

**BIOCHEMICAL AND GENE EXPRESSION STUDIES ON UTERINE  
FIBROID PATIENTS IN IBADAN, OYO STATE, NIGERIA**

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(18PCP01851)**

**OCTOBER, 2022**

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FIBROID PATIENTS IN IBADAN, OYO STATE, NIGERIA**

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COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT  
UNIVERSITY, OTA, OGUN STATE, NIGERIA**

**OCTOBER, 2022**

## **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 in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota.

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## **DECLARATION**

**I, OKESOLA, MARY ABIOLA (18PCP01851)**, declare that I carried out this research under the supervision of Prof. Israel S. Afolabi and Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria and Dr. Folasade A. Bello of the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan. 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.

**OKESOLA, MARY ABIOLA**

**Signature and Date**

## **CERTIFICATION**

We certify that this thesis, titled "**BIOCHEMICAL AND GENE EXPRESSION STUDIES OF UTERINE FIBROID PATIENTS IN IBADAN, OYO STATE, NIGERIA**", is an original research work carried out by **OKESOLA, MARY ABIOLA (18PCP01851)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Israel S. Afolabi, Prof. Olubanke O. Ogunlana, and Dr. Folasade A. Bello. We have examined and found this work acceptable as part of the requirements for the award of a Doctor of Philosophy (Ph.D) degree in Biochemistry.

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## **DEDICATION**

I dedicate this project to God Almighty, my help in ages past. He has been the source of inspiration throughout this programme. On His wings only, I am soaring.

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## LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ACP	Acid Phosphatase
ACT	Actin
BHD	Birt-Hogg Double syndrome
BMI	Body Mass Index
BSA	Bovine Serum Albumin
C <sub>4</sub> H <sub>11</sub> NO <sub>3</sub>	Tris (hydroxymethyl) aminomethane
Ca <sup>2+</sup>	Calcium
CAT	Catalase
CBC	Complete Blood Count
cDNA	Complementary deoxyribonucleic acid
CDNB	Chloro-dinitrobenzine
Cox-1 &-2	Cyclooxygenase-1 & -2
CHREC	Covenant Health Research and Ethics Committee
DTNB	Di-thio-nitrobenzol
ECM	Extra Cellular Matrix
EDTA	Ethylene-Diamine Tetraacetic Acid
EGCG	Epigallocatechin Gallate
EGF	Epidermal Growth Factors
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Estrogen Receptor
FDA	Food and Drug Administration
FH	Fumarase Hydratase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GnRH <sub>a</sub>	Gonadotropin-releasing hormone agonists
GP <sub>x</sub>	Glutathione Peroxidase
GR	Glutathione Reductase
GRAN	Granulocytes
GSH	Reduced Glutathione
GSP	Gene Specific Primer
GST	Glutathione-S-Transferase
Hb	Hemoglobin

HMGA2	High Motility Group AT Hook -2
IGF	Insulin Growth Factor
ILs	Interleukins
K <sup>+</sup>	Potassium
KOH	Potassium Hydroxide
LYM	Lymphocytes
MeCN	Acetonitrile
MED 1 2	Mediator Complex Subunit 12
MeOH	Methanol
Mg <sup>2+</sup>	Magnesium
MRgFUS	Magnetic Resonance Guided Focused Ultrasound
Na <sup>+</sup>	Sodium
NADPH <sup>+</sup>	Nicotinamide Adenine Dinucleotide Phosphate
NIEHS	National Institute of Environmental Health Sciences
PBS	Phosphate Buffer Saline
PCA	Perchloric Acid
PLT	Platelets
PR	Progesterone Receptor
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RQ	Research Questions
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SOD	Superoxide Dismutase
TBA	Total Bile Acid
TBARs	Thiobarbituric Acid Reactive Substances
TCA	Trichloro-Acetate
TCEP	1- octanol and tris (2-carboxyethyl) phosphine hydrochloride
TGF- $\alpha$	Transforming Growth Factors Alpha
TGF- $\beta$	Transforming Growth Factor- $\beta$
TNF- $\alpha$	Tumor Necrosis Factor-Alpha
TSC-2	Tuberous Sclerosis Complex-2
UAE	Uterine Artery Embolization
UCH	University College Hospital
UF	Uterine Fibroid



UPA	Uplistrial Acetate
VDR	Vitamin D Receptor
VDREs	Vitamin D Response Elements
VEGF	Vascular Endothelial Growth Factor

## ABSTRACT

Uterine fibroids (UF) are benign, non-carcinomatous growths that reside in the smooth layer of the uterus and cause many gynaecological problems among reproductive-age women worldwide. Their occurrence infringes on women's biological, sociological, physiological status and quality of lifestyle with an added financial burden, especially in black women. The actual cause of the phenomenon remains undiagnosed despite numerous studies, thereby prompting further investigation on its relationship to some biochemical and molecular markers of cellular growth and development. One hundred and ninety (190) reproductive-aged women (95 each for UF patients and age-matched non-UF participants) were recruited for the study. The experimental design was divided into two phases - Case-control model, and Case-control age-related model [16-25 years (group A), 26-35 years (group B), 36-45 years (group C), 46-55 years (group D) and 56 & above (group E)]. A portion of 5g each of the excised fibroid tissues and normal tissue samples from the adjacent myometrium were collected and sectioned into three parts. The first part was preserved using RNAlater® and kept at -80° C for molecular analysis. The second portion was preserved with 10 % formalin solution for histological examination, while the last portion was rinsed with normal saline and frozen for biochemical analysis. The blood samples (10mL) were also collected; a 2mL portion was used for haematological analysis and the remaining 8mL for biochemical tests. Vitamins A, D, and K concentrations, the female reproductive hormones, and inflammatory markers (oestrogen, progesterone, IL-1, IL-2, CoX-1, CoX-2, and TNF- $\alpha$  respectively) were assessed using Enzyme-linked immunosorbent assay kits. Concentrations of mineral elements in UF and non-UF were examined using atomic absorption spectrophotometry. The supernatant obtained from tissues and serum was utilised to assess the antioxidant status, and vitamins E and C concentrations were investigated spectrophotometrically. Age, reproductive hormones, antioxidant enzymes and micronutrients were identified as contributing factors promoting the incidence or occurrence of the UF. The micronutrient examination in the phase one design model revealed that selenium of all the examined mineral elements and vitamins A, D, and E were significantly reduced while Vitamin K increased significantly in UF cases at ( $p < 0.05$ ) promoting the occurrence of tumour. There was a significant increase ( $p < 0.05$ ) in the level of vitamin K, sodium, magnesium, iron, zinc and IL-2 in group C; Ca in group E, progesterone in groups B and C, reduction of progesterone in group D, estrogen, and cholesterol in group D; the activities of glutathione-s-transferase and over expression of transforming growth factor gene in the fibroid tissues. Conversely, there was a significant reduction of ( $P < 0.05$ ) in the levels of reduced glutathione in groups B, C and E, and SOD and selenium in groups E and D respectively. Similarly, there was a significant ( $P < 0.05$ ) decrease in the levels of vitamin A in groups B and C, vitamin D in groups D, vitamin E in groups D and E, sodium and cholesterol in group B in UF cases. The UF incidence or occurrence were associated with the nutritional influence of vitamin E, A = D, and selenium deficiencies in decreasing order, excess vitamin K intake and with overexpression of TGF- $\beta$ . Thus, biochemical and molecular processes related to dietary lifestyle are strongly associated factors facilitating the occurrence of UF in most Black women.

**Keywords:** *Antioxidant, ELISA Kits, Gene expression, Inflammation, Uterine fibroids, Vitamins.*