CHAPTER ONE
INTRODUCTION

1.1 Introduction:
Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. One of the most common symptoms of cervical cancer is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer has progressed to an advanced stage (Kumar, et al. 2007). Cervical cancer is the third most commonly diagnosed cancer worldwide and the fourth leading cause of cancer death in women (Duenas, et al. 2014). It affects about 16 per 100,000 women per year and kills about 9 per 100,000 per year (Jemal, et al. 2011).
Approximately 80% of cervical cancers occur in developing countries (Kent, 2010). Cervical cancer death rates have been decreasing, but the disease still accounted for 200,000 deaths in 2010; in developing countries, 46,000 of these women were aged 15-49 years, and 109,000 were aged 50 years or older (Forouzanfar, et al. 2011).
Infection with some types of human papilloma virus (HPV) is the greatest risk factor for cervical cancer, followed by smoking. Other risk factors include human immunodeficiency virus, oral contraceptive use, hormone replacement therapy use and previous cancer (Gadducci, et al. 2011).
Not all of the causes of cervical cancer are known, however, several other contributing factors have been implicated (Stuart and Ash, 2006).
The Papa. Nicola smear can be used as a screening test for pre cancers and cancer, but do not make a final diagnosis of cervical cancer. Confirmation of the diagnosis of cervical cancer or pre-cancer requires a biopsy of the cervix; this is often done through colposcopy (Noller, 2012).
Treatment usually consists of surgery (including local excision) in early stages, and chemotherapy and/or radiotherapy in more advanced stages of the disease (Petignat and Roy, 2007).

Bcl-2 is a proto-oncogene situated in the inner mitochondrial membrane. It is a 25 KD protein with 239 amino acids which protects the cells from apoptosis and is localized on the long arm of the 18th chromosome (Hockenbery, et al. 1990).

Bcl2 over-expression is present in premalignant and malignant lesions of cervix. It has been suggested that bcl2 may play a vital role in a relatively early stage of cervical cancer. Bcl2 positivity has also been shown to confer a better 5 year survival rate and prognosis (Dimitrakakis, et al. 2000).

Bcl2 is responsible for the prevention of apoptotic cell death in several situations. Inappropriate expression of bcl-2 may prolong survival of defective and harmful cells, including those involved in HPV infection, thus increasing the probability of malignant conversion (Green, et al. 1994).
1.2 Objectives:

1.2.1 General objective:
-To detect the expression of bcl-2 in cervical tumors using immunohistochemistry.

1.2.2 Specific objectives:
-To correlate between bcl-2 expression with histological diagnosis and cancer grade.
CHAPTER TWO
LITERATURE REVIEW

2.1 Anatomy of the cervix:
The cervix is part of the uterus and is part of the female reproductive system. It is situated between the vagina and the body of the uterus. The cervix has a central canal, which has an external opening to the vagina, and an internal opening to the uterus. These structures are also known as the external os and internal os respectively. The part of the cervix in the vagina is called the ectocervix and the part within beyond the external opening and vagina is known as the endocervix (Darke, et al. 2005). The cervix is about 2–3 centimeters (0.8–1.2 in) in length (kurman and Robert, 1994).

Cells that line the endocervical canal are glandular cells that produce mucus. They are simple columnar cells because they are tall and shaped like columns. Cells that line the ectocervix and vagina are flat and scaly and are stratified squamous cells. The columnar cells join the squamous cells in an area of the cervix called the squamo-columnar junction. This is also called the transformation zone (TZ) because the tall columnar cells are constantly being changed into flat squamous cells, especially during puberty and child-bearing years. It is in this TZ that precancerous changes occur and most cervical cancers start (Randall, et al.2005).

2.2 Benign tumours:
2.2.1 Polyps:
These are the most common benign neoplasms of the cervix (found in 4% of the gynaecological population). These are classified into two types:

2.2.1.1 Endocervical polyps: they are usually found in the fourth to sixth decade of life. They are cherry red lesions which may be single or multiple and may appear as a pedunculated lesion on a stalk of varying length (Stamatellos, et al.2007).
2.2.1.2 Cervical polyps:
They are equally benign and tend to occur as single, smooth grey-white lesions that bleed easily if touched (Stamatellos, et al. 2007).

2.2.2 Fibroids (myoma/leiomyoma):
These smooth, firm masses are often solitary and account for about 3-8% of uterine myomata. A fibroid growing down into the cervix from higher up in the uterus is a more common situation. They tend to be small (5-10 mm in diameter). Enlargement causes upward displacement of the uterus and the fibroid may become impacted. Other symptoms also relate to its size and exact location: dysuria, urgency, obstruction and dyspareunia (Shaw, et al. 2003).

2.2.3 Cervical endometriosis:
Endometriosis in the cervix is relatively common and usually considered to be inoffensive. This may be apparent as blue-red or blue-black lesions 1-3 mm in diameter which may have been implanted during childbirth or surgery. Occasionally, it can cause post coital bleeding and it may present as a mass (Yokota, et al. 2008).

2.3 Malignant tumors of the cervix:
The two main types of cervical cancer are:

2.3.1 Squamous cell carcinoma:
Squamous cell carcinoma (SCC) starts in the flat, scaly cells called squamous cells that line the outer surface of the cervix (ectocervix), mostly in the transformation zone. They account for about 80% to 90% of all cervical cancers. SCC usually develops from precancerous changes in the cervix (Tewari, et al. 2006).

2.3.2 Adenocarcinoma:
Adenocarcinoma starts in the glandular cells of the endocervix, it accounts for about 10% of cervical cancers. They occur more often in younger women.
Adenocarcinoma usually develops from precancerous changes in the cervix and may cause the cervix to become enlarged or barrel-shaped (Tewari, *et al.* 2006).

### 2.3.3 Rare cervical tumors:

Rare malignant cervical tumors make up less than 5% of all cervical cancers such as adenosquamous carcinoma, contains features of both adenocarcinoma and squamous cell carcinoma. It accounts for 3–5% of all cervical cancers and can affect women of any age (Tewari, *et al.* 2006).

### 2.4 Epidemiology of cervical cancer:

Cervical cancer is the third most commonly diagnosed cancer worldwide and the fourth leading cause of cancer death in women (Duenas, *et al.* 2014). Cervical cancer causes around 266,000 deaths worldwide (WHO, 2012). The mortality rate is ten times higher in developing countries compared with the UK. Over a period of 20 years, the incidence of cervical cancer has reduced by a third and mortality by a half. It has been proven that the cervical screening programme is associated with improved rate of cure of cervical cancer (Andrae, *et al.* 2012).

### 2.5 Risk factors of cervical cancer:

#### 2.5.1 Human papilomavirus (HPV):

Virtually all (99.7%) cervical cancers are caused by persistent infection with a high-risk type of HPV. There are approximately 15 high-risk (oncogenic) types of HPV, with just two of these, 16 and 18, responsible for about 70% of all cervical cancers (Munoz, *et al.* 2003). The rate of persistent high-risk infection for women older than age 55 is 50%, compared with a persistence rate of 20% in women younger than age 25 (Castellsagué, *et al.* 2011).

#### 2.5.2 Smoking:

Smokers are susceptible to many cancer-causing chemicals that affect organs other than the lungs. These harmful substances are absorbed through lungs and carried in the blood stream throughout the body. Women who smoke are about twice as
likely as non-smokers to get cervical cancer. Tobacco by products has been found in the cervical mucus of women who smoke. Smoking also makes the immune system less effective in fighting HPV infections (Plummer, et al. 2003).

2.5.3 Oral contraceptives:
The long-term use (5 or more years) of oral contraceptives has been shown to increase the risk of developing cervical cancer. A collaborative analysis of data from 24 epidemiological studies found that risk increases with duration and declines after use ceases. After 10 or more years of cessation, risk appears to return to that of normal (Appleby, et al. 2007).

2.5.4 Sexual activity:
Most women at risk for cervical cancer are those with a history of multiple sexual partners, sexual intercourse at age 17 years or younger, or both. A woman who has never been sexually active has a very low risk for developing cervical cancer (Cherath, et al. 2006).

2.5.5 Age at first full-term pregnancy:
Cervical cancer risk among parous women is 77% higher in those under 17 years old at their first full-term pregnancy, compared with those aged 25 or older (Gierisch, et al. 2013).

2.5.6 Genetic susceptibility:
Women who have an affected first-degree biologic relative have a 2-fold relative risk of developing a cervical tumor compared with women who have a non biologic first-degree relative with a cervical tumor. Genetic susceptibility accounts for less than 1% of cervical cancers (Galloway, 2003).

2.5.7 Previous cancer:
Cervical cancer develops in under 1% of young women with carcinoma in situ per year. Cervical cancer risk is higher in survivors of vaginal and vulval, kidney, urinary tract, or skin cancers (Gadducci, et al. 2011).
2.6 Diagnosis of the cervical cancer:

2.6.1 Papanicolaou test:
The Papanicolaou test is a method of cervical screening used to detect potentially pre-cancerous and cancerous processes in the endocervical canal (transformation zone) of the female reproductive system (Follen, et al. 2004).

2.6.2 Biopsy:

2.6.2.1 Colposcopic biopsy:
This biopsy is done during colposcopy. A local anesthetic (freezing) used to numb the cervix. Biopsy forceps are used to remove small amounts of tissue from suspicious-looking areas, mainly in the lower part of the cervix and vagina. The procedure may cause mild cramping or pain, and there may be some light vaginal bleeding after colposcopic biopsy (Eifel, et al. 2011, Randall, et al. 2013).

2.6.2.2 Endocervical curettage:
This biopsy is done during colposcopy. A local anesthetic may be used to numb the cervix. A narrow, spoon-shaped tool called a curette is used to gently scrape cells and tissue from the endocervical canal. The procedure may cause mild cramping or discomfort (similar to menstrual cramps), and there may be some light vaginal bleeding after endocervical curettage (Eifel, et al. 2011, Randall, et al. 2013).

2.6.2.3 Cone biopsy (cervical conization):
A cone biopsy removes a cone-shaped piece of tissue from the cervix. The cone is formed by removing the outer part of the cervix closest to the vagina and part of the endocervical canal. There are 3 ways to do a cone biopsy: The loop electrosurgical excision procedure (LEEP) uses a thin wire loop heated by an electrical current to remove cervical tissue. Cold-knife excision uses a surgical scalpel to remove cervical tissue. Laser excision uses a laser to remove cervical tissue (Eifel, et al. 2011, Randall, et al. 2013).

2.7 Treatment of the cervical cancer:
2.7.1 Surgery:
An operation to remove the cervix and uterus (hysterectomy) is a common treatment. If the cancer is at an early stage surgery alone can be curative. In some cases, where the cancer is at a very early stage, it may be possible to just remove the part of the cervix affected by the cancer without removing the entire uterus. If the cancer has spread to other parts of the body, surgery may still be advised, often in addition to other treatments. Even if the cancer is advanced and a cure is not possible, some surgical techniques may still have a place to ease symptoms (Petignat and Roy, 2007).

2.7.2 Radiotherapy:
It is a treatment which uses high-energy beams of radiation which are focused on cancerous tissue. This kills cancer cells, or stops cancer cells from multiplying. Radiotherapy alone can be curative for early-stage cervical cancer and may be an alternative to surgery. For more advanced cancers, radiotherapy may be advised in addition to other treatments (Noller, et al. 2012).

2.7.3 Chemotherapy
This is a treatment using anti-cancer drugs which kill cancer cells, or stop them from multiplying. Chemotherapy may be given in addition to radiotherapy or surgery in certain situations (Andrae, et al. 2012).

2.8 Bcl-2:
The bcl-2 gene is located at chromosome 18 q 21 and its product is a 25 kilodalton protein localized in nuclear envelope, endoplasmic reticulum and outer mitochondrial membrane (Zavrides, et al. 2005).

The bcl-2 family of related proteins is one of the key regulators of the apoptotic process. It consists of two opposing groups of proteins: death antagonists (bcl-2, bcl-XL, Mcl-1) and death agonists (bax, bak, bcl-XS) (Gross, et al. 1999). Apoptosis occurs through competing dimerization between the two protein groups.
The relative proportions of which ultimately control the sensitivity or resistance of cells to apoptotic stimuli (Schlesinger, et al. 1997).

The bcl-2 protein is characterized for being an intracellular membrane protein that does not allow cell death (apoptosis) in several situations. An inadequate bcl-2 expression might extend the life-time of the damaged cells, thus increasing significantly the probability of malignant transformation. In addition, the bcl-2 super-expression can interrupt the arrest in G1 stage of the cell cycle, mediated by P53, therefore inhibiting apoptosis (D’Angelo, et al. 2011).

Overexpression of bcl-2 and their prognostic significance have been reported in several epithelial cancers (Schlesinger, et al. 1997).

Bcl2 overexpression is present in premalignant and malignant lesions of cervix. It has been suggested that it play a vital role in a relatively early stage of cervical tumorigenesis in association with HPV infection. Bcl2 positivity has also been shown to confer a better 5 year survival rate (Dimitrakakis, et al. 2000).

Two studies of bcl-2 expression and cervical cancer have been found 61 to 63 percent of invasive cervical cancer to have bcl-2 over expression, and in both studies, this correlated with increase overall survival (Tjalma, et al. 1998).

Other studied of bcl-2 expression and cervical cancer showed; there are 12 of the 27 samples stained positively (44%). This positively increase in early stage (grade one) as 50%, this correlated indicated that Bcl-2 is good to using as prognostic marker for cervical cancer (Sema, et al. 2001). Also Tang et al. showed that the over expression of Bcl-2 protein was activated in the early stage of cervical cancer (Tang, et al. 2003).

Rajkumar et al. studied bcl-2 expression as immunohistochemistry techniques in 40 cases, Bcl-2 was expressed in 65% of cervix squamous cell carcinoma (Rajkumar, et al. 1998).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:
Archived tissue blocks obtained from samples cervical tumors were used in this study.

3.2 Methods:

3.2.1 Study design:
This is a hospital based descriptive retrospective case study aimed to detect expression of bcl-2 tumor marker in cervical tumor using immunohistochemical method.

3.2.2 Study samples:
Forty tissue blocks were obtained from cervical tumors, thirty samples were previously diagnosed as malignant cervical tissues and ten samples were diagnosed as benign cell. Patients identification data were obtained from patients files.

3.2.3 Study area:
This study held in Omdurman Military hospital, Ribat teaching hospital and Soba teaching hospital during period from April to August 2014.

3.2.4 Sample processing:

3.2.4.1 Immunohistochemical staining:
The section of 3µm thickness were obtained from formalin fixed paraffin embedded tissue using a rotary microtome, then immunostained using monoclonal antibodies by new indirect technique as follows:
Sections were dewaxed in hot oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then Ag retrieval by water bath retrieval technique for thirty minutes at 97°C (coplin jar contain citrate buffer PH 6.0), then washed in phosphate buffer saline (PH 7.4) for five minutes, then
section use circulated by Dako pen, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffered saline (PH 7.4) for five minutes, then treated with anti Bcl-2 (Bcl-2 alpha Ab-1) primary antibody for thirty minutes, then rinsed in phosphate buffered saline (PH 7.4), then treated with secondary polymer conjugated antibody for thirty minutes, then rinsed in phosphate buffer saline (PH 7.4), then treated with DAB for seven minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then counter stained in Mayer’s haematoxylin for one minutes, then washed and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn, 2010).

3.2.5 **Result interpretation:**
Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.
All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining.

3.2.6 **Data analysis:**
Data analysis was done using SPSS 11.5 computer program. Frequencies mean and chi-square test values were calculated.

3.2.7 **Ethical consideration:**
Sample collected after taking ethical acceptance from hospital administration.
CHAPTER FOUR  
RESULTS

A total of 40 samples of patients with cervical tumors were investigated, 30 of them were malignant cervical tumors representing 75%, and the remaining 10 (25%) were benign as indicated in table (4.1).

The age of study population ranged between 30 to 85 years with mean of age 55 years. subject less than 70 years were 26 (65%), and older than 70 years were 14 (35%), as indicated in table (4.2).

The description of tumor grade revealed that well differentiated tumor in 4 (13.3%) samples, moderately differentiated tumor in 12 (40%) samples, poorly differentiated tumor in 12 (40%) samples. And not graded in 2 sample (6.7%) as indicated in table (4.3).

Malignant cervical tumors revealed positive expression of Bcl-2 in 12 (40%) samples and negative expression in 18 (60%) samples, while all benign tumors showed negative expression of Bcl-2. This result showed significant statistical association (P.value=0.02) as indicated in table (4.4).

Out of 12 positive samples of Bcl-2, 11 were SCC and one was AC and negative in 18 samples, 17 were SCC and one was AC. With no significant association (P.value=0.78) as indicated in table (4.5).

The comparison between Bcl-2 expression and the grade of tumor showed that Bcl-2 expression was positive in 2 well differentiated tumor, 5 moderately differentiated tumor and 4 poorly differentiated tumor. With insignificant association (P.value=0.55) as indicated in Table (4.6).
Table (4.1): Distribution of sample among the study population:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>30</td>
<td>75%</td>
</tr>
<tr>
<td>Benign</td>
<td>10</td>
<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.2): Distribution of age group among study population:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 70 years</td>
<td>26</td>
<td>65%</td>
</tr>
<tr>
<td>More than 70 years</td>
<td>14</td>
<td>35%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.3): Distribution of cancer grade among malignant cervical tumor:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated tumors</td>
<td>4</td>
<td>13.3%</td>
</tr>
<tr>
<td>Moderately differentiated tumors</td>
<td>12</td>
<td>40%</td>
</tr>
<tr>
<td>Poorly differentiated tumors</td>
<td>12</td>
<td>40%</td>
</tr>
<tr>
<td>Not graded</td>
<td>2</td>
<td>6.7%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.4): Relation between Bcl-2 expression and histopathology diagnosis:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td>Malignant</td>
<td>12</td>
<td>18</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>28</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.5): Relation between Bcl-2 expression and type of cervical cancer:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bcl-2 expression</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>11</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>AC</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>18</td>
<td>30</td>
</tr>
</tbody>
</table>
Table (4.6): Relation between Bcl-2 expression and the grade of cervical cancer:

<table>
<thead>
<tr>
<th>grade</th>
<th>Bcl-2 expression</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Well differentiated tumors</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Moderately differentiated tumors</td>
<td>5</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Poorly differentiated tumors</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>17</td>
<td>28</td>
</tr>
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</table>
Graph (4. 1): Cervical squamous cell carcinoma show positive expression of Bcl-2
Graph (4.2): Cervical squamous cell carcinoma show negative expression of Bcl-2
CHAPTER FIVE
DISCUSSION

In this study patients age ranging between 30-85 years, with mean age 55 years, indicating that increasing age is associated with high risk of developing cervical tumors probably due to hormonal disturbance that may initiate such type of tumors. A similar result was observed by Tjalma et al (1997), their study reported that the mean age of patients ranging between 18-80 years was 51 years.

The samples revealed that 28 (70%) were squamous cell carcinoma, while 2 (5%) were adenocarcinoma, our finding revealed that most malignant cervical tumors were squamous cell carcinoma representing 28/30, this finding was consistent with the result of Tewari et al (2006), who reported that cervical squamous cell carcinoma is the most frequent cervical cancer subtype internationally.

Bcl-2 is a mitochondrial protein associated with anti-apoptotic function. Over expression of Bcl-2 is found to be in a variety of tumor due to degradation of Bcl-2. In this study over expression of Bcl-2 is observed in malignant cervical tumors 12/30, while benign cervical tumors showed no expression of Bcl-2. This relation showed significant association (P.value=0.02) this finding is compatible with results observed by Semaet al (2001), in their study in Bcl-2 expression in carcinoma of the cervix only 12 (44%) out of 27 showed cytoplasmic Bcl-2 expression.

Bcl-2 positive samples were compared with the type of tumor and we found that 11/12 positive samples were diagnosed as cervical squamous cell carcinoma, and 1/12 was adenocarcinoma. With insignificant relationship between each type of malignancy and the Bcl-2 positive results (P.value=0.78) and this was contradicted with results obtained by Shukla et al (2014) who reported that a higher percentage
of adenocarcinomas were positive for bcl-2 compared with squamous cell carcinomas (66.67% vs. 33.33%).

Bcl-2 expression and grade of cancer showed that positive expression in 2/2 of well differentiated tumors, 5/12 of moderately differentiated tumors and 4/12 of poor differentiated tumors, this relation showed insignificant association (P.value=0.55) indicating that rising of cancer grade is not affected by the Bcl-2 dysfunction. This finding is compatible with result observed by Shukla et al (2014) reported that 50% of well differentiated and 33.3% moderately differentiated carcinoma were bcl-2 positive, but the difference was not statistically significant (P.value=0.622). Incompatible result observed by Grace et al (2003) showed an increasing expression of bcl-2 with the rising grade of cervical cancer.
6.1 Conclusion:
On the basis of this study we conclude that:
There is association between Bcl-2 expression and malignant tumors of cervix, with no association with type of cancer.
The expression of Bcl-2 is not associated with histological differentiation of cancer.

6.2 Recommendations:
On the basis of this study we recommend that:
More studies should be conducted with large sample size.
Bcl-2 could be used as marker for differentiation between malignant and benign cervical tumors.
Molecular detection of tumor markers and HPV should be done.
Other tumor markers should be applied for cervical tumors.
References


Ki67 and bcl-2 Identify Two Groups with Different Prognosis. Gynecologic Oncology, 121, 328-333.


Materials and Instruments

Materials and instruments used for processing and staining of the specimens include:

- Disposable gloves
- Rotary microtome
- Microtome knives
- Coated slides
- Cover glasses
- Oven
- Water path
- Paraffin block
- Humidity chamber
- Paraffin block
- Ethanol (100%, 90%, 70%, 50%)
- Xylene
- Mayer’s haematoxylin: (1gm haematoxylin, 50gm aluminum ammonium sulfate, 0.2 sodium iodate, 50gm chloral hydrate, 1gm citric acid and 1 liter distilled water)
- Sodium citrate buffer: (10 Mm Sodium citrate, 0.05% Tween 20, PH 6.0 prepared as the following: Tri-sodium citrate (dehydrate) 2.94 g, 1L DW mix to dissolve and add 0.05 ml of Tween 20 and mix)
- Phosphate buffer (PH 7.4)
- Peroxidase blocking solution
- Primary antibody Bcl-2
- Secondary antibody
- DAB (3.3 diaminobenzidine)
- DAB substrate buffer