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, et al. 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 are two types of treatment for skin cancer radiation or chemotherapy, for provide 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 oncology diagnostics (Barak, et al. 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 investigate the 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.
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 and Gregory, 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 keratosis is a 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 as a 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). It is 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 a non 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 cancer also 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, epidermodyplasiaverruciformis and

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. Incisional biopsy is 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). Cytokeratins are 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 aimed to investigate 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 tetrahydrochloride (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:

<table>
<thead>
<tr>
<th>Histopathology diagnosis</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>27</td>
<td>71.1</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>11</td>
<td>28.9</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (4.2) Distribution of age groups among study population:

<table>
<thead>
<tr>
<th>Age group(years)</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>50&gt;</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td>50&lt;</td>
<td>23</td>
<td>60.5</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (4.3) Distribution of sex among study population:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27</td>
<td>71.1</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>28.9</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>
Table (4.4) Relation between HMWCK expression and histopathology diagnosis:

<table>
<thead>
<tr>
<th>Expression of HMWCK</th>
<th>Histopathology Diagnosis</th>
<th>Total</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC</td>
<td>BCC</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20(52.6%)</td>
<td>8(21.1%)</td>
<td>28(73.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>7(18.4%)</td>
<td>3(7.9%)</td>
<td>10(26.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>27(71.1%)</td>
<td>11(28.9%)</td>
<td>38(100%)</td>
</tr>
</tbody>
</table>
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).
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.
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 cytokeratin cannot 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


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).