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***Research Article***

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

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## ABSTRACT
*Background:* Melanin is a skin pigment that determines skin colour and plays a role in photoprotection. 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 study was aimed at demonstrating and quantifying the melanin in albino skin compared to normal indigenous black skin. The study was conducted at the University Teaching Hospital (UTH), Lusaka, Zambia.
*Methods:* This was an analytical cross-section study in which 12 clinically diagnosed albinos and 12 non-albino black indigenous Zambians were recruited. Skin biopsies from the study participants were sectioned, stained with H&E and Mason-Fontana. The latter was used determine the presence of melanin in the skins. Also, melanin was extracted from the skin and assayed using the Human melanin ELISA kit and measured using a spectrophotometer. Independent t test was use to compare the melanin concentration.
*Results:* Mason-Fontana technique was able to demonstrate melanin in one albino biopsy (n=1) and in the rest the results were negative (n=11). However, using the human melanin Elisa kit all biopsies contained melanin. Melanin concentration of albinos was (102 ±62.5 pg/ml) and the normal indigenous blacks was (127 ±29.0pg/ml), p = 0.223.
*Conclusion:* Not all individuals who have been clinically diagnosed with albinism lack melanin even though phenotypically may appear without any 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. Such variations in clinical diagnosis and the results obtained by the technique used could explain the varied response to sunlight exposure and thus development of cancer.

***Keywords****: Albinism, Melanin, Skin*

## INTRODUCTION
The human skin pigment melanin represents one of the most visible markers of human variation and skin disorders (King *et al.,* 1995).Humans with skin genetically lacking partial melanin or with total lack of melanin are frequently referred to as albinos(Van Dorp, 1987). Albinism is due to a genetic mutation so the inheritance pattern is quite variable (Kamaraj and Purohit, 2014). Oculocutaneous albinism (OCA) is mostly an autosomal recessive disorder, whereas ocular(OA) 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 *et al.,* 2012). 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)(Summers, 2009). 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 (Oetting, 2000) . In the more prevalent OCA2 type there is some level of tyrosinase activity (Summers, 2009). 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 (Lund and Gaigher, 2002, Kagore and Lund, 1995). The prevalence of albinos in Zambia is estimated at 1 in 565 (Central statistics office population census, 2010).

Synthesis of melanin in the melanocytes takes place within highly specialized membrane bound intracellular organelles called melanosomes (Yoo *et al.*, 2007). 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). Thus melanin formation and transformation in human skin is an important mechanism for protection of skin from ultraviolet light (Yoo *et al.*, 2007).

The problems associated with albinism are attributed to a defect in the melanin synthesis pathway, in which melanocytes fail to produce tyrosinase and results in absence or reduced formation of melanin (Okulicz *et al.*, 2003). Individuals with albinism 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). There is an inverse correlation between the degree of constitutive pigmentation (melanin) and the risk of sun-induced skin cancers (Manga *et al.*, 2013). Skin cancers are indeed a major cause of morbidity amongst albinos in the tropics, from a young age these patients face battles against these cancers (Opara and Jiburum, 2010). It can be predicted that the already known medical problems facing people living with albinism will escalate thus qualifying albinism as a public health issue deserving further attention (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 can be used to support other similar studies. The objective of this study is to demonstrate and quantify the melanin in albinos compared to normal indigenous black skin.

## MATERIALS AND METHODS

This was a laboratory- based analytical cross-sectional study conducted at the Pathology and Dermatology Departments at the University Teaching Hospital (UTH), Lusaka, Zambia from January to May 2016. The study included 24 participants: - 12 clinically diagnosed albinos, attending the dermatology clinic and 12 normal-skinned comparison group of indigenous black patients undergoing excisional or incisional biopsy. Our study protocol was approved by Excellence in Research Ethics and Science (ERES) CONVERGE and given the ethical approval number.

Punch biopsies were taken on the radial side of the forearm, of about 3mm to 5mm diameter and were cut into two parts. One part was fixed in 10% neutral buffered formalin, dehydrated using a graded series of alcohol baths, cleared with xylene, infiltrated with paraffin to make blocks, and sectioned 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 ready for staining. Tissue sections were stained with H&E for demonstrating the general morphology, and with Mason Fontana staining technique to demonstrate melanin. The slides were studied using a light microscope. In addition to this process melanin was extracted from unprocessed (fresh) tissue and assayed using the Human melanin ELISA kit, and was measured using a spectrophotometer. The detection range of the kit was from 12.5pg/ml to 1000pg/ml and all procedures were done according to the Manufactures' instruction (Mybiosource USA).

## RESULTS

During the period under review, a total of 12 clinically diagnosed albinos formed the study population all of them where of black African heritage. The ages ranged from 14 to 40 years with a mean and median

age of 23 and 20.5 respectively. The majority were aged below 23 years.

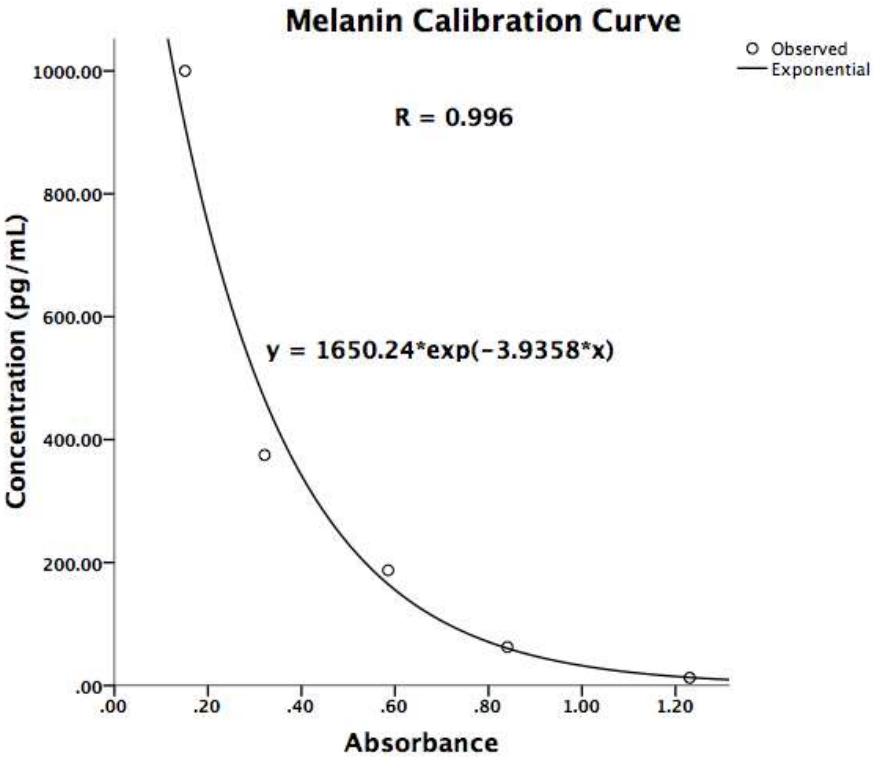


**Figure 1: Calibration Curve for the ELISA test**
**The relationship between melanin concentration and optical density at 450nm.**

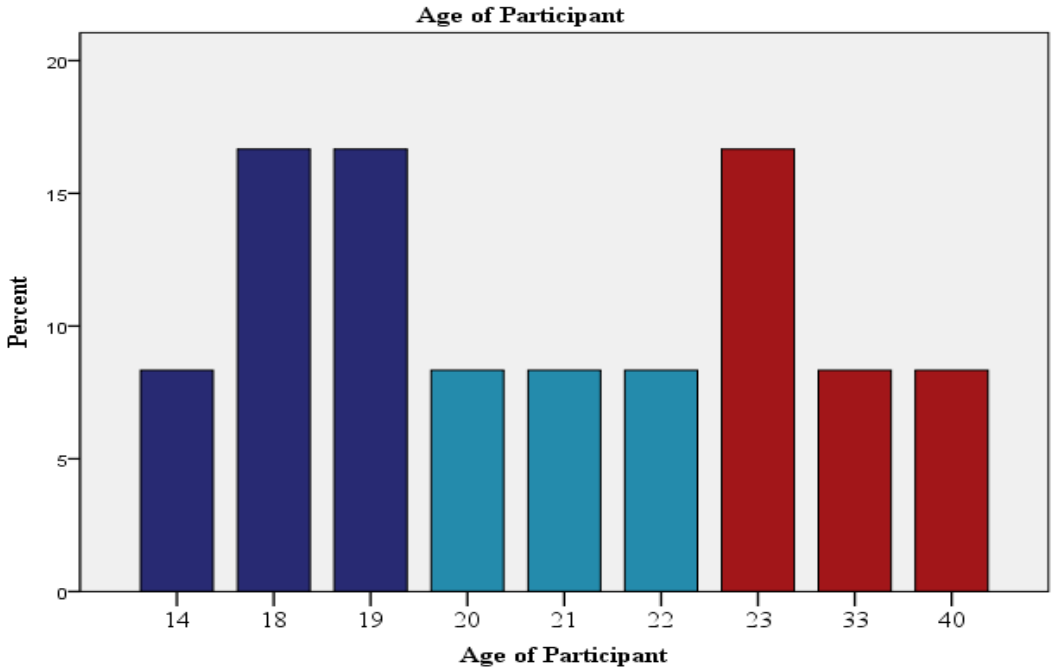


**Figure 2: Age distribution**

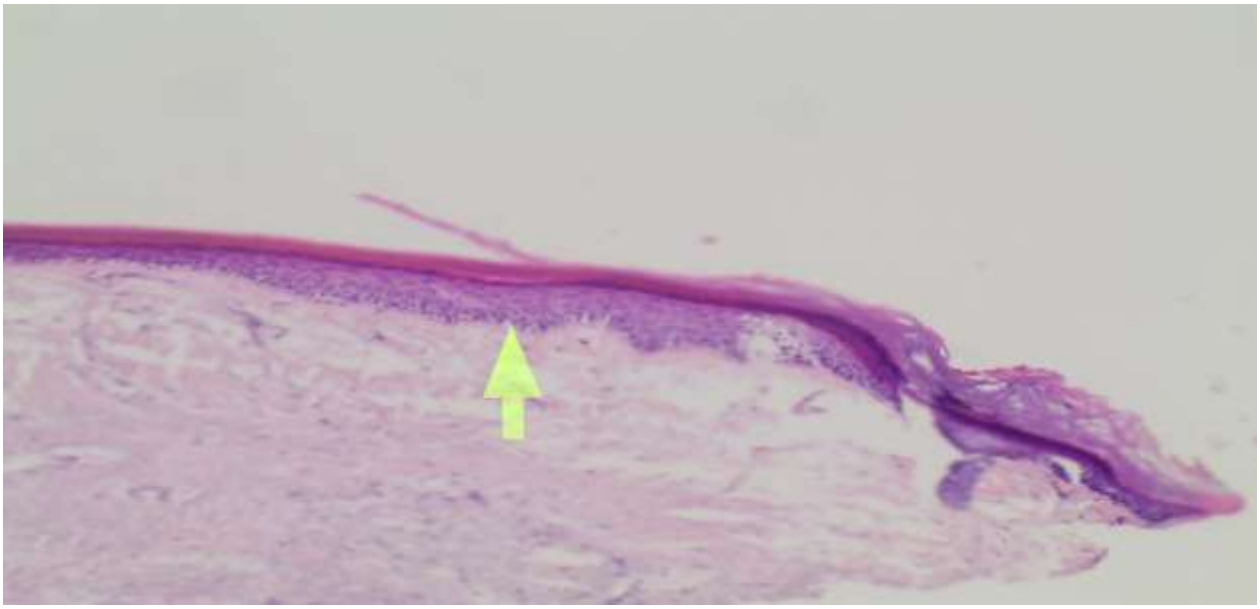

**Figure 3: Non-diseased albino skin (H&E stain, Magnification x10). This shows the epidermis (Arrow pointing at the melanocytes in the basal layer)**

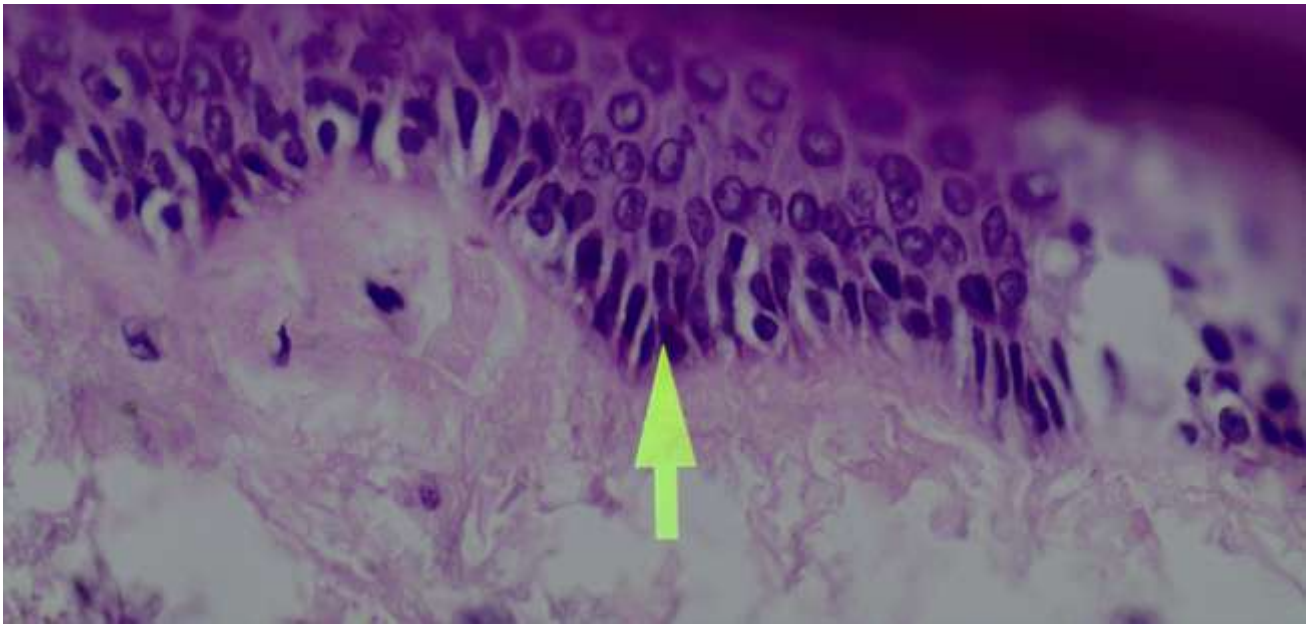

**Figure 4: Non-diseased skin of an albino (H&E stain, magnification x40). This shows the melanocytes scattered in the basal layer of the epidermis. Melanocytes (arrow) have smaller nuclei and inconspicuous cytoplasm compared with the surrounding keratinocytes.**

Mason-Fontana technique was able to demonstrate melanin in one albino biopsy (n=1) and in the rest the results were negative (n=11).In the normal indigenous black skin (n=12) were positive.

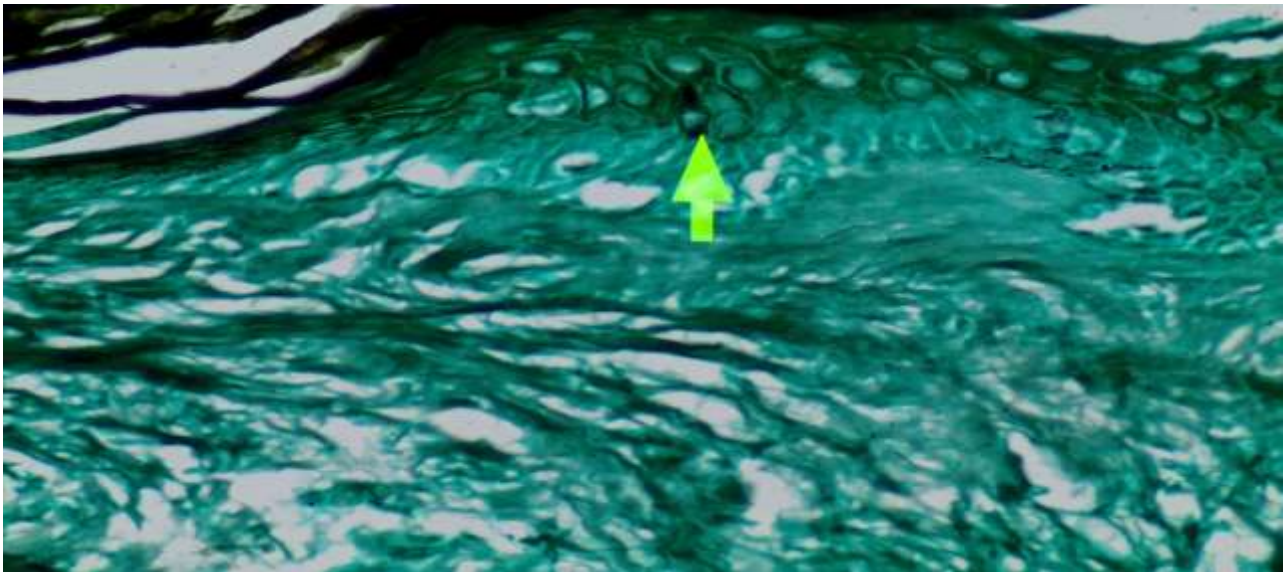

**Figure 5: Non-diseased skin of an albino (Masson-Fontana stain, magnification x40). This shows some melanin in keratinocytes in the epidermis layer.**

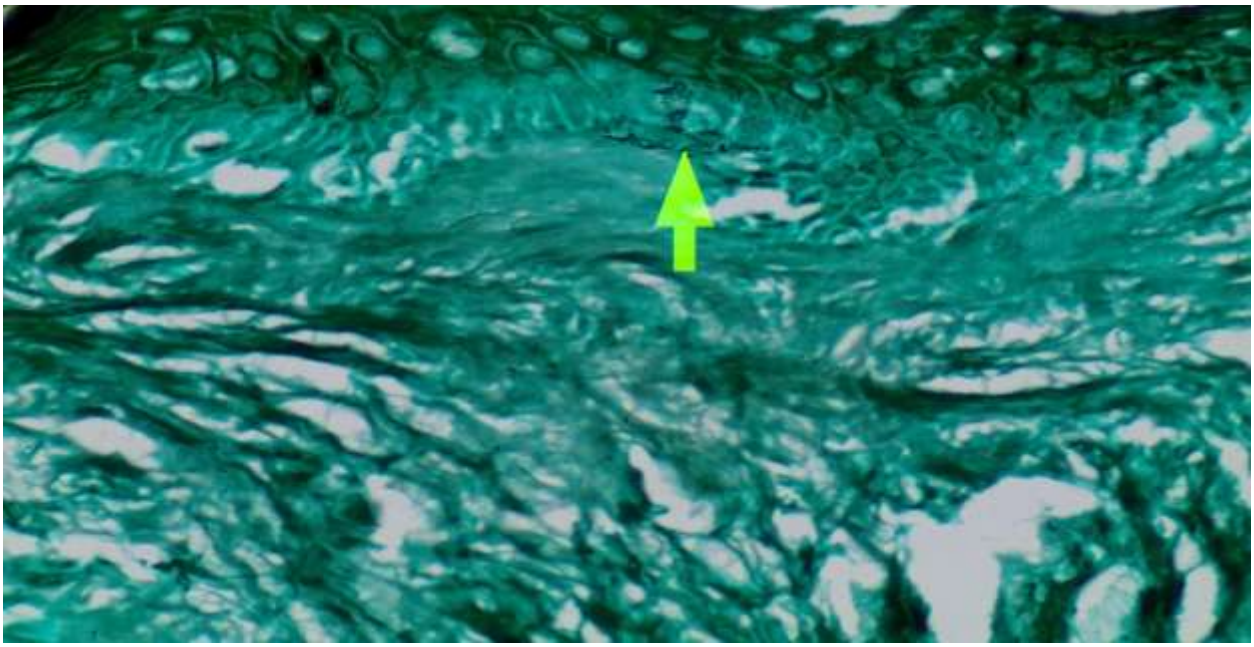

**Figure 6: Non-diseased skin of an albino (Masson-Fontana stain, magnification x40).This shows the presence of the melanin (arrow) in the melanocytes in the basal layer of the epidermis. Any substance with reducing properties (argentaffins) will appear black.**

Our results showed that the melanin levels in albino skins were (102 ± 8.37pg/mL) and non-albino were (127 ± 17.32), p = 0.223. Melanin concentration was compared in both groups using the Independent t-Test

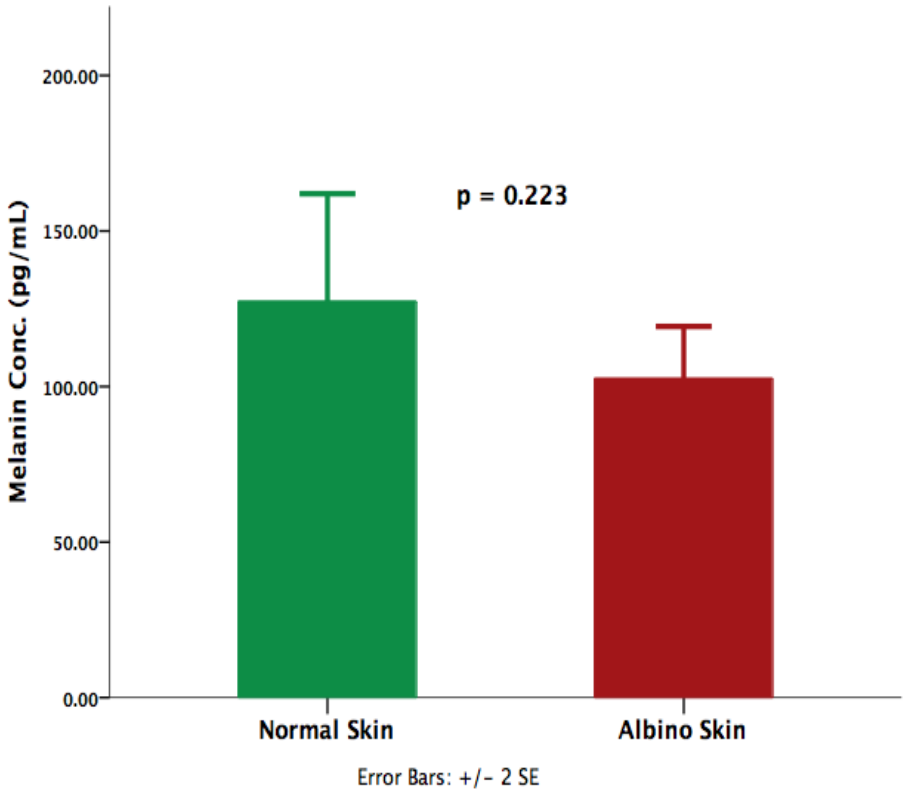


**Figure 7: Mean melanin concentration of normal black skin and albino skin**

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.

**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 |

## 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 or even develop cancer.

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 and Bancroft, 2002). The Masson-Fontana method is not specific for melanin as other reducing substances will also give positive reaction. In a similar a study done by (Broodbakker *et al.,* 1983) on 47 Caucasian albinos and 10 Caucasian controls their findings were variable as 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) 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) .

Using the ELISA method for detection of melanin 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 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 and Hearing, 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, 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 remain 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).

## CONCLUSION

The study established that all the albinos in this study had melanin in their skin. This study revealed that not all individuals who have been clinically diagnosed with albinism lack melanin. 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.

## 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.

Future studies should be done to determine the reliability of clinical diagnosis of albinism.

Future researches should be done on the quality and functionality of melanosomes

## COMPETING INTERESTS

All the authors declare they have no competing interests.

## AUTHORS' CONTRIBUTION

Dailesi Ndhlovu conceived the study, participated in the design, coordination of the study, drafted the manuscript and participated in all the laboratory work.
Krikor Erzingatsian and Ephraim Zulu supervised the study and contributed in data analysis, manuscript writing and editing. Musalula Sinkala participated in extraction, assay procedure and measurement of melanin. Pascal Polepole participated in the histology laboratory work. Elliot Kafumukache, Patience Buumba and Moono Silitongo contributed to manuscript editing. All the authors read and approved the final manuscript.

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