retention and physiological effect of calcium (Ca\(^{2+}\)) which result in negative balance of Mg\(^{2+}\) but very low Mg\(^{2+}\) can inhibit PTH secretion that can reduce calcium absorption in the cell (Norman et al., 1981).

This clearly shows that within both the extracellular and intracellular milieus there is a certain level of Ca\(^{2+}\) needed to keep up with Mg\(^{2+}\) when the cell is under normal ionic equilibrium and physiological control. Cell containing the right amount Ca\(^{2+}\) relative to normal level of Mg\(^{2+}\) is said to have correct Ca\(^{2+}\) and Mg\(^{2+}\) balance or ratio. The ionic balance between Ca\(^{2+}\) and Mg\(^{2+}\) can be impaired by either increase or decrease in the absorption of one which can directly affect the other as noted above. The disturbance in the Ca\(^{2+}\)/ Mg\(^{2+}\) ratio have been shown to alter the normal biological activities such as cell differentiation, proliferation, DNA repair and carcinogenesis in the prostate gland, breast and many other cell (Wolf et al., 2007). The destruction of Ca\(^{2+}\) and Mg\(^{2+}\) balance in these cells have been associated to different cause including abnormal expression of some of the ion channels, undertaken of these ions in a diet and many other factors.

### 2.3.6 Human diseases associated to abnormal Ca\(^{2+}\)/Mg\(^{2+}\) ratio

Development or progression of some human diseases have been shown to influence by imbalance of Ca\(^{2+}\)/ Mg\(^{2+}\) ratio which includes prostate cancer, BPH, Colorectal cancer, Breast cancer etc. High grade prostate cancer development was shown to influence by increase in Ca\(^{2+}\)/Mg\(^{2+}\) ratio that promoted proliferation of the prostate cells. This study associated the increase in Ca\(^{2+}\)/Mg\(^{2+}\) ratio to expression of more TRPM7 channels which promoted influx of
Ca\textsuperscript{2+} on the expense of low Mg\textsuperscript{2+} level in the cell (Sun et al., 2012). Similar work was reported on the same result for development of high grade prostate cancer under condition of low Mg\textsuperscript{2+} and high Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio however, mechanism for imbalance of the Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio was not detected in the study (Dai et al., 2011).

A study also proposed that the reduce uptake of Mg\textsuperscript{2+} with corresponding increase intake of Ca\textsuperscript{2+} in postmenopausal women may lead to alteration in Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio and this can be associated to the development of new and recurrent breast cancer (Sahmoun and Singh, 2010). Population based study later concluded on an independent inverse association between dietary and total Mg\textsuperscript{2+} intake and all-cause of death in breast cancer patients however, there was a stronger mortality risk reduction among breast cancer patients with high Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio after increasing their Mg\textsuperscript{2+} uptake (Tao et al., 2016).

A prospect cohort study from Shanghai, observed a similar inverse association between total Mg\textsuperscript{2+} intake and total cancer mortality among women with high Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio (Dai et al., 2013). Additionally, calcium (Ca\textsuperscript{2+}) and magnesium (Mg\textsuperscript{2+}) intake was inversely associated with the risk of colorectal cancer progression or development in a population with low Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio (Dai et al., 2007). However, a clinical trial later confirmed that Ca\textsuperscript{2+} intake only reduces colorectal cancer risk in those with Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio less than 2.62 (Dai et al., 2012). Asare et al. (2017), recently concluded on the association of imbalance of Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio to the development of BPH in aging male population. Contrast to the above studies where TRPM7 expression and intake of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} were link to changes in Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio that affected the development of the diseases, the mechanism that leads to alteration of Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio in BPH
condition was not detected in the study.

2.4 Transient Receptor Potential (TRP) channels

Cellular communication ensures proper maintenance of tissue function, development and homeostasis. TRP ion channels have emerged as a prominent means by which communication between cells and their environment are carried out in addition to growth factor receptors, G-protein couple receptors, etc. TRP channels comprise of a large and diverse group of voltage independent cation-permeable channel proteins expressed in several organisms, tissues and cell types, encompassing both excitable and non-excitabile cells. TRP superfamily of proteins mediate transmembrane flux of cations down their concentration gradient, which depolarize and regulate cation concentrations in mammals and other organisms (Pedersen et al., 2005). The TRP ion channel was first detected in Drosophila melanogaster flies with mutation in the TRP gene that become visually impaired, due to photoreceptor response, becoming transient upon prolong illumination instead of sustained, hence the name transient receptor potential. These unique spectrum of channels are conserved from worms to mammals and they mediate a variety of sensory and sensory induced signals (Montell and Rubin, 1989).

Mammalian TRP channels constitute about 28 isoforms classified into six subfamilies; TRPC (Canonical), TRPV (Vanilloid), TRPM (Malastatin), TRPML (Mucolipin), TRPA (Ankyrin) and TRPP (polycystin) (Clapham, 2003; Moran et al., 2004). However, TRPN (No mechanopotential) has been discovered in some organisms to make the subfamilies seven but not in mammals (Nilius and Owsianik, 2011). TRP channels are permeable to divalent cations with the exception of TRPM4 and TRPM5, which permit the entrance of only monovalent ions. They
varies in penetrability for the different mono- and divalent cations among isoforms. All TRP subunits also possess six transmembrane segments with a pore region between the fifth and sixth segment, except some polycyseins. The subunits can come together as homo or heterotetramerer to form functional cation channels. TRPC, TRPV, and TRPA N-terminal contain ankyrin repeat at their cytoplasmic domain (Yao et al., 2005; Ramsey et al., 2006; Montell, 2005).

They are mostly localized in plasma membrane and also the basic channel architecture, amino acid sequence homology and cation selectivity are the major features so far employed in the categorization of TRP channels. These channels greatly vary in their biological functions, activation mechanisms and ion selectivity. Ligand binding, changes in voltage or physical/chemical factors (like temperature, pH, ions) and covalent modification of amino acid residues can mediate the stimulation of TRP channels (Yee et al., 2014). Activated TRP channel can lead to depolarization of cell membrane and flux of cations such as Ca$^{2+}$ and Mg$^{2+}$ across the membrane through voltage dependent ion channels as well as modulation of the associated signaling pathway. The channels modulate fundamental biological processes like cell proliferation, differentiation, growth, migration and survival via adjustment of cellular cations concentration and the associated signaling pathways. TRP channels functions as cellular sensor and transducer of various stimuli by mediating a variety of physiological responses including ionic homeostasis, muscular contraction and others (Clapham, 2003).

2.4.1 Transient Receptor potential Melastatin-like (TRPM) subfamily

TRPM are voltage-independent cation permeable channels, which are diverse and abundantly expressed in mammalian cells. TRPM subfamily constitute eight members that were named according to the order of their discovery and based on amino acid sequence homology, the
members have been divided into three groups; TRPM1/3, TRPM4/5 and TRPM6/7 however, contrasting results have been reported for TRPM2 and TRPM8, whiles some studies combined them to make the TRPM groups four others separate TRPM2 and TRPM8 on the bases of not sharing enough properties to guarantee grouping (Fleig and Penner, 2004; Pedersen et al., 2005). TRPM channels shows a varying degree of permeability to Ca\(^{2+}\) and Mg\(^{2+}\) ranging from TRPM4/5 which are impermeable to Ca\(^{2+}\) to more Ca\(^{2+}\) and Mg\(^{2+}\) permeable TRPM6/7 channels (Mederos y Schnitzler et al., 2008). TRPM subfamily lack the ankyrin repeats at their N-terminal domain.

Each of the TRPM monomeric protein is made up of conserve three regions: N-terminal, transmembrane and C-terminal domain. The N-terminal has a regulatory region that may take part in protein assembly and trafficking. Transmembrane region is located between the other two domains and forms a cation permeable channel compose of six membrane traversing portion, with pore between fifth and sixth span (Montell, 2005). The final C-terminal domain is divided in to coiled-coil region (CCR) involved in channel subunit multimerization or connection with regulatory proteins and variable region which may function to regulate channel activity. The C-terminal of three members of TRPM greatly differs in their architectural domain compared to other members. TRPM6 and TRPM7 C-terminals are fused to protein kinase while TRPM2 encompass ADP-ribose phosphohydrolase in their C-terminus. TRPM channels contributes to changes in cation concentrations by acting directly as cation permeation channel or changing membrane potential, modulating the driving force for other cation (Mederos y Schnitzler et al., 2008).
2.4.1.1 Expression and function of TRPM members

2.4.1.1.1 TRPM1

TRPM1 is the founding member of the family and a tumor suppressor protein expressed in melanocyte. The expression of TRPM1 gene in melanocyte inversely correlates with progression of malignant melanoma cells. This channel has been also proposed to conduct Ca\(^{2+}\) into the cell (Miller et al., 2004). The active involvement of TRPM1 in the ON pathway of retinal bipolar cells has been shown and might explain the congenital night blindness seen in patients with TRPM1 mutation.

2.4.1.1.2 TRPM2

TRPM2 is mainly expressed in brain and a variety of peripheral cells. TRPM2 is a nonselective cation channel protein permeable to Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\) and Cs\(^{+}\). TRPM2 channel fuses with ADP-ribose phosphohydrolase (Nudix domain) at C-terminus. It has a splice variant and the channel is activated by necotinamide adenine dinucleotide (NAD) and ADP-ribose in ADP-ribose pyrophosphatase dependent manner (Kraft and Harteneck, 2005). Hydrogen peroxide (H\(_2\)O\(_2\)) has been shown to activate TRPM2 and due to this, the channel could function as cellular redox sensor. Oxidative stress and TNF-\(\alpha\) mediated cellular Ca\(^{2+}\) entry and death have been associated to this channel protein (Pedersen et al., 2005).

2.4.1.1.3 TRPM3

TRPM3 channel protein is closely related to TRPM1 but possess more splice variants. Mostly, this channel is expressed in kidney, brain and few can be seen in testis and spinal cord. The TRPM3 channels are permeable to divalent cations, particularly Ca\(^{2+}\) and Mn\(^{2+}\). The channel is sensitive to endogenous muscarinic receptor activation, decreased extracellular osmolarity and
D-erythro-sphingosine which seems to be TRPM3 specific activator. This channel has been mentioned to be store operated channel (Grimm et al., 2003).

2.4.1.1.4 TRPM4 and TRPM5

TRPM4 and TRMP5 channel proteins share similar properties and are closely related with 50% sequence homology. However, TRPM4 which exist as two splice variant is highly expressed in heart, pancreas and placenta whiles TRPM5 channel protein is found in tongue, lungs, testis, digestive system and brain. These channels contrast with other TRPs by being more permeable to monovalent cations but impermeable to Ca\(^{2+}\). Additionally, they have been suggested to be endogenous calcium-activated cation channels due to their intrinsic voltage-sensitivity and also to be directly gated Ca\(^{2+}\). TRPM5 in the taste receptor also function as essential transducer of sweet, amino acid (umami) and bitter taste (Launay et al., 2002; Pedersen et al., 2005).

2.4.1.1.5 TRPM6 and TRPM7

TRPM6 and TRPM7 channel proteins are also related with about 50% sequence homology. The unique feature about these channels is the combination of channel activity with \(\alpha\)-serine/threonine protein kinase within the C-terminal domain. The \(\alpha\)-kinase in TRPM7 has been found to autophosphorylate and is required for channel activity however, studies have disputed this function of the kinase (Runnels et al., 2001). TRPM6 and TRPM7 assemble to form heteromeric complex which has been observed to be involved in TRPM6 channel localization in the plasma membrane. TRPM6 are predominantly expressed in the intestine and kidney epithelial cells but TRPM7 is ubiquitous in all mammalian cells. Their expression in the prostate gland has been shown.

Additionally, TRPM6 mediate the cellular transport of Mg\(^{2+}\). TRPM6 is also involved in the
regulation Mg\(^{2+}\) homeostasis and the channel is activated by decrease in Mg\(^{2+}\) with active reabsorption shown in kidney and intestine (Voets et al., 2004). TRPM7 on other hand is highly permeable to Mg\(^{2+}\) and Ca\(^{2+}\) in the cell however; the channel can also conduct divalent trace metal ions. TRPM7 expression is shown to be essential for cellular viability and similar to TRPM6, Mg\(^{2+}\) also modulate the permeability of the channel (Yee et al., 2014). TRPM6 and TRPM7 cation channels are very important in the regulation of Mg\(^{2+}\) and Ca\(^{2+}\) homeostasis in both excitable and non-excitable. They also involved in cell growth and proliferation especially TRPM7. Both channels show a large outward rectification current and small inward rectification currents during the movement of the ions (Schmitz et al., 2003).

### 2.4.1.1.6 TRPM8

TRPM8 was initially discovered in prostate cells but high levels of expression have been detected in sensory neuron. Activation of this channel is mediated by cool temperature and cool evoking pharmacological products. TRPM8 channel function as thermosensor in sensory neuron and is associated to homeostasis of Ca\(^{2+}\) in prostate epithelial cells due to it regulation of androgens. It conducts intracellular Ca\(^{2+}\) release and Ca\(^{2+}\) channel in plasmalem (Clapham, 2003; Patapoutian et al., 2003).

### 2.4.2 TRPM6

TRPM6 is a divalent cation selective channel that is permeable and has more affinity for Mg\(^{2+}\) than Ca\(^{2+}\). TRPM6 gene comprises of 39 exons which encodes a large protein of 2022 amino acids (Van Der Wijst et al., 2009). The channel share 50% sequence homology with close family member TRPM7. TRPM6 consist of six transmembrane traversing proteins with a putative pore region between the fifth and sixth segment, a long intracellular amino (N) and carboxyl termini (C) domain. It is a chanzyme and combined channel activity with α-serine/threonine protein
kinase within the C-terminal domain. TRPM6 are predominantly expressed in the intestine and kidney, though its expression in the prostate gland has recently been shown (Voets et al., 2004; Wang et al., 2007). TRPM6 is also involved in the regulation of Mg$^{2+}$ homeostasis and the channel is activated by decrease in Mg$^{2+}$ with active (re)absorption shown in kidney and intestine epithelia cells. TRPM6 and TRPM7 assemble to form heteromeric complex which has been observed to be involved in TRPM6 channel localization in the plasma membrane and function (Li et al., 2006). Micromolar concentration of 2-aminoethoxydiphenylborate increases TRPM6 activity while the same amount inhibit TRPM7 channel activity (Li et al., 2007).

2.4.3 TRPM7

TRPM7 is also a divalent cation selective ion channel that is Mg$^{2+}$ and Ca$^{2+}$ permeable, however the channel can also conduct Zn$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Ni$^{2+}$ and some essential or toxic metals (Monteilh-Zoller et al., 2003; Yee et al., 2014). The TRPM7 channel protein was discovered in 2001 by a multiple independent research groups and it consist of six transmembrane spanning proteins with a pore-forming loop located between the fifth and sixth domain. These assembly into tetramers composed of four subunit that form a functional TRPM7 channel (Kraft and Harteneck, 2005). Human TRPM7 genes is located at locus 15q21 on human chromosome 15 which covers 39 exons and encodes 1865 – amino acid protein with a mass of 212.7 KDa (Clapham et al., 2005). With the exception of its closest relative TRPM6 (shares 50% amino acid sequence homology), TRPM7 channel uniquely fuses cation channel activity with functional C-terminal alpha-type serine/threonine protein kinase domain, though the functional interaction between them remains poorly understood. TRPM7 is ubiquitously distributed throughout the body, however highly expressed in the liver, bone, heart and adipose tissue in human samples (Nadler et al., 2001). TRPM7 is a magnesium-nucleotide-regulated current (MgNum) channel.
controlled by serum Mg\textsuperscript{2+} concentration. TRPM7 channel have been also detected in both normal and cancerous prostate (Sun \textit{et al.}, 2012; Wang \textit{et al.}, 2007). The expression of TRPM7 channel in human starts at early embryonic stage and is vital for embryonic viability which lethality could occur in embryo with TRPM7 gene deletion.

\subsection*{2.4.4 Maintenance of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} homeostasis by TRPM6 and TRPM7}

TRPM6 and TRPM7 channels play a major function in the maintenance of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} homeostasis. TRPM6 regulate the total concentration of body Mg\textsuperscript{2+} via intestinal and kidney (re)absorption however, TRPM7 is more concern with intracellular homeostasis of both Mg\textsuperscript{2+} and Ca\textsuperscript{2+}. Mg\textsuperscript{2+} and Ca\textsuperscript{2+} (re)absorption through both TRPM6 and TRPM7 channels uses the active transcellular pathway as described above. Activated TRPM6 and TRPM7 channel can lead to depolarization of cell membrane which can mediate Ca\textsuperscript{2+} and Mg\textsuperscript{2+} transport across the membrane directly or through voltage dependent ion channels after membrane depolarization (Schmitz \textit{et al.}, 2005). The channels are regulated by different mechanisms discussed below.

\subsection*{2.4.5 Regulation of TRPM6 and TRPM7 channel activities}

TRPM6 and TRPM7 channels activities in the body are tightly regulated by the intracellular concentration of Mg\textsuperscript{2+} and Mg\textsuperscript{2+}-ATP. The rise in level of Mg\textsuperscript{2+} and Mg\textsuperscript{2+}-ATP have been shown in several studies to inhibit the activities of both channels however, opposite happens when cellular Mg\textsuperscript{2+} concentration reduce (Nadler \textit{et al.}, 2001). Phosphatidylinositol 4, 5, bisphosphate (PIP\textsubscript{2}) hydrolysis via direct binding to phospholipase C (PLC) isoform has also been demonstrated to inhibit TRPM7 activation. That is depletion of PIP\textsubscript{2} level following PLC-activation can inhibit TRPM7 activity. Cellular binding of PLC and TRPM7 is important for G-protein couple receptor activation of TRPM7 (Rohacs and Nilius, 2007; Runnels \textit{et al.}, 2002).
Additionally, diet and estrogen in the body modulate both expression and activity of TRPM6 but not TRPM7. High concentration of 17β-estradiol level in the increases the TRPM6 mRNA in the colon and kidney however, the repressor of estrogen activity (REA) binds TRPM6 β-sheet kinase in the absence of 17β-estradiol to inhibit the channel activity. Reduction of dietary magnesium level increase the expression of TRPM6 mRNA in the colon and kidney but increase uptake of magnesium only up regulates colon TRPM6 mRNA expression (Long and Romani, 2014).

Proton (H⁺) level has been shown to also affect the permeability of TRPM7 channel by Ca²⁺ and Mg²⁺. Extracellular high levels of proton (H⁺) inhibited the entry of the ions through TRPM7 channel even under condition of high extracellular concentrations of Ca²⁺ and Mg²⁺ and instead allowed the passage monovalent cations (Romani, 2011). The kinase domain of TRPM7 has been reported to be essential for channel function however, studies have disputed this finding and they have rather suggested modulatory role instead. The kinase domain of the channels can only be influenced by fluctuation in Mg²⁺. TRPM7 and TRPM6 kinase can be autophosphorylate (Schmitz et al., 2003). Increase in cellular angiotensin II and aldosterone have been shown to also up regulate TRPM7 activities (Nilius et al., 2007).

2.4.6 Diseases of prostate and other organs associated to the abnormal expression of TRPM7 and TRPM6 leading to ionic imbalance

These channels are highly expressed in the body and they convey Ca²⁺ and Mg²⁺ to various parts of the body which the prostate gland is no exception. Calcium (Ca²⁺) and magnesium (Mg²⁺) are physiologically active and they perform enormous functions in the body when their levels are within limit. Deregulation of Ca²⁺ and Mg²⁺ balance as a result of dysfunction of channels that mediate their transport can have a detrimental effect on the body. Development of many human
diseases have been suspected or associated to altered activity or expression of TRPM6 and TRPM7 including cancer, neurodegenerative diseases, hypomagnesaemia secondary to hypocalcaemia, cardiovascular defects like hypertension and many others.

Aberrant expression of TRPM7 and its associated deregulation of Ca$^{2+}$ and Mg$^{2+}$ homeostasis have been observed to involved in the development of many human cancer including prostate cancer, Ovarian carcinoma, Leukemia, Colon cancer, Breast carcinoma and Pancreatic adenocarcinoma. TRPM7 channel expression in the above mentioned cancers was compromised which lead to abnormal regulation of one or both ions and this predisposed the cell to uncontrolled proliferation, cell cycle, survival, invasion and migration. The prostate cancer development in the study was associated to increased Ca$^{2+}$/ Mg$^{2+}$ ratio as a result of defect in the channel enhances TRPM-mediated currents which promoted cellular influx of Ca$^{2+}$, leading to increased proliferation (Yee et al., 2014).

Hypomagnesaemia with secondary hypocalcaemia is a rare autosomal-recessive disease characterized by extreme low magnesium concentration accompanied by hypocalcaemia. The development of the disease has been associated to a mutation in TRPM6 gene expression leading to truncated channel TRPM6 proteins. This disease occurs in children and is mediated by intestinal and kidney Mg$^{2+}$ leak as a result of mutation. The low levels of Mg$^{2+}$ inhibit the secretion of parathyroid hormone which contribute to the reduce calcium in the patient. Symptoms normally experience by patients are tetany, muscle spasm, convulsion etc (Schlingmann and Gudermann, 2005).
Both TRPM6 and TRPM7 have been mentioned to contribute to the development of neurodegenerative diseases and cardiovascular abnormalities. TRPM7 genes variation can affect the channel function leading to alteration of Ca\(^{2+}\) and Mg\(^{2+}\) homeostasis. Dysfunction in cellular ionic homeostasis can mediate the production of oxidative reactive species which disrupt the differentiation, function and survival of neurons. This predisposes the neuron to neurodegenerative disease. The involvement both TRPM6 and TRPM7 in hypertension has been studied (Nilius et al., 2007; Sun et al., 2015).

The association between BPH and the regulatory channel proteins (TRPM6 and TRPM7) has not been done or established to the best of my knowledge, though Asare et al., (2017), detected the occurrence of Ca\(^{2+}\)/Mg\(^{2+}\) ratio imbalance in BPH patients. The mechanism that mediates this Ca\(^{2+}\)/Mg\(^{2+}\) ratio imbalance in BPH is unknown. Since TRPM6 and TRMP7 are common channels for Ca\(^{2+}\) and Mg\(^{2+}\) and abnormal regulation could end up in unregulated proliferation, this study seek to measure their level of expression in BPH samples to check whether the Ca\(^{2+}\) and Mg\(^{2+}\) imbalance could be their cause.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The study was a quasi-experimental study. Quasi-experimental design typically allows the researcher to control the assignment to the treatment condition, but using some criterion other than random assignment. Histologically diagnosed BPH patients constituted case and the comparable groups were prostate cancer patients and healthy men without any urological abnormality.

3.2 Study site

The study was conducted at Ghana police hospital and Chemical Pathology laboratory of School of Biomedical and Allied health sciences in Korle-Bu, which is a laboratory under College of Health Sciences of University of Ghana. The Ghana police hospital, Cantonments-Accra was established in 1970. It was a medium-sized hospital but currently undergoing expansion into ultramodern 400-bed facility. The hospital is having almost all the departments and serves as one of the centers that provide treatment to urologic associated problems. The hospital is having urology department with urologist providing their services.

3.3 Participants

The study participants comprised of male population with histologically diagnosed BPH attending Ghana police hospital for treatment and the comparable groups of prostate cancer patients and healthy male population without any known urological condition or abnormality that might interfere with the parameters being measured in the research. The participants who
volunteered for the study were between the ages of 40 and 90 years. Additionally, most of the BPH patients were on treatment and came from all walks of life, however, majority of them were from Accra.

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

The study included men with histologically diagnosed BPH between the ages of 40 and 100 years. These BPH patients were either on treatment or yet to undergo treatment. The same groups of men within the age range were also recruited for comparable groups.

3.4.2 Exclusion criteria

The exclusion criteria were as follows:

- All women were excluded.
- Men with ages outside the above mention range.
- Men taking diet, supplement or medicine with irregular amount of calcium (Ca\(^{2+}\)) or magnesium (Mg\(^{2+}\)) that might affect the result.
- Men with renal abnormality or any other condition known to interfere with the measurement of calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)).

3.5 Sample size

The minimum sample size was determined using Cochrane’s statistical formula as defined by (Charan and Biswas, 2013):

\[
N = \frac{Z^2(P)(1-P)}{E^2} = \frac{1.96^2 * 0.133(1-0.133)}{0.10^2} = 44.298
\]

Where:
N = Sample size

Z, 1.96 is the standard score for confidence interval of 95% or percentile for 95% significance level for normal distribution (1.96).

P, is the sample proportion prevalence of BPH in Ghana (birth preparedness = 0.133 = 13.3%). This prevalence estimation was based on symptomatic BPH.

E, is the accepted error margin = 0.10

Although the calculated sample size was approximately 44, however, 36 BPH patients volunteered to form part of the study.

3.6 Procedure for data collection

A convenient sampling technique was employed for this study. Questionnaire and laboratory assays were the study tools used for data acquisition.

3.6.1 Questionnaire

A well-structured questionnaire to test patient knowledge on BPH and its symptoms and also to exclude patients with conditions that might interfere with the major parameters being measured was used in this study.

3.6.2 Laboratory assays

3.6.2.1 Sample collection

Venous blood samples of 4 ml were taken from patients who met the criteria and consented, into lithium heparin tubes. This was done in participants who had fasted for at least 8 hours as recommended for calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) measuring samples. Samples were then centrifuged at 3500 rpm for 5 minutes, using Heal Force centrifuge [Shanghai Lishen Scientific equipment Co. (Shanghai, China)] to obtain plasma and buffy coat samples, however the buffy
coat samples were obtained after centrifugation of samples at 2200 rpm for 10 minutes. Plasma and buffy coat samples were aliquotted into cryo tube and stored at -20°C until ready for analysis. The buffy coat samples was left at -20°C storage for future use.

The following biochemical assays were performed: calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), albumen, total protein, TRPM6 and TRPM7.

### 3.6.2.2 TRMP6 and TRPM7

TRPM6 and TRPM7 were assayed using ELISA kits from Mybiosource, Inc. (San Diego, CA 92195-3308, USA). All the assays employed a quantitative Sandwich method. The ELISA analytical biochemical techniques of the kits was based on interaction between microelisa strip plate specific antibody that target TRPM6 or TRPM7 antigen in samples (immunosorbency) and also a second antibody, horseradish peroxidase (HRP) conjugated antibody specific to TRPM6 or TRPM7. HRP colorimetric detection system then detects TRPM6 or TRPM7 antigen targets in samples. The ELISA kits are designed to detect native, not recombinant, TRPM6 or TRPM7.

**Procedure:**

An amount of 50µl of the sample diluent, standards and samples were pipetted into assigned wells. The sample diluent was for Blank/Control well.

This was followed by the addition of 100µl of HRP-conjugate reagent to each well, covered and incubated for 60 minutes at 37°C.

The microplate content was discarded after 60 minutes of incubation followed by washing 4x with 350µl of wash solution. Wash solution was also discarded and plate was blotted dry by hitting it on absorbent paper.

An amount of 50µl of chromogen solution A and 50µl of chromogen B were successively added to each well and incubated away from light for 15 minutes at 37°C.
The reaction was stopped by the addition of 50µl of stop solution to each well. Yellow coloration was obtained.

Optical Density (OD) for the reaction was read at 450 nm using Humareader Plus Human microplate reader (Human GmbH, Wiesbaden Germany).

Optical Density for Blank/Control was subtracted from standard and sample OD to get their actual ODs.

A standard curve was drawn with standards and used to calculate the concentration of the samples.

**3.6.2.3 Calcium (Ca\(^{2+}\)) and Magnesium (Mg\(^{2+}\)) determination**

Serum concentration of calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) were assayed by standard analytic methods on the Mindray BS- 200 (Fully automated clinical chemistry analyzer, from New York, USA) with LABKIT reagents. Reagents were purchased from Barcelona in Spain. All study assays included a set of standards, controls and blank. The tests were carried out on already calibrated analyzer and controls and blank were performed before running the samples.

**3.6.2.3.1 Estimation of serum calcium ion levels**

Reaction principle:

Calcium (Ca\(^{2+}\)) with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis(azo)-dibenzenearsonic acid), at neutral pH, yielded a blue or purple color complex which was absorbed at 650nm. The intensity of the color formed is proportional to the calcium (Ca\(^{2+}\)) concentration in the sample.

\[
\text{Ca}^{++} + \text{C}_{22}\text{H}_{18}\text{AS}_{2}\text{N}_{4}\text{O}_{14}\text{S}_{2} \downarrow \rightarrow \text{Calcium-Arsenazo complex (Purple or blue color)}
\]

Procedure:
The analyzer sucked or aspirated some of the serum sample containing the calcium which combined with Arsenazo III to form a purple or blue coloured complex. The coloured complex was absorbed at 650 nm to give the concentration of calcium (Ca²⁺). Everything was automatically done by the machine.

3.6.2.3.2 Estimation of serum Magnesium (Mg²⁺) levels

Principle: Magnesium (Mg²⁺) formed a purple coloured complex that was absorbed at 520nm when reacted with calmagite in an alkaline solution. The intensity of the color formed was proportional to the concentration of magnesium (Mg²⁺) in the sample.

\[
\text{Mg}^{2+} + \text{calmagite} \rightarrow \text{Purple coloured complex}
\]

Alkaline medium

Procedure:

The analyzer sucked or aspirated some of the serum sample containing the magnesium which combined with calmagite to form a purple coloured complex. The coloured complex was absorbed at 520 nm to give the concentration of magnesium (Mg²⁺) automatically.

3.6.2.4 Serum albumin and total protein determination

3.6.2.4.1 Albumin estimation

Principle: Albumin in the presence of bromocresol green at a slightly acid pH, produced a colour change of the indicator from yellow-green to green-blue which was absorbed at 630 nm. The intensity of the colour formed is proportional to the albumin concentration in the serum sample.

\[
\text{Albumin} + \text{bromocresol green} \rightarrow \text{green-blue colour complex}
\]

Slightly acidic medium

Procedure:
The albumin and total protein concentration for the samples were also estimated using the same Mindray BS-200 clinical chemistry analyzer. The analyzer sucked or aspirated some of the serum sample containing the albumin which combined with bromocresol green to produce the green-blue colour complex. The coloured complex was absorbed at 630 nm to give the concentration of albumin automatically.

### 3.6.2.4.2 Estimation of total protein concentration

Reaction principle: Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide was included as an antioxidant. The intensity of the colour formed was proportional to the total protein concentration in the sample. The violet-blue complex was absorbed at 540 nm.

\[
\text{Protein} + \text{copper salts} \downarrow \rightarrow \text{violet blue colour complex}
\]

Alkaline medium

The analyzer aspirated or sucked some of the serum samples containing the protein which combined with copper salts under acidic medium to form the violet-blue colour complex. The coloured complex was absorbed at 540 nm to obtain the concentration of total protein automatically.

### 2.6.2.5 Corrected calcium calculation

Corrected calcium of the samples were calculated from calcium ion and albumin concentration obtained above. It was calculated as follows:

Corrected calcium (mmol/l) = serum Ca\(^{2+}\) (mmol/l) + 0.02(40-Albumin (g/l)), if the albumin concentration was less than 40 or corrected calcium (mmol/l) = serum Ca\(^{2+}\) (mmol/l) - 0.02(Albumin (g/l) - 40), if the albumin concentration was greater than 40.
2.6.2.6 Globulin estimation

The globulin concentration in the samples was obtained from the concentration of albumin and total protein above. The serum globulin concentration was calculated by subtracting the concentration of the albumin from total protein concentration for each sample.

\[
\text{Serum globulin (g/l)} = \text{Total protein concentration (g/l)} - \text{Albumin concentration (g/l)}
\]

3.7 Ethical issues

The project was approved by the Ethical and Protocol Review committee of the School of Biomedical and Allied Health Sciences Ethics number: SBAHS – MD. /10552215/AA/5A/2016-2017. Furthermore, approval was sought from the Police Hospital Administration. Informed consent was also sought from all participants whose information and samples were used.

3.8 Data Analysis

Graphpad Prisms statistics for windows, Version 6.05 and Microsoft Excel 2013 were used for the statistical analysis. Data was presented as mean ± standard deviation (SD). Statistical analysis for unpaired data was determined by student’s t test. Comparing of means between groups was done with one way ANNOVA. Correlation analysis was performed with Pearson correlation test. The p value less than 0.05 was considered statistical significant.
CHAPTER FOUR

RESULTS

Biochemical analysis

4.1 Age and channel regulatory analytes

Table 4.1 shows the ages and plasma levels of calcium (Ca\(^{2+}\)), magnesium (Mg\(^{2+}\)), albumin, total protein and globulin in the various groups. The change in means for calcium (Ca\(^{2+}\)), magnesium (Mg\(^{2+}\)), total protein (TP) and globulin (p= 0.0005, <0.0001, 0.0257 and 0.0138) respectively were statistically significant in BPH subjects whiles albumin and globulin also showed statistical significance in PCa subjects (p= <0.0001 and 0.0138) respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean±SD</th>
<th>BPH Mean±SD</th>
<th>PCa Mean±SD</th>
<th>p-value BPH</th>
<th>p-value PCa</th>
<th>p-value BPH &amp; PCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>54.72±5.83</td>
<td>70.61±8.81</td>
<td>69.88±8.55</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.7779</td>
</tr>
<tr>
<td>Ca(^{2+}) (mmol/l)</td>
<td>2.44±0.36</td>
<td>1.92±0.63</td>
<td>2.09±0.73</td>
<td>0.0005*</td>
<td>0.3328</td>
<td>0.4009</td>
</tr>
<tr>
<td>Mg(^{2+}) (mmol/l)</td>
<td>1.87±0.37</td>
<td>0.83±0.37</td>
<td>1.75±0.44</td>
<td>&lt;0.0001*</td>
<td>0.3328</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>47.30±4.52</td>
<td>46.53±3.37</td>
<td>35.75±10.29</td>
<td>0.4517</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>TP (g/l)</td>
<td>71.89±17.56</td>
<td>62.89±13.09</td>
<td>72.02±7.67</td>
<td>0.0257*</td>
<td>0.9735</td>
<td>0.0104*</td>
</tr>
<tr>
<td>Globulin (g/l)</td>
<td>26.59±9.54</td>
<td>19.23±9.15</td>
<td>36.27±14.18</td>
<td>0.0052*</td>
<td>0.0138*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval. Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa), Total protein (TP) and standard deviation (SD).
Table 4.2: Shows the plasma levels of TRPM6 & TRMP7, A/G ratio, corrected Ca\(^{2+}\) and Ca\(^{2+}\)/Mg\(^{2+}\) ratio. The changes in plasma TRPM6 were significant for both BPH and PCa subjects \((p<= 0.0001)\) in both cases while TRPM7 was significant for only PCa subjects as shown in the table below.

**Table 4.2: Plasma levels of TRPM6 and TRPM7, A/G ratio, Corrected Ca\(^{2+}\) and Ca\(^{2+}\)/Mg\(^{2+}\) ratio**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD</th>
<th>Mean±SD</th>
<th>Mean±SD</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BPH</td>
<td>PCa</td>
<td>BPH</td>
<td>PCa</td>
<td>BPH &amp; PCa</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>2.17±1.35</td>
<td>2.76±1.27</td>
<td>1.06±0.48</td>
<td>0.0980</td>
<td>0.0025*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Corrected Ca(^{2+}) (mmol/l)</td>
<td>2.29±0.33</td>
<td>1.79±0.61</td>
<td>2.17±0.64</td>
<td>0.0004*</td>
<td>0.4314</td>
<td>0.0416*</td>
</tr>
<tr>
<td>TRPM6 (ng/ml)</td>
<td>1.53±0.46</td>
<td>0.94±0.5</td>
<td>0.93±0.40</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>0.9339</td>
</tr>
<tr>
<td>TRPM7 (ng/ml)</td>
<td>0.70±0.18</td>
<td>0.82±0.43</td>
<td>0.48±0.10</td>
<td>0.1937</td>
<td>&lt;0.0001*</td>
<td>0.0023*</td>
</tr>
<tr>
<td>Ca(^{2+})/Mg(^{2+}) ratio</td>
<td>1.35±0.29</td>
<td>2.59±1.09</td>
<td>1.20±0.35</td>
<td>&lt;0.0001*</td>
<td>0.1642</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval. Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa) and standard deviation (SD).
Table 4.3 shows the correlation between TRPM6 and the channel regulatory analytes. TRPM6 positively correlated with plasma calcium in both BPH and PCa (p=0.0084 and 0.0485) respectively. Mg^{2+}, albumin and total protein did not show any significant correlation.

Table 4.3: A correlation between TRPM6 and channel regulatory analytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control r-value</th>
<th>BPH r-value</th>
<th>PCa r-value</th>
<th>Control p-value</th>
<th>BPH p-value</th>
<th>PCa p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^{2+} (mmol/l)</td>
<td>0.3204</td>
<td>0.4329</td>
<td>0.4850</td>
<td>0.1184</td>
<td>0.0084*</td>
<td>0.0485*</td>
</tr>
<tr>
<td>Mg^{2+} (mmol/l)</td>
<td>0.3079</td>
<td>0.0314</td>
<td>0.0249</td>
<td>0.1342</td>
<td>0.8558</td>
<td>0.9242</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>-0.2567</td>
<td>0.1968</td>
<td>-0.0701</td>
<td>0.2154</td>
<td>0.2499</td>
<td>0.7890</td>
</tr>
<tr>
<td>TP (g/l)</td>
<td>-0.1366</td>
<td>-0.0732</td>
<td>-0.3240</td>
<td>0.5149</td>
<td>0.6713</td>
<td>0.2046</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval. Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa), standard deviation (SD), Total protein (TP) and correlation coefficient (r-value).
Table 4.4 shows the correlation between TRPM6 and corrected Ca\(^{2+}\) and the ratios. TRPM6 positively correlated with corrected calcium in both BPH and PCa (p=0.0099 and 0.0145) respectively. Ca\(^{2+}\)/Mg\(^{2+}\) also showed a positive correlation in PCa whiles globulin A/G ratio did not show any significant correlation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>BPH</th>
<th>PCa</th>
<th>p-value</th>
<th>BPH</th>
<th>PCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globulin (g/l)</td>
<td>0.0233</td>
<td>-0.0295</td>
<td>-0.1244</td>
<td>0.9158</td>
<td>0.8704</td>
<td>0.6344</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>-0.1338</td>
<td>0.0488</td>
<td>0.1679</td>
<td>0.5426</td>
<td>0.7875</td>
<td>0.5194</td>
</tr>
<tr>
<td>Corrected Ca(^{2+})</td>
<td>0.4189</td>
<td>0.4243</td>
<td>0.5810</td>
<td>0.0371*</td>
<td>0.0099*</td>
<td>0.0145*</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(^{2+})/Mg(^{2+}) ratio</td>
<td>-0.0976</td>
<td>0.2467</td>
<td>0.5346</td>
<td>0.6424</td>
<td>0.1531</td>
<td>0.0270*</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval. Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa), standard deviation (SD), correlation coefficient (r-value) and albumin/globulin (A/G ratio).
Table 4.5 shows the correlation between TRPM7 and the channel regulatory analytes. TRPM7 positively correlated with plasma calcium and albumin in BPH subjects \((p=0.0012 \text{ and } 0.0268)\) respectively. \(\text{Mg}^{2+}\) and total protein did not show any significant correlation as shown in the table below.

**Table 4. 5: Correlation between TRPM7 and the channel regulatory analytes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r-value</th>
<th>r-value</th>
<th>r-value</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BPH</td>
<td>PCa</td>
<td>Control</td>
<td>BPH</td>
<td>PCa</td>
</tr>
<tr>
<td>Ca(^{2+}) (mmol/l)</td>
<td>-0.0347</td>
<td>0.5195</td>
<td>0.0121</td>
<td>0.8692</td>
<td>0.0012*</td>
<td>0.9633</td>
</tr>
<tr>
<td>Mg(^{2+}) (mmol/l)</td>
<td>0.1076</td>
<td>0.2852</td>
<td>-0.1139</td>
<td>0.6087</td>
<td>0.0918</td>
<td>0.6634</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>-0.2706</td>
<td>0.3690</td>
<td>-0.4101</td>
<td>0.1907</td>
<td>0.0268*</td>
<td>0.1021</td>
</tr>
<tr>
<td>TP (g/l)</td>
<td>0.2120</td>
<td>-0.1866</td>
<td>-0.1337</td>
<td>0.3091</td>
<td>0.2758</td>
<td>0.6090</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa), standard deviation (SD), Total protein (TP) and correlation coefficient (r-value).
Table 4.6 shows the correlation between TRPM7, corrected Ca\textsuperscript{2+} and other ratios. TRPM7 positively correlated with corrected calcium and A/G ratio (p=0.0022 and 0.0164) respectively in BPH.

Table 4.6: Correlation between TRPM7 and corrected Ca\textsuperscript{2+} and the ratios of subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control r-value</th>
<th>BPH r-value</th>
<th>PCa r-value</th>
<th>Control p-value</th>
<th>BPH p-value</th>
<th>PCa p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globulin (g/l)</td>
<td>0.0336</td>
<td>-0.3343</td>
<td>0.2253</td>
<td>0.8791</td>
<td>0.0575</td>
<td>0.3847</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>-0.1414</td>
<td>0.4148</td>
<td>-0.2267</td>
<td>0.5199</td>
<td>0.0164*</td>
<td>0.3816</td>
</tr>
<tr>
<td>Corrected Ca\textsuperscript{2+}</td>
<td>0.0355</td>
<td>0.4946</td>
<td>0.1464</td>
<td>0.8660</td>
<td>0.0022*</td>
<td>0.5751</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}/Mg\textsuperscript{2+} ratio</td>
<td>-0.0421</td>
<td>0.0654</td>
<td>0.1443</td>
<td>0.8416</td>
<td>0.7091</td>
<td>0.5805</td>
</tr>
</tbody>
</table>

*Significance at 95% Confidence Interval. Legend: Benign prostatic hyperplasia (BPH), Prostate cancer (PCa), standard deviation (SD), correlation coefficient (r-value) and albumin/globulin (A/G ratio).

4.1a: Age and TRPM6 correlation

A positive relationship was observed between age and TRPM6 in control subjects but the p-value (p=0.8584) was not statistically significant (figure 4.1a). However, an inverse relationship was observed in both BPH (p=0.3131) and prostate cancer patients (p=0.0545). Although the p-values were not statistically significant in both cases, that of prostate cancer patients was just on the borderline of significance as show in figures 4.1b and 4.1c respectively.
Figure 4.1a: A correlation between Age and TRPM6 in control subject with $r=0.0376$ and $p=0.8584$.

Figure 4.1a: A correlation between Age and TRPM6 in control subjects.

Figure 4.1b shows a negative relationship between age and TRPM6 in BPH patients however, the p-value ($p=0.3131$) was not statistically significant (figure 4.1b).

Figure 4.1b: A correlation between Age and TRPM6 in BPH patients with $r=-0.1730$ and $p=0.3131$.

Figure 4.1b: A correlation between Age and TRPM6 in BPH patients.
A negative relationship was observed between age and TRPM6 in prostate cancer patients with p=0.0545 just on the borderline of significance (figure 4.1c).

![Figure 4.1c: A correlation between Age and TRPM6 in PCa patients with r=-0.4741 and p=0.0545](image)

**Figure 4.1 c:** A correlation between Age and TRPM6 in PCa patients

### 4.2a: Age and TRPM7 correlation

A negative relationship was observed between age and TRPM7 in control subjects with an insignificant p-value (p=0.3222) (figure 4.2a). However, an inverse relationship was observed in the BPH patients with p=0.0532 just on the borderline of significance (figure 4.2b). Although the relationship in prostate cancer group was negative, the p=0.1040 was also not significant (figure 4.2c).
A positive relationship was observed between age and TRPM7 in BPH patients with p-value (p=0.0532) just on the borderline of significance (figure 4.2b).
Figure 4.2c shows a negative relationship between age and TRPM7 in BPH patients however, the p-value (p=0.3131) was not statistically significant (figure 4.2c).

Figure 4.2c: A correlation between Age and TRPM7 in PCa patients with r=-0.4080 and p=0.1040

Figure 4.2c: A correlation between Age and TRPM7 in PCa patients
Benign prostatic hyperplasia (BPH) is a global public health concern and continues to cause more problems in the life of aging male populations especially those that develop the bothersome lower urinary tract symptoms (LUTS). The condition impacts negatively on the quality of life of the patient by interfering with normal daily activities, sleep pattern, sexual relations and compromised feeling of well-being. The factors known to predetermine the onset of the condition is the presence of testis and advancing age however, the exact aetiology or factors that initiates and promotes the proliferative process is unknown. Though mechanisms such as hormonal alterations, defect in apoptotic genes, alterations in the properties of stem cells, abnormal levels of growth factors and others have been implicated to the development of the condition, none of the mechanism has been proven to solely cause BPH.

Cellular abnormal levels and metabolism of trace elements have been associated with the development and progression of many human diseases, especially tumors (Sun et al., 2014). Trace elements under normal regulation and levels are involved in many important functions of the body however; dysfunction in their control can generate increased levels of free radicals, unregulated cell proliferation and differentiation, impairment in the DNA repair system, mutagenesis and carcinogenesis. Ionized calcium (Ca\(^{2+}\)) in the cell is physiologically active and
has been associated with the development of many cellular and organ injury. Disturbances in Ca$^{2+}$ homeostasis have been shown to impact on cytoplasm, endoplasmic reticulum and mitochondria (Paschen, 2000). However cellular Ca$^{2+}$ homeostasis is tightly associated with magnesium (Mg$^{2+}$) which has been shown to share some common transport channel and other regulatory factors. The modulation of calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) has been implicated in the development of tumors like BPH (Asare et al., 2017).

Trace elements are closely connected with proteins such as albumin in a way that includes transport, proliferation and apoptosis. In this study, plasma levels of calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), albumin, total protein and globulin were determined (Table 4.1). The level of calcium (Ca$^{2+}$) was higher in all the groups compared to magnesium (Mg$^{2+}$). This result is similar to the findings in other studies where increased calcium (Ca$^{2+}$) level with reduced magnesium (Mg$^{2+}$) were reported (Asare et al., 2017; Dai et al., 2012; Dai et al., 2007; Sun et al., 2012). The control group recorded the highest followed by PCa and then BPH. Both Calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$) level in BPH group were significant with calcium (Ca$^{2+}$) being high and Mg$^{2+}$ recording the lowest among the groups but Ca$^{2+}$ level in PCa was also significant. The increased calcium (Ca$^{2+}$) level with corresponding decreased in magnesium (Mg$^{2+}$) level in BPH in the study might be involved in the development of the condition as mentioned by Asare et al. (2017). The BPH group recorded the highest mean age followed by PCa and control but there was no significant difference in age among BPH and PCa group.
Calcium and magnesium ratio (Ca\(^{2+}\)/Mg\(^{2+}\)) in the study was high in all the groups but the highest was recorded in BPH subjects followed by PCa and then control. The difference between BPH and PCa was significant but with the individual groups only BPH was significant compared to the control (Table 4.2). The increase in Ca\(^{2+}\)/Mg\(^{2+}\) ratio has been linked to high level of cellular proliferation which could lead to organ enlargement and this may suggest a high level of proliferation in BPH. The high Ca\(^{2+}\)/Mg\(^{2+}\) ratio in the BPH subject may be associated with the very low level of magnesium (Mg\(^{2+}\)) that was obtained compared to the other groups and this is consistent with findings in other studies. Magnesium (Mg\(^{2+}\)) in the body has been shown to compete and antagonize calcium (Ca\(^{2+}\)) in many biological activities including cellular transport, binding site. A decrease in magnesium (Mg\(^{2+}\)) will permit the transport and retention of calcium (Ca\(^{2+}\)) which could account for the high levels of calcium (Ca\(^{2+}\)) that was obtained for all the groups in the study (Dai et al., 2013; Iseri and French, 1984). The surge in calcium (Ca\(^{2+}\)) over magnesium (Mg\(^{2+}\)) leads to loss of homeostasis control which is a pre-requisite for abnormal proliferation and development of many human conditions. In this study, PCa and control results though showed high levels of calcium (Ca\(^{2+}\)) concentration however, their cellular magnesium (Mg\(^{2+}\)) were also high compared to the result obtained for BPH, which might compete a little to inhibit calcium (Ca\(^{2+}\)) movement into the cells. The situation was different with BPH group where calcium (Ca\(^{2+}\)) transport may be less antagonized by the very low level of magnesium (Mg\(^{2+}\)) and this could further account for high Ca\(^{2+}\)/Mg\(^{2+}\) ratio seen in BPH samples.
The level of TRPM6, TRPM7, corrected calcium, albumin/globulin ratio (A/G) and Ca\(^{2+}\)/Mg\(^{2+}\) were determined in the study (Table 4.2). The mean level of TRPM6 measured was higher in the control compared to BPH and PCa groups which were almost the same with no significant difference between them. However, TRPM6 level was significant between controls and both BPH and PCa. The TRPM7 level was rather high in BPH followed by controls and then PCa. There was significant difference between PCa and BPH and also between controls and PCa group. The TRPM6 and TRPM7 are major transport channels for both calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) and both have a greater affinity for Mg\(^{2+}\) transport. While TRPM6 regulate magnesium (Mg\(^{2+}\)) concentration and to a lesser extent calcium (Ca\(^{2+}\)) at the epithelia cell level and are common in the colon and distal convoluted tubule, TRPM7 is more concern with maintaining both calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) homeostasis at the cellular level and thus TRPM6 is totally removed in this contest (Schmitz et al., 2005). However, a finding that was carried out in rat showed the expression of TRPM6 in the prostate though it was the least expressed among TRPM channels. The result of that findings contrast with this studies in that TRPM6 was high in all the groups compared to the values obtained for TRPM7 (Wang et al., 2007).

The major transport channel that might mediate the intracellular transport of Ca\(^{2+}\) leading to high Ca\(^{2+}\)/Mg\(^{2+}\) ratio in BPH in this study is TRPM7 and this was largely expressed in BPH than PCa and the difference between the BPH and PCa was very significant. The result is consistent with a
finding in PCa where expression of TRPM7 channel influence the proliferation of the prostate through increasing Ca\(^{2+}\)/Mg ratio (Sun et al., 2012). The high level of TRPM7 in BPH may activate proliferation leading to prostate enlargement. There seems to be a weaker control of Ca\(^{2+}\) influx into the cells and subsequent lower serum calcium (Ca\(^{2+}\)) as seen in BPH. TRPM7 is a magnesium-nucleotide-regulated current (MgNum) channel controlled by serum Mg\(^{2+}\) concentration. In this study Mg\(^{2+}\) was significantly low in the BPH subjects as mentioned above which suggest increase TRPM7 activity. TRPM7 will transport more Ca\(^{2+}\) into the cell to cause abnormal proliferation and cellular injury. Therefore cell proliferation is altered by concentrations of serum Mg\(^{2+}\) (Rubin, 2010).

However, the channels seem to be influence by the levels of serum calcium (Ca\(^{2+}\)) and corrected Ca\(^{2+}\) in both BPH and PCa because they correlated positively with total serum calcium and corrected calcium and the results were significant (Table 4.3, 4.4, 4.5 and 4.6). This may confirm the weak control of calcium (Ca\(^{2+}\)) in both BPH and PCa subjects. The study identified one parameter that seem to influence the level of TRPM7 but did not have any effect on TRPM6. Albumin in the BPH was significantly high and correlated positively with TRPM7 but no correlation was observed between TRPM7 and low level of albumin in the PCa. Such observation was not seen in TRPM6 when albumin level fluctuation occurred in both BPH and PCa. Therefore albumin in this study may have some influence on the TRPM7 activity that is not present TRPM6. The A/G ratio also correlated positively with TRPM7 in BPH group which
seems to support the effect of albumin in this study. The ratio depends on both globulin and albumin and since albumin was very high in BPH compare to PCa which the level was almost equal to globulin, the ratio value recorded in PCa was low.

Most trace elements are shuttled to different parts of the body by protein carrier- albumin. This indicates how the levels of carrier proteins can directly influence the actions of trace elements in the body. A low albumin level will suggest low level of albumin bounds and hence a higher level of ionized trace element. This is seen in the PCa group in the study where the mean level of albumin was significantly low and could be described as hypoalbuminaemia. This means there will be decreased transport of calcium (Ca$^{2+}$) which may lead to reduced serum calcium (Ca$^{2+}$) concentration as a result of increase calcium loss (Ca$^{2+}$). The role of albumin in tumor progression is therefore critical. Albumin may be utilized by tumor as energy source to accelerate growth because they can be trapped within the interstitium of the tumor with the macromolecules they transport (Greish, 2007; Stehle et al., 1997). This may also be the cause of hypoalbuminaemia in the PCa. The total globulin was significantly higher in the PCa group of this study compared to the BPH group that was at the same level with the control group. Elevation of globulin is found in many types of inflammation, certain infection and in chronic liver disease. The different globulins are elevated in various diseases state and as a compensatory mechanism for reduced albumin level.
The corrected Ca$^{2+}$ value obtained in the study was high in the controls, followed by PCa and then BPH. There was a significant difference between BPH and PCa and also BPH group compared to control. This result also confirms the hypoalbuminemia state in the PCa group when the result is compared to the total calcium (Ca$^{2+}$) measured (Table 4.1). While others were decreasing when the calcium bound to albumin was corrected, the PCa corrected Ca$^{2+}$ was increasing because of the reduced albumin bound to calcium. The albumin and globulin ratio (A/G) was also significant in PCa and also between PCa and BPH. The reduced albumin to increase globulin levels measure in PCa resulted in the reduction of A/G in PCa. The result also shows the effect of reduced albumin in the PCa and high free calcium (Ca$^{2+}$) levels causing more injury in PCa patient.

Age is an important factor when it comes to the development of both BPH and PCa. In this study a positive relationship was seen between the age and TRPM6 in the control group (P=0.8584, r=0.0376) but inverse relationship was observed in both BPH (P=0.3131, r=-0.1730) and PCa (P=0.0545, r=-0.04741) (Fig: 4.1a, 4.1b and 4.1c). The study also showed a negative relationship between age and TRPM7 in control subject (P=0.3222, r=-0.2064) however, the relationship was inverse in BPH group with P=0.0532, r=0.3249 and negative in PCa (P=0.1040, r=-0.4080) (Fig: 4.2a, 4.2b and 4.2c). The association between TRPM channels and age have not been cleared and so far only a study between a close subfamily member TRPV1 and its reversal role of systemic
inflammation from anti–inflammation to pro–inflammation through age modulation was detected (Wanner et al., 2012).
CHAPTER SIX

CONCLUSION AND RECOMMENDATION

Conclusion

The findings confirmed the presence of calcium and magnesium imbalance in the BPH patients. The ion channel in the study that may mediate this imbalance of calcium and magnesium is TRPM7. It was not clear the role of TRPM6 in the ionic imbalance in BPH subjects. Albumin and globulin in this study seems to have effect on the channels especially TRPM7.

Recommendation

- A research into this ionic imbalance in BPH that will involve the use of molecular method and direct prostate tissue homogenate but not serum sample will be needed.
- A study that will investigate the effects of albumin and globulin on this channel function is also recommended.

Limitation

- The major limitation that is leading to the suggestion of the use of prostate tissue direct instead of serum sample is that, these ion channels are not specific to the prostate and could be found at different part of the body. This means any destruction or obstruction to the channel at a place different from the prostate could directly affect our results.
- Total calcium and magnesium were measured instead of the ions.
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Appendix

UNIVERSITY OF GHANA
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PROJECT TITLE: THE INVOLVEMENT OF TRPM6 AND TRPM7 CHANNEL REGULATORY PROTEINS AND IMBALANCE OF Ca^{2+} AND Mg^{2+} IONS IN THE AETIOLOGY OF BPH

Dear Potential Research Participant,

I am a student of the University of Ghana in the Department of Medical Laboratory Science. This questionnaire is designed to solicit information from you for a study I am conducting on the topic above. The study is part of the requirements for a Professional Masters in Medical Laboratory Science degree. It would be appreciated if you could make time to complete the questionnaire. Please be assured that your responses will be treated with strict confidentiality and anonymity and will be used for academic purpose only. Please your name is not needed and you can stop the interview at any time.

Researcher’s Contact Details
NAME: Illias Suleman
TEL: 0248190408/0503739715
EMAIL: sulemanillias@yahoo.com

Supervisor’s Contact Details
NAME: Prof. George Awuku Asare
(Department of Medical Laboratory Science) TEL: 0244627456 EMAIL: gasare@chs.edu.gh

NAME: Dr. Seth Amanquah
(Department of Chemical Pathology) TEL: 0244293987 EMAIL: nii.dortey@yahoo.com

The questions have been categorized into sections. Section A to C, each addressing a different facet of the study. Kindly read and answer the questions according to the instructions for each question.

SECTION A: PERSONAL DATA
1. Age - 
2. Educational Level
   - Non formal education [ ]
   - Primary level [ ]
   - Junior high/JSS [ ]
   - Senior high/SSS/Secondary [ ]
   - Tertiary/Post-secondary [ ]
3. Marital status
   - Single [ ]
   - Married [ ]
   - Divorce [ ]
   - Widow [ ]
4. Number of Children

5. Religion  Christian [ ]  Islam [ ]  Traditional [ ]

Other, specify

SECTION B: KNOWLEDGE ABOUT BPH

6. Have you heard about BPH before? Yes [ ]  No [ ]

7. Do you know what BPH is? Yes [ ]  No [ ]

8. If YES, what is it?

9. Which class of humans does it affect? Males [ ]  Females [ ]  Both [ ]

10. Do you know some of the symptom of BPH? Yes [ ]  No [ ]

11. Can you tick BPH symptoms you know among the list of symptoms?

A. Urinary hesitancy [], B. delay in initiating micturition [], C. intermittency [], D. involuntary interruption of voiding [], E. weak urinary stream [], F. straining to void [], G. a sensation of incomplete emptying [], H. terminal dribbling [], I. urinary frequency [], J. nocturia [], K. urgency [], L. incontinence []

12. When did you get to know about the BPH?

13. Have you been diagnosed of BPH? Yes [ ]  No [ ]

14. When did you know that you are having BPH condition?

15. Which among the following list of BPH symptoms, were you experiencing before diagnoses?

A. Urinary hesitancy [], B. delay in initiating micturition [], C. intermittency [], D. involuntary interruption of voiding [], E. weak urinary stream [], F. straining to void [], G. a sensation of incomplete emptying [], H. terminal dribbling [], I. urinary frequency [], J. nocturia [], K. urgency [], L. incontinence []

SECTION C: Conditions that can affect results

16. Have you ever taken any medicine to treat the condition since you started experiencing the symptoms? Yes [ ]  No [ ]  I don’t have BPH []

17. If YES, please indicate the name of the medicine?

18. Are you currently taking any medicine to treat the BPH? Yes [ ]  No [ ]

19. If YES, please indicate the name of the medicine?

20. Are you taking medicine to treat any other condition apart from BPH? Yes [ ]  No [ ]

21. If YES, please indicate the name of the medicine?
21. Are you taking any mineral supplement? Yes [ ] No [ ]
22. If YES, please indicate the type of the mineral supplement? ................................................
23. Have you been assigned by your health professional to take in a special kind of diet? Yes [ ] No [ ]
24. If YES, please indicate the kind of diet? .............................................................
25. Have you been restricted by your health professional from taking some foods? Yes [ ] No [ ]
26. If YES, please indicate the type of food? .............................................................
27. Have you been diagnosed of any of the conditions listed below? Chronic heart disease [ ], Renal disease [ ], Hypertension [ ], Atherogenesis [ ], Hepatic fibrosis [ ], Diabetes mellitus type 2 [ ], Tetany [ ], Seizure [ ], Cardiac arrhythmias [ ], Parkinson disease [ ], Alzheimer [ ], prostate cancer [ ], prostatitis [ ].
28. What other condition(s) apart from the above (27) have you been diagnosed of? ..........................................................

CONSENT FORM

The nature and the purpose of the above research study have been openly explained to me in a language that I understand. I have also been made known the benefit and possible risk that is involved in the study. I have also been made aware that the study is entirely voluntarily and I can withdraw at any time with/without any reason without attracting any penalty or being denied maximum treatment.

Signing or thumbprinting below therefore indicates that I have been duly informed about the research study and voluntarily agree to participate. I have asked questions to clear any doubt and the information given to me has permitted me to make an informed and free decision about participation in the study. By signing this consent form, I do not forgo any legal rights against the investigators and I can also withdraw from this study at any time. A copy of this consent form will be provided to me.

Signature/ Thumbprint of participant______________________ Date____________
Signature of Person Obtaining Consent/Authorization___ Date ___

THANK YOU FOR YOUR TIME