Chapter One

Introduction

1.1 Introduction:

Skin cancer is a malignant tumor that starts in cell of the skin and it can be metastasize to other part of the body (Thomas, *etal.* 2008). Skin cancer is divide into two major types melanoma and non melanoma((basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) (Beers and Robert,2004).

Worldwide incidence of non melanoma skin cancer varies widely with the highest rates in Australia and the lowest rates in parts of Africa (Lomas, *et al.* 2012). Non melanoma skin cancer has the highest incidence of all cancers and rise in the rate of cutaneous melanoma exceeds all other preventable cancers. In 2002 an estimated 1.3 million Americans were diagnosed with skin cancer (Geller and Annas, 2003). In Sudan squamous cell carcinoma is the commonest skin malignancy followed by basal cell carcinoma (Samie, *et al*. 2012).

Ultraviolet radiation from sun exposure is the primary cause of skin cancer. Other factors includes smoking tobacco, HIV infections, some genetic syndromes, chronic non-healing wounds, ionizing radiation, environmental carcinogens, artificial UV-radiation, aging and light skin color (Saladi and Persaud, 2005).

Diagnosis of basal cell carcinoma and squamous cell carcinoma growth can be biopsied by various ways, part or all of the growth can be taken with a scalpel for examination under microscope (Jennifer and Robinson, 2012).

They aretwo types of treatment for skin cancerradiation or chemotherapy, forprovide adequate controlin low-risk disease used radiation therapy or topical chemotherapy and cryotherapy, other

treatment can be used like photodynamic therapy, topical chemotherapy, electro desiccation and curettage (Hill, *et al*. 2014).

CK are the intermediate filament (IF) protein family are particularly useful tools in o

ncology diagnostics (Barak,*etal*. 2004). There are two types of cytokeratins basic and acidic or neutral (Levy, *et al*. 2011).

Keratins is long and extensively used as immunohistochemical markers in diagnostic tumor pathology (Moll and Langbein. 2008). HMWCK immunohistochemistry is very helpful in diagnosis of difficult cases in epithelial skin cancer especially basal cell carcinoma and squamous cell carcinoma (Alhumaidi, 2012).

Objectives:

General Objective:

To investigatethe comparison between the expression of high molecular weight cytokeratin (HMWCK) in basal cell carcinoma and squamous Cell carcinoma of skin

Specific Objectives:

1- To detect high molecular weight cytokeratin in skin cancer using immunohistochemical methods.

2- To correlate the expression of high molecular weight cytokeratin with skin cancer types.

Chapter Two

Literature Review

2. Literature Review:

2.1. Scientific background:

Skin cancer is the uncontrolled growth of abnormal cells in the skin (Lewandowski, *et al.* 2014). The incidence of non melanoma skin cancer (NMSC) can determine and the majority of it is basal cell carcinoma which comprises 75% of all NMSC cases, squamous cell carcinoma accounts 20% of NMSC cases (Julie, *et al.* 2007). The average annual number of adults treated for skin cancer increased from 3.4 million in 2002-2006 to 4.9 million in 2007-2011(Gery, *et al.* 2014). Basal and squamous cell carcinoma the most common forms of skin cancer are highly curable if detected early and treated promptly (Neville, *et al.* 2007).

2.2. Anatomy and physiology of skin:

There are three layers of the skin, first epidermis is a stratified squamous epithelium that consists primarily of keratinocytes in progressive stages of differentiation from deeper to more superficial layers(Bardia andGregory,2013). It is harbors a number of cell populations such as melanocytes, langerhans cells and Merkel cells (James, *et al.* 2006). Second dermis is an integrated system of fibrous and the matrix components including collagen and elastic connective tissue (Chu,*et al.* 2008). Third hypodermis is a lobules of fat cells or lipocytes that separated by fibrous septa made up of large blood vessels and collagen (James, *et al.* 2006), it's also contain adipose-derived stem cells that act as source of adipose tissue(Heimburg, *et al.* 2005). Skin have many function include effective barrier to the external environment, allow the transmission of sensory information, thermoregulation, control of

evaporation, storage and synthesis of lipid and water and absorption of gases (Madison, 2003).

2.3. Abnormalities of skin:

2.3.1. Inflammation of skin:

Moles are a pigmented spots on the outer layer of the skin, it can be round, oval, flat or raised, they occur singly or in cluster on any part of the body (Beers and Robert,2004). Eczema is an inflammatory skin disorder affecting the scalp, face, and torso, typically presents with scaly, flaky, itchy and red skin. It particularly affects the sebaceous gland rich areas of skin (Dessinioti and Katsambas, 2013). Acne is a long-term skin condition characterized by areas of blockhead, whitehead, pimples, greasy skin and possibly scarring (Adityan, *et al*. 2009).

2.3.2. Precancerous skin lesions:

Actinic keratosisis common intraepidermal neoplasm mainly occur in areas of chronic sun exposure (Sherrif, *et al*. 2009). Bowen disease is called squamous cell carcinoma in situ which appear asa plaque in covered as well as uncovered skin areas, histopathologically its features full thickness dysplasia and incorporating loss of polarity of the epidermis (Wang, *et al*. 2012).

2.3.3. Malignant lesions of skin:

Non-melanoma skin cancer (NMSC) is one of the most common cancers, although it is a much less dangerous form of skin cancer than malignant melanoma (Madan and Szeimies. 2010). Exposure to sunlight may cause DNA damage (thymine dimer formation) and DNA repair removes most UV-induced damage not all cross-links are excised (Wood, *et al*. 2008).

2.3.3.1. Squamous cell carcinoma:

Squamous cell carcinoma (SCC) is amongst the top three common skin cancers, ranking behind basal cell carcinoma (BCC) and ahead of melanoma (Diepgen and Mahler, 2002). Squamous cell cancer is occur in

undamaged skin or in skin that has been injured or inflamed. The earliest form of squamous cell cancer is called Bowen disease or squamous cell carcinoma in situ. This type does not spread to nearby tissues, actinic keratosis is a precancerous skin lesion that may become a squamous cell cancer (Bhambri, *et al*. 2011).

2.3.3.2. Basal cell carcinoma:

Basal cell carcinoma is starts in the upper layer of the skin called the epidermis. Most skin cancers are basal cell cancer, it is almost always a slow-growing form of skin cancer (Cockerell, *et al.* 2011). Itis a locally invasive malignant epidermal skin tumor, the lesion infiltrates tissues in a three-dimensional fashion, basal cell carcinoma has morbidity results from local tissue invasion and destruction, particularly on the face, head and neck (Braun, *et al*. 2005).

2.3.3.3. Other skin cancer:

2.3.3.3.1 Melanoma:

It is a dark pigmented malignant cell that arising from a skin cell capable of making the pigment melanin (Berrs and Robert, 2004). Melanoma is the most dangerous type of skin cancer. Symptoms of this lesion a mole, sore, lump or growth on the skin can be a sign of melanoma or other skin cancer. A sore or growth that bleeds or changes in color can also be a sign of skin cancer (Bichakjian and Halpern. 2011).

2.3.3.3.2 Kaposi's sarcoma:

It is a malignancy of endothelial skin cells with multifocal localization on the skin, lymph nodes and visceral organs. It is associated with human herpes virus-8 infection (Mesri, *et al*. 2010).

2.4. Signs and symptoms of skin cancer:

Basal cell cancer grows slowly and usually painless. It is appear as skin bump or growth (Pearly or waxy, white or light pink and flesh-colored or brown), in some cases the skin is just slightly raised or even flat

(Cockerell, *et al*. 2011), also it is appears in dome-shaped skin growth with visible blood vessels (Carucci and Leffell. 2008).

The main symptom of squamous cell cancer is a growing bump that may have a rough, scaly surface and flat reddish patches. The earliest form (squamous cell carcinoma in situ) can appear as a scaly, crusted, and large reddish patch that can be larger than 1 inch (Bhambri, *et al.* 2011), also it is appears as a bump or lump that may become dome-shaped or crusty and can bleed, a sore it not heal or heal and return flat (Grossman,*etal*. 2008).

2.5. Risk factor of skin cancer:

2.5.1. Ultraviolet light

Ultraviolet light exposure either from the sun or from tanning beds can damage skin, causes DNA damage and genetic mutations, which subsequently lead to skin cancer (Narayanan, *et al*. 2010). Non melanoma skin cancer is by far the most common human malignancy and nearly 30% of white people living in areas of exposure to high ultraviolet radiation will develop anon melanoma skin cancer in their life time (Mackenzie, *et al*. 2012).

2.5.2. Immunosuppression

A chronically suppressed immune system from underlying diseases such as HIV infection or cancer or from some medications such as prednisone or chemotherapy (Saladi and Persaud, 2005).

2.5.3. Ionizing radiation

It is a potential cause of non melanoma skin canceralso certain types of wart virus infections can cause the same type of skin cancer (Saladi and Persaud, 2005).

2.5.4. Other factors

Other risk factors of skin cancer included basal cell nevus syndrome, xerodermapigmentosum, albinism, epidermodysplasiaverruciformis and

Gardner's syndrome(Lang, *etal*. 2005). Also alcohol consumption, tobacoo smoking (Flavia, *et al*. 2011). Genetic mutation are an important cofactor in development non melanoma skin cancer, discovery of mutation of gene on chromosome 9 underlying basal cell carcinoma (Venura and Vishal, 2012).

2.6. Diagnosis of skin cancer:

2.6.1. Skin biopsy:

Skin biopsies are usually under taken to confirm a clinical diagnosis, to remove a lesion and to determine the adequacy of excised tissue margin. Skin biopsy has many types include shave biopsy is a horizontal section of the skin, removed either for diagnosis or treatment. Punch biopsy is 2-6 mm of tissue removed for diagnostic purpose. Excisional biopsy is a completely cut out lesion for diagnosis and treatment. Incisionalbiopsyis a segment of the lesion removed for diagnosis only. Curette biopsy is a consists of fragments of tissue removed using a spoon shaped bone, its primary for treatment (Richa, *et al*. 2014).

2.6.2. Imaging techniques:

2.6.2.1. Multi photon laser scanning microscopy (MPLSM):

This technique is one of optical imaging technique, it is based on the nonlinear process of 2-photon excitation of endogenous fluorophores, which can be used to acquire horizontal optical sectioning of intact biological tissue samples. Is provides high-resolution fluorescence imaging, allowing visualization of cellular and subcellular structures of the epidermis and upper dermis (Paoli, *et al.* 2008).

2.6.2.2. Real time roman spectroscopy:

It is a noninvasive optical technique capable of measuring vibrational modes of biomolecules within viable tissues, it is also be used to distinguish malignant from benign skin lesions with good diagnostic

accuracy comparable with clinical examination and other optical-based methods (Lui, *et al.* 2012).

2.6.3. Immunohistochemistry:

Is a technique of detecting antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues (Ramos and Miller, 2014), the site of antibody binding can identify either by direct labeling of antibody or by use of a secondary labeling method (Bancroft and Marilyn, 2008).

2.6.4. Tumor marker:

2.6.4.1. High Molecular Weight Cytokeratin:

Keratin intermediate filament are present in essentially all epithelial cells and in neoplasm derived from it. Keratin positivity is a sensitive marker for carcinomas (Bancroft and Marilyn, 2008). They are filamentous proteins which together with other filaments form the eukaryotic cytoskeleton. They have numerous functions including maintenance of epithelial structure, protection from injury and communication with other cytoplasmic components (Folpe and Cooper, 2007).Cytokeratinsare expressed specifically in the cytoplasm of epithelial cells (Apaydin, *et al.* 2005). Basal cell carcinoma has positive HMWCK expression especially in small skin biopsy samples in which morphologic differentiation is difficult (Bedir, *et al.* 2015).

2.7. Staging of skin cancer:

Non melanoma skin cancer with or without the clinical, radiological, pathological evidence of regional or distant metastases are staged into four stages include stage I tumor size 2 cm or less with no metastases, stage II tumor size more than 2 cm with no metastases, stage III any tumor size with one involved lymph node metastases, stage IV any tumor size with two or more involved lymph nodes or multiple and distant metastases (Edge, *et al.* 2010).

2.8. Management of skin cancer:

Choice of treatment approach depends on the tumor's location, size, borders and growth rate. The standard treatment approaches are superficial ablative techniques (electro-desiccation, curettage and cryotherapy) used primarily for low-risk tumors, full-thickness techniques (Mohs micrographic surgery, Excisional surgery and radiotherapy) used to treat high-risk tumors. Removal of the entire tumor is essential to limit and prevent tumor recurrence (Juan, *et al*. 2001).

Chapter Three

Materials and Methods

3. Materials and Methods:

3.1. Materials:

Archive tissue blocks previously diagnosed as skin cancer were used in this study.

3.2. Methods:

3.2.1. Study design:

This is hospital based descriptive retrospective case study aimedtoinvestigate the comparison between the expression of high molecular weight cytokeratin (HMWCK) in basal cell carcinoma and squamous Cell carcinoma of skin

3.2.2. Study samples:

Thirty eight skinblocks were obtained from tissues previously diagnosed as skin cancer at Omdurman Teaching Hospital during the period from January 2015 to July 2016. Patient identification data including age and sex was obtained from patient`s file.

3.2.3. Sample processing:

Section of 3µ in thickness was obtained from each block using rotary microtome, then dewaxed in hot plate oven.

3.2.4. Staining Method:

Immunohistochemical staining:

Paraffin sections were immunostained using modified biogenex indirect technique. Sections were put in hot plate oven and cleared in two change of xylene for two minutes. Then rehydrated through descending concentration of ethanol (100%, 90%, 70%, 50%) and water two minutes each. Then antigen retrieved by temperature buffer for forty minutes, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffer saline (PH7.4) for five minutes. Then treated with

high molecular weight cytokeratin primary antibody for twenty minutes, then rinsed in phosphate buffer saline then treated with secondary polymer conjugate for thirty minutes, then rinsed in phosphate buffer saline, then treated with substrate and 3,3-diaminobenzidene tetra hydrochloride (DAB) chromogen for seven minutes, then washed in phosphate buffer saline, then counterstained in Mayer's haematoxylin for one minute, then washed and blued in running tap water for ten minutes, then dehydrated through ascending concentration of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn, 2008).

3.2.5. Result interpretation:

All quality control measures were adopted during sample staining for immunohistochemical results assessment.

3.2.6. Statistical analysis:

Data were analyzed using version 11.5 SPSS computer program. Frequencies, means and Chi-square test were calculated.

3.2.7. Ethical considerations:

Hospital administration agreements were taken ethically for archive samples and patient data collection.

Chapter Four

4. Results

Thirty eight blocks previously diagnosed as skin cancer were used in this study. 27(71.1%) of samples were squamous cell carcinoma whilst 11(28.9%) samples were basal cell carcinoma (table 4.1). The patient ages ranged between 4-93 years with mean age 55 years, most of them 23(60.5%) were above 50 years and the remaining 15(39.5%) were less than 50 years(table 4.2). The patient sex revealed that 11(28.9%) patients were female and 27(71.1%) patients were male (table 4.3). Positive expression of HMWCK is common among squamous cell carcinoma with frequency 20(52.6%) and less in basal cell carcinoma with frequency 8(21.1%) with insignificant correlation between HMWCK and cancer type (P. value 0.932), negative expression of HMWCK seen in squamous cell carcinoma with frequency 7(18.4%) and basal cell carcinoma with frequency 3(7.9%) (table 4.4). Squamous cell carcinoma of skin shows positive cytoplasmic expression of HMWCK (photograph 4.1) and squamous cell carcinoma of skin shows negative cytoplasmic expression of HMWCK (photograph 4.2).

Table (4.1) Distribution of histopathology diagnosis among study population:

Histopathology diagnosis	Frequency	Percent
Squamous cell carcinoma	27	71.1
Basal cell carcinoma	11	28.9
Total	38	100

Table (4.2) Distribution of age groups among study population:

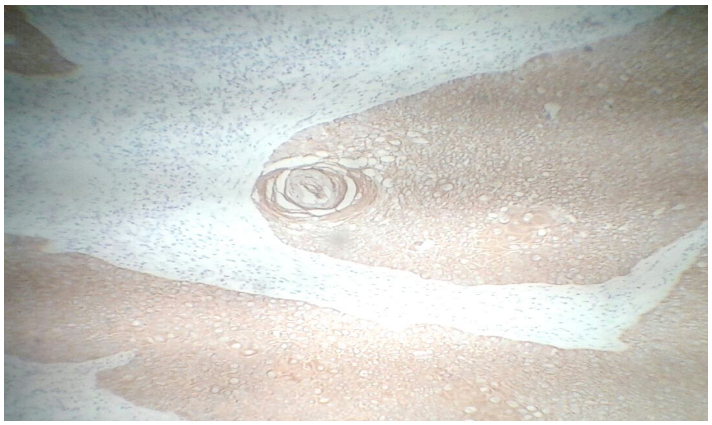
Age group(years)	Frequency	Percent
50>	15	39.5
50<	23	60.5
Total	38	100

Table (4.3) Distribution of sex among study population:

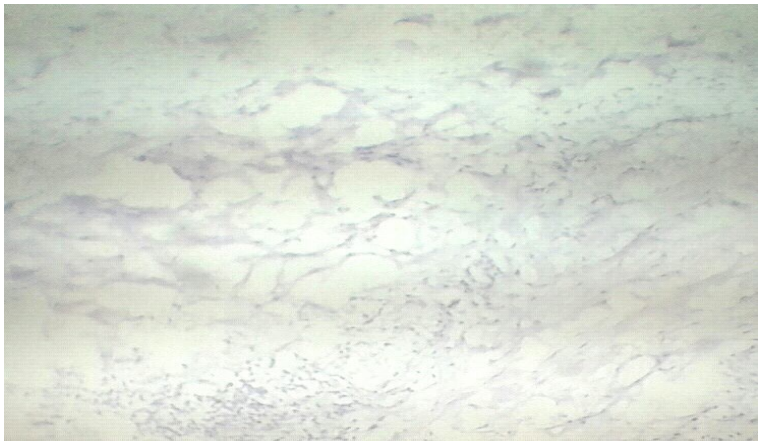
Sex	Frequency	Percent
Male	27	71.1
Female	11	28.9
Total	38	100

Table (4.4) Relation between HMWCK expression and histopathology diagnosis:

Expression of HMWCK	Histopathology Diagnosis		Total	P. Value
	SCC	BCC		
Positive	20(52.6%)	8(21.1%)	28(73.7%)	0.932
Negative	7(18.4%)	3(7.9%)	10(26.3%)	
Total	27(71.1%)	11(28.9%)	38(100%)	



Photograph (4.1): Squamous cell carcinoma of skin shows positive cytoplasmic expression of HMWCK (40x).



Photograph (4.2): Squamous cell carcinoma of skin shows negative cytoplasmic expression of HMWCK (40x).

Chapter Five
5. Discussion

Non melanoma skin cancer (NMSC) represents the most common form of cancer in Caucasians, with continuing increase in incidence worldwide Porcia, (2009). Thirty eight patients previously diagnosed with skin cancer by histopathology were used in this study. In this study the commonest skin cancer is squamous cell carcinoma, this finding is similar to finding described by Samie, *et al.,* (2012), they reported that squamous cell carcinoma was the commonest skin malignancy seen in Sudan constituting for 42.6% of cases followed by basal cell carcinoma it was seen in 32% of patient. The age of patients was aggregate above 50 years would explain that risk of skin cancer increase by age, similar result described by Vries, *et al*., (2005) they reported that number of patients with SCC increase overall by 80% mainly among older males and females, also compatible with the study described by Kumar, *et al*., (2014) they reported that 95% of the skin cancer occur in patients aged more than 40 years. In this study the most patientswere male, this result similar to finding described by Lang, *et al.,* (2005) they reported that men are about twice as likely as women to have basal cell carcinoma and about three times as likely to have squamous cell carcinoma of the skin. The study found that there is insignificant relation between HMWCK expression and histopathological diagnosis, that indicating there is no different in HMWCK expression in squamous and basal cell carcinoma due to HMWCK are expressed specifically in cytoplasm of epithelial cells. This result similar to finding described by Rodney and Miller, (2004) they reported the both SCC and BSC characteristically express strong and diffuse HMWCK.

Chapter Six

6. Conclusion and recommendation

Conclusion:

On basis of this study we conclude the follow:

Squamous cell carcinoma is the most common type of skin cancer in Sudan.

The expression of high molecular weight cytokeratincannot be used for differentiation between squamous and basal cell carcinoma.

Recommendation:

On basis of this study we recommend the follow:

- More efficient marker should be used in order to get better result when using the immunohistochemical method for the comparison between the squamous and basal cell carcinoma of skin.

References

- Adityan, B.,Kumari, R., &Thappa, D.M., (2009). Scoring systems in acne vulgaris, *Indian Journal of Dermatology Venereology and Leprology*, **75** (3):323–326.
- Alhumaidi, A., (2012). Practical immunohistochemistry of epithelial skin tumor, *Indian journal for dermatology verenology and leprology*, **78**(6):698-708.
- Apaydin, R.,Gurbuz, Y.,Bayramgurler, D., &Bilen, N., (2005). Cytokeratin contents of basal cell carcinoma. Epidermis overlying tumorand associated stromal amyloidosis: an immunohistochemical study, *British Journal Dermatology*, **12**(1):41-70.
- Bancroft, J. D., &Marilyn, G., (2008).Theory and practice of histological techniques, 6th edition, London, New York, Oxford Philadelphia St Louis Sydney Toronto, PP.**423-538**.
- Bardia, A., & Gregory G.C., (2013).Textbook of Dermatology, 7th edition, Malden, Blackwell Science, PP.**201**.
- Barak, V.,Goike, H.,Panaretakis, K.W., &Einarsson,R., (2004). Utility of cytokeratins as tumor markers, *ClinBiochemistry*, **37**(7):529-540.
- Bedir, R., Sehitoglu, I.,Yurdakul, C., Saygin, I., Ustuner, P., &Dilek, N., (2015). The Importance of Cytokeratin 19 Expression in the Differentiation of Basal Cell Carcinoma and Trichoepithelioma, *Journal of clinical diagnostic research*, **9**(1): 01-04.
- Beers, M.H., & Robert, B., (2004). The Merck Manual of diagnosis and therapy, section 10, chapter 126, Whitehouse station, Merck Research Laboratories, USA, Rahway, PP.**2752-2758**.

- Bhambri, S., Dinehart, S.,Bhambri, A., Rigel, D.S., Robinson, J.K., &Ross, M., (2011). Cancer of the Skin, 2nd edition, chapter 12, Philadelphia, Elsevier Saunders, PP.**124-139**.
- Bichakjian, C.K., & Halpern, A.C., (2011).Guidelines of care for the management of primary cutaneous melanoma, *Journal American Academy Dermatology,* **65**:1032-1047.
- Braun, R.P., Klumb, F., & Girard, C., (2005). Three-dimensional reconstruction of basal cell carcinomas, *Dermatologic Surgery*. **31**(5):562–566.
- Carucci, J.A., &Leffell,D.J., (2008).Dermatology in General Medicine, *7th edition,* USA, McGraw Hill Medical, pp.**1036-1042**.
- Chu, D.H.K., Wolff, L. A., Goldsmith, S. I., Katz, B. A., Gilchrest, A. S., Paller, D. J., &Leffell.,(2008). Fitzpatrick's dermatology in general medicine, 7th edition, New York, McGraw-Hill, pp.**57-73**.
- Cockerell, C.J., Tran, K.T.,Caruuci, J.,Rigel, D.S., Robinson, J.K., &Ross, M.,(2011). Cancer of the Skin, 2nd edition, chapter 11, Philadelphia, Elsevier Saunders, PP.**99-122**.
- Dessinioti, C., &Katsambas, A., (2013). Seborrheic dermatitis: etiology, risk factors, and treatments: facts and controversies,*Clinic Dermatology*,**31** (4): 343–51.
- Diepgen, T.L., & Mahler, V., (2002). The epidemiology of skin cancer, *British Journal of Dermatology,* **146**(6):1–6.
- Edge, S.B., Byrd, D.R., Compton, C.C., Fritz, A.G., &Greene, F.L., (2010). American Joint Committee Cancer Staging Manual,7thedition, Berlin, Springer, PP.**237-246**.
- FLavia, R.F., luizF.C.N., &Cimento, O. R., (2011). Risk factors for non-melanoma skin cancer in Taubate, Sao Paulo, Brazil a case control study, *Revista da AssociacaoMedicaBrasileira journal*, **57**(4):424-30.

- Folpe, A.l., & Cooper, K., (2007). Best practices in diagnostic immunohistochemistry pleomorphic cutaneous spindle cell tumors,*Archives of Pathology and Laboratory Medicine*, **131**:1517-1524.
- Geller, A.C., &Annas, G.D., (2003). Epidemiology of melanoma and nonmelanoma skin cancer, *Seminars in Oncology Nursing*, **19** (1):2-11.
- Gery, P., Guy, J.R., Steven, R.M.,Donatus, U.E.,& Robin, Y., (2014). Prevalence and cost of skin cancer treatment in U.S, *American Journal of Prevalence Medicine*, **48**(2):183-187.
- Grossman, D.,Leffell, D.J., &Wolff, K., (2008). Squamous cell carcinoma, Dermatology in general medicine, 7th edition, USA, McGraw Hill Medical, pp.**1028-1036**.
- Heimburg, D.V.,Hemmrich, K., Zachariah, S.,Staiger, H., &Pallua, N., (2005). Oxygen consumption in undifferentiated versus differentiated adipogenicmesenchymal precursor cells,*Respiratory Physiology and Neurobiology*, **146**(3):107–116.
- Hill, R.,Healy, B., Holloway, L.,Kuncic, Z.,Thwaites, D., &Baldock, C., (2014). Advances in kilovoltage x-ray beam dosimetry, *Physics in medicine and* biology, **59** (6):183–231.
- James, W. D., Berger, T. G., &Elston, D. M., (2006). Andrews' diseases of the skin, Clinical dermatology, 10th edition, Philadelphia, Elsevier Saunders, PP.**343-352**.
- Jennifer, J.M., & Robinson, A., (2012). Diagnosis and treatment of basal and squamous cell carcinoma, *American Family Physician journal*, **86**(2): 161-168.
- Juan, C., Martinez C.C., &Otly., (2001). The Management of Melanoma and Nonmelanoma Skin Cancer, *AReview for the Primary Care Physician*, **76**(12):1253–1265.

- Julie, A., Neville, EW. D.J., &Leffell., (2007).Management of no melanoma skin cancer By the American Society for Dermatologic Surgery, *Nature Clinical Practice Oncology*, pp.**462-469**.
- Kumar, S., Bharat, B.M., Sandeep, K., Ashish, Y., Navtej, S., &Amarbir, S., (2014). A Study of Basal Cell Carcinoma in South Asians for Risk Factor,*Journal of Skin Cancer*,**9**(17):35-82.
- Lang, P.G., and Maize, J.C.,Rigel, D.S., Friedman, R.J.,&Dzubow, L.M., (2005). Basal cell carcinoma, *Cancer of the skin*, Philadelphia, Elsevier Saunders, pp.**101–132**.
- Levy, G., Purcel, K., DeCherney., Alan, N.,Lauren,G.T., Murphy, L. N., Roman., & Ashley., (2011). Premalignant and Malignant Disorders of the Ovaries and Oviducts, *Current Diagnosis and Treatment Obstetrics & Gynecology*, pp.**848–858**.
- Lewandowski, R., Soyer, P.H., Carole, A., & Mohamed, M., (2014). *Understanding skin cancer*, 2thedition, Australia, pp.**10**.
- Lomas, A.,Leonardi, B.J., &Bath, H.F.B.J., (2012).A systematic review of worldwide incidence of nonmelanoma skin cancer, *Journal of Dermatology*, **166**(5):1069-1080.
- Lui, H., Zhao, J., Mclean, D., &Zeng, H., (2012). Real time Raman spectroscopy for in vivo skin cancer diagnosis, *Journal of Dermatology*, **72**(10):2491-2500.
- Mackenzie, R. W., Melissa, L. S., Mary,M.C., Jiali, H., Abrar, A. Q., &Eleni L., (2012).Indoor tanning and non-melanoma skin cancer systematic review and meta-analysis,*British Journal of Dermatology*,**345**(10):1136.
- Madan, V. L.,&Szeimies, R.M., (2010) Non Melanoma skin cancer, *Lancet oncology*, **375**(975):673-685.
- Madison, K.C., (2003). Barrier function of the skin, *Journal Invest Dermatology*, **121** (2): 231–41.

- Mesri, E.A., Cesarman, E., &Boshoff, C., (2010). Kaposi sarcoma and its associated herpesvirus, *Journal Natures reviews cancer*, **10**:707-719.
- Moll, R., &Langbein, L., (2008).The human keratins biology and pathology, *HistochemestiryCell Biology*, pp.**705–733**.
- Narayanan, D.L.,Saladi, R.N., & Fox, J.L., (2010). Ultraviolet radiation and skin cancer, *International journal of dermatology*, **49**(9):978-986.
- Neville, J.A., Welch, E., &Leffell, D.J., (2007). Management of nonmelanoma skin cancer, *Journal Natures Clinical Practice Oncology*, **4**(8):462-479.
- Paoli, J., Smedh, M., & Ericson, M.B., (2008). Multi photon laser scanning microscopy a novel diagnostic method for superficial skin cancers, *British Journal dermal*, **28**(3):190-195.
- Porcia, T., (2009). Skin cancer in skin color, *Dermatology Nursing*, **206** (4): 170 – 77.
- Ramos V.J.A., &Miller, M.A., (2014). When Tissue Antigens and Antibodies Get Along Revisiting the Technical Aspects of Immunohistochemistry The Red, Brown, and Blue Technique, *Veterinary Pathology Journal*, **51**(1):42-87.
- Richa, R., Lavleen, S.,Sudheer, K. A., &Manoj K.S., (2014). Margins in skin excision principles and guidelines, *Indian journal of dermatology*, **59**(6):567-570.
- Rodney, T., &Miller, M.B., (2004). Immunohistochemistry in the differential diagnosis of cutaneous basal cell carcinoma and cutaneous squamous cell carcinoma, *Modern Pathology*, **8**(8): 854-858.

- Saladi, R.N., &Persaud, A.N., (2005).The causes of skin cancer: a comprehensive review, journal *Drugs of today, Barcelona, Spain,* **41** (1): 37–53.
- Samie, A., Abdalla, M., Shaddad M.M., Muawia, A.H., Mohammed, O.H.E., & Kamal E.M. H., (2012).Skin cancer in dark skin in Sudan, *Journal of advance management science,***02**(01):08-12.
- Sherrif, F., Ibrahim, A., Marc,D., &Brown, C., (2009). Actinic keratosis, *journal clinical and aesthetic dermatology*, **2**(7):43-48.
- Thomas, V.D., Aasi, S.Z., & Wilson, L.D., (2008). Principles and practice of oncology, 8th edition, Philadelphia PP.**1863-1887**.
- Vries, E.D., Van, D.P.,Louwman, F.R.,Gruijl, D., &Coebergh, J.W., (2005). Prediction of skin cancer incidence in the Netherlands up to 2015, *British Journal of Dermatology*, **152**(3):481-488.
- Venura, S., &Vishal, M., (2012). Non melanoma skin cancer, *journal of cutaneous and aesthetic surgery*, **5**(1):3-10.
- Wang, E.C.,Kwah, Y.C., Tan, W.P., Lee, J.S., & Tan, S.H., (2012). Extramammary Paget disease immunohistochemistry is critical to distinguish potential mimickers,*Dermatology Online Journal*, **18**(9):4.
- Wood, G.S.,Gunkel, J., Stewart, D.,Abeloff, M.,Armitage, J.O.,&Niederhuber, J.E., (2008). No melanoma skin cancers basal cell and squamous cell carcinomas, Abeloff's clinical oncology, 4th edition, Philadelphia,Churchill Livingstone, pp.**1253–1270**.

Appendices

Instrument and Materials:

Instrument:

Rotary microtome.

Caplin jar.

Oven.

Staining racks.

Coated slides.

Water bath.

Cover glass.

Dako pen.

Materials:

Mayer`s haematoxylin component:

Haematoxylin powder 1gm

Potassium alum or ammonium alum 50gm

Sodium iodate 0.2gm

Citric acid 1gm

Chloral hydrate 50gm

Distilled water 1000ml

Ammoniated water:

Concentrated ammonia 0.05ml

Tap water 99.95ml

1% Eosin.

Xylene.

Ethyl alcohol (absolute, 90%, 70%, 50%).

Distell water.

Peroxidase blocker.

Anti HMWCK antibodies (primary antibody).

Dextran polymer conjugate secondary antibodies and Horse Reddish Peroxidase.

3.3 di amino benzidine tetra hydrochloride in substrate buffer.

DPX mounting media.

Phosphate buffer (PH7.4).

Citrate buffer (PH6.8).