STUDY OF THE POLYMORPHISM *BsmI* AND *TaqI* IN VITAMIN D RECEPTOR GENE IN EGYPTIAN PATIENTS WITH CHRONIC KIDNEY DISEASE

Thesis

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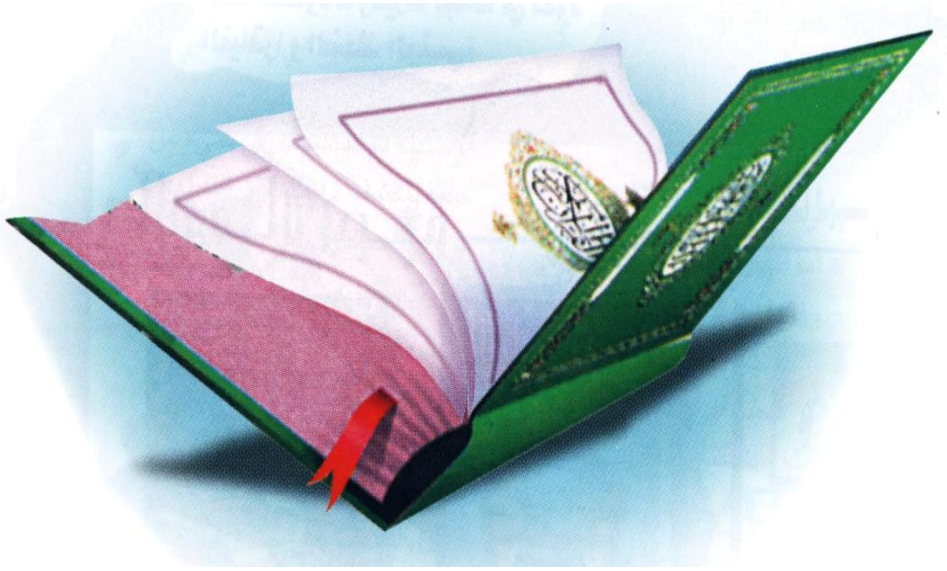
بسم الله الرحمن الرحيم

إِنَّا فَتَحْنَا لَكَ فَتْحًا مُّبِينًا



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Farida Mohamed Khanany

List of Contents

List of Contents cont...

List of Tables

List of Tables cont...

List of Figures

List of Figures cont...

List of Abbreviations

Abb.	*Full term*
25-OH VIT D	*25- -hydroxy vitamin D*
AAS	*Atomic absorption spectrophotometry*
ACR	*Albumin / creatinine ratio*
AE	*Acridinium ester*
AER	*Albumin excretion rate*
AMP	*2amino-2-methyl-1-propanol*
bALP	*Bone alkaline phosphatase*
BCP	*Bromcresol purple*
BGP	*Bone gla protein*
BMD	*Bone mineral density*
bp	*Base pair*
Ca	*Calcium*
CaR	*Calcium sensing receptors*
CDC	*Centres for disease control and prevention*
Cdx	*Caudal-related homeodomain protein*
CI	*Confidence intervals*
CKD	*Chronic kidney disease*
CKD-MBD	*Chronic kidney disease –mineral bone disorder*
Conc	*Concentration*
CPBA	*Competitive protein binding assay*
Cr	*Creatinine*
CT	*Computed tomography*
CTX	*Products of type I collagen C-telopeptide*
CVD	*Cardiovascular disease*
DBD	*DNA binding domain*
DM	*Diabetes mellitus*
DNA	*Deoxy ribonucleic acid*
DHPLC	*Denaturing high-performance liquid chromatography*
dNTPs	*Deoxynucleotide triphosphates*
DXA	*Dual X-ray absorptiometry*
EDTA	*Ethylene diamine tetra-acetic acid*
eGFR	*Estimated glomerular filtration rate*
EGIPT-CKD	*Egypt Information, Prevention, and Treatment of Chronic Kidney Diseases Program*
EIA	*Enzymatic assay*
ELISA	*Enzyme-linked immunosorbent assay*
ESRD	*End-stage renal disease*

List of Abbreviations cont...

Abb.	Full term
FBG	*Fasting blood glucose*
FGF23	*Fibroblast growth factor 23*
FITC	*Anti-fluorescein labeled*
GFR	*Glomerular filtration rate*
GLDH	*Glutamate dehydrogenase*
HCC	*Hepatocellular carcinoma*
HPLC	*High-performance liquid chromatography*
HVDRR	*Hereditary vitamin D-resistant rickets*
ICTP	*Products of type I collagen C-telopeptide*
ID-MS	*Isotope dilution–mass spectrometry*
iPTH	*Intact Parathyroid hormone*
IQR	*Inter-quartile range*
IRMA	*immunoradiometric assay*
ISE	*Ion-selective electrodes*
K/DOQI	*Kidney Disease Outcome Quality Initiative*
KCl	*Potassium Chloride*
kda	*Kilo Dalton*
KDIGO	*Kidney Disease Improving Global Outcomes*
KDIGO-MBD	*Kidney Disease Improving Global Outcomes-Mineral Bone Disease*
LBD	*Ligand-binding domain*
LC/MS	*Liquid chromatography mass spectrometry*
LVH	*Left ventricular hypertrophy*
MA	*Microalbuminuria*
MDRD	*Modification of diet in renal disease formula*
$MgCl_2$	*Magnesium Chloride*
MS	*Mass spectrometry*
NA	*Nucleic acid*
NGS	*Next-generation" or "second-generation" sequencing*
NKDEP	*National Kidney Disease Education Program*
NKF	*National kidney foundation*
NTX	*Products of type I collagen N-telopeptide*
OC	*Osteocalcin*
OR	*Odds ratio*

List of Abbreviations cont...

Abb.	*Full term*
PCR- RFLP	*Polymerase chain reaction-restriction fragment length polymorphism*
Pcr	*Plasma creatinine*
PCR	*Polymerase Chain Reaction*
Pi	*Phosphorus*
PICP	*Procollagen type 1 C propeptides*
PINP	*Procollagen type 1 N propeptide*
PMP	*Paramagnetic particles*
QCT	*Quantitative computed tomoghraphy*
RANKL	*Receptor activator of nuclear factor κ-B ligand*
RFLP-PCR	*Restriction fragment length polymorphism-polymerase chain reaction*
RFLPs	*Restriction Fragment Length Polymorphisms*
RIA	*Radioimmunoassay*
RO	*Renal Osteodystrophy*
RT	*Room temperature*
RXR	*Retinoid X receptor*
SNP	*Single nucleotide polymorphism*
SPSS	*Statistical package for the social science*
T.Bil	*Total Bilirubin*
TAE	*Tris-acetate EDTA buffer*
TBE	*Tris-borate EDTA buffer*
TRAP5b	*Tartrate resistant acid phosphatase*
Ucr	*Urinary creatinine*
UK	*United Kingdom*
URL	*Upper reference limit*
UTR	*Untranslated region*
UV	*Ultraviolet*
V	*Volume of urine*
VDDR2A	*Vitamin D-dependent rickets type 2A*
VDR	*Vitamin D receptor*
VDRE	*Vitamin D response elements*
WHO	*World health organization*

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem and a leading cause of morbidity and mortality worldwide, accounting for 60% of all deaths, affecting 5–10% of the world population with an ever-increasing prevalence. Estimated Prevalence of CKD (Stages 3-5) in the World is nearly 200-333 million ***(Kidney Disease Improving Global Outcomes (KDIGO), 2012)***, while the prevalence of end-stage renal disease patients in Egypt per million population is about 522 ***(Soliman et al., 2012)***.

Chronic kidney disease mineral and bone disorders (CKD-MBD) is a common complication of CKD and an important cause of morbidity and decreased life quality ***(Delanaye et al., 2014)***. CKD-MBD comprises a group of inter-related abnormalities of either one or a combination of the following: *i)* serum bone biomarkers: calcium, phosphorus, parathyroid hormone (iPTH), or vitamin D metabolism, *ii)* bone: bone turnover, mineralization, volume, linear growth or strength and *iii)* the vasculature: arterial calcification ***(Al Rukhaimi et al., 2014).*** It is clinically detectable as early as stage (2) CKD. Up-to 20% of death in dialysis patients may be associated with poorly controlled CKD-MBD, illustrating the importance for its management efficiently ***(Evenepoel et al., 2015)***.

Serum levels of 25-hydroxy vitamin D and iPTH provide an accurate picture of bone turnover and mineralization states. Therefore, they could be used with other serum bone biomarkers as non-invasive sensitive bone markers to help management of MBD in CKD patients as recommended by the Kidney Disease Improving Global Outcome- mineral and bone disorders (KDIGO.MBD) ***(Sprague et al., 2015***).

The kidney is the main site for the conversion of 25-hydroxyvitamin D to circulating 1, 25-dihydroxyvitamin D. Vitamin D receptor (VDR) is a transcription factor that mediates the genomic effects of vitamin D. It is activated by 1, 25-dihydroxyvitamin D to induce/repress genes that maintain mineral homeostasis and skeletal integrity, and prevent secondary hyperparathyroidism, hypertension, immune disorders, renal and cardiovascular damage as the VDR is widely expressed in many tissues and cells such as heart, kidney, immune cells, brain and muscle ***(Dusso, 2011; Zhou et al., 2014)***.

The VDR gene is located on chromosome 12q12.14. The VDR-regulated gene products tightly coordinate intestinal calcium and phosphate absorption, bone metabolism, renal calcium and phosphate reabsorption, and parathyroid function to prevent or slow the development of secondary hyperparathyroidism, phosphate retention, and soft-tissue calcifications ***(Khadijeh et al., 2014).***

There have been several VDR gene polymorphisms named according to the restriction sites, such as VDR *BsmI (rs1544410), Fok1 (rs2228570), ApaI (rs7975232) and TaqI (rs731236).* The VDR gene polymorphisms have been shown to drive the progression toward secondary hyperparathyroidism and influence the response to calcitriol and the survival in CKD patients ***(Zhou et al., 2014)***.

Due to the complex role played by vitamin D in kidney disease, the genotyping for VDR variants in CKD patients has been regarded as a way for improving the management of the disease, given that some VDR genotypes have been proven to influence the responsiveness to therapeutical approaches ***(Santoro et al., 2015)***.