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DETERMINATION OF THE MELANIN CONTENT IN THE SKIN OF ALBINOS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

BY

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DECLARATION

I **Dailesi Ndhlovu** declare that this Dissertation is my own work and that all the sources l have cited have been indicated and acknowledged using complete references. I further declare that this Dissertation has not previously been submitted for diploma, degree or for any other qualifications at this or any other university. It has been written according to the guidelines for Masters of Science in Human Anatomy degree dissertations of the University of Zambia.

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I, **Dr Elliot Kafumukache,** 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 approve it for final submission.

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CERTIFICATE OF APPROVAL

This dissertation by **Dailesi Ndhlovu** is approved as fulfilling part of the requirement for the award of the **Degree of Master of Human Anatomy** by the University of Zambia.

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ABSTRACT

BACKGROUND: Melanin is a skin pigment that determines skin colour and plays a role in photoprotection. It represents one of the most visible markers of human variation and skin disorders. Lack of Melanin or presence of very little melanin or albinism makes the skin susceptible to a wide range of damages and diseases such as skin cancers caused by ultraviolet rays from the sun. The aim of this study was to demonstrate and quantify the melanin in albinos compared to normal indigenous black skin.

METHODS: This was a case-controlled study which was conducted at the University Teaching Hospital Dermatology and Pathology departments. The study recruited 12 cases' who were clinically diagnosed albinos and 12 normal black indigenous Zambians who were the controls. Skin biopsies from the study participants were taken from the radial aspect of the forearm and were cut into two parts. One part was fixed in 10% neutral buffered formalin, processed, and sectioned using a microtome then one tissue section was stained with H&E stain and the other tissue section was stained with the Mason-Fontana staining technique to determine the presence of melanin in the skins. The other second part, melanin was extracted assayed using the Human melanin ELISA kit and measured using a spectrophotometer.

RESULTS: In this study, 12 albino participants were recruited. The majority were males (n = 7, 58.3%) and 5 were females who represented 41.6%. The youngest was 14 years and the oldest was 40 years old, the mean age was 23. Melanin was only present in a biopsy of only one female case (n=1) and all the remaining skin biopsies (n=11) had no melanin as demonstrated using the Mason-Fontana technique. However in all normal indigenous black skin it was present; all the biopsies contained melanin after being assayed using the human melanin Elisa kit. The mean standard deviation of the melanin concentration of albinos was (102 ±62.5 pg/ml) and the normal indigenous blacks was (127 ±29.0pg/ml), p = 0.223.

CONCLUSION: This study has showed that not all individuals who have been clinically diagnosed with albinism lack melanin even though phenotypically may appear without any melanin. The presence of melanin has some clinical significance, though albinos phenotypically appear to lack melanin, susceptibility to skin cancers differ because of their variation in the amount of melanin in their skin.

KEY WORDS: Albinism, Melanin, Skin

DEDICATION

This work is dedicated to all People Living with Albinism in Zambia, my husband Roy Chikwanda, Micheal and Chika Chikwanda.

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DEFINITION OF TERMS USED

Biopsy - Excision of tissue to provide a sample for microscopic examination to establish a precise diagnosis.

Optical Density - It is the measurement of how much light a sample absorbs and how much of the light passes through the sample.

Melanin - Pigment found in the skin

Melanocyte - Cells in the skin that produce melanin.

Melanogenesis - The process by which melanin is formed in the melanosomes within the melanocyte.

Melanosomes - Highly specialized membrane bound intracellular organelles found in the melanocytes.

Normal Skin - For the purpose of this study normal skin is defined as showing no evidence of active disease such as infection, inflammation, tumours or trauma.

ACRONYMS/ABBREVIATIONS

NOAH	-	National Organization for Albinism and Hypopigmentation
OA	-	Ocular Albinism
OCA	-	Oculocutaneous Albinism
OCA1	-	Oculocutaneous Tyrosinase Negative
OCA2	-	Oculocutaneous Tyrosinase Positive
PLWA	-	People Living With Albinism
UTH	-	University Teaching Hospital
UV	-	Ultraviolet
UVR	-	Ultraviolet Radiation

CHAPTER ONE

1.0 INTRODUCTION

The human skin pigment melanin represents one of the most visible markers of human variation and skin disorders and has long fascinated Scientists and Human biologists (King et al, 1995).

Melanin in the skin determines the skin pigmentation and hair colour (Sturm et al, 1998), dark skin coloured individuals have a large content of melanin in their skin while light skin coloured ones have less (De Leeuw et al, 2001). Humans with skin genetically lacking partial melanin (hypomelanism or hypomelanosis) or with total lack of melanin (amelaninism or amelanosis) are frequently referred to as albinos (National Organization for Albinism and Hypopigmentation (NOAH), 2012). This condition affects all races worldwide, there is no cure for lack of melanin and it is estimated that there is one in 17-20,000 based on the world population (United Nations, 2013). Albinism is also due to a genetic mutation so the inheritance pattern is also quite variable (Kamaraj et al, 2014). Oculocutaneous albinism is mostly an autosomal recessive disorder, whereas ocular albinism is transmitted as a sex-linked or autosomal recessive disorder (Carden et al, 1998). Consequently the condition is passed on by both parents to their offspring resulting in affected individuals having fair skin, fair hair and most often blue eyes that look purple in bright sunlight (Pooe-Monyemore, 2012). While Oculocutaneous albinism (OCA) is found throughout the world the prevalence distribution varies widely between different population groups and societies, being most prevalent among indigenous people in Southern Africa (Gaigher et al, 2002 and Kagore et al, 1995). Christianson et al (2005) reported that the prevalence of Oculocutaneous albinism is fairly uniform across countries in Sub-Saharan Africa ranging from approximately 1 in 1429 in Tanzania, in Zimbabwe estimates have ranged from 1 in 1000 to 1 in 4182 and 1 in 4000 in South Africa have OCA, which is strikingly disparate from the prevalence rates in the United States and Europe among the individuals of Caucasian descent being approximately 1 in 37,000 and globally 1 in 20,000(Greaves, 2014).

The prevalence of albinos in Zambia is estimated at 1 in 565 (Central statistics office population census, 2010).

The skin is the largest organ of the body, constituting 16% -20% of total body weight and in adults presenting surface area of 1.5 to 2 m^2 (Young et al, 2013 and Mescher, 2013). It has three major layers the superficial epidermis, the dermis and subcutaneous layer (Singh, 2011). The epidermis is a stratified epithelium that forms the interface between the organism and its environment (Waster, 2007). It is composed of 5 layers from the deepest to the most superficial consisting of the stratum basale, the stratum spinosum, the stratum granulosum, the stratum lucidum (Singh, 2011) and stratum corneum which is composed of several layers of flattened, keratin-containing dead cells called squames (Gartner et al, 2007). Interposed between keratinocytes are, melanin producing cells the melanocytes with the cell body in the stratum basale with its dendrites stretching into the interspaces of keratinocytes of the stratum spinosum(Figure 1).

Melanocytes are dendritic cells of neural crest origin that produce melanin (Borovansky et al, 2011) and found within the basal layer of the epidermis (Osawa, 2009). The melanocytes are also located in the iris, the inner ear, the leptomeninges, bones, heart and hair follicles (Nasr 2013).

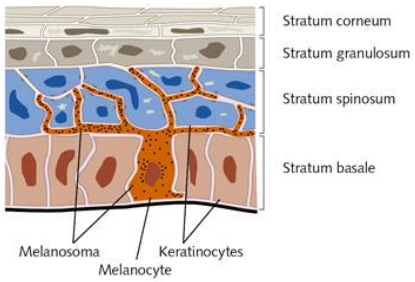
Synthesis of melanin in the melanocytes takes place within highly specialized membrane bound intracellular organelles called melanosomes (Yoo et al, 2007). The process by which melanin is produced is called melanogenesis; it is catalysed by tyrosinase a transmembrane enzyme, whose role is to convert tyrosine into 3,4dihydroxyphenylalanine (DOPA) which is further transformed and polymerized into the different forms of melanin (Mescher, 2013). Two distinct type of melanin pigments produced are eumelanin, a brown or black pigment and pheomelanin, a yellow or red-brown pigment (Stepien et al, 2009).Eumelanin is more abundant in dark-skinned people (Solano, 2014) while light skin has more pheomelanin (De Leeuw et al, 2001). The melanosomes(Figure 2) are secreted through the dendritic processes of the melanocyte cell to the surrounding keratinocytes (Borovansky et al, 2011), within the keratinocytes melanosomes localize over the nucleus and in this way protect genetic material from ultraviolet(UV) irradiation(Waster, 2007).

Although melanocytes produce melanosomes, the keratinocytes are the melanin depot and contain more melanin pigment than the melanocytes (Mescher, 2013). In the human skin, one epidermal melanocyte is attached to up to 36- 40 neighbouring keratinocytes and one Langerhans cell, the complex is called epidermal melanin unit (Tolleson, 2005). The type of melanin produced in the melanosomes and the number, size and distribution of melanosomes within keratinocytes not only determine skin colour but play a role in the photoprotection (Rees, 2004). In general, lighter-skinned people are believed to have low basal levels of melanogenesis and skin exposure to short wavelength ultraviolet B (UV-B) radiation causes an increased melanogenesis which leads to sunburn a reaction to injury (Borovansky et al, 2011).Thus melanin formation and transformation in human skin is an important mechanism for protection of skin from UV light(Yoo et al, 2007).This is achieved by limiting the penetration of UV(Ultraviolet) rays through the epidermal layers, thus preventing damage to DNA, by forming a cup like structure covering the side of the nucleus exposed to sunlight (Borovansky et al, 2011).

However, the absence or decreased melanin production resulting from a genetic defect in tyrosinase synthesis leads to a condition called albinism, melanosomes are present but the melanocytes fail to produce tyrosinase (Gartner et al, 2007).

Albinism (a term derived from Latin Albus meaning white) is a genetic hereditary disorder characterized by complete or reduced skin pigmentation, but with normal numbers and disposition of melanocytes in the skin (Greaves, 2014). This disorder is heterogeneous and can be separated clinically into those types that primarily involve the eyes ocular albinism (OA) and those types that involve the skin, hair as well as the eyes oculocutaneous albinism (OCA)(Mohammed et al, 2013). The clinical spectrum of OCA varies according to type and there are seven genetic heterogeneities, of which the tyrosinase negative (OCA1) and tyrosinase positive (OCA2) are the most frequent (Raju et al, 2013). In OCA1 there is little or no melanin production due to the lack of a functional tyrosinase, the critical enzyme required in the melanin biosynthetic pathway (NOAH, 2012). In the more prevalent OCA2 type there is some level of tyrosinase activity, thereby producing some red-yellow pheomelanin pigment that gives rise to sandy coloured hair and light brown irises (Summers, 2009).

When the skin is exposed to sunlight, melanocytes increase production of melanin so as to protect it from the heat and harmful ultraviolet rays (UVR) (Nasr, 2010).Borovansky et al (2011) look upon this function as a sunscreen, however in People living with Albinism (PLWA), there are not enough melanosomes with melanin available in the skin cells to shield off the harmful rays leaving the skin vulnerable to sun damage, increasing the risk of sunburn (Nasr, 2010). These individuals are intensely sensitive to the effects of (UVR): they develop acute erythema, swelling, blistering, and pain in response to what would be trivial amounts of UVR to others (Rees, 2004). In the long term this population is susceptible to developing skin lesions that have both cosmetic and health complications and leads to a greatly increased risk of developing some forms of skin cancer (Hong et al, 2006, Lund, 1996 and Lund, 2005).



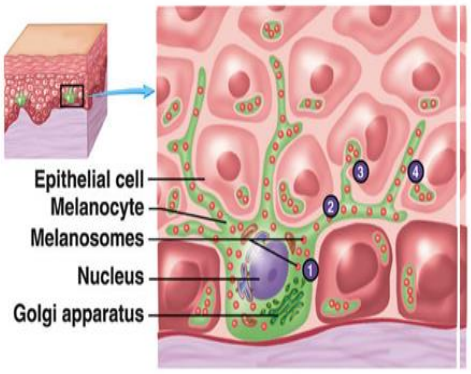


Figure: 1 **Figure: 2**

Figure 1 and Figure 2: Show melanocytes in the basal layer containing melanosomes being distributed to the keratinocytes in the epidermis (Nasr, 2010)

Phenotypic differences in constitutive pigmentation are not related to melanocyte numbers but to levels of melanogenetic activity, ratio of eumelanin to pheomelanin and distribution to the keratinocytes (Brenner et al, 2008). However, there are between 1000 and 2000 melanocytes per square millimetre of skin varying in different body sites and melanocytes constitute 5% to 10% whereas keratinocytes 90% of the cells in the epidermis. While the size can vary, melanocytes are typically 7um in length (Tollenson, 2005). In the developed

world and some countries in sub-Saharan region like South Africa ultrastructure studies on albino skins have been reported and studied (Broodbakker et al, 1983 and Kidson et al, 1993). This has not been done in Zambia. This study will endeavour to bridge the gap by providing information for our population.

1.1 Statement of the Problem

The problems associated with albinism are attributed to a defect in the melanin synthesis pathway which results in absence or reduced formation of melanin (Okulicz et al, 2003). Individuals with albinism have little or no protective melanin pigment in their skin and are extremely sensitive to sunburn; they are more susceptible to the harmful effects of ultraviolet radiation exposure leading to skin lesions and cancers especially in Africa (Gaigher et al, 2002 and Ngazi, 2009). In Lusaka, records from the University Teaching Hospital Dermatology Clinic from 21/12/13 to 21/01/15 attended to 102 PLWA with various problems like keratosis, elastosis, lentigines, skin ulcers, erythema, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Manga et al (2013) states that there is an inverse correlation between the degree of constitutive pigmentation (melanin) and the risk of sun-induced skin cancers those with completely no melanin are more likely to develop cancers at a faster rate and early age. Skin cancers are indeed a major cause of morbidity amongst albinos in the tropics, these patients from a young age face a raging battle against these cancers (Opara et al, 2010). The variation of melanin content in the skins of albino population in Zambia has not been described.

1.2 Significance of Study

Most studies on albinism in Lusaka have focused attention on the social challenges affecting people living with albinism. No microscopic or ultrastructure studies on the skin of albinos have been done. Microscopy or histological examination of the skin is important for verification and confirmation that the patient is in fact an albino as clinical methods of evaluating the colour of the skin based on visual assessments is often subjective and inaccurate((Petrovajova et al, 2014)).

The melanin level assessment is important in clinical dermatology for exact diagnosis of pigmentary disorders and also for objective estimation of skin phototypes for adequate photoprotection. Furthermore, methods are needed to quantify the melanin content because the concentration of melanin and its depth distribution is affected by ultraviolet radiation (Nielsen et al, 2006). In untanned skin or those diagnosed with albinism, where melanin pigments can be found only in the basal layer of the epidermis it is vital to offer prophylaxis such as appropriate attire, hats, clothing and sunscreens (Nielsen et al, 2006). Melanin concentration measurement in albinos can help us to categorize them into those having more and those with less amounts. This can be a used as a guide in prescribing the right type of sunscreen. Those with less amounts of melanin will need specialized care and will have to use broad- spectrum sunscreen lotions that protect them from both UV-A and UV-B radiation, and has a sun protection factor(SPF)(Mader,2004). There is a SPF number on the sunscreen e.g. SPF15 (This means that if one burns 15-20 minutes after exposure ,it will take 15 times longer or 5 hours before you burn). However those with low melanin concentration should use sunscreen with a higher SPF such as 30 or 45, also known as sun block (Mader, 2004). These measures help to reduce the incidence of skin malignancies (Petrovajova et al, 2014). Melanin protects individuals from developing skin cancers, determining the quantity of melanin in an individual will predict the chances of developing skin cancers. Currently, microscopic study of albino skins and quantification of melanin using a spectrophotometer has not been done in Zambia. A study of this nature which is designed to determine the presence and quantify melanin in the skin of albinos is important because the population of albinos is increasing as the population of Zambia is increasing. It can be predicted that the already known medical problems facing people living with albinism will escalate thus qualify albinism as a public health issue deserving further attention to increase the awareness of and information about this condition (Hong et al, 2006). In addition, this study has a bearing on policy makers, health institutions and researchers on the management of albinism. The information that has been generated will be used to support studies both on small and larger scale, hence adding to the body of knowledge.

1.3 Research Question

Do the skins of albinos at the University Teaching Hospital of Lusaka, Zambia contain melanin?

1.4 General Objective

To demonstrate and quantify the melanin in albinos compared to normal indigenous black skin.

1.5 Specific Objectives

1. To determine the presence of melanin in the skin of albinos.
2. To measure the melanin content in albino skins.

CHAPTER TWO

2.0 LITERATURE REVIEW

Investigations on melanocytes and melanin dates back as far as 1917 based on the work of Bloch who demonstrated the formation of a pigment resembling melanin in normal epidermal melanocytes (Kugelman et al, 1961). And in 1952 Becker et al first demonstrated amelanotic melanocytes in the skins of albinos using gold impregnations and concluded that population density of melanocytes were the same as those of a normally pigmented skin .Other microscopy investigations established the presence of melanocytes in the skins of albinos beyond reasonable doubt but they lacked the enzyme tyrosinase essential for melanogenesis (Kugelman et al, 1961). Bischitz et al (1983) argued that the amount of melanin in the skin and hair was subject to considerable variation and was dependent on the numbers of melanocytes present and activities in these cells. However, recent studies have also established that differences in skin pigmentation do not result from difference in the number of melanocytes in the skin, but from differences in the melanogenic activity, the type of melanin produced in the melanosomes and the size, number and packing of melanosomes with melanin content of melanosomes ranging from17.9%-72.3 %(Brenner et al, 2008).

Rees (2004) has observed that studies of epidermal melanin rather than hair melanin are few because they require skin biopsies. In most of these studies heavy metals were used for staining such as uranyl and lead, using an electron microscope, particular attention was paid to the amount of pigmented granules in the keratinocytes, presence of distinct maturational stages of melanosomes in the melanocytes and keratinocytes, occurrence of abnormal melanosomes and packing of melanosomes in the keratinocytes (Broodbakker et al, 1983).

A study done on 7 albinos and 4 controls in South Africa by Kidson et al (1993) examined the hair bulb and skin melanocytes of albinos with an electron microscope observed that the skin melanocytes had melanosomes at various stages of melanization and incompletely melanized melanosomes. On average, albino melanosomes were 30%

smaller than normal black skin melanosomes. In the keratinocytes, the melanosomes were packaged into distinct aggregations, whereas in normal black skin, they occurred singly.

A study done on skin biopsies of 47 Caucasian albinos and 10 Caucasian controls in the Netherlands by Broodbakker et al (1983) to describe the ultrastructure of Caucasian albinos observed that 15 non-pigmented albinos completely lacked pigmented granules in the epidermis. Whereas 29 variably pigmented albinos were characterized by the presence of melanized granules in the melanocytes and keratinocytes. These albinos had variable amounts of pigmented granules, some had few while others had relative abundance. There were 3 albinos who were characterized by the presence of macromelanosomes which were aggregated or lacked aggregation of the pigmented granules in the epidermis. The authors were trying to prove if diagnosis of albinism clinically was accurate, the study revealed disparities as those who were classified as Oculocutaneous type 2 (OCA2) on clinical examination (based on having some moles) turned out to have complete absence of melanin on microscopic examination.

In a similar study by Breathnach et al (1965) using an electron microscope demonstrated that hypopigmentation of oculocutaneous albinism was due to the absence of melanin biosynthesis and in this disorder unmelanized organelles premelanosomes were present within the melanocytes, which appeared to be otherwise normal. But the cytoplasm of normal humans melanocytes contained melanosomes in various stages of melanization but in human albinos had a few partially melanized organelles (melanosomes).

In a study conducted on 98 Caucasians in the Netherlands by Van Dorp (1987) discovered that 12 apparently normally looking pigmented Caucasians were in fact albinos. While the skins of these albinos appeared to have normal pigment clinically they had various eye problems consistent with ocular features of albinism. Van Dorp et al (1987) concluded that there may be many normally pigmented albinos who have not been diagnosed as such, since most clinicians' diagnosis of albinism is primarily clinical examination of the skin. When a microscopic study of the skin biopsy of these patients was done, it revealed no presence of macromelanosomes and that in Ocular albinism it was important to use a microscope to see if the skin is involved. Van Dorp et al (1987) also found that patients with autosomal recessive albinism can be normally pigmented and

those with sex-linked albinism can be hypopigmented. Comparative studies on concentration of melanin in albinos and other races has not been found.

CHAPTER THREE

3.0 METHODOLOGY

3.1.1 Study Design

This was a laboratory based case-control study.

3.1.2 Study Setting

The study was conducted at the Pathology & Microbiology Department, Karposis Sarcoma (KS) Laboratory and Dermatology Department at the University Teaching Hospital (UTH), Lusaka, Zambia. Biopsy collection from participants was done at the dermatology clinic. The Histopathology component of this study (tissue sectioning, H & E staining, Mason-Fontana staining technique, cover slipping and microscopic examinations) was done at the UTH histopathology laboratory whereas melanin extraction and quantification was done at the KS Laboratory, UTH. The sites were selected purposely because of the convenience and ease of access to facilities.

3.1.3 Target and Study Population

The target population included all albinos attending the dermatology clinic at the University Teaching Hospital (UTH), Lusaka.

The total study population was 24 participants, which included 12 clinically diagnosed albinos, attending dermatology clinic and 12 normal-skinned comparison group of indigenous black patients undergoing excisional or incisional biopsy.

3.1.4 Inclusion Criteria

Albinos and normal indigenous black individuals who have no skin lesions or diseases.

3.1.5 Exclusion Criteria

Normal indigenous black individuals who have bleached the skin, patients diagnosed with vitiligo and albinos with cancers.

3.1.6 Sampling Method

Convenience sampling method was used to enrol clinically diagnosed albinos attending the dermatology clinic. The participants in the control were matched with study group for age, sex.

3.1.7 Sample Size Calculation

Based on albino population in Lusaka at 0.20 percent and 99.8 percent for indigenous blacks; the sample size was 3 participants from each group in order to have 80 percent power using $\alpha = 0.05$

This was calculated using the formula indicated below;

$$N = \frac{[u\sqrt{\pi_1(1-\pi_1)}+\pi_0(1-\pi_0) + v\sqrt{2\pi(1-\pi)}]^2}{(\pi_0-\pi_1)^2}$$

N = size of **each** group

π_0=0.20%=**0.002**, π_1=99.8%=**0.998** Proportions, π=Average of the proportions=**0.5**

u = **1.28** for 90% power, v = Z statistic = **1.96** if α =0.05

N = 2.5=**3**

3 participants were in each group (study and control); in order to augment the power of the study the sample size was increased to 12 in each group.

3.2 Materials and methods for determination of the presence of melanin in the skin of albinos

3.2.1 Specimen Collection

Ethical procedures were followed, the participants were educated about the research, upon agreeing to participate in the research they signed the informed consent, assent and parental/guardian form. A qualified doctor was recruited to take the punch biopsies however, prior to that the participants were examined for any skin infections and lesions. The punch biopsies were taken on the radial side of the forearm, of about 3mm to 5mm

diameter and local anaesthesia was administered to reduce on the pain prior to the procedure. After the biopsies were taken, they were cut into two parts; one was for staining with H& E and Mason-Fontana technique and the other was frozen at -80 degrees Celsius for melanin extraction.

3.2.2 Specimen labelling

Each skin biopsy was given a new identification code and number for easier identification. The letter "Z" (was for an albino biopsy) and ''N'' (was for normal black skin) plus a three digit numbering system (i.e. 000) was used. Hence specimen numbers 1, 2, 12, were labelled as Z001, Z002, Z010 and N001, N002 and N012 respectively.

3.2.3 Tissue preparation

The tissues for staining were fixed in 10% buffered formalin after which water was removed from the tissues using a graded series of alcohol baths, beginning with 50% alcohol progressing in graded steps to 100% alcohol and cleared with xylene. The tissues were placed in an automated tissue processor that gave several changes in wax before making blocks. Blocks were trimmed and mounted for sectioning using a microtome with thickness of about 5-10um.The wax was removed from the section using ethanol and the tissue rehydrated. Finally they were mounted on glass slides and ready for staining to make the cells visible (Gartner, 2007).

3.2.4 Specimen sectioning

Before the specimens were sectioned on the microtome the blocks to be sectioned were placed face down on an ice-cold plate for 20 minutes. The water bath was turned on and the temperature was set at 35-37degrees Celsius. The paraffin block were placed in the block holder of the microtome machine and advanced it closer to the microtome blade. The dial was set to cut 5-10µm sections. The blade was angled 4-6º. The rough trimming of the paraffin section was done until a complete section was seen in the block. By utilizing a clean section of the blade, a ribbon was obtained. The ribbons were picked using the forceps and transferred to the water bath. The ribbons were laid on the water bath to allow the sections to stretch for a few seconds. The sections were carefully separated and each section picked on a glass slide. The sections were picked at an angle to

allow the water to exit the slide and section. The slide sections were allowed to drain for a few minutes before putting the specimen slide on the hot plate. To remove some more water from the tissue section, the tissue section was put on the hot plate. The glass slides were then placed in a warm plate for about 15 minutes to help the section adhere to the slide.

3.2.5 Ehrlich's Haematoxylin and Eosin (H&E Stain) staining

Sections were taken to water, that is deparaffinized in two changes of xylene (2 minutes each), washed in two or three changes of absolute alcohol (2 minutes each) then in water for 2 minutes. The slides were then stained with Ehrlich's Haematoxylin solution for 25 minutes. After staining the slides were washed in running tap water for 5 minutes then differentiated in 1% hydrochloric acid in 70% alcohol (1% acid alcohol) for 15-30 seconds. The slides were further blued in running tap water for 10 minutes and counter stained in 1% aqueous eosin for 2-5 minutes. Finally, the slides were rinsed in 95% alcohol (10 dips), dehydrated in 2 changes of absolute alcohol for 2 minutes in each, cleared in 2 or 3 changes of xylene for 2 minutes each. This was done to demonstrate the general tissue morphology and served as the main diagnostic staining method to ensure that the biopsies had no cancer which would distort the melanin quantity results (Crookham et al, 1991 and Gamble et al, 2002).

3.2.6 Cover slipping

The slides were placed on a clean horizontal bench surface. A drop of DPX Mountant was applied to each tissue section and at the far end of the slide away from the frosted end. The cover-slips were then carefully applied by placing one end of the slip on top of the drop of DPX Mountant at the far end of the slide. Slowly and gently the cover slip were rolled down the side, only using enough pressure to allow the medium to spread evenly. The slides were placed flat upon the bench for 2 hours to eliminate bubbles under the cover slip. When the slides were dry they were removed on the bench and ready for microscopic examination.

3.2.7 Masson- Fontana staining technique

The sections were taken to distilled water. Sections were treated with the ammonical silver solution in a Coplin jar for 30-40 minutes at 56 degrees Celsius or at room temperature overnight. The slides were washed well in 3 changes of distilled water followed by treatment with 0.5% gold chloride for 5 minutes. The sections were rinsed in water for 1 minute, fixed with 5% sodium thiosulphate for 5 minutes and then counterstained with neutral red for 2 minutes. The slides were then rinsed in distilled water, dehydrated, cleared and mounted in DPX. (Carson, 1990, Crookham et al, 1991 and Gamble et al, 2002).

3.2.8 Cover slipping

The slides were placed on a clean horizontal bench surface. A drop of DPX Mountant was applied to each tissue section and at the far end of the slide away from the frosted end. The cover-slips were then carefully applied by placing one end of the slip on top of the drop of DPX Mountant at the far end of the slide. Slowly and gently the cover slip were rolled down the side, only using enough pressure to allow the medium to spread evenly. The slides were placed flat upon the bench for 2 hours to eliminate bubbles under the cover slip. When the slides were dry they were removed on the bench and ready for microscopic examination.

3.2.9 Microscopic examination and identification of melanin

The histological examination of slides to determine presence of melanin was done by a qualified Histopathologist. Any substance with reducing properties (argentaffin) will appear black like melanin. These cellular structures have the ability to take up silver nitrate and reduce it to metallic silver without the aid of an external reducing agent.

3.3 Materials and methods for measuring melanin in the skin of albinos

3.3.1 Melanin extraction for measuring melanin

Manufacturer's (MybiosourceUSA) instructions were followed to achieve the desired results as elaborated below.

Tissue Homogenates: 100mg tissue was rinsed with 1X Phosphate buffered saline (PBS), homogenized in 1ml of 1X PBS and stored overnight at 4 degrees Celsius. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2-8 degrees Celsius. The supernate was removed and were stored at -20 degrees Celsius.

3.3.2 Assay Procedure

The reagents were brought to room temperature before use and samples were thawed and centrifuged again before the assay. Thirty wells were used and blank wells were set without any solution. Fifty (50) ul of standard or sample was added per well. 50ul of HRP-conjugate was added to each well and 50ul Antibody. It was mixed well and then incubated for one hour at 37 degrees Celsius. Each well was aspirated and washed, repeating the process twice for a total of three washes. Each well was washed filling with 200ul of Wash Buffer using a multi-channel pipette and was left to stand for 10 seconds, complete removal of liquid at each step was essential to good performance. After the last wash, the remaining Wash Buffer was removed by aspirating ordecanting. The plate was inverted and blotted against paper towels. 50ul of Substrate A and 50ul of Substrate B was added to each well and mixed, and then it was incubated for 15 minutes at 37 degrees Celsius. The plate was kept away from drafts and other temperature fluctuations in the dark. Stop Solution of about 50ul was added to each well and gently tapping the plate to ensure thorough mixing. The optical density of each well was determined within 10 minutes, using a microplate reader set to 450nm. The minimum detectable dose of human melanin is typically less than 15.6 pg/ml. The detection range of the kit was from 12.5pg/ml to 1000pg/ml.

3.4 Research Variables

3.4.1 Dependent Variables

Melanin

3.4.2 Independent Variable

Age
Gender

3.5 Data Management

3.5.1 Data Collection Tool

Data collection sheet was used to collect demographic data, histological and melanin quantity results.

3.5.2 Validity

To ensure validity, the reagents were tested on one of the samples to ensure that they were effective and were used with the expiration date of the kit.

3.5.3 Reliability

The same data information sheet and method of collecting data was used on all the participants.

3.5.4 Data Analysis

Following data collection, the pre-coded data information sheet was double checked for completeness, consistency, legibility and accuracy daily. Data from the collection sheet was then entered in IBM SPSS version 20 for analysis. Data was described using percentages and bar charts. The two groups which were being compared i.e. albinos (cases) and normal indigenous blacks (reference controls). Melanin quantity was analysed as continuous variables; the mean for melanin concentration was compared in both groups using Independent sample t-test. P-value of 0.05 was chosen to indicate statistical significance.

3.5.5 Data Presentation

Data was processed in frequency,tables and graphs.

3.6 Ethical Consideration

Prior to the commencement of this study, Excellence in Research Ethics and Science (ERES) CONVERGE reviewed and approved the protocol. Permission to conduct the study was obtained from the Senior Medical Superintendent and the Head of the Departments of Dermatology and Pathology and Microbiology at UTH.

Written informed Consent and assent 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. 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.

CHAPTER FOUR

4.0 RESULTS

This chapter represents the results demonstrating the sex, age, histology and the melanin quantity. Thereafter the results show the differences in mean melanin concentration among albinos and Normal indigenous blacks. The mean difference was determined by the Independent sample t -Test.

4.1 Gender of Participants

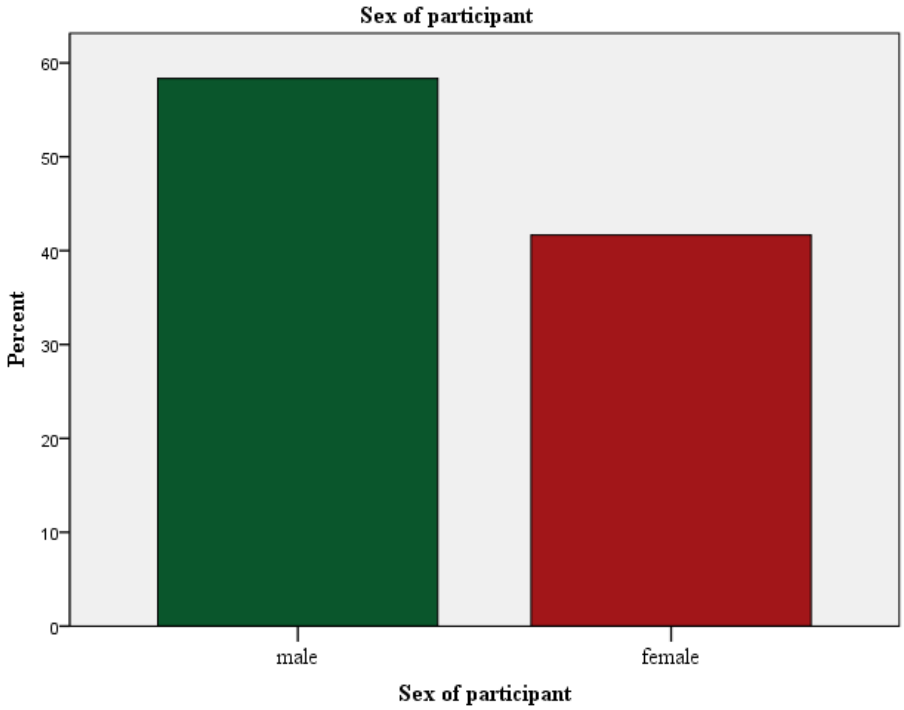


Figure 3: Sex of participants.

Out of the 12 participants recruited, 5 were female while 7 were male.

4.2 Distribution of Age

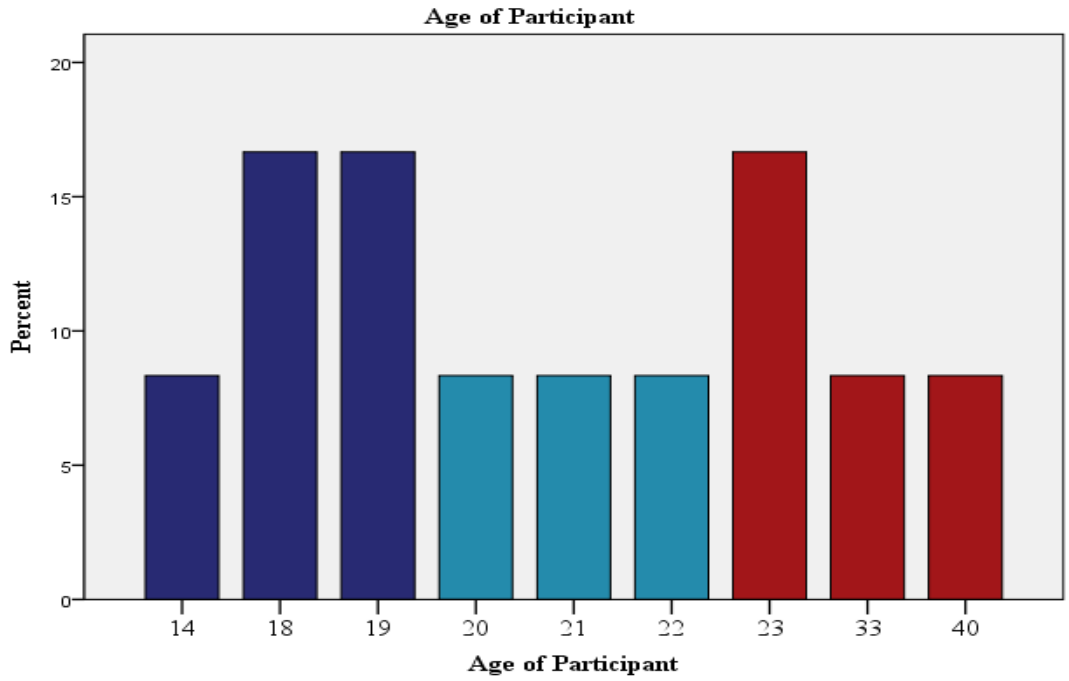


Figure 4.0 Age distribution

It shows the age of albinos ranging between 14 and 40 years with mean and median age of 23 and 20.5 respectively. The majority were aged below 23 years.

4.3 Histology Results

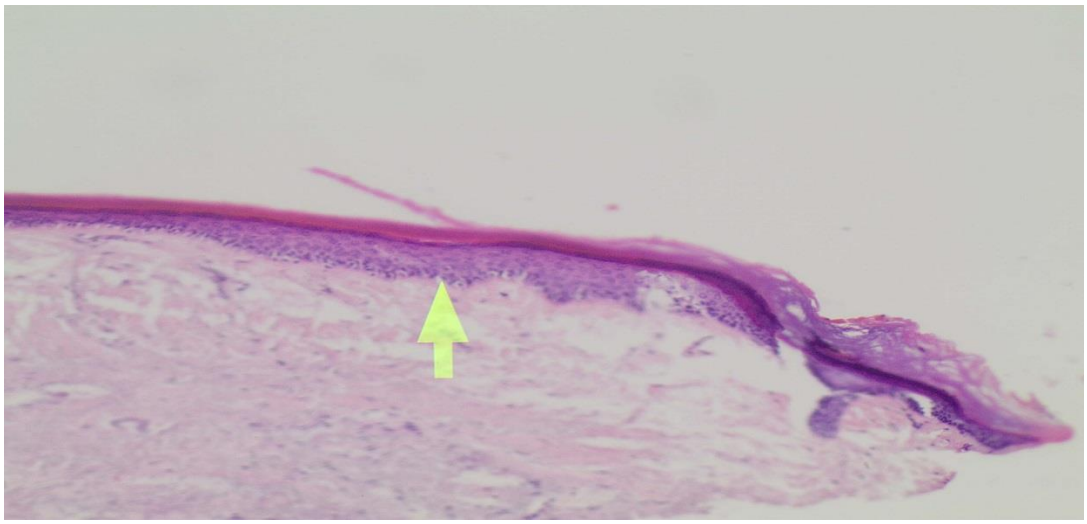


Figure 5.0 Non-diseased albino skin (H&E stain, Magnification x10)
This shows the epidermis (Arrow pointing at the melanocytes in the basal layer)

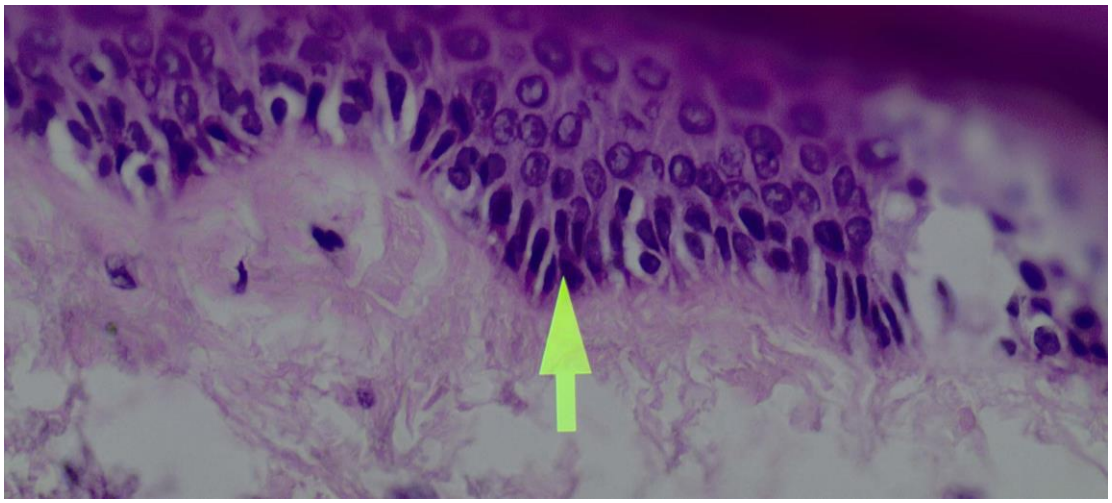


Figure 6.0 Non-diseased albino skin (H&E stain, magnification x40). Same specimen as in figure 5; this shows the melanocytes scattered in the basal layer of the epidermis. Melanocytes (arrow) have smaller nuclei and inconspicuous cytoplasm compared with the surrounding keratinocytes which contain large nuclei, which stain blue.

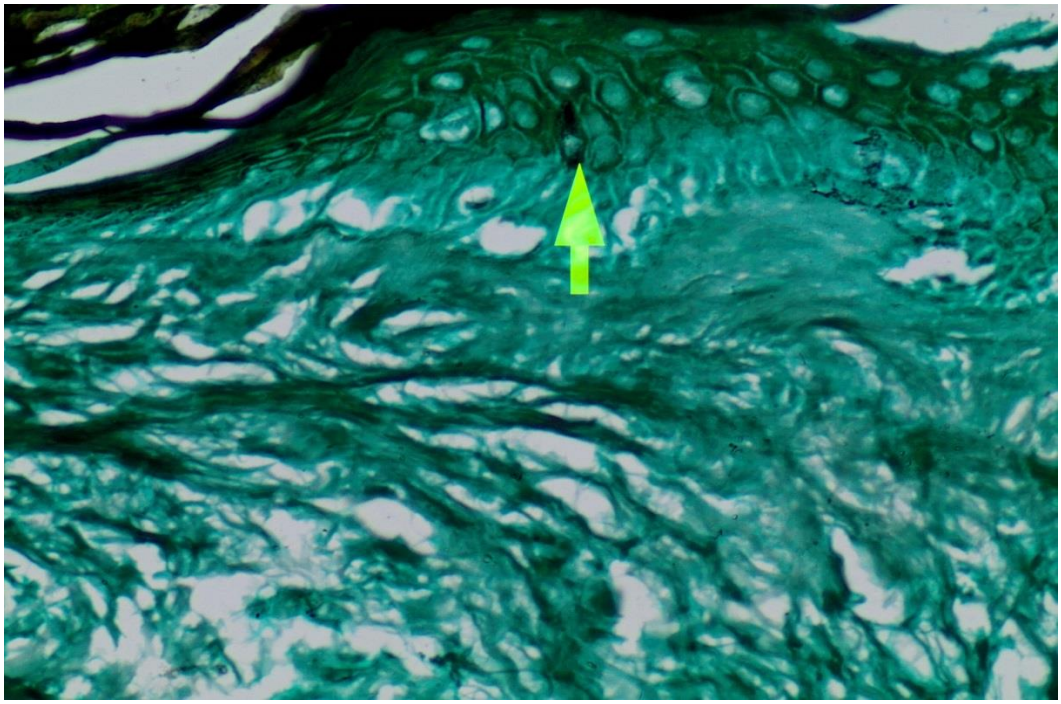


Figure 7.0 Non-diseased skin of an albino (Masson-Fontana stain, magnification x40). Same specimen as in figure 5; it shows some melanin in keratinocytes in the epidermis layer.

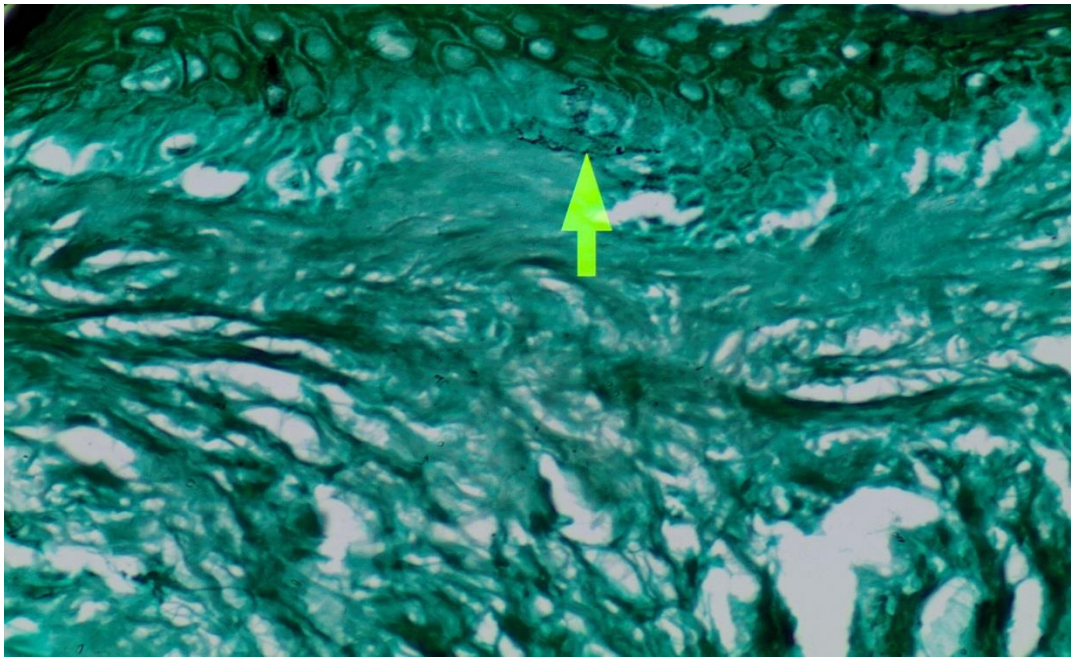


Figure 8.0 Non-diseased skin of an albino (Masson-Fontana stain, magnification x40). Same specimen as in figure 5; it shows the presence of the melanin in the melanocytes in the basal layer of the epidermis (arrow pointing at the melanin). Any substance with reducing properties (argentaffins) will appear black.

4.4 Melanin Quantification

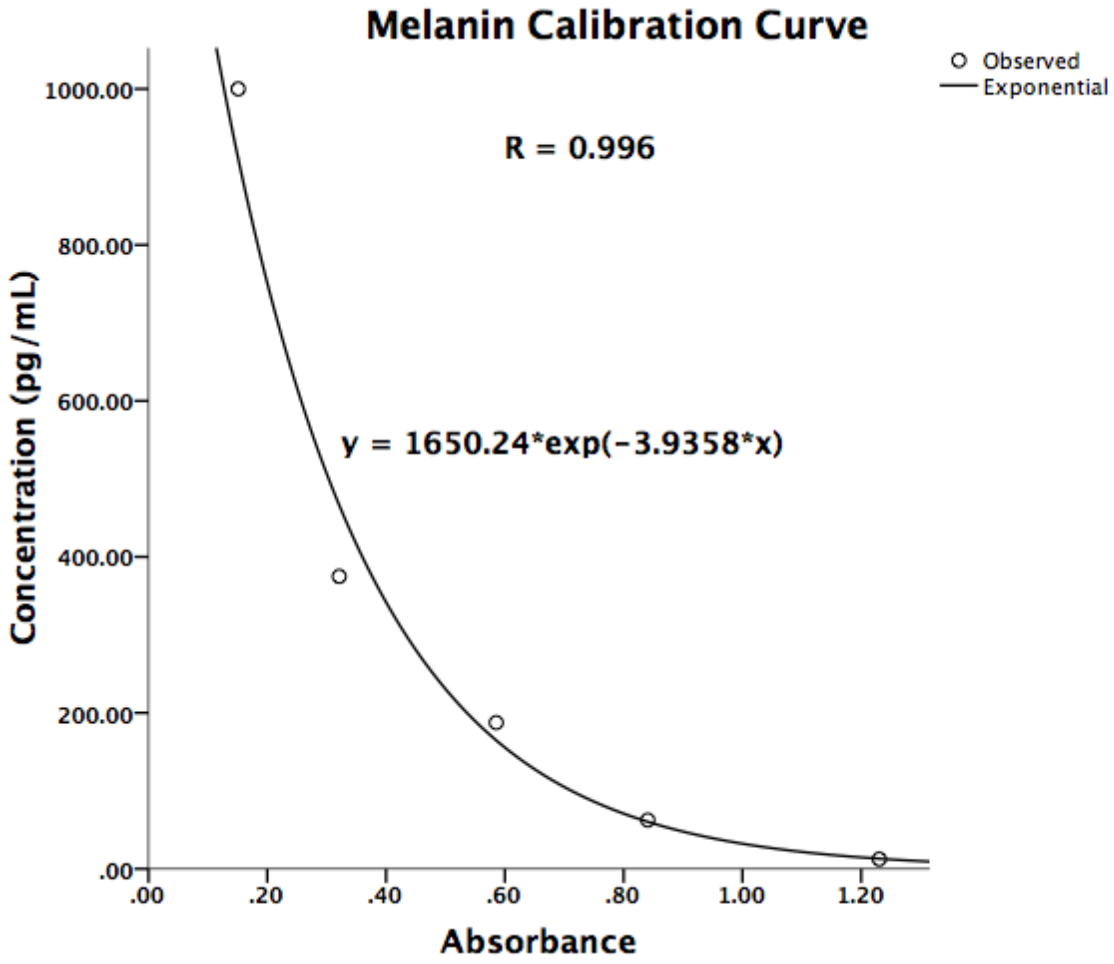


Figure 9.0 Calibration Curve for the ELISA test

The relationship between melanin concentration and optical density at 450nm.

4.5 Melanin concentration for normal indigenous blacks and albinos

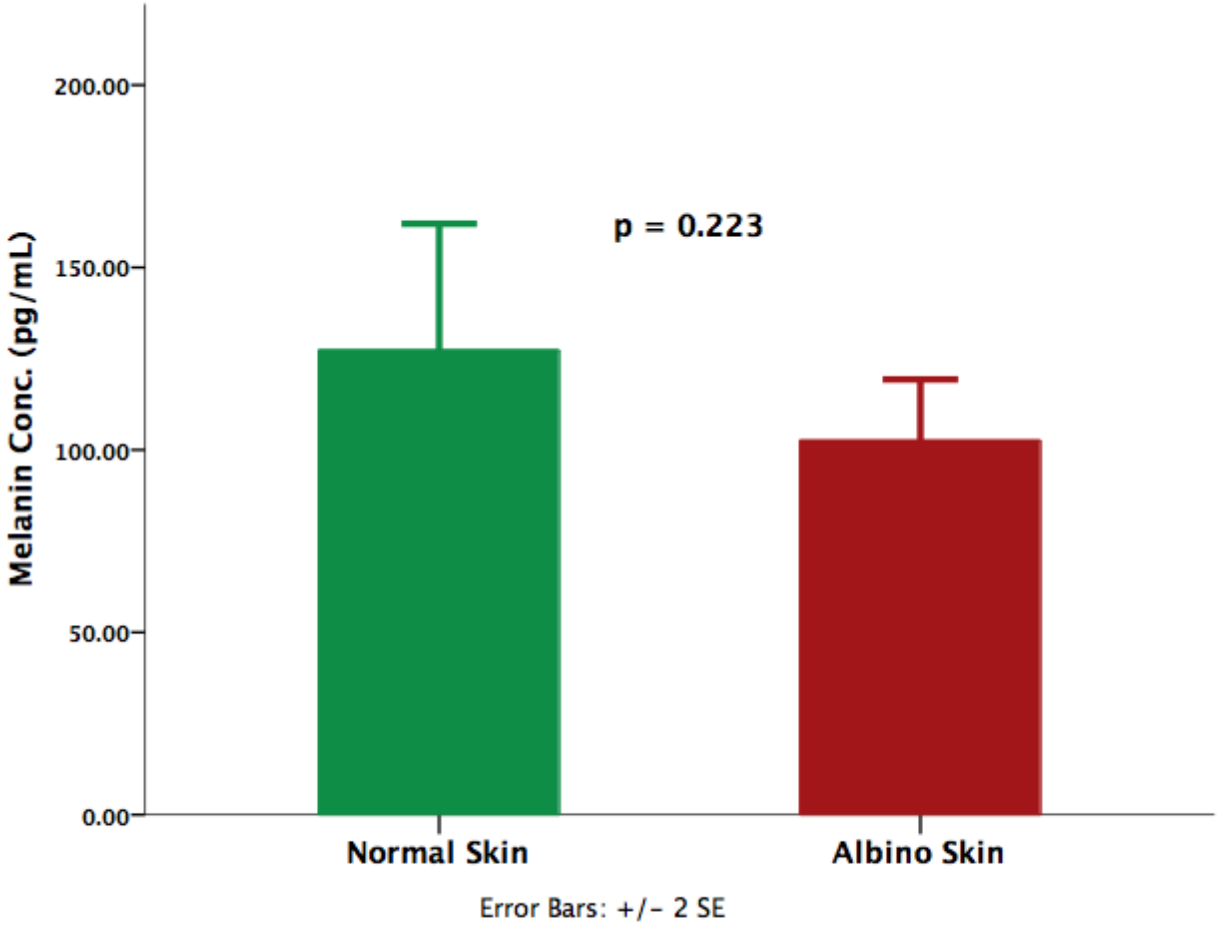


Figure 10.0 graph for T-test results.

The melanin levels in albino skin biopsies (102 ± 8.37pg/mL) compared to normal skin biopsies (127 ± 17.32)

4.6 Table 1. Variables

Specimen ID	Age (years)	Sex	Melanin Status (MF Stain)	Melanin Quantity (pg/mL)
Z1	18	female	positive	96.6
Z2	19	female	negative	147.1
Z3	40	female	negative	171.5
Z4	22	male	negative	108.3
Z5	33	male	negative	84.7
Z6	20	male	negative	77.1
Z7	14	female	negative	98.0
Z8	23	male	negative	98.1
Z9	19	male	negative	79.7
Z10	18	male	negative	94.1
Z11	23	female	negative	149.1
Z12	21	male	negative	112.0

The table 1 summarizes the results of the study according to age, sex, histological status and melanin quantity which were found in the albino skin. The total number of albino participants were 12 , out of which 5 were female and 7 were male. A biopsy of one female (n=1) was positive to the Mason-Fontana technique while the rest (n=11) were negative.Whereas using the human melanin Elisa Kit all the albinos had melanin in their skins with quantities which ranged from 77.1pg/l to 171.5pg/ml with the mean quantity of 109.6pg/ml. The oldest female albino 40 years had the highest quantity of melanin while the lowest was in a male aged 20 years.

CHAPTER FIVE

5.0 DISCUSSION

Melanin is an important pigment in the skin; it plays an important role in the protection of the skin from harmful ultraviolet rays which can cause skin damage. This study focuses on melanin demonstration and quantification on albino skins.

5.1 Determination of the presence of melanin in the skin of albinos

On all the biopsies that were analysed in this study, only one was melanin positive to the Masson-Fontana technique while the remaining 11 were negative, this may be due to the low sensitivity of the Masson-Fontana method (Gamble et al, 2002). The Masson-Fontana method is not specific for melanin as other reducing substances will also give positive reaction. In a similar study that was done by Broodbakker et al (1983) on 47 Caucasian albinos and 10 Caucasian controls their findings were variable some albinos had melanin pigments and while others had no melanin pigments in the skin. The findings by Broodbakker et al (1983)was compared to those by Breathnach et al (1965) in the United States of America on Caucasian albinos who also found unmelanized melanosomes and partially melanized melanosomes in the melanocytes implying that they had no melanin and partial melanin respectively. Similarly Kidson et al (1993) in South Africa on Black 7 albinos and 3 normal black individuals found melanosomes at various stages of melanization and incompletely melanized melanosomes in the skin. Mescher (2013) pointed out that the variability in the melanin pigment seen in the skin of albinos could be attributed to the melanogenetic activity of some melanocytes and those that were able to transfer melanosomes with melanin to the neighbouring keratinocytes. In this study the only biopsy in which melanin was detected using the Masson-Fontana technique did not have highest level of melanin. The reason for this observation is not known. However the oldest albino had the highest quantity of melanin. This observation could be explained in line with literature that states that albinos are born with some pigmentation and there is a slight increase in pigmentation with age (Manga et al, 2013).

5.2 Melanin Measurement

In this study melanin was extracted from skin biopsies, it was assayed using the Human melanin ELISA kit and quantified using a spectrophotometer. All the samples had melanin including those which were negative using the Masson-Fontana technique. This suggests that the technique was more sensitive than the Masson-Fontana as it was able to detect melanin even in biopsies which did not demonstrate melanin with the Masson-Fontana technique. There was a variation in the quantity of melanin present in the skin biopsies which ranged from the lowest 77.1pg/ml to the highest 171.9pg/ml. This study is in agreement with the study by Brenner et al (2008) who found out that phenotypic differences in constitutive pigmentation are not related to melanocyte numbers but from differences in the levels of melanogenic activity, number of melanosomes with melanin, the type of melanin produced and the distribution to the keratinocytes. In this study female albinos had more melanin than males. The reasons for this sex difference remains unclear. The presence of melanin in all the biopsies implied that the there was some melanogenetic activity in the melanocytes and that these albinos could be classified as those with oculocutaneous type two (OCA2) albinism which has some level of tyrosinase activity (summers, 2009). In this study the mean melanin concentration between albinos and blacks with normal skin was statistically insignificant, this implies that albino skins do not lack melanin but it is the amount of melanin that is produced.

CHAPTER SIX

6.0 CONCLUSION

The study established that all the albinos in this study had melanin in their skin. It implies that though albinos phenotypically appear to lack melanin they do actually contain melanin. This study revealed that not all individuals who have been clinically diagnosed with albinism lack melanin. The human melanin Elisa kit should be used to determine the presence of melanin in the skin as the Mason-Fontana technique can give false negative results. The presence of melanin has some clinical significance, the variation in the quantity of melanin in their skins means that they have different susceptibility to development of skin cancers when exposed to the ultraviolet rays from the sun.

6.1 Study Limitations

Albinism is a rare disorder therefore large sample numbers were not attainable and the study was expensive as it required the use of a lot of reagents.

6.2 Dissemination and utilization of findings

The findings of the study were presented to the Department of Anatomy, School of Medicine, and UNZA. Then, the results were later presented at the postgraduate seminar week and an article was published with a recognised journal. The University Teaching Hospital which was the study site was given a copy of the study results report so that the hospital would use them to render evidence based care to people living with albinism.

6.3 RECOMMENDATIONS

- It is recommended that other similar studies be carried out perhaps on a larger sample size and over a longer period of time to confirm the findings of this study.
- Studies should be done to determine whether autosomal recessive or Sex –linked albinism is more prevalent in our population.
- Future studies should be done on clinical diagnosis of albinism.

- And further research on melanin characterization should be done to establish the type of melanin found in Zambian albinos. Knowing the eumelanin and pheomelanin ratio expected in black people is important as this will have an impact on future research on albinism in line with gene therapy (Kariuki, 2014).This will help in melanin production in albinism.
- Future researches should be done on the quality and functionality of melanosomes

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8.0 APPENDICES

APPENDIX 1

BUDGET

item	Description	Quantity	Rate	Amount
1	ERES submission fee - For ethical approval	1	1000.00	1000.00
2	Stationary and printing - Specialist copyist/secretary to edit monographs from first post-data analysis to final submission	1	2,000.00	2,000.00
3	Transport costs - To and from place of data collection	1	8,000.00	8,000.00
4	Participants	20	200.00	4,000.00
5	Personal research assistants - Biopsy taking and Processing tissues	1	5,000.00	5,000.00
6	Data analysis	1	2,000.00	2,000.00
7	Thesis printing	1	3,000.00	3,000.00
8	Surgical Blades	4	10.00	40.00
9	10 litre Formalin	1	1,000.00	1,000.00
10	Sterile containers -Box of 50	1	1,000.00	1,000.00
11	Silver Nitrate-50gm	1	800.00	800.00
12	Ammonium Hydroxide-50gm	1	1,000.00	1,000.00
13	Gold chroride-100ml	1	1,500.00	1,500.00
14	Neutral Red-120ml	1	260.00	260.00
15	Ethanol-5liters	1	1,200.00	1,200.00
16	Xylene-5l	1	1,200.00	1,200.00
17	Slides-10 boxes of 50	10	25.00	250.00
18	Cover slips-1 box of 100(24by 40'')	1	50.00	50.00
19	DPX-100g	1	500.00	500.00
20	Melanin extraction and analysis kit-48tests	1	5,400.00	5,400.00
	Sub-Total			38,700.00
	Add Contingency @ 10%			3,870.00
	Grand Total ZK			43,070.00

APPENDIX 2

PLAN OF ACTIVITIES

ACTIVITY	TIME FRAME IN MONTHS											
	September 2014	October 2014	November 2014	March 2015	January 2015	February 2016	May 2016	June l 2016	June 2016	June 2016	July 2016	August 2016
Finding a Research Topic												
Proposal Development												
Writing of Proposal												
Submission of proposal to the Department												
Presentation of proposal to the graduate forum												
Submission of proposal to ERES converge												
ERES converge review of proposal												
Data collection												
Data Analysis												
Writing of Dissertation												
Submission of final dissertation												
Presentation of findings												

APPENDIX 3

INFORMATION SHEET

TITLE OF STUDY: Determination of the melanin content in the skin of albinos at the University Teaching Hospital-Lusaka Zambia

Dear Participant

This is to inform you about the study and to request you to take part in this study being carried out by Dailesi Ndhlovu, a student with the University of Zambia, School of Medicine, Department of Anatomy.

The study will endeavour to find out if the pigment found in the skin (melanin) is present or not, to measure the amount and type of melanin. Melanin in the skin acts as a sunscreen thus shielding off ultraviolet rays and preventing skin cancers. Therefore, efforts should be made in establishing the pigment (melanin) content in the skin of albinos and non-albinos in the Zambian population. This will assist the dermatologist to confirm, and diagnose that you are really an albino thus preventing complications such as skin cancers. Also help on how to best handle patients living with albinism and prescribe adequate photoprotection. The participants also will know how best to take care of themselves after knowing their levels of melanin in their skin.

A small skin will be cut from the lateral aspect of the forearm, skin anaesthesia will be injected on the part to be cut to reduce on the pain or discomfort, and the cut skin will be transferred into the specimen container. The cutting of the skin will be done by a qualified doctor in the skin clinic. The specimen will then be coded and later subjected to light microscopic analysis in the laboratory.

Your participation in this study is entirely voluntary and therefore, you are eligible to withdraw should you decide and your action will not affect your acquisition of health service. The information collected from you in this research will be kept strictly confidential and all the data collection tools used will be destroyed thereafter.

There will be no direct monetary gain to you by participating in this research. The skin melanin (pigment) findings will be used to gather information which will contribute to the management of people living with albinism.

In the event that the participant is injured during the procedure, the researcher will take full responsibility of the consequences to correct the situation.

If you have any questions about the study please contact the principal investigator or the chairperson for ERES CONVERGE at the following addresses and contact numbers;

10. Contact Details of Principal Investigator

Dailesi Ndhlovu
The University of Zambia
School of Medicine
Department of Human Anatomy
P.O. Box 50110
Ridgeway Campus
Cell No: +260969754663
Email: dnheaven@yahoo.com
LUSAKA
ZAMBIA

11. Contact Details of Ethics Committee

The Chairperson
ERES CONVERGE IRB
33 Joseph Mwilwa Road
Rhodes Park
LUSAKA
Tel: 0955 155633/4
LUSAKA
ZAMBIA

TRANSLATED INFORMATION SHEET

BEMBA

ICHIPEPA CHA IFYO ABALEIBIMBA MULI UKU KUFWAILISHA PA NKANDA YABA MWABI BAFWILE UKWISHIBA

1. Ukuilondolola

Kafwailisha afwile aeba abaleibimbamo muli uku kufwailisha ishina lyakwe, efyo acita elo nomulimo wakwe muli uku kufwailisha.

2. Umutwe Wa Uku Kufwailisha

“Ukulengula ifilenga inkanda ukufita munkanda yabamwabi pa chipatala chikalamba icha UTH muno Lusaka mu chalo cha Zambia”

3. IchoTulefwailisha

Ukulengula ifilenga inkanda ukufita munkanda yapantu aba bamwabi elo nokumona ngemofili.

4. Ifyo Tulechita

Akankanda tususula utulingilefye twalabulwa ukufuma mutumipaipi twankanda elo nokutwala utu tunkanda ku kupima nabamashini pakuti tumone ifilenga inkanda ukufita.

5. Ukuipelesha

Ukuibimba muli uku kufwailisha kwa sugar mu mulopa kuipelesha elo ngatamulefwaya kuti mwakana. Kaili ngamwakana ukuibimbamo mulu uku kufwailisha temulandu iyoo nangula teti ababomfi bamuchipatala bamuchite wanyawanya mu kundapo. Nga chakweba ati mwachinja amano, namukwata insambu ishyakukana konkanyapo muli uku ukuibimbamo nangula namutampako kale ukwabula uku londolola ifilifyonse. Elo namukwata insambu shaku kana yasuka amepusho eyo tamulefwaya ukwasuka muli uku kufwailisha.

6. Ichilayo Cha Nkâma

Ndemweba ukuti fyonse ifyo twalalandishyanya pamo namepusho mulu uku kufwailisha nalafisunga munkâma elo nama pepala yonse eyo twala lemba po tuka yocha nga twapwisha ukuyabonfya.

7. Ubusuma No Ububi Bwa Uku Kufwailisha

Ukususula inkanda yapakuboko mu tumipaipi twankanda takwakwata amafya elo tachikalipa sana. Ubusuma bwa uku kufwailisha bwakeba ati, ukulengula nga kwapwa, twalaishiba ifilenga ukuti inkanda ifite nokuchingila kukasuba ifyaba mumubili yenu ,elo no kumyeba ifya kuisunga. Ichi chikankala sana mukwafwilishako bashingânga uku ishiba ati chachine niwe bamwabi ,tamwakwata ifififitisha inkanda.

Muku palanya nokulengula kumbi ukufwaya ukufumya inkanda, limo limo kuti kwaba ukumfwa ulunshingwa, ukufimba elo nokukalipa kumulandi wa kutungaulwa ne nshindano elyo bashinganga balelwisha ukufumya inkanda.

8.Amafuto

Ukuibimba muli uku kufwailisha ukishiba ifififitisha inkanda takulemipela insambu sha ku mufuta mu musango uli onse.

9.Amasanso Muli Uku Fwailisha

Ngachakweba ati abaibimbile muli uku kufwailisha bachenekwa, kafwailisha aka bombesha ukumona ukuti mwaloleshewapo bwino pakuti tachitwlele ku bubi.

Ngachakweba ati namukwata amepusho pali uku kufwailisha, kuti mwamona ka fawailisha mukalamba nangula umukalamba wakabungwe akalolesha pa milandu yakufwailisha pe sukukulu ya masambililo yakalamba pe tweyala uti pe samba;

10.Akeyala Ka Kafwailisha Mukalamba

Dailesi Ndhlovu

The University of Zambia

School of Medicine

Department of Anatomical Sciences

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Cell No: +260969754663

Email: dnheaven@yahoo.com

LUSAKA, **ZAMBIA**

11. Akeyala Ka Umukalamba Uwulolesha Pa Kabungwe Ka Kufwailisha

The Chairperson

ERES CONVERGE IRB

33 Joseph Mwilwa Road

Rhodes Park

Tel: 0955 155633/4

LUSAKA, ZAMBIA

Ngacakweba ati mwasumina ukuibimbamo saineni akapepala aka pesamba;

TRANSLATED INFORMATION SHEET

ICHINYANJA

Mutu wa kafukufuku: kuona pakunchuluka kwa zomwe zimalengetsa nkhanda kuda mu adangwaleza pa chipatala cha University Teaching Hospital- Lusaka, Zambia

Kwa otengako mbali

Ichi ndi chokuziwitsani pali kafukufuku ndi kukupemphani kuti mutengeko mbali pali kufukufuku komwe kaza tsongoleledwa ndi Dailesi Ndhlovu, omwe ali mwana wa sukulu pa University of Zambia, school of Medicine, Department of Anatomy.

Kafukufuka aka kadzafunitsitsa ku pedza ngati kala yakuda yocedwa kuti melanin muchizungu, ipezeka munkhanda kapena ai, ndiyochulika kapena ai, ndiya mtundu wanji. Kala yakuda yocedwa kuti melanin isebeza ngati chitetedzo cha dzuwa ku nkhanda ndiposo ithandiza kupewetsa matenda ya Skin cancer. Chifukwa chaichi, chifunika kuyesetsa kuona pakunchuluka Kwa zomwe zimalengetsa nkhanda kuda mu adangwaleza mukuchuluka Kwa anthu amu dziko la Zambia. Ichi chidza thandiza omwe oyang'anira pa matenda ya nkhanda ochedwa a dermatologist kudziwa ndi kupedza njira yo nthandiza mwanayo wachidangwaleza kumutetedza ku matenda ya Skin cancer, Ndiposo kukuthandiza monkhalira ndi adangwaleza. Otengako mbali aza phunzira mozisungira ngati aziwa muyeso wa nkhanda ya kuda.

Ka nkhanda kakang'ono kadza dulidwa kuzanja lanu ndiposo muzalasidwa nsingano pomwe azacheka nkhanda yanu kuchepetsa kuwawa. A dotolo akuchipatala cha nkhanda kapena skin clinic muchizungu, azakudulani nkhanda yanu ndikuipima.

Ngati nichifunilo chanu utengako mbali mukafukufuku aka, mulinayo mphamvu yoziletsa utengako mbali ndiposo simuzaletsedwa ai. Mayankho anu azasungidwa mwa chinsinsi

Kudzankkala kulibe malipilo ngati muzatengako mbali ku kafukufuku aka. Ndiposo zotuluka za kafukufuku akaziza thandiza monkhalira ndikusunga zidangwaleza. Mwatsoka ngati kwapezaka ngozi kuli otengako mbali, omwe atsogolera kafukufuku aka azakuthandizani.

Ngati muli ndimafunso alionse pali kafukufuku aka, mungathe utuma lamya, kalata olo email kuli atsongoleri a ERES CONVERGE panambala ndi keyala ili pansi apa:

10. Contact Details of Principal Investigator

Dailesi Ndhlovu
The University of Zambia
School of Medicine
Department of Human Anatomy
P.O. Box 50110
Ridgeway Campus
Cell No: +260969754663
Email: dnheaven@yahoo.com
LUSAKA
ZAMBIA

11. Contact Details of Ethics Committee

The Chairperson
ERES CONVERGE IRB
33 Joseph Mwilwa Road
Rhodes Park
LUSAKA
Tel: 0955 155633/4
LUSAKA
ZAMBIA

APPENDIX 4

INFORMED CONSENT FORM

Dear participant,

My name is Dailesi Ndhlovu, am a student enrolled in the Master of Science in Anatomy Programme at the School of Medicine, University of Zambia.

In partial fulfilment of the Master of Science in Anatomy Programme, I am required to undertake a research project .My study topic is ‘‘Determination of the melanin content in the skin of albinos at the University Teaching Hospital-Lusaka Zambia’’

I have read (or have had explained to) the information about this study as contained in the participant information sheet. I have had the opportunity to ask questions about the research and any questions I have asked have been answered to my satisfaction.

I now consent voluntarily to participate in this study and understand that I have the right to withdraw my participation at any time if I so wish, and to choose not to answer particular questions that are asked in the study.

My signature below signifies that I am willing to participate in this study:

Name of participant (Print): ……..……..….....….

Signature of participant: ……..…..…… Consent Date: …….……

Participant’s right thumb print if unable to write: …….……..……..……

Name of researcher conducting voluntary consent (Print):……..……..……..…..

Signature of researcher: …….…….…….…… Date: …….....……

Name of witness (Print): ……..…….

Signature of witness: ……..…….. Date: ……...……..…….

TRANSLATED INFORMED VOLUNTARY CONSENT FORM

BEMBA

ICHIPEPALA CHAKUSUMINA MU KUIPELESHYA

SOSA PALWALALA

Nimbelenga (nangula naba nondondolwela) amashiwi ayalekuma ukulengula sugar mubalwele ba bulwele bwa sugar ngafilya fine yalembelwe mu chipepa cha ifyo abaleibimba muli uku kufwailisha pa bulwele bwa sugar bafwile ukwishiba. Kaili nachipelwa akashita aka kwipusha amepusho pali uku kufwailisha elo nabanjasuka fye bwino amepusho nachipusha.

Nomba nasumina mu kuipelesha ukuti ningaibimbamo muli uku kufwailisha. Elo ninjishiba ukuti ninkwata insambu shaku kana yasuka amepusho eyo nshilefwaya ukwasuka muli uku kufwailisha.

Uku saina kwandi pe samba kulepilibula uku ninsumina ukuibimbamo muli uku kufwailisha:

Ishina lyenu (Lembeni bwino): ……..……..….....….

Saineni: ……..…… Ubushiku bwa kusumina: ……..……

Fwatikeni ne chikumo cha kukulyo ngatamwaishiba ukulemba: ……..……..…..

Ishina yaba kafwailisha (Lembeni bwino): …….……..…….……

Saineni: …….……..…… Ubushiku: …….....……..

Ishina yaba kamboni (Lembeni bwino): …….……..……

Saineni: ……...……..…….. Ubushiku: ……...…….....

TRANSLATED INFORMED VOLUNTARY CONSENT FORM ICHINYANJA

Kwa otengako mbali,

Ine dzina langa ndine Dailesi Ndhlovu, ndine mwana wa sukulu pa University of Zambia, school of Medicine, Department of Anatomy. Kuti ndithe ma phunzira aga Ndichofunikira kuti ndichite kafukufuku aka komwe mutu wake unena kuti: kuona pakunchuluka Kwa zomwe zimalengetsa nkhanda kuda mu adangwaleza pa chipatala cha University Teaching Hospital- Lusaka, Zambia.

Ndawerenga zonse zomwe zipedzeka kuli otengako mbali pali kafukufuku aka. Ndinalinawo mwayi wo funsa mafunso ndiposo mayako yanalandildwa yanili yokodweletsa.

Ndiloledza utengako mbali ku kafukufuku aka ndiposo ndiziwa kuti ndilinayo mphamvu yoleka utengako mbali ndi kuyankha mafunso mwachifuniro changa.

Chisindikizo changa pansi apa chionesa kuti nifuna kutengako mbali kukafukufuku aka.

Dzina la otengako mbali…….

Chisindikizo cha otengako mbali……

Tsiku la chiloledzo…….

Chisindikizo cha chala la zanja la manja la otengako mabli ngati saziwa kulemba…….

Dzina la atsongoleri……

Chisindikizo cha atsongoleri……..

Tsiku……

Dzina la mboni……

Chisindikizo cha mboni……...

Tsiku…….....

APPENDIX 5

ASSENT FORM

Paediatric Patients (5-17 years)

Dear participant,

My name is Dailesi Ndhlovu, am a student enrolled in the Master of Science in Anatomy Programme at the School of Medicine, University of Zambia.

In partial fulfilment of the Master of Science in Anatomy Programme, I am required to undertake a research project .My study topic is ‘‘Determination of the melanin content in the skin of albinos at the University Teaching Hospital-Lusaka Zambia’’

I have read (or have had explained to) the information about this study as contained in the participant information sheet. I have had the opportunity to ask questions about the research and any questions I have asked have been answered to my satisfaction.

I now consent voluntarily to participate in this study and understand that I have the right to withdraw my participation at any time if I so wish, and to choose not to answer particular questions that are asked in the study.

Participant

I_____ (participant’s name)

have been informed about the study. I volunteer to participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/Thumb _____

Date (dd/mm/yy) ____/____/____

Researcher

I have explained this research study to the subject. I am available to answer any questions now or in the future regarding the study and the subject's rights.

Signature of Investigators & Printed Names

Name _____ Signature _____

Date (dd/mm/yy) ____/____/_____

TRANSLATED ASSENT FORM
ICHIPEPALA CHA KUSUMINA

Umutwe Wa Uku Kufwailisha: "Ukufywa ukwishiba ifilenga inkanda ukufita munkanda yabamwabi nga fya likwanina pa chipatala chikalamba icha UTH muno Lusaka mu chalo cha Zambia"

Kafwailisha: Dailesi Ndhlovu

Tulefwailisha ukwishiba ngaemofyba ifififitisha inkanda mukanda yabamwabi. Ukufwailisha ninshila eyo tusambililapo ifingi mu bantu.Ngachakweba ati ulefwaya ukuibimbamo muli uku kufwailisha, ba kafwailisha balakwipusha amepusho pa mibele yobe elo nakananda akanonofye kalabulwa ukufuma mutumipaipi twa nkanda.Kaili ufwile ukwishiba ati utukanda utwalabulwa twalatutwala kukupima pakuti tulengula ifyo inkanda ili.

Nkanda walasendwafye umukumo kaili ukubulwa nkanda kusendafye ba minute basano. Ukufumya inkanda mu tumipaipi twankanda takwakwata amafya elo tachikalipa sana. Muku palanya nokulengula kumbi ukufwaya ukufumya inkanda, limo limo kuti kwaba, ukufimba elo nokukalipa kumulandi wa kususula bamunofu. Ubusuma bwa uku kufwailisha bwakeba ati, ukulengula nga kwapwa, twalaishiba ngomofwaba ifififitisha inkanda yamuntu. Ichi chikankala sana mukwafwilishako bashingânga uku mundapo bwino no kwishiba ngachachine nimwe ba mwabi no kumipususha kuti ba cancer beyamba.

Ngatwapwisha ukufwailisha pa bulwele bwa sugar, tukalemba ifyo tukasambililapo. Efyo tukalemba tafyakakukume munshila iyiliyonse.

Temulandu ngataulefwaya ukuibimbamo muli uku kufwailisha elo ngataulefwaya ukwasuka amepusho ayo tausekelemo wiyasuka. Kaili ngawachinja amano nangula nauibimbamo kale muli uku kufwailisha, kuti wafumamo temulandu iyoo. Abafyashi bobe natubeba nabo pali uku kufwailisha.

Ngachakweba ati ulefwaya ukuibimbamo muli uku kufwailisha, saina ishina lyobe pe samba.

Ine, _____, ndefwaya ukuibimbamo muli uku kufwailisha.

Saina ishina lyobe apa _____ ubushiku: _____

Ukusaina kwa ka kafwailisha _____ Ubushiku: _____

TRANSLATED ASSENT FORM (ICHINYANJA)

Banabangono (5-17 years)

Mutu wa kafukufuku: Kuona pakunchuluka Kwa zomwe zimalengetsa nkhanda kuda mu adangwaleza pa chipatala cha University Teaching Hospital- Lusaka, Zambia.

Mutsongoleri wa kafukufuku: Dailesi Ndhlovu

Ichi ndi chokuziwitsani pali kafukufuku Ka kuona pakunchuluka Kwa zomwe zimalengetsa nkhanda kuda mu zidangwaleza. Kafukufuku Kama thandiza kuziwa zambiri pali anthu osiyanasiyana. Ngati muzafuna utengako mabli, muzafunsidwa mafunso ndiposo nkhanda kakang'ono kaza dulidwa ku dzanja lanu.

Zina zomwe mufunika kuziwa Pali kafukufuku aka ndiza kuti nkhanda yomwe yadulidwa iza pimidwa kuona muyeso wa kuda Kwa nkhanda yanu. Kadulidwe ka nkhanda yanu kazachitika kamodzi chabe ndi a dotolo aku dermatology clinic, izi siziza tenga nthawi ai. Ndondomeko yakuduidwe iyi ndiyosabvuta ai ndiposo yotetedzeka. Muza lasidwa nsingano yochepetsa kuwawa.

Sionse otengako mbali aza penzamo zotuluka za bwino. Zotuluka za bwino chitathauza kuti za bwino za kuchitikirani. Ngati mwa pimidwa kuda Kwa nkhanda yanu. Zotulukamo za bwino ndi izi; a dotolo aza ku thandizani kupewa matenda ya cancer.

Zotuluka za kafukufuku aka ziza lebedwa ndiposo dzina lanu liza nkhalamo mu bukuli. Muli nalo mphamvu lokana ngati simufuna dzina lanu kuti ikapedzeke mu bukuli. Ndiposo muli nalo mphamvu yoleka utengako mbali Ndondomeko itayamba kale.

Ngati mufuna utengako mbali, mulembe dzina lanu pansi apa:

Ine……..ndifuna kutengako mbali ku kafukufuku aka.

Chisindikizo…….Tsiku……

Chisindikizo cha munthu wokuloledzani……..……Tsiku……

APPENDIX 6

PARENTAL/GUARDIAN INFORMED CONSENT FORM

Dear parent/guardian

My name is Dailesi Ndhlovu, am a student enrolled in the Master of Science in Anatomy Programme at the School of Medicine, University of Zambia.

In partial fulfilment of the Master of Science in Anatomy Programme, I am required to undertake a research project .My study topic is ‘‘Determination of the melanin content in the skin of albinos at the University Teaching Hospital-Lusaka Zambia’’

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

Permission for a Child to Participate in Research

As parent or legal guardian, I authorise _____
(child’s name) to become a participant in the research study described in this form.

Child’s date of birth: _____

Parent or Legal guardian’s signature: _____Date: _____

Signature of person obtaining consent: _____Date: _____

Signature of Investigators & Printed Names
Name _____ Signature _____

Date ____/____/_____

Contacts for Questions or Problems?

Contact Details of Principal Investigator

Dailesi Ndhlovu
The University of Zambia
School of Medicine
Department of Anatomical Sciences
P.O. Box 50110
Ridgeway Campus
Cell No: +260969754663
Email: dnheaven@yahoo.com
LUSAKA
ZAMBIA

Contact Details of Ethics Committee

The Chairperson

ERES CONVERGE IRB

33 Joseph Mwilwa Road

Rhodes Park

LUSAKA

Tel: 0955 155633/4

LUSAKA

ZAMBIA

TRANSLATED PARENTAL/GUARDIAN INFORMED CONSENT FORM

BEMBA

ICHIPEPALA CHAKUSUMINA MU KUIPELESHYA NO MUFYASHI NAGULA UMULINSHI WA MWANA

Umutwe Wa Uku Kufwailisha: "Ukufywa ukwishiba ifilenga inkanda ukufita munkanda yabamwabi nga fya likwanina pa chipatala chikalamba icha UTH muno Lusaka mu chalo cha Zambia"

Kafwailisha: Dailesi Ndhlovu

Ichantanshi

Umwana wenu naitwa ukuibimbamo muli uku ukufwailisha ukumona ifilenga inkanda ukufita ngomofyaba mukanda yakwe. Fwayeni inshita iya kuti mwingalanshyanya nendupwa shyenu nangula abanenu pali uku kufwailisha. Chilikuli imwe ukuti umwana wenu engaibimbamo muli uku kufwailisha.

Muli uku kufwailisha, tulefwaya ukwuishiba ififitisha inkanda ngemo fyaba muliena.

Efyo Mufwile Ukuwishiba Pali Uku Kufwailisha?

Umwana wenu bala mwipushako amepusho pa nkanda ywakwe elo akanda akanono utulingilefye twalabulwa ukufuma mutumipaipi nkanda. Utu nkanda twala tutwala ku kupima nabamashini pakuti tumone ififitisha inkanda.Inkanda walasendwafye umukumo kaili ukusenda inkanda kusendafye ba minute basano.

Bakafwailisha kuti baleka uku kuwailisha nangula kuti ba mufumyamo umwana wenu muli uku kufwailisha ngabamona ukuti chalaleta ubwafya ku mwana wenu. Kuti bachita ifi ukwabula ukumyeba.

Umwana wenu ngachakweba ati bamufumyamo muli uku kufwailisha, tachilepilibula ukuti ninshi tabamutangate bwino iyoo.

Ububi Bwa Uku Kufwailisha

Muku palanya nokulengula kumbi ukufwaya ukususula inkanda, limo limo kuti kwaba uku kalipa no kufimba elyo balesusula.

Ubusuma Bwakuibimba Muli Uku Kufwailisha?

Ubusuma bwa uku kufwailisha bwakeba ati, ukulengula nga kwapwa, twalaishiba nga emo yaba ifififitisha inkanda and kumichingilila kukasuba pakutimilaluala cancer. Ichi chikankala sana mukwafwilishako bashingânga ukumundapo bwino ukishiba nga nimwe ba mwabi zoona elo nokulesha ba cancer ukwisa. Telyonse elyo umwana wenu enganonkelamo muli ububusmuma nomba bambi kuti banonkelamo umuyenshiku.

Inkama

Ishina lya mwana wenu tatwaibomye muli uku kufwailisha iyoo. Kaili tuleesha namaka ukuti fyonse ifipepala ifyotulebomya tulefisunga munkama. Ngatwapwa uku kufwailisha tukafyocha fyonse ifipepala tulebomya.

Amalipilo

Ukuibimba muli uku kufwailisha ubwingi bwa sugar mumulopa takulemipela insambu sha kumulipila mu musango uli onse.

Insambu Shenu Ngabaleibimba Muli Uku Kufwailisha?

Ukuibimba muli uku kufwailisha kuipelesha. Umwana wenu nakwata insambu shakukana yibimbamo muli uku kufwailisha. Kaili nakwata insambu shakufumamo muli uku kufwailisha inshita iiliyonse. Umwana wenu ngafumamo muli uku kufwailisha tachilepilipbula ukuti twala leka ukumundapo bwino pamo ngabakafwailisha nagula ababomfi bamuchipatala.

Insambu Shakusuminisha Umwana Wenu Ukuibimbamo Mulu Uku Kufwailisha

Ngomufwashi nangula umulinshi wa uyu mwana, ndemipela amaka ayakuti _____ (ishina lyamwana) engaibimbamo muli uku kufwailisha ngafilyafine mulondolwele muli ichi chipepala.

Ubushiku umwana afyelwe: _____

Ukusaina kwa mufyashi nangula umulinshi: _____

Ubushiku: _____

Ukusaina kwa ka kafwailisha: _____ Ubushiku: _____

Utweyala Nganamukwata Amaepusho Nangula Amafya?

Ngachakweba ati namukwata amepusho pali uku kufwailisha nangula amafya, kuti mwamona ka fawailisha mukalamba nangula umukalamba wakabungwe akalolesha pa milandu yakufwailisha pe sukukulu ya masambililo yakalamba pe tweyala uti pe samba;

Akeyala Ka Kafwailisha Mukalamba

Dailesi Ndhlovu
The University of Zambia
School of Medicine
Department of Anatomical Sciences
P.O. Box 50110
Ridgeway Campus
Cell No: +260969754663
Email: dnheaven@yahoo.com
LUSAKA
ZAMBIA

Akeyala Ka Umukalamba Uwulolesha Pa Kabungwe Ka Kufwailisha

The Chairperson
ERES CONVERGE IRB

33 Joseph Mwilwa Road

Rhodes Park

LUSAKA

ZAMBIA

TRANSLATED PARENTAL/GUARDIAN INFORMED CONSENT FORM

ICHINYANJA

Mutu wa kafukufuku: Kuona pakunchuluka Kwa zomwe zimalengetsa nkhanda kuda mu zidangwaleza pa chipatala cha University Teaching Hospital- Lusaka, Zambia.
Mutsongoleri wa kafukufuku: Dailesi Ndhlovu

Chiziwitso

Mwana wanu itanidwa kuti aka tengeko mbali kukafukufuku kali kuona pali kuda kwa nkhanda. Ndichofunkira ukambitsana ngati a banja, azibwezi kapena ndi aliwonse omwe mufuna kukamba nao. Ichi chidza ku thandizani kuaona ngati munga mulole mwana wanu kuti a katengako mbali.
Mu kafukufuku aka tidza funa kuziwa kuchuluka kwa kuda ndi mtundu ya nkhanda ya mwana wanu.

Ndondomeko ya kafukufuku

Mwana wanu aza funsidwa mafunso ndiposo ka nkhanda kakang'ono kaza dulidwa ku Dzanja Lake. Nkhanda yomwe yadulidwa iza pimidwa kuona muyeso wa kuda Kwa nkhanda yanu. Kadulidwe ka nkhanda yanu kazachitika kamodzi chabe ndi a dotolo aku dermatology clinic, izi siziza tenga nthawi ai.
Mwana wanu anga letsedwe utengako mbali nthawi ili lonse kulingana ndi kufuna kwa a dotolo. A dotolo anga chitizimenezi kopanda chiloledzo chanu. Mwana wanu anga leke utengako mbali nthawi ili lonse ndiposo sazataya mwayi ulionse.

Zoopsa

Kuwawa kwa pamalo pomwe nkhanda iza dulidwa.
Kutupa kwa pamalo pomwe nkhanda iza dulidwa.
Kumvela chizwezwe

Zotuluka za bwino zotengelako mbali kukafukufuku.

Ndichofunikira kuziwa Zotulukamo za bwino mu kafukufuku: Ngati mwa pimidwa kuda Kwa nkhanda yanu. Zotulukamo za bwino ndi izi; a dotolo aza ku thandizani kuziwa ngati ndinu chidangwaleza olo ai ndiposo kupewa matenda ya cancer. Mwaichi sitingathe kukulonjezani kuti mwana wanu azapezamo zotuluka za bwino. Ena angapezemo umwai kutsongolo.

Za chinsinsi

Dzina la mwana wanu sizatomoledwe mu kafukufuku aka ndiposo polemba buku la zo tuluka. Zonse ziza sungidwa mwa chinsinsi.

Malipilo

Kulibe malipilo alionse ya mwana wanu potengako mbali.

Mphamvu za otengako mbali

Mungathe utengako mbali mwachifuniro chanu. Mwana wanu alinayo mphamvu yosatengako mbali ndiposo anga leke utengako mbali nthawi ili lonse, sazataya mwayi ulionse.

Chiloledzo cha mwana wanu kuti atengeko mbali.

Ngati kholo, ndiloledza……. (Dzina la mwana) kuti atengeko mbali.

Tsiku ana badwilapo……

Chisindikizo cha

kholo……Tsiku……..

Chisindikizo chaomwe alutenga chiloledzo…….……Tsiku……..

Kofunsa mafunso

Ngati muli ndimafunso alionse, mabvuto olo kapena mwana wanu aluoneka kudwala atantha utengako mabli mungathe kutuma lamya, kalata olo email kuli atsongoleri a ERES CONVERGE panambala ndi keyala ili pansi apa:

Contact Details of Principal Investigator

Dailesi Ndhlovu
The University of Zambia
School of Medicine
Department of Anatomical Sciences
P.O. Box 50110
Ridgeway Campus
Cell No: +260969754663
Email: dnheaven@yahoo.com
LUSAKA
ZAMBIA

APPENDIX 7

APPROVAL LETTER FROM ASSISTANT DEAN (POSTGRADUATE)

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

Telephone : +260211252641
Telegram: UNZA, Lusaka
Telex: UNZALU ZA 44370

P.O Box 50110
Lusaka, Zambia

Email: assistantdeanpgmedicine@unza.zm

13th April, 2015

Ms. Dailesi Ndhlovu
Department of Anatomy
School of Medicine
UNZA
LUSAKA

Dear Ms. Ndhlovu,

RE: GRADUATE PROPOSAL PRESENTATION FORUM

Following the presentation of your dissertation entitled **"Determination of Melanin Content in the skin of Albinos at the University Teaching Hospital – Lusaka, Zambia";** your supervisor has confirmed that the necessary corrections to your research proposal have been done.

You can proceed and present to the Research Ethics.

Yours faithfully,

Dr. S.H. Nzala
ASSISTANT DEAN, POSTGRADUATE
CC: HOD, Anatomy

UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
14 APR 2015
ASSISTANT DEAN (PG)
P.O. BOX 50110, LUSAKA

APPENDIX 8

APPROVAL LETTER FROM ERES CONVERGE

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

2nd November, 2015

Ref. No. 2015-May-015

The Principal Investigator
Ms. Dailesi Ndhlovu
University of Zambia
School of Medicine
Dept of Human Anatomy
P.O. Box 50110,
LUSAKA.

Dear Ms. Ndhlovu,

RE: DETERMINATION OF MELANIN CONTENT IN THE SKIN OF ALBINOS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

Reference is made to your resubmission dated 26th October, 2015. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2015-May-015**
Approval and Expiry Date	Approval Date: 2nd November, 2015	Expiry Date: 1st November, 2016
Protocol Version and Date	Version-Nil	1st November, 2016
Information Sheet, Consent Forms and Dates	• English, Bemba, Nyanja.	1st November, 2016
Consent form ID and Date	Version-Nil	1st November, 2016
Recruitment Materials	Nil	1st November, 2016
Other Study Documents	-	1st November, 2016
Number of participants approved for study	20	1st November, 2016

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. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

APPENDIX 9

LETTER OF PERMISSION TO CONDUCT RESEARCH

The University of Zambia
School of Medicine
Department of Anatomy
P. O. Box 50110
Ridgeway Campus
LUSAKA

25th April, 2015

REPUBLIC OF ZAMBIA HEAD CLINICAL CARE 15 MAY 2015 UNIVERSITY TEACHING HOSPITAL P/BAG RW 1X, LUSAKA

REPUBLIC OF ZAMBIA SENIOR MEDICAL SUPERINTENDENT 29 APR 2015 UNIVERSITY TEACHING HOSPITAL PRIVATE BAG RW1X, LUSAKA

Approved

The Managing Director
University Teaching Hospital
P/B RW1
LUSAKA

UFS: The Head - Department of Anatomy

Dear Sir / Madam,

RE: PERMISSION TO CONDUCT RESEARCH

I am a postgraduate student pursuing Master of Science in Human Anatomy (MSc. PGY) degree programme at the University of Zambia, School of Medicine, Department of Anatomical Sciences. As part of the programme requirements I have to undertake a dissertation. It is in this premise that I write to seek permission to undertake a research at your institution. The title of the research is "**Determination of melanin content in the skin of albinos at the University Teaching Hospital-Lusaka".** I intend to carry out the study from June, 2015 to July, 2015 at the Histopathology and microbiology Department and Dermatology clinic.

It is my hope that the findings will help in strengthening the management of People with Albinism at UTH and the country as a whole.

Your favourable response to my request will highly be appreciated.

Yours faithfully,

Dailesi Ndhlovu (Computer No. 513802313)
Contacts: Email:dnheaven@yahoo.com
Cell: +260969754663

APPENDIX 10

DATA COLLECTION SHEET

New ID	Age	Sex	Melanin type	Melanin Status	Melanin Quantity