THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF ANATOMY

THE COLOUR OF THE IRIS IN PATIENTS PRESENTING AT THE OPHTHALMOLOGY UNIT UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

BY

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A RESEARCH STUDY SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN HUMAN ANATOMY AT THE UNIVERSITY OF ZAMBIA

The University of Zambia
School of Medicine
Lusaka

NOVEMBER, 2015

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I, Professor Erzingatsian Krikor, having supervised and read this dissertation is satisfied that this is the original work of the author under whose name it is being presented. I confirm that the work has been completed satisfactorily and is ready for presentation to the examiners.

Signature……. **Date**……

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Department of Anatomy, School of Medicine, University of Zambia.

CERTIFICATE OF APPROVAL

This dissertation of Mulipilwa Martin has been approved in partial fulfillment of the requirements for the award of the Degree of Master of Science in Human Anatomy by the University of Zambia.

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Examiner II

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Examiner III

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ABSTRACT

Background

The iris is the coloured part around the black pupil. It is a flat structure and each iris is unique in its colour, patterns and texture. Its colour varies in different individuals, ethnic groups and is largely affected by geographical factors. The colour of the iris in the Zambian population is not known.

Aims and objectives

The study aimed at determining the Colour of the Iris in patients presenting to the Ophthalmology unit University Teaching Hospital (UTH), Lusaka, Zambia.

Materials and Methods

This was a cross sectional analytical study, done in Ophthalmology unit at UTH, from June to August, 2014. A total of 384 randomly sampled patients were physically examined for the colour of the Iris using the Martin-Schultz scale.

Results

Out of 384 participants, 372 had brown eyes representing 96.9% and 12 had black eyes representing 3.1%. The sex distribution among the 372 with brown eyes were 189 males and 183 females. Moreover, among the 12 participants with black eyes, 8 were females while 4 were males. It was also observed that refractive errors in 181 (47.1%) were the most common conditions in patients presenting at the UTH eye clinic, followed by cataract 77 (20%); diseases affecting the cornea and conjunctiva 67 (17.4 %) and glaucoma 59 (15.4%) respectively.

Conclusion

It was established that the most common eye colour in the Zambian population is Brown 272/384 (96.9%) followed by black 12/384 (3.1%). The most common eye conditions patients present with at the eye clinic were refractive errors 181 (47.1%); cataract 77 (20.0%), glaucoma 59 (15.4%) and diseases affecting the cornea and conjunctiva 67 (17.4%) which included uveitis, conjunctivitis, Trachoma and age related macular degeneration.

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Keywords: Colour of the Iris, melanin, melanocyte

DEDICATION

To the people with Ocular pathologies.

My Mother Rhodah Ndhlovu who has wholly supported me and fought for my education.

ACKNOWLEDGEMENTS

My heartfelt gratitude goes to my research supervisors, Prof. Erzingatsian Krikor and Dr Grace Mutati whose diligence and unwavering guidance saw me through the research process.

My sincere thanks also go to the following:-

- The University of Zambia through the Staff Development Office, for sponsoring my studies.
- The Dean, School of Medicine; Assistant Dean, Postgraduate; Departments of Human Anatomy and Biomedical Sciences for their encouragement and support throughout my period of study.
- The Managing Director, University Teaching Hospital; the Head, Ophthalmology unit; the Medical Doctors, Nurses and Support staff at the Ophthalmology unit University Teaching Hospital.
- My family, for their continued support, understanding and encouragement throughout my period of study.
- My colleague, Mutemwa Sikhanyiso for the encouragement, moral support and academic sharing which I needed.
- The participants for their cooperation as without them this study would have been impossible.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF ACRONYMS

AMD : Age related Macular Degeneration.

ERES : Excellence in Research and Ethics and Science

IBM : International Business Machines

NY : New York

SPSS : Statistical Packages for Social Sciences

USA : United States of America

UTH : University Teaching Hospital

DEFINITION OF OPERATIONAL TERMS

Iris - A coloured part of the eye with a central opening called a pupil**.**

Melanin - Pigment synthesised by melanocytes responsible for the colour of the iris, skin and hair.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background

The eye's **iris** and **pupil** are the most visible or obvious eye structures that people see immediately when they take a closer look at an individual's eye. The iris is the coloured part around the black pupil. It is a flat structure and each iris is unique in its colour, patterns and texture. The two irises (Irides) can identify an individual as definitely as the fingerprints do (Anderson *et al,* 1998).

The study of the iris is known as Iridology. Iridology is the scientific analysis of the iris (the coloured part of the eye) that studies the colour, the structure and markings. The eye is one of the most complex tissue structures in the human Anatomy. The state of the eye can be an indicator of our relative state of health and the iris can reveal the level of hereditary deficiencies and the general state of our health. The iris reflexes could be a harbinger of diseases in other parts of the body. The iris also displays the activity within our organs, glands and tissues according to the way we live our lives (Sturm and Larsson, 2009).

Iridology provides an accurate analysis of structure and pigmentation and provides vital information that is not always available through other methods. Furthermore, Iridology concerns itself with the integrity of the body's tissues and can determine under or over activity in various organs of the body. (Catherine 2003).

The stromal melanocytes of the iris that contain melanin are believed to be the principal factor in determining iris colour (Albert et al, 2003). However, controversy has long existed as to whether the number of melanocytes, distribution of melanocytes, or the melanin content of individual melanocytes is the determining factor that varies with iris colour, or whether these factors are complementary. Eagle RC Jr, 1988).

The Eye colour is due to the colour of the iris, which can be green, blue, brown or heterochromatic. In some cases it can be hazel (a combination of light brown, green and gold) or grey. Eye colour is determined by the type of melanin present, the density and distribution of melanosomes located within the melanocytes of the iris stroma (Sturm and Larsson, 2009). However, there is a relationship between the colour of the eye; the skin and the hair as the three structure's entire colour depends on the amount and distribution of the melanin contained in them (Anderson J.L *et al,* 1998. In response to the amount of light entering the

eye, muscles attached to the iris dilate or constrict the aperture at the centre of the iris, known as the pupil. Therefore the larger the pupil, the more the light can enter the eye. There is a relationship between different eye diseases and the colour of the iris as most eye diseases affect the blood supply to the eyes and affect the intra ocular pressure. Studies done in Europe showed that diseases like Diabetes mellitus, Age related macular degeneration affect the eye colour. This study was aimed at establishing the colour of the iris and the relationship between several eye diseases presenting to the eye clinic at UTH. Such information in Zambia is lacking at the moment.

1.2 Anatomy of the iris of the Eye.

The Eye is composed of three concentric layers namely: (1) a tough external fibrous layer consisting of the sclera and transparent cornea, (2) a middle vascular layer that includes the choroid, the ciliary body and the Iris and (3) an inner sensory layer, the retina which communicates with the cerebrum through the posterior optic nerve. (Anthony L, 2013)

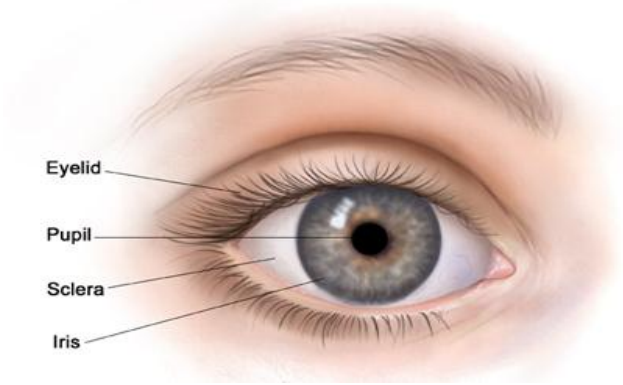
The **iris**, a component of the uveal tract partially covers the lens, leaving a round opening in the center called the **pupil.** It is a thin, contractile, pigmented diaphragm suspended in the aqueous humor between the cornea and the lens. The periphery of the iris is attached to the anterior surface of the ciliary body. It divides the space between the lens and the cornea into an anterior and a posterior chamber. (Snell R.S, 2008).

The anterior surface of the iris, exposed to the anterior chamber, is not covered by epithelium. However, it consists of an irregular, discontinuous layer of fibroblasts and melanocytes, densely packed and with interdigitating processes (Anthony L, 2013). Deeper in the iris the stroma is more typical loose connective tissue with microvasculature. The posterior surface of the iris is smooth, with a two-layered epithelium continuous with that covering the ciliary body and its processes. However, the epithelial cells in direct contact with the posterior chamber are filled with melanin granules, which obscure most cellular features. The highly pigmented epithelium of the iris prevents light from entering the interior of the eye except through the pupil. The underlying epithelial layer is composed of myoepithelial cells which are also at least partially pigmented. Radially extended processes from these myoepithelial cells make up the very thin dilator pupillae muscle along the posterior side of the iris. The abundant melanocytes in the vascular layer of the eye act collectively to keep stray light rays from interfering with image formation. Melanocytes of the iris stroma contain melanin which

provide the colour of the eyes. In individuals with very few lightly pigmented cells in the stroma, light with a blue colour is reflected back from the black pigmented epithelium on the posterior iris surface. As the number of melanocytes and amount of collagen increases in the stroma, the iris colour changes through various shades of green, gray, and brown (Anthony L, 2013). Individuals with albinism have almost no pigment and the pink colour of their irises is due to the reflection of incident light from the blood vessels of the stroma. (Anthony L, 2013) The muscle fibers of the iris are involuntary and consist of circular and radiating fibers. The circular fibers form the sphincter pupillae and are arranged around the margin of the pupil. The radial fibers form the dilator pupillae and consist of a thin sheet of radial fibers that lie close to the posterior surface.

- **Arterial supply and venous drainage:** The iris receives blood from the ophthalmic artery via the long posterior ciliary arteries and anterior ciliary arteries. The venous drainage from the iris is directed posteriorly into the choroid and thence into the vena vorticosa.
- **Innervation:** The sphincter pupillae is supplied by parasympathetic fibers from the oculomotor nerve. After synapsing in the ciliary ganglion, the postganglionic fibers pass forward to the eyeball in the short ciliary nerves. The dilator pupillae is supplied by sympathetic fibers, which pass forward to the eyeball in the long ciliary nerves.
- **Action**: The sphincter pupillae constricts the pupil in the presence of bright light and during accommodation. The dilator pupillae dilates the pupil in the presence of light of low intensity or in the presence of excessive sympathetic activity such as occurs in fright. (Snell R.S, 2008)

Figure 1: Surface Anatomy of the eye



Acknowledgement: Terese Winslow, 2007.

1.3 Statement of the problem

There are several ocular pathologies presented at the University Teaching Ophthalmology unit. Some of which affect the intra-ocular pressure and affect blood supply to the eyes. Therefore such diseases affect the normal colour of the iris and can also affect the clinical diagnosis of the eye diseases. Most diseases affecting the eyes and the impact they have on the colour of the iris have not been studied in the Zambian population hence posing difficulties in proper clinical diagnosis of the eye. Diabetes mellitus and age related macular disease and their effects on the colour of the eyes in Zambian patients are not known despite being common in eye patients presenting at the University Teaching Hospital.

1.4 Rationale for the study

The colour of the iris of the eye in Zambia and its distribution is not well established. Several diseases that affect the eyes have an impact on the colour of the iris and such information is lacking in Zambia at present. This study established the colour of the iris in patients that present to the eye clinic at UTH and the association between the eye conditions and the colour of the eyes. It also established the common eye diseases that patients present with at Ophthalmology unit at UTH.

1.5 Research question

What is the colour of the iris in the patients presenting to the Ophthalmology Unit of the University Teaching Hospital in Lusaka, Zambia?

1.6 Objectives

1.6.1 General Objectives

The aim of this research project was to establish the distribution of the colour of the iris in patients attending the eye clinic at the Ophthalmology Unit of the University Teaching Hospital Lusaka, Zambia.

1.6.2 Specific objectives

1. To determine the colour of the Iris in patients attending the eye clinic at University Teaching Hospital.
2. To find out the most common eye diseases presenting to the eye clinic at the University Teaching Hospital.

CHAPTER TWO
LITERATURE REVIEW

Iris pigmentation exhibits a variable global distribution. In most populations, eye colour is primarily limited to varying shades of brown. However, individuals of European, and to a lesser extent, North African, Middle Eastern, Central Asian, and South Asian ancestry, express a wide range of colours that include shades of brown, green, and blue. A similar study done in the Danish population revealed that the dominant eye colour was blue with the 'fair' eye colour constituting the majority in both male and females (Anderson J.L *et al,* 1998) gender difference with more females having blue eye colour was also observed. In the study done by Fuchs 1913, the number of melanocytes in the anterior border layer was found to be the principal determinant in the colour of the iris, with darkly pigmented irides having greater numbers of melanocytes. In contrast to these findings, Wobmann and Fine 1972.observed that the number of melanocytes in the anterior border layer is relatively constant, irrespective of iris colour, but that the amount of pigment within the anterior border layer is greater in darkly pigmented irides. Eagle (1988), in his American Ophthalmological Society thesis, studied 21 irides, spanning the spectrum of iris colour by means of light and electron microscopy. He noted that melanocyte numbers remain relatively constant but melanosome numbers and size increase with darkening of the iris (Albert *et al*, 2003).

There are three cell types that contain pigment within the iris: a) stromal melanocytes, which are derived from the neural crests; b) iris pigment epithelium, which is derived from the neuroectoderm of the optic cup; c) and clump cells, which are thought primarily to be of histiocytic in origin (Albert *et al*, 2003). The epithelial pigmentation does not vary between different iris colours (Eagle 1988) and the clump cells are relatively few in number (Kahn *et al* 1983). Consequently, the various shades of iris colour have been mainly attributed to the variability in number and distribution of stromal melanocytes (Albert *et al*, 2003). Another study done by Wobmann and Fine in 1972 in which he studied the clump cells of Koganei using both light and electron microscope showed that melanocyte numbers, total cellularity, and percentage of melanocytes do not vary significantly among the various Caucasian iris colour groups and consequently are not major contributors to iris colour. However, Wolfrum 1922 established that iris colour is determined by the area and the number of melanosomes within the superficial iris stromal melanocytes and not the number of melanocytes.

In the last several decades, claims have been made to the effect that there is an association between iris colour and various ocular pathological conditions. These conditions include glaucoma, age-related macular degeneration and pigmented tumours of the uvea (Hiller *et al* 1982). Much attention has also been paid to geo-ethnic variations in ocular disease, and this has stimulated interest in their underlying aetiology. A study conducted by Caroline *et al* in 2003, titled "Iris colour, ethnic origin and progression of Age-related Macular Degeneration" (AMD), the colour of the iris in different ethnic groups was noted and a significant relationship observed between colour and AMD. Homocysteine, a sulfur-containing, non-proteinogenic amino acid, is an intermediate in methionine metabolism. Elevation of homocysteine, termed hyperhomocysteinemia, has been implicated in the pathogenesis of a variety of diseases including cardiovascular disorders (Wierzbicki AS, 2007). There is evidence that hyperhomocysteinemia may play a role in diseases of the visual system including maculopathy, open-angle glaucoma, and optic atrophy (Roedl JB, *et* al 2007). Severe hyperhomocysteinemia due to methionine synthase deficiency appears to decrease rod photoreceptor responses and induce retinal ganglion cell loss based on electrophysiological findings (Poloschek CM *et al* 2005). Given the potential relationship between hyperhomocysteinemia, vascular disease and neurodegeneration it is not surprising that clinical studies have examined also the relationship of hyperhomocysteinemia, diabetic retinopathy and eye colour. Diabetic retinopathy is a complex disease characterized by vascular dysfunction and neuronal cell loss. (Antonetti DA, *et al* 2005). There are reports in the clinical literature suggesting a link between excess homocysteine, colour of the iris and Diabetic retinopathy (Ndrepepa G, *et al* 2008. In the similar way, the colour of the iris in a Zambian population amongst different ethnic groups attending a Lusaka specialist ophthalmology hospital will be studied. This research will help to provide information about the distribution of iris colour in a population attending eye clinic at the University Teaching Hospital Lusaka Zambia and the relationship between the eye diseases and the colour of the iris of the eye. To the best knowledge of the researcher such a study has not been conducted in Zambia before.

CHAPTER THREE
3.0 METHODS AND MATERIALS

3.1 Research design

This was a hospital based cross sectional analytical study.

3.2 Study setting

The study was conducted from the Ophthalmology Unit of the department of Surgery at UTH, Lusaka, Zambia.

3.3 Study Period

The study was done for the period of one year from the time it was approved by the Ethics Committee in February 2014 to January 2015.

3.4 Study population

The study target population were randomly sampled from the patients attending the eye clinic from June 2014 to August 2014

3.5 Study Sample

The study sample for this research project was the target population who met the inclusion criteria.

3.5.1 Inclusion Criteria

In this research project, human subjects 18 years and older presenting at the Ophthalmology unit, UTH, Lusaka, Zambia were enrolled. This age was chosen because by Zambian law such individuals are considered adults who can make decisions on their own, in this case whether to take part in the study or not.

3.5.2 Exclusion criteria

Patients with skin, hair or eye colour deficiency such as in albinos were not considered in this research project.

3.5.3 Sample size calculation

Based on the facts that the distribution of the iris colour in Zambian population is not known, the prevalence of 50% was used to enrol a large number of participants. Therefore the formula below was used:-

$$N = \frac{Z^2 \times P(1-P)}{(E)^2}$$ where;

N= Sample required

Z= Z statistic at confidence=1.96, using 95 per cent Confidence Interval

P= 0.5, since expected prevalence is not known.

E= Confidence Interval=0.05, accuracy range (+/- 5 per cent)

Therefore, the calculated sample size= **384**.

3.6 Sampling Techniques

Non probability convenience sampling was used in this study.

3.7.0 Data Management

3.7.1. Instruments of Data Collection

The instruments used to collect data were:-

i. Torch
ii. Martin Schultz scale chart
iii. A data information sheet used to collect demographic data, clinical data, and the colour of the iris observed in a participant.
iv. Computer laptop for data analysis

3.7.2 Validity

All examiners underwent colour blindness testing by the Ishihara chart and the same examiners determined iris colour in all patients to mitigate interexaminer related variability in colour interpretation. To ensure validity, all the independent variables as well as the

confounders were considered in this study by capturing them in the interview schedule during data collection and data analysis.

3.7.3 Reliability

The same data information sheet and method of collecting data was used on all the participants. The same Martin-Schultz scale colour charts were used on all participants.

3.7.4 Procedure for data collection/Data Retrieval

Two Nurses were recruited as research assistants and oriented for two days on data collection using the interview schedule. However, the colour of the iris identification was done by the principle investigator with the help of the Ophthalmologist.

The research assistants and other staff at eye clinic were sensitised on the whole process of data collection so as to reduce the waiting time of the participants. This also ensured that normal standard of care of the participants was not compromised in any way

On arrival of the participants at eye clinic, the clerk sorted out the files of all the patients to be attended to by Ophthalmologists, the same patients were scrutinised for those who met the inclusion criteria by the researcher and research assistants. The demographic data was collected and those of interests were the sex and age of the participants, there after the colour of the iris was also observed and recorded and then the patient was screened by the Doctors at the clinic for their routine follow up and the conditions or disease of the patients that made the patient visit the clinic was also recorded either from what is indicated in the patients file or from the doctor's finding at that time..

The methods used in this research project was the physical examination of the iris to observe the colour using the light source i.e. the subject's eyes were subjected to the source of light from the torch and the observed colour of the iris of the subject was compared to the standard colours of the irides on the charts. This type of method of examining the colour of the iris is called the Martin-Schultz scale (D. A. Mackey *et al*, 2011) which comprises a chart with 16 different irides colours. The association between the eye disease and colour of the iris if any was also established by noting the colour of the iris of the eye against the disease suffered by the subject. To rule out issues of mistaking colours, the investigator was tested for colour blindness using the Ishihara colour test which is an example of a colour perception test for red-green colour deficiencies named after its designer Dr Shinobu Ishihara and eye specialists

from Ophthalmology unit were involved for verification of the colours found.

3.8. Data Analysis

Following data collection, the pre-coded data information sheet was double checked daily for completeness, consistency, legibility and accuracy. Numerical codes were used on the data collection sheet. The data collected was entered and stored into the data editor of IBM SPSS and statistically analyzed using IBM SPSS Statistics for Windows Version 16 .0 (IBM Corp. Armonk, NY, and USA). The software statistical package enabled the researcher to obtain a data set for the colour of the iris of the participants and the common eye pathologies presented to the eye clinic. It also allow to find the relationship between the colour of the iris and the disease of the participant

3.9. Detail of ethical considerations

3.9.1 Confidentiality

The participant was assured of confidentiality and that no names or any form of identification was to appear on the data information sheet. Moreover, each participant was assigned a unique confidential study number, which was used when collecting and reporting data.

3.9.2 Approval

The research project involved live human beings therefore approval was sourced from the ERES. Permission to conduct the study at UTH was obtained from the senior medical superintendent of UTH and the head of unit Ophthalmology unit. The participation by the recruits was voluntary and the discomforts expected to be experienced by the participant was only illumination of the light in the patients eye, though the type of light used did not have any negative effects on the participants as the intensity and type of light was regulated by qualified Ophthalmologists.

Written informed consent was obtained from the participants before the study. The researcher/research assistants introduced themselves and explained to participant the purpose and nature of the study.

CHAPTER FOUR
4.0 RESULTS

The research findings are presented in frequency tables, charts and contingency tables.

Table 4.1 Demographics characteristic of participants

Variable	Frequency	Percent
Age		
25 years and below	45	11.7
26 – 40 years	124	32.3
41 – 55 years	120	31.2
56 years and above	95	24.7
Total	384	100
Sex		
Male	193	50.3
Female	191	49.7
Total	384	100

In Table 1: 45 (11.7 %) of the participants were aged 25 years and below. 124 (32.3%) were aged between 26 – 40 years, 120 (31.2%) were aged between 41 – 55 years. 95 (24.7 %) were aged 56 years and above. The participants were aged between 18 and 78 years with193 being males and 191 females.

Table 4.2: The colour of the iris observed in participants

Sex	Colour		Total
	Brown	Black	
Male	189	4	193
Female	183	8	191
Total	372	12	384

In Table 2: Out of the 193 males, 189 (97.9%) had brown eyes while 4 (2.1 %) had black eyes. Out of the 191 females, 183 (95.8%) had brown eyes while 8 (4.1%) had black eyes.

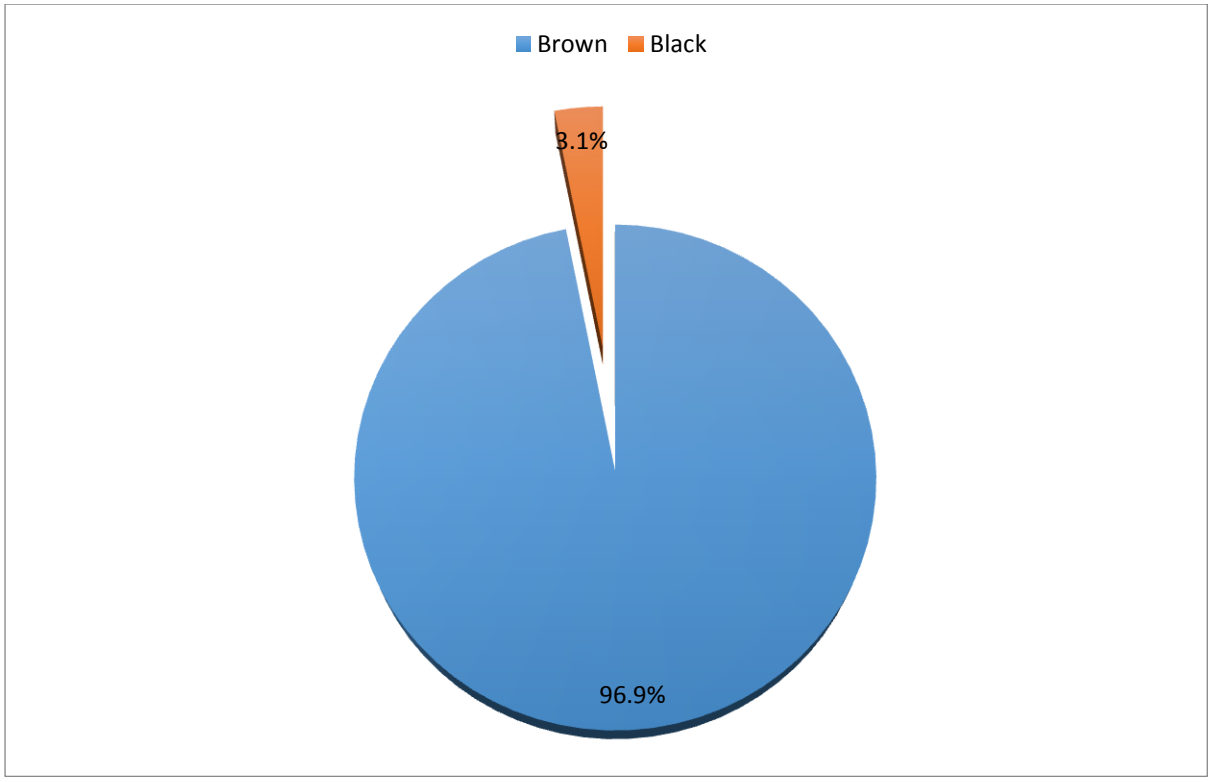


Figure 2: Pie chart presenting the colour of the iris observed in participants, 372 (96.9%) were brown and 12 (3.1%) were black.

Table 4.3: Eye pathologies of the participants

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Refractive errors	181	47.1	47.1	47.1
	Cataract	77	20.1	20.1	67.2
	Glaucoma	59	15.4	15.4	82.6
	Diseases affecting cornea and conjunctiva	67	17.4	17.4	100.0
	Total	384	100.0	100.0	

The Table above (3) shows the most common conditions` affecting patients presenting at eye clinic at UTH were Refractive errors 181 (47.1%), Cataract was 77 (20.1%) glaucoma was 59 (15.4%) and diseases affecting the cornea, conjunctiva or both were 67 (17.4 %.

Table 4.4: Pathologies affecting cornea, conjunctiva and Uveal tract 17.4 % of the entire sample studied.

	Frequency	Percent
Uveitis	26	**6.8**
Conjunctivitis	19	**4.9**
Age related macular degeneration	9	**2.3**
Trachoma	13	**3.4**
Total	67	

In table 4.4, the data for the diseases affecting the cornea and conjunctiva have been presented and majority was Uveitis comprising 26 (6.8 %) , conjunctivitis were 19 representing 4.9% .age related macular degeneration were 9 (2.3%) and trachoma were 13 (3.4%)

Table 4 5: Colour of the iris and the ocular pathologies of the participant.

	Refractive errors	Cataract	Glaucoma	Diseases affecting cornea and conjunctiva	Total
Brown	181	77	48	66	372
Black	0	0	11	1	12
Total	181	77	59	67	384

Table 4.6: The colour of the iris corresponding with the pathologies affecting the eye participants.

Colour of the Eye	Refractive errors	Cataract	glaucoma	Diseases affecting the cornea and conjunctiva	Total
BROWN					
Count	181	77	48	66	372
% within colour	48.7%	20.7%	12.9%	17.7%	100.0%
BLACK					
Count	0	0	11	1	12
% within colour	0.0%	0.0%	91.7%	8.3%	100.0%
TOTAL					
Count	181	77	59	67	384
% within colour	47.1%	20.1%	15.4%	17.4%	100%

Table 4.7: Statistical tests showing the association between the colour of the iris and the ocular pathologies

	Value	Degree of freedom	Asymptotic significance (2-sided)	Exact significance. (2-sided)	Point probability
Pearson chi-square	55.850	3	0.000	0.000	
Likelihood ratio	39.644	3	0.000	0.000	
Fishers exact test	34.474		0.000		
Linear-by-linear association	10.345	1	0.001	0.002	0.001
No of valid associations	384				

Three cells (37.5%) have expected count less than 5, so we used Fishers exact test to calculate the significance of the study. The minimum expected count is 1.84. The standard statistic is 3.216.

CHAPTER FIVE

Discussion

The present study determined the Colour of the Iris detected during physical examination in patients presenting to the Ophthalmology unit at the University Teaching Hospital. It also aimed to establishing the most common ocular pathologies patients present with at eye clinic. Lastly it determined the relationship between the colour of the iris and the common eye pathologies patient present with at the Ophthalmology unit.

In this study three hundred and eighty four (384) participants were recruited, 372 (96.9%) had brown eyes while 12 (3.1%) had black eyes. The sex distribution among the 372 participants with brown eyes was 189 males and 183 females. Out of the 12 participants with black eyes, 4 were males and 8 were females. The eye colour depends on the distribution of the melanosomes in the stromal cells of the iris and the type of melanin synthesised (either eumelanin or phoemelanin) by melanocytes (Anderson J.L *et al,* 1998). Excessive exposure to sunlight, as is the case in tropical countries such as Zambia stimulates melanocytes to synthesise more eumelanin which in turn will affect the colour seen in Zambians of black ancestry. As established by Wobmann and Fine in 1972, the colour of the iris depends on the amount of the melanin, type of melanin and its distribution. The brown colour of the eyes mostly observed in this study could be due to the eumelanin and its high concentration in the stromal cells of the iris thus offering protection against UV light which has a damaging effect on DNA. The literature reviewed in this study was from the studies done in Europe. We could not trace publications dealing with the subject from this part of the world thus a comparison was not possible. Nevertheless, the findings of this study are in contrast to that reported from Europe where a similar study done in the Danish population revealed that the dominant eye colour was blue (Anderson J.L *et al,* 1998). The findings in this study revealed that the commonest iris colour in the Zambian population is brown (96.9%) and a few individuals have black eyes (3.1%). The colour of the iris and its distribution in males and females was also noted in this study. Black eyes were observed to be twice the number in females compared to males - females 8/191 versus males 4/193. Similar differences were also noted by Anderson J.L *et al,* 1998) in his study in a Danish population where males and females did not have similar eye colour distribution, with more females having blue eye colour.

The study also established the common ocular pathologies which are seen at the clinic. The

results showed that most patients presenting to the eye clinic had refractive errors (181/384 (47.1%). These comprised of (near-sightedness), hyperopia (farsightedness), astigmatism (imperfectly shaped cornea or lens) and presbyopia (adult eye – difficulties to focus on near objects). Most of these refractive errors were observed in patients who had diabetes.

Cataract was observed in 77/384 representing 20% of the participants with most people affected being above 40 years while glaucoma was seen in 59/384 participants representing 15.4 %.

Pathologies affecting either the cornea, conjunctiva and uveal tract were studied together and were 67/384 representing 17.4%. These included uveitis 26 (6.8%), conjunctivitis 19 (4.9%), age rerated macular degeneration 9 (2.3%) and Trachoma 13 (3.4%).

The association between the colour of the iris and the eye pathology was also noted; it was observed that the colour of the iris had an association with the condition presented by the patients as most of those who had black eyes suffered from glaucoma with a Chi-square p-value of 0.000. The Literature supports this association. (Hiller R *et al,* (1982). None the less, in view of small number observed, this association could have been a chance finding. No association was observed in brown eyes as there was no statistical significance between the condition of the eyes and having brown eyes.

CHAPTER SIX
6.0. CONCLUSION AND RECOMMANDATIONS

6.1 Conclusion

1. This study established the colour of the iris in patients presenting to the eye clinic detected by physical examinations of the eyes and comparing them to the standard colours using the Martin-Schultz scale. It was observed that 272 (96.9%) had brown eyes while 12 (3.1%) had black eyes.

2. The most common ocular pathologies with which patients presented with at the eye clinic were refractive errors 181 (47.1%); cataract 77 (20.0%), glaucoma 59 (15.4%) and diseases affecting the cornea and conjunctiva 67 (17.4%) which included uveitis, conjunctivitis, Trachoma and age related macular degeneration. With reference to Glaucoma, in view of the small numbers observed, no valid conclusion can be reached about the association of the colour of the iris and Glaucoma.

6.2 Recommendations

1. A larger community based study should be done to determine the true colour of the iris in the Zambian population.
2. A Molecular based Research should be carried out to investigate the type and the amount of melanin contained in the melanocytes which is responsible for the iris colour.
3. Further studies should be undertaken to determine the content of melanosomes in the iris, skin and hair.

CHAPTER SEVEN

BUDGET

7.1 Budget for the study

No	Item	Quantity	Price
1	Colour charts	2	K 6000
2	Transport, accommodation and food	-	K 5000
3	Stationary	-	K 1000
4	Secretarial services		K 2000
5	Torch	1	K 200
5	Camera	1	K 6 000
	Total		**K 20,200**

CHAPTER EIGHT

TIME FRAME

8.1 Time Frame

Below is a timeline for the research project:

	July 2013	August 2013	February 2014		February 2014	February 2014	June 2014	June 2014	June 2014	January 2015	Feb - April 2015	May 2015	November 2015
Present to department	■												
Submit proposal to Asst Dean (PG) office		■											
Present at GPPF		■											
Submit proposal to REC			■										
REC review and approval			■	■	■	■							
Enroll patients and collect data							■	■	■				
Analyze data										■			
Write dissertation										■	■	■	
Submit final dissertation for Marking												■	
Submit bound copies to DGRS												■	■

CHAPTER NINE
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CHAPTER TEN

10.0APPENDICES

10.1 Martin-Schultz scale

10.2 Data collection sheet

1. Participants Details

First Name: ……

Surname: …….....

Sex: …….....

Age: ……..

Marital Status: ……..

2. Clinical Details

CLINICAL DIAGNOSIS	OUTCOME
Clinical findings	
Temperature	
Blood Pressure	
Others	

3. Clinical history

CLINICAL DIAGNOSIS	COMMENTS
Diabetes (type)	
Cataract	
Glaucoma	
Strabismus	
Amblyopia	
Refractive errors	
Colour blindness	

Ocular hypertension
Uveitis
Age related macula degeneration
Trachoma
Conjunctivitis

4. Eye colour confirmed by colour chart

EYE COLOUR: …….. COMMENT:

……

10.3. Information Sheet

Title: Colour of the Iris in Zambian Population.

Dear Participant,

This is to inform you that this study is being carried out by Mr Mulipilwa Martin D. a student with the University of Zambia, School of Medicine, Department of Anatomy. The study is going to be conducted from the department of Ophthalmology at the University Teaching Hospital. The Colour of the iris is responsible for the Colour of the Eyes and it has been associated with several diseases such as the Age related Macular Degeneration (Nerve damage). Glaucoma (high pressure in the eye) and childhood sugar disease (Diabetes Type 1) to mention but a few. Therefore, efforts should be made in establishing the colour of the iris in the Zambian population to serve as the baseline information for more research to be conducted to establish this relationship between the colour of the iris and diseases like Diabetes type 1 which has been reported to be on the increase in Zambia of late.

The Eyes of the participants are going to be examined by first observing the participants eyes using noninvasive methods which will be just looking into the eyes of the participants and recording the observed colour of the eyes. Secondly the photographs of the eyes are going to be taken so that the colour of the iris of the eye can be established. This process is going to be taking approximately about 2 - 5 minutes depending on the cooperation of the participant. All this is going to be done by the principle investigator Mr Martin Mulipilwa with the assistance of the presence of the Ophthalmologist on duty. There may be some physical discomfort when examining the eyes; however you should not worry because you will be attended to by qualified Health personnel who will be there at the point of Eye Examinations. There will be no direct or monetary rain to you by participating in this research, your participation in this research is purely voluntary and therefore you are eligible to withdraw if you are not interested and your action will not affect your acquisition of health services.

Please seek clarification where you do not understand.

All the information you will provide will be kept under lock and key and will remain confidential. The research information will be disseminated to the relevant authorities and with no direct link to you since anonymity shall be maintain.

10.4. Informed consent form

The purpose of the study has adequately been explained to me and I understand the aim, benefits, risks and confidentiality of the study. I further understand that; if I agree to take part in this study, I can withdraw at any time without having to give explanation and that taking part in this study is purely voluntary.

I..... (Names)

Consent to participate in this study

Signed.....; Date..... (Participant)

Participant's Signature or thumb print

Signed.....; Date.....; (witness)

Witness's Signature or thumb print

Name of the interviewer;

Signed.....; Date.....;

Persons to Contact for Problems

1. Mulipilwa Martin D. (Mr) University of Zambia, School of Medicine, Department of Anatomy. P.O. Box 50110, Lusaka, Zambia. Mobile Phone; 0979175304.
2. The Chairperson, ERES CONVERGE, 33 JOSEPH MWILA RD RHODES PARK, LUSAKA. ZAMBIA. Tel +260955155633, +260955155634.

10.5 Mutuwakafukufuku: *Maonekedwe a masoyaodwalaameneabwela kuchipatalachachikulu cha university teaching hospital, lusaka, Zambia*.

Kwa otengakombali,

Tikudziwitsani ndikukupephani kutengakombali ku kafufuku ocitidwa ndi Abambo a Martin Mulipilwa, omweachita ma phuziro akuya (Masters of Science in Human Anatomy) pa sukulu ya University of Zambia, School of Medicine, ku Department of Anatomy. Kafukufuku kaona pa kupima maonekedwe a matso a odwala amene aliku bwela ku chipatala chachikulu cha UTH. Pali mabvuto ambiri omwe amalengetsa kuti matso aodwala matso absinthe maonekedwe, enamwaiwo ndiawa, matenda a sugar, matendaothamangamwazi ndi ena otere.

Ndichofunikira kuziwa maonekedwe a maso a odwala mosokuti a dotolo a matso aziwe kupelekachithandizo choyenera.

Choyamba ndikuona pa umoyo wanu paku kufusani mafunso okhudza inu monga zaka zanu, matenda omwe muna dwalapo ndi zina zace kuti ndi dziwe zanthanzi lanu.

Dziwani kuti Kulibe malipilo amutundu uli onse monga ndalama kuli omwe atengako mbali pa fukufuku uyu pakuti kutengako mbali ndi kodzipereka kwa ulele. Mulinayo danga yozi chotsa pa ku tengako mbali pa kafukufuku uyu pa nthawi ili yonse mwafunira kopanda chifukwa chilichonse. Zopezeka mu kafukufuku zi zathandiza pakuyang'anira odwala matenda a matso.

Mau ali onse ndi zotulukamo zizasungidwa mwa chisinsi, ndiponso zizaperekedwa kuoyang'anira apamwamba kopanda kukutchulani maina anu munjira iliyonse.

10.6. Kuvomekeza Kwa otengakombali,

Ine dzinalanga ndine **Martin Mulipilwa.** Ndikuchita maphuziro *a Masters muAnatomy* ku *school of medicine,* pa *University of Zambia.*

Ndi chofunikira pa maphuziro anga kuchita kafukufuku. Kafukufuka kaona pama onekedwe a

masto a anthu odwala matso obwela ku chipatala cha UTH.

Mwasakhidwa kutengako mbali kukafukufuku uyu. Ndifunanso kukudziwitsani kuti kusankhidwa kwanu ndikozipereka pa inu nokha ndipo mulinalo danga yozi chotsapo pa maphuziro aya pa nthawi ili yonse mwafuna. Mudzafusidwa mafunso okhuza umoyo wanu. Dziwani kutinso ngaili yonse kapena mau alionse dzasungidwa mwa chisinsi ndiposo dzinalanu sidzatchulidwa ngakhale kulembedwa papela iriyonse.

Simudzalandila malipiro munjira iriyonse ngakhale ndalama.

Ngatimulindimafunso tumani lamya pa 0979175304 kapena pa 0967175304.

Ine ..... Ndamvetsetsa ndondomeko zonse

Ndiponso ndineofunitsitsa kutengako mbali kukafukufukuuyu.

Tsikulino la ....., 2013.

Chitsimikizo cha oyakha.....

Chitsimikizo cha of unsa.....

Maina a anthuomwe mukhoza kufunsako ngati pali vuto.

1. Mulipilwa Martin D. (Mr) University of Zambia, School of Medicine Department of Anatomy. P.O. Box 50110, Lusaka, Zambia. Mobile Phone; 0979175304. Email: mmuiipilwa@yahoo.com.
2. The Chairperson, ERES CONVERGE, 33 JOSEPH MWILA RD RHODES PARK, LUSAKA. ZAMBIA. Tel +260955155633, +260955155634.

10.7. Ethical clearance form

C/o Mr Martin Mulipilwa
University of Zambia,
Department of Anatomy,

08th October, 2013.

The Managing Director/Senior Medical Superintendent,
University Teaching Hospital,
LUSAKA.

Dear Sir/Madam,

RE: REQUEST TO CONDUCT A STUDY AT THE UNIVERSITY TEACHING HOSPITAL

With reference to the above, I wish to ask for permission to carry out a study titled,"The Colour of the Iris in patients presenting at the Department of Ophthalmology University Teaching Hospital, Lusaka, Zambia.

I am a postgraduate, MSc Human Anatomy student at the University of Zambia, School of Medicine. My target population are patients presenting to Ophthalmology Department. Therefore, it is prudent that permission is sought from your office before any study of this nature is carried out. The brief description of the study is as reflected in the copy that is attached to this letter.
Your consideration of this letter will be highly appreciated.
Yours faithfully,

Mr Mulipilwa Martin D.33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260955155633
+ 260 955 155 634
Cell: +260966765503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948

EVV.A.No.00011697

28thFebruary, 2014

Ref. No. 2013-Nov-008

The Principal Investigator

Mr. Martin Mulipilwa

The University of Zambia, School of Medicine

Dept. of Anatomy

P.O. Box 50110,

LUSAKA.

Dear Mr. Mulipilwa,

RE: The Colour of the iris in patients presenting at the Department of Ophthalmology, UTH Lusaka, Zambia.

Reference is made to your corrections dated 18th February, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. 20 13-Nov-008
Approval and Expiry Date	Approval Date: 28L February, 2014	Expiry Date: 2ih February, 2015
Protocol Version and Date	Version-Nil	271n February, 2015
Information Sheet, Consent Forms and Dates	• English, Nyanja.	27U1February, 2015
Consent form ID and Date	Version-Nil	27U1 February, 2015
Recruitment Materials	Nil	2tll February, 2015
Other Study Documents	-	2tll February, 2015
Number of participants approved for study	384	27tl1 February, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully

ERES CONVERGE IRB

Dr. Munalula-Nkandu

BSe (Hons), MSc, MA Bioethics, PgD RJ Ethics, PhD

CHAIRPERSON

10.8. Permission letters

C/O Mr Martin Mulipilwa
University of Zambia,
Department of Anatomy,

08th October, 2013.

The Managing Director/Senior Medical Superintendent,
University Teaching Hospital,
LUSAKA.

Dear Sir/Madam,

RE: REQUEST TO CONDUCT A STUDY AT THE UNIVERSITY TEACHING HOSPITAL

With reference to the above, I wish to ask for permission to carry out a study titled,"The Colour of the Iris in patients presenting at the Department of Ophthalmology University Teaching Hospital, Lusaka, Zambia.

I am a postgraduate, MSc Human Anatomy student at the University of Zambia, School of Medicine. My target population are patients presenting to Ophthalmology Department. Therefore, it is prudent that permission is sought from your office before any study of this nature is carried out. The brief description of the study is as reflected in the copy that is attached to this letter.
Your consideration of this letter will be highly appreciated.
Yours faithfully,

Mr Mulipilwa Martin D.

ERES CONVERGE
33Joseph Mwilwa Road
Rhodes Park
LUSAKA

+260-955-155-633
+260-955-155-634

IRB No. 00005948
FWA No. 00011697

PROPOSAL REVIEW APPLICATION FORM

Please note that 4 copies this form attached to proposals **should be submitted** with a letter **from the PI or CO-PI requesting ERES CONVERGE IRB to review your proposal. Address all correspondence to the Secretary.**

1 **TITLE OF STUDY**:
The Colour of the Iris in patients presenting at the Department of Ophthalmology Lusaka Zambia

---

2. **PRINCIPAL INVESTIGATOR:**

Name: MULIPILWA MARTIN .D.

Qualifications: BSc BIOMEDICAL SCIENCES.

Present Appointment/Affiliations: Student Development Fellow University of Zambia

FOR STUDENTS: Qualification being pursued: MASTERS OF SCIENCE IN HUMAN ANATOMY

3a **OTHER INVESTIGATORS: N/A**

Name:

Qualifications:

Present Appointment/Affiliations:

Name:

Qualifications:

Present Appointment/Affiliations:
(Other names to be included on a separate page.)

3b. **SUPERVISORS: FOR STUDENTS ONLY**

1. Name: PROFESSOR ERZINGATSIAN KRIKOR

Qualifications:FRCSI; Hon FCS (ECSA)

Present Appointment/Affiliations: MSc ANATOMY CORDINATOR/ PROFESSOR OF SURGERY

Institution where supervisor is based: UNIVERSITY OF ZAMBIA

2. Name: Dr GRACE MUTATI,

Qualifications: (BSc, MBCHB, MRCOPH, FRCS Edinburgh, MPH Eye Health).

Present Appointment/Affiliations: HEAD OF DEPT OPHTHALMOLOGY

Institution where supervisor is based: UNIVERSITY TEACHING HOSPITAL

3. **FOR STUDENTS REGISTERED IN INSTITUTIONS OUTSIDE ZAMBIA (WHERE APPLICABLE)**

Name of local supervisor N/A

END