In silico AND BIOCHEMICAL STUDIES ON THE MODULATORY EFFECTS OF CALCIFEROL ON PROSTATE AND LIVER CANCER

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SEPTEMBER, 2021

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, NIGERIA.

SEPTEMBER, 2021

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota

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DECLARATION

I, **ADELANI, ISAACSON BABABODE (07CP05662),** declare that this research was carried out by me under the supervision of Prof. Emmanuel N. Maduagwu (FAS, FAMedS) of the Department of Biochemistry, Chrisland University, Abeokuta, Ogun State, Nigeria and Dr. Solomon O. Rotimi of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

ADELANI, ISAACSON BABABODE ……

Signature and Date

CERTIFICATION

We certify that this thesis titled **"*In silico* and Biochemical Studies on the Modulatory Effects of Calciferol on Prostate and Liver Cancer"** is an original research work carried out by **ADELANI, ISAACSON BABABODE (07CP05662)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Emmanuel N. Maduagwu and Dr. Solomon O. Rotimi. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Biochemistry.

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DEDICATION

I dedicate this thesis to God, the author and finisher of my faith who, in his infinite mercies, kept me through the journey.

ACKNOWLEDGEMENTS

I appreciate God almighty for his provision, kindness, protection, guidance, and mercies during this journey.

I acknowledge the Chancellor of Covenant University, Dr. David O. Oyedepo, for his motivation, mentorship, and leadership during my stay at the University. I also appreciate the University management for their financial support and publications of this thesis' scientific findings.

My sincere appreciation also goes to the Vice-Chancellor of Covenant University, Prof. Abiodun H. Adebayo, for his leadership roles and provision of necessary scholarly access. I acknowledge the Registrar, Dr. Oluwasegun P. Omidiora; the Dean, School of Postgraduate Studies, Prof. Akan B. Williams; the Sub-Dean, School of Postgraduate Studies, Dr. Emmanuel O. Amoo, as well as the Dean, College of Science and Technology, Prof. Temidayo V. Omotosho.

I appreciate my supervisor, Prof. Emmanuel N. Maduagwu (FAS, FAMedS), for his motivation, assistance, and contributions to this research. I am highly indebted and register my sincere appreciation to my co-supervisor, Dr. Solomon O. Rotimi, for his mentorship, ideas, and immense contributions to this research work. My gratitude will be incomplete without appreciating the tremendous efforts of Prof. Clayton C. Yates of Tuskegee University, Alabama, USA, and Dr. Moray J. Campbell of Ohio State University, Columbus, Ohio, USA in mentoring, assisting, and contributing towards this research.

My appreciation goes to the present Head of Department of Biochemistry, Prof. Israel S. Afolabi. I also appreciate and thank the immediate past Head of Department of Biochemistry, Prof. Olubanke O. Ogunlana, for their encouragement and advice during the programme. I also appreciate Professors and Senior Faculty in the Department of Biochemistry for their input during the PhD programme – Prof. Shalom N. Chinedu, Prof. Emeka E. J. Iweala, Dr. Titilope M. Dokunmu, Dr. Omolola E. Omotosho, Dr. Omolara F. Yakubu, and Dr. Wisdom O. Joel for their support. Special appreciation goes to Dr. Oluwakemi A Rotimi, Dr. Tolulope D. Olawole and Dr. Opeyemi C. De Campos for their constant assistance and support.

I also thank the laboratory staff of the Department of Biochemistry for their assistance at any given time – Late Mrs. Julie O. Akinyele, Mr. Alaba O. Adeyemi, Mrs. Deborah K.

Akinlabu, Mrs. Omowunmi R. Afolabi, Mrs. Juliet Nwabuenu, Mr. Daniel U. Okere, and Miss. Bose E. Adegboye.

I appreciate my wonderful colleagues and friends for their support and encouragement – Mr. Abiola O. Kehinde, Mr. Olufemi O. Osho, Mrs. Adeola E. Benson and Mrs. Iyanuoluwa O. Ademola. I also appreciate my senior colleagues, colleagues, and friends in Tuskegee University and Ohio State University for the kindness, support, and assistance during my stay in the USA – Dr. Balasubramanyam Karanam, Dr. Honghe Wang, Dr. Huixian Lin, Dr. Joakin Mori, Mr. Jason Whites, Mr. Chris Andrews, Mr. Ahamd Salam, Mrs. Isra Elhussin, Mr. Lecarde Webb, Mr. Kawsar Chowdhury, Ms. Rania Mohamedelhassan, Dr. Sajad Wani, Ms. Alby Wu, Ms. Jafari Hedieh, Ms. Jaimie Gray, Dr. Joel Tumwebaze, Dr. Joel O. Wao, and Mr. Gilbert Adjoyi.

I will be ungrateful if I fail to acknowledge the USA's Fulbright commission operated under the International Institute of Education for the scholarship and exposure given to me. I also appreciate Fulbright Nigeria under the US Consulate and its staff, including Mr. Clemson O. Ayegbusi and Mrs. Doyinsola O. Apalara, for their immense contributions and assistance.

I appreciate my senior colleagues, office mates, friends, and colleagues for their assistance and encouragement – Dr. Opeyemi I. Ayanda Mr. Emmanuel A. Omonigbehin, Mr. Oluwadurorotimi S. Aworunse, Dr. Samuel E. Ejoh, Dr. Jacob O. Popoola, Ms. Faith O. Akinbo, Ms. Evarista A. Ebigwai, Ms. Omoremime E. Dania, Dr. Franklyn N. Iheagwam, and Mrs. Gloria N. Okenze.

I sincerely appreciate my parents for their support, motivation, and encouragement even through the tough times. To Engr. Stephen A. Adelani and Mrs. Mojisola A. Adelani, I say thank you for providing me with the necessary foundation to get this far. The seeds you planted in my life will never be forgotten. I also appreciate my brother, Mr. Boluwatife J. Adelani, for his love, care, and support during this academic journey. You all made it easy for me with your encouraging and kind words of support. You are simply the best.

I register my appreciation to my family members for their love and encouragement – my late grandmother, Mrs. Florence T. Jiboku. Appreciation also goes to every family member - Mrs. Omobolanle M. Sogeke, Mrs. Oluwakemi M. Adebiyi, Mr. and Mrs. Sylvanus O. Daniel, Mr. and Mrs. Tunde A. Jayeola, Mr. and Dr (Mrs). Akinkunmi A. Jiboku, Mr. and Mrs. Samuel O. Sogeke, Mr. and Mrs. Temitope J. Adenokun, Mr. and Mrs. Oluwajubelo

T. Idowu, Ms. Omolara D. Adebiyi, Ms. Hannah O. Adebiyi, Ms. Ayomide G. Jayeola, Ms. Omolola B. Jayeola, Ms. Stella O. Daniel, Ms. Suzan O. Daniel, Ms. Ewaoluwa O. Jiboku, Mr. Inioluwa O. Jiboku, Mr. Oreofeoluwa I. Jiboku, Mr. and Mrs. Kayode D. Adelani, Ms. Tofunmi E. Adelani, Mr. and Mrs. Ademuyiwa E. Adelani.

I thank Arch. and Mrs. Semeon A. Alofun for always supporting and encouraging me. I also appreciate Engr. and Mrs. Stephen O. Kehinde, and Dr. (Mrs.) Morenike B. Fajimi for their encouragement. I duly appreciate the assistance of Barr. and Mrs. Babatunde L. Oke, Mr. Gbolahan E. Alofun and Mr. Olujimi S. Alofun.

To my wonderful son, Isaacson A. Adelani, I say thank you for your endurance and patience. From the day you arrived, you have been a source of joy for me, and I will always appreciate God for gifting you to me.

Lastly, my sincere appreciation goes to my wife and support system, Mrs. Adeola F. Adelani. I appreciate you for your love, dedication, and care during this journey. Thank you for all the encouragement, assistance, sacrifices and provisions you made in the past few years. I cannot thank you enough for all you have done and sacrificed for me in this time. I will always cherish all your sacrifices and love. Thank you.

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LIST OF ABBREVIATIONS

AA	African American
AANAT	Aralkylamine N-acetyltransferase
ADP	Adenosine diphosphate
ADT	Androgen deprivation therapy
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Androgen receptor
ARNTL	Aryl Hydrocarbon Receptor Nuclear Translocator Like
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BAZ1A	Bromodomain adjacent to zinc finger domain protein 1A
BMAL 1	Brain and Muscle ARNT-Like 1
BPH	Benign prostatic hyperplasia
CaP	Prostate cancer
CDC	Centers for Disease Control and Prevention
CDNB	1-chloro-2,4-dinitrobenzene
CHO	Cholesterol
CLOCK	Circadian locomotor output cycles protein kaput
CNT-VDD	Normal control with vitamin D deficient diet
CNT-V	Normal control with normal diet
CR	Circadian Rhythm
CRP	C-reactive protein

CRPC	Castration-resistant prostate cancer
CRY	Cryptochrome Circadian Regulator
CSNK1E	Casein kinase I isoform epsilon
CTNNB1	Catenin Beta 1
CYP24A1	Cytochrome P450 Family 24 Subfamily A Member 1
CYP27B1	Cytochrome P450 Family 27 Subfamily B Member 1
CYP2E1	Cytochrome P450 Family 2 Subfamily E Member 1
CYP2R1	Cytochrome P450 Family 2 Subfamily R Member 1
CYTO	Cytoplasm
DAB	3'-Diaminobenzidine
DBP	Vitamin D binding protein
DEN	Diethylnitrosamine
DMN	Dimethylnitrosamine
DNA	Deoxyribonucleic acid
DN-V	DEN-induced with normal diet
DN-VDD	DEN-induced with vitamin D deficient diet
DTNB	5,5'-Dithiobis (2-nitrobenzoic acid)
EA	European American
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal Growth Factor
EGTA	Ethylene glycol tetraacetic acid
ELISA	Enzyme-linked immunoassay
EMT	Epithelial-Mesenchymal Transition
ERG	Erythroblast transformation-specific–related

FGF23	Fibroblast growth factor 23
FIG	Figure
FKBP5	FK506-binding protein 51
FOXO1	Forkhead Box O1
GGT/ϒ-GT	Gamma-glutamyl transferase
GR	Glucocorticoid receptor
GSH	Glutathione
GST	Glutathione S-transferase
H & E	Haematoxylin and Eosin
H_2O_2	Hydrogen peroxide
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein
IFN	Interferons
IFN-ϒ	Interferon gamma
IHC	Immunohistochemistry
IL	Interleukin
KAISO/ ZBTB33	POZ/BTB family protein
KEAP 1	Kelch-like ECH-associated protein 1
LIHC	Liver hepatocellular carcinoma
MAdCAM-1	Mucosal Vascular Addressin Cell Adhesion Molecule 1
miRNA	microRNA
MTOR	Mammalian target of rapamycin
NAMPT	Nicotinamide phosphoribosyltransferase

NCI	National Cancer Institute
NPAS2	Neuronal PAS domain protein 2
NUCL	Nucleus
PER	Period
PHLPP	PH domain and Leucine rich repeat Protein Phosphatases
PPC	Positive pixel counts
PRAD	Prostate Adenocarcinoma
PSA	Prostate-specific antigen
PTEN	Phosphatase and tensin homolog
PTH	Parathyroid hormone
Px	Peroxidase
RXR	Retinoid X
sICAM-1	Intercellular adhesion molecule 1
SIRT	Sirtuin, Silent mating type information regulation 2 homolog
SMARCA5	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 5
SOD	Superoxide dismutase
TB	Total bilirubin
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TCGA	The Cancer Genome Atlas
TG	Triglycerides
TGF	Tumour growth factor
TLR	Toll-like receptor

TMPRRS2	Transmembrane protease, serine 2
TNF	Tumour necrosis factor
TP53	Tumour protein p53
UV	Ultra violet
VCAM-1	Endothelial vascular cell-adhesion molecule 1
VD	Vitamin D
VDR	Vitamin D Receptor
VDRE	Vitamin D response element
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization

ABSTRACT

Cancer is a large group of diseases characterised by the rapid proliferation of abnormal cells. This disease group contributes to global mortality, with prostate cancer (CaP) and hepatocellular carcinoma (HCC) at the forefront. The liver is one of the sites of synchronous distant metastases where metastatic prostate cancer spreads to. Incidentally, the increased prevalence of vitamin D (VD) deficiency is also a global public health challenge. VD is a lipid-soluble vitamin known for primary roles in skeletal mineralisation. However, it is crucial to understand VD's role in carcinogenesis. This study examined VD metabolism in prostate cancer (CaP) and hepatocellular carcinoma (HCC). Bioinformatics techniques were used to evaluate dysregulated genomic networks in CaP and HCC. The expression of prostate cancer-related transcriptional factors was analysed with immunohistochemistry techniques. Also, *in silico* antioxidant and anti-inflammatory potentials of VD were evaluated using molecular docking. In the HCC *in vivo* study, rats were divided into four experimental groups. Groups one and two were administered 30 mg/kg diethylnitrosamine (DEN) for eleven weeks, with groups three and four receiving normal saline. Before DEN administration, endogenous VD was depleted. Additionally, groups one and three received VD-deficient diet, while groups two and four took VD diet. Using enzyme-linked immunosorbent assay (ELISA), various inflammatory cytokines and cancer biomarkers were evaluated, while quantification of antioxidant parameters and lipids were carried out using spectrophotometric methods. Findings from this study showed synergistic network of events between circadian rhythm (CR), inflammation, oxidative stress, and VD metabolism. In CaP and HCC, VD metabolic gene disruption resulted in significant ($p < 0.05$) alteration of CR genes. Also, significant ($p < 0.05$) correlations between the disrupted VD metabolic genes and CR genes, inflammatory, and oxidative stress genes were observed. Meanwhile, racial differences in the expression and correlations of CR gene networks were observed in CaP. Results from CaP studies showed significant ($p < 0.05$) differential expression of VD and CR genes in African Americans (AA) in comparison to European Americans (EA), which could account for more aggressive subtypes in AA. *In silico* studies showed varying types of VD are strong antioxidant and anti-inflammatory agents *via* respective binding to KEAP1, Interleukin 1 (IL-1β), and Tumour necrosis factor (TNF-α). Following the *in silico* analysis, *in vivo* rat experimental results also showed ameliorative effects of VD in oxidative stress and inflammation. In the rats, dietary VD significantly ($p < 0.05$) reduce oxidative stress through increased antioxidant enzyme activities, including glutathione S-transferase and nitric oxide. Furthermore, inflammatory effects were reduced with the inclusion of the VD diet. Increased IL-1β and TNF-α production observed in VD deficient group was systematically reduced ($p < 0.05$) with dietary VD. In line with other results from this study, histopathological examinations indicate dietary VD could prevent cancer progression at the inflammation stage. Therefore, VD deficiency as a part activates and triggers cancer deterioration through alteration of non-classical pathways. In conclusion, increased vitamin D uptake in deficient cases could play integral roles in mitigating cancer progression hence a possible cancer preventive regime.

Keywords: Vitamin D, hepatocellular carcinoma, circadian rhythm, inflammation, oxidative stress