EVALUATION OF ANTIWOLBACHIAL TREATMENT IN PATHOGENESIS OF LYMPHEDEMA DEVELOPMENT

A THESIS

PRESENTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY, SCHOOL OF MEDICAL SCIENCES, COLLEGE OF HEALTH SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI, IN PARTIAL FULLFILLMENT OF THE REQUIREMENT FOR THE AWARD OF

M'PHIL. CLINICAL MICROBIOLOGY

BY

ALEXANDER KWARTENG (BSC. HONS)

DEPARTMENT OF CLINICAL MICROBIOLOGY

MAY, 2010

DECLARATION

I hereby declare that this submission is my own work toward the award of an MPhil degree in Clinical Microbiology and that to the best of my knowledge, it contains no material previously published by another person nor material which had been accepted for the award of any other degree of the university, except where due acknowledgement had been made in the text.

ALEXANDER KWARTENG	……..	……...
	Signature	Date
Prof Ohene Adjei		
	……..	……...
(SUPERVISOR)	Signature	Date
Prof. Adu Sarkodie	…….	……
(HEAD OF DEPARTMENT)	Signature	Date

DEDICATION

This thesis is dedicated to Madam Abena Badu, Ms. Sarah Kwarteng not forgetting my dear friend Ms. Evelyn Efiba Vidda.

ACKNOWLEDGEMENTS

But thanks be to God who gives us victory through our Lord Jesus Christ. Therefore, my brethren, be steadfast, immovable, always abounding in the work of the Lord, knowing that your labor is not in vain in the Lord.(1st Corinthians 15:57-58)

This thesis would not have been a success without the advice of Prof. Ohene Adjei my supervisor, Prof Adu Sarkodie, the Head of the Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science Technology (KNUST) and Thomas van Kampen, Director of Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR). Big thanks goes to Prof. Dr. Achim Hoerauf, Dr. Sabine Mand, Dr. Sabine Specht, Dr. Susane Denninger all of the Department of Immunology, Microbiology,and Parasitology at the University of Bonn, Germany as well as Dr. Peter Konadu of the Komfo Anokye Teaching Hospital (KATH).

To Dr. Alex Yaw Debrah and Mrs. Linda Debrah of KCCR, I want to say thank you for your unquantifiable support during my period of study at KCCR/SMS on the filariasis projects. My appreciation also goes to the following supporting staff and students Mr. Yebaoh Marfo Debrekyei, Mr. Henry Hanson, Mr. Yusif Mubarak, Mr. Jubin Osei- Mensah, Mr. Kenneth Otabil-Bentum, Ms. Lilian Duku, at KCCR, project drivers especially Mr. Paul Marfo Bekyie, Mr. Seth Wiredu, Mr. Philip Frimpong, Mr. Emmanuel Laare, the cook Miss. Ruth Boateng as well as all village the health workers and the Health Authorities in the Nzema East and Ahanta West districts of the Western Region

My deepest appreciation goes to my father, the late Mr. Yaw Kwarteng and my dear mother Madam Abena Badu for their parental support and care. I also want to thank Mr. Yarhands Dissour Arthur, Mr. Alex Aboagye, Mr. Kenneth Fletcher and Miss Mercy Ayittey who played diverse role in achieving this success, I am grateful to you all.

ABSTRACT

Globally, filarial LE affects more than 16 million individuals. Registered antifilarial drugs do little to mitigate the pathology. Currently, there is no definite drug for treating subjects who develop the pathology because the Global Programme to Eliminate Lymphatic Filariasis (GPELF) relies only on hygiene management practices as the only source of relieve for this group. Antiwolbachial therapy is therefore believed to be the most promising approach for treating lymphedema. To elucidate the efficacy of anti-wolbachial treatment with antibiotics in lymphedema, 180 individuals were recruited from 25 endemic communities of the Nzema East and Ahanta West Districts of the Western Region of Ghana for a double blind placebo-controlled trial. In all, 119 patients were stratified according to circulating filarial antigen (CFA) status, randomized to receive 200mg/d of doxycycline (n=46), 1000mg/d of amoxicillin (n=36) and placebo (n=38) for 42days in a daily observed treatment. Although minimal significant improvements were seen for almost all parameters measured in the CFA-positive treated with doxycycline, there were remarkable improvement in the CFA-negative doxycycline-treated patients particularly in the area of decreased mossy lesions, healed sores, reduced knobs, regressed leg stage, decreased ultrasound measurements ($p=0.0001$), reduced filarial acute attacks, halt of disease progression, significant reduction in antigenaemia levels ($p=<0.00$). In the majority of the patients who received 6 weeks doxycycline treatment, there was a highly significant improvement (43.9%) in the leg stage at the end of the study. Although there was halt of disease progression (61.9%) as well as decreased filarial attacks in the amoxicillin treated group, there was no significant improvement in the amoxicillin as well as the placebo-treated patients regarding all other parameters assessed. The study suggests that doxycycline as the first therapy for treating lymphedema and recommends its use as individual drug administration.

Table of content

List of content	page
Title	i
Declaration	ii
Dedication	iii
Acknowledgement	iv
Abstract	v
List of content	vi
List of tables	xii
List of figures	xiii
List of plates	xiv
List of abbreviation	xv

CHAPTER ONE

1.0 BACKGROUND 1

1.1 General Overview of Lymphatic Filariasis 2

CHAPTER TWO

2.0 LITERATURE REVIEW 7

2.1 THE PARASITES AND VECTORS TRANSMISSION 7

2.1.1 Life Cycle of the Parasite 7

2.2 THE SPECTRUM OF CLINICAL MANIFESTATIONS 9

2.2.1 Filarial Attacks 9

2.2.1.1 Acute Attacks 9

2.2.1.2 Acute Filarial Lymphangitis (AFL) 10

2.2.1.3 Acute dermatolymphangioandenitis (ADLA) 10

2.2.1.4 Lymphedema and Elephantiasis 10

2.3 MECHANISM OF PATHOGENESIS IN LYMPHEDEMA 12

2.3.1 Economic and social burden of lymphedema 13

2.4 CHEMOTHERAPY 14

2.4.1 Ivermectin 14

2.4.2 Mechanism of action, pharmacokinetics, safety and efficacy of ivermectin 14

2.4.3 Diethylcarbamazine 15

2.4.4 Mechanism of action, pharmacokinetics, safety and efficacy of DEC 15

2.4.5 Albendazole 16

2.4.6 Mechanism of action, pharmacokinetics, safety and efficacy of albendazole 16

2.4.7 Lymphedema and antibiotics- tetracycline in focus 17

2.4.8 Absorption and extraction of tetracycline 17

2.4.9 Contraindications of tetracyclines 18

2.4.10 Anti-wolbachial treatment in lymphatic filariasis-lymphedema 18

2.4.11 The role of *Wolbachia* in the development of lymphedema 19

2.5 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGFS) AND LYMPHEDEMA DEVELOPMENT 21

CHAPTER THREE

3.0 MATERIALS AND METHODS 22

3.1 Study area 22

3.2 Ethical clearance 24

3.3 Selection of villages 24

3.4 Selection of Lymphedema Patients 25

3.4.1 Exclusion and inclusion criteria 25

3.5 Clinical chemistry test 25

3.6 Determination of circulating filarial antigens 26

3.7 Assessment of microfilaraemia in lymphedema 26

3.8 Limb measurement of lymphedema patients 27

3.9 Staging lymphedema legs 28

3.10 Ultrasound examination 30

3.11 Treatment regimen 30

3.12 Follow -up examinations of patients 30

3.13 Statistical Analysis 30

CHAPTER FOUR

4.0 RESULTS 32

4.1 Adherence to treatment, stratification and drop–out 32

4.2 Adverse effects of doxycycline, amoxicillin and placebo treatment 34

4.3 Microfilaraemia and Antigenaemia values of *Wuchereria bancrofti* from start of treatment 35

4.4 Lymphedema staging- sum of both legs 38

4.4.1 Assessment of lymphedema staging-improvement, halt of progression, deterioration 24 months after treatment 42

4.5 Circumference-measurement of the lymphedema legs 43

4.5.1 Effects of treatment of on mean leg circumference measurement-sum of both legs (10-30cm) comparing pretreatment versus study time points 44

4.6 Malleolus lateralis and medialis measurements 45

4.6.1 Effects of treatment on malleolus measurement at various time points 46

4.6.2 Efficacy of treatment on *Malleolus lateralis*sum of both legs split by CFA status at study time points 47

4.6.3 Efficacy of treatment on*Malleolus medialis*- sum of both legs according to CFA status at study time points 49

4.7 Incidence of acute attacks during the study period 50

CHAPTER FIVE

5.0 DISCUSSION 52

5.1 Anti-wolbachial treatment in lymphedema 52

5.2 Microfilariae and Antigenaemia 55

5.2.1 Effect of treatment on microfilariae and antigenaemia levels 55

5.3 Lymphedema staging 57

5.3.1 Effect of treatment on legs staging 57

5.4 Effects of treatment on leg measurements 62

5.5 Effects of treatment on *Malleolus* split by CFA status 63

5.6 Incidence of acute attacks during study period 65

5.7 Assessment of lymphedema staging–improvement, halts of progression,

progression at 24 months after treatment 67

CHAPETR 6

6.0 CONCLUSIONS 70

6.1 RECOMMENDATIONS 72

REFERENCES 73

APPENDIX I 92

APPENDIX II 93

LIST OF TABLES

Table **Page**

Table 1.0: Demographic data and stratifications of various treatment groups 35

Table 2.0: Microfilaraemia and antigenaemia levels of *W. bancrofti* at study time points 37

Table 3.0: The mean and median of lymphedema staging of sum of both legs 40

Table 4.0: Difference within treatment groups of LE staging at various time points 41

Table 5.0: Performance of treatment on improvement, halt of progression and progression at 24 months 42

Table 6.0: Circumference measurement of sum of both legs 10-30cm 44

Table 7.0: *Malleolus* measurement of both legs in treatment arms at various time points 46

Table 8.0: The Geometric mean of the sum of *Malleolus lateralis* of both legs staging stratified CFA status in at various time points 47

Table 9.0: The Geometric mean of the sum of *Malleolus medialis* of both legs stratified by CFA status at various time points 49

Table 10.0: Incidence of acute attacks during the study period 50

LIST OF FIGURES

Figure		Page
Fig.1.0	Diagram of the life cycle of the parasite	8
Fig.2.0	Flowchart of the LE patient participation	33
Fig. 3.0	Circumferential measurements at various positions of the leg	43
Fig. 4.0	Diagram of the human leg showing *Malleolus lateralis* and *medialis*	45

LIST OF PLATES

Plate		Page
Plate 1.0	The onset of folds development in a lymphedema patient	11
Plate 2.0	Map of study area	23
Plate 3.0	Measuring a lymphedema patient's leg	27
Plate 4.0	Stages of lymphedema legs	28,29

List of Abbreviations

LF	Lymphatic Filariasis
LE	Lymphedema
CFA	Circulatiing Filaria Antigen
USG	Ultrasonography
MDA	Mass Drug Administration
GPELF	Global Programme to Eliminate Lymphatic Filariasis
AFL	Acute Filarial Lymphangitis
ADLA	Acute dermatolymphangioandenitis
DEC	Diethylcarbamazine
IVM	Ivermectin
ALB	Albendazole
VEGF	Vascular Endothelial Growth Factors
LEC	Lymphatic Endothelial Cells
GPT	Glutamate-pyruvate transaminase
GGT	Gamma-Glutamate transpeptides
CREA	Creatinine
ELISA	Enzyme link immunosorbent assay
DOT	Direct Observed Treatment
Mf	Microfilaria
MMP	Matrix Metalloproteinase