

A DESCRIPTIVE STUDY OF MELANOMA SKIN CANCERS IN PATIENTS
ATTENDING CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL
AND HELEN JOSEPH HOSPITAL FROM JULY 2013 TO JUNE 2016

TEMITOPE AWOTEDU

1325081

Submitted to the Faculty of Health Sciences, University of the Witwatersrand, in
partial fulfillment of the requirements for the degree of Master of Medicine in
Dermatology

Johannesburg ,2018

Declaration

I, Temitope Awotedu, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Dermatology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this, or any other University.

.....

.....day of, 2018

Dedication

In loving memory of my Father, Prof. A.A. Awotedu

24/02/1949 – 26/07/2017

Abstract

Introduction

Melanoma is a malignant tumour that arises from melanocytic cells, which are neural crest derived cells. They are located in the basal layer of the epidermis, uvea of the eye, vaginal epithelium, meninges, bone and inner ear.

Although more common in fair skinned individuals, melanomas are still one of the most common skin cancers seen in dark skinned people and are the most aggressive of all the skin cancers, amongst all racial groups.

Aims

The aim of this research was to describe the clinicopathological features of melanoma skin cancer in patients attending Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital.

Methods

This study was a retrospective record review of 128 patients, with histologically confirmed melanoma skin cancers, based on biopsies submitted to the National Health Laboratory Service (NHLS). The histology report provided the demographic data, confirmation of melanoma skin cancer, histologic subtype, other risk factors associated with development of melanoma skin cancers as well as the site of these tumours.

Results

The mean age ($\pm$ SD) age of the patient population was 60 (15.6) years with a significant difference between men (63.2 years) and women (57.5 years) ($p = 0.0364$)

The most common type of melanomas in males in all age groups were on the trunk (29%) and in females on the lower extremities (36%).

The main clinical subtypes found, were nodular melanoma (NM), acral lentiginous melanoma (ALM) and lentigo maligna melanoma (LMM), with an incidence of 63.4% in NM and 11,7% in both ALM and LMM.

Females presented with a higher percentage of invasive tumours (36%) than men (31%).

Conclusion

The frequency of melanoma was the same in both sexes and there was a notable difference in the anatomic site in males and females. The anatomic sites of melanoma also differed between the older and younger age group. The most common subtypes documented in our patient population were nodular melanoma, acral lentiginous melanoma and lentigo maligna melanoma.

Acknowledgements

Thank You Heavenly Lord, the Author and Finisher of my faith.

My thanks go to the following people:

- Prof. M.J. Hale for his supervision and expertise on the subject matter and to Prof. D. Modi, my supervisor.
- Dr. Alison Bentley for her assistance with statistical analysis.
- Ms. Fadila Ebrahim Ibrahim for their help in retrieving archived material.
- My dear husband, Ayo, for his constant support and encouragement throughout this process and our darling daughter Tiwalade Leah for making it all worth it.
- To my mum and sisters, the best cheerleaders anyone could ask for.

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Abbreviations

AAD - American Academy of Dermatology

AJCC - American Joint Committee on Cancer

AIDS - Acquired Immunodeficiency Syndrome

ALM – Acral Lentiginous Melanoma¹¹¹

ASR - Age Standardised Rate

AMS - Atypical Mole Syndrome

BCC - Basal Cell Carcinoma

BRAF - Serine protein kinase

CDKN2A - Cyclin-Dependent Kinase Inhibitor 2A

CGH - Comparative Genomic Hybridization

c-KIT - Receptor tyrosine kinase

CM - Cutaneous Melanoma

CMJAH - Charlotte Maxeke Johannesburg Academic Hospital

CMM - Cutaneous malignant melanoma

CPD - Cylopyrimidine Dimer

CT – Computed Tomography

DNA – Deoxyribonucleic acid

FAMM - Familial atypical multiple mole syndrome

FDA - Food and Drug Administration

FISH - Fluorescence in situ Hybridization

HIV - Human Immunodeficiency Virus

HJH - Helen Joseph Hospital

LDH - Lactic Dehydrogenase

MAPK – Mitogen- Activated Protein Kinase

MC1R - Melanocortin 1 Receptor

MPNSTs - Malignant Peripheral Neuroectodermal Tumours

M0 - No distant metastasis

M1 - Distant metastasis

M1a - Metastasis to skin, subcutaneous tissues, or distant lymph nodes

M1b - Metastasis to lung M1c - Metastasis to all other visceral sites or distant metastasis at any site associated with elevated levels of serum

Mx - Distant metastasis cannot be assessed.

NBLP - Neonatal Blue Light Phototherapy

NCCN – National Comprehensive Cancer Network

NF1 – Neurofibromatosis type 1

NHLS – National Health Laboratory Service

NRAS - Neuroblastoma RAS Viral Oncogene homolog

PTEN - phosphatase and tensin homolog

PUVA - Psoralen and ultraviolet A radiation

pRb - Retinoblastoma Protein

ROS - reactive oxygen species

SCC - Squamous cell carcinoma

SEER - Surveillance Epidemiology and End Results

SLNB - Sentinel Lymph Node Biopsy

SSM – Superficial Spreading Melanoma

SUNMEL - Study Using Ultrasound to Examine lymph glands after melanoma removal

UK - United Kingdom

USA - United States

UV - Ultraviolet UVR – Ultraviolet Radiation

UVA - Ultraviolet -A rays

UVB - Ultraviolet -B rays

UVC - Ultraviolet -C rays

WHO - World Health Organization

6,4-PP - 6,4 photoproducts

CHAPTER ONE: INTRODUCTION

1.1. Introduction

A melanoma is a malignant tumour that arises from melanocytes ,primarily the skin (Garbe et al., 2016). Melanoma can also arise in the eye (uvea, conjunctiva and ciliary body), meninges and on various mucosal surfaces.

Skin melanoma is the most rapidly increasing cancer in white populations (Garbe et al., 2001).

1.2. Background and History

The first recorded descriptions of melanoma (derived from the Greek words *melas*, "dark" and *oma* "tumor") in history appeared in the writings of Hippocrates of Cos in the 5th century, B.C., and later in those of the Greek physician Rufus of Ephesus (Urteaga & Pack, 1966).

Although archeological evidence of cancer has been quite scant, tumours can be diagnosed from the skeletons of those afflicted by osteolytic bone metastases or bony osteosarcomas. The first evidence of melanoma comes from the diffuse melanotic metastases found in the skeletons of Pre-Colombian mummies (radiocarbon dated to be 2400 years old) from Chancay and Chingas in Peru (Urteaga & Pack, 1966).

European medical literature, including the work of Bonet (1651), Henrici and Northangel (1757), made numerous references to “fatal black tumours with metastases and black fluid in the body”. Isaac Parrish documented the first North American case of melanoma in 1837. His patient, a 43-year old widow, was admitted to Wills Hospital in Philadelphia, PA with a “fungous tumor upon her toe”.

The melanoma was described to have originated from a “purple mark or mole about the size of a mulberry, which was supposed to have been congenital”. Despite the application of a poultice of ground elm, purgatives and leeches to the groin, the patient soon succumbed to her disease (Parrish. I. 1837).

Pathologist, Sir Robert Carswell, coined the word melanoma in 1838. He contributed illustrations of various diseases in his book titled, “*Illustrations of the Elementary Forms of Disease*”, among them were melanomas .

CHAPTER TWO

2.1 Aims and Objectives

The aim of this research is to describe the clinicopathological features of cutaneous melanoma in patients attending Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital. There is no report of other studies being conducted at either of these two hospitals. Determining the clinicopathological features of cutaneous melanoma will help determine the morbidity associated with this condition, as well as the burden this cancer places on the health care resources. This in turn will facilitate in the re-allocation of health care resources and implementation of preventative strategies that include wearing protective clothing, sunscreens and avoidance of excessive sun exposure.

STUDY OBJECTIVES

- I. To calculate the number and percentages of patients with cutaneous melanomas in the different genders, age groups, tumour sites (head and neck, upper and lower extremities, trunk), and racial groups.
- II. To compare the numbers and percentages of melanoma skin cancer in males and females according to the tumour sites (head and neck, upper extremities, lower extremities, trunk or other sites).
- III. To calculate the number and percentages of the different histologic subtypes of melanoma skin cancer (e.g. superficial spreading, nodular etc.).
- IV. To calculate the number and percentages of patients with other risk factors associated with development of melanomas e.g. genodermatoses (xeroderma pigmentosum, oculocutaneous albinism), immunosuppression, fair skin etc..

CHAPTER THREE: LITERATURE REVIEW

3.1. Epidemiology

3.1.1. Global Incidence and Prevalence of Melanoma

The melanoma prevalence in the United States was reported to be 6 per 100 000 cases in the early 1970s and 18 per 100,000 cases a year at the beginning of the year 2000 (Garbe et al., 2001). Incidence rates in central Europe increased in the same time period, from 3-4 cases to 10-15 cases per 100,000 inhabitants in similar time frames, which is very similar to the increase in the United States (Garbe et al., 2001). The highest incidence rates in Europe were in Scandinavian countries (Mansson-Brahme et al., 2003) and this is postulated to be as a result of increased hours to sun exposure and increased sun seeking behavior. Significant increases in melanoma incidences were also found in central and southern Europe (Osterlind et al., 1988). The Mediterranean countries had the lowest incidence rates, suggested to be due to their darker skin type (Balzi et al., 2003). The highest incidence rates were reported in Australia and New Zealand, with 30-60 cases per 100,000 inhabitants per year (Marrett et al., 2001).

Melanoma is the most common type of cancer in 25-29 year olds and the second most common malignancy in 15-29 year olds in the U.S (Jemal et al., 2009). This malignancy strikes individuals almost a decade before solid organ tumours arise (Erdel et al., 2010).

As shown in Table 1, Australia diagnosed 13,941 new cases of cutaneous melanomas (8,392 males and 5,549 females), in 2017.

Cancer type	New cases 2017	% of all new cancers 2017
Breast	17,730	13.2
Breast (among females)	17,586	28.4
Colorectal (bowel)	16,682	12.4
Prostate (among males)	16,665	23.1
Melanoma	13,941	10.4
Lung	12,434	9.3

Table 1: Estimated most common cancers diagnosed in 2017 - Australian Institute of Health and Welfare 2017

3.1.2. Incidence and Prevalence of Melanoma in Africa

There is scant data existing on the prevalence of melanomas in Africa, particularly outside of South Africa, however, globally, the incidence of melanomas amongst Africans, African-Americans, Indians and the Chinese, have remained stable over the past thirty years (Swerdlow et al., 1990).

Melanomas are rare among black populations in Africa and most cases are of the acral lentiginous subtype (Asuquo & Ebughe, 2009). This begs the following question: 'What is the aetiology of cutaneous melanomas in dark-skinned populations?'. Mutations in NRAS and BRAF seen in Caucasians, resulting from intermittent solar exposure, were infrequent in cutaneous melanomas affecting black Africans, suggesting a different aetiology (Asklen et al., 1990).

Chronic ulcers, oculocutaneous albinism, inflammation and UV exposure were identified risk factors for both melanoma and non-melanoma skin cancers in a small study in Nigeria, published in 2009 by Asuquo & Ebughe. This retrospective study conducted in Port Harcourt, Nigeria, identified a mere 15 cases of melanoma at the University Hospital over an 11-year period (Seleye-Fubara and Etebu ,2005). The small number of patients in this particular study could be attributed to: delays in diagnosis, resulting from the incorrect sentiment that dark skinned population groups do not get melanomas.

In this study, the lower limbs and feet were the most common anatomic site of melanoma; these melanomas were diagnosed at stage 3 or higher, and the majority of the cases were female. Africans have greater levels of melanin than Caucasians which provide protection from the carcinogenic effects of solar radiation. The acral lentiginous melanomas seen in Africans and African-Americans, develop on body sites such as the nail beds, palms and soles (Seley-Fubara & Etebu,2005).

3.1.3. Incidence and prevalence of melanoma in South Africa

South Africa has a multi-racial population. A census conducted in 2001 revealed a population of 79.4% blacks, 9.2% whites, 8.8% coloureds and 2.6% Asians (Norval et al., 2014).

South Africa's geographical location, an average annual temperature of 22° C and high ultraviolet index, predispose individuals to the development of skin cancer (Norval et al., 2014). The incidence of skin cancer in South Africans, especially in whites, is amongst the highest in the world (Wright et al, 2012). A retrospective analysis of melanomas in black South Africans revealed that in most age groups women were affected more than men (Lodder et al., 2010). The data also revealed that the majority of these melanomas were acral lentiginous, ulcerated and > 2 mm in Breslow thickness; all of which are poor prognostic indicators.

Age standardized incidence rates of cutaneous melanomas in white males and females (15.88/100000 and 12.68/100000 respectively), have been reported in South Africa (Sewram, 2016). The incidence rates in the city of Cape Town, are in excess of 60/100000. Cutaneous melanomas have the highest incidence in White, followed by the Coloureds (persons of mixed ancestry) and a considerably lower incidence in both the Black and Asian population (Sewram, 2016).

According to the National Cancer Registry of South Africa in 2013, malignant melanoma was the 8th most common histologically diagnosed cancer in both men and women (Table 2 and 3) and ranked the 3rd most common cancer in white males (Table 4). According to the WHO, the incidence of both non-melanoma and melanoma skin cancers has been increasing over the past decades, and the WHO estimates that a 10 % decrease in ozone levels will result in an additional 4 500 cases of cutaneous melanomas worldwide (WHO International Agency for Research in Cancer,2013).

Of note, a retrospective analysis of cutaneous melanomas in the Coloured population of South Africa, revealed histological subtypes and anatomic distributions that were characteristic in black populations, yet the 5-year survival rates in these patients were similar to those seen in white populations (Swan et al., 2003). These findings further suggest the influence of genetics in the progression and development of melanomas.

Type of Cancer for All Men 2013	Actual No of Cases	Estimated Lifetime Risk	Percentage of All Cancers
Prostate Cancer	6 778	1:18	18,86%
Colorectal Cancer	1 906	1:39	5,30%
Lung Cancer	1 766	1:76	4,91%
Cancer of Unknown Primary*	1 712	1:86	6.63%
Kaposi Sarcoma	986	1:315	2.74%
Cancer of the Bladder	952	1:147	2,65%
Non-Hodgkin's Lymphoma	882	1:221	2,45%
Cancer of the Oesophagus	844	1:167	2,35%
Malignant Melanoma	819	1:183	2,28%
Cancer of the Stomach	743	1:194	2,07%
All Cancers	35 940	1:6	

Table 2: Top 10 Most Common Cancers in Men in South Africa. National Cancer Registry 2013

Type of Cancer for All Women 2013	Actual No of Cases	Estimated Lifetime Risk	Percentage of All Cancers
Breast Cancer	8 132	1:28	22,22%
Cervical Cancer	5 701	1:44	15,58%
Cancer of Unknown Primary (CUP)*	1 741	1:123	4,76%
Colorectal Cancer	1 542	1:145	4,21%
Cancer of the Uterus	1 152	1:159	3,15%
Lung Cancer	923	1:205	2,52%
Non-Hodgkin's Lymphoma	802	1:327	2,19%
Kaposi Sarcoma	728	1:503	1,99%
Malignant Melanoma	723	1:349	1,98%
Cancer of the Oesophagus	651	1:319	1,78%
All Cancers	36 599	1:9	

Table 3: Top Ten Most Common Cancers of Women in South Africa. National Cancer Registry 2013

Type of Cancer for White Men 2013	Actual No of Cases	Estimated Lifetime Risk	Percentage of All Cancers
Prostate Cancer	3 114	1:9	15,42%
Colorectal Cancer	1 041	1:30	5,15%
Malignant Melanoma	663	1:47	3,28%
Cancer of the Lung	636	1:47	3,15%
Cancer of the Bladder	623	1:54	3,09%
Cancer of Unknown Primary (CUP)*	482	1:62	3,09%
Cancer of the Stomach	332	1:93	1,64%
Non-Hodgkin's Lymphoma	254	1:123	1,26%
Cancer of the Kidney	180	1:160	0,89%
Cancer of the Oesophagus	156	1:178	0,77%
All Cancers	20 201	1:3	

Table 4: Top 10 Most Common Cancers in White Men in South Africa according to the National Cancer registry of 2013

3.2 Risk Factors

Risk factors for developing melanoma can be divided into extrinsic and intrinsic factors (Table 5). These factors include geographic factors, exposure to UV radiation, e.g. via sunlight or indoor tanning salons, heavy metal and chemical exposures, skin phenotype family history of cancer and presence of nevi or some medical disorders (Rigel, D., 2010).

Extrinsic Risk Factors		Intrinsic Risk Factors	
Ultraviolet Radiation	UV exposure, especially blistering sunburns in childhood, correlates with melanoma risk.	History of Skin Cancer	A family and personal history of melanoma and non-melanoma skin cancer increases risk of melanoma
	Countries located closer to the equator with increased sun exposure have a higher melanoma incidence rate	Nevi	A large number of congenital nevi, nevi with large diameter, and dysplastic nevi are all associated with an increased risk
	Indoor tanning bed use increases incidence and mortality rate of melanoma	Medical History	Immunosuppressive states and a past medical history of non-cutaneous skin cancer increase the risk of melanoma
Medication	Psoralen, UVA light therapy and neonatal blue light phototherapy are associated with an increased risk of melanoma	Defective DNA Repair	Individuals diagnosed with xeroderma pigmentosum cannot repair UV induced DNA damage
Environmental Exposure	Polyvinyl chloride, heavy metals and pesticides are associated with an increased risk of melanoma	Skin Complexion	Fair skin, inability to tan, and increased susceptibility to UV induced sunburn increase risk for melanoma

Table 5: Risk Factors for Melanoma Skin Cancers (Adapted from Wolf-Horrel et al. 2015)

3.2.1. Geographical Factors

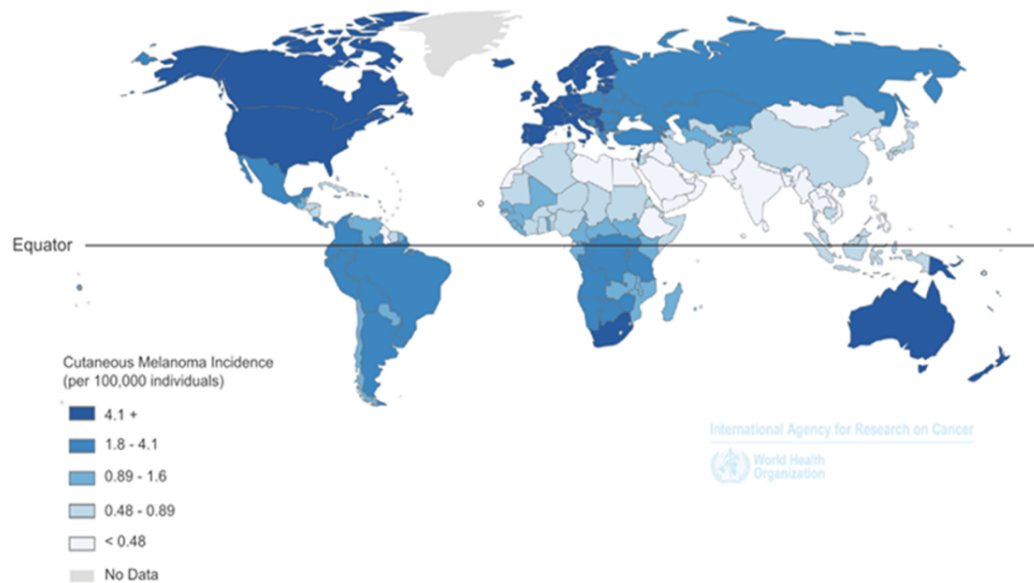
The worldwide distribution of cutaneous melanomas, demonstrates the importance of UV radiation in the aetiology. Countries that have a predominantly white population and a close proximity to the equator have higher incidences of melanomas (Lens and Dawes, 2004). Australia being sub - equatorial, has the highest global incidence of cutaneous melanomas, with a risk of 1 in 50 individuals being affected (Figure 1).

The World Health Organization (WHO) reports that both these countries have an age adjusted incidence of 35.1/100,000 people (GLOBACON ,2010).

In spite of also having a predominantly fair-skinned population of Scandinavian ancestry, Norway has a relatively low incidence of melanoma, which may be due to the lower UV index and high altitude (Moan et al., 2009).

Comparatively, Central America has a significantly lower incidence rate (1.5/100 000) than North America (13.8/100,000), presumably due to having a largely dark-skinned population (Figure 1)

A



B

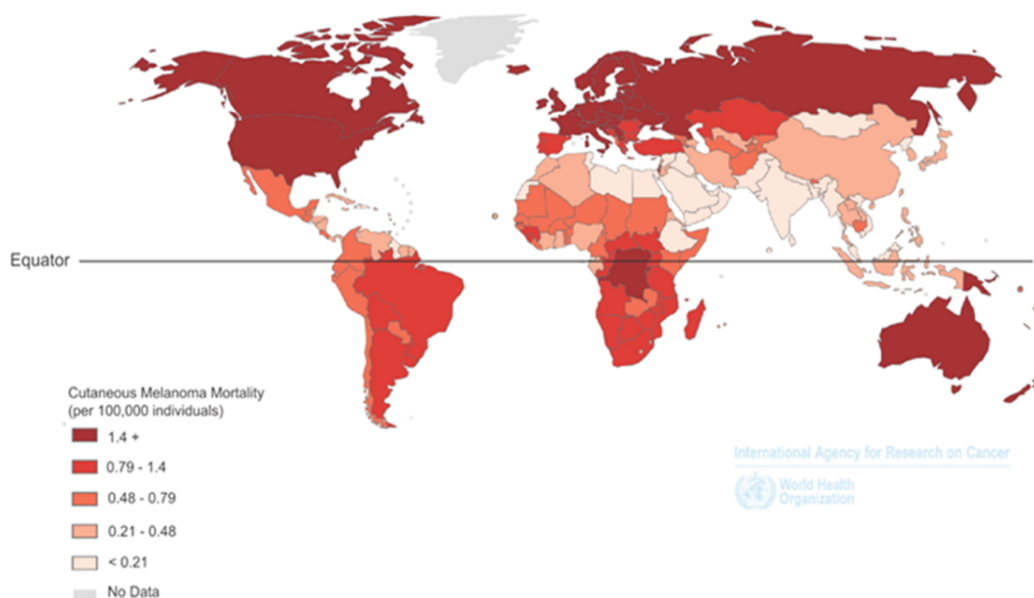


Figure 1: Geographical Variation in Melanoma Incidence (A) and Mortality (B) - Adapted from GLOBACON 2010, Cancer Incidence and Mortality Worldwide

3.2.2. Ultraviolet Radiation

Ultraviolet (UV) radiation, likely the most important environmental carcinogen in melanomagenesis, is found in natural sunlight and artificial tanning sources (Wolf - Horrel et al., 2015). UV energy is conventionally divided into three wavelengths: UVA (320-400 nm), UVB (290-320 nm), and UVC (100-280 nm). Each type of UV energy has its own distinctive biophysical characteristics, energy profile and effects on biologic tissue. UVA and UVB contribute to the pathogenesis of melanoma via several mechanisms (Wolf - Horrel et al., 2015). Both UVA and UVB cause DNA damage indirectly through the generation of oxidative injury and free radicals. Free radicals change DNA in ways that ultimately affect base-pairing, which can lead to mutations. Oxidation of guanine, causes formation of 8-oxo-dG which pairs with A instead of C bases; this change leads to point mutations if not repaired (Wolf- Horrel et al.,2015). It is thought that up to 80% of cutaneous melanomas may result from indirect DNA damage, buttressing the importance of decreased UV radiation in the pathogenesis of melanoma (Wolf-Horrel et al., 2015). The link between melanoma risk and UV exposure is particularly strong with the UVA component, providing further evidence that UV-A is a significant causative factor in melanomagenesis (Moan et al., 1999).

UV-B radiation, in comparison to UV-A radiation, is higher in energy and directly affects pyrimidines in the double helix, causing a photochemical reaction. By the absorption of UV-B radiation by pyrimidines, two major photo products are generated: cyclopyrimidine dimers (CPD's) and 6,4 photo products (Bhatia &Thompson, 2009). Both photo products distort the DNA double helix and if left unrepaired, may lead to ultraviolet signature transition mutations (G →T and G→C mutations), (Dumaz et al., 1997).

It is mostly through the identification of these UV signature mutations that we know that UV radiation is a major risk factor for skin cancers, including cutaneous melanomas. (Hodis E et al., 2012).

UV-C, the UV component with the highest energy per photon, can cause significant damage to cells, however, the majority of UVC is absorbed by the ozone layer, thus not thought to be a very important contributor to the disease. Melanomas typically occur on sun exposed skin, and exposure to UV radiation correlates with an increased incidence of cutaneous melanomas and mortality rate, seen in Figure 1.

Intermittent sun exposure confers a higher risk for developing melanoma than continual exposure (Walter & Marrett, 1999), but the age at which the exposure causes the greatest damage is still controversial. Some studies suggest that sun exposure at a younger age is more likely to be associated with the development of melanoma while other studies suggest that age of exposure is less important than the cumulative dose of UV radiation (Pfahlberg et al., 2001). Regardless of age, sunburn is a major risk factor in the development of melanoma, and the risk doubles, with more than 5 sunburns according to Pfahlberg et al (2001), or one or more blistering sunburns. Some studies have estimated that up to 80% of malignant cutaneous melanomas may result from indirect DNA damage, highlighting the significance of lower UV energy in the pathogenesis of melanoma (Davies et al., 2002).

There has been an increase in the widespread use of indoor tanning beds since their invention in the early 1970's. More than 30 million individuals use indoor tanning salons and over 70% of these individuals are female (Swerdlow & Weinstock, 1998).

The prevalence of artificial tanning is concerning, especially among young adolescents. The earlier tanning begins, the more risk there is for carcinogenesis. In individuals between 18-29 years of age diagnosed with melanoma, 76% of individuals had previously used artificial tanning devices (Boniol et al., 2012). One tanning session increases the chance of an individual developing melanoma by 20%, and each additional session per year increases risk by another 2% (Boniol et al., 2005). The International Agency for Research on Cancer classifies UV tanning devices in Group 1 - the most dangerous oncogenic substances (El Ghissassi et al., 2009). Among salon establishments, artificial tanning beds vary in their delivered dose and the amount of UVA and UVB radiation is not regulated (Balk et al., 2013) making artificial tanning very dangerous. Experts have suggested that the increased use of artificial tanning salons over the last three decades may be a major contributing factor to the continued increase in the incidence of melanoma.

3.2.3. Medication and Chemical Exposure

Medications that have great benefit for disease treatment also increase susceptibility to cancer. Psoralen and ultraviolet A radiation (PUVA) is an effective treatment for psoriasis and other dermatologic conditions. Psoralen increases reactivity to UV radiation, therefore the combination of psoralen and UVA causes a substantial degree of cellular damage (Stern, 2001). Patients who receive PUVA have a 10-fold increase in risk of developing melanoma 15 years after treatment and the risk increases with number of treatment sessions (>250) and time interval between treatments (Stern, 2001).

Neonatal blue light phototherapy (NBLP) is another example of photo therapy that may lead to an increased risk for the development of melanoma. NBLP is used to treat neonates with elevated bilirubin levels. Reports are conflicting as to whether exposure to NBLP causes an increase in nevi number and melanoma susceptibility. One study on 8112 Caucasian children showed no correlation between the number of nevi and exposure to NBLP (Bauer et al., 2004).

However another study demonstrated that treatment with NBLP correlated with size of nevus (nevi > 2mm in diameter significantly correlated with exposure to NBLP) or with presence of atypical nevi (Matichard et al., 2006),.

Exposure to heavy metals and certain chemicals are associated with an increased risk of melanoma, presumably through mutagenic changes to the DNA in melanocytes. Exposure to polycyclic hydrocarbons (e.g. individuals who work in industries associated with printing, petroleum and electronics), ionizing radiation, polyvinyl chloride, heavy metals and pesticides may all increase the risk of developing melanoma (Fortes and de Vries., 2008). Both heavy metals and polycyclic aromatic hydrocarbons react with UVA to generate free radicals which can damage DNA (Fortes & de Vries, 2008).

3.2.4. Phenotype

The amount of melanin pigment present in the skin determines skin complexion and decreased pigmentation (having a fair-skinned phenotype) poses a major risk factor for the development of melanoma. Melanin pigments, all derived from the amino acid tyrosine, are synthesized by melanocytes, transported to the keratinocytes where they absorb UV radiation and prevent damage to the skin (Mitra et al.,2012).

Melanocytes produce two distinct forms of melanin; eumelanin, a dark brown/black chemically inert pigment that potently blocks penetration of UV rays into the skin and pheomelanin, a lightly coloured pigment less effective at blocking UV rays and may even potentiate oxidative damage (Mitra et al., 2012).

The amount of eumelanin in the epidermis largely determines skin complexion. The Fitzpatrick skin phototype was developed by a dermatologist at Harvard University Medical School, to classify an individual's UV susceptibility based on basal pigment levels, tendency to burn, and tanning ability (Fitzpatrick, 1975). Individuals with a lower Fitzpatrick score have fair skin, red or blonde hair, burn easily and are unable to tan, while individuals with a higher Fitzpatrick score have a darker complexion, do not burn, and tan easily (Table 6).

A recent study demonstrated that pigment and race were not sufficient to predict an individual's Fitzpatrick score and sun sensitivity; which highlights the importance of physician-based counseling to all patients regarding prevention of sun-induced malignancies and sun safety (He et al., 2014).

Phototype	Skin color	MED (mJ/cm ² UV)	UV Response	Melanoma Risk
I	Ivory/pale white	15-30	Burns easily and strongly, never tans	++++
II	Very white	25-40	Burns easily, tans minimally with difficulty	+++ /++++
III	White	30-50	Burns moderately, tans somewhat	+++ /++
IV	Light brown, beige, olive	40-60	Burns minimally, tans moderately	++
V	Moderate brown	60-90	Rarely burns, tans well	+
VI	Dark brown/black	90-150	Never burns, tans profusely	+/-

Table 6: The Fitzpatrick Scale of Skin Complexion. MED represents the minimal erythematous dose (the least amount of UV required to cause a sunburn)

3.2.5. History of Skin Cancer

Family or personal history of skin cancers is associated with a higher melanoma risk. Malignancies incited by UV radiation such as squamous cell carcinomas, basal cell carcinomas, (Marghoob, 1995), may indicate cumulative sun exposure, but other skin malignancies not thought to be UV-related (e.g. mycosis fungoides) may also increase the risk of melanoma (Pielop et al., 2003).

First degree relatives of an individual with melanoma also have a higher risk of developing melanoma than the general population (Hemminki et al., 2003). A past medical history of cutaneous melanoma also increases the risk of subsequently developing another melanoma. The majority of melanomas are sporadic, although 10% of cutaneous melanomas occur in the setting of familial syndromes (Begg et al., 2005). Individuals diagnosed with Dysplastic Nevus Syndrome, also known as the Familial Atypical Multiple-Mole Melanoma Syndrome (FAMMM syndrome), an autosomal dominant genodermatosis characterized by >50 melanocytic nevi and a family history of melanoma, have a 48.9% risk of developing melanoma by age 50 and an 82% risk by age 72 (Tucker et al., 1993). The most significant cause of FAMMM syndrome is a mutation in the cyclin dependent kinase inhibitor 2A (CDKN2A) gene, which regulates the cell cycle (Tucker et al., 1993). However increased melanoma prevalence within a family may also represent shared environmental factors e.g. geography and chemical exposure rather than genetic mutations.

3.2.6. Presence of Nevi

Nevi can precede the development of melanoma (Clark, 1984). In 1978 two independent studies reported an association between nevi and melanoma for individuals with familial melanoma syndromes. Reimer et al (1978), reported that there was “a syndrome of pigmented lesions in melanoma-prone families”, while Lynch et al (1978), described melanomas that were linked to individuals with a large number of “moles of color and size”.

The majority of benign nevi and melanoma share a common mutation in the BRAF gene which results in a gain of function in BRAF signaling (Yazdi, 2003). This mutation activates the mitogen activated protein kinase cascade leading to the deregulation of the cell cycle and an increase in cell division.

While the BRAF mutation may be sufficient for the formation of a benign nevus, additional mutations are needed for the nevus to become a malignant melanoma (Yazdi , 2003).

The number of nevi (50 or more), the presence of atypical or large nevi, and the development of new nevi all correlate with melanoma risk (Yazdi, 2003). Melanomas generally arise from dysplastic nevi and the risk of a normal nevus converting to melanoma is quite low (Tsao et al., 2003). The presence of one dysplastic nevus increases the risk of melanoma 2-fold, however, more than 10 dysplastic nevi can increase the risk up to 12-fold (Tucker, 1997) and dysplastic nevi are present in 34-56% of melanoma cases.

The risk associated with congenital nevi vary both with quantity and size. Small congenital nevi are not associated with an increase in risk (Tucker, 1997) whilst larger congenital nevi covering more than 5% of the total body surface area confers an increased risk of melanoma (Swerdlow,1995).

Individual large congenital nevi more than 20 cm in diameter increase an individual's lifetime risk of melanoma to 10% (Krengel et al.,2006). Reports suggest that if a melanoma is going to arise from a congenital nevi, most will occur before the age of 10, pointing out the importance for screening in the pediatric population (Marghoob ,2006).

3.2.7. Medical History

Diseases associated with immunodeficiency or those managed with immunosuppressive agents can trigger melanoma. Patients diagnosed with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) have a 50% increased risk of melanoma (Olsen et al., 2014). Because antiretroviral treatment for HIV/AIDS has increased the life span of HIV positive patients, these individuals should be closely monitored and be screened regularly throughout their life for the development of melanomas.

Patients who receive an organ transplant not only have 2.4 times greater risk of developing melanoma (Dahlke ,2014), they also have more aggressive cancer and a poorer prognosis than the general population (Frankenthaler et al., 2010). Transfer of melanoma from donor to recipient is possible if the donor was previously afflicted with the condition (Zwald , 2010).

A previous medical history of both cutaneous and non-cutaneous cancer is also associated with an increased risk of developing melanoma. In one study, individuals who were previously diagnosed with Kaposi sarcoma, prostate cancer, breast cancer, lymphoma, thyroid malignancies and leukemia had an increased risk of subsequently developing cutaneous melanoma (Yang et al., 2011).

Childhood cancer survivors have a 2.5 - fold increased risk of developing melanoma and are diagnosed earlier than the general population (Hayat et al.,2007). Studies speculate that the increased risk following malignancy may be due to chemotherapy or germ-line mutations in oncogenes and the usage of radiation to treat the prior malignancy (Pappo, 2013).

3.3 Pathogenesis of Melanoma

3.3.1 NF1 mutations

The NF1 gene is a tumour suppressor gene mutated in the germline of those individuals with neurofibromatosis type 1 (NF1) and is one of the genetic syndromes with mutations in the RAS/MAPK pathways (Bottillo et al., 2009). Somatic mutations in NF1 are present in sporadic cancers and are accountable for 12-18% of melanoma cases (Hodis et al., 2012). While many mutations have been described in NF1 melanoma (Cerami et al., 2012) the overall frequencies of these mutations are yet to be established. Melanomas characterized by NF1 mutations usually occur on chronically sun exposed skin and also in older individuals (Hodis et al., 2012). These melanomas typically show a high mutation rate and ultraviolet signature mutation (Cerami et al., 2012). Melanomas with mutations in NF1 (tumor suppressor gene), have shown to be resistant to treatment with BRAF inhibitors. NF1 mutations are also found in approximately 45–93% of desmoplastic melanomas (Wiesner et al., 2015), a rare variant.

3.3.2 BRAF mutations

Studies have shown that around 40–60% of all melanoma cases exhibit an activated BRAF mutation (Flaherty et al., 2010). BRAF is a proto-oncogene that encodes a serine/threonine protein kinase as part of the RAS-RAF-MEK-ERK kinase pathway, which promotes proliferation and cell growth (Houben et al., 2004). BRAF participates in homo- or hetero-dimerization with another RAF kinase in response to growth signaling.

An activating mutation, however, leads BRAF to act as a continuously activated and self-sufficient monomer (Solit & Sawyers, 2010), which in turn leads to uncontrolled cell proliferation which plays a role in tumour development and growth (Solit & Sawyers, 2010).

The V600E mutation, that changes valine into glutamic acid, is the most common BRAF alteration and constitutes up to 90% of BRAF activating mutations and occurs in 50% of all metastatic melanomas (Flaherty et al., 2010). The V600K is the second most common mutation that results in a change from valine to lysine. BRAF mutations, such as the V600E amino acid substitution are also shown in 68% of benign nevi (Pollock et al., 2003). This evidence raises the prospect that BRAF mutations may not be involved in the pathogenesis of all cases of cutaneous melanoma.

As nevi are usually stable after their formation, some researchers suspect that BRAF mutations instead play a part in melanocytic neoplasm formation (Pollock et al., 2003).

Further research has revealed that the early stage of melanoma formation, known as the radial growth phase, exhibits a low 10% BRAF mutation rate, which supports the hypothesis that BRAF does not play a part in initiating melanoma (Dong et al., 2003). At the same time, 60–70% of vertical growth lesions and metastasized melanomas possess BRAF mutations, which suggests that this mutation may be involved in cancer progression (Pollock et al., 2003).

The existence of activating BRAF in metastasized melanoma is consistent with the BRAF function of cell growth promotion and the high prevalence of BRAF mutation in melanoma patients, making this a prime target for anti-melanoma therapy (Pollock et al., 2013).

3.3.3 NRAS mutations

Another source of molecular changes that may allow for melanoma initiation is found in the NRAS GTPase or neuroblastoma RAS oncogenes. NRAS GTPase mutations are found in 15–20% of melanoma patients (Kelleher & McArthur, 2012). NRAS is a constituent of the RAS-RAF-mitogen activating kinase pathway and is part of the class of RAS proteins that modulate RAF protein activity (Houben et al., 2004), therefore, both NRAS and BRAF are found on the same pathway. Interestingly, concurrent mutations in both BRAF and NRAS are not frequently found in the same patient (Azvolinsky, 2013), although some melanoma patients who had BRAF mutations and did not respond to BRAF inhibitor treatment, did display NRAS mutations in melanomas that had progressed. Patients with NRAS mutations possess thick vertically growing tumours that researchers attribute to the heightened cell proliferations rates induced by the NRAS mutation (Kelleher & McArthur, 2012). These characteristics of melanoma tumours in NRAS mutations are similar to those of BRAF and like BRAF mutations, NRAS mutations are also found in high numbers in benign nevi, which may limit the possibility that NRAS mutations act in melanoma initiation (Davies, 2002).

The mutation in primary and metastatic tumour samples show that metastatic tumors exhibit a higher incidence of NRAS and BRAF mutations compared to primary tumors (Houben et al., 2004).

In addition, Houben et al (2004) also found that BRAF and NRAS mutations in metastatic melanomas were correlated with lower survival rates. These findings collectively, suggest that both of these mutations in the mitogen- activated protein kinase (MAPK) pathway may be involved in promoting the transition of primary melanoma into the metastatic variant.

NRAS mutations are also linked to uncontrolled melanomagenesis, and may also serve as a good target to develop anti-melanoma therapeutics.

3.3.4 PTEN mutations

The PTEN pathway is a separate mechanistic route that also plays a role in cell survival and proliferation (Fresno et al., 2004). Similarly, to the RAS-RAF-MEK-ERK pathway, activation of PI3K-AKT leads to a competitive growth advantage that allows for melanoma metastases (Hennessy et al., 2005). The PI3K-AKT gains advantage of function changes through point mutations in chromosome copy number for genes that encode key proteins in the pathway as seen in PIK3CA and AKT1 (Davies, 2012). Analysis of melanoma tumour samples have identified a 3% PI3K missense mutation rate (Omholt et al., 2006). This decreased rate makes this pathway appear an unlikely contributor to melanoma development.

There are other ways the PI3K-AKT pathway can achieve hyper activation; high levels of PI3K-AKT activity may also result from activation via an NRAS mutation (Davies, 2012). Phosphatase and tensin homologues (PTEN), is a tumor suppressor gene that produces PIP3, a product of PI3K (Fresno et al., 2004). PTEN activity is lost by deletions, mutations or promoter methylation silencing in many cancers, including melanoma (Fresno et al., 2004).

One of PI3K-AKT pathway's functions to maintain cell viability; is to inhibit cell apoptosis. When this pathway is hyper-active or has lost its negative feedback regulation system, then chemotherapy and radiation therapy may lose their therapeutic potencies (Krasilnikov et al., 1999).

3.3.5 CDK4 and CDKN2A

Cyclin-dependent kinase 4 (CDK4) and an oncogene and tumor suppressor gene CDKN2A have been implicated in familial melanoma development (Liu et al., 1999). It has been observed that about 5–10% of melanoma patients have a relative who has also suffered from this cancer (Fitzgerald et al., 1996). The proposed form of genetic inheritance of this predisposition is through an autosomal dominant pattern, with a 53% penetrance rate by the 8th decade (Fitzgerald et al., 1996). Along with other D-type cyclins, CDK6, and the retinoblastoma protein (pRb), CDK4 regulates cell cycle progression from the G1 to the S phase (Bartkova et al., 1996). Upon activation by Cyclin D1, CDK4 acts as a proto-oncogene that promotes progression from the G1 to the S phase of the cell cycle, allowing cell growth (Bartkova et al., 1996). Studies have shown that approximately 25–60% of melanoma patients with a family history of melanoma, have an inactivated p16INK mutation, found on 9p21, while CDKN4 gene mutations occur at a lower rate (Goldstein et al., 2000).

A very small number (2–5%) of melanoma cases consist of the subtype acral lentiginous melanoma (Bradford, 2009). Acral lentiginous melanoma is characterized as melanoma found on the palms and the soles, affecting Blacks and Asian/Pacific Islanders at higher rates than Caucasian populations (Bradford, 2009). Mutations in the c-KIT gene have been detected in about 23% of acral melanomas (Beadling et al., 2008) and activated c-KIT utilizes PI3K signaling, as the predominant effector of both cell survival and growth (Rivera et al., 2008).

c-KIT encodes a tyrosine kinase transmembrane receptor and in the case of melanocytes it is believed to contribute to melanocyte cell division and melanogenesis (Rivera et al., 2008).

Point mutations and gene duplications of c-KIT frequently lead to increased expression of the KIT protein, which may alter melanocyte proliferation, apoptosis, chemotaxis and adhesion, contributing to tumorigenesis (Rivera et al., 2008).

3.3.6 MC1R

Melanocortin 1 receptor (MC1R) acts as a G-protein coupled receptor in melanocytes and plays an important role in determining skin pigmentation (Reed , 1976). Exposure to ultraviolet radiation leads to generation of alpha-melanocyte stimulating hormone, which activates MC1R to generate melanin, via the process of melanogenesis (Landi et al., 2006).

The full functions of melanin remain to be elucidated. Originally believed to shield from photo-radiation and protect the skin from DNA damage, melanin also possesses properties of a thermoregulator and an antioxidant (Riley, 1997). UV radiation and DNA mutagenesis, lead to the production of reactive oxygen species (ROS), which can lead to oxidative stress within melanocytes and genetic mutations (Meyskens, et al., 2001). In normal conditions, both melanin and glutathione pathways, acts as antioxidants that neutralize the dangerous effects of ROS. The decreased melanin production in MC1R variant individuals, could lead in increased oxidative stress and thus DNA damage, triggering the mechanisms of melanoma initiation (Meyskens et al., 2001).

A case control study analyzing MC1R variants that were detected in melanoma patients and matched control subjects, found that 72% of melanoma patients had a MC1R polymorphism, compared to 56% of the controls (Palmer et al., 2000).

There are over 60 MC1R polymorphisms that have been grouped based upon their strong correlation to the red hair phenotype (Palmer et al., 2000). Each MC1R allele variant, nearly doubles the risk for melanoma, but does not appear to be sufficient to initiate the development of melanoma (Hacker & Hayward, 2008).

MC1R variants that decrease MC1R signaling capabilities increase the propensity to acquire additional genetic mutations that may promote melanoma development (Landi et al., 2006). Those individuals with MC1R variant alleles that lead to decreased melanin production are more vulnerable to UV-induced DNA mutagenesis, making them susceptible to acquiring mutations in genetic loci of NRAS, BRAF or CDKN2A (Palmer et al., 2000).

Mutations in CDKN2A are present at birth, transmitted between parent and child and it cannot definitely be concluded that MC1R variants increase the chances of melanocytes developing the CDKN2A mutation (Palmer et al., 2000). Instead, it has been found that the MC1R variants may influence the rate at which patient with the CDKN2A mutation develop melanoma, by increasing the penetrance (Palmer et al., 2000). Taking advantage of these trends may allow for improved diagnostic procedures in the future, using MC1R variant allele analysis to provide information on an individual's likelihood of developing melanoma (Sturm, 2002).

3.4. Clinical Features

The 'ABCDE' rule describes the five clinical features of melanoma: Asymmetry, Border irregularity, Colour variation, Diameter greater than 6mm and Evolving skin lesion (Rigel et al., 2004).

A recent and novel method for clinically diagnosing melanoma, is the "ugly duckling sign" (Mascaro, 1998). Correlation of common lesion characteristics is made and lesions that greatly deviate from the common characteristics are labeled an "Ugly Duckling" (Mascaro, 1998).

The "Little Red Riding Hood" sign (Mascaro, 1998), suggests that individuals with light-colored hair and fair skin might have difficult-to-diagnose amelanotic melanomas. Extra care is required when examining such individuals, as they might have multiple melanomas and severely dysplastic nevi. A dermatoscope must be used to detect these "ugly ducklings", as many melanomas in these individuals resemble non-melanomas or "wolves in sheep's clothing" (Mascaro, 1998). These fair-skinned individuals often have lightly pigmented melanomas that do not present as easily visible lesions.

3.5. Subtypes

In 1969, Clark and Bernardino were the first to divide melanoma into subtypes depending on clinical and histologic features that were later used by other researchers. The majority of all melanomas fall into the following four subtypes according to the World Health Organization (Table 7):

Melanoma subtypes according to the WHO classification	
Melanoma subtypes	ICD-O-3 codes
Malignant melanoma – major subtypes	M8720/3
Superficial spreading melanoma	M8743/3
Nodular melanoma	M8721/3
Lentigo maligna melanoma	M8742/3
Acral lentiginous melanoma	M8744/3

Table 7: Melanoma subtypes according to the WHO classification. ICD-O-3, International Classification of Diseases for Oncology, 3rd Edition; NOS, not otherwise specified; WHO, World Health Organization. LeBoit et al 2003.

- Superficial spreading
- Nodular
- Lentigo maligna melanoma
- Acral lentiginous

Precursor lesions with no penetration of the basal membrane but with a high risk of transforming into melanoma are called lentigo maligna (Schadendorf et al., 2013). The cells of the primary lesion, either intraepidermal or below the basement membrane, determine the classification of melanoma.

Lentigo maligna begin as a tan irregular macule that extends peripherally, with differing shades throughout the lesion.

They occurs on atrophic, sun-damaged skin in the elderly and occurs equally in men and women, usually in the seventh and eighth decades of life (Odom et al., 2000). The exact percentage of lentigo maligna that may progress to invasive lentigo maligna melanoma is unknown, but is estimated to be less than 30% and according to Odom and colleagues (2000), the lesion can grow slowly for 5 to 15 years in the precursor form, before invasion.

Superficial spreading melanoma (SSM), represent almost 70% of all melanomas and are the most common type of cutaneous melanoma occurring in fair-skinned people (Odom et al.,2000). It affects adults of all ages, with the peak incidence in the fourth and fifth decades of life (Odom et al., 2000). SSM can arise in a preexisting melanocytic nevus, with a clinical history of a slowly changing mole over 1 to 5 years (Odom et al.,2000). SSM most commonly affects intermittently sun-exposed areas with the greatest density of nevi, such as the lower legs of women and upper backs of men (Odom et al., 2000).

Clinically, SSM starts as a deeply pigmented macule or plaque with intact skin markings and according to Odom and colleagues (2000), the earliest changes can be a focal area of darkening within a pre-existing nevus. Pigment variegation ranges from and blue-gray/black to pink or gray-white (Odom et al.,2000).

The second most common subtype of melanoma is nodular melanoma(NM) and represents 15% of all melanomas (Odom et al., 2000). The median age at onset is 53 years and clinically manifests as a uniform blue-red, blue-black, or amelanotic nodule (Odom et al., 2000).

The most common sites for nodular melanoma are the trunk, head, and neck and it is more common for nodular melanoma to begin in normal skin rather than in a preexisting lesion (Odom et al., 2000).

Acral lentiginous melanoma (ALM) accounts for approximately 10% of melanomas overall; however, they are the most common types among African, Japanese, African Americans, Native Americans, and Latin Americans (Odom et al., 2000). According to Odom and colleagues (2000), the median age for occurrence is 65 years, with equal gender distribution.

The most common site of melanoma in Africans and African Americans is the feet, with 60% of patients having subungual or plantar lesions (Odom et al., 2000). Overall, ALM can occur on the palms, soles and subungually, the sole being the most common site in all racial groups (Odom et al., 2000). Clinically, the lesion is characterized by a brown-to-black macule with colour variegation and irregular borders; unlike lentigo maligna melanoma, development of ALM is not dependent on sun exposure (Odom et al., 2000).

Sub-ungual melanoma is a rare variant of ALM and mostly involves the thumb or big toe and generally arise from the nail matrix (Odom et al., 2000). Hutchinson's sign is the finding of pigmentation on the posterior nail fold and is associated with advanced subungual melanoma (Odom et al., 2000).

Other more uncommon variants of melanoma include melanoma of the mucosa and desmoplastic melanoma and patients may present with a tumour or bleeding (Odom et al., 2000). When melanoma occurs on the mucosa, it usually develops on the mucosal surfaces of the oral and nasal cavities, genital, or anorectal mucosa (Odom et al., 2000).

Nodular and acral lentiginous melanomas have the poorest 5-year survival rates among all histological subtypes (69.4% and 81.2%, respectively) because of their higher tumor thickness at the time of diagnosis (Pollock et al., 2011).

Diagnosis

Apart from initial visual inspection of the lesion, a variety of tools have been developed for accurate diagnosis of melanomas. These tools include dermoscopy, photography, histopathology and analysis of molecular biomarkers.

3.6.1. Dermoscopy

Dermoscopy, also known as skin surface microscopy or epiluminescence microscopy (ELM), is a non-invasive, helpful tool for diagnosing pigmented skin lesions and for recognizing cutaneous melanoma (Argenziano & Soyer, 2001). Clinical diagnostic sensitivity is dramatically enhanced by the use of dermoscopy and the correct diagnosis of melanoma is improved by up to 50% by using this diagnostic technique (Kittler et al, 2002). The major dermoscopic criteria for melanoma can be broken down into morphological and associated histopathological changes and a combination of these features can aid in making a clinical diagnosis of melanoma (Table 8).

DERMOSCOPIC CRITERIA AND THEIR CORRESPONDING HISTOPATHOLOGIC FEATURES			
Criterion	Morphologic definition	Associated histopathologic changes	Diagnosis
Pigment network	Network of brownish lines over a diffuse tan background	Pigmented rete ridges	Melanocytic lesion
Typical network	Brown-colored, regularly meshed and narrowly spaced network	Regular and elongated rete ridges	Benign melanocytic lesion
Atypical network	Black, brown or gray network with irregular meshes and thick lines	Irregular and broadened rete ridges	Melanoma
Dots/globules	Black, brown and/or gray, round to oval, variably sized structures regularly or irregularly distributed within the lesion	Pigment aggregates within the stratum corneum, epidermis, dermal-epidermal junction or papillary dermis	If regular: benign melanocytic lesion; if irregular: melanoma
Streaks	Irregular, linear structures not clearly combined with pigment network lines at the margins	Confluent junctional nests of melanocytes	Melanoma
Blue-whitish veil	Irregular, confluent, gray-blue to whitish-blue diffuse pigmentation	Acanthotic epidermis with focal hypergranulosis above sheets of heavily pigmented melanocytes in the dermis	Melanoma
Blotches	Black-, brown- and/or gray-colored areas with regular or irregular shape/distribution	Hyperpigmentation throughout the epidermis and/or upper dermis	If regular: benign melanocytic lesion; if irregular: melanoma
Regression structures	White (scar-like) areas, blue (pepper-like) areas or combinations of both	Thickened papillary dermis with fibrosis and/or variable amounts of melanophages	Melanoma
Milia-like cysts	White-yellowish, roundish dots	Intraepidermal horn globules, also called horn pseudocysts	Seborrheic keratosis (occasionally observed in papillomatous melanocytic nevi)
Comedo-like openings	Brown-yellowish, round to oval or even irregularly shaped, sharply circumscribed structures	Keratin plugs situated within dilated follicular openings	Seborrheic keratosis
Leaf-like areas	Brown-gray to gray-black patches revealing a leaf-like configuration	Pigmented, solid aggregations of basaloid cells in the papillary dermis	Basal cell carcinoma
Red-blue lacunas	Sharply demarcated, roundish to oval areas with a reddish, red-bluish, or red-black color	Dilated vascular spaces situated in the upper dermis	Vascular lesion
Vascular structures	Comma-like vessels Arborizing vessels Hairpin vessels Dotted or irregular vessels		Benign melanocytic lesion Basal cell carcinoma Seborrheic keratosis Melanoma

Table 8: Dermoscopic criteria and their corresponding histopathologic features. With permission from Argenziano & Sover, 2001;

3.6.2. Photography

In high-risk patients with complex mole patterns, baseline photographs can be a helpful addition in the identification of new and changing lesions. (Halpern et al., 2003). Digital photography and computerized image archives have improved the practicality of this approach. The availability of baseline images for comparison, including dermoscopic images, allows the detection of melanomas that are growing or changing (Halpern et al., 2003).

3.6.3. Histopathology

When a suspicious skin lesion is identified, a histological examination of the tissue sections is warranted. Histological samples can be obtained via tangential/shave biopsy, punch biopsy, fine needle aspiration, incisional and excisional biopsy, sentinel lymph node biopsy or surgical lymph node biopsy (Garbe et al., 2009).

The choice of biopsy type indicated depends on the extent of the tumor. Tissue sections are stained with haematoxylin and eosin and examined by a dermatopathologist; whose expert opinion is considered as a gold standard for the diagnosis of melanoma (Ruiter et al., 2002). In addition to details of diagnosis and clinicopathologic type, the histopathologic report should include information on tumor thickness in mm (Breslow depth), presence or absence of ulceration, presence of microsatellites, mitotic rate, the deep and lateral excision margins (Carlson et al., 2003), as these features are all prognostic factors in melanoma. Histopathological diagnosis is limited by the absence of reproducible and objective criteria that can be applied to all melanomas. Furthermore, the subset of lesions with borderline or contradictory findings adds to the limitations of histopathology in the diagnosis of melanoma (Lodha et al., 2008).

3.6.4. Molecular Diagnosis

In cases where histologic diagnosis is unclear, analysis of molecular biomarkers (S-100 protein, Melan-A, HMB 45, MIB-1, Ki-67) using immunohistochemical staining could be of benefit (Ohsie et al., 2008).

Among several markers, expression of S-100 protein, a low molecular weight calcium binding protein that is involved in cell division and differentiation, is considered the most sensitive marker for melanocytic lesions. (Ohsie et al., 2008).

Serum levels of S-100 protein were also found to correlate with progression of metastatic melanoma in some patients (Henze et al., 1997).

While S-100, HMB45 and Melan-A are useful for the confirmation of the melanocytic nature of the tumour, Ki-67 and MIB-1 are indicators of tumour growth (Ohsie et al., 2008). Apart from immunohistochemical analysis, genomic and analytical techniques like comparative genomic hybridization (CGH) analysis and fluorescence in situ hybridization (FISH) assay can be used when the diagnosis of melanoma poses a challenge, however this is not routinely done.

Additional staging examinations such as ultrasound of regional lymph nodes, total body CT scans or PET-CT scans are also used in patients with primary melanomas at initial diagnosis and follow-up examinations to identify the high-risk patients (Ohsie et al., 2008). Serum LDH and S100 protein levels are also routinely used as markers for relapse (Jackel et al., 1999).

3.7. Melanoma Staging

The revised Melanoma Staging System was published in the 8th edition of the American Joint Committee on cancer Cancer (AJCC) in 2017.

This system makes use of the TNM classification; where 'T' is tumour size and thickness in millimeters, 'N' is presence and number of lymph nodes and 'M' is presence and site of metastases (Table 9).

Table 9: AJCC Guidelines Staging Categories for Cutaneous Melanoma 2017. Bichakjian et al 2016

Table 5 Eighth Edition of the AJCC TNM Staging Manual for Melanoma (Cutaneous).

Classification System for Melanoma (Eighth Edition)			
T	TX	Primary tumor cannot be assessed (after curettage...)	
	T0	No evidence of primary tumor	
	Tis	Melanoma in situ	
	T1	= 1 mm	a (< 0.8 without ulceration) b (< 0.8 mm with ulceration or 0.8-1 mm with or without ulceration)
	T2	> 1 mm-2 mm	a (without ulceration) b (with ulceration)
	T3	> 2 mm-4 mm	a (without ulceration) b (with ulceration)
	T4	> 4 mm	a (without ulceration) b (with ulceration)
N	NX:	Nearby lymph nodes cannot be assessed (prior resection for another reason, body habitus...)	
	N0	No involvement of nearby lymph nodes as determined clinically/radiologically	
	N1	N1a: micrometastasis in a lymph node (clinically occult)	
		N1b: clinically detected lymph node	
	N2	N1c: in transit, satellite, or microsatellite metastasis without lymph node involvement	
		N2a: micrometastasis in 2 or 3 lymph nodes (clinically occult)	
		N2b: metastasis in 2 or 3 lymph nodes N2c: metastasis in a lymph node (occult or clinically detected) and in transit, satellite, or microsatellite metastasis	
N3	N3a: 4 or more micrometastases in lymph nodes		
	N3b: 4 or more metastases in lymph nodes, at least one of them clinically evident or presence of matted lymph nodes		
	N3c: 2 or more micrometastases or clinically detectable lymph node metastases or presence of matted lymph nodes and in transit, satellite, or microsatellite metastasis		
M	M0	No evidence of lymph node metastasis	
	M1	M1a: distant metastasis in skin, soft tissue (including muscle), and/or nonregional lymph nodes	
		- M1a(0): Normal LDH	
		- M1a(1): Elevated LDH	
		M1b: pulmonary metastasis with/without M1a	
		- M1b(0): Normal LDH	
		- M1b(1): Elevated LDH	
		M1c: distant metastasis in organs other than the CNS with/without M1a and M1b	
		- M1c(0): Normal LDH	
		- M1c(1): Elevated LDH	
M1d: metastasis of the CNS with/without M1a, M1b, or M1c			
- M1d(0): Normal LDH			
- M1d(1): Elevated LDH			
AJCC TNM Staging System for Melanoma (Eighth Edition)			
Tis	N0	M0	Stage 0
T1a-T1b	N0	M0	Stage IA
T2a	N0	M0	Stage IB
T2b-T3a	N0	M0	Stage IIA
T3b-T4a	N0	M0	Stage IIB
T4b	N0	M0	Stage IIC
T0	N1b/1c	M0	Stage IIIB
T0	N2b/2c, N3b/3c	M0	Stage IIIC
T1a/b-T2a	N1a, N2a	M0	Stage IIIA
T1a/b-T2a	N1b/1c, N2b	M0	Stage IIIB
T2b/T3a	N1a-N2b	M0	Stage IIIB
T1a/T3a	N2c/N3a,b,c	M0	Stage IIIC
T3b/T4a	N1-N3	M0	Stage IIIC
T4b	N1a-N2c	M0	Stage IIIC
T4b	N3a/b/c	M0	Stage IIID
Any T, Tis	Any N	M1	Stage IV

In boldface, aspects most relevant to staging.

Approximately 90% of melanomas are diagnosed as primary tumours with no evidence of metastasis and the tumour specific 10-year survival for such tumours is 75-85% (Balch et al., 2009).

The most important histological prognostic factors for primary melanoma without metastases are described as :

- a) Vertical tumour thickness (Breslow's depth) as measured on tissue specimen with an optical micrometer scale and defined as the histologic depth of the tumour from the base of the keratinous layer of the epidermis, to the deepest level of tumour invasion (Balch et al., 2009). Tumour thickness is the most important prognostic factor. In patients with melanomas with a tumor thickness ≤ 1.00 mm, the 10-year survival rate is approximately 92%, compared with 80% in patients with melanomas of 1.01–2.00-mm thickness, 63% in patients with melanomas of 2.01–4.00-mm thickness, and 50% in patients with melanomas of >4.00 -mm thickness (Balch et al., 2009).
 - b) Presence of histologically recognized ulceration. Melanoma ulceration is defined as the combination of the following features: thinning, full-thickness epidermal defect, evidence of host response (i.e. fibrin deposition, neutrophils) and reactive hyperplasia of the surrounding epidermis (Spatz et al., 2003). Ulceration has a significant influence on survival and is an important prognosticator (Spatz et al., 2003). Patients with an ulcerated T4 melanoma have a 5-year survival rate of 53%, while the survival rate for patients with a non-ulcerated T4 primary, is about 71% (Balch et al., 2009).
- Mitotic rate (number of mitosis/mm²) appears as an independent prognostic factor in several population studies and is used for sub-classification of thin melanomas in the 2017 AJCC classification (1a, 1b)).

Mitotic rate is a marker for growth of the primary melanoma (Balch et al., 2010). A highly significant correlation between increasing mitotic rate and declining survival rates was demonstrated. The most significant correlation with survival was identified at a threshold of a mitotic rate of at least 1/mm². Survival rates of patients with an ulcerated primary or increased mitotic rate are lower than those of patients with a non ulcerated melanoma of equivalent T-category (Balch et al., 2009).

When the invasive component of the melanoma is <1/mm², the number of mitoses in 1 mm² of dermal tissue that includes the tumour, should be recorded as a number of mitoses per mm². In tumours whose invasive component consists of an area <1 mm², it is recommended to designate the presence or absence of a mitosis as “at least 1/mm²” (i.e., “mitogenic”) or as 0/mm² (i.e., “non-mitogenic”), respectively (Balch et al., 2010). According to Balch et al (2010), the mitotic count has been described as “<1/mm², when mitotic figures are not found after examining.

The updated AJCC Melanoma staging database demonstrated that an elevated serum LDH level is an independent, highly significant predictor of survival outcome amongst patients with stage IV melanoma. When increased LDH levels are found, patients are classified as M1c regardless of the location of distant metastases.

The AJCC 2017 melanoma guideline also recommends that sentinel lymph node biopsy be performed as a staging procedure in patients requiring subsequent follow up and treatment. Nodular involvement-related survival rates differ due to heterogeneity. Tumor burden at the time of staging was shown to be a further prognosticator. Five-year survival rates within stage III were 78%, 59%, and 40% for patients with stage IIIA, IIIB, and IIIC melanoma, respectively (Balch et al., 2009).

Level of tumour invasion (Clark's level) is no longer part of the 2009 AJCC staging system, as it is not an independent prognosticator when the mitotic rate is included in the analysis.

Prognosis is worse in patients with distant metastases. Patient one-year survival rates are approximately 62% (M1a), 53% (M1b), and 33% (M1c), respectively, according to AJCC 2009 guidelines. Survival rates after 10 years range between 5% and 20% (Balch et al., 2009).

3.8. Treatment

Surgery is the definitive treatment for early stage melanoma. Wide local excision with completion lymph node dissection in patients with positive sentinel lymph node biopsy results is considered the gold standard of treatment for patients with primary melanoma (McWilliams et al., 2008).

According to the National Cancer Institute (NCI) in 2017, the definitive treatment of cutaneous melanoma is surgery, and medical management is reserved for adjuvant therapy of patients with advanced melanomas. Less than 50% of patients with deep primaries (>4 mm) or regional lymph node involvement have long-term disease-free survival; consequently, these patients are classified as high risk and should be considered for adjuvant therapy (NCI, 2017).

According to the National Cancer Institute (2017), The standard treatment options for melanoma are:

- Stage 0 - Excision
- Stage I - Excision, with or without lymph node management
- Stage II - Excision, with or without lymph node management
- Resectable stage III - Excision, with or without lymph node management; adjuvant therapy and immunotherapy
- Unresectable stage III, stage IV, and recurrent melanoma – immunotherapy, intralesional therapy, chemotherapy, signal transduction inhibitors and palliative local therapy

3.8.1. Surgery

Excisional biopsies may remove the tumor, but further surgery is often necessary to reduce the risk of recurrence. Complete surgical excision with adequate surgical margins and assessment for the presence of detectable metastatic disease along with short and long term follow up is standard procedure (Clark et al., 2008). This is done by means of wide local excision with 1-2 cm margins (Clark et al., 2008). Wide excisions aim to reduce the rate of tumour recurrence at the site of the original cancer and extensive research has aimed to clarify appropriate margins for excision with a trend toward less aggressive treatment (Balch et al.,1993).

Lentigo maligna are treated with narrower surgical margins, usually 0.2 to 0.5 cm and many surgeons consider 0.5 cm the standard of care for excision of lentigo maligna (Clark et al., 2008). A 0.2 cm margin might be acceptable for margin controlled surgery, i.e. the double-bladed technique with margin control or Moh's microsurgery (Saunders, 2016). Mohs surgery has been reported to have a cure rate as low as 77% and as high as 98.0% for lentigo maligna (Saunders,2016).

Melanomas that metastasize, typically spread to the regional lymph nodes first and attempts to improve survival by removing lymph nodes surgically, have been associated with many complications and no overall survival benefit (SUNMEL, 2009).

Recently, the technique of sentinel lymph node biopsy has been developed to reduce the complications of lymph node surgery while allowing assessment of nodes involved with tumour (SUNMEL,2009).

Sentinel lymph node biopsy (SLNB) is a widely used procedure when treating cutaneous melanoma (Crowson and Haskell, 2013). Diagnostic tests including SLNBs should not be performed to evaluate early, thin melanoma, including melanoma in situ, T1a or T1b melanoma $\leq 0.5\text{mm}$ (Bichakjian, 2011) These individuals are unlikely to have melanoma spread to their lymph nodes or anywhere else and have a 97% 5-year survival rate (Bichakjian, 2013) deeming SLNB unnecessary.

SLNB is more commonly performed for T1b/T2+ tumors, mucosal tumors, ocular melanoma and tumors of the limbs (Bichakjian,2011). A procedure called lymphoscintigraphy is performed in which a radioactive tracer is injected at the tumor site to localize the sentinel node. Further precision is provided using a blue tracer dye, and surgery is performed to biopsy the node. Routine hematoxylin and eosin and immunoperoxidase staining is usually adequate to rule out nodal involvement. Polymerase chain reaction tests on nodes, usually performed to test for entry into clinical trials, now demonstrate that many patients with a negative SLNB in actual fact, had a small number of positive cells in their nodes. (Bichakjian, 2011) Alternatively, a fine-needle aspiration biopsy can be performed and is often used to test tumours.

If a lymph node is positive, and depending on the extent of nodal spread, a radical lymph node dissection can be performed and if the disease is completely resected, the patient will be considered for adjuvant therapy (Swanson et al., 2002). In this case, the suspicious lesion is completely excised with 1-2 mm margins of surrounding skin and tissue (Swanson et al., 2002).

To avoid disruption of the local lymphatic drainage, the preferred surgical margin for the initial biopsy should be narrow (1 mm) and the biopsy should include the epidermal, dermal, and subcutaneous layers of the skin. (Swanson et al., 2002). This enables the dermatopathologist to determine the thickness of the melanoma by microscopic examination. The initial biopsy should not include the final surgical margin, as a misdiagnosis can result in excessive scarring and morbidity from the procedure and a large initial excision will disrupt the local lymphatic drainage and can affect further lymphangiogram-directed lymph node dissection (Swanson et al., 2002).

Radiographic imaging studies and baseline blood tests should not be performed only based on identifying metastatic melanoma, as these tests often have high false-positive rates (AAD, 2013).

3.8.2. Chemotherapy and Immunotherapy

Current guidelines from the National Comprehensive Cancer Network (NCCN) in 2012, recommend treating unresectable or metastatic disease with immunotherapy, or with targeted therapy if a BRAF V600 activating mutation is present. Targeted therapy is preferred if clinically needed for early response. Current targeted therapies can slow tumor growth (eg, BRAF inhibition) or release the brakes on the immune response, resulting in tumor lysis (eg, programmed cell death–1 [PD-1] inhibition).

For immunotherapy, the NCCN recommends PD-1 inhibitor monotherapy with pembrolizumab or nivolumab, or the combination of nivolumab plus ipilimumab.

Interleukin-2 was the first new therapy approved (1990 Europe, 1992 USA) for the treatment of metastatic melanoma in 20 years and studies have demonstrated that interleukin-2 offers the possibility of a complete and long-lasting remission in this disease, although only in a very small percentage of patients (Buzaid, 2004).

3.8.3. Radiation therapy

Radiation therapy is often used after surgical resection for patients with locally or regionally advanced melanoma or in patients with unresectable metastatic disease. Kilo-voltage x-ray beams are often used for these treatments and have the property of delivering the maximum radiation dose close to the skin surface (Hill et al., 2014). Radiotherapy may reduce the rate of local recurrence but does not prolong survival (Bastiaannet et al., 2005). Radio- immunotherapy of metastatic melanoma is currently under investigation. Radiotherapy also has a palliative role in metastatic melanoma (Boyer, 1999).

3.9. Follow Up

The 2010 American Academy Dermatology (AAD) Guidelines/Outcomes Committee formed a task force to evaluate and give recommendations regarding the guidelines of care for primary cutaneous melanoma. The recommendations advise clinicians to evaluate the patient individually at least annually and possibly every 3 to 12 months based on factors affecting the risk of recurrent and new primary melanoma (Bichakjian et al., 2011).

These modifying factors include: history of multiple melanomas, tumour stage, presence of atypical nevi, family history of melanoma, patient anxiety, and the patient's ability to recognize signs and symptoms of melanoma (Bichakjian et al., 2011).

The AAD Melanoma guidelines state that patients be educated on performing monthly self-skin and self-lymphnode examinations. Routine surveillance blood tests and imaging studies are not recommended in asymptomatic patients, however imaging studies can be considered in patients with high risk for recurrence, but is not recommended after five years.

Chapter Four: Patients and Methods

4.1. Study Design

A retrospective review of laboratory reports of histologically confirmed cutaneous melanoma of patients attending Charlotte Maxeke Hospital Johannesburg Academic Hospital (CMJAH) and Helen Joseph Hospital (HJH) was performed. These skin biopsy specimens were submitted to the National Health Laboratory Service (NHLS) at CMJAH from July 2013 to June 2016. The histology reports provide the demographic data, the confirmation of melanoma skin cancer, the histologic subtype, other risk factors associated with development of melanoma skin cancers as well as the site of these tumours. In total 128 patient reports were reviewed.

CMJAH and HJH hospitals are quaternary and tertiary hospitals respectively, servicing the Johannesburg Central Business District (CBD), Northern Johannesburg suburbs and surrounding areas. These hospitals offer secondary, tertiary and specialized services to the Gauteng province and neighboring provinces. The NHLS is accredited by the South African National Accreditation System (SANAS) and meets all international standards.

4.1.1. Study aims

The aim of this research is to describe the clinicopathological features of cutaneous melanomas in patients attending CMJAH and HJH Hospital. This type of study has not been conducted at either of these two hospitals.

Determining the clinicopathological features of cutaneous melanoma will assist in establishing the morbidity and mortality associated with this malignancy, as well as the burden it places on the health care system.

This in turn will help in allocation of health care resources and implementation of melanoma preventive strategies. These strategies would include: regular skin check-ups, counseling on the use of protective clothing and sunscreens, as well as avoiding sun exposure.

4.1.2. Study Objectives

1. To calculate the number and percentages of patients with melanoma skin cancers in the various sexes, age groups, tumour sites (head and neck, upper extremities, lower extremities, trunk or other sites), and racial groups. The calculations will be based on the results of histology reports. These histology reports will provide demographic data, the clinical type and histologic subtypes of melanoma skin cancer as well as the site of the tumours.
2. To calculate the number and percentages of patients with other risk factors associated with development of skin cancers (e.g. multiple nevi, family or personal history of previous melanoma, genetic factors, immunosuppression, etc).

4.2. Patients

4.2.1. Inclusion Criteria

Histologically confirmed melanoma skin cancers recorded from 1 July 2013 to 30 June 2016.

4.2.2. Exclusion Criteria

- I. Cases with insufficient data to confirm a histological diagnosis of cutaneous melanoma.
- II. Other skin cancers.
- III. In the case where multiple specimens were taken from the same patient, only the first report confirming melanoma was included for statistical analysis and the subsequent reports excluded.

4.2.3. Ethical Considerations

An ethics clearance certificate No. M170455 (Appendix 2) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, in order to conduct this study.

Permission to use the data was obtained from the Head of Department of Pathology at the NHLS laboratory based at CMJAH (Appendix 3). To protect participant's confidentiality, the data was saved in an anonymized format to avoid identification of patients and was stored on a password protected device.

4.3. Methods of data collection

Demographic, clinical and histological data was collected from the laboratory reports.

A data capture sheet was used.

The following variables were recorded from the laboratory reports:

a. Demographic data:

Race, age and sex

b. Type of skin cancer:

Melanoma skin cancer

c. Site of skin cancer:

Head and neck, upper and lower extremities, trunk, other sites, not-specified

d. Histologic subtypes of melanoma skin cancers:

Superficial spreading melanoma, nodular melanoma, lentigo maligna, acral
lentiginous, unclassifiable, others

e. Risk factors:

Genetic (e.g. Oculocutaneous Albinism, Xeroderma Pigmentosa),
environmental (e.g. HIV, immunosuppression).

4.4. Statistical Methods

The number and percentages of patients with melanoma in the various sexes, age groups and tumour sites were recorded and a comparison was made between the different anatomic sites, in both males and females. The presence of histological tumour invasion was calculated, as was the different histologic subtypes of melanoma.

Descriptive statistics on the general demographics of the patients such as age and gender were performed. Numerical variables were described using mean and standard deviations. Associations between pairs of categorical variables were determined using Pearson's Chi-Square test and, where applicable, Fisher's exact test was used. To test for association with continuous variables like the age of the patient, the unpaired T-test was used. Statistical significance was ascertained at the 5% level, thus a p-value of less than 0.05 was significant.

Chapter Five: Results

5.1. Demographics of Patient Population

There was a total of 359 biopsy specimens submitted for histopathological examination to the NHLS at the Charlotte Maxeke Hospital over a 3-year period, between June 2013 - July 2016 and 173 biopsy reports were found to have a histopathological diagnosis of melanoma.

The histopathological reports of these cases were confirmed by the research supervisor and the researcher. 18 of the confirmed cases of cutaneous melanoma were repeat biopsies of recurrent lesions. In these cases, only the first biopsy report was included in the statistical analysis. Furthermore, data with regard to ethnicity and family history of cancer was incomplete or unreliable and was therefore not used for statistical analysis. After re-examination and exclusion of the above- mentioned cases, a total of 128 cases were included in the study.

The demographic data for these 128 patients is given in Table 10 and Figure 2.

Gender % (n)	Male	50 (64)
	Female	50 (64)
Age	Mean (SD)	60.4 (15.6)
Site % (n)	Head and neck	17.6 (22)
	Upper extremities	9.6 (12)
	Lower extremities	41.6 (52)
	Trunk	31.2 (39)
	Other sites	0
	Not specified	3
No. of specimens	1	85.9 (110)
	2	12.5 (16)
	3	0.8 (1)
	4	0.8 (1)
Invasive	Yes	52.3 (67)
	No	47.7 (60)

Table 10: Demographic Data

There was an equal number of male and female patients (50% males and 50% females). The most commonly affected sites for both genders were the lower extremities at 41.6%. The majority (52.3%) of specimens were invasive in nature. The ethnicity of all the patients could not be determined as it was not stated on the report. The ages ranged from 21 to 90 years and the mean (SD) age was 60.4 years at the time of diagnosis. There was a significant difference in age between males and females with the males being older (63.2 years) than the females (57.5 years, $p=0.0364$). There was a bimodal frequency distribution for age (Figure 2).

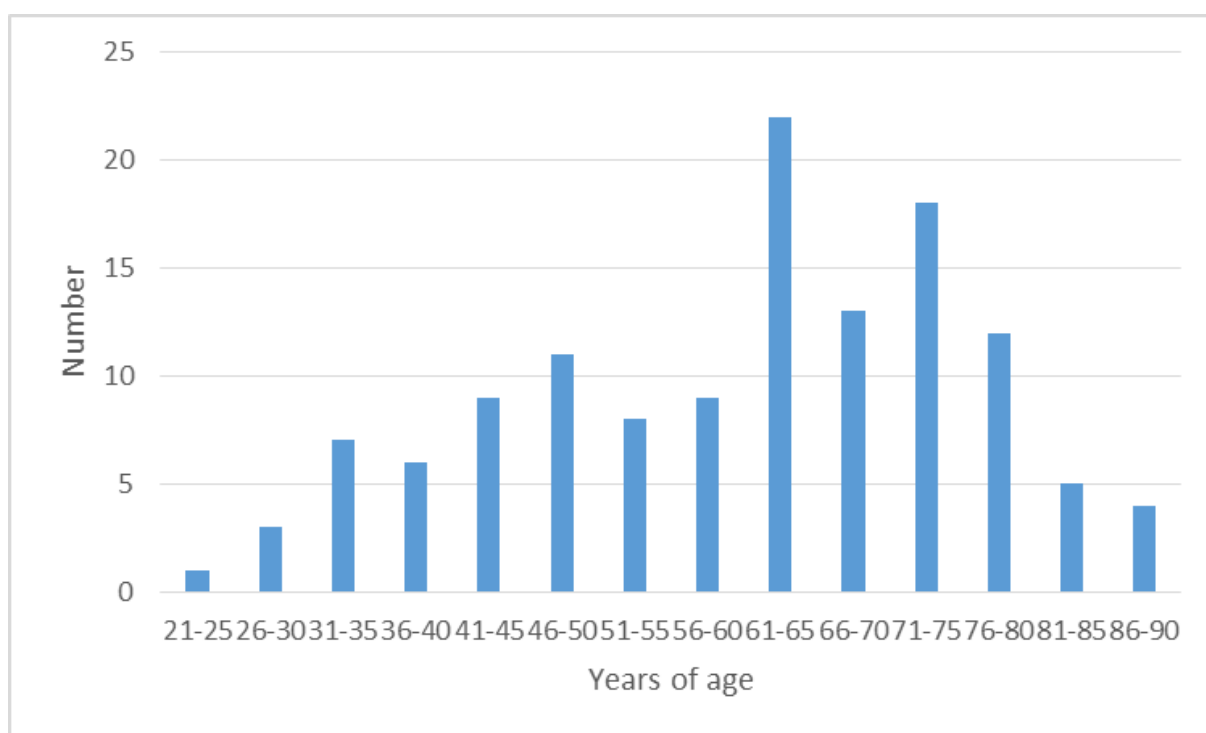


Figure 2: Frequency Distribution for Age: 5 Year bins

5.3. Site of Melanoma

The most common site of tumour involvement in both genders were the lower extremities, affecting 41,6% of the patients (Table 11).

There was a significant difference between men and women for site of primary melanoma. Males were also likely to have lesions on their trunk (45% in males and 16% in females) as compared to females who mostly had lesions on their lower extremities (56% in females and 25% in males).

The presence of invasive melanoma differed between males and females, with males having a decreased incidence of invasive melanoma at diagnosis, compared to females (n-28 and n-32 respectively).

Variable (n)	Male	Female	P value
Head and neck	13	9	0.0004
Upper extremities	5	7	0.0004
Lower extremities	16	36	0.0004
Trunk	29	10	0.0004
Invasive (Y/N)	28	32	P=0.4792

Table 11: Site of Melanoma in both genders and presence of tumour invasion

5.4. Tumour Invasiveness

Patients over the age of 60 years are significantly more likely to have melanomas on the head, neck and lower extremities compared to younger patients who were likely to have them on the trunk. There was no difference seen in whether the lesions were invasive or not (Table 12).

		≤ 60 years	> 60 years	P value
Site %(n)	Trunk	45.3(24)	20.8(15)	0.0088
	Lower extremities	39.7 (21)	43.1(31)	0.0088
	Upper extremities	7.5 (4)	11.1(8)	0.0088
	Head and neck	7.5(4)	25.0 (18)	0.0088
Invasive %(n)	Yes / no	53.7/46.3 (29/25)	51.4/48.6 (38/35)	P = 0.8596

Table 12: Comparing <60 to over 60 years

5.5. Histological Subtypes

Nodular melanoma was the most commonly diagnosed subtype, at 63.4%. This category included both malignant melanoma and nodular melanoma, as they have the same histological features. followed by acral lentiginous melanoma and lentigo maligna, both at 11.7% (Table 13).

Superficial spreading and amelanotic melanoma ranked 4th most common subtypes at 3.1% and 7% of the specimens submitted consisted of other unclassified subtypes.

	% (n)
Nodular	63.4% (81)
Acral Lentiginous	11.7(15)
Lentigo Maligna	11.7 (15)
Amelanotic Melanoma	3.1 (4)
Superficial Spreading	3.1(4)
Others	7.0 (9)

Table 13: Histological Subtypes

Risk factors were documented for 10 of the 128 individuals. There were 4 individuals (3.1%) infected with HIV and 6 individuals (4.6%) who were previously diagnosed with melanoma, as per the histology reports. However, information regarding recurrence was not indicated in the reports.

5.6. Ethnicity

There was no clinical information available regarding race in the histology reports.

Chapter Six: Discussion

6.1. Discussion

The data from this study on cutaneous melanoma has been obtained from a sample of patients who had consulted medical practitioners within the public health care system for assessment of suspicious skin lesions and had their biopsies microscopically examined. In this retrospective review of patients with melanoma at Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital, we describe the prevalence of cutaneous melanoma in 128 patients with histologically confirmed melanoma.

From an epidemiological perspective, our study revealed that melanoma had an equal incidence in both men and women (64 males and 64 females) amongst all age groups.

The mean age at diagnosis for the patients in our study was 60.4 years.

The average age in the USA for a melanoma diagnosis is 63 years (American Cancer Association, 2017), which was in keeping with our findings.

Our study showed a significant difference between men and women for site of primary melanoma, in that the males had truncal lesions predominantly (45% in males and 16% in females), while females had lesions mainly on their lower extremities (56% in females and 25% in males).

These findings in our study corresponded to a study conducted by Clarke et al in 2004 and Pruthi et al in 2009.

Clark et al (2007) compared the distribution of melanomas seen at their institution (University of Pennsylvania, USA) in the 1970s and 2004 to determine whether the anatomic distribution of melanoma changed over time. Males in the 1970s had a higher odds of developing melanoma on the trunk and females on the extremities (Clark et al., 2007).

In a 2004 study involving 152 patients with cutaneous melanoma conducted by Clark et al, women had an increased odds ratio of developing melanoma on the chest and men on their lower legs, when compared with a study they conducted 1970s (Clark et al., 2007). These new patterns in distribution were attributed to new changes and trends in clothing and lifestyle. Pruthi et al (2009) also suggested that these changes in body site distribution of melanoma over time could be caused by marked changes in the influence of risk factors for melanoma, increased sun- protective practices and skin cancer awareness as well as differences in sun-exposure behaviours.

Interestingly, our study showed that the presence of invasive melanoma differed between males and females, with males having a decreased incidence of invasive melanoma at diagnosis, compared to females (31% and 36% respectively). This particular finding differed from an international retrospective melanoma study, where invasion was significantly more prominent in males than females (Scoggins et al., 2010). A large cohort study of 1906 patients, conducted in the US, showed a Breslow's depth of >2mm in 64% males and >2mm in 36% females, showing a disparity in tumour invasion between the different genders at presentation (Scoggins et al., 2010). 44.6% of our histologically proven cases of melanoma were classified simply as 'malignant melanoma' in the histology reports and were not further subtyped. Of the specimens that had a subtype, Nodular melanoma, which included the subtype malignant melanoma accounted for 63,4% of melanomas diagnosed, and acral lentiginous and lentigo maligna melanoma , both accounted for 11.7%). Nodular melanoma was the most common subtype, by a large margin.

In the city of Johannesburg, Black Africans account for 76.4% , 12.3% are White, 5.6% are Coloured and 4.9% are Asian (Statistics South Africa, 2017). We postulated that this may account for the higher incidence of ALM seen in our study as it is the subtype most common in darker skinned tones (Seleye-Fubara and Etebu ,2005).

Unfortunately, information with respect to race was not available for the present study in order to confirm or exclude this hypothesis. Statistics regarding the racial demographics of individuals attending CMJAH and HJH hospitals were also not available from the hospitals themselves or from online government resources.

Amelanotic melanoma and invasive superficial spreading (SS) accounted for 3.1% of the histologically confirmed melanoma biopsies.

This was out of keeping with most literature published from Europe, Australia and American where SSM, represent almost 70% of all melanomas (Odom et al., 2000).

Only 10 of the histology reports had documented risk factors, which were HIV infection (4 patients) and a previous history of melanoma (6 patients). Immunosuppression as a result of HIV infection, chronic ulceration, inflammation, albinism and UV exposure are identified risk factors for skin cancer in Africans (Seleye-Fubara & Etebu, 2005).

Data on ethnicity was not documented on the histology reports and was thus unavailable; this formed a major limitation of the current study. Drawing conclusions based on race with respect to prevalence would have been very informative, especially when comparing our study with those conducted internationally.

6.2. Limitations

As with all retrospective studies, we were limited by the data recorded in the laboratory reports. Missing data, inconsistencies in data recording by different clinicians and differences in interpretation of cutaneous signs, may lead to under or over biopsy-ing of tumors. Certain subtypes of melanoma, especially lentigo maligna may be easily missed and not biopsied.

Ideally, all patients should have a thorough history taken and the information documented both in the clinical patient file as well as the laboratory report. Any lack of such data is not possible to correct in a retrospective review. The gold standard however, still remains histologic confirmation of cutaneous melanoma.

Data regarding sun exposure was not included in this study, although this is a very important risk factor for development of melanoma. The presence and degree of sun exposure in particular, is open to interpretation. Unfortunately, this also means that this important risk factor was not included.

This review may also be prone to statistical error and some sub- categories that were analyzed, contained small numbers, leading to decreased statistical accuracy.

Comparison of this data, with results of other studies of cutaneous melanoma by other selection criteria (history taking and physical examination etc.) may not be applicable; and the incidence of melanoma in the CMJAH and HJH hospitals should be interpreted with caution, as they may reflect a referral bias.

Chapter Seven: Conclusion

This study was a retrospective record review of 128 patients, with histologically confirmed melanoma skin cancers, based on biopsy specimen results from the National Health Laboratory Service (NHLS).

The mean age of the patient population was 60 years, which is in keeping with international data, where the mean age of diagnosis is between the 5th and the 7th decade. There was a significant difference in the age at diagnosis between men (63.2 years) and women (57.5 years), this was also in keeping with international literature, where females were diagnosed at an earlier age than males.

The anatomic site of melanoma was predominantly on the lower extremities in females as compared to males who predominantly had lesions on their trunk. The numerous studies on melanoma conducted in Australia, Europe and the United States had similar outcomes where women had lesions on the lower extremities and men on the trunk, which was attributed to factors such as protective clothing, sun seeking behavior etc. Of particular interest in our study, was the high prevalence of the ALM and nodular subtypes, which were jointly ranked the most common subtypes.

This is the first study to investigate cutaneous melanomas within the population of CMJAH and HJH hospitals to date, thus providing valuable new information from a South African perspective.

Chapter Eight Recommendations

8.1. Recommendations

8.1.1. Implications for practice

It is recommended that all patients with a clinical suspicion of melanoma, undergo a skin biopsy. All patients with a confirmed diagnosis of melanoma should be notified to the National Cancer Registry of South Africa, in order to have accurate nation wide statistics. Correct documentation of all patient demographics and risk factors in the histopathology forms by the clinician is essential and would enable a more thorough prospective study on melanoma in CMJAH and HJH hospitals.

8.2. Implications for research

The study provides a stimulus to undertake other comparative studies of cutaneous melanoma in South Africa and Africa.

Knowledge of the genetics of melanoma, may assist in the understanding as to why some melanoma subtypes (i.e. ALM) is known to be aggressive and have a worse prognosis. This may form a basis for further studies where targeted molecular studies may be carried out on all patients diagnosed with melanoma in CMJAH and HJH hospitals.

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APPENDICES

Appendix A: Data Collection sheet

Data Collection Sheet

Unique	Sex	Age	Race	Tumour	Histo type	Other risks

Abbreviations

Histo type	Histologic subtypes of melanoma (e.g. Nodular, superficial spreading)
Other risks	Other risk factors for melanoma (e.g. genodermatoses, HIV infection, Immunosuppression), excluding ultraviolet light exposure

Appendix B: Ethics approval- Human Research Ethics Committee (Medical)



R14/49 Dr Awotedu Temitope

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170455

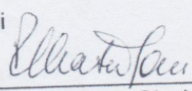
NAME: Dr Awotedu Temitope
(Principal Investigator)
DEPARTMENT: Dermatology
Charlotte Maxeke Johannesburg Academic Hospital
Helen Joseph Hospital
National Health Laboratory Services

PROJECT TITLE: A Descriptive Study of Melanoma Skin Cancers in Patients Attending Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital from July 2013 to June 2016

DATE CONSIDERED: 05/05/2017

DECISION: Approved unconditionally

CONDITIONS:
SUPERVISOR: Prof Deepak Modi

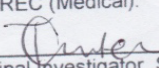
APPROVED BY: 
Professor P. Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 14/06/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

Date

15/6/17

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C: Consent for access to NHLS database

 NATIONAL HEALTH LABORATORY SERVICE
UNIVERSITY OF THE WITWATERSRAND – JOHANNESBURG

SCHOOL OF PATHOLOGY
Division of Anatomical Pathology

 NATIONAL HEALTH
LABORATORY SERVICE

P.O. Box 1038, Johannesburg 2000
Tel : +27-11-489-8477
+27-11- 489-8479
Fax: +27-11-489-8512

Division of Anatomical Pathology
Faculty of Health Sciences
York Road
Parktown e-mail : martin.hale@nhls.ac.za

Professor MJ Hale MBChB (Rhodesia) FCPATH (SA), LRCP, LRCS, LRCP&S (Edinburgh & Glasgow)
Professor & Head: Division of Anatomical Pathology.

Human Research Ethics Committee (Medical)
University of the Witwatersrand
Johannesburg
20000

March 24, 2017

Re: Consent for access to NHLS database

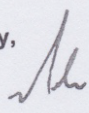
This letter serves to confirm that the Department of Anatomical Pathology at the University of the Witwatersrand and NHLS is happy to assist Dr Temitope Awotedu with her project entitled "A descriptive study of melanoma skin cancers in patients attending Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital from July 2013 to June 2016".

Notwithstanding the requirement that research projects should comprise the researchers work only, it is recognized that publication of such work is encouraged. In the event that the information used comprises the diagnosis only then joint authorship from a member of staff in the Department of Anatomical Pathology would not be expected. However should additional information be extracted from the report for purposes of further interpretation such as morphological details and immunohistochemical profiles, it would be expected that this would be done in conjunction with a member of staff in the Department of Anatomical Pathology and that joint authorship would follow in resulting publications. Dr Awotedu will be in contact with the Department of Anatomical Pathology in respect of this.

Assuring you of the Department of Anatomical Pathology's co-operation in this and future research projects.

With best wishes.

Yours sincerely,



Professor MJ Hale
Head: Department of Anatomical Pathology